RESEARCH ARTICLE



# Pseudanthias hangapiko, a new anthiadine serranid (Teleostei, Serranidae, Anthiadinae) from Rapa Nui (Easter Island)

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Academic editor: David Morgan   Received 16 February 2021   Accepted 15 June 2021   Pu	ıblished 3 August 2021
http://zoobank.org/0629BFCB-ADBC-41DB-B6F5-298BE719AA7C	

**Citation:** Shepherd B, Pinheiro HT, Phelps TAY, Pérez-Matus A, Rocha LA (2021) *Pseudanthias hangapiko*, a new anthiadine serranid (Teleostei, Serranidae, Anthiadinae) from Rapa Nui (Easter Island). ZooKeys 1054: 1–13. https://doi.org/10.3897/zooKeys.1054.64508

# Abstract

*Pseudanthias hangapiko* **sp. nov.** (Teleostei, Serranidae, Anthiadinae) is herein described from three specimens collected from a depth of 83 m in a mesophotic coral ecosystem off Hanga Piko, Rapa Nui (Easter Island), Chile. *Pseudanthias hangapiko* **sp. nov.** can be distinguished from its congeners in live coloration and by the following combination of characters: dorsal-fin rays X, 17; anal-fin rays III, 8; pectoral-fin rays 16 (left side of one specimen 17); vertebrae 10+16; scales relatively large, two scales above lateral-line to base of fifth dorsal spine, and 16–17 circumpeduncular scales; gill rakers 11+23; and a slender body, with greatest body depth 3.6 (3.4–3.8) in SL. The most similar DNA barcodes (mitochondrial COI gene) are from *Pseudanthias ventralis* Randall, 1979 and *Pseudanthias hawaiiensis* Randall, 1979, with 16.8% and 17.0% uncorrected divergence, respectively. This fish is one of four new species that were documented from a pair of technical dives at a single location in Rapa Nui, emphasizing the high number of undescribed species likely still unknown in mesophotic coral ecosystems, especially in geographically remote locations. *Pseudanthias hangapiko* **sp. nov.** adds to the Rapa Nui ichthyofauna, which hosts the second-highest level of endemism in both shallow and deep-water fishes.

#### Keywords

Biodiversity, coral-reef twilight zone, ichthyology, island, mesophotic coral ecosystem, reef fish, taxonomy

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# Introduction

The genus Pseudanthias Bleeker, 1871 (Teleostei, Serranidae, Anthiadinae) currently comprises more than sixty valid species found globally in temperate and tropical oceans (Allen and Erdmann 2012). Many members of the genus are protogynous hermaphrodites, and most can be easily identified by the sexually dichromatic coloration of living adult specimens (Kuiter 2004). They are conspicuous inhabitants of coral reef environments and mesophotic coral ecosystems (MCEs), occurring in large haremic aggregations and feeding on zooplankton (Allen and Erdmann 2012). The highest diversity of *Pseudanthias* species is recorded in the coral triangle, with nearly 30 described species occurring in Indonesia and the Philippines alone (Allen and Erdmann 2012). Moving eastward out of the coral triangle and into the South Pacific, diversity within the genus decreases, and the relative proportion of endemic species increases, following the general pattern observed in other groups (Randall 1998; Allen and Erdmann 2012). Most Pseudanthias species occur on MCEs (Rocha et al. 2018), where several new species have been recently described (Gill et al. 2017; Gill and Psomadakis 2018; Victor et al. 2020), a trend in discoveries that results from increased exploration of the deeper sections of coral reef habitats (Rocha et al. 2018; Pinheiro et al. 2019; Pimentel et al. 2020).

Through a partnership between the Pontificia Universidad Católica de Chile and the Hope for Reefs Initiative of the California Academy of Sciences, our team conducted surveys using technical diving and closed-circuit rebreathers (Hollis Prism 2) to depths of 110 m to identify and document the fish communities associated with MCEs at Rapa Nui. Here we describe a new species of *Pseudanthias* collected in a MCE off Hanga Piko, Rapa Nui (Easter Island), Chile in March 2017. This is the fourth species description resulting from this expedition to Rapa Nui, and the sixth member of the subfamily Anthiadinae to be found at the island. Caprodon longimanus Günther, 1859, Plectranthias parini Anderson & Randall, 1991, P. ahiahiata Shepherd, Phelps, Pinheiro, Pérez-Matus & Rocha, 2018, and Luzonichthys kiomeamea Shepherd, Pinheiro, Phelps, Pérez-Matus & Rocha, 2019 are the other described Anthiadinae known from Rapa Nui; a presumed Tosanoides has also been observed with remotely operated vehicles but has yet to be captured and examined (Easton et al. 2017). These species, along with Chromis mamatapara Shepherd, Pinheiro, Phelps, Easton, Pérez-Matus & Rocha, 2020, and the new anthias presented here, appear to be restricted to MCEs, as is the case for much of the ichthyological biodiversity being revealed by scientists exploring these ecosystems (Rocha et al. 2018; Pinheiro et al. 2019).

# Materials and methods

Three individuals of the new species were collected with hand nets while diving on mixed-gas, closed-circuit rebreathers (Hollis Prism 2) around Rapa Nui, in March 2017.

Specimens were collected and immediately transported to a field laboratory where they were photographed, tissue sampled, fixed in 10% formalin, and preserved in 75% ethanol. Measurements and x-radiographs were made at the California Academy of Sciences three and one-half years later. Counts were performed with the aid of a stereomicroscope. Morphological characters were measured to the nearest 0.01 mm with digital calipers following the conventions described in Anderson et al. (1990), Anderson and Heemstra (1980, 2012), and Gill et al. (2017). Morphometric data for the holotype and paratypes are presented in Table 1. Proportional measurements in the text are rounded to the nearest 0.1 mm. We measured standard length (SL) as the straight-line distance from the anteriormost point of the upper lip to the base of the caudal fin. Head length (HL) was measured from the anteriormost point of the upper lip to the posterior end of the opercular flap. Body depth (BD), was measured as the maximum depth from the origin of the spinous dorsal fin. Counts of principal caudal-fin rays follow Gill et al. (2016), and are presented in the form upper + lower, where the upper rays are those associated with hypurals 3-5, and the lower rays are those associated with hypurals 1-2 and the parhypural. Procurrent caudal-fin rays are those dorsal and ventral to the prin-

Pseudanthias hangapiko sp. nov.	Holotype CAS 247252	Paratype USNM 443821	Paratype CAS 247254
Standard length (mm)	45.2	28.1	33.2
Head length	30.8	33.3	31.4
Greatest body depth	27.6	26.3	29.1
Body width	14.2	10.8	14.8
Snout length	7.3	8.9	9.2
Bony interorbital width	7.5	9.1	8.7
Orbit diameter	7.8	10.9	9.4
Upper jaw length	12.9	15.7	14.8
Maxilla width	3.9	4.8	5.6
Caudal peduncle length	11.7	10.0	10.1
Caudal peduncle depth	12.7	12.8	14.2
Predorsal length	29.8	31.1	35.1
Preanal length	55.6	65.1	64.8
Prepelvic length	33.8	34.7	30.8
Dorsal fin base length	57.9	55.7	61.6
First dorsal spine	4.9	5.0	4.3
Longest dorsal spine (number)	13.4 (4)	16.4 (3)	15.2 (4)
First segmented dorsal ray	12.7	16.7	14.6
Longest segmented dorsal ray (number)	15.6 (4)	18 (4)	15.7 (4)
Anal fin base length	21.6	18.0	19.2
First anal spine	6.2	4.9	4.8
Second anal spine	13.4	13.6	12.1
Third anal spine	12.6	11.6	9.9
First segmented anal ray	13.6	13.8	14.0
Longest segmented anal ray (number)	20.4 (4)	15.5 (4)	15.3 (4)
Caudal fin length	32.4	25.0	26.4
Pectoral fin length	26.3	29.7	28.1
Pelvic spine length	18.3	19.7	17.1
Pelvic fin length	31.1	26.8	23.7

**Table 1.** Morphometric data for the three specimens of *Pseudanthias hangapiko* sp. nov. expressed as percentage of standard length.

cipal rays. Elongated filaments on the fin rays were not included in the measurements. Vertebral counts include the last vertebra fused to the hypural plate; vertebral counts are presented as precaudal + caudal, where the first caudal vertebra is the anteriormost vertebra bearing a haemal spine. Gill raker counts were made on the left side first arch, and are presented as upper (epibranchial) + lower (ceratobranchial) rakers on the anterior face of the first arch; the angle raker is included in the lower limb count. In the description, data are given first for the holotype, followed by a range of values for the paratypes, in parentheses, where variation was noted. Where counts are recorded bilaterally, both counts are given and separated by a vertical line (]). Where there is some degree of uncertainty, counts are followed by a question-mark (?). Comparisons with related species were based on literature accounts, particularly morphometric and meristic data from *Pseudanthias ventralis* Randall, 1979 and *Pseudanthias hawaiiensis* Randall, 1979, the more recently described *Pseudanthias tequila* Gill, Tea & Senou, 2017, *Pseudanthias emma* Gill & Psomadakis, 2018, *Pseudanthias timanoa* Victor, Teitelbaum & Randall, 2020, Allen and Erdman (2012), and Anderson's (2018) checklist of the Anthiadinae.

Mitochondrial cytochrome c oxidase subunit I (COI) DNA was sequenced and analyzed for the new species. DNA extraction and PCR amplification of the COI were performed following protocols described by Arango et al. (2019), using BOLFishF1/ BOLFishR1 primers. Alignments of DNA sequences were done using a standard Geneious global alignment with free end gaps and 65% similarity in the program Geneious Prime 2020.0.3 (Kearse et al. 2012). Because taxon sampling for *Pseudanthias* in public databases and our lab is not sufficient for a detailed phylogenetic analysis, and we only sequenced one mitochondrial DNA marker, we did not attempt a phylogenetic reconstruction. For our comparisons, genetic distances are uncorrected. COI sequences were compared to sequences of all other available Anthiadinae in GenBank, which include 29 species of Pseudanthias as well as representatives of the following genera: Anthias, Baldwinella, Choranthias, Hemanthias, Luzonichthys, Meganthias, Nemanthias, Odontanthias, Plectranthias, Sacura, Serranocirrhitus, and Tosanoides. The GenBank accession numbers for Pseudanthias hangapiko sp. nov. are MZ087699, MZ087670, and MZ087671. The holotype is deposited in the California Academy of Sciences ichthyological collection (CAS 247252) and the paratypes are deposited at the California Academy of Sciences ichthyological collection (CAS 247254) and the Smithsonian National Museum of Natural History (USNM 443821).

# Taxonomy

*Pseudanthias hangapiko* sp. nov. http://zoobank.org/4F39F82B-8465-4694-9A4E-A674D800C33E Figs 1–3, Table 1

**Type locality.** Hanga Piko, Rapa Nui (Easter Island), Chile *Holotype*. CAS 247252 (Field number LAR2642). Male, 45.2 mm SL, GenBank accession number MZ087699. Location: Hanga Piko, Rapa Nui, Chile (27°9'12"S, 109°26'52"W). Collected by B.



**Figure 1.** Holotype CAS 247252, male specimen (upper), 45.2 mm SL. Paratype CAS 247254, female specimen (lower), 33.2 mm SL. Photos by LA Rocha.

Shepherd, L.A. Rocha, T.A.Y. Phelps, and M.V. Bell using a hand-net at 83 m depth, 7 March 2017 (Figs 1, 2). *Paratypes.* USNM 443821 (Field number LAR2643). Male, 28.1 mm SL, GenBank accession number MZ087700. CAS 247254 (Field number LAR2645). Female, 33.2 mm SL, GenBank accession number MZ087701. Both from the same location as the holotype. Collected by B. Shepherd, L.A. Rocha, T.A.Y. Phelps, and M.V. Bell using a hand-net, 7 March 2017 (Fig. 1).

**Diagnosis.** The following combination of characters distinguishes *Pseudanthias hangapiko* sp. nov. from congeners: dorsal rays X, 17; anal rays III, 8; pectoral rays 16 (left side of one specimen 17); vertebrae 10+16; scales relatively large, two scales between lateral line and base of fifth dorsal spine, and 16 (17) circumpeduncular scales; gill rakers 11+22–23; body very slender and compressed, the greatest body depth 3.4–3.8 in SL; caudal peduncle short, its length 2.6–3.3 in HL; sexually dichromatic, with male coloration red dorsally, yellow laterally, silvery-pink on throat and belly; females pink, silvery-pink on operculum, throat and belly; both sexes dark red on top of head, along anterior two-thirds of dorsal fin base; both sexes with rows of irregularly-spaced metallic magenta spots laterally, and red dorsal and caudal fins with yellow highlights.

**Description.** Dorsal fin X, 17; anal fin III, 8; pectoral rays 16 (one paratype with 17 rays on left pectoral fin), upper two and lowermost unbranched; pelvic fin I, 5; principal caudal-fin rays 9 + 8 (7 + 6 branched); upper procurrent caudal-fin rays 9 (10); lower procurrent caudal rays 9 (9, 10?); tubed lateral-line scales 40 | 41



Figure 2. Radiograph of holotype, CAS 247252. Photo by J Fong.

(40–43); scales above lateral line to origin of dorsal fin 5 (4); scales above lateral line to base of fifth dorsal spine 2; scales below lateral line to origin of anal fin 11 (12); circumpeduncular scales 16 (17); gill rakers 11+23 (11+22-23); pseudobranchial filaments 10 (9); branchiostegal rays 7; vertebrae 10+16; supraneurals 2; predorsal formula 0/0/2/1+1; main shaft (proximal component) of first dorsal pterygiophore inclined slightly backwards; dorsal pterygiophores in interneural spaces 9-13 1/1/1+1/1+1/1; terminal dorsal pterygiophore in interneural space 19 (18); no trisegmental pterygiophores associated with dorsal fin; proximal tip of first anal-fin pterygiophore near distal tip of haemal spine on first caudal vertebra; terminal anal pterygiophore in interhaemal space 6; no trisegmental pterygiophores associated with anal fin; ribs present on vertebrae 3 through 10; epineurals present on vertebrae 1 through 13 (12?); no paired parapophyses on first caudal vertebra; parhypural and hypurals autogenous; well-developed hypurapophysis on parhypural; epurals 3; single uroneural (posterior uroneural absent); ventral tip of cleithrum with well-developed posteroventral process.

Body very slender, compressed, its depth 3.6 (3.4-3.8) in SL, the width 2.0 (2.0-2.4) in depth; head length 3.2 (3.0-3.2) in SL; snout length 4.2 (3.4-3.7) in HL; snout and front of upper lip rounded, lacking fleshy anterior extension; diameter of orbit 3.9 (3.1-3.3) in head; posterior edge of orbit with 13 (12) fleshy papillae; interorbital space smooth, the bony width 4.1 (3.6-3.7) in HL; least depth of caudal peduncle 2.4 (2.2-2.6) in HL; caudal peduncle length 2.6 (3.1-3.3) in HL.

Mouth moderately large, slightly oblique, the posterior margin of the maxilla reaching a vertical through the center of the pupil; lower jaw does not protrude when mouth is closed; maxilla width 2.0 (1.7–2.3) in orbit diameter. Upper jaw with two pairs of slightly enlarged canines directed ventrally; a band of small conical teeth, three rows wide at symphysis, reducing to two rows on sides of jaw, with the outer row teeth much larger and slightly curved dorsally, and the inner pair of teeth anteriorly nearest symphysis enlarged and caniniform; dentary with two rows of small conical teeth narrowing to one row and becoming larger posteriorly; lower jaw with one to two enlarged, curved, forward-projecting canine teeth on either side of symphysis; vomer with triangular patch of small conical teeth; palatine with a narrow band of small conical teeth, five rows wide, decreasing to one row posteriorly; tongue small, triangular, pointed, and edentate.



**Figure 3.** An aggregation of *Pseudanthias hangapiko* sp. nov. on a rocky mesophotic coral ecosystem at Rapa Nui (Easter Island) at 80 m depth.

Anterior nostril positioned at middle of snout, with a short fleshy flap on posterior margin; posterior nostril at mid-upper anterior border of orbit, covered by a thin, narrow membrane anteriorly. Opercle with three flat spines, all stout and acute; the middle opercle spine largest and level with center of eye; the upper smallest; ventral margin of preopercle smooth; vertical margin of preopercle with 14 acute spines (11), the largest almost the same size as the inferior opercle spine; posterior margin of subopercle with two strong spines; posterior corner of interopercle with one strong, acute spine.

Scales ctenoid, relatively large, without basal cteni; head and preopercle scaled; distal portion of maxilla covered with scales, head fully scaled except for lips and areas in front of and immediately below nostrils; dorsal fin and anal fin without scales; proximal one-third of pelvic fin scaled; caudal fin with scales extending approximately three quarters distance to posterior margin; scales cover the central portion of the proximal one-fifth of the pectoral fin. Lateral line complete, smoothly curved, mostly follows dorsal contour of body reaching its highest point below the fifth dorsal spine.

Origin of dorsal fin at vertical through base of pectoral fin, the predorsal length 3.4 (2.8-3.2) in SL; first dorsal spine 6.3 (6.6-7.2) in HL; fourth dorsal spine longest (third in smaller paratype), 2.3 (2.0-2.1) in HL; first dorsal ray 2.4 (2.0-2.2) in HL, longest dorsal ray the fourth, 2.0 (1.8) in HL; origin of anal fin below base of third dorsal soft ray, the preanal length 1.8 (1.5) in SL; first anal spine 5.0 (6.6-6.8) in HL; second anal spine the longest, nearly three times the length of the first, 2.3 (2.5-2.6)

in HL; third anal spine 2.4 (2.9–3.2) in HL; posterior margin of anal fin rounded, the first segmented ray 1.4 (2.4–3.2) in HL, the longest segmented ray the fourth, 1.5 (2.0–2.1) in HL. Caudal fin lunate with trailing filaments, longer in males, the caudal concavity 3.1 (3.8–4.0) in SL. Pectoral fins 3.8 (3.4–3.6) in SL, extending to a vertical below base of first dorsal soft ray. Pelvic fins moderately long, 5.4 (5.1–5.9) in SL reaching second anal spine.

Color in life: Pseudanthias hangapiko sp. nov. is sexually dichromatic. Males (Fig. 1): body pink, with yellow and dark red obscuring most of the ground color, except on belly and throat. Rows of metallic magenta spots, about one per scale, cover body, creating an irregularly spotted pattern starting from behind orbit and extending to base of caudal fin; upper third of body dark red, sides yellow. Dorsal fin dark red, with thin yellow stripe following upper margin; posterior half of soft dorsal-fin base with region of less-pronounced color, extending approximately one quarter of the height of dorsal fin; dark gray region on upper posterior margin of dorsal fin, spanning last five to six fin rays; pectoral fins hyaline; pelvic and anal fins yellow on anterior half, hyaline posteriorly; caudal fin red with yellow-orange filaments; yellow patch at ventral origin of caudal fin. Head pale orange, red along snout and between eyes, operculum yellow. Eye red, darker along outer edge. Females (Figs 1, 3): body predominantly pink with less-pronounced red and yellow markings, silvery-pink on operculum, throat and belly; spotting pattern of metallic magenta scales more widelyspaced than in males; anterior dorsal third of head dark red, pale silvery-pink below; faint orange on tip of snout and lower jaw. Dorsal fin red, with thin yellow stripe following upper margin, region of lighter color on posterior half of soft dorsal-fin base more pronounced in females than in males, same pink as body ground color; dark gray region on upper margin of dorsal fin more pronounced than in males, spanning posteriormost ten to twelve fin rays; pectoral fins hyaline; pelvic and anal fins hyaline with faint yellow markings anteriorly, thin magenta line on distal edge of anal fin; outermost caudal-fin rays yellow with red, especially on upper and lower margins and near base of caudal fin, centermost caudal-fin rays hyaline distally, thin dark gray lines on distal edges of upper and lower caudal fin filaments. Eye silver, darker along outer edge.

**Color in alcohol:** All specimens straw-colored, except dorsally, where all specimens are darkly pigmented above lateral line where red in life; dorsal fin translucent with dark pigment, all other fins translucent.

**Etymology.** The species is named for the location where it was collected, Hanga Piko, meaning "hidden bay" in the Rapa Nui language. To be treated as a noun in apposition.

Common name. Rapa Nui Fairy Basslet.

**Distribution and habitat.** The new species is currently known only from Rapa Nui. The holotype and paratypes were collected at a depth of 83 m at a small, rocky patch reef surrounded by a large sandy area (Fig. 3). Other species collected at this location and recently described by our team include *Plectranthias ahiahiata*, *Luzonichthys kiomeamea*, and *Chromis mamatapara* (Shepherd et al. 2018, 2019, 2020). Due to

geographical isolation and the high degree of endemism (21.7%) among the shore fishes of Rapa Nui (Delrieu-Trottin et al. 2019), it is likely that *Pseudanthias hangapiko* sp. nov. is endemic to the island.

Remarks. Coloration is important for the identification of Pseudanthias (Randall and Pyle 2001). Pseudanthias hangapiko sp. nov. is distinguished from all congeners in coloration of adult individuals: males with red and yellow mostly obscuring a pink ground color; females mostly pink; both sexes silvery-pink on throat and belly, with rows of irregular metallic magenta spots, a dark red region along the anterior-dorsal portion of the body, and predominantly red and yellow dorsal and caudal fins. In morphology, Pseudanthias hangapiko sp. nov. most resembles Pseudanthias connelli Heemstra & Randall, 1986, Pseudanthias randalli Lubbock & Allen, 1978, and P. squamipinnis, sharing overlapping counts in the number of spines and rays on the dorsal and pectoral fins, the number of lateral line scales, and the number of gill rakers. However, it can be differentiated from all of these species by coloration and morphology, especially in the number of segmented rays on the anal fin (8, vs. 7 in P. connelli, P. randalli, and *P. squamipinnis*), by having a very slender body (greatest body depth 3.4-3.7 in SL), and in the small number of circumpeduncular scales (16-17, vs. 22-25 in P. connelli, P. randalli, and P. squamipinnis). Pseudanthias hangapiko sp. nov. lacks the scaly dorsal and anal fins and auxiliary scales present in P. squamipinnis, and has only two supraneurals, not three as in P. squamipinnis. Male Pseudanthias hangapiko sp. nov. lack the pennant-like extension on the third dorsal spine occurring in *P. squamipinnis*, and male Pseudanthias hangapiko sp. nov. do not possess a fleshy, protruding upper lip, present in the subgenus Mirolabrichthys, but rather have a rounded snout. It should be noted that the diagnostic validity of this latter character is questionable, as some species exhibit various degrees of hypertrophy and the character may have risen independently multiple times within the anthiadine fishes (Gill et al. 2017).

The most similar DNA barcodes (mitochondrial COI gene) are from *Pseudanthias ventralis* and *P. hawaiiensis*, with 16.8% and 17.0% uncorrected divergence, respectively. These distances are much higher than average divergences between sister species (Rocha 2004), and even between genera, so it is not surprising that *P. hangapiko* can be differentiated from these two species by several characters, including the number of anal-fin rays (III, 8 vs. III, 9–10 in *P. ventralis* and *P. hawaiiensis*), body depth (3.4–3.8 vs 2.3–3.0), and also by body coloration.

# Discussion

The presence of *Pseudanthias hangapiko* sp. nov. in Rapa Nui extends the known geographic range of the genus *Pseudanthias* by nearly 2,000 km eastward in the South Pacific. *Pseudanthias ventralis*, the species with the smallest uncorrected genetic distance at the mtDNA COI gene, is also the one occurring nearest, with the southeasternmost edge of its range occurring at Pitcairn Island (Randall 1979). Although the type specimens for *P. ventralis* are from Pitcairn, it likely comprises a species flock with at least three distinct groupings based on coloration and geographical range (Kuiter 2004), and many phylogeographic studies have found evidence of strong biogeographic partitions in the Pacific resulting in genetically distinct conspecifics (Bowen et al. 2016). A closely related species, *P. hawaiiensis*, marks the north-easternmost edge of the genus' distribution in Hawai'i (Randall 1979). Not surprisingly, diversity within the genus *Pseudanthias* decreases moving eastward out of the coral triangle: there are at least 30 species of *Pseudanthias* in the Coral Triangle (Allen and Erdmann 2012), however, aside from *P. ventralis*, only an additional nine species of *Pseudanthias* occur in French Polynesia (Bacchet et al. 2017). Only three species, all presumed to be endemic, occur in the Marquesas (Williams et al. 2013; Delrieu-Trottin et al. 2015). There are no *Pseudanthias* known from the Eastern Pacific, which is relatively well-explored even at depth (Cortés 2019). As such, *Pseudanthias hangapiko* sp. nov. likely marks the easternmost distribution for the genus.

Taxonomy within the anthiadine fishes is problematic. Several genera, including *Pseudanthias*, may be well-documented and easily recognized groups of reef fishes, but they are poorly diagnosed, likely polyphyletic, and greatly in need of revision (Gill et al. 2017). With more complete sampling of extant taxa using multiple molecular markers, several members of the genus *Pseudanthias* will likely be assigned to other genera (Gill et al. 2017). Additionally, the high genetic distance between *P. hangapiko* and its closest sequenced relatives (>16%) indicates that the placement of this new species within the genus *Pseudanthias* is uncertain. Nevertheless, creating new genera without a complete understanding of the phylogenetic and morphological variation within *Pseudanthias* today would likely only generate more taxonomic instability. Thus, the generic placement of *P. hangapiko* should be considered provisional.

Understanding remote island ecosystems is critical to advancing scientific knowledge of speciation and ecology (Pinheiro et al. 2017; Quimbayo et al. 2019). However, the challenging logistics of conducting mesophotic exploration in geographically isolated locations such as Rapa Nui has undoubtedly limited knowledge of the deep reef biodiversity occurring in such places. With recent advances in technology, such as remote operated vehicles (ROV), consumer-grade closed-circuit rebreathers (CCR), baited remote underwater video (BRUV) employing inexpensive, low-light digital cameras, and autonomous underwater vehicles (AUV), scientists now have multiple tools to use for exploring deeper habitats that were not available to us even a decade or two ago (Pimentel et al. 2020). These studies remain expensive and administratively complex, requiring both significant funding and effective collaboration in order to be successful. Our 2017 expedition to Rapa Nui resulted in descriptions of four new species, all of which were collected on a pair of dives at a single location near the island. This remarkable discovery rate demonstrates the magnitude of the biodiversity waiting to be unveiled in Rapa Nui and elsewhere through deep reef exploration and purposeful collaboration.

# Acknowledgements

This work was funded by the generous support of donors to the California Academy of Sciences' Hope for Reefs Initiative, and Fondecyt # 1151094 granted to APM. This research was conducted under permit 2231 from the Ministerio de Economía Fomento Y Turismo de Chile and exported under certificate 669081 from the National Fisheries Service of Chile (SERNAPESCA). We are grateful to colleagues who helped in the field, lab, and with discussions: M. Bell, C. Castillo, D. Catania, J. Fong, V. (Tuto) Garmendia, E. Hey, I. Hinojosa, K. Jewett, M. Lane, A. Mecho, J. McCosker, C. Rocha, and W. Teao. We thank A. Gill for reviewing the first draft of the manuscript and for his assistance with verifying osteological characters. Logistical and equipment support was provided by Orca Diving Center and Hollis Rebreathers. We would like to thank J. Fong for taking the radiograph presented here. This work was approved by the Institutional Animal Care and Use Committee of the California Academy of Sciences (CAS IACUC approval number 2016-01).

# References

- Allen GR, Erdmann ME (2012) Reef Fishes of the East Indies Vol. I–III. Tropical Reef Research, Perth, 1292 pp.
- Anderson WD, Heemstra PC (1980) Two new species of western Atlantic Anthias (Pisces: Serranidae), redescription of A. asperilinguis and review of Holanthias martinicensis. Copeia 1980(1): 72–87. https://doi.org/10.2307/1444135
- Anderson WD, Heemstra PC (2012) Review of Atlantic and Eastern Pacific Anthiine fishes (Teleostei: Perciformes: Serranidae), with descriptions of two new genera. Transactions of the American Philosophical Society, New Series 102(2): 1–173. https://www.jstor.org/stable/41507695
- Anderson WD, Parin NV, Randall JE (1990) A new genus and species of anthiine fish (Pisces: Serranidae) from the eastern South Pacific with comments on anthiine relationships. Proceedings of the Biological Society of Washington 103(4): 922–930.
- Bacchet P, Zysman T, Lefèvre Y (2016) Guide des poissons de Tahiti et ses îles. Éditions Au vent des îles, Tahiti, Polynésie Française, 446 pp.
- Bowen BW, Gaither MR, DiBattista JD, Iacchei M, Andrews KR, Grant WS, Toonen RJ, Briggs JC (2016) Comparative phylogeography of the ocean planet. Proceedings of the National Academy of Sciences 113(29): 7962–7969. https://doi.org/10.1073/pnas.1602404113
- Cortés J (2019) Isla del Coco, Costa Rica, Eastern Tropical Pacific. In: Loya Y, Puglise KA, Bridge TCL (Eds) Mesophotic Coral Ecosystems. Springer, New York, 465–475. https:// doi.org/10.1007/978-3-319-92735-0\_26
- Delrieu-Trottin E, Williams JT, Bacchet P, Kulbicki M, Mourier J, Galzin R, Lison de Loma T, Mou-Tham G, Siu G, Planes S (2015) Shore fishes of the Marquesas Islands, an updated checklist with new records and new percentage of endemic species. CheckList 11(5): 1–13. https://doi.org/10.15560/11.5.1758

- Delrieu-Trottin E, Brosseau-Acquaviva L, Mona S, Neglia V, Giles EC, Rapu-Edmunds C, Saenz-Agudelo P (2019) Understanding the origin of the most isolated endemic reef fish fauna of the Indo-Pacific: Coral reef fishes of Rapa Nui. Journal of Biogeography 46(4): 723–733. https://doi.org/10.1111/jbi.13531
- Easton EE, Sellanes J, Gaymer CF, Morales N, Gorny M, Berkenpas E (2017) Diversity of deep-sea fishes of the Easter Island ecoregion. Deep-Sea Research II 137: 78–88. https:// doi.org/10.1016/j.dsr2.2016.12.006
- Friedlander AM, Ballesteros E, Beets J, Berkenpas E, Gaymer CF, Gorny M, Sala E (2013) Effects of isolation and fishing on the marine ecosystems of Easter Island and Salas y Gomez, Chile. Aquatic Conservation: Marine and Freshwater Ecosystems 23: 515–531. https://doi.org/10.1002/aqc.2333
- Gill AC, Tea YK, Senou H (2016) *Plectranthias takasei*, new species of anthiadine fish from southern Japan (Teleostei: Serranidae). Zootaxa 4205(4): 349–356. https://doi.org/10.11646/ zootaxa.4205.4.3
- Gill AC, Tea YK, Senou H (2017) *Pseudanthias tequila*, a new species of anthiadine serranid from the Ogasawara and Mariana Islands. Zootaxa 4341(1): 67–76. https://doi. org/10.11646/zootaxa.4341.1.5
- Gill AC, Psomadakis PN (2018) *Pseudanthias emma* new species, with notes on a collection of anthiadine serranid fishes from off Myanmar (eastern Indian Ocean). Zootaxa 4455(3): 525–536. https://doi.org/10.11646/zootaxa.4455.3.8
- Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199
- Kuiter RH (2004) Basslets, Hamlets and their relatives. A Comprehensive Guide to Selected Serranidae and Plesiopidae. TMC Publishing, Chorleywood, 216 pp.
- Pimentel CR, Rocha LA, Shepherd B, Phelps TAY, Joyeux J, Martins AS, Stein CE, Teixeira JB, Gasparini JL, Reis-filho JA, Garla RC, Francini-filho RB, Delfino SDT, Mello TJ, Giarrizzo T, Pinheiro HT (2020) Mesophotic ecosystems at Fernando de Noronha Archipelago, Brazil (South-western Atlantic), reveal unique ichthyofauna and need for conservation. Neotropical Ichthyology 18: e200050. https://doi.org/10.1590/1982-0224-2020-0050
- Pinheiro HT, Bernardi G, Simon T, Joyeux J-C, Macieira RM, Gasparini JL, Rocha C, Rocha LA (2017) Island biogeography of marine organisms. Nature 549: 82–85 https://doi. org/10.1038/nature23680
- Pinheiro HT, Shepherd B, Castillo C, Abesamis RA, Copus JM, Pyle RL, Greene BD, Coleman RR, Whitton RK, Thillainath E, Bucol AA, Birt M, Catania D, Bell MV, Rocha LA (2019) Deep reef fishes in the world's epicenter of marine biodiversity. Coral Reefs 38: 985–995. https://doi.org/10.1007/s00338-019-01825-5
- Quimbayo JP, Dias MS, Kulbicki M, Mendes TC, Lamb RW, Johnson AF, Aburto-Oropeza O, Alvarado JJ, Bocos AA, Ferreira CEL, Garcia E, Luiz OJ, Mascareñas-Osorio I, Pinheiro HT, Rodriguez-Zaragoza F, Salas E, Zapata FA, Floeter SR (2019) Determinants

of reef fish assemblages in tropical oceanic islands. Ecography 42: 77-87. https://doi. org/10.1111/ecog.03506

- Randall JE (1979) A review of the Serranid fish genus *Anthias* of the Hawaiian Islands, with descriptions of two new species. Natural History Museum of Los Angeles County Contributions in Science 302: 1–13.
- Randall JE (1998) Zoogeography of shore fishes of the Indo-Pacific region. Zoological Studies 37: 227–268.
- Randall JE, Lubbock R (1981) A revision of the serranid fishes of the subgenus *Mirolabrichthys* (Anthiinae: *Anthias*), with descriptions of five new species. Natural History Museum of Los Angeles County Contributions in Science 333: 1–27.
- Randall JE, Pyle RL (2001) Four new serranid fishes of the anthiine genus *Pseudanthias* from the South Pacific, Raffles Bulletin of Zoology 49(1): 19–34.
- Randall JE, Cea A (2010) Shore Fishes of Easter Island. University of Hawai'i Press, Honolulu, 154 pp. https://doi.org/10.1515/9780824861001
- Rocha LA (2004) Mitochondrial DNA and color pattern variation in three western Atlantic *Halichoeres* (Labridae), with the revalidation of two species. Copeia 2004(4): 770–782. https://doi.org/10.1643/CG-04-106
- Rocha LA, Pinheiro HT, Shepherd B, Papastamatiou YP, Luiz OJ, Pyle RL, Bongaerts P (2018) Mesophotic coral ecosystems are threatened and ecologically distinct from shallow water reefs. Science 361: 281–284. https://doi.org/10.1126/science.aaq1614
- Shepherd B, Phelps T, Pinheiro HT, Pérez-Matus A, Rocha LA (2018) *Plectranthias ahiahiata*, a new species of perchlet from a mesophotic ecosystem at Rapa Nui (Easter Island) (Teleostei, Serranidae, Anthiadinae). ZooKeys 762: 105–116. https://doi.org/10.3897/zookeys.762.24618
- Shepherd B, Pinheiro HT, Phelps T, Pérez-Matus A, Rocha LA (2019) Luzonichthys kiomeamea (Teleostei: Serranidae: Anthiadinae), a new species from a mesophotic coral ecosystem of Rapa Nui (Easter Island). Journal of the Ocean Science Foundation 33: 17–27. https://doi. org/10.5281/zenodo.3237914
- Shepherd B, Pinheiro HT, Phelps TA, Easton EE, Perez-Matus A, Rocha LA (2020) A new species of *Chromis* (Teleostei: Pomacentridae) from mesophotic coral ecosystems of Rapa Nui (Easter Island) and Salas y Gómez, Chile. Copeia 108(2): 326–332. https://doi. org/10.1643/CI-19-294
- Victor BC, Teitelbaum A, Randall JE (2020) *Pseudanthias timanoa*, a new fairy basslet from New Caledonia, South Pacific (Teleostei: Serranidae: Anthiadinae). Journal of the Ocean Science Foundation 36: 6–15. https://doi.org/10.5281/zenodo.4050419
- Williams JT, Delrieu-Trottin E, Planes S (2013) Two new fish species of the subfamily Anthiinae (Perciformes, Serranidae) from the Marquesas. Zootaxa 3647(1): 167–180. https:// doi.org/10.11646/zootaxa.3647.1.8

RESEARCH ARTICLE



# On the Lathrobium fauna of the Nanling National Nature Reserve, southern China (Coleoptera, Staphylinidae, Paederinae)

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Academic editor: Volker Assing	Received 21 May 2021	Accepted 1 July 2021	Published 3 August 2021
http://z	oobank.org/A1637949-C460	-431D-8768-91EE3690207	77

**Citation:** Lin X-B, Zheng Y-D, Peng Z (2021) On the *Lathrobium* fauna of the Nanling National Nature Reserve, southern China (Coleoptera, Staphylinidae, Paederinae). ZooKeys 1054: 15–24. https://doi.org/10.3897/zooKeys.1054.68991

#### Abstract

Material of the paederine genus *Lathrobium* Gravenhorst, 1802 from the Nanling National Nature Reserve, southern China, is examined. Four species are identified, one of them described previously. Three species are described and illustrated for the first time, all of them micropterous and locally endemic: *L. yangyihani* Lin & Peng **sp. nov.**, *L. jiaxingyangi* Lin & Peng **sp. nov.**, and *L. wangxingmini* Lin & Peng **sp. nov.** The female sexual characters of *L. guangdongense* Peng & Li, 2014 are described and illustrated for the first time. Including the new taxa, 224 *Lathrobium* species are currently known from mainland China.

#### Keywords

Guangdong, new species, taxonomy

# Introduction

From mainland China, 221 species of the genus *Lathrobium* Gravenhorst have been reported, with the vast majority of them locally endemic (Zhao and Peng 2021). One micropterous species was previously recorded from Guangdong and six species from Hunan: *L. guangdongense* Peng & Li, 2014 (Guangdong: Nanling), *L. badagongense* Peng & Li, 2014 (Hunan: Badagong Shan), *L. bamianense* Peng & Li, 2016 (Hunan: Bamian Shan), *L. fumingi* Peng & Li, 2016 (Hunan: Bamian Shan), *L. hunanense* 

Watanabe, 2011 (Hunan: Longshan), *L. jinyuae* Peng & Li, 2016 (Hunan: Nanfengmian; Jiangxi: Bijia Shan), and *L. kishimotoi* Watanabe, 2011 (Hunan: Longshan) (Watanabe 2011; Peng et al. 2014, 2016).

Covering an area of 58,400 hm<sup>2</sup>, the Nanling National Nature Reserve is situated at the border between Guangdong and Hunan provinces and includes the easternmost part of the Nanling range. The highest peak of the Nanling National Nature Reserve is the Shikengkong at 1,902 m (Liu et al. 2018).

In recent years, we conducted several collecting trips and obtained numerous *Lathrobium* specimens. Four species were recognized, including three new species and the previously unknown females of *L. guangdongense*.

# Material and methods

The following abbreviations are used in the text, with all measurements in millimeters:

Body length (**BL**) from the anterior margin of the mandibles (in resting position) to the abdominal apex; length of forebody (**FL**) from the anterior margin of the mandibles to the posterior margin of the elytra; head length (**HL**) from the anterior margin of the frons to the posterior margin of the head; head width (**HW**): maximum width of head; length of antenna (**AnL**); length of pronotum (**PL**) along midline; maximum width of pronotum (**PW**); elytral length (**EL**) at the suture from the apex of the scutellum to the posterior margin of the elytra (at the sutural angles); length of aedeagus (**AL**) from the apex of the ventral process to the base of the aedeagal capsule.

The type material is deposited in the Insect Collection of Shanghai Normal University, Shanghai, China (**SNUC**).

# Results

# Lathrobium yangyihani Lin & Peng, sp. nov.

http://zoobank.org/8FACCDF3-F4DA-4688-99D9-FA3819B4DD2E Figures 1A, 2, 5

**Type material.** *Holotype:*  $\mathcal{J}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Hamashi, 24°55'37"N, 112°59'21"E, 1,750 m, 01.V.2015, Peng, Tu & Zhou leg.' (SNUC). *Paratypes:* 3  $\mathcal{Q}\mathcal{Q}$ , same data as the holotype (SNUC); 2  $\mathcal{Q}\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Guangdong Di Yi Feng, 24°55'29.62"N, 112°59'31.42"E, 1,538–1,784 m, 28.VI.2020, Xia, Zhang, Yin & Lin leg.' (SNUC); 2  $\mathcal{Q}\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'33"N, 112°59'29"E, 1,820 m, 30.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\mathcal{Q}$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Laopengyidui,

24°56'21"N, 113°01'21"E, 1,260 m, 02.V.2015, Peng, Tu & Zhou leg.' (SNUC); 1 ♂, 1 ♀, labeled 'China: Guangdong Prov., Shixing County, Chebaling, 24°43'12.27"N, 114°11'18.03"E, 904–1,004 m, 23.VI.2020, Xia, Zhang, Yin & Lin leg.' (SNUC).

**Description.** Measurements (in mm) and ratios: BL 5.34–6.67, FL 2.19–2.67, HL 0.76–0.98, HW 0.78–0.87, AnL 1.49–1.74, PL 1.00–1.09, PW 0.82–0.93, EL 0.41–0.65, AL 0.81–0.83, HL/HW 0.97–1.13, HW/PW 0.90–1.00, HL/PL 0.75–0.92, PL/PW 1.15–1.27, EL/PL 0.41–0.60.

Habitus as in Figure 1A. Body brown, legs, and antennae light brown.

Head approximately as long as broad; punctation moderately coarse and sparse, sparser in median dorsal portion; interstices with shallow microreticulation; eyes small and composed of approximately 40 ommatidia.

Pronotum nearly parallel-sided; punctation somewhat sparser than that of head; impunctate midline broad; interstices without microsculpture.

Elytral punctation moderately dense, shallow, and ill-defined. Hind wings completely reduced.

Abdomen with fine and moderately sparse punctation, that of tergite VII somewhat sparser than that of anterior tergites; interstices with shallow microsculpture; posterior margin of tergite VII without palisade fringe.

Male. Sternites III–VI unmodified; sternite VII (Fig. 2D) with very shallow postero-median impression without distinctly modified setae, posterior margin weakly con-



Figure 1. Male habitus of *Lathrobium* spp. A *L. yangyihani* B *L. jiaxingyangi* C *L. wangxingmini*. Scale bars: 1.0 mm.

cave in the middle; sternite VIII (Fig. 2E) with symmetric, subtriangular emargination and shallow impression; aedeagus as in Figure 2F and G, with stout ventral process of highly distinctive shape; dorsal plate with moderately long apical portion and very short basal portion; internal sac without sclerotized spines.

**Female.** Posterior margin of tergite VIII (Fig. 2A) strongly convex. Posterior margin of sternite VIII (Fig. 2B) strongly convex and with moderately dense micropubescence; tergite IX (Fig. 2C) with very short, medially undivided antero-median portion and moderately long postero-lateral processes; tergite X (Fig. 2C) 2.5 times as long as antero-median portion of tergite IX.

**Comparative notes.** The new species resembles *L. guangdongense* Peng & Li, 2014 in habitus and the similarly derived morphology of Sternites VII–VIII but differs from



**Figure 2.** *Lathrobium yangyihani*. **A** female tergite VIII **B** female sternite VIII **C** female tergites IX–X. **D** male sternite VIII **F** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

this species by the stouter ventral process and the simple internal sac of the aedeagus. For illustrations of *L. guangdongense*, see Figure 3A–C and Peng et al. (2014).

**Etymology.** The species is named after Yi-Han Yang, who supported us on our field trips.

**Distribution and natural history.** The species was found in six adjacent localities in western Ruyuan County to southeastern Shixing County. A specimen was sifted, together with *L. guangdongense*, from moist leaf litter of a secondary mixed and deciduous forest at an altitude of 1,260 m (Fig. 5).

# Lathrobium guangdongense Peng & Li, 2014

Figure 3A–C

Material studied. 3 33, 6 99, CHINA: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Laopengyidui, 24°56'21"N, 113°01'21"E, 1,260 m, 02.V.2015, Peng, Tu & Zhou leg. (SNUC); 4 ♂♂, 3 ♀♀, Сніма: Guangdong Prov., Ruvuan County, Nanling National Nature Reserve, Laopengkeng, 24°56'29"N, 113°00'27"E, 1,360 m, 29.V.2015, Peng, Tu & Zhou leg. (SNUC); 1 ♀, CHINA: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Walkway, 24°55'57"N, 113°00'18"E, 1,220 m, 28.IV.2015, Peng, Tu & Zhou leg. (SNUC); 2 33, CHINA: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Disilindao, 24°55'47"N, 112°59'50"E, 1,500 m, 05.V.2015, Peng, Tu & Zhou leg. (SNUC); 1 Å, CHINA: Hunan Prov., Yizhang County, Mangshan Nature reserve, 24°56'26"N, 112°59'18"E, 1400 m, 26.IV.2015, Peng, Tu & Zhou leg. (SNUC); 3 33, 4 99, CHINA: Guangdong Prov., Ruyuan County, Nanling National Nature reserve, 24°55'43.67"N, 113°00'58.50"E, 1,020 m, sifted, 27.VI.2020, Lin, Xia, Yin & Zhang leg. (SNUC); 1 3, 2  $\bigcirc$ , CHINA: Hunan Prov., Xinning County, Shunhuang Shan Nature Reserve, 26°22'33.82"N, 110°59'32.11"E, 1,112 m, sifted, 24.VIII.2020, leaf litter, sifted, Chong Li leg. (SNUC); 2 ♂♂, 1 ♀, CHINA: Guangdong Prov., Ruyuan County, Nanling Nature reserve, 24°55'42.9"N, 113°00'59.05"E, 1,020–1,250 m, 04.V.2021, Hu, Lin, Zhou & Li leg. (SNUC); 2 ♀♀, CHINA: Guangdong Prov., Ruyuan County, Nanling Nature reserve, 24°56'16.20"N, 113°00'8.43"E, 980-1,350 m, 01.V.2021, Hu, Lin, Zhou & Li leg. (SNUC); 2 🖧, 2 ♀, Сніла: Guangdong Prov., Ruyuan County, Nanling Nature reserve, Xiaohuangshan, 24°53'44.7"N, 113°01'26.9"E, 1,270–1,570 m, 02.V.2021, Hu, Lin, Zhou & Li leg. (SNUC).

**Comment.** The original description is based on four males. The previously unknown female sexual characters are as follows: posterior margin of tergite VIII (Fig. 2A) convex; posterior margin of sternite VIII (Fig. 2B) strongly convex and with moderately dense micropubescence; tergite IX (Fig. 2C) with median suture and long postero-lateral processes; tergite X (Fig. 2C) nearly reaching anterior margin of tergite IX. The above records from Hunan represent new province records. For illustrations of the habitus and the male sexual characters, see Peng et al. (2014).

**Figure 3.** *Lathrobium guangdongense* (**A–C**) and *L. jiaxingyangi* (**D–G**) **A** female tergite VIII **B** female sternite VIII **C** female tergites IX–X. **D** male sternite VII **E** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

# Lathrobium jiaxingyangi Lin & Peng, sp. nov.

http://zoobank.org/A2AC29DE-EA0E-438B-BEE4-1D131CFE0B1F Figures 1B, 3D–G, 6

**Type material.** *Holotype*: ♂, labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'33"N, 112°59'29"E, 1,820 m, 30.IV.2015, Peng, Tu & Zhou leg.' (SNUC). *Paratypes:* 1 ♂, same data, but '24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg' (SNUC).

**Description.** Measurements (in mm) and ratios: BL 7.95–8.34, FL 3.12–3.24, HL 1.09–1.14, HW 1.09, AnL 1.99–2.16, PL 1.34–1.37, PW 1.09–1.14, EL 0.68–

0.73, AL 1.55, HL/HW 1.00–1.04, HW/PW 0.96–1.00, HL/PL 0.82–0.83, PL/PW 1.20–1.22, EL/PL 0.51–0.53.

Habitus as in Figure 1B. Body dark reddish brown, legs reddish brown, antennae dark to light reddish brown.

Head punctation moderately fine and moderately dense, not sparser in median dorsal portion; interstices with shallow microsculpture. Eyes moderately small and composed of approximately 60 ommatidia.

Pronotum nearly parallel-sided; punctation somewhat sparser than that of head; impunctate midline broad; interstices without microsculpture.

Elytral punctation moderately dense and shallow. Hind wings completely reduced.

Abdomen with fine and moderately dense punctation, that of tergite VII somewhat sparser than that of anterior tergites; interstices with shallow, but distinct microsculpture; posterior margin of tergite VII without palisade fringe.

**Male.** Sternites III–VI unmodified; sternite VII (Fig. 3D) strongly transverse and symmetric, with shallow median impression with modified short black setae, posterior margin broadly and very weakly concave; sternite VIII (Fig. 3E) approximately as long as broad, with moderately extensive median impression posteriorly, this impression with numerous distinctly modified, stout black setae, posterior excision relatively deep; aedeagus as in Figure 3F and G; ventral process weakly hooked apically in lateral view; dorsal plate with long apical portion and very short basal portion; internal sac with one sclerotized spine.

Female. Unknown.

**Comparative notes.** *Lathrobium jiaxingyangi* resembles *L. wangxingmini* sp. nov. in having the similarly derived chaetotaxy of the asymmetric male sternite VIII and the long dorsal plate of the aedeagus. It is distinguished from *L. wangxingmini* by the lighter coloration, the smaller body size, the chaetotaxy of the male sternite VII, the shallower posterior excision of the male sternite VIII, and the morphology of the aedeagus (shape of ventral process; internal sac with one shorter sclerotized spine).

**Etymology.** The species is named after Xing-Yang Jia, who supported us on our field trips.

**Distribution and natural history.** The type locality is situated in the Nanling National Nature Reserve to western Ruyuan County, eastern Guangdong. The specimens were sifted from leaf litter and grass roots in shrub habitats at an altitude of 1,850 m (Fig. 6).

# Lathrobium wangxingmini Peng & Lin, sp. nov.

http://zoobank.org/A47F4DF3-D990-4DEB-A7C9-0F2F3891E52D Figures 1C, 4, 7

**Type material.** *Holotype:*  $\Diamond$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Walkway, 24°55'57"N, 113°00'18"E, 1,220 m, 28.IV.2015, Peng, Tu & Zhou leg.' (SNUC). *Paratypes:* 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'33"N, 112°59'29"E, 1,820 m, 30.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'33"N, 112°59'29"E, 1,820 m, 30.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'33"N, 112°59'29"E, 1,820 m, 30.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 24°55'33"N, 112°59'29"E, 1,820 m, 30.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Shikengkong, 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Nature Reserve, Nature Reserve, Shikengkong, 1  $\heartsuit$ , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, Nature Re

Reserve, Shikengkong, 24°55'38"N, 112°59'30"E, 1,850 m, 27.IV.2015, Peng, Tu & Zhou leg' (SNUC); 1  $\bigcirc$ , labeled 'China: Hunan Prov., Yizhang County, Mangshan Nature reserve, 24°56'26"N, 112°59'18"E, 1,400 m, 26.IV.2015, Peng, Tu & Zhou leg.' (SNUC); 1  $\bigcirc$ , 2  $\bigcirc$  , labeled 'China: Guangdong Prov., Ruyuan County, Nanling National Nature Reserve, 1,090 m, 18.VI.2007, Huang & Xu leg.' (SNUC).

**Description.** Measurements (in mm) and ratios: BL 8.62–10.56, FL 3.48–4.27, HL 1.30–1.67, HW 1.30–1.52, AnL 2.32–2.70, PL 1.57–1.85, PW 1.33–1.57, EL 0.61–0.83, AL 2.25, HL/HW 1.00–1.15, HW/PW 0.96–0.98, HL/PL 0.82–0.95, PL/PW 1.18–1.19, EL/PL 0.39–0.45.

Habitus as in Fig. 4C. Body blackish brown, legs brown, antennae dark to light brown. Head transverse; punctation coarse and dense, sparser in median dorsal portion; interstices with distinct microsculpture.



**Figure 4.** *Lathrobium wangxingmini.* **A** female tergite VIII **B** female sternite VIII **C** female tergites IX–X. **D** male sternite VII **E** male sternite VIII **F** aedeagus in ventral view **G** aedeagus in lateral view. Scale bars: 0.5 mm.

Pronotum nearly parallel-sided; punctation somewhat sparser than that of head; impunctate midline broad; interstices without microsculpture. Eyes moderately big and composed of approximately 80 ommatidia.

Elytral punctation dense and defined. Hind wings completely reduced. Protarsi without appreciable sexual dimorphism, distinctly dilated.

Abdomen with fine and dense punctation, punctation of tergite VII slightly less dense than that of anterior tergites; interstices with shallow microsculpture; posterior margin of tergite VII without palisade fringe.

**Male.** Sternites III–VI unmodified; sternite VII (Fig. 4D) strongly transverse, with shallow median impression posteriorly with numerous distinctly modified, short black setae, posterior margin nearly truncate; sternite VIII (Fig. 4E) strongly modified and of distinctive shape and chaetotaxy, with deep and extensive median impression, middle of this impression with unmodified pubescence, laterally with dense short and very stout black setae, posterior margin deep and weakly asymmetric; aedeagus as in Figure 4F and G; ventral process asymmetric and apically acute; dorsal plate with long apical portion and very short basal portion; internal sac with one long sclerotized spine.



**Figures 5–7.** Collecting sites on the Nanling National Nature Reserve **5** type localities of *Lathrobium yangyihani* (1,260 m) **6** type locality of *Lathrobium jiaxingyangi* (1,850 m) **7** type locality of *Lathrobium wangxingmini* (1,090 m).

**Female.** Posterior margin of tergite VIII (Fig. 4A) strongly convex. Posterior margin of sternite VIII (Fig. 4B) strongly convex and with moderately dense micropubescence; tergite IX (Fig. 4C) with short antero-median portion and slender postero-lateral processes; tergite X (Fig. 4C) 2.5 times as long as antero-median portion of tergite IX.

**Comparative notes.** *Lathrobium wangxingmini* resembles *L. jiaxingyangi* sp. nov. in having the similarly derived chaetotaxy of the asymmetric male sternite VIII and the long dorsal plate of the aedeagus. It is distinguished from *L. jiaxingyangi* by the darker coloration, the larger body size, more dense short black setae in the impression of the male sternite VIII, the deeper posterior excision of the male sternite VIII and the morphology of the aedeagus (shape of ventral process; internal sac with one longer sclerotized spine).

**Etymology.** The species is dedicated to Xing-Min Wang, who supported us on our field trips.

**Distribution and natural history.** The species was found in five adjacent localities in western Ruyuan County to southern Yizhang County. Some specimens were sifted from the leaf litter of mixed deciduous forests at an altitude of 1,090 m (Fig. 7).

# Acknowledgements

We are indebted to all the collectors mentioned above for their fieldwork and to Yi-Han Yang (Guangdong, China), Xing-Yang Jia (Guangdong, China), and Xing-Min Wang (Guangdong, China) for their extensive support during our collecting trips. We thank two anonymous reviewers for their comments on a previous version of the manuscript. The present study was supported by the National Natural Science Foundation of China (no. 31872965), and Science and Technology Commission of Shanghai Municipality, China (19QA1406600).

# References

- Liu Z-F, Yang C-T, Gong Y-N (2018) Forest bird diversity investigation on the Guangdong Nanling National Nature Reserve. Tropical Geography 38(3): 328–336.
- Peng Z, Li L-Z, Zhao M-J (2014) Seventeen new species and additional records of *Lathrobium* (Coleoptera, Staphylinidae) from mainland China. Zootaxa 3780(1): 1–35. https://doi. org/10.11646/zootaxa.3780.1.1
- Peng Z, Li L-Z, Zhao M-J (2016) On the *Lathrobium* fauna of the Luoxiao Mountains, Central China. Zootaxa 4158(3): 385–402. https://doi.org/10.11646/zootaxa.4158.3.5
- Watanabe Y (2011) Three new species of brachypterous *Lathrobium* (Coleoptera, Staphylinidae) from Central China. Elytra, New Series 1(2): 177–186.
- Zhao Q-H, Peng Z (2021) New species and new records of the genus *Lathrobium* Gravenhorst (Coleoptera: Staphylinidae: Paederinae) from Zhejiang, East China. Zootaxa 4990(1): 172–181. https://doi.org/10.11646/zootaxa.4990.1.11

RESEARCH ARTICLE



# The ichthyofauna of a poorly known area in the middlesouthern Espinhaço mountain range, state of Minas Gerais, Brazil: diagnostics and identification keys

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Academic editor: Caleb McMahan   Received 19 April 2021   Accepted 17 June 2021   Publ	olished 3 August 2021
http://zoobank.org/BAEC6089-8F87-4A56-BAD0-8E2922E22F60	

**Citation:** dos Santos SA, Britto MR (2021) The ichthyofauna of a poorly known area in the middle-southern Espinhaço mountain range, state of Minas Gerais, Brazil: diagnostics and identification keys. ZooKeys 1054: 25–66. https://doi.org/10.3897/zookeys.1054.67554

# Abstract

Knowledge about the taxonomy and fish composition from the upper rio Paraúna (rio São Francisco basin) and upper rio Santo Antônio (rio Doce basin) in the middle portion of the Southern Espinhaço mountain range, state of Minas Gerais, Brazil is still incipient. Only few studies focusing on ichthyo-faunistic diagnostic and species descriptions in the lower stretches of the rio Santo Antônio are available. Herein the aim was to provide a species list of the freshwater ichthyofauna from the headwaters of both basins in such region, and to verify the occurrence of threatened, exotic, and potentially new species. Sixty species were registered, with 34 associated to the upper rio Paraúna, and 40 to the upper rio Santo Antônio. Two species are included in some threatened category, three are exotics, and 14 represent potentially new species. An identification key of the fish species recorded in the area is also provided.

#### Keywords

Headwater, inventory, rio Doce, rio Paraúna, rio Santo Antônio, rio São Francisco, taxonomy

# Introduction

The Espinhaço mountain range is one of the most diverse areas in Brazil, presenting a poorly known fauna with high degree of endemism and records of new fish species in recent years (Alves et al. 2008). This scene is particularly reinforced in the headwaters

of the rio Doce and rio São Francisco basins. This mountain range acts as important watershed divide of three of the main hydrographic systems from the central-south region of the state of Minas Gerais: rio São Francisco, rio Doce, and rio Jequitinhonha basins. A rich and diversified ichthyofauna is found in these basins. Recent studies show an estimated richness of ca. 240 native species in the rio São Francisco basin (Barbosa et al. 2017), 110 native species in the rio Doce basin (Bueno et al. 2021), and numbers varying between ca. 50 to 70 native species in the rio Jequitinhonha basin (Andrade Neto 2010; Bueno et al. 2021).

The complex of mountains in such a region presents a considerable diversity of fishes that, among other reasons, primarily supported the inclusion of such basins in the list of priority areas for fish conservation in the state of Minas Gerais (Drummond et al. 2005). Despite the great importance of the area, the lack of information about the fish taxonomy occurring there can impact attempts for biodiversity conservation. Additionally, there has been an increase of anthropogenic pressure in the region, especially due to large projects, such as mining and hydroelectric power plants (Vieira 2006, 2010; pers. obs. 2011). Such pressures bring out the need for more studies in an attempt to reduce the knowledge gaps about species taxonomy, enabling a better understanding of continental freshwater fish distribution patterns, and proposition of conservation measures (Menezes et al. 2007).

Herein we aim to present a species list of the ichthyofauna from the headwaters of the middle portion of southern Espinhaço mountain range: the upper rio Paraúna (rio São Francisco basin) and the upper rio Santo Antônio (rio Doce basin). In addition, we propose a dichotomous identification key for the fish species found in the region.

# Material and methods

# Study area

The middle-southern Espinhaço mountain range (SEMR) is located in the centralsouth region of the state of Minas Gerais, Brazil, in an area between the municipalities of Conceição do Mato Dentro, Alvorada de Minas, Congonhas do Norte, Presidente Kubitschek, and Santana de Pirapama. The rio Paraúna is an affluent of the rio das Velhas, and the latter is one of the most important tributaries of the rio São Francisco on its right bank. In its turn, the rio Santo Antônio is one of the most important tributaries of the rio Doce basin on its left bank. Throughout these drainages we sampled 40 localities, which drain the watershed of the upper rio Paraúna and upper rio Santo Antônio (Table 1; Figs 1, 2).

# Ichthyofaunistic sampling

Two field expeditions were carried out in March and July-August 2016, under collecting permits 8142-1 and 52362-1, issued by the Instituto Chico Mendes de Conservação da Biodiversidade and 041-2016, by the Instituto Estadual de Florestas (IEF-

Site	Locality	Drainage	Basin	Coordinates
1	Rio Parauninha	Santo Antônio	Doce	18°56'16"S; 43°38'29"W
2	Ribeirão Congonhas	Rio das Velhas	São Francisco	18°53'07"S; 43°40'52"W
3	Ribeirão Congonhas	Rio das Velhas	São Francisco	18°52'20"S; 43°40'33"W
4	Rio Lambari ou Cachoeira do Jacu	Santo Antônio	Doce	18°53'43"S; 43°36'34"W
5	Rio Lambari ou Cachoeira do Jacu	Santo Antônio	Doce	18°52'43"S; 43°37'24"W
6	Córrego Santa Maria	Rio das Velhas	São Francisco	18°49'03"S; 43°39'51"W
7	Ribeirão Gurutuba	Rio das Velhas	São Francisco	18°46'20"S; 43°36'57"W
8	Ribeirão Gurutuba	Rio das Velhas	São Francisco	18°44'07"S; 43°36'29"W
9	Córrego Santa Maria	Rio das Velhas	São Francisco	18°50'11"S; 43°38'28"W
10	Ribeirão Santo Antônio ou Cruzeiro	Santo Antônio	Doce	18°48'39"S; 43°33'32"W
11	Rio Parauninha	Santo Antônio	Doce	18°56'31"S; 43°37'59"W
12	Rio Paraúna	Rio das Velhas	São Francisco	18°41'42"S; 43°34'14"W
13	Ribeirão Santo Antônio ou Cruzeiro	Santo Antônio	Doce	18°49'26"S; 43°32'21"W
14	Rio Paraúna	Rio das Velhas	São Francisco	18°42'23"S; 43°31'38"W
15	Ribeirão Congonhas	Rio das Velhas	São Francisco	18°52'49"S; 43°40'21"W
16	Córrego Ponte Nova	Rio das Velhas	São Francisco	18°42'18"S; 43°31'37"W
17	Córrego sem nome	Santo Antônio	Doce	18°46'46"S; 43°33'22"W
18	Ribeirão Santo Antônio ou Cruzeiro	Santo Antônio	Doce	18°47'01"S; 43°33'38"W
19	Córrego Pica-pau	Santo Antônio	Doce	18°46'58"S; 43°33'11"W
20	Córrego Ponte Nova	Rio das Velhas	São Francisco	18°41'59"S; 43°31'27"W
21	Córrego Pica-pau	Santo Antônio	Doce	18°47'01"S; 43°33'23"W
22	Córrego sem nome	Rio das Velhas	São Francisco	18°41'16"S; 43°34'14"W
23	Ribeirão Santo Antônio do Norte	Santo Antônio	Doce	18°47'55"S; 43°31'01"W
24	Ribeirão Santo Antônio do Norte	Santo Antônio	Doce	18°48'24"S; 43°30'47"W
25	Córrego sem nome	Rio das Velhas	São Francisco	18°52'13"S; 43°40'23"W
26	Córrego sem nome	Rio das Velhas	São Francisco	18°53'14"S; 43°39'44"W
27	Córrego dos Esteios	Rio das Velhas	São Francisco	18°40'57"S; 43°46'08"W
28	Córrego dos Esteios	Rio das Velhas	São Francisco	18°40'51"S; 43°46'28"W
29	Córrego sem nome	Rio das Velhas	São Francisco	18°39'08"S; 43°45'49"W
30	Córrego sem nome	Rio das Velhas	São Francisco	18°38'37"S; 43°45'46"W
31	Córrego do Sítio	Rio das Velhas	São Francisco	18°38'33"S; 43°45'11"W
32	Ribeirão do Tijucal	Rio das Velhas	São Francisco	18°40'04"S; 43°36'16"W
33	Ribeirão do Tijucal	Rio das Velhas	São Francisco	18°40'27"S; 43°36'35"W
34	Córrego Santa Maria	Rio das Velhas	São Francisco	18°47'49"S; 43°42'35"W
35	Córrego Capão	Santo Antônio	Doce	19° 00'55"S; 43°35'10"W
36	Córrego Capão	Santo Antônio	Doce	19° 01'20"S; 43°35'37"W
37	Ribeirão das Pedras	Santo Antônio	Doce	18°45'50"S; 43°27'36"W
38	Ribeirão das Pedras	Santo Antônio	Doce	18°45'44"S; 43°25'45"W
39	Calha principal do Rio Cipó	Rio das Velhas	São Francisco	18°41'38"S; 43°59'24"W
40	Córrego sem nome	Rio das Velhas	São Francisco	18°41'04"S; 43°59'18"W

**Table 1.** Sampled localities in the middle-southern Espinhaço mountain range, hydrographic basins, and coordinates.

MG). For this purpose, fishing artifacts commonly employed in ichthyological studies were used, which included aluminum ring sieves and 1 mm-mesh mosquito net, aluminum cord and hoop socks and 1 mm mesh mosquito net, 15 mm- and 20 mm-mesh netting and bamboo rods with nylon line, and worm used as bait. Samples were taken during the day and occasionally in the early evening.

The care and use of experimental animals complied with animal welfare laws, guidelines and policies under Collecting Permit by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (SISBIO #8142-1). Afterwards, the collected specimens were preserved in 10% formalin solution and transferred to 70% ethanol solution. The sampled material was deposited at the Ichthyological Collection of the



**Figure 1.** Sample localities in the middle-southern Espinhaço mountain range, Minas Gerais state, Brazil. Numbers matches sample localities in Table 1. Upper rio Paraúna drainages (black circle); upper rio Santo Antônio drainages (blue square); black arrow indicates study area.

Museu Nacional, Universidade Federal do Rio de Janeiro (**MNRJ**, Rio de Janeiro, Brazil). In order to increase recorded species richness and more reliable sampling of actual diversity, specimens available in different scientific collections were analyzed, such as the Museu de Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (**MCP**, Porto Alegre); Museu de Zoologia da Universidade de São Paulo (**MZUSP**, São Paulo); Museu de Zoologia da Universidade Estadual de Campinas (**ZUEC**, Campinas); Museu de Ciências Naturais da Pontifícia Universidade Católica de Minas Gerais (**MCNIP**, Belo Horizonte); and Naturhistorisches Museum (**NMW**, Wien) (Table 2). Comparative material was also verified in these institutions. Geographical distribution of species was based on Fricke et al. (2021).

### Identification keys

The identification keys are exclusive for identifying species that occur in the upper rio Paraúna (rio São Francisco basin) and upper rio Santo Antônio (rio Doce basin), state of Minas Gerais, Brazil. We first present a dichotomous key based on morphological characters to identify fish orders. Registered orders that have representatives of a single family, have their respective names given in parentheses, including the number of genera and species associated with the family. A second dichotomous key is organized sequentially, following the classification adopted by Buckup et al. (2007), except for



**Figure 2.** Sample localities in the middle-southern Espinhaço mountain range, Minas Gerais state, Brazil. Numbers follow Figure 1 and Table 1.



Figure 2. Continued.

some taxonomic updates, such as order Perciformes, which was treated as Cichliformes, following Nelson et al. (2016), subfamily Stethaprioninae (Téran et al. 2020), and family Bryconidae (Fricke et al. 2021). Families which have only one species present in the study area are identified in the second dichotomous key. For those families which have more than one species present, another key is presented (one key per family).

Species were identified through available publications and comparisons with reference material in fish collections. Meristic and morphometric data were taken point to point, whenever possible, on the left side of specimens. Morphometric data were taken using digital calipers under a stereomicroscope. Standard length of the specimens was abbreviated as SL and measurements were taken in millimeters. Whenever necessary, analysis of branchial arch, teeth, procurrent rays of caudal fin, and vertebrae were obtained from cleared and stained material, according to Taylor and Van Dyke (1985). Whenever possible, we also made X-Ray images of some specimens for bone structure analyses - such images were made at the Laboratório de Radiografia from Departamento de Vertebrados, Museu Nacional (Faxitron X-ray, model MX-20 DC12). Osteological terminologies were based on specific bibliographies for each group. Some diagnostic characters shown in the identification key such as number of fin rays and pored scales in lateral line, may present some overlap between different species. However, those characters aim to complement the diagnosis of each species. General distributions of species were based on the published literature for each taxon, and represent their respective ranges of occurrence in the Neotropical region. Distributions of species shown in the identification key were solely based on the records of the study area. Supplementary file 1 summarizes voucher information and comparative material with institutional acronyms, following Sabaj (2019).

# Results

# Material examined

**Parodontidae:** Apareiodon ibitiensis Amaral Campos, 1944 (n = 3); **Curimatidae:** Cyphocharax gilbert (Quoy & Gaimard, 1824) (n = 1); **Prochilodontidae:** Prochilodus costatus Valenciennes, 1850 (n = 1); **Anostomidae:** Hypomasticus mormyrops (Steindachner, 1875) (n = 8); Hypomasticus thayeri Borodin, 1929 (n = 24); Leporellus vittatus (Valenciennes, 1850) (n = 1); Leporinus amblyrhynchus Garavello & Britski, 1987 (n = 1); Leporinus copelandii (Steindachner, 1875) (n = 1); Leporinus marcgravii Lütken, 1875 (n = 1); Leporinus taeniatus Lütken, 1875 (n = 2); Megaleporinus obtusidens (Valenciennes, 1837) (n = 1); **Crenuchidae:** Characidium fasciatum Reinhardt, 1867 (n = 24,); Characidium zebra Eigenmann, 1909 (n = 1 paratype); Characidium sp. A (n = 91); Characidium sp. B (n = 22); Characidium sp. C (n = 26); **Bryconidae:** Brycon opalinus Cuvier, 1819 (n = 9); **Characidae:** Phenacogaster franciscoensis Eigenmann, 1911 (n = 1), Astyanax lacustris (Lütken, 1875) (n = 221); Astyanax sp. (n = 10); Deuterodon sp.

(8); Deuterodon giton (Eigenmann, 1908) (n = 41); Deuterodon intermedius (Eigenmann, 1908) (n = 449); Deuterodon pedri Eigenmann, 1908(n = 123); Deuterodon aff. taeniatus(n = 395); Psalidodon sp. (n = 62); Psalidodon fasciatus (Cuvier, 1819) (n = 17); Psalidodon rivularis (Lütken, 1875) (n = 765); Hasemania nana (Lütken, 1875)(n = 335); Hasemania sp. (n = 84); Knodus moenkhausii (Eigenmann & Kennedy, 1903) (n = 662); Oligosarcus argenteus Günther, 1864 (n = 153); Piabina argentea Reinhardt, 1867 (n = 8); Serrapinnus heterodon (Eigenmann, 1915) (n = 2); Erythrinidae: Hoplias intermedius (Günther, 1864) (n = ) Aspredinidae: Bunocephalus hartti Carvalho, Cardoso, Friel & Reis, 2015 (n = 4); Trichomycteridae: Cambeva variegata (Costa, 1992) (n = 60); Trichomycterus alternatus (Eigenmann, 1917) (n = 522); Trichomycterus auroguttatus Costa, 1992 MZUSP 43341 (n = 6); Trichomycterus brasiliensis Lütken, 1874 (n = 27); Trichomycterus caudofasciatus Alencar & Costa, 2004 (n = 21); Trichomycterus immaculatus (Eigenmann & Eigenmann, 1889) (n = 13); Trichomycterus itacambirussu Triques & Vono, 2004 (n = 1); Trichomycterus jequitinhonhae Triques & Vono, 2004 (n = 3); Trichomycterus melanopygius Reis, dos Santos, Britto, Volpi & de Pinna, 2020 (n = 27); Trichomycterus novalimensis Barbosa & Costa, 2010 (n = 27); Trichomycterus pauciradiatus Alencar & Costa, 2006 (n = 7); Trichomycterus pradensis Sarmento-Soares, Martins-Pinheiro, Aranda & Chamon, 2005 (n = 43); Trichomycterus reinhardti (Eigenmann, 1917) (n = 9); Trichomycterus sp. A (n = 16); Trichomy*cterus* sp. B (n = 4); **Callichthyidae:** *Callichthys callichthys* (Linnaeus, 1758) (n = 20); Hoplosternum littorale (Hancock, 1828) Uncatalogued; Loricariidae: Euryochus thysanos Pereira & Reis, 2017 (n = 10); Neoplecostomus doceensis Roxo, Silva, Zawadzki & Oliveira, 2014 (n = 40); Neoplecostomus franciscoensis Langeani, 1990 (n = 9); Neoplecostomus paranensis Langeani, 1990 (n = 73); Neoplecostomus sp. A (n = 36); Neoplecostomus sp. B (n = 13); Harttia carvalhoi Miranda Ribeiro, 1939 (n = 47); Harttia gra*cilis* Oyakawa, 1993 (n = 4); *Harttia intermontana* Oliveira & Oyakawa, 2019 (n =1); Harttia leiopleura Oyakawa, 1993 (n = 2); Harttia longipinna Langeani, Oyakawa & Montoya-Burgos, 2001 (n = 1); Harttia loricariformis Steindachner, 1877 (n = ); Harttia novalimensis Oyakawa, 1993 (n = 6); Harttia torrenticola Oyakawa, 1993 (n = 81); Harttia cf. gracilis (n = 4); Harttia cf. longipinna (n = 13); Harttia sp. (n = 5); Hypostomus francisci (Lütken, 1874) (n = 4); Hypostomus sp. (n = 1); Pareiorhaphis mutuca (Oliveira & Oyakawa, 1999) (n = 4); Pareiorhaphis nasuta Pereira, Vieira & Reis, 2007 (n = 3); Pareiorhaphis scutula Pereira, Vieira & Reis, 2010 (n = 119); Pareiorhaphis vetula Pereira, Lehmann & Reis, 2016 (n = 25); Pareiorhaphis sp. MNRJ 48424 (3); Heptapteridae: Phenacorhamdia tenebrosa (Schubart, 1964) (n = 1); Rhamdia quelen group (n = 7); **Pimelodidae:** *Duopalatinus emarginatus* (Valenciennes, 1840) (n = 1); *Pimelodus fur* (Lütken, 1864) (n = 1); **Gymnotidae:** *Gymnotus carapo* group (n = 85); Sternopygidae: Eigenmannia virescens (Valenciennes, 1836) (n = 1); Poeciliidae: Phalloceros harpagos Lucinda, 2008 (n = 269); Phalloceros uai Lucinda, 2008 (n = 719); Poecilia reticulata Peters, 1859 (n = 234); Synbranchidae: Synbranchus marmoratus group (n = 2); Cichlidae: Australoheros mattosi Ottoni, 2012 (n = 7); Australoheros sp. (n = 1); Geophagus brasiliensis (Quoy & Gaimard, 1824) (n = 115).

We recorded 60 species which were distributed in six orders and 17 families (Table 2). Characiformes and Siluriformes were predominant on both sides of the mountain **Table 2.** Fish species found in the middle-southern Espinhaço mountain range, Minas Gerais state, Brazil. Legend: (+) presence of species in drainages. CG = Ribeirão Congonhas; CP = Rio Cipó; PA = Rio Paraúna; PD = Ribeirão das Pedras; PH = Rio Parauninha; SA = Rio Santo Antônio; <sup>1</sup> – threatened species, according to COPAM (2010) and/or (MMA, 2018); <sup>2</sup> – endemic species from Rio São Francisco basin; <sup>3</sup> – endemic species from Rio Doce basin; <sup>4</sup> – exotic species to Rio São Francisco and/or Rio Doce basin. The order sequence follows Buckup et al. (2007), except for the updates in Bryconidae, Cichliformes and Stethaprioninae (see Material and methods). Genera and species sequences are given in alphabetic order.

Species	Upper Rio Paraúna		Upper Rio Santo Antônio			
	CG	СР	PA	PD	PH	SA
Order Characiformes						
Family Prochilodontidae						
Prochilodus costatus <sup>2</sup> Valenciennes, 1850		+				
Family Anostomidae						
Hypomasticus mormyrops (Steindachner, 1875)				+		
Hypomasticus thayeri1 (Borodin, 1929)				+		
Leporellus vittatus (Valenciennes, 1850)		+				
Leporinus amblyrhynchus <sup>4</sup> Garavello & Britski, 1987		+				
Leporinus copelandii Steindachner, 1875						+
Leporinus marcgravii <sup>2</sup> Lütken, 1875		+				
Leporinus taeniatus <sup>2</sup> Lütken, 1875		+				
Megaleporinus obtusidens (Valenciennes, 1837)		+				
Family Crenuchidae						
Characidium fasciatum Reinhardt, 1867			+			
Characidium sp. A				+	+	
Characidium sp. B				+		+
Characidium sp. C				+		+
Family Bryconidae						
Brycon opalinus <sup>1</sup> (Cuvier, 1819)				+		
Family Characidae						
Phenacogaster franciscoensis <sup>2</sup> Eigenmann, 1911		+				
Astyanax lacustris Lütken, 1875	+	+	+		+	+
Astyanax sp.	+				+	
Deuterodon giton Eigenmann, 1908				+	+	+
Deuterodon intermedius Eigenmann, 1908				+		
Deuterodon pedri <sup>3</sup> Eigenmann, 1908				+	+	+
Deuterodon aff. taeniatus				+	+	+
Deuterodon sp.						+
Psalidodon rivularis <sup>2</sup> (Lütken, 1875)	+		+		+	
Psalidodon sp.			+			+
Hasemania nana <sup>2</sup> (Lütken, 1875)	+		+	+		+
Hasemania sp.	+		+			
Knodus moenkhausii Eigenmann & Kennedy, 1903				+		+
Oligosarcus argenteus Günther, 1864	+			+	+	+
Piabina argentea Reinhardt, 1867		+				
Family Erythrinidae						
Hoplias intermedius (Günther, 1864)	+	+	+	+	+	+
Order Siluriformes						
Family Aspredinidae						
Bunocephalus hartti <sup>2</sup> Carvalho, Cardoso, Friel & Reis, 2015		+				
Family Trichomycteridae						
Trichomycterus alternatus (Eigenmann, 1917)			+	+	+	+
Trichomycterus melanopyeius Reis, dos Santos, Britto, Volpi & de Pinna, 2020				+	+	+
Trichomycterus sp. A				+		
Trichomycterus sp. B			+	+	+	+
Family Callichthyidae						
Callichthys callichthys (Linnaeus, 1758)	+				+	+

Species	Upp	Upper Rio Paraúna		Upper Rio Santo Antônio		
	CG	СР	PA	PD	PH	SA
Hoplosternum littorale (Hancock, 1828)			+			
Family Loricariidae						
Euryochus thysanos Pereira & Reis, 2017				+		
Neoplecostomus sp. A				+	+	+
Neoplecostomus sp. B			+			
Harttia intermontana <sup>3</sup> Oliveira & Oyakawa, 2019						+
Harttia sp.				+		
Hypostomus francisci (Lütken, 1874)		+				
Hypostomus sp.				+		
Pareiorhaphis scutula <sup>3</sup> Pereira, Vieira & Reis, 2010				+	+	+
Pareiorhaphis vetula3 Pereira, Lehmann & Reis, 2016					+	+
Pareiorhaphis sp.			+			
Family Heptapteridae						
Phenacorhamdia tenebrosa (Schubart, 1964)		+				
Rhamdia quelen group	+	+	+	+	+	
Family Pimelodidae						
Duopalatinus emarginatus <sup>2</sup> (Valenciennes, 1840)		+				
Pimelodus fur <sup>2</sup> (Lütken, 1874)		+				
Order Gymnotiformes						
Family Gymnotidae						
Gymnotus carapo group	+		+	+	+	+
Family Sternopygidae						
Eigenmannia virescens (Valenciennes, 1836)		+				
Order Cyprinodontiformes						
Family Poeciliidae						
Phalloceros harpagos Lucinda, 2008					+	
Phalloceros uat <sup>2</sup> Lucinda, 2008					+	+
Poecilia reticulata <sup>4</sup> Peters 1859	+		+			+
Order Synbranchiformes						
Family Synbranchidae						
Synbranchus marmoratus group						+
Order Cichliformes						
Family Cichlidae						
Australoheros mattosi <sup>2</sup> Ottoni, 2012			+			
Australoheros sp.					+	
Geophagus brasiliensis (Ouoy & Gaimard ,1824)	+		+	+	+	+

range: 20 and 13 species from the upper rio Santo Antônio, and 17 and 12 species from the upper rio Paraúna. Cyprinodontiformes and Cichliformes were also recorded (three representatives from each of them), followed by Gymnotiformes (two species), and Synbranchiformes (one species). The most representative families were Characidae and Loricariidae, with 12 and seven species, respectively, from the upper rio Santo Antônio; and nine and three species, respectively, from the upper rio Paraúna. The other recorded families were: Anostomidae (eight species); Crenuchidae and Trichomycteridae (four species), Poeciliidae and Cichlidae (three species), Heptapteridae, Pimelodidae and Callichthyidae (two species), Prochilodontidae, Bryconidae, Erythrinidae, Aspredinidae, Gymnotidae, Sternopygidae, and Synbranchidae (one species). Thirty-four species were associated to headwaters of the upper rio Paraúna, while 40 species were attributed to the upper rio Santo Antônio. A total of 14 species was recorded for both drainages. The highest species richness was registered from the ribeirão das Pedras (26 species) and the rio Cipó (17 species) drainages. In the latter was confirmed the highest number of exclusive species (14 species). Three migratory species (Pimelodus fur, Prochilodus costatus, and Megaleporinus obtusidens); two endangered (Brycon opalinus and Hypomasticus thayeri); 17 endemic (Australoheros mattosi, Bunocephalus hartti, Deuterodon pedri, Duopalatinus emarginatus, Harttia intermontana, Hasemania nana, Leporinus marcgravii, L. taeniatus, Prochilodus costatus, Pareiorhaphis scutula, P. vetula, Phalloceros uai, Phenacogaster franciscoensis, P. fur, Psalidodon rivularis, Trichomycterus alternatus, and T. melanopygius); and two exotic species (Leporinus amblyrhynchus, and Poecilia reticulata) were registered. Four of the aforementioned endemic species (H. nana, P. uai, P. rivularis, and T. alternatus) were found out in different basin instead of their original ones. Nineteen species presented some taxonomic inaccuracy and 14 are possibly new species (Characidium sp. A, Characidium sp. B, Characidium sp. C, Astyanax sp. A, Astyanax sp. B, Astyanax sp. C, Hasemania sp., Trichomycterus sp. A, Trichomycterus sp. B, Harttia sp., Neoplecostomus sp. A, Neoplecostomus sp. B, Pareiorhaphis sp., and Australoheros sp.). Other four species are possibly related to species complex (Deuterodon aff. taeniatus, Gymnotus carapo group, Rhamdia quelen group, and Synbranchus marmoratus group). Historical records of 15 species were obtained exclusively during visits to fish collections (B. opalinus, B. hartii, D. emarginatus, Eigenmannia virescens, Hoplosternum littorale, Hypostomus francisci, L. amblyrhynchus, L. marcgravii, L. taeniatus, M. obtusidens, P. franciscoensis, Phenacorhamdia tenebrosa, Piabina argentea, P. fur, and P. costatus).

#### Order Characiformes

# Prochilodus costatus Valenciennes, 1850

#### Distribution. Rio São Francisco basin.

**Diagnosis.** *Prochilodus costatus* is diagnosed from its congeners by having 44–47 perforated scales in the lateral line; 8–9 scales between the origin of dorsal fin and lateral line.

#### Family Anostomidae

#### Hypomasticus mormyrops (Steindachner, 1875)

Distribution. Rio Paraíba do Sul, rio Piabanha, and rio Doce basins, Brazil.

**Diagnosis.** *Hypomasticus mormyrops* differs from *H. thayeri* by the moderate lips; mouth ventral; premaxillary and dentary teeth anteriorly oriented when mouth is closed; first teeth (close to the symphysis in the premaxilla and dentary) larger than the others.

# Hypomasticus thayeri (Borodin, 1929)

Fig. 3A

Distribution. Rio Paraíba do Sul and rio Jequitinhonha basins, Brazil.

**Diagnosis.** *Hypomasticus thayeri* differs from *H. mormyrops* by the upper lip developed; mouth subterminal, not facing down; premaxillary teeth posteriorly oriented and dentary teeth anteriorly oriented when mouth is closed; three anterior teeth of premaxilla and dentary with similar size.

#### Leporellus vittatus (Valenciennes, 1850)

**Distribution.** Rio Amazonas, rio Paraná-Paraguay, and rio São Francisco basins: Brazil, Ecuador, Colombia, Bolivia, Paraguay, and Peru.

**Diagnosis.** *Leporellus vittatus* is diagnosed by having two longitudinal dark stripes on upper and lower lobes and one on the caudal fin median rays.

#### Leporinus amblyrhynchus Garavello & Britski, 1987

Distribution. Rio Paraná and upper rio São Francisco basins, Brazil.

**Diagnosis.** *Leporinus amblyrhynchus* differs from its congeners by a longitudinal dark band on the sides of the body and 8–10 transversal dark bands on the dorsal region.

#### Leporinus copelandii Steindachner, 1875

Distribution. Rio Paraíba do Sul and rio Doce basins, Brazil.

**Diagnosis.** *Leporinus copelandii* differs from its congeners by having three rounded or slightly rectangular spots conspicuously distributed in median line of the body, respectively below dorsal fin, below adipose fin and at the end of caudal peduncle; and all fins presenting reddish color.

#### Leporinus marcgravii Lütken, 1875

Distribution. Rio São Francisco basin, Brazil.

**Diagnosis.** *Leporinus marcgravii* differs from its congeners by having several conspicuous maculae throughout the lateral line and smaller maculae above and below lateral line; hyaline or slightly darkened fins base.

# Leporinus taeniatus Lütken, 1875

Distribution. Rio das Velhas, rio São Francisco basin, Brazil.

**Diagnosis.** *Leporinus taeniatus* differs from its congeners by dark macula in the maxilla and reddish pigmentation under the longitudinal dark band.
#### Megaleporinus obtusidens (Valenciennes, 1837)

**Distribution.** Upper rio Paraná, rio Jacuí, rio São Francisco, rio Paraguay, and rio Uruguay basins.

#### Family Crenuchidae

*Characidium fasciatum* Reinhardt, 1867 Fig. 3B

#### Distribution. Rio São Francisco basin.

**Diagnosis.** *Characidium fasciatum* differs from *Characidium* sp. A by the high or little tapered snout; adult specimens with vertical bars without defined shape or almost missing in the lateral of the body; narrow longitudinal dark band occupying less than one scale; pigmentation on caudal fin rays not forming conspicuous bands or just forming narrow bands. Distinguished from *Characidium* sp. B by having 36 or 37 perforated scales in the lateral line; four series of scales below lateral line.

#### Characidium sp. A

Fig. 3C

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Characidium* sp. A differs from its congeners of this study by having very tapered snout; wide and conspicuous vertical bars in the lateral of body in both juveniles and adults specimens; longitudinal dark band occupying one or more scales; one-two dark, wide and conspicuous bands in half of caudal fin rays and another in the base of first and posterior caudal fin ray.

**Remarks.** In this study, we refer *Characidium* sp. A such as a first putative new species from the rio Doce basin, due to differences in morphology and color pattern between this species and another similar ones from Southeastern Brazil river basins such as *C. alipioi*, *C. grajahuense*, *C. lagosantense*, *C. lauroi*, and *C. timbuiense*.

#### Characidium sp. B

Fig. 3D

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Characidium* sp. B differs from its congeners from the study area by predorsal length less than 45% of total length; lateral vertical bars absent or without defined shape; dark maculae on caudal fin not forming defined bands.

**Remarks.** In this study, we refer *Characidium* sp. B such as a second putative new species from the rio Doce basin, due to differences in morphology and color pattern



Figure 3. Characiformes species from the middle-southern Espinhaço mountain range, Minas Gerais state, Brazil A Hypomasticus thayeri, MNRJ 43577, 91.1 mm SL B Characidium fasciatum, MNRJ 48435, 68.8 mm SL C Characidium sp. A, MNRJ 46861, 65.5 mm SL D Characidium sp. B, MNRJ 48460, 56.2 mm SL E Characidium sp. C, MNRJ 46911, 42.3 mm SL F Astyanax lacustris, MNRJ 48521, 52.6 mm SL G Deuterodon giton, MNRJ 48129, 47.5 mm SL H Deuterodon intermedius, MNRJ 47840, 42.1 mm SL I Deuterodon pedri, MNRJ 48381, 65.5 mm SL J Deuterodon aff. taeniatus, MNRJ 45824, 55.5 mm SL K Hasemania nana, MNRJ 48440, 28.4 mm SL L Hasemania sp., MNRJ 48416, 25.1 mm SL M Oligosarcus argenteus, MNRJ 48393, 82.6 mm SL N Piabina argentea, MZUSP 110200, 44.2 mm SL O Psalidodon rivularis, MNRJ 48516, 46.8 mm SL P Psalidodon sp. MNRJ 48128, 59.4 mm SL Q Hoplias intermedius MZUSP 54696, 40.4 mm SL.

between this species and another from Southeastern Brazil river basins such as *C. alipioi*, *C. grajahuense*, *C. lagosantense*, and *C. lauroi*.

*Characidium* sp. C

Fig. 3E

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Characidium* sp. C differs from its congeners from the study area by predorsal length up to 55% of total length; vertical bars always arranged above and below the lateral line in a "y" or "yy" shape; weak of narrow dark band on caudal fin.

**Remarks.** In this study, we refer *Characidium* sp. C such as a third putative new species from the rio Doce basin, due to differences in morphology and color pattern between this species and another from Southeastern Brazil river basins such as *C. cricarense* and *C. litorale*.

#### Family Bryconidae

#### Brycon opalinus (Cuvier, 1819)

Distribution. Rio Paraíba do Sul and rio Doce basins.

**Diagnosis.** *Brycon opalinus* is diagnosed from its congeners by having one humeral spot and another in the caudal peduncle, never extending up to median caudal fin rays; tubules of the lateral line without secondary branches.

#### Family Characidae

#### Astyanax lacustris (Lütken, 1875) Fig. 3F

Distribution. Rio São Francisco basin, Southeastern Brazil.

**Diagnosis.** Astyanax lacustris differs from Astyanax sp. by the absence of teeth in maxillary bone; a conspicuous oval humeral spot arranged horizontally; hyaline fins usually yellowish, more evident in the caudal fin. It is also diagnosed by having 33–36 perforated scales in lateral line; 26–29 branched rays in anal fin; 6.5–7.5 scales above and 5.5–6.5 scales below lateral line.

#### *Astyanax* sp.

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** Astyanax sp. differs from A. lacustris by having teeth in maxillary bone; conspicuous humeral spot vertically oriented; hyaline fins slightly reddish. It is also diagnosed by having teeth tetracuspidate to heptacuspidate in the inner series of pre-

maxillary bone forming a notch; dentary teeth decreasing abruptly in size from fourth tooth; 6.5 scales above the lateral line; iii+19 or 20 anal fin rays.

**Remarks.** In this study, we refer *Astyanax* sp. such as putative new species from the study area, due to differences in morphology and color pattern between this species and another from Southeastern Brazil river basins such as *A. microschemos* and *A. turmalinensis*.

#### Deuterodon giton Eigenmann, 1908

Fig. 3G

Distribution. Rio Paraíba do Sul, Brazil.

**Diagnosis.** *Deuterodon giton* differs from its congeners of the study area by having dentary teeth decreasing gradually in size until the sixth or seventh tooth; dentary with more than five cusps (usually seven or eight); infraorbital 3 totally exposed, with almost no naked area prior to preopercle; infraorbital 3 shiny due to high concentration of guanine crystals and low concentration of chromatophores.

**Remarks.** *Deuterodon giton* is described from the rio Paraíba do Sul basin. However, in the present study it was found in the rio Doce basin, confirming the first record of the species for this basin. The difference observed in the specimens between both morphotypes is a tendency of longer length in adult specimens from the rio Paraíba do Sul basin.

#### Deuterodon intermedius Eigenmann, 1908

Fig. 3H

**Distribution.** Rio Paraíba do Sul basin and coastal drainages in state of Rio de Janeiro, Brazil.

**Diagnosis.** *Deuterodon intermedius* can be distinguished from its congeners of the study area by the absence of space in the symphysis of dentary; five tetracuspidate to hexacuspidate teeth in the inner series of the premaxillae; infraorbital 3 without chromatophores; small humeral spot, sometimes slightly rounded in smaller specimens; no more than 1.5 scales below the lateral line; 35–37 perforated scales in the lateral line

**Remarks.** *Deuterodon intermedius* is described from the rio Paraíba do Sul basin. However, in the present study it was found in the rio Doce basin. The only difference observed in the specimens between both morphotypes is a tendency of longer length in adult specimens from the rio Paraíba do Sul basin.

## Deuterodon pedri Eigenmann, 1908

Fig. 3I

Distribution. Rio Doce basin.

#### Deuterodon aff. taeniatus

Fig. 3J

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Deuterodon* aff. *taeniatus* differs from its congeners of the study area by the presence of a space in the symphysis of dentary; infraorbital 3 with high concentration of chromatophores; usually verticalized humeral spot with a lower comma-shaped feature, reaching 2.5 scales below the lateral line; 32–39 perforated scales in the lateral line; iii-v+17–24 anal fin rays.

**Remarks.** *Deuterodon taeniatus* is described from the rio São João and rio Macaé basins (rio Paraíba do Sul basin), in state of Rio de Janeiro, Brazil. The presence of the species in different basins has been notified in literature (Vieira 2006; Alves and Pompeu 2010) and sometimes with imprecise taxonomy (e.g., Alves et al. 2008; Vieira et al. 2015). In this study, *A. taeniatus* was recorded only for the rio Doce basin. However, it was observed that specimens from the rio Doce basin have lower body depth when compared to the morphotypes from rio Paraíba do Sul basin.

#### Deuterodon sp.

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Deuterodon* sp. differs from its congeners from the study area by having five hexacuspidate to heptacuspidate teeth in the inner series of the premaxillae; cusps straight, not forming notch; infraorbital 3 with naked area anteriorly, and below it; low concentration of chromatophores in the infraorbital 3; inconspicuous humeral spot slightly verticalized, straight anteriorly and straight or half-moon shaped posteriorly; 5.5 scales above lateral line; iii+21 anal fin rays.

**Remarks.** In this study, we refer *Deuterodon* sp. such as putative new species from the rio Doce basin, due to differences in morphology and color pattern between this species and another from southeastern Brazil river basins such as *D. giton*, *D. interme-dius*, and *D. taeniatus*.

#### Hasemania nana (Lütken, 1875)

Fig. 3K

#### Distribution. Rio São Francisco basin.

**Diagnosis.** *Hasemania nana* differs from *Hasemania* sp. by having 13–19 branched rays in anal fin and absence of rounded blotch in the median caudal fin rays.

**Remarks.** *Hasemania nana* is an endemic species from the rio São Francisco basin. However, in the present study it was found in the rio Doce basin, confirming the first record of the species for this basin. The only difference observed in the specimens between both morphotypes is a tendency of higher number of anal fin rays in the specimens from the rio Doce basin (13–19 vs. 13–16). The higher number of anal fin rays is congruent to the *H. nana* morphotype from the rio Paraopeba (rio São Francisco basin).

#### Hasemania sp.

Fig. 3L

Distribution. Upper rio Paraúna, rio São Francisco basin.

**Diagnosis.** *Hasemania* sp. differs from *H. nana* by having 11–14 branched rays in anal fin and presence of rounded blotch in the base of median caudal fin rays.

**Remarks.** In this study, we refer *Hasemania* sp. such as putative new species and second record from the rio São Francisco basin, due to differences in morphology and color pattern between this species and another from Southeastern Brazil river basins such as *H. bilineata*, *H. crenuchoides*, *H. nana*, and *H. uberaba*. In the taxonomic revision carried out by Serra (2003) there is no mention of the morphotype. The same have occurred in Vieira et al. (2015) resulting here in the first record of the taxon for the basin.

#### Knodus moenkhausii (Eigenmann & Kennedy, 1903)

**Distribution.** Rio Doce, rio Paraíba do Sul, upper rio Paraná, rio Paraguay, and rio Jequitinhonha basins, in Brazil, and some drainages in Bolivia and Paraguay.

**Remarks.** Occurrence of *K. moenkhausii* in the rio Doce basin was already confirmed. Different studies have mentioned about such record (dos Santos 2015; Vieira et al. 2015; Sales et al. 2018). In Vieira et al. (2015) the species was identified such as *K. cf. moenkhausii*. In tributaries of the rio Santo Antônio basin is quite common to collect it. However, it may be an exotic species which was introduced on the basin in the past (Vieira et al. 2015), but future research will be needed to confirm how the species reached the basin. Souza et al. (2015) revealed through DNA barcoding technique that *K. moenkhausii* has been shared throughout rio São Francisco, rio Paraíba do Sul and upper rio Paraná basins, through recent interchange. According to the authors, the species represent a single panmitic species, and its sharing in those basins may have occurred due to different human activity processes, such as intentional introduction, transposition of natural barriers or accidental escape in ornamental fish trade.

## Oligosarcus argenteus Günther, 1864

Fig. 3M

Distribution. Rio Doce, rio das Velhas, and upper rio Paraná basins, Brazil.

**Diagnosis.** *Oligosarcus argenteus* is diagnosed by having 17–24 teeth in maxillary bone; 44–48 perforated scales in lateral line; 8–9 series of scales above and 6–8 below lateral line; iv-v+20–25 branched rays in the anal fin; 17–20 scales around caudal peduncle.

#### Phenacogaster franciscoensis Eigenmann, 1911

Distribution. Rio São Francisco basin, Brazil.

#### Piabina argentea Reinhardt, 1867

Fig. 3N

**Distribution.** Upper rio Paraná, rio São Francisco, rio Itapicuru, rio Paraíba do Sul, and rio Itapemirim basins: Brazil and Paraguay.

**Diagnosis.** *Piabina argentea* is diagnosed by having longitudinal dark band in the lateral of the body; dark spot in caudal peduncle absent; 18–21 rays in anal fin.

#### Psalidodon rivularis (Lütken, 1875)

Fig. 3O

Distribution. Rio São Francisco basin, Southeastern Brazil.

**Diagnosis.** *Psalidodon rivularis* differs from its congeners of the study area by having premaxilla aligned with dentary in lateral view; four or five wide teeth in the inner series of premaxilla (if present, the fifth tooth is too small or not aligned with others); chromatophores surrounding abdominal scales and in higher concentration on the base of scales; developed scales in pectoral, pelvic, and anal fins.

**Remarks.** *Psalidodon rivularis* was originally described as an endemic species from the rio São Francisco basin. However, in the present study it was found in the rio Doce basin, confirming record of the species in this basin. Oliveira (2017) also confirmed the occurrence of *P. rivularis* as such as from the headwaters of rio Doce plus the headwaters of rio Jequitinhonha basin, while suggesting the synonymy of *Astyanax turmalinensis* (Triques, 2003) with *Psalidodon rivularis*.

#### Psalidodon sp.

Fig. 3P

**Distribution.** Upper rio Paraúna, rio São Francisco basin, and upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Psalidodon* sp. can be distinguished from its congeners from the study area, except of *P. rivularis*, by higher body anteriorly to dorsal fin origin; from *A. rivularis* by having premaxilla slightly in front of dentary in lateral view; five narrow teeth aligned in the inner series of premaxillary bone; two narrow vertical lines of chromatophores surrounding border of abdominal scales; small hooks in pectoral and anal fins in mature males.

**Remarks.** In this study, we refer *Psalidodon* sp. such as putative new species from the study area, due to differences in morphology and color pattern between this species and another Stethaprioninae from Southeastern Brazil river basins, such as *P. fasciatus*, *P. rivularis*, *A. scabripinnis*, and *A. turmalinensis*.

## Hoplias intermedius (Günther, 1864)

Fig. 3Q

**Distribution.** Rio São Francisco and rio Paraná basins plus tributaries of the rio Doce, Brazil.

**Diagnosis.** *Hoplias intermedius* is diagnosed by having 4–6 pores in lateral sensory system of the ventral surface of dentary; 42–46 perforated scales in lateral line; dark or light brown color in head and body.

#### **Order Siluriformes**

#### Family Aspredinidae

#### *Bunocephalus hartii* Carvalho, Cardoso, Friel & Reis, 2015 Fig. 4A

U

Distribution. Middle rio São Francisco basin, Minas Gerais, Brazil.

**Diagnosis.** *Bunocephalus hartii* is diagnosed by the absence of hooks throughout anterior margin of spine of pectoral fin; posterior ray of dorsal fin completely or almost adnate to dorsum.

#### Family Trichomycteridae

#### Trichomycterus alternatus (Eigenmann, 1911)

**Distribution.** Rio Doce basin, in the states of Minas Gerais and Espírito Santo, Brazil. **Diagnosis.** *Trichomycterus alternatus* differs from its congeners of the rio Doce and rio São Francisco basins by having seven branchiostegal rays; yellowish to light brown body color; rectangular or rounded sequential dark maculae at the midline of the body, sometimes fused and with a vermicular pattern, or forming a narrow stripe from the post-opercular region to the base of caudal fin; a row of rectangular sequential middorsal maculae, round or fused to maculae of the midlateral of the body; subtruncate caudal fin.

## *Trichomycterus melanopygius* Reis, dos Santos, Britto, Volpi & de Pinna, 2020 Fig. 4B

#### **Distribution.** Tributaries from rio Doce basin.

**Diagnosis.** *Trichomycterus melanopygius* differs from its congeners of the study area by the absence of evident maculae, spots, streaks and/or stripes on the flanks and dorsum of the body; i+7 (rarely i+8) pectoral fin rays; dark band in the median caudal fin rays.

#### Trichomycterus sp. A

Fig. 4C

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Trichomycterus* sp. A is distinguished from its congeners of the study area by the spotted body due to high concentration of large maculae with no defined shape; caudal fin strongly truncated; eight branchiostegal rays; few dorsal procurrent rays (14 or 15).

**Remarks.** In this study, we refer *Trichomycterus* sp. A such as a putative new species from the rio Doce basin, due to differences in morphology and color pattern between this species and another with similar color pattern from Southeastern Brazil river basins such as *T. auroguttatus*, *T. albinotatus*, *T. brasiliensis* group, *T. caipora*, *T. argos*, *T. novalimensis*, and *T. rubiginosus*.

#### *Trichomycterus* sp. B Fig. 4D

**Distribution.** Upper rio Paraúna, rio São Francisco basin, and upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Trichomycterus* sp. B differs from its congeners of the study area by having nine branchiostegal rays; high concentration of rounded dark spots on the head and sides of the trunk, dorsum, and belly, which may fuse and form small vermiculations; rounded caudal fin.

**Remarks.** In this study, we refer *Trichomycterus* sp. B such as a putative new species from the rio Doce basin, due to differences in morphology and color pattern between this species and another with similar color pattern from Southeastern Brazil river basins such as *T. brasiliensis* group, *T. argos, T. landinga, T. novalimensis*, and *T. rubiginosus*.

#### Family Callichthyidae

#### *Callichthys callichthys* (Linnaeus, 1758) Fig. 4E

Distribution. Drainages from Colombia to the Río de La Plata basin, South America.

#### Hoplosternum littorale (Hancock, 1828)

Distribution. Widespread in South America.

#### Family Loricariidae

Euryochus thysanos Pereira & Reis, 2017

Fig. 4F

**Distribution.** Coastal rivers in Eastern Brazil, from the rio Itapemirim, and including the larger basins of the rio Doce and Mucuri, in Espirito Santo and Minas Gerais states, to the rio Frades, state of Bahia.

**Diagnosis.** *Euryochus thysanos* is diagnosed by having rounded and short inferior lip, leaving a large naked area in the ventral portion of head; inferior lip with barbel developed; 30–35 bicuspidate teeth in the premaxillary and dentary bones; absence of hypertrophied odontodes.

#### Harttia sp.

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Harttia* sp. differs from *H. intermontana* by inferior region of orbit straight; compressed and narrow plates with developed odontodes in the dorsal and ventral region of caudal peduncle.

**Remarks.** In this study, we refer *Harttia* sp. such as a putative new species from the rio Doce basin, due to differences in morphology between this species and another from Southeastern Brazil river basins such as *H. carvalhoi*, *H. garavelloi*, *H. leiopleura*, *H. loricariformis*, *H. novalimensis*, and *H. torrenticola*.

#### *Harttia intermontana* Oliveira & Oyakawa, 2019 Fig. 4G

115.10

**Distribution.** Upper rio Doce basin, Brazil.

**Diagnosis.** *Harttia intermontana* differs from *Harttia* sp. by having orbit rounded; short and wide plates with poorly developed odontodes in the dorsal and ventral region of caudal peduncle.

#### Hypostomus sp.

Distribution. Upper rio Santo Antônio, rio Doce basin.



Figure 4. Siluriformes species from the middle-southern Espinhaço mountain range, Minas Gerais state, Brazil A Bunocephalus hartti, MZUSP 064227, 44.7 B Trichomycterus melanopygius, MNRJ 47902, 85.2 mm SL C Trichomycterus sp. A, MNRJ 47901, 87.4 mm SL D Trichomycterus sp. B, MNRJ 46932, 57.0 mm SL E Callichthys callichthys, MNRJ 48501, 58.2 mm SL F Euryochus thysanos, MNRJ 47897, 74.4 mm SL G Harttia intermontana, MNRJ 4863, 42.4 mm SL H Hypostomus francisci, MZUSP 37162, 66.8 mm SL I Neoplecostomus sp. A, MNRJ 46935, 73.0 mm SL J Neoplecostomus sp. B, MNRJ 48431, 43.0 mm SL K Pareiorhaphis scutula, MNRJ 48471, 88.1 mm SL L Pareiorhaphis vetula, MNRJ 46936, 40.4 mm SL.

**Diagnosis.** *Hypostomus* sp. differs from *H. francisci* by having black and large spots in the head and throughout the body.

**Remarks.** The only juvenile specimen collected in the study area was analyzed in such a way that is not possible to mention about species level identity or if it configures into a new species.

#### Hypostomus francisci (Lütken, 1874)

Fig. 4H

Distribution. Rio São Francisco and rio Paraná basins.

**Diagnosis.** *Hypostomus francisci* can be distinguished from *Hypostomus* sp. by pale small, rounded spots in the whole body, including in the fins; spine of the dorsal fin slightly smaller than predorsal distance.

#### Neoplecostomus sp. A

Fig. 4I

Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Neoplecostomus* sp. A differs from *Neoplecostomus* sp. B by the maxillary barbels poorly developed; premaxillary teeth and dentary with separate cusps and large concavity between them; lateral and central cusps with similar size; no developed papillae between branches of dentary; plates between dorsal and adipose fin meeting on the back of the dorsum.

**Remarks.** In this study, we refer *Neoplecostomus* sp. A such as a putative new species from the rio Doce basin, due to differences in morphology between this species and another from the rio Doce basin such as *N. doceensis*, and *N. pirangaensis*.

# Neoplecostomus sp. B

Fig. 4J

Distribution. Upper rio Paraúna, rio São Francisco basin.

**Diagnosis.** *Neoplecostomus* sp. B differs from *Neoplecostomus* sp. A by the maxillary barbels developed; premaxillary teeth and dentary with close cusps; median cusp more developed than lateral one; plates between dorsal and adipose fin not meeting.

**Remarks.** In this study, we refer *Neoplecostomus* sp. B such as a putative new species from the rio São Francisco basin, due to differences in morphology between this species and another from rio São Francisco basin such as *N. franciscoensis*.

#### Pareiorhaphis scutula Pereira, Vieira & Reis, 2010

Fig. 4K

#### **Distribution.** Upper rio Doce basin, Brazil.

**Diagnosis.** *Pareiorhaphis scutula* differs from its congeners of the study area by having abdomen with small plates covered by skin from the pectoral fin region to insertion of pelvic fins; fins with pale yellow and light brown spots.

#### Pareiorhaphis vetula Pereira, Lehmann & Reis, 2016

Fig. 4L

#### Distribution. Rio Doce basin, Brazil.

**Diagnosis.** *Pareiorhaphis vetula* can be distinguished from its congeners of the rio Doce basin by inferior lip with maxillary barbels completely adnate; elongated and pointed urogenital papilla.

### Pareiorhaphis sp.

Distribution. Upper rio Paraúna, rio São Francisco basin.

**Diagnosis.** Pareiorhaphis sp. differs from Pareiorhaphis scutula by the abdomen without plates; from *P. scutula* and *P. vetula* by the pectoral, pelvic and anal fins with clear color; dark caudal with clear borders. Pareiorhaphis sp. can still be distinguished from Pareiorhaphis vetula by inferior lip with developed maxillary barbels; urogenital papilla with normal size, not elongated.

**Remarks.** In this study, we refer *Pareiorhaphis* sp. such as a putative new species from the rio Doce basin, due to differences in morphology between this species and another from rio Doce basin such as *P. nasuta*, and *P. proskynita*.

#### Family Heptapteridae

#### Phenacorhamdia tenebrosa (Schubart, 1964)

Distribution. Upper rio Paraná and rio São Francisco basins, Argentina and Brazil.

#### Rhamdia quelen group

**Distribution.** Coastal river drainages from state of Rio de Janeiro to state of Santa Catarina, Brazil (Angrizani & Malabarba, 2020).

**Remarks.** A redescription of *R. quelen* was made, and the original locality where it comes from is rio Macacu drainage, a tributary of rio Soarinho, in the municipality of Cachoeira de Macacu, state of Rio de Janeiro, Brazil.

#### **Family Pimelodidae**

#### Pimelodus fur (Lütken, 1874)

Distribution. Rio das Velhas drainages in rio São Francisco basin, Brazil.

#### Duopalatinus emarginatus (Valenciennes, 1840)

Distribution. Rio São Francisco basin, Brazil.

Order Gymnotiformes

Family Gymnotidae

Gymnotus carapo group

Fig. 5A

**Distribution.** Upper rio Paraúna, rio São Francisco basin, and upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Gymnotus carapo* group is diagnosed by having the mouth upturned, pronate; rictus curved ventrally; eyes positioned below half of median line of the head; branchial opening throughout the posterior margin of opercle; oblique and conspicuous dark bands in the lateral of the body, from dorsal region to ventral surface of preanal; longitudinal band reaching the base of the anal fin.

**Remarks.** Although *G. carapo* is widespread from Trinidad and Tobago to Argentina, in this study we refer the species as *G. carapo* group, since the taxonomic status of *G. carapo* is uncertain for the Southeastern and Southern Brazil and may represent more than one species. A taxonomic review of the *G. carapo* group in Central and South America is needed.

#### Family Sternopygidae

#### Eigenmannia virescens (Valenciennes, 1836)

Distribution. Widespread from rio Orinoco to rio de La Plata basin.

**Diagnosis.** *Eigenmannia virescens* is diagnosed by having small branchial opening, smaller than the snout; body light brown, maculae absent.

#### Order Synbranchiformes

Family Synbranchidae

## Synbranchus marmoratus group

Fig. 5B

#### Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Synbranchus marmoratus* group is diagnosed by having dark-brown color on dorsal region of body and light brown below the midline and head; many rounded dark spots or irregularly shaped dark spots throughout the whole body; dark stripe composed by fusion of many dark spots in ventrolateral region of head.

**Remarks.** Albeit the true *S. marmoratus* have a wide distribution in the Neotropical region, in this study we refer the species as *S. marmoratus* group, since the taxonomic status of *S. marmoratus* may represent more than one species. A taxonomic review of *S. marmoratus* from Central and South America is needed to clarify this problematic issue.

#### Order Cyprinodontiformes

#### Family Poeciliidae

#### Phalloceros harpagos Lucinda, 2008

**Distribution.** Rio Paraná-Paraguay basin and coastal rivers from rio Itabapoana to rio Araranguá, in Brazil, Paraguay, and Argentina.

**Diagnosis.** *Phalloceros harpagos* differs from *P. uai* by having gonopodium with hook in the two gonopodial appendages in males positioned close to the appendages; distal half of the appendage narrower than proximal half; urogenital papilla centralized in females, positioned between anus and the base of first anal fin ray.

**Remarks.** Albeit *P. harpagos* presents a relatively wide distribution in coastal drainages of Brazil, here we present the first record of the species in the rio Santo Antônio basin.

#### Phalloceros uai Lucinda, 2008

Fig. 5C

Distribution. Rio das Velhas, rio São Francisco basin, Brazil.

**Diagnosis.** *Phalloceros uai* differs from *P. harpagos* by having gonopodium with small left hook facing down or up and forward in males, situated near to gonopodial appendage base; right hook absent; urogenital papilla directed to the right side in females, positioned laterally; anal opening in contact with first anal fin ray or close to it.

**Remarks.** *Phalloceros uai* is an endemic species from rio São Francisco basin and, in this study, we present the first record for the rio Santo Antônio basin. Vieira et al. (2015) recorded the species in the rio Piracicaba, another important drainage from the rio Doce basin. The distribution of the species in adjacent basin deserves further research, and it is still being carefully investigated.

### Poecilia reticulata Peters, 1859

**Distribution.** Trinidad and Tobago in Central America and Northern South America. **Remarks.** *Poecilia reticulata* was widely introduced in aquatic environments in Brazil, so its occurrence in many rivers of the country is quite common nowadays.

## Order Cichliformes

## Family Cichlidae

*Australoheros mattosi* Ottoni, 2012 Fig. 5D

Distribution. Rio São Francisco basin, Southeastern Brazil.

**Diagnosis.** *Australoheros mattosi* differs from *Australoheros* sp. by having XVI–XVII+9–11 dorsal fin rays; VII–VIII+9 anal fin rays.

*Australoheros* sp. Fig. 5E

#### Distribution. Upper rio Santo Antônio, rio Doce basin.

**Diagnosis.** *Australoheros* sp. differs from *A. mattosi* by having XVIII+7 dorsal fin rays; VIII+7 anal fin rays.



**Figure 5.** Fish species from the middle-southern Espinhaço mountain range, Minas Gerais state, Brazil **A** Gymnotiformes (*Gymnotus carapo* group, MNRJ 48407, 130.5 mm SL) **B** Synbranchiformes (*Synbranchus marmoratus* group, MNRJ 48448, 116.8 mm SL) **C** Cyprinodontiformes (*Phalloceros uai*, MNRJ 48408, 32.0 mm (female – above) and 16.3 mm (male – below) SL) **D** Cichliformes (*Australoheros mattosi*, MNRJ 48454, 54.6 mm SL **E** Cichliformes (*Australoheros* sp., MNRJ 48659, 39.3 mm SL).

**Remarks.** In this study, we refer *Australoheros* sp. such as putative new species due to differences in morphology between it and another from rio Doce basin, since *A. perdi* and *A. ipatinguensis* seems to be restricted to small areas such as lagoons and small rivers in the rio Doce basin. However, a higher number of specimens with difference sizes is needed and a taxonomic review of the cichlid *Australoheros* genus as well.

## Geophagus brasiliensis (Quoy & Gaimard, 1824)

Distribution. coastal drainages in eastern Brazil.

An identification key for its species from the study area is provided.

## Identification key to orders

1	body covered by scales
_	scales absent, naked body covered by skin or bony plates5
2	pelvic, dorsal, and caudal fins absent GYMNOTIFORMES
_	pelvic, dorsal, and caudal fins present
3	presence of spines in pelvic, dorsal, and anal fins CICHLIFORMES
_	spines in pelvic, dorsal, and anal fins absent4
4	premaxilla protractible; anal fin modified in gonopodium in males; adipose fin
	absent CYPRINODONTIFORMES
_	premaxilla non-protractible; anal fin not modified in gonopodium in males;
	adipose fin usually present CHARACIFORMES
5	barbels present on anterior portion of head (at least, one pair); two branchial
	openings located in variable position in the head; pelvic and pectoral fins pre-
	sentSILURIFORMES
_	barbels absent; single branchial opening, located ventrally in the head; pelvic
	and pectoral fins absentSYNBRANCHI-
	FORMES (Synbranchidae: 1 gen., 1 sp.) (Synbranchus marmoratus group)

## Identification keys to families

#### CHARACIFORMES

Small teeth implanted in thick moving lips in premaxillary and dentary
bones Prochilodontidae (Prochilodus costatus)
Teeth with different shapes, sizes, and numbers in premaxilla and dentary, im-
planted in bones of the maxillae
Fontanel absent in head
Fontanel present

## SILURIFORMES

body totally or partially covered by bone plates
body covered by skin; bony plates absent
Mouth terminal or subterminal; double series of plates in the sides of body;
nuchal plate meeting parieto-supraoccipital bone in the midline of predorsal
region
Mouth ventral, forming an oral disc; presence of plates on each side of body
arranged in three longitudinal series or moreLoricariidae
presence of patch of odontodes in preopercle and opercle Trichomycteridae
odontodes absent
head and body severely depressed, its maximum width at posterior region of skull
and pectoral girdle; adipose fin absentAspredinidae (Bunocephalus hartti)
slightly depressed or rounded head and high body; adipose fin present5
head higher than wide; first ray of pectoral and dorsal fins modified in an acute
and penetrating spine Pimelodidae
head as wide as high; first ray of pectoral and dorsal fins modified in a hard
spine, but not exactly an acute and penetrating spineHeptapteridae

## **GYMNOTIFORMES**

## CYPRINODONTIFORMES

- 1 Poecillidae (1 subfamily)
- Third, fourth and fifth rays of the anal fin modified in an intromittent organ (gonopodial structure) ...... Poecillinae

## CICHLIFORMES

## Identification keys to genera and species

## FAMILY ANOSTOMIDAE

1	dark blotch on anterior dorsal fin rays
_	Absence of dark blotch on dorsal fin rays and dark stripes on caudal fin
	lobes
2	dark longitudinal band present; absence of three or more large maculae in the
	sides of the body
_	dark longitudinal band absent; three or more large maculae in the lateral of
	body
3	dark macula in the maxilla; reddish pigmentation under the longitudinal
	band
_	dark macula in the maxilla absent; 8-10 transversal dark bands in the dorsal
	region Leporinus amblyrhynchus
4	terminal mouth5
_	Mouth subterminal or ventral; premaxilla ventrally oriented7
5	premaxilla and dentary with three teeth Megaleporinus obtusidens
_	premaxilla and dentary with 4 teeth; red macula in the mouth commissure6
6	three rounded or slightly rectangular spots conspicuously distributed in median
	line of the body, respectively below dorsal fin, below adipose fin and at the end
	of caudal peduncle; all fins presenting reddish color Leporinus copelandii
_	several conspicuous maculae throughout the lateral line and smaller macu-
	lae above and below lateral line; hyaline fins or slightly darkened on
	base Leporinus marcgravii
7	anterior region of snout convex in lateral view, moderate lips; mouth ventral;
	premaxillary and dentary teeth anteriorly oriented when mouth closed; first
	teeth (close to the symphysis in the premaxilla and dentary) larger than the
	others
_	anterior region of snout straight in lateral view, upper lip developed; mouth
	subterminal, not facing down; premaxillary teeth posteriorly oriented and den-
	tary teeth anteriorly oriented when mouth closed; three anterior teeth of pre-
	maxilla and dentary with similar size Hypomasticus thayeri

## FAMILY CRENUCHIDAE

1	very tapered snout; wide and conspicuous vertical bars in the lateral of the
	body in both juveniles and adult specimens; longitudinal dark band occupy-
	ing one or more scales; one to two dark, wide and conspicuous bands in half
	of caudal fin rays and another in the base of first and posterior caudal fin
	ray Characidium sp. A
_	high or little tapered snout; adult specimens with vertical bars without defined
	shape or almost missing in the lateral of the body; narrow longitudinal dark
	band occupying less than one scale; pigmentation on caudal fin rays not form-
	ing conspicuous bands or just forming narrow bands2
2	36-37 perforated scales in the lateral line; four series of scales below lateral
	line
_	34-36 perforated scales in the lateral line; two scales below lateral line3
3	predorsal length less than 45% of total length; lateral vertical bars absent
	or without defined shape; dark maculae on caudal fin not forming defined
	bands Characidium sp. B
_	predorsal length up to 55% of total length; vertical bars always arranged above
	and below the lateral line in a "y" or "yy" shape; weak of narrow dark band on
	caudal fin Characidium sp. C

## FAMILY CHARACIDAE

three series of teeth in premaxillary bone2
1 or 2 series of teeth in premaxillary bone with one or more cusps; one series
of teeth in dentary
obtuse snout; teeth in premaxillary bone arranged in three series and two series
in dentary; posterior series composed by a pair of small symphysial conic teeth
on each corner
short and sharp snout; intermediate series of premaxillary teeth not totally
separated from external one
pseudotimpanum present; very large scales covering preventral
area
pseudotimpanum absent; preventral area with scales of small size
scales reaching half of the caudal fin rays
scales just in the caudal fin base
adipose fin absent
adipose fin present
13–19 branched rays in anal fin: absence of rounded blotch in the median
caudal fin rays.
11–14 branched rays in anal fin: presence of rounded blotch in the base of
median caudal fin rays

7	a single series of conic teeth in the premaxillary, maxillary and den-
	tary bones; premaxilla aligned or slightly anterior to dentary in lateral
	view
_	two series of tricuspidate to multicuspidate teeth in the inner series of premax-
	illa; one series of tricuspidate to multicuspidate teeth in dentary
8	teeth in the inner series of premaxillary bone forming a notch
_	teeth in the inner series of premaxillary bone not forming a notch
9	absence of teeth in the maxillary bone; a conspicuous oval humeral spot ar-
	ranged horizontally; hvaline fins usually vellowish, more evident in the caudal
	fin: more than 20 branched rays in the anal fin
_	presence of teeth in maxillary bone: conspicuous humeral spot vertically ori-
	ented: hvaline fins slightly reddish: jij+19 to 20 anal fin rays
10	Greater body height roughly in the middle of the pectoral fin
_	body higher at the dorsal fin origin
11	premaxilla slightly in front of dentary in lateral view: five narrow teeth aligned
	in the inner series of premaxillary bone: two parrow vertical lines of chroma-
	tophores surrounding border of abdominal scales: small books in pectoral and
	anal fins in mature males
_	premaxilla aligned with dentary in lateral view: 4 or -5 wide teeth in the inner
	series of premavilla (if present, the fifth teeth is too small or not aligned with
	sches of premasing (in present, the first feed is too small of not angled with others), chromatophores surrounding abdominal scales and in higher concern
	tration on the base of the scales; developed scales in the pectoral pelvic and
	anal fins
12	anal inis
12	teeth of dentary decreasing graduary in size until sixth of seventh tooth
_ 12	infraorbital 2 with paled area prior to proceed a bioh concentration of abro
15	mitaoronal 5 with haked area prior to preopercie, high concentration of chro-
	matophores; conspicuous numeral spot vertically oriented, its similar width sith an above and below lateral line lateral line $28 / (1 (\bar{a} - 20))$ performed acales
	either above and below lateral line; lateral line $36-41$ ( $x = 39$ ) periorated scales
	In the lateral line
_	infraorbital 5 totally exposed, with almost no naked area prior to preoper-
	cle; infraorbital 5 sniny due to the high concentration of guanine crystals and
	low concentration of chromatophores; conspicuous numeral spot vertically
	oriented, larger above lateral line; 3/ or less perforated scales in the lateral
- /	line
14	presence of space in the symphysis of dentary; infraorbital 3 with high concen-
	tration of chromatophores Deuterodon aff. taeniatus
_	absence of space in the symphysis of dentary; infraorbital 3 with low concen-
	tration of chromatophores15
15	five hexacuspidate to heptacuspidate teeth in the inner series of the premaxilla;
	infraorbital 3 with naked area anteriorly, and below it; low concentration of chro-
	matophores in the infraorbital 3; inconspicuous humeral spot slightly verticalized,
	straight anteriorly and straight or half-moon shaped posteriorlyDeuterodon sp.
-	five tetracuspidate to hexacuspidate teeth in the inner series of the premaxilla;
	naked area anteriorly, below, and posteriorly; absence of chromatophores in

the infraorbital 3; small humeral spot, no regular shaped ,sometimes slightly rounded in juveniles; no more than 1.5 scales below the lateral line; 35–37 perforated scales in the lateral line......*Deuterodon intermedius* 

## FAMILY CALLICHTHYIDAE

coracoid bones covered by thick skin; caudal fin lobed ...... *Callichthys callichthys* coracoid bones exposed; caudal fin bifurcated ..............*Hoplosternum littorale*

## FAMILY TRICHOMYCTERIDAE

1 absence of evident maculae, spots, streaks and/or stripes the flanks and dorsum of the body; i+7 (rarely i+8) pectoral fin rays; dark band in the median caudal body with round or rectangular spots; stripes and/or vermiculations on the flanks and/or dorsum of the body; i+6 or i+7 pectoral fin rays ......2 Body spotted due to a high concentration of large maculae with no defined 2 shape; caudal fin strongly truncated; eight branchiostegal rays; few dorsal procurrent rays (14 or 15) ...... Trichomycterus sp. A body with round or rectangular spots; stripes and/or vermiculations on flanks and/or back of the body; 7-9 branchiostegal rays; more than 20 dorsal procur-rounded head in dorsal view; nine branchiostegal rays; high concentra-3 tion of rounded dark spots on the head and sides of the trunk, dorsum and belly, which may fuse and form small vermiculations; rounded caudal subtriangular head in dorsal view; seven branchiostegal rays; yellowish to light brown body color; rectangular or rounded sequential dark maculae at the midline of the body, sometimes fused and with a vermicular pattern, or forming a narrow stripe from the post-opercular region to the base of caudal fin; a row of rectangular sequential middorsal maculae, round or fused to maculae of the midlateral of the body; subtruncate caudal fin...... Trichomycterus alternatus

## FAMILY LORICARIIDAE

1	depr	essed snout a	nd caudal j	pedur	ncle; ad	dipose fin	absent		Loricari	inae
_	caud	al peduncle n	ot depress	ed; ad	ipose	fin preser	1t			2
2	func	tional spinele	t of the do	rsal sp	oine; i	+7 dorsal	fin rays	H	ypostom	inae
_	no	functional	spinelet	of	the	dorsal	spine;	i+7	dorsal	fin
	rays		•••••	•••••			•••••	Neop	ecostom	inae

## Subfamily Loricariinae

## Subfamily Hypostominae

black and large spots in the head and throughout the body....*Hypostomus* sp.
pale small rounded spots in the whole body, including in the fins; spine of the dorsal fin slightly smaller than predorsal distance......*Hypostomus francisci*

### Subfamily Neoplecostominae

1	large eyes (until 19.7% in HL); flat body between posterior dorsal fin ray and
	adipose origin; flat abdomen with no plates Euryochus (Eurochus thysanos)
_	small (less than 19% in HL); body usually rounded; abdomen plated or not;
	rounded or oval inferior lip, leaving small naked area in the ventral portion of
	the head
3	odontodes well developed in the first ray of pectoral fins and on the sides of
	head in nuptial males; odontodes with normal size in no nuptial males and
	females Pareiorhaphis
4	inferior lip with maxillary barbels completely adnate; elongated and pointed
	urogenital papilla Pareiorhaphis vetula
_	inferior lip with free and conspicuous maxillary barbels; papilla not devel-
	oped in males5
5	abdomen without plates; pectoral, pelvic and anal fins with clear color; dark
	caudal with clear borders
_	abdomen with small plates covered by skin, from the pectoral fin
	to insertion of pelvic fins; fins with pale yellow and light brown
	spotsPareiorhaphis scutula
_	odontodes poorly developed in the first ray of pectoral fins in mature and
	not nuptial males; odontodes with normal size on the lateral margin of head;
	abdomen with a large number of plates
1	maxillary barbels poorly developed; premaxillary teeth and dentary with
	separate cusps and large concavity between them; lateral and central cusps
	with similar size; no developed papillae between branches of dentary;
	plates between dorsal and adipose fin meeting on the back of the dor-
	sumNeoplecostomus sp. A
_	maxillary barbels developed; premaxillary teeth and dentary with close cusps;
	median cusp more developed than lateral one; plates between dorsal and adi-
	pose fin not meeting

## FAMILY HEPTAPTERIDAE

## FAMILY PIMELODIDAE

## FAMILY POECILIIDAE - subfamily Poecilliinae

1 modified anal fin in a short gonopodium in males (not exceeding or reaching the tip of the dorsal fin rays, in a vertical); 1-2 dark maculae in the sides of the body, anteriorly to a vertical through dorsal fin origin...... Poecilia reticulata long gonopodium in males (reaching and even surpassing in a vertical trough the tip of the dorsal fin rays); vertical or slightly rectangular macula at the dorsal fin rays height or slightly posterior to them......2 2 gonopodium with hook in the two gonopodial appendages in males positioned close to the appendages; distal half of the appendage narrower than proximal half; urogenital papilla centralized in females, positioned between anus and the base of first anal fin ray ..... Phalloceros harpagos gonopodium with small left hook facing down or up and forward in males, situated near to gonopodial appendage base; right hook absent; urogenital papilla directed to the right side in females, positioned laterally; anal opening in contact with first anal fin ray or close to it ..... Phalloceros uai

## FAMILY CICHLIDAE

1	upper branch of fir	st branchia	l arch v	vith lobe.	Geophage	us brasili	iensis
_	lobe absent in first	branchial a					2
2	XVI-XVII+9–11	dorsal	fin	rays;	VII-VIII+9	anal	fin
	rays				Australo	beros ma	ittosi
_	XVIII+7 dorsal fin	rays; VIII+	-7 anal :	fin rays	Aus	tralohero	os sp.

60

## Discussion

The predominance of Characiformes and Siluriformes in the study area is consistent with the pattern observed among freshwater fishes in the Neotropical region (Lowe-McConnell 1999; Alves et al. 2008; Camelier and Zanata 2014).

Although taxonomic (e.g., Menezes et al. 2007; Buckup et al. 2014; Vieira et al. 2015) and ecological approaches (e.g., Castro 1999; Sabino 1999; Castro et al. 2004; Ferreira and Casatti 2006; Felipe and Súarez 2010) to study stream fishes have increased considerably in recent years, studies of taxonomy and biology of small species in a wide area of the Neotropical region are still limited. The compilation of regional records of taxonomic and ecological diversity may support conservation plans and generate data for biogeographic analyses (Winemiller et al. 2008). We found a considerable number of small and medium sized fish species arranged in populations (sensu Vazzoler 1996) that use headwaters of the upper rio Paraúna and upper rio Santo Antônio as living and developmental areas.

Almost 32% of the whole ichthyofauna from the rio das Velhas (Alves and Pompeu 2005) and ca. 56% of the ichthyofauna from the rio Santo Antônio (Vieira 2006) were recorded in this study. When compared to the species previously registered from the upper rio Santo Antônio, this percentage is even higher than that recorded by dos Santos (2015) (40 species in the present study vs. 39 in dos Santos 2015). The ichthyofaunal richness of headwaters is usually known as low and endemic (Lowe-McConnell 1999), with species that have limited ability to travel great distances (Castro 1999).

The highest species richness was registered at ribeirão das Pedras and rio Cipó, which are in lower altitude areas (Fig. 1). As observed by Castro et al. (2003) for the species from rio Parapanema basin, species richness is associated with the longitudinal gradient in the location of sampling sites. Furthermore, it is combined with the fish regional diversity plus the physical extension of the sampling environment and biogeographic patterns of ichthyofaunistic diversity. rio Cipó presented a substantial number of exclusive species from this drainage. Since it was noted the characteristic of fastwater environments nearby the mouth of the rio Paraúna and downstream of the Paraúna waterfall, we suggested that some species prefer such environments. Some of them with migratory habits (i.e., *Salminus franciscanus* Lima & Britski, 2007 and *Pimelodus maculatus* Lacépède, 1803) and highly appreciated in artisanal fishery in regions among municipalities of Conceição do Mato Dentro, Congonhas do Norte, Presidente Kubitschek, Santana de Pirapama, Gouveia, and Presidente Juscelino were registered.

As expected for the Southern Espinhaço mountain range, and corroborating Alves et al. (2008), several endemic and/or endangered species were found. Two of these (*Hypomasticus thayeri* and *Brycon opalinus*) are listed in Brazil as "Endangered" and "Vulnerable", respectively (Akama et al. 2018), or "Critically Endangered", according to the state list for endangered fish species in state of Minas Gerais (Minas Gerais 2010). Fifteen species are endemic to the studied hydrographic basins. From the total of en-

demic species, 11 of them come from the rio São Francisco basin (Prochilodus costatus, Leporinus marcgravii, L. taeniatus, Psalidodon rivularis, Hasemania nana, Phenacogaster franciscoensis, Duopalatinus emarginatus, Pimelodus fur, Bunocephalus hartti, Phalloceros uai, and Australoheros mattosi) and four come from the rio Doce basin (Deuterodon pedri, Harttia intermontana, Pareiorhaphis scutula, and P. vetula). Three endemic species (H. nana, P. uai, and P. rivularis) from the rio São Francisco basin were found in the rio Santo Antônio basin (Table 2). These were the first records of H. nana and P. rivularis in the adjacent basin. The occurrence of P. uai in a different basin instead of rio São Francisco was already mentioned in the literature (Vieira et al. 2015). On the other hand, T. alternatus was originally described from the rio Doce basin (Reis and de Pinna 2019), and it was registered for the first time to the rio Paraúna basin. Triques and Vono (2004) extended its distribution to the rio Jequitinhonha basin and Fricke et al. (2021) summarized its distribution to the Atlantic coastal rivers in the states of Rio de Janeiro, Minas Gerais, and Espírito Santo. However, the new species records in different basins will need further investigations to elucidate the possibilities of sharing basins or even an introduction problem due to human actions. Two species (Leporinus amblyrhynchus and Poecilia reticulata) are exotic to the studied basins. However, another three registered species (Deuterodon giton, D. intermedius, and Knodus moenkhausii) are also usually considered exotic for such basins, but there is no investigation into the validity of such status.

Considering our results, we reinforce the importance of headwater environment conservation, as pointed out by Drummond et al. (2005), who defined such areas as priority for fish conservation in state of Minas Gerais. Furthermore, Menezes et al. (2007) highlighted the need of studies and surveys in order to increase the knowledge about fish species which inhabit those areas and to recognize conservation priorities in aquatic environments in the Atlantic rainforest region. In addition, records for 25% of the species were based exclusively on material from scientific collections. These results support the substantial importance of zoological collections in sampling and archiving biological diversity, and also allows the development of knowledge in research facing the conservation of biodiversity (Zaher and Young 2003; Ohara et al. 2015).

The substantial number of taxonomically inaccurate identifications (ca. 30%) and potentially new species (almost 22%) recorded herein, added to the lack of data on distributional patterns reinforces the need of studies in such areas. The considerable number of potentially new species indicates the large knowledge gap in the Espinhaço mountain range. It is important to mention that the aforementioned species have been studied by different researchers and descriptions have been made, such as the currently described *H. intermontana* and *T. melanopygius*. Also, the occurrence of many large ventures in the region, such as mining and hydroelectric power plants, make such areas high priorities for biodiversity studies, to minimize the possibility of populations and species extinctions even before they are properly recognized. The increase of knowledge about such fishes may contribute to future assessments of the conservation status and the encouragement of exploratory field expeditions of remote areas, as in the case of this study. The new results shown here can provide a better understanding about biogeographic patterns and evolution of fish at the Espinhaço mountain range and adjacent basins.

## Acknowledgements

Most of the present study was conducted at the Setor de Ictiologia, Museu Nacional, Universidade Federal do Rio de Janeiro, as a requirement for a Master's degree in Zoology at the Programa de Pós-graduação em Ciências Biológicas (Zoologia) - PPGZoo. Financial support to SAS was provided by CAPES (Coordenação de Aperfeicoamento de Pessoal de Nível Superior - Brazilian Federal Government). We are indebted to D.F. Moraes Jr and P.R. Menezes (MNRJ), Tarcísio Sousa, and Roberta Miranda for helping during the fieldwork. We would also like to thank Gilmar B. Santos and Tiago Pessali (MCNIP); Carlos de Lucena, Roberto Reis (MCP-PUCRS); Cristiano Moreira, Paulo Buckup, and Giovana Vignoli (MNRJ); Aléssio Datovo, Mário de Pinna, Oswaldo Oyakawa, Michel Gianeti, and Naércio Menezes (MZUSP); Flávio Lima (ZUEC); and Anja Palandacic (NHM) for friendly reception, curatorial assistance, and for the loan of comparative material. We are grateful to Miguel Andrade, Luciana Nascimento, Vinícius Espíndola, Willian Ohara, Flávio Lima, Luiza Aguiar, Suellen Andreas, M. Lúcia Fonseca, and Roberto Murta for hosting SAS in their homes during field expeditions or visits to the fish collections. We thank Marcos dos Santos (IEF-MG) for his help in obtaining the collecting permits, to Instituto Estadual de Florestas (IEF-MG), Insituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO) for providing the collecting permits. We also thank Cristiano Moreira (MNRJ), Daniel Fernandes (UFRJ), Felipe Talin (Opyta), Ricardo Campos da Paz (UNIRIO), Roberta Murta-Fonseca (UFMS), Tiago Pessali (PUC/MG), Luisa Sarmento-Soares, Caleb McMahan, and one anonymous reviewer for all suggestions and improvements in the manuscript. We also thank Marcelo Soares for the support with X-ray images in the Laboratório de Radiografia of the Departamento de Vertebrados, Museu Nacional; Gilberto Salvador for helping with the shapes of the map. Finally, to all colleagues from the Fish Laboratory of the Museu Nacional/UFRJ for encouraging us with the project. Financial support to MRB was provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grant # 309285/2018-6) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, grant #200.103/2019).

## References

Akama A, Netto-Ferreira AL, Paixão AC, Zanata AM, Calegari BB, Figueiredo CAA, Caramaschi EMP, Vieira F, Martins FO, Carvalho FR, Langeani F, Birindelli JLO, Silva LVV, Ingenito LFS, Rapp Py-Daniel LH, Montag LFA, Mehanna MN, Britto MR, Oyakawa OT, Albornoz PCL, Catelani PA, Buckup PA, Carvalho PH, Reis RE, Souza-Lima R, Santos VLM, Wosiacki WB (2018) Peixes. In: Instituto Chico Mendes de Conservação da Biodiversidade (Org.) Livro vermeho da fauna brasileira ameaçada de extinção: Volume VI – Peixes. ICMBio, Brasília, 39–92.

- Alves CBM, Pompeu PS (2010) Peixes do rio das Velhas: passado e presente. Argvmentvm Editora, Belo Horizonte 2: e196.
- Alves CBM, Leal GC, Brito MFG, Santos ACA (2008) Biodiversidade e conservação de peixes no Complexo do Espinhaço. In: Azevedo AA, Machado RB (Eds) Megadiversidade: Cadeia do Espinhaço: avaliação do conhecimento científico e prioridades de conservação. Conservação Internacional, Belo Horizonte 4: 177–196.
- Alves CBM, Pompeu PS (2005) Historical changes in the rio das Velhas fish fauna Brazil. American Fisheries Society Symposium 45: 587–602.
- Andrade Neto FR (2010) Estado atual do conhecimento sobre a fauna de peixes da bacia do Jequitinhonha. MG. Biota 2(5): 23–35.
- Angrizani RC, Malabarba LR (2020) Genetic diversity and species delimitation in *Rhamdia* (Siluriformes: Heptapteridae) in South America, with a redescription of *R. quelen* (Quoy & Gaimard, 1824). Zootaxa 4801(1): 85–104. https://doi.org/10.11646/zootaxa.4801.1.3
- Barbosa JM, Soares EC, Cintra IHA, Hermann M, Araújo ARR (2017) Perfil da ictiofauna da bacia do rio São Francisco. Acta of Fisheries and Aquatic Resources 5(1): 70–90.
- Buckup PA, Britto MR, Souza-Lima R, Pascoli JC, Villa-Verde L, Ferraro GA, Salgado FLK, Gomes JR (2014) Guia de identificação das espécies de peixes da bacia do rio das Pedras, município de Rio Claro, RJ. The Nature Conservancy, Rio de Janeiro, 79 pp.
- Buckup PA, Menezes NA, Ghazzi MS (2007) Catálogo das espécies de peixes de água doce do Brasil. Museu Nacional, Rio de Janeiro, 195 pp.
- Bueno ML, Magalhães ALB, Andrade Neto FR, Alves CBM, Rosa DM, Junqueira NT, Pessali TC, Pompeu PS, Zenni RD (2021) Alien fish fauna of southeastern Brazil: species status, introduction pathways, distribution and impacts. Biol Invasions 2021. https://doi. org/10.1007/s10530-021-02564-x
- Castro RMC, Vari RP, Vieira F, Oliveira C (2004) Phylogenetic analysis and redescription of the genus *Henochilus* (Characiformes: Characidae). Copeia (3): 496–506. https://doi.org/10.1643/CI-03-202R2
- Castro RMC, Casatti L, Santos HF, Ferreira, KM, Ribeiro AC, Benine RC, Dardis GZP, Melo ALA, Stopiglia R, Abreu TX, Bockmann FA, Carvalho M, Gibran FZ, Lima FCT (2003) Estrutura e composição da ictiofauna de riachos do rio Paranapanema, Sudeste e Sul do Brasil. Biota Neotropica 3(1): 1–31. https://doi.org/10.1590/S1676-06032003000100007
- Castro RMC (1999) Evolução da ictiofauna de riachos sul-americanos: padrões gerais e possíveis processos causais. In: Caramaschi EP, Mazzoni R, Peres-Neto, PR (Eds) Ecologia de peixes de riachos – Série Oecologia Brasiliensis. PPGE-UFRJ, Rio de Janeiro, 139–155. https://doi.org/10.4257/oeco.1999.0601.04
- Drummond GM, Martins CS, Machado ABM, Sebaio FA, Antonini Y (2005) Biodiversidade em Minas Gerais: um atlas para sua conservação, 2ed. Fundação Biodiversitas, Belo Horizonte, 222 pp.
- Felipe TRA, Súarez YR (2010) Caracterização e influência dos fatores ambientais nas assembléias de peixes de riachos em duas microbacias urbanas, alto rio Paraná. Biota Neotropica 10(2): 143–151. https://doi.org/10.1590/S1676-06032010000200018
- Ferreira CP, Casatti L (2006) Influência da estrutura do habitat sobre a ictiofauna de um riacho em uma micro-bacia de pastagem, São Paulo, Brasil. Revista Brasileira de Zoologia 23(3): 642–651. https://doi.org/10.1590/S0101-81752006000300006

- Fricke R, Eschmeyer WN, Van der Laan R [Eds] (2021) Eschmeyer's catalog of fishes: genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/ fishcatmain.asp [Electronic version accessed 01 March 2021]
- Lowe-McConnell RH (1999) Estudos ecológicos de comunidades de peixes tropicais. Edusp, São Paulo, 535 pp.
- Menezes NA, Weitzman SH, Oyakawa OT, Lima FCT, Castro RMC, Weitzman MJ (2007) Peixes de água doce da Mata Atlântica: lista preliminar das espécies e comentários sobre conservação de peixes de água doce Neotropicais. Museu de Zoologia – Universidade de São Paulo, 408 pp.
- Minas Gerais (2010) Minas Gerais. http://www.siam.mg.gov.br/sla/download.pdf?idNorma = 13192 [Accessed on: 2020-4-21]
- Nelson JS, Grande TC, Wilson MVH (2016) Fishes of the world. John Wiley & Sons, Hoboken, 707 pp. https://doi.org/10.1002/9781119174844
- Ohara WM, Queiroz LJ, Zuanon J, Torrente-Vilara G, Vieira FG, Doria CRC (2015) Fish collection of the Universidade Federal de Rôndonia: its importance to the knowledge of Amazonian fish diversity. Acta Scientiarum 37(2): 251–258. https://doi.org/10.4025/act-ascibiolsci.v37i2.26920
- Oliveira CAM (2017) Revisão taxonômica do complexo de espécies *Astyanax scabripinnis sensu* Bertaco & Lucena (2006) (Ostariophysi: Characiformes: Characidae). PhD Thesis, Universidade Estadual de Maringá, Maringá, Brazil.
- Reis VJC, dos Santos SA, Britto MR, Volpi AT, de Pinna MCC (2020) Iterative taxonomy reveals a new species of *Trichomycterus* Valenciennes 1832 (Siluriformes, Trichomycteridae) widespread in rio Doce basin: a pseudocryptic of *T. immaculatus*. Journal of Fish Biology 2020: 1607–1623. https://doi.org/10.1111/jfb.14490
- Sabaj MH (2019) Standard symbolic codes for Institutional resource collections in herpetology and ichthyology: An online reference. Version 7.1 (21 March 2019). American Society of Ichthyologists and Herpetologists, Washington. https://asih.org/sites/default/ files/2019-04/Sabaj\_2019\_ASIH\_Symbolic\_Codes\_v7.1.pdf
- Sabino J (1999) Comportamento de peixes em riachos: métodos de estudo para uma abordagem naturalística. In: Caramaschi EP, Mazzonni R, Peres-Neto PR (Eds) Ecologia de peixes de riachos – Série Oecologia Brasiliensis. PPGE-UFRJ, Rio de Janeiro, 183–208. https://doi.org/10.4257/oeco.1999.0601.06
- Sales NG, Mariani S, Salvador GN, Pessali, TC, Carvalho DC (2018) Hidden diversity hampers conservation efforts in a highly impacted Neotropical river system. Frontiers in Genetics 9(271): 1–11. https://doi.org/10.3389/fgene.2018.00271
- dos Santos SA (2015) Peixes. In: Pimenta BVS, Câmara T (Orgs) Fauna: Leste Atlântico do Espinhaço Meridional. Bicho do Mato Editora, Belo Horizonte, 106–131.
- Serra JP (2003) Análise filogenética e revisão taxonômica de *Hasemania* Ellis 1911 (Characiformes, Characidae). Master thesis, Universidade Estadual Paulista, São José do Rio Preto, Brazil.
- Souza CS, Oliveira C, Pereira LHG (2015) *Knodus moenkhausii* (Characiformes: Characidae): one fish species, three hydrographic basins – a natural or anthropogenic phenomenon? DNA Barcodes 3: 129–138. https://doi.org/10.1515/dna-2015-0016
- Taylor WR, van Dyke GC (1985) Revised procedures for staining and clearing small fishes and other vertebrates for bone and cartilage study. Cybium 9: 107–119.

- Triques ML, Vono V (2004) Three new species of *Trichomycterus* (Teleostei: Siluriformes: Trichomycteridae) from the rio Jequitinhonha basin, Minas Gerais, Brazil. Ichthyological Exploration of Freshwaters 15: 161–172.
- Vazzoler AEAM (1996) Biologia da reprodução de peixes teleósteos: teoria e prática. EDUEM, Maringá, 169 pp.
- Vieira F (2010) Em destaque: Leporinus thayeri Borodin, 1929. MG.Biota 2(5): 44-48.
- Vieira F (2006) A ictiofauna do rio Santo Antônio, bacia do rio Doce, MG: proposta de conservação. PhD Thesis, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.
- Vieira F, Gomes JPG, Maia BP, Martins LG (2015) Peixes do Quadrilátero Ferrífero guia de identificação. Fundação Biodiversitas, Belo Horizonte, 208 pp.
- Winemiller KO, Agostinho AA, Caramaschi EP (2008) Fish ecology in tropical streams. In: Dudgeon D (Ed.) Tropical stream ecology. Elsevier, New York, 107–146. https://doi. org/10.1016/B978-012088449-0.50007-8
- Zaher H, Young PS (2003) As coleções zoológicas brasileiras: panorama e desafios. Ciência e Cultura 55(3): 24–26.

#### Supplementary material I

# Summarizes voucher information and comparative material with institutional acronyms, following Sabaj (2019)

Authors: Sérgio Alexandre dos Santos, Marcelo Ribeiro de Britto

Data type: species data

Explanation note: Number in brackets represent the total number of specimens.

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Link: https://doi.org/10.3897/zookeys.1054.67554.suppl1

RESEARCH ARTICLE



## DNA Barcoding of Portuguese Lacewings (Neuroptera) and Snakeflies (Raphidioptera) (Insecta, Neuropterida)

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Academic editor: S. Winterton	Received 18 February 2021	Accepted 25 May 2021	Published 3 August 2021
http://	lzoobank.org/D2A38983-2C5A-4	56A-B5F8-2CE3F7E3121A	

**Citation:** Oliveira D, Chaves C, Pinto J, Paupério J, Fonseca N, Beja P, Ferreira S (2021) DNA Barcoding of Portuguese Lacewings (Neuroptera) and Snakeflies (Raphidioptera) (Insecta, Neuropterida). ZooKeys 1054: 67–84. https://doi.org/10.3897/zookeys.1054.64608

#### Abstract

The orders Neuroptera and Raphidioptera include the species of insects known as lacewings and snakeflies, respectively. In Portugal, these groups account for over 100 species, some of which are very difficult to identify by morphological analysis. This work is the first to sample and DNA sequence lacewings and snakeflies of Portugal. A reference collection was built with captured specimens that were identified morphologically. DNA barcode sequences of 658 bp were obtained from 243 specimens of 54 species. The results showed that most species can be successfully identified through DNA barcoding, with the exception of seven species of Chrysopidae (Neuroptera). Additionally, the first published distribution data are presented for Portugal for the neuropterans *Gymnocnemia variegata* (Schneider, 1845) and *Myrmecaelurus* (*Myrmecaelurus*) trigrammus (Pallas, 1771).

#### **Keywords**

Cytochrome c oxidase subunit I (COI), DNA barcode, mitochondrial DNA, Portugal, taxonomy

## Introduction

Neuropterida is a superorder of insects which encompasses the orders Neuroptera, Raphidioptera and Megaloptera. The present work focuses on DNA barcoding of the first two orders in Portugal, while DNA barcoding of Megaloptera in the country was addressed in Ferreira et al. (2019).

The order Neuroptera includes the holometabolous insects commonly known as lacewings. With at least 6000 species worldwide, more than 300 of which occur in Europe, Neuroptera accounts for most of the diversity of the Neuropterida (Aspöck 2002b; Aspöck et al. 2015). For the almost 200 species known in the Iberian Peninsula, around half have been recorded in Portugal, spanning 10 families (Aspöck et al. 2001; Letardi and Almeida 2013; Monserrat and Triviño 2013; Oliveira and Ferreira 2020; this work).

The small order Raphidioptera Latreille, 1810, groups about 260 species of insects worldwide (Aspöck 2002a), which are commonly known as snakeflies. From the 16 species of Raphidioptera present in the Iberian Peninsula, six species are known to occur in Portugal (Monserrat and Papenberg 2015; Papenberg 2015). The family Raphidiidae is represented by five species: *Atlantoraphidia maculicollis* (Stephens, 1836), *Harraphidia laufferi* (Navás, 1915), *Hispanoraphidia castellana* (Navás, 1915), *Ohmella bolivari* (Navás, 1915) and *Subilla aliena* (Navás, 1915). In contrast, Inocelliidae is represented by a single species: *Fibla hesperica* Navás, 1915 (Monserrat and Papenberg 2015; Papenberg 2015).

The monophyly of the three orders of Neuropterida (Megaloptera as a sister group of Neuroptera + Raphidioptera) has been solidly established. Nonetheless, taxonomy of the groups is incompletely resolved and internal relationships are not yet established, despite recent studies, especially in the case of Neuroptera (Aspöck 2002b; Winterton et al. 2010; Wang et al. 2017; Engel et al. 2018). Most notably, recent evidence has been mounting for the integration of Ascalaphidae as a subfamily of Myrmeleontidae (Winterton et al. 2018; Machado et al. 2019; Vasilikopoulos et al. 2020).

DNA barcoding was proposed in 2003 as a method to rapidly and accurately identify species (Hebert et al. 2003; Hebert and Gregory 2005). This method relies on the existence of comprehensive databases of short DNA sequences (the DNA barcodes), which are attributed to previously identified specimens and used for comparison with DNA barcode sequences obtained from unidentified specimens or even environmental samples. For insects, the typical DNA barcode consists of a 658 bp sequence of the cytochrome c oxidase subunit I (COI) (Folmer et al. 1994), also known as the "Folmer region". DNA barcoding has been used in studies involving Neuropterida, namely in the construction and analysis of DNA barcode databases for the fauna of certain regions, including Central Europe (Morinière et al. 2014) and Beijing, China (Yi et al. 2018), in the description of new species (Pantaleoni and Badano 2012; Badano et al. 2016), and to resolve taxonomic questions (Price et al. 2015). It is important to accurately identify species, especially the ones with agricultural applications, such as those in Chrysopidae and Hemerobiidae, as misidentifications may compromise biological control. Hitherto, DNA barcoding studies of Neuroptera and Raphidioptera in Portugal were non-existent, despite the considerable number of species known to occur in the country.

In this work, we present a contribution to the DNA barcode library for the Portuguese species of Neuroptera and Raphidioptera representing about 50% of known species in the country, alongside new and interesting distributional data. While most species were found to be identifiable through the use of the obtained DNA barcodes, this was not true for some cases in Chrysopidae. This work was conducted within the frame of the InBIO Barcoding Initiative, which aims at producing a comprehensive DNA barcode database for the Portuguese terrestrial invertebrate biodiversity.

## Materials and methods

#### Sampling of specimens

Specimens were collected during field expeditions throughout continental Portugal, from 2006 to 2019, and stored in 96% ethanol at the InBIO Barcoding Initiative reference collection (Vairão, Portugal). Specimens were captured during direct searches of the environment or lured by light trapping, the latter with UV LEDs or mercury vapour lamps. Morphological identification was done based on the most recent literature on Iberian Neuroptera and Raphidioptera (Monserrat and Acevedo 2012a, b, 2013; Monserrat 2014a, b, c, 2016a, b; Monserrat et al. 2014; Monserrat and Papenberg 2015), and using an Olympus SZX2-ILLT Stereozoom microscope when necessary. From each specimen, one tissue sample (a leg) was removed and stored in 96% ethanol for DNA extraction.

#### DNA extraction, amplification and sequencing

For each species, we selected six specimens for DNA sequencing based on their location of capture, attempting to maximize the geographical coverage of the study. For species with less than six specimens, all were selected for sequencing.

DNA was extracted from most tissue samples using the EasySpin Genomic DNA Microplate Tissue Kit. For specimens belonging to species of smaller sizes (such as those from the Hemerobiidae and Coniopterygidae families), the QIAmp DNA Micro Kit was used, as it is designed to extract higher concentrations of genetic material from samples with small amounts of DNA.

Amplification of the DNA was performed using three different primer pairs, that amplify three overlapping fragments of the same 658 bp region of the COI mitochondrial gene. Initially, we used two primer pairs, LCO1490 (Folmer et al. 1994) + Ill\_C\_R (Shokralla et al. 2015) and Ill\_B\_F (Shokralla et al. 2015) + HCO2198 (Folmer et al. 1994) (henceforth referred to as LC and BH, respectively) to amplify two overlapping fragments of 325 bp and 418 bp, respectively. Following publication of the third primer pair, BF2 + BR2 (422 bp fragment), by Elbrecht and Leese (2017), this started to be used instead of Ill\_B\_F + HCO2198 due to higher amplification efficiency.

PCRs were performed in 10  $\mu$ l reactions, containing 5  $\mu$ l of Multiplex PCR Master Mix (Qiagen, Hilde, Germany, 0.3 (BF2-BR2) – 0.4 mM of each primer, and 1–2  $\mu$ l of DNA, with the remaining volume in water. For DNA amplification, an initial denaturation at 95 °C for 15 min was performed followed by 5 cycles at 95 °C for 30 sec, 47 °C for 45 sec, 72 °C for 45 sec (only for LC and BH); then 40 cycles at 95 °C for 30 sec, 51 °C for 45 sec (48 °C for 60 sec for BF2 + BR2), 72 °C for 45 sec; and a final elongation step at 60 °C for 10 min. DNA amplification was performed in T100 Thermal Cycler (Bio-Rad, California, USA).

All PCR products were analysed by agarose gel electrophoresis and samples selected for sequencing were then organised for assignment of sequencing 'indexes'. One of two types of index were used for each run. For Illumina indexes, samples were pooled into one plate, as described in Shokralla et al. (2015). When using custom indexes (designed based on (Meyer and Kircher (2010)) no pooling was required. The latter allow for a maximum of 1920 unique index combinations. A second PCR was then performed where the 'indexes' and Illumina sequencing adapters were attached to the DNA extract. The index PCR was performed in a volume of 10 µl, including 5 µL of Phusion High-Fidelity PCR Kit (New England Biolabs) or KAPA HiFi PCR Kit (KAPA Biosystems, USA), 0.5 µL of each 'index' and 2 µL of diluted PCR product (usually 1:4). This PCR reaction is only of 10 cycles and performed at an annealing temperature of 55 °C. The amplicons were purified using AMPure XP beads (New England Biolabs) before quantification using NanoDrop 1000 (Thermo Scientific). This step allows for a normalization of concentrations between samples before the final quantification step with a qPCR using the KAPA Library Quantification Kit Illumina Platforms (KAPA Biosystems, USA) (Paupério et al. 2018).

Sequencing was performed at the CIBIO facilities on an Illumina MiSeq benchtop system, using a V2 MiSeq sequencing kit (2× 250 bp).

#### Bioinformatic processing and data analysis

Sequences were filtered and processed with OBITools (Boyer et al. 2014) and the fragments were assembled into their consensus 658 bp-long sequences using Geneious 9.1.8 (https://www.geneious.com). The obtained DNA sequences were then compared against the BOLD database (Ratnasingham and Hebert 2007) using the built-in identification engine, based on the BLAST algorithm. Sequences were submitted to the BOLD database and the Barcode Index Numbers (BIN) for every sequence were retrieved and analysed (Suppl. material 1: Table S1).

All DNA barcode sequences were aligned in Geneious 9.1.8. with the CLUSTALW (Thompson et al. 1994) plugin. Nucleotide composition of all sequences, as well as intra and interspecific p-distances were calculated in MEGAX (Kumar et al. 2018). Neighbour-joining trees were constructed in PAUP\* 4.0a167 (Swofford 2003), with 1000 bootstrap replicates, as a simple way of visualizing genetic distance between sequences,

while detecting possible misidentifications and incongruences. First, a tree with all obtained DNA barcode sequences of Neuroptera and Raphidioptera was constructed. For this, the outgroup sequences IBIMP001-19 and AGRID020-10 from the BOLD database (of *Sialis fuliginosa* Latreille, 1803 and *Agriotes proximus* Schwarz, 1891, respectively) were used to root the tree. These outgroups refer, respectively, to a species of Megaloptera (the third order within the Neuropterida) and a species of Coleoptera, the closest order to Neuropterida (Wang et al. 2017). Additionally, a NJ tree was constructed for Chrysopidae and Hemerobiidae, utilizing the sequences FBNE073-11 and FBNE001-11 (of *Osmylus fulvicephalus* (Scopoli, 1763) and *Sisyra nigra* (Retzius, 1783), respectively) as outgroups. The latter set of outgroups was used for family-level trees as representative of Osmylidae Linnaeus, 1758 and Sisyridae Banks, 1905.

An analysis of the data with the Automatic Barcode Gap Discovery (ABGD) method (Puillandre et al. 2012) was performed at the dedicated website (https://bioinfo. mnhn.fr/abi/public/abgd/abgdweb.html), as a test of the existence of a barcoding gap between species, which is fundamental to species identification using DNA barcodes (Hebert et al. 2003, 2004).

### Results

DNA barcode sequences of 658 bp were obtained for 243 specimens of Neuropterida, representing 54 of the 104 species known to occur in continental Portugal (Fig. 1; Suppl. material 1: Table S1). These species are representative of 9 of 10 families of Neuroptera, and one of two families of Raphidioptera recorded in the country. These sequences represent 21 new species of Neuroptera and one of Raphidioptera for the BOLD database. Furthermore, of the already available sequences in BOLD only six originate from continental Portugal (accessed on 19/01/2021).

#### Neuroptera Linnaeus, 1758

Neuroptera specimens were collected from 67 sampling locations, in 12 districts (Fig. 1 and Suppl. material 1: Table S1). From the 51 species, 12 were captured only once and are therefore represented by a single DNA barcode sequence in the dataset. Two of the species were hitherto without published records in scientific literature for the country: *Gymnocnemia variegata* and *Myrmecaelurus trigrammus* (Suppl. material 1: Table S1), despite being widespread in the whole Euro-Mediterranean area and their presence well known in Spain.

For the DNA barcode sequences of Neuroptera, average nucleotide composition is 39.4% thymine (T), 16.2% cytosine (C), 28.6% adenine (A) and 15.8% guanine (G). Base frequencies analysis revealed GC-contents of 32% for the DNA barcode fragment. Average genetic p-distances between captured species ranged from 0.46% between *Pseudomallada picteti* (McLachlan, 1880) and *Pseudomallada flavifrons* (Brauer, 1851) to 25.91% between *Dilar meridionalis* Hagen, 1866 and *Aleuropteryx iberica* 



**Figure 1.** Map of continental Portugal with sampling locations **A** sampling locations of the 8 captured specimens of Raphidioptera (N = 8) **B** sampling locations of the 235 captured specimens of Neuroptera (N = 67).

Monserrat, 1977 (Suppl. material 2: Table S2). Intraspecific distances ranged from 0% in *Palpares hispanus* Hagen, 1860 (N = 3), *Cunctochrysa baetica* (Hölzel, 1972) (N = 5) and *Italochrysa italica* (Rossi, 1790) (N = 2) to 3.6% in *Dilar meridionalis* (N = 4) (Suppl. material 2: Table S2).

Regarding the neighbour-joining analysis (Fig. 2), most species were recovered as monophyletic except for seven species of Chrysopidae, which were separated into two polyphyletic groups of morphologically identified species. One group encompassing *P. picteti* and *P. flavifrons*, another encompassing *Chrysoperla carnea* (Stephens, 1836), *Chrysoperla lucasina* (Lacroix, 1912), *Chrysoperla pallida* Henry et al., 2002, *Chrysoperla agilis* Henry et al., 2003 and *Chrysoperla mediterranea* (Hölzel, 1972) (Fig. 3).

The ABGD method yielded partitions generally congruent with morphological identification. Nonetheless, some exceptions were noted. Regarding the Chrysopidae, the ABGD analysis yielded 15 partitions (P = 0.0055) (Fig. 3). While congruent with the NJ analysis (by considering the aforementioned polyphyletic groups of species as two separate species), it also grouped the DNA barcoding sequences of *Pseudomallada prasinus* and *Pseudomallada abdominalis* (Brauer, 1856), which NJ analysis separates into three clades (in congruence with three detected morphospecies; see Discussion), into one single "species". In the Hemerobiidae family, the ABGD analysis recovered only eight partitions (P = 0.0492), grouping *Wesmaelius subnebulosus* (Stephens, 1836) and *Wesmaelius nervosus* (Fabricius, 1793) (Fig. 4).


**Figure 2.** Neighbour-joining tree of all obtained DNA sequences for Portuguese Neuroptera and Raphidioptera. Neighbour-joining tree constructed in PAUP\* 4.0a167. Non-highlighted terminal branches represent the two outgroup sequences.

Similar to the other two methods used, BIN allocation using BOLD Systems yielded congruent results for most species, with some particular cases of incongruence. In Ascalaphidae, the sequences belonging to the two species of *Libelloides* were grouped under the same BIN. For Chrysopidae, the BIN framework clustered sequences simi-



**Figure 3.** Neighbour-joining tree of Chrysopidae DNA barcode sequences. Neighbour-joining tree constructed in PAUP\* 4.0a167 and contrasted with the results from the ABGD analysis and BIN attribution. Bootstrap values under 90% omitted.



**Figure 4.** Neighbour-joining tree of Hemerobiidae DNA barcode sequences. Neighbour-joining tree constructed in PAUP\* 4.0a167 and contrasted with the results from the ABGD analysis and BIN attribution. Bootstrap values under 90% omitted. Subtrees were collapsed for the monophyletic morphologically identified species. Triangle size for each species is proportional to the intraspecific distance.

larly to ABGD, except for two sequences of *Pseudomallada prasinus* (INV10273 and INV07344), which were assigned BINs different from each other and the other sequences for the species, as well as one sequence from both *Pseudomallada genei* and *Pseudomallada venosus* which were not grouped in the same BIN as the other sequences of the same species (Fig. 3 and Suppl. material 1: Table S1). The sequences of Hemerobiidae yielded 10 BINs, one more than the number of morphologically identified species, as sequences of *Sympherobius pygmaeus* are in two BINs (Fig. 4).

## Raphidioptera Latreille, 1810

DNA barcode sequences were obtained for eight specimens of Raphidioptera, accounting for three of the six species known to occur in Portugal.

Average nucleotide composition of all DNA barcode sequences of Raphidioptera was calculated as 37.2% thymine (T), 18.1% cytosine (C), 29.6% adenine (A) and

15.1% guanine (G). Base frequencies analysis revealed GC-contents of 33% for the DNA barcode fragment. Genetic distances between species ranged from 12.3% between *A. maculicollis* and *H. castellana* to 15.9% between *H. laufferi* and *H. castellana*. Intraspecific distances ranged from 0.2% in *H. castellana* to 1.2% in *A. maculicollis* (Suppl. material 2: Table S2). The NJ tree constructed with the calculated genetic distances recovered all species as monophyletic (Fig. 2). Analysis with the BOLD BIN system yielded three BINs, congruent with the morphological identification. Similarly, three partitions were recovered from ABGD analysis.

The eight specimens of Raphidioptera were captured in six sampling locations in Bragança and Leiria (Fig. 1 and Suppl. material 1: Table S1)

## Discussion

In this work, DNA barcode sequences and their respective analyses, as well as novel distributional data are provided based on 235 specimens of 51 species of Neuroptera and 8 specimens of 3 species of Raphidioptera. This is the first study focusing on DNA barcoding for these orders in Portugal.

The main goal of this work was to compile a DNA barcode reference collection for the Portuguese species of Neuroptera and Raphidioptera. About 50% of the faunal diversity of the groups is represented in the collection, and DNA barcode sequences were added to the BOLD database for species hitherto unrepresented. The analyses conducted suggest that most of the encompassed species can be identified with the COI gene-based DNA barcodes. This is the case for the Ascalaphidae, Berothidae, Mantispidae, Myrmeleontidae and Nemopteridae families. For the other families, Chrysopidae and Hemerobiidae, further scrutiny is necessary.

Interestingly, despite the congruence of taxonomy and the obtained DNA barcodes for the families Ascalaphidae and Myrmeleontidae, the genetic distances and phylogenetic tree (Fig. 2) show the latter group as paraphyletic. These results may provide further evidence for the integration of current Ascalaphidae species into the family Myrmeleontidae, a taxonomic change that has seen growing support in recent years (Winterton et al. 2018; Machado et al. 2019; Vasilikopoulos et al. 2020)

Regarding the Chrysopidae, the results show four groups of species with conflicting results between morphological identification, NJ and ABGD analysis, and BIN attribution. The first consists of the DNA barcode sequences belonging to *P. flavifrons* and *P. picteti*, whose sequences were recovered as a single clade (NJ) and placed by ABGD analysis into a single group. Despite possessing distinctive morphological characteristics these are closely-related species with high degree of morphological variation (Aspöck et al. 2001; Monserrat 2016b; Duelli et al. 2017). The obtained results suggest that *P. picteti* and *P. flavifrons* share mitochondrial haplotypes, which may be due to incomplete lineage sorting or mitochondrial genome capture as a result of introgressive hybridization.

The morphospecies *P. venosus* and *P. genei* were recovered as monophyletic and ABGD considered each of the species as single units, although two different BINs were attributed to each species.

The Pseudomalla "prasinus" species complex, where P. prasinus and P. abdominalis are included, is the third group with conflicting results between NJ, ABGD and morphological analysis, and has been a subject of interest and contention in Neuropterology for over a century (McLachlan 1886). Recent molecular genetics works have supported the existence of a species complex (Duelli et al. 2017), showing cryptic diversity in the group. One of the prasinoid morphotypes is known as "marianus" and was previously considered as a valid species. The specimens INV10273 and INV07344 were identified as *Pseudomallada marianus*, by utilizing the key available for the Iberian Peninsula in Monserrat (2016b). Duelli and Obrist (2019) established the synonymy of P. marianus with P. prasinus, previously proposed by Hölzel (1973), based on Central European specimens. In the former, authors state that the morphological characters previously attributed to the "marianus" morph (i.e., larger size and bundled egg placement) are the ones that define the "real" P. prasinus. As such, smaller specimens belonging to the "prasinus" species complex can't yet be identified conclusively to species level until the prasinoid morphotypes are well-defined and described as a species (Duelli and Obrist 2019). However, the implications of this work on the Iberian Peninsula's specimens of the "prasinus" species complex are not clear and require further research. In the present work, the NJ analysis was congruent with the morphological identification based on the characteristics described in Monserrat (2016b) since it separately grouped INV7344 and INV10273, which were identified as the "marianus" morphotype, but failed to retrieve *P. prasinus* as monophyletic. In contrast, the ABGD analysis grouped all specimens of *P. prasinus* and *P. abdominalis*. Additionally, the intraspecific distance between DNA barcode sequences of P. prasinus (2.25%) was higher than expected relative to the other species in our dataset. Our results, albeit limited, provide additional support to the existence of cryptic diversity in P. prasinus. Identification through DNA barcoding may prove problematic until the taxonomy of the group is better resolved, and will likely benefit of the use of other DNA markers.

A more complex situation is that of *Chrysoperla carnea*, *C. lucasina*, *C. pallida*, *C. agilis* and *C. mediterranea*, in which all obtained sequences are grouped by NJ, ABGD and BIN analysis in a single unit. The five species belong to the so-called *C. carnea* species complex (Thierry et al. 1998; Henry et al. 2002, 2013). So far, the most reliable way to identify the species in this group is by their substrate-borne vibrational songs, produced by tremulation (Henry and Wells 2015). Even though these are not used for attraction of mates at long-distances as in many other animals, these signals are produced for close-range recognition of sexually receptive mates (Henry et al. 2002, 2003, 2012). The obtained results for the species of the group are congruent with previous studies (Lourenço et al. 2006; Morinière et al. 2014) and might be a result of the pre-copulatory reproductive isolation and the recent and rapid speciation of this group of species (Henry et al. 2013). Considering the obtained data and the available literature, a COI-based DNA barcode is not a feasible tool for species identification in this species complex.

The analysis of the sequences obtained from Dilaridae specimens yielded the highest intra and interspecific genetic distances of all studied species. The intraspecific genetic diversity in *Dilar meridionalis* was 3.67% (N = 4), while the genetic distance between the

*D. meridionalis* and *D. saldubensis* was 17.7%. Since previous works on DNA barcoding of Neuroptera have poorly (Yi et al. 2018) or not represented (Morinière et al. 2014) the family at all, further sampling and sequencing would be needed to access the validity of DNA barcoding based on the COI gene for identification of species in this family.

The two species of *Wesmaelius* were separated in the NJ analysis as by morphology, though ABGD failed to recover two distinct groups. Furthermore, both the ID engine and BIN analysis in BOLD systems clearly separated the species and grouped the sequences in BINs with other sequences available in the BOLD database of the same two species. Considering these results, we suggest that COI DNA barcode sequences may be used in the identification of these two species.

Another species that presents more than one BIN is *Sympherobius pygmaeus*. The genetic diversity observed is congruent with previous work (Morinière et al. 2014) and further research is needed to verify if it is a case of cryptic diversity.

In our dataset, all species of Raphidioptera showed relatively low intraspecific divergence when compared with the respective interspecific distances. Despite the low number of DNA barcode sequences available and the absence of three of the six species in the dataset, the obtained results suggest that a DNA barcoding approach using a COI gene fragment may be used to discern between species of Portuguese Raphidioptera. This assumption is reinforced by the fact that all six species in the country belong to six different genera and are, as such, predicted to show relatively high interspecific distances between them.

For the large majority of encompassed species, DNA barcoding appears to be a reliable method of identification. While DNA barcoding cannot replace morphological taxonomy experts entirely, especially in taxa where the taxonomy still needs revision, it can aid in species identification in cases where morphology cannot be used. For example, in diet analyses, where only small body parts (or none at all) can be retrieved, using DNA barcoding may be the only method suitable for species identification, allowing the understanding of species interactions and their roles in the ecosystems.

Currently 73 species of Neuropterida present in Portugal have DNA barcoding data available, comprising the 54 species encompassed in this work and the 19 already available in the BOLD database from other countries. Nonetheless, 29 species known to occur in Portugal remain without DNA barcode available and further efforts are needed to fill this gap.

# Conclusion

This work provides novel data on the DNA barcoding and geographical distribution of Neuroptera and Raphidioptera species in Portugal. Our results suggest that DNA barcoding using COI Folmer region may be used to identify the great majority of species of Neuroptera and Raphidioptera species recorded in the country. It is not, however, suitable for identification of several species of the Chrysopidae family. In total, there were 22 cases where the first publicly available DNA barcode sequence for a species was obtained but further sampling and sequencing efforts are still needed for many. The completion of DNA barcode databases is an ongoing effort and, in the cases of Neuroptera and Raphidioptera, still require much work, including in Europe, where several species are not yet sequenced. The future, however, looks bright as international initiatives are promoting and aiding in the development of DNA barcode sequences databases for particular regions worldwide (Letardi 2019).

# Acknowledgements

InBIO Barcoding Initiative is funded by the European Union's Horizon 2020 Research and Innovation programme under grant agreement No 668981 and by the project PORBIOTA – Portuguese E-Infrastructure for Information and Research on Biodiversity (POCI-01-0145-FEDER-022127), supported by Operational Thematic Program for Competitiveness and Internationalization (POCI), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (FED-ER). SF was supported by individual research contract (2020.03526.CEECIND) funded by FCT. The fieldwork benefited from EDP Biodiversity Chair, the project "Promoção dos serviços de ecossistemas no Parque Natural Regional do Vale do Tua: Controlo de Pragas Agrícolas e Florestais por Morcegos" funded by the Agência de Desenvolvimento Regional do Vale do Tua, and includes research conducted at the Long Term Research Site of Baixo Sabor (LTER\_EU\_PT\_002).

# References

- Aspöck H (2002a) The biology of Raphidioptera: A review of present knowledge. Acta Zoologica Academiae Scientiarum Hungaricae 48: 35–50.
- Aspöck H, Hölzel H, Aspöck U (2001) Kommentierter Katalog der Neuropterida (Insecta: Raphidioptera, Megaloptera, Neuroptera) der Westpaläarktis. Denisia 2: 1–606.
- Aspöck U (2002b) Phylogeny of the Neuropterida (Insecta: Holometabola). Zoologica Scripta 31: 51–55. https://doi.org/10.1046/j.0300-3256.2001.00087.x
- Aspöck U, Aspöck H, Letardi A, de Jong Y (2015) Fauna Europaea: Neuropterida (Raphidioptera, Megaloptera, Neuroptera). Biodiversity Data Journal 3: e4830. https://doi. org/10.3897/BDJ.3.e4830
- Badano D, Acevedo F, Pantaleoni RA, Monserrat VJ (2016) Myrmeleon almohadarum sp. nov., from Spain and North Africa, with description of the larva (Neuroptera Myrmeleontidae). Zootaxa 4196: 210–220. https://doi.org/10.11646/zootaxa.4196.2.2
- Boyer F, Mercier C, Bonin A, Taberlet P, Coissac E (2014) OBITools: a Unix-inspired software package for DNA metabarcoding. Molecular Ecology Resources 16(1): 176–182. https:// doi.org/10.1111/1755-0998.12428
- Duelli P, Obrist MK (2019) In search of the real *Pseudomallada prasinus* (Neuroptera, Chrysopidae). Zootaxa 4571: 510–530. https://doi.org/10.11646/zootaxa.4571.4.4

- Duelli P, Henry CS, Hayashi M, Nomura M, Mochizuki A (2017) Molecular phylogeny and morphology of *Pseudomallada* (Neuroptera: Chrysopidae), one of the largest genera within Chrysopidae. Zoological Journal of the Linnean Society 180: 556–569. https://doi. org/10.1093/zoolinnean/zlw008
- Elbrecht V, Leese F (2017) Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment. Frontiers in Environmental Science 5: 1–11. https://doi.org/10.3389/fenvs.2017.00011
- Engel MS, Winterton SL, Breitkreuz LCV (2018) Phylogeny and Evolution of Neuropterida: Where Have Wings of Lace Taken Us? Annual Review of Entomology 63(1): 531–551. https://doi.org/10.1146/annurev-ento-020117-043127
- Ferreira S, Paupério J, Grosso-Silva JM, Beja P (2019) DNA barcoding of *Sialis* sp. (Megaloptera) in Portugal: the missing tool to species identification. Aquatic Insects 40: 1–12. https://doi.org/10.1080/01650424.2019.1571612
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology 3: 294–299.
- Hebert PDN, Gregory TR (2005) The Promise of DNA Barcoding for Taxonomy. Systematic Biology 54: 852–859. https://doi.org/10.1080/10635150500354886
- Hebert PDN, Cywinska A, Ball SL, DeWaard JR (2003) Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270: 313–321. https://doi.org/10.1098/rspb.2002.2218
- Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM (2004) Identification of birds through DNA barcodes. PLoS Biology 2(10): e312. https://doi.org/10.1371/journal.pbio.0020312
- Henry CS, Wells MLM (2015) Courtship songs of green lacewings filmed in slow motion: how a simple vibrating structure can generate complex signals (Neuroptera: Chrysopidae: Chrysoperla). Journal of Insect Behavior 28: 89–106. https://doi.org/10.1007/s10905-015-9484-6
- Henry CS, Brooks SJ, Duelli P, Johnson JB (2002) Discovering the true *Chrysoperla carnea* (Insecta: Neuroptera: Chrysopidae) using song analysis, morphology, and ecology. Annals of the Entomological Society of America 95: 172–191. https://doi.org/10.1603/0013-8746(2002)095[0172:DTTCCI]2.0.CO;2
- Henry CS, Brooks SJ, Duelli P, Johnson JB (2003) A lacewing with the wanderlust: The European song species "Maltese", Chrysoperla agilis, sp.n., of the carnea group of Chrysoperla (Neuroptera: Chrysopidae). Systematic Entomology 28: 131–147. https://doi.org/10.1046/j.1365-3113.2003.00208.x
- Henry CS, Brooks SJ, Duelli P, Johnson JB, Wells MM, Mochizuki A (2012) Parallel evolution in courtship songs of North American and European green lacewings (Neuroptera: Chrysopidae). Biological Journal of the Linnean Society 105: 776–796. https://doi.org/10.1111/ j.1095-8312.2011.01845.x
- Henry CS, Brooks SJ, Duelli P, Johnson JB, Wells MM, Mochizuki A (2013) Obligatory duetting behaviour in the *Chrysoperla carnea*-group of cryptic species (Neuroptera: Chrysopidae): Its role in shaping evolutionary history. Biological Reviews 88: 787–808. https://doi. org/10.1111/brv.12027

- Hölzel H (1973) Zur Revision von Typen europäischer *Chrysopa*-Ar ten. Revue suisse de zoologie 80: 65–82. https://doi.org/10.5962/bhl.part.75938
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547– 1549. https://doi.org/10.1093/molbev/msy096
- Letardi A (2019) Preliminary results of NEUIT (Barcoding of Italian Neuropterida) project. https://doi.org/10.5281/zenodo.3569411
- Letardi A, Almeida JM (2013) Contributing to a checklist of Neuropterida in Portugal: the Naturdata project. Açoreana 9: 29–38.
- Lourenço P, Brito C, Backeljau T, Thierry D, Ventura MA (2006) Molecular systematics of the *Chrysoperla carnea* group (Neuroptera: Chrysopidae) in Europe. Journal of Zoological Systematics and Evolutionary Research 44: 180–184. https://doi.org/10.1111/j.1439-0469.2006.00352.x
- Machado RJP, Gillung JP, Winterton SL, Garzón-Orduña IJ, Lemmon AR, Lemmon EM, Oswald JD (2019) Owlflies are derived antlions: anchored phylogenomics supports a new phylogeny and classification of Myrmeleontidae (Neuroptera). Systematic Entomology 44(2): 418–450. https://doi.org/10.1111/syen.12334
- McLachlan R (1886) Notes concerning *Chrysopa ventralis*, *prasina*, *abdominalis*, *aspersa*, and *zelleri*. The Entomologist's Monthly Magazine 23: 33–36.
- Meyer M, Kircher M (2010) Illumina sequencing library preparation for highly multiplexed target capture and sequencing. Cold Spring Harbor Protocols 5. https://doi.org/10.1101/pdb.prot5448
- Monserrat VJ (2014a) Los berótidos de la Península Ibérica (Insecta: Neuropterida: Neuroptera: Berothidae). Heteropterus Revista de Entomología 14: 31–54. https://doi.org/10.3989/ graellsia.2014.v70.111
- Monserrat VJ (2014b) Los diláridos de la Península Ibérica (Insecta: Neuropterida: Neuroptera: Dilaridae). Heteropterus Revista de Entomología 14: 187–214. https://doi.org/10.3989/ graellsia.2014.v70.111
- Monserrat VJ (2014c) Los mantíspidos de la Península Ibérica y Baleares (Insecta, Neuropterida, Neuroptera, Mantispidae). Graellsia 70: e012. https://doi.org/10.3989/graellsia.2014.v70.115
- Monserrat VJ (2016a) Los coniopterígidos de la Península Ibérica y Islas Baleares (Insecta Neuropterida, Neuroptera: Coniopterygidae). Graellsia 72: e047. https://doi.org/10.3989/ graellsia.2016.v72.157
- Monserrat VJ (2016b) Los crisópidos de la Península Ibérica y Baleares (Insecta, Neuropterida, Neuroptera: Chrysopidae). Graellsia 72: e037. https://doi.org/10.3989/graellsia.2016.v72.143
- Monserrat VJ, Acevedo F (2012a) Los ascaláfidos de la Península Ibérica y Baleares (Insecta : Neuroptera: Ascalaphidae). Heteropterus Revista de Entomología 12: 33–58.
- Monserrat VJ, Acevedo F (2012b) Los nemoptéridos y crócidos de la Península Ibérica (Insecta: Neuroptera: Nemopteridae, Crocidae). Heteropterus Revista de Entomología 12: 231–255.
- Monserrat VJ, Acevedo F (2013) Los mirmeleóntidos (hormigas-león) de la Península Ibérica y Islas Baleares (Insecta, Neuropterida, Neuroptera, Myrmeleontidae). Graellsia 69: 283–321. https://doi.org/10.3989/graellsia.2013.v69.098

- Monserrat VJ, Papenberg D (2015) Los rafidiópteros de la Península Ibérica (Insecta, Neuropterida: Raphidioptera); The snake-flies from the Iberian Peninsula (Insecta, Neuropterida: Raphidioptera). Graellsia 71(1): e024. https://doi.org/10.3989/graellsia.2015.v71.116
- Monserrat VJ, Badano D, Acevedo F (2014) Nuevos datos de ascaláfidos para la Península Ibérica, con una nueva especie para la fauna europea (Insecta: Neuropterida: Neuroptera: Ascalaphidae). Heteropterus Revista de Entomología 14: 147–167.
- Monserrat VJ, Triviño V (2013) Atlas of the Iberian and Balearic lacewings (Insecta, Neuroptera: Megaloptera, Raphidioptera, Planipennia) Sociedad Entomologica Aragonesa. Monografias S.E.A. 13: 1–154.
- Morinière J, Hendrich L, Hausmann A, Hebert PD, Haszprunar G, Gruppe A (2014) Barcoding fauna bavarica: 78% of the neuropterida fauna barcoded! PLoS ONE 9(10): e109719. https://doi.org/10.1371/journal.pone.0109719
- Oliveira D, Ferreira S (2020) *Wesmaelius (Kimninsia) nervosus* (Fabricius, 1793) (Neuroptera, Hemerobiidae) a new species of brown lacewing for the Portuguese fauna. Boletín de la Asociación Española de Entomología 44: 1–4.
- Pantaleoni RA, Badano D (2012) *Myrmeleon punicanus* n. sp., a new pit-building antlion (Neuroptera myrmeleontidae) from sicily and pantelleria. Bulletin of Insectology 65: 139–148.
- Papenberg D (2015) Revisión de los rafidiópteros (insectos neuropteroides, raphidiopteros) de la Península Ibérica. Universidad Complutense de Madrid.
- Paupério J, Fonseca N, Egeter B, Galhardo M, Ferreira S, Oxefelt F, Aresta S, Martins F, Mata V, Veríssimo J, Puppo P, Pinto JC, Chaves C, Garcia-Raventós A, Peixoto S, Vasconcelos LP da SS, Gil P, Khalatbari L, Jarman S, Beja P (2018) EnvMetaGen Deliverable 4.4 (D4.4) Protocol for next-gen analysis of eDNA samples. https://doi.org/10.5281/zeno-do.2586885
- Price BW, Henry CS, Hall AC, Mochizuki A, Duelli P, Brooks SJ (2015) Singing from the grave: DNA from a 180 year old type specimen confirms the identity of *Chrysoperla carnea* (Stephens). PLoS ONE 10(4): e0121127. https://doi.org/10.1371/journal.pone.0121127
- Puillandre N, Lambert A, Brouillet S, Achaz G (2012) ABGD, Automatic Barcode Gap Discovery for primary species delimitation. Molecular Ecology 21: 1864–1877. https://doi. org/10.1111/j.1365-294X.2011.05239.x
- Ratnasingham S, Hebert PD (2007) BOLD: The Barcode of Life Data System. Molecular Ecology Notes 7: 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x
- Shokralla S, Porter TM, Gibson JF, Dobosz R, Janzen DH, Hallwachs W, Golding GB, Hajibabaei M (2015) Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. Scientific Reports 5: e9687. https://doi.org/10.1038/ srep09687
- Swofford DL (2003) PAUP\*. Phylogenetic analysis using parsimony (\* and other methods). Sinauer Associates.
- Thierry D, Cloupeau R, Jarry M, Canard M (1998) Discrimination of the West-Palaearctic *Chrysoperla* Steinmann species of the *carnea* Stephens group by means of claw morphology (Neuroptera, Chry sopidae), 255–262.
- Thompson DJ, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive position-specific gap penalties and weight matrix choice multiple sequence

alignment through sequence weighting, Julie. European Molecular Biology Laborator 22: 4673–4680. https://doi.org/10.1093/nar/22.22.4673

- Vasilikopoulos A, Misof B, Meusemann K, Lieberz D, Flouri T, Beutel RG, Niehuis O, Wappler T, Rust J, Peters RS, Donath A, Podsiadlowski L, Mayer C, Bartel D, Böhm A, Liu S, Kapli P, Greve C, Jepson JE, Liu X, Zhou X, Aspöck H, Aspöck U (2020) An integrative phylogenomic approach to elucidate the evolutionary history and divergence times of Neuropterida (Insecta: Holometabola). BMC Evolutionary Biology 20: e64. https://doi. org/10.1186/s12862-020-01631-6
- Wang Y, Liu X, Garzón-Orduña IJ, Winterton SL, Yan Y, Aspöck U, Aspöck H, Yang D (2017) Mitochondrial phylogenomics illuminates the evolutionary history of Neuropterida. Cladistics 33(6): 617–636. https://doi.org/10.1111/cla.12186
- Winterton SL, Hardy NB, Wiegmann BM (2010) On wings of lace: Phylogeny and Bayesian divergence time estimates of Neuropterida (Insecta) based on morphological and molecular data. Systematic Entomology 35: 349–378. https://doi.org/10.1111/j.1365-3113.2010.00521.x
- Winterton SL, Lemmon AR, Gillung JP, Garzon IJ, Badano D, Bakkes DK, Breitkreuz LCV, Engel MS, Lemmon EM, Liu X, Machado RJP, Skevington JH, Oswald JD (2018) Evolution of lacewings and allied orders using anchored phylogenomics (Neuroptera, Megaloptera, Raphidioptera). Systematic Entomology 43: 330–354. https://doi.org/10.1111/ syen.12278
- Yi P, Yu P, Liu J, Xu H, Liu X (2018) A DNA barcode reference library of Neuroptera (Insecta, Neuropterida) from Beijing. ZooKeys 807: 127–147. https://doi.org/10.3897/zookeys.807.29430

# Supplementary material I

# Summary table of all used sequences and specimens, with country of origin

Authors: Daniel Oliveira, Sónia Ferreira

Data type: Occurences and access codes to DNA barcodes

- Explanation note: For captured specimens, sex, latitude and longitude coordinates (WGS 84), date of capture and IBI reference collection code (IBI) are presented. BOLD accession numbers and BINs (when available) presented for all DNA sequences used..
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1054.64608.suppl1

# Supplementary material 2

# Genetic distances

Authors: Daniel Oliveira, Sónia Ferreira

Data type: Genetic distances between analysed specimens

- Explanation note: Estimates of average genetic divergence (uncorrected p-distances) for species of Neuropterida. Values under the diagonal refer to interspecific divergence while values in the diagonal and in bold represent intraspecific divergence.
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/zookeys.1054.64608.suppl2

RESEARCH ARTICLE



# New species of the genus *Inversidens* Haas, 1911 (Unionoida, Unionidae, Gonideinae) from Jiangxi Province, China

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Academic editor: Graham Oliver   Received 24 May 2021   Accepted 9 July 2021   Pr	ublished 3 August 2021
http://zoobank.org/BA743F4E-2452-432A-9796-E1DEBC1D4CAD	

Citation: Wu R, Liu X, Kondo T, Ouyang S, Wu X (2021) New species of the genus *Inversidens* Haas, 1911 (Unionoida, Unionidae, Gonideinae) from Jiangxi Province, China. ZooKeys 1054: 85–93. https://doi.org/10.3897/zookeys.1054.69075

## Abstract

We diagnose and describe a new freshwater mussel species of the genus *Inversidens, I. rentianensis* **sp. nov.** from Jiangxi Province, China based on morphological characters and molecular data. This paper includes a morphological description and photograph of the holotype, and partial sequences of mitochondrial COI as DNA barcode data.

## Keywords

COI, freshwater mussel, genetic distances, morphology, taxonomy

# Introduction

The genus *Inversidens* Haas, 1911 belongs to the subfamily Gonideinae in the family Unionidae. The genus was first depicted by Haas (1911) as a subgenus of *Nodularia* with two species, i.e., *Unio brandtii* Kobelt, 1879 and *Nodularia parcedentata* Haas, 1911, both restricted to Japan. Later, Haas (1969) further classified *Unio reinianus* Kobelt, 1879, *Unio haconensis* Ihering, 1893, *Unio japanensis* Lea, 1859, *Unio pantoensis* Neumayr, 1899 within *Inversidens*. All species were restricted to Japan, except for

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*U. pantoensis*, which was distributed in China. By comparing the conchological characters, Kondo (1982) believed that *U. brandtii* was not morphologically distinct from *N. parcedentata*, and regarded *N. parcedentata* as a variety of *U. brandtii*. Based on the morphology of the glochidium, Habe (1991) removed *U. reinianus* from *Inversidens*, and established a new genus *Inversiunio*. Based on morphological characteristics of the shell, Kondo (1998) moved *Unio haconensis*, which was regarded as a synonym of *Unio jokohamensis* (Ihering, 1893), into *Inversiunio*. Furthermore, Starobogatov (1970) used *Unio japanensis* as the type species for his newly established genus *Pronodularia*.

Currently, only two species are recognized within *Inversidens*, the Japanese endemic *I. brandtii* and *I. pantoensis* in China (Fig. 1A, B; Kondo 2008; He and Zhuang 2013; Lopes-Lima et al. 2020; Graf and Cummings 2021a, b; MolluscaBase eds. 2021).

In this study, we diagnose and describe a new *Inversidens* species from Jiangxi Province, China. In addition, we provide estimations of the intraspecific and interspecific genetic distances within *Inversidens* based on the mitochondrial COI barcode to examine species validity.

## Materials and methods

## Specimen collection and identification

In March 2018, four samples were collected from the Mianshui River, Rentian Town, Ganzhou City, Jiangxi Province, China (25.989557°N, 116.131333°E). All type and voucher specimens are deposited at the Biological Museum of Nanchang University, China (NCFM180325-NCFM180328).

#### DNA extraction and COI amplification

Of the four individuals, only two samples had tissues. Total genomic DNA was extracted from dissected somatic tissue using TIANamp Marine Animals DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions.

Mitochondrial cytochrome oxidase subunit I (COI) gene sequences have been widely used for species delimitation of freshwater mussels based on genetic distance and the criteria of monophyly (Elderkin et al. 2016; Lopes-Lima et al. 2019; Smith et al. 2019). Polymerase chain reaction (PCR) amplification of the COI gene with a 680-base pair fragment was performed using a primer pair consisting of LCO1490 and HCO2198 (Folmer et al. 1994). Thermal cycling conditions were 98 °C for 10 s, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1–2 min, and a final extension of 72 °C for 7 min, following the TaKaRa Ex manufacturer's protocol. The amplified PCR products were purified and sequenced by Sangon Biotech (Shanghai). The PCR product size for the COI amplicon was 680 bp. The sequences obtained in this study have been uploaded to GenBank.

# DNA barcode dataset construction

We constructed a mitochondrial COI dataset with the newly obtained sequences from this study and the available *Inversidens brandtii* sequences from GenBank.

Previously published sequences were downloaded from GenBank and added to the dataset, i.e., 17 species of the subfamily Gonideinae and four species of the subfamily Unioninae for the ingroup, and one species of the subfamily Parreysiinae as the outgroup.

As a result, a total of 29 COI sequences were used for this study. Sequence details and GenBank accession numbers are shown in Table 1.

All COI nucleotide sequences were translated to amino acid sequences using MEGA 5.0 (Tamura et al. 2011) and aligned based on the amino acid sequences using the program MUSCLE (Edgar 2004) with default settings. We calculated and compared inter-and intraspecific distances with MEGA 5.0 using the uncorrected *p*-distance. Standard error was assessed using 1000 bootstrap replicates.

Taxa	GenBank accession number
UNIONIDAE Rafinesque, 1820	
Parreysiinae Henderson, 1935	
Indonaia andersoniana (Nevill, 1877)	KX865835
Unioninae Rafinesque, 1820	
Acuticosta chinensis (Lea, 1868)	MG462919
Inversiunio jokohamensis (Ihering, 1893)	LC518985
Inversiunio reinianus (Kobelt, 1879)	LC518976
Nodularia douglasiae (Griffith & Pidgeon, 1833)	NC_026111
Gonideinae Ortmann, 1916	
Pseudodon bogani Bolotov, Kondakov & Konopleva in Bolotov et al. 2017	MF352216
Pseudodon manueli Konopleva, Kondakov & Vikrev in Bolotov et al. 2017	MF352228
Monodontina cambodjensis (Petit de la Saussaye, 1865)	KP795028
Pilsbryoconcha exilis (Lea, 1838)	KP795024
Chamberlainia hainesiana (Lea, 1856)	KX822635
Sinohyriopsis cumingii (Lea, 1852)	NC_011763
Sinohyriopsis schlegelii (Martens, 1861)	NC_015110
Lamprotula caveata (Heude, 1877)	KX822646
Lamprotula leaii (Griffith & Pidgeon, 1833)	NC_023346
Potomida littoralis (Cuvier, 1798)	JN243905
Pronodularia japanensis (Lea, 1859)	KX822659
Gonidea angulata (Lea, 1838)	DQ272371
Leguminaia wheatleyi (Lea, 1862)	KX822651
Microcondylaea bonellii (Férussac, 1827)	KX822652
Sinosolenaia carinata (Heude, 1877)	KX822669
Ptychorhynchus pfisteri (Heude, 1874)	KY067440
Parvasolenaia rivularis (Heude, 1877)	KX966393
Inversidens brandtii (Kobelt, 1879)	AB040827
Inversidens brandtii (Kobelt, 1879)	MT020598
Inversidens brandtii (Kobelt, 1879)	MT020597
Inversidens brandtii (Kobelt, 1879)	LC519005
Inversidens brandtii (Kobelt, 1879)	LC519004
Inversidens rentianensis sp. nov. 1*	MZ073336
Inversidens rentianensis sp. nov. 2*	MZ073337

 Table 1. List of sequences used in this study. (\*) Sequenced from this study.

#### Phylogenetic analysis

Bayesian inference (BI) analyses were inferred in MrBayes Version 2.01 (Ronquist et al. 2012), using GTRGAMMAI model of nucleotide substitution. Four chains were run simultaneously for 10 million generations and trees were sampled every 1000 generations. The first 25% of these trees were discarded as burn-in when computing the consensus tree (50% Majority Rule). Sufficient mixing of the chains was considered to have been reached when the average standard deviation of split frequencies was below 0.01. Additionally, IQ-TREE was run for Maximum Likelihood (ML) tree reconstruction, using partition models with 1000 ultrafast bootstraps (Minh et al. 2013).

## Taxonomy

#### Inversidens rentianensis Wu & Wu, sp. nov.

http://zoobank.org/62424717-9514-4C7D-9C0E-240F1D95F03E Fig. 1C

**Type specimens.** *Holotype.* CHINA • Jiangxi Province, Ganzhou City, Rentian Town (壬田镇), Mianshui River (25.989557°N, 116.131333°E), 13 March 2018, coll. Xiongjun Liu (NCFM180325). *Paratypes.* Same data as holotype (NCFM180326-NCFM180328).

**Diagnosis.** *Inversidens rentianensis* sp. nov. is morphologically distinct from the other two recognized species within the genus by shell shape, beak position and nacre colour (Table 2). Diagnostic characteristics: shell irregularly subtriangular; curvature of the ventral margin slight, nearly straight; umbo situated 1/2 of shell length; nacre reddish.

**Description.** Shell irregularly subtriangular, medium thickness, and quite inflated. Anterior margin regularly rounded; ventral margin nearly straight; posterior margin obliquely arc-shaped. Umbo prominent and slightly eroded. Umbo sculptured with feebly wavy wrinkles. Posterior slope formed by the ventral margin and posterior margin low, triangular. Epidermis shining black or with brownish-yellow hue. Only one cardinal tooth in each valve, shape triangular. Laterals thick, a little curved, 2 in each valve. Nacre reddish-bronze in colour.

Length 43–52 mm, height 29–36 mm.

**Etymology.** The specific epithet is derived from the type locality, Rentian Town.

**Distribution.** The species is known only from Mianshui River, Rentian Town, Ganzhou City, Jiangxi Province, China (present study) (Fig. 2).

GenBank accession number. Holotype, NCFM180325: MZ073336; paratypes, NCFM180326: MZ073337.

**Molecular analyses.** Pairwise COI sequence divergences from *Inversidens brandtii* and *Inversidens rentianensis* sp. nov. were conducted using MEGA 5.0. Based on the uncorrected *p*-distance model, the intraspecific divergences of *I. brandtii* and *I. rentianensis* sp. nov. were both 0.00%. The interspecific divergence of *I.* 



Figure 1. Photographs of *Inversidens* taxa **A** *I. brandtii* **B** *I. pantoensis* **C** *I. rentianensis* sp. nov. Photos: **[A, B]** from the MUSSEL Project, **[C]** from this study, NCFM180325 (holotype), scale is 2 cm.

*brandtii* and *I. rentianensis* sp. nov. was 10.1%. Both BI and ML trees obtained a completely consistent topology. Consistent topology relationships are shown in Figure 3. In the phylogenetic trees, *I. rentianensis* sp. nov. formed a well-supported

**Table 2.** Conchological characters of *Inversidens rentianensis* sp. nov., *Inversidens brandtii*, *Inversidens pantoensis*. Characteristic descriptions of *I. brandtii* and *I. pantoensis* are referenced from Kondo (1982, 2008) and He and Zhuang (2013).

	I. rentianensis sp. nov.	I. brandtii	I. pantoensis
Shell shape	Irregularly subtriangular	Ovate	Inequilateral, quadrate
Umbo position	1/2 of shell length	1/4 of shell length	1/3 of shell length
Umbo sculpture	Feebly wavy wrinkles	Rippled	Angularly wrinkled
Surface sculpture	Concentric ridges	Concentric ridges	Irregular growth lines
Nacre colour	Reddish	Milk-white	Bluish
Posterior slope	Sharp	Blunt	Blunt
Ventral margin	Nearly straight	Arc-shaped	Long and straight



Figure 2. Photograph of sampling site of Inversidens rentianensis sp. nov. in China.

sister-group relationship with *Inversidens brandtii* (PP = 1.00, BS = 100; Fig. 3). The genera *Pronodularia* and *Inversiunio* belong to different clades well-separated from *Inversidens* (Fig. 3).

**Remarks.** Species delineation can be problematic in the presence of morphological ambiguities due to phenotypic plasticity and convergence (e.g., cryptic species), especially in mollusks (Zieritz et al. 2010; Inoue et al. 2013). The use of molecular genetics can aid species delineation in the case of phenotypic plasticity and/or convergence (Pieri et al. 2018; Wu et al. 2018). *Inversidens rentianensis* sp. nov. can be



**Figure 3.** Phylogenetic tree of freshwater mussels inferred from Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of COI barcode. Support values above the branches are posterior probabilities (PP)/bootstrap support (BS). Red font indicates the new species from this study.

distinguished from congeneric species based on diagnostic characteristics of the shell. In this study, we also analyzed the interspecific divergence between *Inversidens brandtii* and *Inversidens rentianensis* sp. nov. based on the COI barcode. The results showed that the average interspecific divergence between the two species was 10.1%, which was much higher than intraspecific divergences. Genetic analysis conducted in this study supports *I. rentianensis* sp. nov. as a valid species, which can be easily distinguished by the COI barcode.

# Acknowledgments

We thank the reviewer Dr Manuel Lopes-Lima, the other anonymous reviewer and the Subject Editor Dr Graham Oliver for valuable comments that have greatly improved this manuscript. This work was supported by the National Natural Science Foundation of China under Grant No.31772412.

# References

- Doucet-Beaupré H, Blier PU, Chapman EG, Piontkivska H, Dufresne F, Sietman BE, Mulcrone RS, Hoeh WR (2012) *Pyganodon* (Bivalvia: Unionoida: Unionidae) phylogenetics: a male- and female-transmitted mitochondrial DNA perspective. Molecular Phylogenetics and Evolution 63: 430–444. https://doi.org/10.1016/j.ympev.2012.01.017
- Edgar RC (2004) Muscle: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797. https://doi.org/10.1093/nar/gkh340
- Elderkin CL, Clewing C, Wembo Ndeo O, Albrecht C (2016) Molecular phylogeny and DNA barcoding confirm cryptic species in the African freshwater oyster *Etheria elliptica* Lamarck, 1807 (Bivalvia: Etheriidae). Biological Journal of the Linnean Society 118(2): 369–381. https://doi.org/10.1111/bij.12734
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrialcytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology & Biotechnology 3: 294–299.
- Graf DL, Cummings KS (2021a) A 'big data' approach to global freshwater mussel diversity (Bivalvia: Unionoida), with an updated checklist of genera and species. Journal of Molluscan Studies 87(1): 1–36. https://doi.org/10.1093/mollus/eyaa034
- Graf DL, Cummings KS (2021b) The Freshwater Mussels (Unionoida) of the World (and other less consequential bivalves). MUSSEL Project Web Site. http://www.mussel-project.net/
- Haas F (1969) Superfamilia Unionacea. Berlin: Walter de Gruyter.
- Haas F (1911) Die Unioniden. [in] H.C. Küster, Systematisches Conchylien-Cabinet von Martini und Chemnitz 9 (pt. 2, h. 45): 89–112.
- Haas F (1920) Die Neumayrschen Najaden aus der Ausbeute des Grafen Széchenyi. Senckenbergiana 2: 146–151.
- Habe T (1991) Catalogue of non-marine molluscs in Japan. Hitachiobi 56: 3-7.
- He J, Zhuang Z (2013) The Freshwater Bivalves of China. ConchBooks.
- Inoue K, Harris JL, Robertson CR, Johnson NA, Randklev CR (2019) A comprehensive approach uncovers hidden diversity in freshwater mussels (Bivalvia: Unionidae) with the description of a novel species. Cladistics 36(1): 88–113. https://doi.org/10.1111/cla.12386
- Inoue K, Hayes DM, Harris JL, Christian AD (2013) Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (B ivalvia: Unionidae). Ecology and Evolution 3(8): 2670–2683. https://doi. org/10.1002/ece3.649
- Jones JW, Neves RJ, Ahlstedt SA, Hallerman EM (2006) A holistic approach to taxonomic evaluation of two closely related endangered freshwater mussel species, the Oyster mussel *Epioblasma capsaeformis* and Tan Riffleshell *Epioblasma florentina* walker (Bivalvia: Unionidae). Journal of Molluscan Studies 72(3): 267–283. https://doi.org/10.1093/mollus/ eyl004
- Kondo T (1982) Taxonomic revision of Inversidens (Bivalvia: Unionidae). Venus 41(3): 181–198.
- Kondo T (1998) Revision of the genus Inversiunio (Bivalvia: Unionidae). Venus 57(2): 85-93.
- Kondo T (2008) Monograph of Unionoida in Japan (Mollusca: Bivalvia). Special Publication of the Malacological Society of Japan (3): 32–34.

- Lopes-Lima M, Burlakova L, Karatayev A, Gomes-dos-Santos A, Zieritz A, Froufe E, Bogan AE (2019) Revisiting the North American freshwater mussel genus *Quadrula sensu lato* (Bivalvia Unionidae): Phylogeny, taxonomy and species delineation. Zoologica Scripta 48(3): 313–336. https://doi.org/10.1111/zsc.12344
- Lopes-Lima M, Hattori A, Kondo T, Hee Lee J, Ki Kim S, Shirai A, Hayashi H, Usui T, Sakuma K, Toriya T, Sunamura Y, Ishikawa H, Hoshino N, Kusano Y, Kumaki H, Utsugi Y, Yabe S, Yoshinari Y, Hiruma H, Tanaka A, Sao K, Ueda T, Sano I, Miyazaki J-I, Gonçalves DV, Klishko OK, Konopleva ES, Vikhrev IV, Kondakov AV, Gofarov MY, Bolotov IN, Sayenko EM, Soroka M, Zieritz A, Bogan AE, Froufe E (2020) Freshwater mussels (Bivalvia: Unionidae) from the rising sun (Far East Asia): phylogeny, systematics, and distribution. Molecular Phylogenetics and Evolution 146: e106755. https://doi.org/10.1016/j. ympev.2020.106755
- Minh BQ, Nguyen MAT, von Haeseler A (2013) Ultrafast approximation for phylogenetic bootstrap. Molecular biology and evolution 30(5): 1188–1195. https://doi.org/10.1093/ molbev/mst024
- MolluscaBase eds (2021) MolluscaBase. http://www.molluscabase.org [on 2021-06-21.]
- Pieri AM, Inoue K, Johnson NA, Smith CH, Harris JL, Robertson C, Randklev CR (2018) Molecular and morphometric analyses reveal cryptic diversity within freshwater mussels (Bivalvia: Unionidae) of the western Gulf coastal drainages of the USA. Biological Journal of the Linnean Society 124(2): 261–277. https://doi.org/10.1093/biolinnean/bly046
- Ronquist F, Teslenko M, Mark PV, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Marc A, Suchard MA, Huelsenbeck JP (2012) Mrbayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/molbev/mst024
- Simpson CT (1914) A descriptive catalogue of the naiades, or pearly fresh-water mussels. Parts I-III. Bryant Walker, Detroit, Michigan. https://www.biodiversitylibrary.org/page/11344898
- Smith CH, Johnson NA, Inoue K, Doyle RD, Randklev CR (2019) Integrative taxonomy reveals a new species of freshwater mussel, *Potamilus streckersoni* sp. nov. (Bivalvia: Unionidae): implications for conservation and management. Systematics and Biodiversity 17(4): 331–348. https://doi.org/10.1080/14772000.2019.1607615
- Starobogatov Y (1970) Fauna of Molluscs and Zoogeographic Division of Continental Waterbodies of the Globe. Leningard Nauka.
- Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) Mega5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731–2739. https://doi.org/10.1093/molbev/msr121
- Wu RW, Liu YT, Wang S, Liu XJ, Zanatta DT, Roe KJ, Song XL, An CT, Wu XP (2018) Testing the utility of DNA barcodes and a preliminary phylogenetic framework for Chinese freshwater mussels (Bivalvia: Unionidae) from the middle and lower Yangtze River. PLoS ONE 13: e0200956. https://doi.org/10.1371/journal.pone.0200956
- Zieritz A, Hoffman JI, Amos W, Aldridge DC (2010) Phenotypic plasticity and genetic isolation-by-distance in the freshwater mussel *Unio pictorum* (Mollusca: Unionoida). Evolutionary Ecology 24(4): 923–938. https://doi.org/10.1007/s10682-009-9350-0

RESEARCH ARTICLE



# A new genus and ten new species of spiders (Arachnida, Araneae) from Iran

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Academic editor: Gergin Blagoev   Received 20 June 2021   Accepted 12 July 2021   Published 3 August 2021	Gergin Blagoev   Received 20 June 2021   Accepted 12 July 2021   Published 3 August 2021
http://zoobank.org/B67B23AA-E055-4911-BAC5-00C32921139D	http://zoobank.org/B67B23AA-E055-4911-BAC5-00C32921139D

**Citation:** Zamani A, Marusik YM (2021) A new genus and ten new species of spiders (Arachnida, Araneae) from Iran. ZooKeys 1054: 95–126. https://doi.org/10.3897/zookeys.1054.70408

## Abstract

One new genus (*Sestakovaia* gen. nov.; Liocranidae) and 10 new species of five families of spiders are described from different provinces of Iran: *Brigittea avicenna* sp. nov. ( $\overset{\circ}{\bigcirc} \heartsuit$ , Alborz and Kurdistan provinces) (Dictynidae), *Micaria atropatene* sp. nov. ( $\overset{\circ}{\oslash}$ , East Azerbaijan Province), *Zagrotes borna* sp. nov. ( $\overset{\circ}{\oslash}$ , Hormozgan Province), *Z. parla* sp. nov. ( $\overset{\circ}{\oslash}$ , Kerman Province) (Gnaphosidae), *Sestakovaia hyrcania* sp. nov. ( $\overset{\circ}{\oslash}$ , Golestan Province), *Mesiotelus patricki* sp. nov. ( $\overset{\circ}{\oslash}$ , Golestan Province) (Liocranidae), *Palpimanus carmania* sp. nov. ( $\overset{\circ}{\oslash}$ , Kerman Province), *P. persicus* sp. nov. ( $\overset{\circ}{\oslash} \heartsuit$ , Hormozgan Province) (Palpimanidae), *Rhysodromus genoensis* sp. nov. ( $\overset{\circ}{\oslash}$ , Hormozgan Province), and *R. medes* sp. nov. ( $\overset{\circ}{\oslash}$ , Hormozgan Province) (Philodromidae). Furthermore, *Sestakovaia annulipes* (Kulczyński, 1897), comb. nov. (ex. *Mesiotelus*) and *Zagrotes bifurcatus* (Zamani, Chatzaki, Esyunin & Marusik, 2021), comb. nov. (ex. *Berinda*) are proposed as new combinations.

## Keywords

Middle East, new combination, Sestakovaia, taxonomy

# Introduction

Currently, 890 species in 321 genera and 54 families of spiders are known from Iran (Zamani et al. 2021b; unpublished data). Although there have been many recent taxonomic revisions and large-scale faunistic surveys focusing on Iranian spiders (e.g., Montemor et al. 2020; Zamani and Bosselaers 2020; Zamani et al. 2020; Zamani and Marusik 2021a, b; Zamani et al. 2021a), the araneofauna of Iran nevertheless remains inadequately known, with much of the country poorly sampled and new species and distribution records found regularly. In this paper we contribute to the knowledge about spiders in Iran by describing a new genus and 10 new species, raising the number of spider species known from this country to 900 species in 322 genera.

# Material and methods

Specimens were photographed using a Canon EOS 7D camera attached to an Olympus SZX16 stereomicroscope and a JEOL JSM-5200 scanning electron microscope at the Zoological Museum of the University of Turku. Specimens were photographed in dishes with paraffin on the bottom holding the specimens in desired position. Digital images were montaged using CombineZP and Helicon focus 3.10 image stacking software programs and edited using CorelDraw graphic design software. Lengths of palp and leg segments were measured on the dorsal side and are listed as: total length (femur, patella, tibia, metatarsus [missing on the palp], tarsus).

# Abbreviations

ALE	anterior lateral eye;
AME	anterior median eye;
PLE	posterior lateral eye;
PME	posterior median eye;
RTA	retrolateral tibial apophysis.

Depositories (with curators' names in parentheses)

MHNG Muséum d'histoire naturelle, Genève, Switzerland (Peter J. Schwendinger);NHMW Naturhistorisches Museum Wien, Vienna, Austria (Christoph Hörweg).

# Taxonomy

Family Dictynidae O. Pickard-Cambridge, 1871

# Genus Brigittea Lehtinen, 1967

**Comments.** *Brigittea* is a small genus with six nominal species distributed in the Western Palaearctic, all of which were previously classified in *Dictyna* Sundevall, 1833. Males have a highly elevated cephalic region and chelicerae with lateral condyles and deeply concaved mesal margins. Females differ from those of the related genera by their spaced receptacles (vs touching each other). http://zoobank.org/D03A116C-9C98-48B0-BD23-B8B36B6EC07E Figures 1A–E, 2A–D, 3A–D

**Type material.** *Holotype* ♂ (MHNG), IRAN: Kurdistan Province: S of Divandareh, 35°45'N, 47°05'E, 23.6.1975 (A. Senglet). *Paratypes:* 3♀ (MHNG), same collection data as the holotype; 2♀ (MHNG), Alborz Province: Asara, 36°02'N, 51°14'E, 1900 m, 4.7.1975 (A. Senglet).



**Figure 1.** Male (**A**, **C–E**) and female (**B**) of *Brigittea avicenna* sp. nov. **A**, **B** habitus, dorsal **D**, **E** same, frontal and lateral, respectively **C** cribellum and spinnerets, ventral.



**Figure 2.** Male palp (**A**, **B**) and epigyne (**C**, **D**) of *Brigittea avicenna* sp. nov. **A**, **C** ventral **B** retrolateral **D** dorsal. Abbreviations: *Er* – epigynal ridge, *Re* – receptacle. Scale bars: 0.2 mm.

**Etymology.** The new species is named after Ibn Sina, also known as Avicenna (ca 980–June 1037), a Persian polymath who is regarded as the father of early modern medicine; noun in apposition.

**Diagnosis.** The male of the new species differs from all congeners by its thick embolus (vs filamentous) and the posterior tip of conductor (*Ct*) directed mesally (vs



**Figure 3.** SEM images of the male palp of *Brigittea avicenna* sp. nov. **A**, **B** ventral **C**, **D** retrolateral. Abbreviations: Ca – anterior part of conductor, Ct – posterior part of conductor, Ts – tibial socket. Scale bars: 0.1 mm.

retrolaterally). The female can be distinguished by the almost round copulatory openings and ridges (Er) spaced by more than 8 diameters of copulatory openings (vs copulatory openings not round and ridges spaced by less than 3 diameters). **Description. Male.** Habitus as in Figure 1A, D, E. Total length 2.60. Carapace 1.25 long, 0.55 wide at pars cephalica, 0.85 at pars thoracica, pars cephalica very high, higher than <sup>1</sup>/<sub>2</sub> (ca 0.65) of carapace length. Eye sizes: AME: 0.05, ALE: 0.06, PME: 0.06, PLE: 0.06. Carapace, labium, chelicera, maxilla and sternum dark reddish brown, without any pattern. Pars cephalica and sternum with sparse coating of long white setae. Legs light brown, without annulations. Abdomen dark grey, with coating of dense short white setae. Spinnerets brownish, unicolourous. Cribellum undivided (Fig. 1C). Measurements of legs: I: 3.50 (1.04, 0.34, 0.85, 0.81, 0.46), II: 3.07 (0.94, 0.33, 0.70, 0.68, 0.42), III: 2.23 (0.71, 0.27, 0.42, 0.50, 0.33), IV: 2.55 (0.80, 0.29, 0.54, 0.59, 0.33).

Palp as in Figures 2A, B, 3A–D; tibia with dorso-retrolateral socket (*Ts*); cymbium almost 2 times longer than wide; anterior part of conductor (*Ca*) terminates at about 11:30 o'clock position; posterior part (*Ct*) covered with fine granulation, tip directed mesally; embolic base large, about  $\frac{1}{2}$  of cymbium's length; embolus thick, originates at 10 o'clock position, tip modified with fine hook terminally.

**Female** (*paratype* from Kurdistan). Habitus as in Figure 1B. Total length 3.60. Carapace 1.15 long, 0.58 wide at pars cephalica, 0.94 at pars thoracica. Coloration, general somatic features and eye sizes as in male. Measurements of legs: I: 3.57 (1.06, 0.40, 0.77, 0.79, 0.55), II: 3.09 (0.96, 0.36, 0.63, 0.71, 0.43), III: 2.36 (0.75, 0.33, 0.46, 0.52, 0.30), IV: 3.02 (0.95, 0.39, 0.66, 0.68, 0.34).

Epigyne as in Figure 2C, D; epigynal field about 3 times wider than long; copulatory opening almost round, spaced by 1.6 diameters, lateral ridges (Er) located 2 diameters of copulatory openings apart from copulatory openings, their length about diameter of copulatory openings; copulatory ducts gradually tapering, making a course of about 90°, about 2 times longer than copulatory opening's diameter; receptacles (Re) smaller than copulatory openings, spaced slightly by more than one width.

**Comments.** At first look, the male palp is similar to those of the species of *Emblyna* Chamberlin, 1948 due to the modified embolus (thick and with complex tip). Current generic placement is due to the strongly raised cephalic region, which is also documented in the generotype, *Brigittea latens* (Fabricius, 1775), and modification of chelicera (cf. Fig. 1D and Miller and Svatoň 1978: pl. IV, figs 1, 2). All species assigned to *Brigittea* similarly have the posterior tip of conductor covered with fine teeth (cf. Marusik et al. 2015: figs 31, 35). Another similarity with the generotype is spaced receptacles (vs touching each other in *Emblyna* and *Dictyna*). Although *B. latens* has a filamentous embolus, its tip is modified in a similar way as in *B. avicenna* sp. nov. (cf. Marusik et al. 2015: fig. 36). There is another character that is different in the new species and the generotype – the structure of the cribellum. In the new species the cribellum is integral, while in *B. latens* it is bipartite (cf. Lehtinen 1967).

**Distribution.** Known only from the listed localities in Alborz and Kurdistan provinces, northern and western Iran.

#### Family Gnaphosidae Pocock, 1898

#### Genus Zagrotes Zamani, Chatzaki, Esyunin & Marusik, 2021

**Comments.** This genus was recently described as monotypic from southwestern and southern Iran, with *Zagrotes apophysalis* Zamani, Chatzaki, Esyunin & Marusik, 2021 as the type species. In the same paper, Zamani et al. (2021a) described another similar species in another genus, namely *Berinda bifurcata* Zamani, Chatzaki, Esyunin & Marusik, 2021. The two new species described here are closely related to *B. bifurcata*, and to a lesser degree to *Z. apophysalis*. All species possess a bifurcated RTA and similar conformation of the bulb. For this reason, we propose a new combination, *Zagrotes bifurcatus* (Zamani, Chatzaki, Esyunin & Marusik, 2021), comb. nov., despite the fact that this species and the two new ones described here lack modifications on palpal patella and swollen tibia that are present in the type species.

**Composition.** Four species: *Z. apophysalis, Z. borna* sp. nov., *Z. bifurcatus* comb. nov., and *Z. parla* sp. nov.

**Distribution.** Endemic to Iran, distributed along the southwestern and southern slopes of Zagros Mountains, from Kohgiluyeh and Boyer-Ahmad to Hormozgan.

#### Zagrotes borna sp. nov.

http://zoobank.org/DA557472-6803-4C50-A165-BF60F41828AC Figures 4A, 5A, B, 6A, B, 7A, B

**Type material.** *Holotype* ∂ (NHMW), IRAN: Hormozgan Province: 40 km NW of Bandar Abbas, 7.4.1972 (G. Pretzmann).

Etymology. The specific epithet is a Persian masculine given name meaning "young".

**Diagnosis.** The new species is most similar to *Z. parla* sp. nov. and can be distinguished by the RTA longer than ½ of the cymbium's length (vs shorter) and less deeply bifurcated (cf. Fig. 6B and D), and the relatively shorter palpal tibia (4 times shorter than cymbium vs 2 times shorter), and by the shape of the bulb. The two species differ also by the shape of the sperm duct and tegular apophysis (cf. Fig. 5A and C).

**Description. Male.** Habitus as in Figure 4A. Total length 4.85. Carapace 2.20 long, 1.43 wide. Eye sizes: AME: 0.11, ALE: 0.09, PME: 0.10, PLE: 0.09. Carapace, labium, chelicera, maxilla and sternum light brown, without any pattern. Legs yellowishbrown, without annulations. Abdomen cream-colored, with a tuft of dark brown long setae anteriorly and sparse lighter setae; ventrally with distinct tracheal marks. Spinnerets unicolourous. Measurements of legs: I: missing, II: 5.35 (1.45, 0.90, 1.12, 1.10, 0.78), III: 4.36 (1.20, 0.63, 0.87, 1.01, 0.65), IV: 7.06 (1.80, 1.00, 1.52, 1.81, 0.93).

Palp as in Figures 5A, B, 6A, B, 7A, B; tibia as long as wide, RTA almost twice longer than tibia, bifurcated in anterior 1/3, ventral arm rounded and dorsal one sharply pointed; cymbium 2 times longer than wide, and 4 times longer than tibia;



**Figure 4.** Males of *Zagrotes borna* sp. nov. (**A**), *Z. parla* sp. nov. (**B**), *Micaria atropatene* sp. nov. (**C**), *Sestakovaia hyrcania* sp. nov. (**D**) and *Mesiotelus patricki* sp. nov. (**E**, **F**) **A–D**, **F** habitus, dorsal **E** chelicerae, ventral. Scale bars: 0.2 mm, unless stated otherwise.

tegular apophysis claw-like, directed laterally, with abrupt tip in lateral view; sperm duct with characteristic coil prolaterally.

Female. Unknown.

**Distribution.** Known only from the type locality in Hormozgan Province, southern Iran.

# Zagrotes parla sp. nov.

http://zoobank.org/0B3CDEE8-E686-4CA2-8FE8-7167C9BB0FF7 Figures 4B, 5C, D, 6C, D, 7C, D

**Type material.** *Holotype*  $\stackrel{\sim}{\bigcirc}$  (NHMW), IRAN: Kerman Province: SE of Sirjan, 6.1972 (G. Pretzmann).



**Figure 5.** Male palps of *Zagrotes borna* sp. nov. (**A**, **B**) and *Z. parla* sp. nov. (**C**, **D**) **A**, **C** ventral **B**, **D** prolateral. Abbreviation: *Ta* – tegular apophysis. Scale bars: 0.2 mm.



Figure 6. Male palps of *Zagrotes borna* sp. nov. (**A**, **B**) and *Z*. *parla* sp. nov. (**C**, **D**) **A**, **C** dorsal **B**, **D** retrolateral. Scale bars: 0.2 mm.



**Figure 7.** SEM images of the male palps of *Zagrotes borna* sp. nov. (**A**, **B**) and *Z. parla* sp. nov. (**C**, **D**) **A** ventral **B**, **D** retrolateral **C** retroventral. Abbreviation: *Ta* – tegular apophysis. Scale bars: 0.1 mm.

Etymology. The specific epithet is a Persian feminine given name meaning "glowing".

**Diagnosis.** The new species is most similar to *Z. borna* sp. nov. and can be distinguished by the RTA shorter than ½ of the cymbium's length (vs longer), the more deeply bifurcated RTA (cf. Fig. 6D and B), relatively longer palpal tibia (2 times shorter than cymbium vs 4 times shorter), and the shape of the bulb. The two species differ also by the shape of the sperm duct and tegular apophysis (cf. Fig. 6C and A).

**Description. Male.** Habitus as in Figure 4B. Total length 4.93. Carapace 2.20 long, 1.63 wide. Eye sizes: AME: 0.10, ALE: 0.09, PME: 0.10, PLE: 0.08. Carapace, labium, chelicera, maxilla and sternum light brown, without any pattern. Legs yellow-ish-brown, without annulations. Abdomen cream-colored, with a tuft of dark brown long setae anteriorly and sparse lighter setae; ventrally with distinct tracheal marks. Spinnerets unicolourous. Measurements of legs: I: 6.83 (1.83, 1.17, 1.51, 1.32, 1.00), II: 5.29 (1.41, 0.93, 1.10, 1.05, 0.80), III: 4.47 (1.20, 0.70, 0.88, 1.04, 0.65), IV: 7.26 (1.84, 1.07, 1.57, 1.90, 0.88).

Palp as in Figures 5C, D, 6C, D, 7C, D; tibia 3 times shorter than cymbium; RTA as long as tibia, deeply bifurcated in middle part, ventral arm rounded on the tip and dorsal arm sharply pointed; cymbium 1.7 times longer than wide; tegular apophysis (*Ta*) with large base, about ½ of tegulum's length, directed ventrally, sharply pointed in lateral view; sperm duct with characteristic loop originated in mesal part of tegulum.

Female. Unknown.

**Distribution.** Known only from the type locality in Kerman Province, southern Iran.

#### Genus Micaria Westring, 1851

#### Micaria atropatene sp. nov.

http://zoobank.org/8E5579B5-A267-4AB2-86CA-028D610E26A5 Figures 4C, 8A, B

**Type material.** *Holotype* ♂ (NHMW), IRAN: East Azerbaijan Province: 20 km SE of Miyaneh, 1970 (K. Bilek).

**Etymology.** The specific epithet (a noun in apposition) refers to an ancient kingdom established in ca 323 BC by the Persian satrap Atropates, centered in present-day northern and northwestern Iran.

**Diagnosis.** The new species is most similar to *M. rossica* (Thorell, 1875) by having a small tibial apophysis, vestigial tegular apophysis (*Ta*), a similar course of the sperm duct, and the distal tegular process (Dp) extending to the anterior edge of the tegulum. *Micaria atropatene* sp. nov. can be easily distinguished by the shape of the tegular process, which has an almost transverse anterior edge and a small spine-like tip (vs gradually tapering).

**Description. Male.** Habitus as in Figure 4C. Total length 4.50. Carapace 2.07 long, 1.38 wide. Eye sizes: AME: 0.08, ALE: 0.08, PME: 0.05, PLE: 0.07. Carapace, labium, chelicera, maxilla and sternum reddish-brown, without any pattern. Legs



**Figure 8.** Male palp of *Micaria atropatene* sp. nov. **A** ventral **B** retrolateral. Abbreviations: Dp – distal tegular process, Ta – tegular apophysis. Scale bar: 0.2 mm.

yellowish-brown, without annulations. Abdomen glossy dark gray, with a coat of short setae. Spinnerets light grey, unicolourous. Measurements of legs: I: 6.01 (1.48, 0.72, 1.37, 1.21, 1.23), II: 5.28 (1.42, 0.65, 1.16, 1.00, 1.05), III: 5.01 (1.28, 0.61, 1.04, 1.11, 0.97), IV: 5.29 (1.84, 0.74, 1.44, 1.78, 1.27).

Palp as in Figure 8A, B; tibia long, more than 3 times longer than wide, 0.83 times of cymbium's length, with fine retrolateral apophysis; cymbium more than 2 times longer than wide; tegulum oval in ventral and lateral views, 1.8 times longer than wide; tegular apophysis (Ta) vestigial; distal tegular process (Dp) large, with abrupt anterior edge and spine-like projection mesally.

Female. Unknown.

**Distribution.** Known only from the type locality in East Azerbaijan Province, northwestern Iran.

#### Family Liocranidae Simon, 1897

## Genus Sestakovaia gen. nov.

http://zoobank.org/C44399C8-5DA7-46D3-84C9-6182AA18ABB6

#### Type species. Sestakovaia hyrcania sp. nov.

**Etymology.** The new genus is named after our colleague and friend, Anna Šestáková (Western Slovakian Museum, Trnava, Slovakia); the gender is feminine.

**Diagnosis.** The new genus differs from other Liocraninae genera by the bent RTA (vs straight) with tip directed antero-dorsally. It differs from *Mesiotelus* Simon, 1897 by unmodified (not elongated) male chelicera, short palp (not longer than carapace), large and complex embolus (cf. Figs 9A, D, 10A with Fig. 11A) and epigyne lacking fine anterior hood (cf. Chyzer and Kulczyński 1897: pl. 9, fig. 75 with Bosmans and El-Hennawy 2018: fig. 1).

**Description.** Medium-sized, carapace ca 2.0 long, 1.5 wide, length of chelicera ca 1/3 of carapace length. Carapace and abdomen with distinct pattern. For details, see Chyzer and Kulczyński (1897) and the description below.

**Comments.** Homology of anterior tegular apophysis (*Aa*) is not clear, as such structure is not known in other genera of the family.

**Composition.** *Sestakovaia hyrcania* sp. nov. and *S. annulipes* (Kulczyński, 1897), comb. nov.

Distribution. Central Europe, Near East and northern Iran.

#### Sestakovaia hyrcania sp. nov.

http://zoobank.org/FEA79A4C-71F9-4080-8EEE-A02F4937F08E Figures 4D, 9A–C, 10A–D

**Type material.** *Holotype* ♂ (NHMW), IRAN: Golestan Province: 10 km SW of Shahpasand, 26.4.1972 (G. Pretzmann).

**Etymology.** The species name (a noun in apposition) refers to the historical region in south-east of the Caspian Sea in modern-day Iran and Turkmenistan which lies between the coastal line to the north-west, the Alborz Mountains to the south, and the Kopet Dag Mountains to the east, in which the type locality of the new species is situated.


**Figure 9.** Male palps of *Sestakovaia hyrcania* sp. nov. (**A–C**) and *Sestakovaia annulipes* comb. nov. (**D**) **A**, **D** ventral **B** retrolateral **C** dorsal **D** after Dimitrov and Naumova (2021). Abbreviations: Aa – anterior apophysis, Co – conductor, Em – embolus, Pe – embolic process, Ra – radix, Ta – tegular apophysis. Scale bars: 0.2 mm.

**Diagnosis.** The new species differs from *S. annulipes* comb. nov. by having the RTA bent almost at a right angle and with a pointed tip (vs RTA bent at a lesser angle and its tip rounded), a wider embolus (*Em*) and process of the embolus (*Pe*), and the embolus located prolaterally in relation to the process (vs embolus mesal and process prolateral).

**Description. Male.** Habitus as in Figure 4D. Total length 4.37. Carapace 2.06 long, 1.58 wide. Eye sizes: AME: 0.13, ALE: 0.11, PME: 0.09, PLE: 0.12. Carapace, labium, chelicera, maxilla and sternum light brown. Carapace with darker submarginal longitudinal bands. Legs yellowish-brown, with numerous darker patches. Abdomen dorsally greyish with lighter chevron markings, ventrally lighter without any markings. Spinnerets light grey, unicolourous. Measurements of legs: I: 6.54 (1.72, 0.85, 1.69, 1.32, 0.96), II: 5.73 (1.31, 0.66, 1.66, 1.30, 0.80), III: 5.82 (1.46, 0.71, 1.38, 1.63, 0.64), IV: 8.25 (2.10, 0.88, 1.96, 2.25, 1.06).

Palp as in Figures 9A–C, 10A–D; RTA about 2 times shorter than tibia, bent almost at a right angle, tip pointed; cymbium 1.6 times longer than wide; bulb ca 1.3 times longer than wide; tegulum with 2 apophyses: long tegular apophysis (*Ta*) with hooked tip, and broad anterior apophysis (*Aa*); conductor (*Co*) lamellar/membranous, large, almost as wide as radix; embolus complex: with broad radical part (*Ra*), broad embolus proper (*Em*) and mesal embolic process (*Pe*).

Female. Unknown.

Distribution. Known only from the type locality in Golestan Province, northern Iran.

### Sestakovaia annulipes (Kulczyński, 1897), comb. nov.

Figure 9D

*Liocranum annulipes* Kulczyński in Chyzer and Kulczyński 1897: 240, pl. 9, figs 54, 56 (♂♀).

*Mesiotelus annulipes*: Dimitrov and Naumova 2021: 695, figs 5A, B, 6A, B (♂).

**Comments.** This species transferred to this new genus due to the similarity of the male palp to the generotype. Although this species has a relatively large range (reported from Slovakia, Hungary, Croatia, Serbia, Bulgaria, Ukraine and Turkey; WSC 2021), the female characters have not been redescribed and the structure of the endogyne remains unillustrated.

# Genus Mesiotelus Simon, 1897

*Mesiotelus patricki* sp. nov. http://zoobank.org/3D4AD9D0-D4D9-4097-86FC-983C99FC643C Figures 4E, F, 11A–E, 12A–C

**Type material.** *Holotype*  $\stackrel{>}{\circ}$  and *paratypes*  $2\stackrel{>}{\circ}$  (MHNG), IRAN: Golestan Province: Gol-e Loweh, 37°20'N, 55°44'E, 21.8.1975 (A. Senglet).



**Figure 10.** SEM images of the male palps of *Sestakovaia hyrcania* sp. nov. **A**, **D** ventral **B** retroventral **C** apical. Abbreviations: Aa – anterior apophysis, Co – conductor, Em – embolus, Pe – embolic process, Ra – radix, Ta – tegular apophysis. Scale bars: 0.1 mm.

**Etymology.** This new species is named after our colleague and friend L. Brian Patrick (Dakota Wesleyan University, USA), in recognition of his efforts in popularizing taxonomy via his podcast "New Species".



**Figure 11.** Male palp of *Mesiotelus patricki* sp. nov. **A–D** ventral, retrolateral, prolateral and dorsal, respectively **E** whole palp, prolateral. Scale bars: 0.2 mm, unless stated otherwise.

**Diagnosis.** The new species differs from the generotype, *Mesiotelus tenuissimus* (L. Koch, 1866), by having a relatively longer, thinner and gradually tapering RTA which is about 3 times longer than its basal width (vs RTA with subparallel margins and less



**Figure 12.** SEM images of the male palp of *Mesiotelus patricki* sp. nov. **A**, **B** ventral **C** retroventral. Abbreviations: Aa – anterior apophysis, Co – conductor, Cs – curved setae, Em – embolus, Ta – tegular apophysis. Scale bars: 0.1 mm.

than 2 times longer than wide), a much longer median apophysis, about  $\frac{1}{2}$  of tegulum's length (vs about 4 times shorter than tegulum), and a longer embolus terminating anteriorly from the tegulum (vs embolus short, spine-like and terminating beyond the anterior edge of the tegulum). The two species also differ by the course of sperm duct and the shape of the anterior tegular apophysis (*Aa*).

**Description. Male** (*holotype*). Habitus as in Figure 4F. Total length 5.22. Carapace 2.00 long, 1.85 wide. Eye sizes: AME: 0.12, ALE: 0.13, PME: 0.10, PLE: 0.10. Carapace, labium, chelicera, maxilla and sternum light brown. Chelicera with 2 pro- and 3 retromarginal teeth (Fig. 4E). Legs yellowish-brown, without annulations. Abdomen dark grey, dorsally with indistinct chevron markings, ventrally lighter with distinct tracheal marks. Spinnerets light grey, unicolourous. Measurements of palp and legs: palp: 4.77 (1.74, 1.08, 1.08, 0.87), legs: I: 10.86 (2.77, 1.39, 2.95, 2.45, 1.30), II: 7.67 + missing tarsus (2.31, 1.08, 2.30, 1.98, missing), III: 7.38 (1.94, 0.91, 1.67, 1.95, 0.91), IV: 10.38 (2.63, 1.10, 2.48, 3.06, 1.11).

Palp as in Figures 11A–E, 12A–C; palp very long, almost as long as body, patella as long as tibia; tibia with 3 times longer than wide and gradually tapering RTA; cymbium twice shorter than femur, and 2 times longer than wide, tip as long as RTA, prolateral side with few very long and curled setae (Cs, Fig. 12A, B); bulb oval, 1.67 times longer than wide; sperm duct almost reaching posterior margin of tegulum; median apophysis long, almost as long as  $\frac{1}{2}$  of tegulum's length; anterior tegular apophysis

(*Aa*) bifid; embolus (*Em*) originates at about ca 6:30 o'clock position, free part long, bent in terminal part and directed anteriorly.

Female. Unknown.

**Distribution.** Known only from the type locality in Golestan Province, northern Iran.

### Family Palpimanidae Thorell, 1870

# Genus Palpimanus Dufour, 1820

**Comments.** *Palpimanus* is the most species-rich genus in Palpimaninae, with 35 currently recognized species (WSC 2021). Although the Mediterranean species have been the subject of two revisions by Kulczyński (1909) and Platnick (1981), the genus remains poorly studied: male palps were illustrated very schematically and endogynes were not illustrated at all. Additionally, there is no proper terminology for the sclerites in the male palp; Platnick (1981) used the neuter terms 'prong' and 'flange'. Within Palpimaninae, the endogynes are very weakly sclerotized and difficult to observe, hence the lack of proper illustrations. The set of illustrations in Platnick (1981: figs 10–18) is very schematic and in some cases appears to be misinterpreted. Both new species found in Iran have male palps rather different from that of the type species (*P. gibbulus* Dufour, 1820) and most likely belong to a different, currently undescribed genus.

### Palpimanus carmania sp. nov.

http://zoobank.org/868D7059-4D36-47BB-9C6B-45B3065CB57B Figures 13A, 14A, B, 15A–C, 16E–G

**Type material.** *Holotype* ♂ (NHMW), IRAN: Kerman Province: 41 km SE of Sirjan, 18.4.1972 (G. Pretzmann).

**Etymology.** The specific epithet is a noun in apposition, referring to a historical region that approximately corresponds to the modern Iranian province of Kerman, where the type locality of the new species is situated.

**Diagnosis.** The new species differs from the related *P. persicus* sp. nov. by the more distal position of the base of the "embolic stalk" (*Es*) and wider stalk with sharply pointed tip (vs more proximal, thinner and without sharply pointed tip; cf. Fig. 16E and A) and also by having wider than long palpal tibia (vs as wide as long). It is most similar to *P.* cf. *sogdianus* from Azerbaijan illustrated by Marusik and Guseinov (2003). Both have a sharply pointed embolic stalk (*Es*), but in the new species this originates from the distal half of the bulb (vs proximal half).

**Description. Male.** Habitus as in Figures 13A, 14A. Total length 5.50. Carapace 2.65 long, 1.92 wide. Eye sizes: AME: 0.17, ALE: 0.08, PME: 0.07, PLE: 0.10. Carapace, labium, chelicera, maxilla and sternum dark reddish, coated with



**Figure 13.** Lateral habitus of *Palpimanus carmania* sp. nov. (**A**) and *P. persicus* sp. nov. (**B, C**) **A, C** males **B** female.



**Figure 14.** Dorsal habitus (**A**, **C**, **E**) and ventral anterior portion of abdomen (**B**, **D**, **F**) of *Palpimanus carmania* sp. nov. (**A**, **B**) and *P. persicus* sp. nov. (**C–F**) **A–D** males **E**, **F** female. Scale bars: 0.2 mm, unless stated otherwise.

white setae. Leg I dark orange, legs II–IV yellowish-brown, without annulations. Metatarsus I and metatarsi and tarsi II–IV with a distinct ventral hair tuft. Abdomen cream-colored, with scattered long dark setae and a ventral scutum, two long diagonal and two dot-like scutula (Fig. 14B). Spinnerets unicolourous. Measurements of legs: I: 6.05 (1.90, 1.58, 1.34, 0.58, 0.65), II: 4.59 (1.26, 0.90, 1.12, 0.74, 0.57), III: 4.05 (1.16, 0.80, 0.87, 0.76, 0.46), IV: 5.18 (1.35, 0.86, 1.28, 1.08, 0.61).

Palp as in Figures 15A–C, 16E–G; tibia swollen, ca 1.1 times wider than long, wider than bulb; cymbium 1.5 times longer than tibia; embolic stalk (*Es*) originates in anterior half, stalk tapering, tip sharply pointed.

Female. Unknown.

**Distribution.** Known only from the type locality in Kerman Province, southern Iran.



**Figure 15.** Male palps of *Palpimanus carmania* sp. nov. (**A–C**) and *P. persicus* sp. nov. (**D–F**) **A**, **D** ventral **B**, **E** retrolateral **C**, **F** proventral and prolateral. Abbreviation: *Es* – embolic stalk. Scale bars: 0.2 mm.

# Palpimanus persicus sp. nov.

http://zoobank.org/9666664CC-AAFC-42AC-B3F8-D0BD28E26BA2 Figures 13B, C, 14C-F, 15D-F, 16A-D, 17A-F

**Type material.** *Holotype*  $\stackrel{>}{\circ}$  (NHMW), IRAN: Hormozgan Province: 40 km NW of Bandar Abbas, 7.4.1972 (G. Pretzmann). *Paratype:* 1 $\stackrel{>}{\circ}$  (NHMW), Hormozgan Province: 26 km S of Minab, 7.1974 (G. Pretzmann).



**Figure 16.** SEM images of the male palps of *Palpimanus persicus* sp. nov. (**A–D**) and *P. carmania* sp. nov. (**E–G**) **A, E** ventral **B, G** proventral **C, F** apical **D** proapical. Abbreviation: *Es* – embolic stalk. Scale bars: 0.1 mm, unless stated otherwise.



**Figure 17.** Endogyne of *Palpimanus persicus* sp. nov. **A–D**, **F** dorsal **E** posterior. Boxes in **C** and **D** refer to detailed views presented in **D** and **F**, respectively. Abbreviations: Co – copulatory opening, Ft – fine threads, Gg – grape-shaped glands, Re – receptacle. Scale bars: 0.2 mm.

**Other material examined.** IRAN: Hormozgan Province: 1<sup>Q</sup> (NHMW), 28 km N of Bandar Abbas, 28.3.1972 (G. Pretzmann).

**Etymology.** The specific epithet is an adjective of "Persian" or "of Persia", referring to the historical region of the Middle East, located in the east of Mesopotamia (nowadays Iran).

**Diagnosis.** The male of this species differs from the closely related *Palpimanus carmania* sp. nov. by having the embolic stalk originating in the proximal half of the bulb (vs distal) and the stalk wider, not tapering, and its tip not pointed (cf. Fig. 16A and E). Endogyne of the new species is similar to that of *P. sogdianus* Charitonov, 1946 and specimens identified as *P.* cf. *sogdianus* from Azerbaijan (see Marusik and Guseinov 2003: figs 35–36), but it differs by having the anterior and posterior halves of the receptacles subequal in length (vs anterior halves longer). In addition, females of the new species differ from specimen from Azerbaijan by having the copulatory opening (*Co*) more arched.

**Description. Male** (*holotype*). Habitus as in Figures 13C, 14C. Total length 5.20. Carapace 2.60 long, 1.95 wide. Eye sizes: AME: 0.21, ALE: 0.11, PME: 0.07, PLE: 0.13. Carapace, labium, chelicera, maxilla and sternum dark reddish, coated with white setae. Leg I dark orange, legs II–IV yellowish-brown, without annulations. Metatarsus I and metatarsi and tarsi II–IV with a distinct ventral hair tuft. Abdomen cream-colored, with scattered long dark setae and a ventral scutum, two long diagonal and two dot-like scutula (Fig. 14D). Spinnerets unicolourous. Measurements of legs: I: 5.57 (1.77, 1.46, 1.27, 0.57, 0.50), II: 4.74 (1.44, 0.92, 1.10, 0.72, 0.56), III: 4.23 (1.29, 0.76, 1.03, 0.70, 0.45), IV: 5.01 (1.53, 0.93, 1.20, 0.91, 0.44).

Palp as in Figures 15D–F, 16A–D; tibia swollen, as wide as long; cymbium 1.6 times longer than wide; embolic stalk (*Es*) originates in proximal half of bulb, wide, not tapering, tip not pointed.

**Female.** Habitus as in Figures 13B, 14E. Total length 6.55. Carapace 3.00 long, 2.27 wide. Eye sizes: AME: 0.17, ALE: 0.11, PME: 0.07, PLE: 0.11. Coloration and somatic features as in male. Postgaster with 3 pairs of scutula (Fig. 14F). Measurements of legs: I: 6.78 (2.17, 1.80, 1.62, 0.61, 0.58), II: 5.71 (1.76, 1.16, 1.32, 0.83, 0.64), III: 5.06 (1.50, 0.93, 1.17, 0.86, 0.60), IV: 6.77 (2.04, 1.19, 1.66, 1.32, 0.56).

Endogyne as in Figure 17A–F; copulatory opening (Co) arched in ventral view and squared in posterior view; endogyne weakly sclerotized, receptacles about 3.5 times longer than wide, each with cylindrical posterior part and subglobular anterior part, posterior parts converging, receptacles lacking distinct accessorial or pore glands; fine threads (Ft) originate near copulatory opening; grape-shaped glands (Gg) poorly distinct, about 5 on each side.

**Comment.** As the single female specimen treated here was collected separately from the males and in a different locality (although all from the same province and from sites relatively close to one another), it was not considered within the type material; the conspecificity of these specimens shall be confirmed when both sexes are collected together.

**Distribution.** Known only from the listed localities in Hormozgan Province, southern Iran.

### Family Philodromidae Thorell, 1870

### Genus Rhysodromus Schick, 1965

**Comments.** Considered a relatively large genus, *Rhysodromus* includes 26 valid species, the majority of which are distributed in the Palaearctic. The genus is relatively well studied due to several revisions, especially that by Szita and Logunov (2008).

http://zoobank.org/D83478E1-738C-4556-A2A5-CAAB97E559C0 Figures 18A, 19A, B, 20A-F

**Type material.** *Holotype*  $\stackrel{\circ}{\bigcirc}$  (NHMW), IRAN: Hormozgan Province: Geno, 38 km NW of Bandar Abbas, 3.4.1972 (G. Pretzmann).

**Etymology.** The specific epithet refers to the type locality of the new species in Geno Biosphere Reserve.

**Diagnosis.** This species differs from all congeners by the lack of the RTA (vs present in all species) and bifurcated tip of tegular apophysis (vs not bifurcated).

**Description. Male.** Habitus as in Figure 18A. Total length 3.80. Carapace 1.65 long, 1.60 wide. Eye sizes: AME: 0.11, ALE: 0.10, PME: 0.06, PLE: 0.10. Carapace, labium, chelicera and maxilla brown, without any pattern; pars cephalica and median part of pars thoracica lighter in color; sternum pale, with a grayish marginal band. Legs yellowish-brown, with numerous dark spots and small patches. Abdomen grayish, dorsally with distinct cardiac mark and darker margins and light spots and patches; ventrally with slightly darker median band. Spinnerets brown, unicolourous. Measurements of legs: I: 7.86 (2.27, 0.89, 1.95, 1.74, 1.01), II: 9.15 (2.66, 0.88, 2.31, 2.05, 1.25), III: 5.55 (1.72, 0.65, 1.29, 1.16, 0.73), IV: 7.06 (2.26, 0.74, 1.69, 1.61, 0.76).

Palp as in Figures 19A, B, 20A–F; tibia as long as bulb's width, lacking distinct apophysis; cymbium with long tip (partially extending tegulum), about 0.9 of tegulum's length; tegulum oval, about 1.5 times longer than wide; sperm duct with small transverse loop in mesal part of tegulum; tegular apophysis long, located anteriorly along longitudinal axis, tip bifid; embolus long, >0.5 of tegulum's length, straight, with tip gently bent retrolaterally.

Female. Unknown.

**Comments.** The new species has a pattern typical for the genus and is particularly similar to *R. alascensis* (Keyserling, 1884) and *R. histrio* (Latreille, 1819).

There are two *Rhysodromus* species in the region (both from Caucasus) known from females only, *R. rikhteri* (Logunov & Huseynov, 2008) and *R. naxcivanicus* (Logunov & Huseynov, 2008), but it is very unlikely that either of them would be conspecific



Figure 18. Male dorsal habitus of *Rhysodromus genoensis* sp. nov. (A) and *Rhysodromus medes* sp. nov. (B).



**Figure 19.** Male palps of *Rhysodromus genoensis* sp. nov. (**A**, **B**) and *Rhysodromus medes* sp. nov. (**C**, **D**) **A**, **C** ventral **B**, **D** retrolateral. Abbreviation: *Ta* – tegular apophysis. Scale bars: 0.2 mm.



**Figure 20.** SEM images of the male palp of *Rhysodromus genoensis* sp. nov. **A**, **E** retroventral **B**, **F** ventral **C** proventral **D** retrolateral. Abbreviation: *Ta* – tegular apophysis. Scale bars: 0.1 mm, unless stated otherwise.

with *R. genoensis* sp. nov., as their type localities are located more than 1000 km distant from that of the new species.

**Distribution.** Known only from the type locality in Hormozgan Province, southern Iran.

# Rhysodromus medes sp. nov.

http://zoobank.org/6BABB45F-31F2-4526-9CC1-9C4CFF99F9D2 Figures 18B, 19C, D, 21A, B

**Type material.** *Holotype*  $\stackrel{\circ}{\supset}$  (NHMW), IRAN: Hormozgan Province: 26 km S of Minab, 7.1974 (G. Pretzmann).



**Figure 21.** SEM images of the male palp of *Rhysodromus medes* sp. nov. **A** ventral **B** retroventral. Abbreviation: *Ta* – tegular apophysis. Scale bars: 0.1 mm.

**Etymology.** The specific epithet is a noun in apposition, referring to an ancient Iranian people who spoke the Median language and inhabited an area known as Media between western and northern Iran.

**Diagnosis.** The new species differs from all congeners (as well as all other members of *Philodromus* sensu lato) by having 2 parallel, unspaced tibial apophyses of the same length (vs apophyses either absent, or 1 or 2 spaced and not of equal length) and sperm duct thick at proximal-prolateral part, as thick as retrolaterally (vs prolateral part gradually tapering and thinner than retrolateral part).

**Description. Male.** Habitus as in Figure 18B. Total length 4.07. Carapace 1.87 long, 1.60 wide. Eye sizes: AME: 0.11, ALE: 0.07, PME: 0.10, PLE: 0.11. Carapace, labium, chelicera, maxilla and sternum yellowish-brown. Legs pale, without annulations. Abdomen pale, with scattered thick dark setae. Spinnerets pale, unicolourous. Measurements of legs: I: 8.97 (2.52, 0.96, 2.41, 2.06, 1.02), II: 11.58 (3.20, 1.19, 3.10, 2.78, 1.31), III: 4.97 (1.52, 0.52, 1.07, 1.18, 0.68), IV: 8.54 (2.65, 0.75, 1.95, 2.23, 0.96).

Palp as in Figures 19C, D, 21A, B; tibia ca 1.3 times longer than wide, with two apophyses, not spaced and equal in length, about  $\frac{1}{2}$  of tibia's length, ventral one membranous and transparent, retrolateral one well sclerotized; cymbium 1.7 times longer than wide, with tip equal in length to tibial apophyses; tegulum oval, ca 1.8 times longer than wide; sperm duct thick, only 2.5 times thinner than tibia's diameter, proximal-prolateral part as thick as retrolateral part, sharply tapering in distal half of tegulum; tegular apophysis (*Ta*) small, claw-like, located antero-retrolaterally; embolus large, about 0.63 of tegulum's length, base as wide as  $\frac{1}{2}$  of tegulum, gradually tapering.

Female. Unknown.

**Comments.** This species is tentatively placed in *Rhysodromus*, as it has a different pattern and a very thick sperm duct which is unknown in other members of the genus. The generic placement is due to the presence of tegular apophysis and two closely placed tibial apophyses, similar to *R. mysticus* (Dondale & Redner, 1975).

**Distribution.** Known only from the type locality in Hormozgan Province, southern Iran.

# Acknowledgements

We are grateful toward Peter J. Schwendinger (MHNG) and Christoph Hörweg (NHMW) for providing us with the spider material deposited in their respective collections, and Sergei L. Zonstein and Anna A. Nekhaeva for providing constructive suggestions on an earlier draft of the manuscript.

# References

- Bosmans R, El-Hennawy HK (2018) *Mesiotelus alexandrinus* (Simon, 1880) is a junior synonym of *Mesiotelus tenuissimus* (L. Koch, 1866) (Araneae: Liocranidae). Serket 16(2): 100–104.
- Chyzer C, Kulczyński W (1897) Araneae Hungariae. Tomus II. Academia Scientarum Hungaricae, Budapest, 147–366.
- Dimitrov D, Naumova M (2021) A faunistic survey of the spiders (Arachnida: Araneae) of Sakar Mountain, Bulgaria. Arachnology 18(7): 689–699. https://doi.org/10.13156/ arac.2020.18.7.689
- Dondale CD, Redner JH (1975) The fuscomarginatus and histrio groups of the spider genus Philodromus in North America (Araneida: Thomisidae). The Canadian Entomologist 107(4): 369–384. https://doi.org/10.4039/Ent107369-4
- Kulczyński W (1909) Fragmenta Arachnologica. XIV, XV. Bulletin International de l'Academie des Sciences de Cracovie 1909: 667–687.
- Lehtinen PT (1967) Classification of the cribellate spiders and some allied families, with notes on the evolution of the suborder Araneomorpha. Annales Zoologici Fennici 4: 199–468.

- Marusik YM, Esyunin SL, Tuneva TK (2015) A survey of Palaearctic Dictynidae (Araneae). 1. Taxonomic notes on *Dictynomorpha* Spassky, 1939, *Brigittea* Lehtinen, 1967 and *Lathys* Simon, 1884. Zootaxa 3925(1): 129–144. https://doi.org/10.11646/zootaxa.3925.1.9
- Marusik YM, Guseinov E (2003) Spiders (Arachnida: Aranei) of Azerbaijan. 1. New family and genus records. Arthropoda Selecta 12(1): 29–46.
- Miller F, Svatoň J (1978) Einige seltene und bisher unbekannte Spinnenarten aus der Slowakei. Annotationes Zoologicae et Botanicae Bratislava 126: 1–19.
- Montemor VM, West RC, Zamani A, Moradmand M, Wirth V von, Wendt I, Huber S, Guadanucci JPL (2020) Taxonomy of the genus *Ischnocolus* in the Middle East, with description of a new species from Oman and Iran (Araneae: Theraphosidae). Zoology in the Middle East 66(1): 76–90. https://doi.org/10.1080/09397140.2020.1675994
- Platnick NI (1981) A review of the spider subfamily Palpimaninae (Araneae, Palpimanidae), I. Bulletin of the British Arachnological Society 5(4): 169–173.
- Szita É, Logunov D (2008) A review of the *histrio* group of the spider genus *Philodromus* Walckenaer, 1826 (Araneae, Philodromidae) of the eastern Palaearctic region. Acta Zoologica Academiae Scientiarum Hungaricae 54: 23–73.
- WSC (2021) World Spider Catalog. Version 21.5. Natural History Museum Bern. http://wsc. nmbe.ch [accessed on 20.06.2021]
- Zamani A, Bosselaers J (2020) The spider family Oecobiidae (Arachnida: Araneae) in Iran, Afghanistan and Turkmenistan. European Journal of Taxonomy 726: 38–58. https://doi. org/10.5852/ejt.2020.726.1173
- Zamani A, Chatzaki M, Esyunin SL, Marusik YM (2021a) One new genus and nineteen new species of ground spiders (Araneae: Gnaphosidae) from Iran, with other taxonomic considerations. European Journal of Taxonomy 751: 68–114. https://doi.org/10.5852/ ejt.2021.751.1381
- Zamani A, Dimitrov D, Weiss I, Alimohammadi S, Rafiei-Jahed R, Esyunin SL, Moradmand M, Chatzaki M, Marusik YM (2020) New data on the spider fauna of Iran (Arachnida: Araneae), Part VII. Arachnology 18(6): 569–591. https://doi.org/10.13156/arac.2020.18.6.569
- Zamani A, Marusik YM (2021a) New taxa of six families of spiders (Arachnida: Araneae) from Iran. Zoology in the Middle East 67(1): 81–91. https://doi.org/10.1080/09397140.2021 .1877382
- Zamani A, Marusik YM (2021b) Revision of the spider family Zodariidae (Arachnida, Araneae) in Iran and Turkmenistan, with seventeen new species. ZooKeys 1035: 145–193. https://doi.org/10.3897/zookeys.1035.65767
- Zamani A, Mirshamsi O, Marusik YM, Moradmand M (2021b) The Checklist of the Spiders of Iran. Version 2021. http://www.spiders.ir [accessed 12.07.2021]

RESEARCH ARTICLE



# Selva Zoque, Mexico: an important Mesoamerican tropical region for reptile species diversity and conservation

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Academic editor: Anthony Herrel   Received 27 April 2021   Accepted 8 July 2021   Published 3 August 20	21							
http://zoobank.org/F1D9569A-E45E-4B64-857F-9832164D0DB0								

**Citation:** Aguilar-López JL, Luría-Manzano R, Pineda E, Canseco-Márquez L (2021) Selva Zoque, Mexico: an important Mesoamerican tropical region for reptile species diversity and conservation. ZooKeys 1054: 127–153. https://doi.org/10.3897/zookeys.1054.67916

### Abstract

The Selva Zoque region is characterized by a great variety of ecosystems for which there is little information about reptile species diversity and their conservation status. This study is the first assessment of the species richness, composition, and conservation status of reptiles of this region. Additionally, this information is compared with that of seven other tropical regions in northern Mesoamerica. In total, 141 native reptile species belonging to 81 genera and 29 families are recorded for the Selva Zoque region. Sixty species (42% of the total) recorded in Selva Zoque are in high-risk categories according to the Mexican Ministry of the Environment, the highest number for the Mexican regions of Mesoamerica. According to the IUCN, six species are in high-risk categories, seven species are in Data Deficient, and 23 (16%) have not been evaluated yet. According to the Environmental Vulnerability Scores approach, 28 species (20%) are in the high vulnerability category. The Selva Zoque species composition is most similar to Los Tuxtlas and Lacandona regions, and most dissimilar to Sian Ka'an Biosphere Reserve. The reptilian fauna of Selva Zoque has a distinctive composition, with the highest number (11 species) of endemic reptiles in the northern Mesoamerican, and species from two biogeographic provinces: the Gulf of Mexico and

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the Mexican Pacific Coast. These results indicate that the Selva Zoque is the most diverse region in native reptile species in northern Mesoamerica, highlighting it as extremely important for the conservation of the reptile fauna at local (southern Mexico) and regional levels (northern Mesoamerica).

#### **Keywords**

Compositional similarity, conservation value, Data Deficient, reptile fauna, species composition, species richness

# Introduction

The 25 biodiversity hotspots identified by Myers et al. (2000) share two characteristics: each one harbors endemic plant species representing at least 0.5% of the global total, and have lost  $\geq$  70% of their primary vegetation. Of these regions, Mesoamerica ranks fifteenth in relation to the latter characteristic (80% of primary vegetation lost; Myers et al. 2000), and ranks third in deforestation rate among the 13 hotspots for which information is available (Brooks et al. 2002). Despite this scenario, some areas still remain covered by primary vegetation (FAO 2011), highlighting the ongoing conservation value of this region.

One such region is the Selva Zoque, composed by the Uxpanapa-Chimalapas zone (UC) and El Ocote Biosphere Reserve, is located on the Isthmus of Tehuantepec in the states of Veracruz, Oaxaca, and Chiapas in southern Mexico. The Selva Zoque region is the second largest extension of well-conserved tropical forest in northern Mesoamerica, is a Pleistocene refuge with high number of endemic species (Pérez-Farrera et al. 2016), contains a large variety of vegetation types (Peterson et al. 2003) and has a broad range of elevation spanning 100 to 2300 m a.s.l. Additionally, the Selva Zoque region, together with the protected area La Sepultura Biosphere Reserve, in the state of Chiapas, make up the La Selva Zoque-La Sepultura Priority Conservation Area (Arriaga et al. 2000b). Currently, detailed knowledge of the vertebrate species diversity in the entire Selva Zoque, or a large portion of it, is available only for birds (Peterson et al. 2003), mammals (Lira-Torres et al. 2012), and amphibians (Aguilar-López et al. 2016a). For other groups such as reptiles, our knowledge of species richness, species composition, and conservation status is limited to El Ocote Biosphere Reserve, where the reptile diversity has been revised on several studies (Reynoso et al. 2011; Luna-Reyes et al. 2017; Muñoz-Alonso et al. 2017) and scarce in UC zone. Herpetological expeditions have been carried out in the UC zone since at least the middle of the last century (Taylor 1951; Duellman 1960; Lynch and Wake 1989) but have focused mainly on amphibians. Moreover, herpetofaunal collection has been limited to few localities, and extensive portions of the region remain with no information.

Worldwide, habitat modification represents the most common threat to terrestrial reptile species, with one in five species included in high-risk categories of extinction (Vulnerable, Endangered, or Critically Endangered) by the IUCN. A further one in five species is listed in the Data Deficient category, and four of ten have not been evaluated according to the criteria of the Red List (Uetz et al. 2018; IUCN 2021). In Mexico, it is estimated that 13% of reptile species are threatened and for another 16% there is insufficient information (in Data Deficient category) to evaluate its extinction risk level (IUCN 2021). On the other hand, about half of the species are included in the high-risk of extinction categories on the species list compiled by the Mexican Ministry of the Environment (NOM-059-SEMARNAT-2010). Given the variety of tropical forests that cover the Selva Zoque region, along with its geographic location and environmental heterogeneity (Wendt 1987; de Teresa 2000; SEMARNAT 2001), a high reptile diversity may inhabit in this region, with a significant portion of species under high risk of extinction.

Based on a comprehensive review of databases, scientific literature, and fieldwork, we provide the first assessment of species richness, species composition and distribution, and conservation status of the reptile fauna inhabiting the Selva Zoque region. Additionally, we compared this information with other tropical regions on the northern end of Mesoamerica.

# Materials and methods

### Study site

The Selva Zoque region is located in southern Mexico east of the Isthmus of Tehuantepec (Fig. 1). Mountains and hills dominate the region (Wendt 1987; SEMAR-NAT 2001; Ortiz-Pérez et al. 2004). The region is covered by several vegetation types, the main ones are evergreen tropical forest (100–1000 m a.s.l.), semi-evergreen tropical forest (600–1200 m a.s.l.), deciduous tropical forest (100–600 m a.s.l.), tropical montane cloud forest (1100–1800 m a.s.l.) and pine-oak forest (1800–2300 m a.s.l.) (Wendt 1987; SEMARNAT 2001). Mean annual temperature ranges from 12 to 23 °C and mean annual rainfall ranges from 800 to 4400 mm (Vidal-Zepeda 1990; SEMARNAT 2001). We delimited the study area using the polygon set by Arriaga et al. (2000a) for the Selva Zoque-La Sepultura Priority Conservation Area but excluded La Sepultura Biosphere Reserve (Fig. 1).

### Data collection

We consulted two databases between January 2017 and May 2021: the National Information System on Biodiversity (**SNIB**) curated by the National Commission for the Knowledge and Use of Biodiversity (**CONABIO**) and the Global Biodiversity Information Facility (GBIF; www.gbif.org). We also reviewed the scientific literature on reptiles from the study region (Navarro-Singüenza and Meave-Castillo 1998; Espinoza et al. 1999; Reynoso et al. 2011; Carmona-Torres 2013; Aguilar-López et al. 2014; Canseco-Márquez and Ramírez-González 2015; Scarpetta et al. 2015; Aguilar-López et al. 2016; Gray et al. 2016; Luna-Reyes et al. 2017; Muñoz-Alonso et al. 2017;



**Figure 1.** Location of the Selva Zoque region. The yellow line denotes our study area limits, the thin black line indicates state boundaries, and black circles denote localities in which reptiles have been recorded.

Campbell et al. 2018; del Moral-Flores et al. 2019). Additionally, we conducted fieldwork during 2013 and 2014 in surroundings of Arroyo Zarco, Uxpanapa, in Veracruz (17°11'N, 94°28'W), and San Francisco La Paz (17°5'N, 94°8'W), La Fortaleza (17°9'N, 94°13'W), and La Esmeralda (17°9'N, 94°46'W), in Santa María Chimalapa, Oaxaca. We surveyed areas with evergreen tropical forest and semi-evergreen tropical forest using standard visual encounter survey techniques (Crump and Scott 1994) during the day and at night. Cumulative sampling effort was 3250 person-hours.

We cross-checked records obtained from the three sources of information to avoid duplication, since the databases consulted might have records of the same specimens. Only records with precise geographic coordinates or detailed information about the collection and observation site were included, and from these records we compiled a general database.

To compare the data we compiled for the Selva Zoque region with the data from seven other tropical regions, we obtained information about reptile species richness and species composition from: 1) Los Tuxtlas in Veracruz (López-Luna 2017), 2) Las Choapas municipality in Veracruz (Aguilar-López and Canseco-Márquez 2006), 3) La Sepultura Biosphere Reserve in Chiapas (Nuñez-Orantes and Muñoz-Alonso 2000; Reynoso et al. 2011; Clause et al. 2020a, b), 4) the Lacandona rainforest in Chiapas (Hernández-Ordóñez et al. 2015), 5) the Calakmul Biosphere Reserve in Campeche (Calderón-Mandujano et al. 2010; Colston et al. 2015), 6) the Sian Ka'an Biosphere Reserve in Quintana Roo (Calderón-Mandujano et al. 2008), and 7) the Mayan Forest in Guatemala (Lee 1996; Campbell 1998).

# Data processing and analysis

To identify the spatial distribution of reptile records in the study region, we projected all geo-referenced records onto our study area polygon using ArcGIS software, version 10.0 (ESRI 2010). To determine the distribution and taxonomically standardize the data set of species that inhabit the Selva Zoque and the other tropical regions, we consulted the specialized literature documenting taxonomic changes and descriptions of new species (Wüster et al. 2005; Castoe et al. 2009; Köhler 2010; Linkem et al. 2011; Cadle and Savage 2012; Hedges and Conn 2012; Iverson et al. 2013; Porras et al. 2013; Köhler et al. 2014; Ruane et al. 2014; Blair et al. 2015; Meza-Lázaro and Nieto-Montes de Oca 2015; Card et al. 2016; Gray et al. 2016; Köhler et al. 2016; McCranie and Hedges 2016; Wallach 2016; Nieto-Montes de Oca et al. 2017; Campbell et al. 2018; Carbajal-Márquez et al. 2020; Jadin et al. 2020; McCranie et al. 2020; Reyes-Velasco et al. 2020; Ramírez-Reyes et al. 2021). Using the compiled data, we defined four distribution categories: species distributed outside Mesoamerica as widely distributed species (WD), species restricted to Mesoamerica (MA), species restricted to northern Mesoamerica (MAMx), and species restricted to one of the eight regions considered. We delimited Mesoamerica as suggested by Campbell (1999) and considered northern Mesoamerica as the zone corresponding to Mexico. To determine the extinction risk category for each species, we consulted the list of Species at Risk published by SEMARNAT, updated in 2018 (NOM-059-SEMAR-NAT-2010), the Red List maintained by the International Union for the Conservation of Nature (IUCN 2021), and the Environmental Vulnerability Score (EVS) proposed by Wilson et al. (2013). SEMARNAT's categories are: Subject to Special Protection (Pr), Threatened (A), and Endangered (P). The IUCN's three high-risk categories are: Vulnerable (VU), Endangered (EN) and Critically Endangered (CR); its low-risk categories are: Least Concern (LC) and Near Threatened (NT). We also included species in the Data Deficient (DD) category, and those Not evaluated (NE) by the IUCN. In addition, we consulted the EVS of Mexican reptile species that have been evaluated and assigned to one of three categories of vulnerability to environmental degradation: low (3-9), medium (10-13) and high (14-19). For the Mayan Forest in Guatemala, we were only able to assign the IUCN categories since the area lies outside of SEMARNAT's jurisdiction and there is not an evaluation of EVS for reptiles of Guatemala.

We compared reptilian faunal composition between regions using Jaccard's similarity index (Magurran 2004), which uses presence-absence data and is expressed as:

$$C_{j} = \frac{a}{a+b+c}$$

where a = the number of species shared between the two sites under comparison, b = number of species exclusive to the first site, and c = number of species exclusive to the second site. The index ranges from zero to one, with zero indicating that no species are shared between the sites being compared, and one indicating that all species are found in both sites. We plotted a dendrogram using PAST software version 2.17c (Hammer et al. 2001) to represent the relationship between sites in terms of their similarity in species composition according to the Jaccard index. For this analysis, we only included native species.

## Results

### Species richness, distribution, and conservation status

A total of 141 native reptile species belonging to 81 genera and 29 families has been recorded at the Selva Zoque region. These comprise 62 species of lizards, 70 snakes, seven turtles, and two crocodilians (Appendix 1). The best represented families are Dipsadidae and Colubridae, with 29 and 25 species, respectively, followed by Dactyloidae with 18 species, Phrynosomatidae with nine species, and Viperidae with seven species. The families with the fewest species in the region are Eublepharidae, Helodermatidae, Mabuyidae, Phyllodactylidae, Scincidae, Boidae, Natricidae, Sybinophiidae, Leptotyphlopidae, Loxocemidae, Dermatemydidae, Emydidae, and Geoemydidae, with one species each one. Three non-native species have been recorded in the region, the lizards *Anolis sagrei* Duméril & Bibron *Gehyra mutilata* (Wiegmann) and *Hemidac-tylus frenatus* Duméril and Bibron. During our fieldwork (2013–2014), we recorded 48 species (Appendix 1), although all of them had been previously recorded.

Reptiles have been recorded mostly on the periphery of the study region, notably on western, southern and eastern end portions. In the northwest, northeast, and west, reptile collections are located below 1000 m a.s.l., while in the southeast, most of collections are between 1000 and 2000 m a.s.l. The central portion of Chimalapas, the mountainous zone known as Espinazo del Diablo in Uxpanapa and a zone between El Ocote Biosphere Reserve and UC zones corresponding to the northwest extreme of Cintalapa in Chiapas, remain with no collection of reptiles (Fig. 1). The distribution of eleven of the 141 native species (8%) is restricted to the Selva Zoque region, 26 species (18%) are distributed in the northern part of Mesoamerica that corresponds to Mexico, 66 species (47%) are distributed on Mesoamerica, and 38 species (27%) have a wide distribution, extending beyond Mesoamerica (Appendix 1).

Of the reptile species recorded in the Selva Zoque region, 60 species (42%) are in high-risk categories according to SEMARNAT: 39 species in the Subject to Special Protection category (Pr), 18 species are in the Threatened category (A) and three are Endangered (P). According to the IUCN Red List, six species (4%) are included in high-risk categories: three are Vulnerable (VU), two are Endangered (EN), and one is Critically Endangered (CR). Additionally, seven species are in the Data Deficient category (DD) and 23 species have not been evaluated (NE). The remaining 105 species are in low-risk categories. According to the EVS system, 28 species (20%) are in the high vulnerability category (Appendix 1).

# Comparison of the richness, composition, and conservation status of the reptile species from the Selva Zoque region with that of other tropical regions

With 141 native species recorded, the Selva Zoque region harbors the highest reptile species richness among the tropical regions considered in this study, surpassing Los Tuxtlas (113 species). The Mayan Forest ranks third (107 species), followed by La Lacandona (89 species), La Sepultura (79 species), Calakmul (73 species), Sian Ka'an (63 species), and finally Las Choapas (56 species; Table 1). The Los Tuxtlas Biosphere Reserve, with four species, has the highest diversity of non-native species, followed by Selva Zoque and Calakmul with three. La Sepultura, Sian Ka'an, and Las Choapas have two non-native species, and the Mayan Forest and Lacandona each have only one.

The dendrogram indicates that La Sepultura has the most dissimilar species composition of the nine regions (Fig. 2). This Biosphere Reserve is followed by Selva Zoque, Las Choapas and Los Tuxtlas in terms of their dissimilarity in relation to the remaining regions. The four remaining regions in turn form a group with a value of Jaccard index  $[C_j] = 0.5$  (Fig. 2). The composition analysis by pairs indicates that Los Tuxtlas had the species composition most similar to that of Selva Zoque, with 44% shared species ( $C_j = 0.44$ ), followed by Lacandona ( $C_j = 0.39$ ), whereas the region with the least similar species composition to Selva Zoque was Sian Ka'an ( $C_j = 0.20$ ). The regions with the most similar species composition were Lacandona and Mayan Forest in Guatemala ( $C_j = 0.72$ ), followed by Sian Ka'an and Calakmul ( $C_j = 0.58$ ). The least similar regions were Sian Ka'an and La Sepultura ( $C_i = 0.14$ ).

The Selva Zoque and Los Tuxtlas regions have the highest number of endemic species to the regions, with eleven each, followed by La Sepultura (three species) and Mayan Forest with one endemic species while the rest of regions do not have endemic species (Appendix 1). Also, the Selva Zoque region has the highest number of species in high-risk categories of extinction according to the NOM-059 criteria, with 60 spe-

**Table 1.** Taxonomic composition of native reptile species recorded in the Selva Zoque region and seven other regions in northern Mesoamerica. Non-native species are not included in the taxonomic composition data.

Tropical region	Orders	Families	Genera	Lizards	Snakes	Turtles	Crocodilians	Native species	Non-native species
Selva Zoque	3	29	81	62	70	7	2	141	3
Los Tuxtlas	3	29	75	35	63	14	1	113	4
Mayan Forest	3	26	70	35	61	9	2	107	1
Lacandona	3	25	64	28	52	7	2	89	1
La Sepultura	2	25	59	29	47	3	0	79	2
Calakmul	3	21	51	24	39	9	1	73	3
Sian Ka'an	3	23	51	25	26	10	2	63	2
Las Choapas	3	20	41	20	28	7	1	56	2



Figure 2. Similarity in reptile species composition for eight tropical areas in northern Mesoamerica.

cies, followed by Los Tuxtlas with 46 species. The other regions have from 33 species (La Sepultura) to 25 species (Las Choapas) in high-risk categories (Fig. 3A). Based on the IUCN Red List, the number of reptiles in high-risk categories of extinction is highest in Los Tuxtlas, with ten species, and the other regions have from one to six species in these categories. The Selva Zoque region has the highest number of species classified as Not evaluated (NE) and with Data Deficient (DD) on the IUCN Red List with 30 species, followed by Los Tuxtlas (24 species); the remaining regions have from eight to 14 NE and DD species (Appendix 1; Fig. 3B). The Selva Zoque has the highest number of species (28) included in the high vulnerability category to environmental degradation, followed by Los Tuxtlas with 18 species; the remaining regions have between five and ten species (Fig. 3C).

# Discussion

Our results show that the Selva Zoque region harbors the greatest reptile species richness in northern Mesoamerica, with a distinctive species composition and several species inhabit exclusively on Selva Zoque region. Furthermore, one in every two reptile species that inhabit in Selva Zoque is threatened, is highly vulnerable or there is not sufficient information to know its extinction risk level. All this underscore the importance of the Selva Zoque region for reptile conservation in Mexico and Mesoamerica. This region also offers opportunities to study unexplored well-preserved tropical forest areas, as well as species for which little is known about their biology, ecology, and conservation status.

The Selva Zoque region is more diverse in native reptile species than even Los Tuxtlas, which has 28 fewer native species (López-Luna 2017). In the Selva Zoque region three exotic species (*Anolis sagrei*, *Gehyra mutilata*, *Hemidactylus frenatus*) have



**Figure 3.** Number of reptile species in high extinction-risk categories according to **A** NOM-059-SE-MARNAT-2010 **B** IUCN Red List, and **C** the Environmental Vulnerability Score, for eight tropical regions in northern Mesoamerica. The black bars in the three plots represent the number of species in high extinction-risk categories, and the grey bars in the graph B represent the number of species not evaluated or in the Data Deficient category by the IUCN.

A

been recorded, fewer than other regions such as Los Tuxtlas, which is located near the coast. The occurrence of the highest diversity of native species in some portions of Selva Zoque over that of other tropical regions has been previously observed in amphibians (Aguilar-López et al. 2016a). The 141 native reptile species recorded in the Selva Zoque region represent 16% of the 864 reptile species recorded in Mexico (Flores-Villela and García-Vázquez 2014), 52% of the 270 reptile species recorded in south-eastern Mexico (Johnson et al. 2010), and 11% of the 1284 species recorded in Mesoamerica (Johnson et al. 2018).

That said, the inventory of reptile species in the Selva Zoque region is far from complete, particularly in UC zones. In the last three years alone, three new species have been described (*Anolis purpuronectes* [Gray et al. 2016], *Chersodromus australis* [Canseco-Márquez et al. 2018] and *Cenaspis aenigma* [Campbell et al. 2018]), and more species await formal description. Furthermore, several zones within our study area lack reptile records entirely and remain unexplored, and hence additional surveys could reveal reptile species unrecorded for the region and perhaps altogether new species to science. As such, the species richness we report here is likely an underestimate, highlighting the need for additional sampling effort to complete the species inventory of the Selva Zoque region.

The high species richness in the Selva Zoque region may be the result of a series of factors. One is the notably complex orography with lowland zones, both on the Gulf of Mexico and on the Pacific versant, and also a series of mountain ranges of intermediate elevation–the Sierra Atravesada, the Espinazo del Diablo, the Sierra Tres Picos, and Cerro La Colmena (Wendt 1987; Ortíz-Pérez et al. 2004; SEMARNAT 2001)–with elevations from 100 to 2300 m a.s.l. There is a range of 11 °C in mean annual temperature across this elevation gradient, and a difference of 3600 mm in mean annual rainfall across the Selva Zoque (Beard 1955; SEMARNAT 2001). In addition, at least seven vegetation types exist in the region (Arriaga et al. 2000b; SEMARNAT 2001). Together, these factors create a wide variety of habitats for a large diversity of reptile species with different eco-physiological requirements and evolutionary histories.

Although the Selva Zoque did not result clustered with any of the regions in the similarity analysis, Los Tuxtlas and Lacandona Biosphere Reserves are the most similar tropical regions to the Selva Zoque. This pattern has been observed for amphibians in a comparison between Uxpanapa-Chimalapas zone and the same tropical regions (except from La Sepultura) used in this study, and can be explained by the high number of recorded species in these three sites and the high number of species that they share. The Selva Zoque reptile fauna has a combined component of species from two different biogeographic provinces (Morrone 2005): species found in the province of Gulf of Mexico (e.g., *Anolis sericeus* Hallowell, *Holcosus amphigrammus* (Smith and Laufe), and those in the province of Mexican Pacific Coast (e.g., *Loxocemus bicolor* Cope, *Porthidium dunni* (Hartweg and Oliver), *Rhinoclemmys rubida* (Cope)). The high percentage (8%) of endemism of reptile species to the Selva Zoque region may be due to its stable Pleistocene climate conditions, which allowed for the diversification of different biological groups (Lira-Torres et al. 2012; Rodríguez-Gómez et al. 2013). A pattern

of high endemicity of the whole Selva Zoque region has been observed for mammals (Escalante 2003), but also in parts of Selva Zoque region like Uxpanapa-Chimalapas for other vertebrates as amphibians (Aguilar-López et al. 2016) or for Chimalapas zone in the case of birds (Peterson et al. 2003).

Our results suggest that Selva Zoque is a priority conservation area for the reptile fauna of Mexico because a relatively high proportion (43%) of the species that inhabit there are in high-risk of extinction categories in the NOM-059; equivalent to 33% of all the Mexican reptile species included in this initiative (SEMARNAT 2010). Additionally, a moderate number of reptile species are in the high vulnerability category of environmental degradation (20%). In contrast, Selva Zoque does not harbor a high number of species in high-risk categories of extinction according to the IUCN. The differences in the number of species in high risk of extinction among initiatives is presumably because the NOM-059 does not consider the entire distribution of the species, it only takes into account the distribution of the species within the Mexican territory, which may lead to a restricted distribution, but only within Mexico, this does not necessarily reflect the entire distribution of the species. Additionally, populations of some reptile species that occur within Mexico may be scarce or may be declining, while populations of those species but outside of Mexico may be stable. Even so, the importance of Selva Zoque region lies in the relatively high proportion (21%) of species that are classified as Not evaluated (NE) and Data Deficient (DD). Because of the conservation status of vegetation in some areas of the region, it represents an opportunity for gathering information on the biology of these species, and this could contribute to their being assigned a category. This is the case for Abronia bogerti Tihen, Anolis alvarezdeltoroi Nieto Montes de Oca, Xenosaurus arboreus (Lynch and Smith), and Tantilla briggsi Savitsky and Smith (Fig. 4A, B, E, G), endemic species to the Selva Zoque region, or species with distribution in Mesoamerica as Trimorphodon biscutatus (Duméril, Bibron and Duméril), and Epictia phenops (Cope) (Fig. 4H, I). In any case, though widely distributed, some reptile species are considered threatened under three classification systems, such as Anolis pygmaeus Alvarez del Toro & Smith Bothriechis rowleyi (Bogert), Dermatemys mawii Gray and Crocodylus acutus (Cuvier) (Fig. 4C, J, K, L), all of which have been reported for the other regions (Appendix 1; Fig. 3).

The extensive areas of well-preserved forest in Selva Zoque are mostly located in the Chimalapas zone and El Ocote Biosphere Reserve (Flamenco-Sandoval et al. 2007; Lira-Torres et al. 2012), and offer an opportunity not only for the conservation of reptile diversity, but also for all the biotic diversity that inhabits the Selva Zoque region. Conservation is under the aegis of community initiatives in Chimalapas and has been successful in recent decades (Monterrubio-Solís and Newing 2013); however, it is necessary to implement and subsequently strengthen conservation efforts in areas without any protection. For Uxpanapa and the intermediate zones between Uxpanapa and El Ocote Biosphere Reserve, the constant and accelerated transformation of the original vegetation cover into crops and pastureland (Flamenco-Sandoval et al. 2007; Hernández et al. 2013) underscores the urgent need to stop the advance of the agricultural frontier (Arriaga et al. 2000b).



Figure 4. Reptile species in high extinction-risk categories by IUCN, NOM or EVS initiatives, or listed as Data Deficient or Not evaluated by the IUCN red list (see text) A Abronia bogerti B Anolis alvarezdeltoroi
C Anolis pygmaeus D Lepidophyma tuxtlae E Xenosaurus arboreus F Xenosaurus rackhami G Tantilla briggsi H Trimorphodon biscutatus I Epictia phenops J Bothriechis rowleyi K Dermatemys mawii and L Crocodylus acutus.

The implementation of activities proven to be effective in the conservation of reptiles in transformed landscapes in the study region is desirable. These could include: 1) ecological restoration (Smith et al. 2015) to facilitate the recovery of the reptile communities over time, 2) the protection of vegetation remnants (Pulsford et al. 2017) that can harbor important components of overall species diversity, and 3) protection of buffer zones around water bodies (Semlitsch and Bodie 2003) that maintain populations of several species, and also constitute biological corridors (Burbrink et al. 1999). Finally, the protection of Uxpanapa portion of Selva Zoque can contribute to the connection of natural protected areas of different governance types in the western (community protected areas of the Chimalapas, in Oaxaca) and eastern (federal protected area El Ocote, in Chiapas) of Selva Zoque (García-Bañuelos et al. 2019). This idea fits completely with the objective of the Mesoamerican Biological Corridor, an international initiative that aims to maintain biological diversity, decrease habitat fragmentation, improve the connectivity of the landscapes and of the ecosystems in Mesoamerica and to impulse social and economic development (Miller et al. 2001).

# Acknowledgements

The authors thank A. Vinalay, C. Nochebuena, D. Aportela, L. Vázquez, L. Alcaide, J. López, M. Oropeza, R. Flores, J. Pelayo, I. Caviedes, F. Vázquez, C. Hernández, and U. García for helping with the fieldwork. Policarpo Ronzón, L. Feria, and A. Vázquez provided logistical support for the trips to the field. The authors are grateful to M. López-Luna for providing the photograph of Crocodylus acutus, to I. Ahumada for providing the photograph of *Bothriechis rowleyi*, and to L. Badillo for providing the photograph of *Epictia phenops*. P. Bañuelos prepared the map and B. Delfosse and A. Clause revised the English of the manuscript. The Toala and Chacha families provided accommodation and were our field guides during our work in Uxpanapa. J. Campbell kindly reviewed an early version of the manuscript and offered helpful suggestions, and Roberto Luna-Reves made useful suggestions on an advanced version of the manuscript. JLAL was awarded graduate studies scholarship No. 344651 by CONACYT. Permits for this study were issued by the Mexican Wildlife Agency, Dirección General de Vida Silvestre of the Secretaría de Medio Ambiente y Recursos Naturales (collecting permit numbers: SGPA/DGVS/03665/06 and SGPA/DGVS/03444/15). This study was funded by project JF-212-CONABIO.

### References

- Aguilar-López JL, Canseco-Márquez L (2006) Herpetofauna del municipio de Las Choapas, Veracruz, México. Boletín de la Sociedad Herpetológica Mexicana 14: 20–37.
- Aguilar-López JL, Canseco-Márquez L, Pineda E, Luría-Manzano R (2014) Aporte al conocimiento de la distribución de la culebra de cola corta de Linton, *Tantillita lintoni* en México. Revista Mexicana de Biodiversidad 96: 1292–1294. https://doi.org/10.7550/rmb.46781
- Aguilar-López JL, Luría-Manzano R, Pineda E, Aportela D (2016b) *Celestus rozellae*. Distribution note. Mesoamerican Herpetology 3: 764–765.
- Aguilar-López JL, Pineda E, Luría-Manzano R, Canseco-Marquez L (2016a) Species diversity, distribution and conservation status in a Mesoamerican region: Amphibians of the Uxpanapa-Chimalapas region, Mexico. Tropical Conservation Science 9: 1–16. https:// doi.org/10.1177/1940082916670003
- Arriaga L, Espinoza JM, Aguilar C, Martínez E, Gómez L, Loa E [Coordinators] (2000a) Mapa de regiones terrestres prioritarias de México. Escala de trabajo 1:1 000 000. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México.
- Arriaga L, Espinoza JM, Aguilar C, Martínez E, Gómez L, Loa E [Coordinators] (2000b) Regiones terrestres prioritarias para la conservación. México, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

- Beard JS (1955) The classification of tropical American vegetation types. Ecology 36: 89–100. https://doi.org/10.2307/1931434
- Blair C, Méndez de la Cruz FR, Law C, Murphy RW (2015) Molecular phylogenetics and species delimitation of leaf-toed geckos (Phyllodactylidae: *Phyllodactylus*) throughout the Mexican tropical dry forest. Molecular Phylogenetics and Evolution 84: 254–265. https:// doi.org/10.1016/j.ympev.2015.01.003
- Brooks TM, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Rylands AB, Konstant WR, Flick P, Pilgrim J, Olfield S, Magin G, Hilton-Taylor C (2002) Habitat loss and extinction in the hotspot of biodiversity. Conservation Biology 16: 909–923. https://doi. org/10.1046/j.1523-1739.2002.00530.x
- Burbrink FT, Phillips CA, Heske EJ (1999) A riparian zone in southern Illinois as a potential dispersal corridor for reptiles and amphibians. Biological Conservation 86: 107–115. https://doi.org/10.1016/S0006-3207(98)00054-8
- Cadle JE, Savage JM (2012) Systematics of the *Dendrophidion nuchale* complex (Serpentes: Colubridae) with the description of a new species from Central America. Zootaxa 3513: 1–50. https://doi.org/10.11646/zootaxa.3513.1.1
- Calderón-Mandujano RR, Bahena-Basave H, Calmé S (2008) Anfibios y reptiles de la Reserva de la Biósfera de Sian Ka'an y zonas aledañas (2<sup>nd</sup> edn.). COMPACT, ECOSUR, CONA-BIO, S.H.M, Quintana Roo, 111 pp.
- Calderón-Mandujano RR, Pozo de la Tijera C, Cedeño-Vázquez JR (2010) Guía Rústica de los Reptiles de la Región de Calakmul, Campeche, México. ECOSUR, CONABIO, Quintana Roo, 57 pp.
- Campbell JA (1984) A new species of *Abronia* (Sauria: Anguidae) with comments on the herpetogeography of the highlands of southern Mexico. Herpetologica 40: 373–381. https:// www.jstor.org/stable/3892089
- Campbell JA (1998) Amphibians and reptiles of Northern Guatemala, the Yucatan, and Belize. Vol. 4, Animal Natural History Series. University of Oklahoma Press, Norman, 400 pp.
- Campbell JA (1999) Distribution patterns of amphibians in Middle America. In: Duellman WE (Ed.) Patterns of distribution of amphibians, a global perspective. The Johns Hopkins University Press, Baltimore, 111–210.
- Campbell JA, Smith EN, Hall AS (2018) Caudals and calyces: the curious case of a consumed Chiapan colubroid. Journal of Herpetology 52: 459–472. https://doi.org/10.1670/18-042
- Canseco-Márquez L, Ramírez-González CG (2015) New herpetofaunal records for the state of Oaxaca, Mexico. Mesoamerican Herpetology 2: 364–367.
- Canseco-Márquez L, Ramírez-González CG, Campbell JA (2018) Taxonomic review of the rare Mexican snake genus *Chersodromus* (Serpentes: Dipsadidae), with the description of two new species. Zootaxa 4399: 151–169. https://doi.org/10.11646/zootaxa.4399.2.1
- Carbajal-Márquez RA, Cedeño-Vázquez JR, Martinez-Arce A, Neri-Castro E, Machkour-M'Rabet SC (2020) Accessing cryptic diversity in Neotropical rattlesnakes (Serpentes: Viperidae: *Crotalus*) with the description of two new species. Zootaxa 4729: 451–481. https://doi.org/10.11646/zootaxa.4729.4.1
- Card DC, Schield DR, Adams RH, Corbin AB, Perry BW, Andrew AL, Pasquesi GIM, Smith EN, Jezkova T, Boback SM, Booth W, Castoe TA (2016) Phylogeographic and population genetic analyses reveal multiple species of *Boa* and independent origins of insular dwarf-

ism. Molecular Phylogenetics and Evolution 102: 104–116. https://doi.org/10.1016/j. ympev.2016.05.034

- Carmona-Torres HF (2013) Diversidad herpetofaunística en remanentes de selva alta perennifolia y su relación con áreas conservadas. Ms thesis, National Autonomous University of Mexico, Mexico.
- Castoe TA, Daza JM, Smith EN, Sasa MM, Kuch U, Campbell JA, Chippindale PT, Parkinson CL (2009) Comparative phylogeography of pitvipers suggests a consensus of ancient Middle American Highland biogeography. Journal of Biogeography 36: 88–103. https://doi. org/10.1111/j.1365-2699.2008.01991.x
- Clause AG, Luna-Reyes R, Nieto-Montes de Oca A (2020a) A new species of *Abronia* (Squamata: Anguidae) from a Protected Area in Chiapas, Mexico. Herpetologica 76: 330–343. https://doi.org/10.1655/Herpetologica-D-19-00047
- Clause AG, Luna-Reyes R, Jimenez-Lang N, Nieto-Montes de Oca A, Martínez-Hernández LA (2020b) Problems with imperfect locality data: distribution and conservation status of an enigmatic pitviper. Amphibian & Reptile Conservation 14: 185–197.
- Colston TJ, Barao-Nóbrega JA, Manders R, Lett A, Willmott J, Cameron G, Hunter S, Radage A, Littlefair E, Williams RJ, López-Cen A, Slater K (2015) Amphibians and reptiles of the Calakmul Biosphere Reserve, México, with new records. Check List 11: 1–7. https://doi. org/10.15560/11.5.1759
- Crump ML, Scott Jr NJ (1994) Visual encounter surveys. In: Heyer RW, Donnelly MA, McDiarmid RW, Hayek LC, Foster MS (Eds) Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Smithsonian Institution Press, Washington and London, 84–92.
- de Teresa AP (2000) Los Vaivenes de la Selva: el Proceso de Reconstitución del Territorio Zoque de los Chimalapas. México. Universidad Autónoma Metropolitana, Consejo Nacional de Ciencia y Tecnología y Secretaría de Medio Ambiente, Recurso Naturales y Pesca, México, 180 pp.
- del Moral-Flores LF, Vázquez-Nuñez LG, Hernández-Arellano T (2019) Primer registro del cocodrilo de pantano *Crocodylus moreletii* Duméril & Bibron 1851, en la selva de Los Chimalapas, Oaxaca, México. Revista Latinoamericana de Herpetología 2: 51–54.
- Duellman WE (1960) A distributional study of the amphibians of the Isthmus of Tehuantepec, Mexico. University of Kansas publications, Museum of Natural History 13: 19–72.
- Escalante-Espinosa T (2003) Determinación de prioridades en las áreas de conservación para los mamíferos terrestres de México, empleando criterios biogeográficos. Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología 74: 211–237.
- Espinoza ME, Núñez H, González P, Luna R, Navarrete GD, Cruz E, Guichard C (1999) Lista preliminar de los vertebrados terrestres de la selva "El Ocote", Chiapas. Publicación Especial del Instituto de Historia Natural 2: 1–40.
- ESRI (2010) ArcGIS (Version 10.0). Environmental Systems Research Institute, Redlands.
- FAO (2011) State of the World's Forests. Food and Agriculture Organization of the United Nations, Rome, 164 pp.
- Flamenco-Sandoval A, Martínez-Ramos M, Masera OR (2007) Assessing implications of landuse and land-cover change dynamics for conservation of a highly diverse tropical rain forest. Biological Conservation 138: 131–145. https://doi.org/10.1016/j.biocon.2007.04.022
- Flores-Villela O, García-Vázquez UO (2014) Biodiversidad de Reptiles en México. Revista Mexicana de Biodiversidad 85: S467–S475. https://doi.org/10.7550/rmb.43236

- García-Bañuelos P, Rovito SM, Pineda E (2019) Representation of threatened biodiversity in protected areas and identification of complementary areas for their conservation: pleth-odontid salamanders in Mexico. Tropical Conservation Science 12: 1–15. https://doi.org/10.1177/1940082919834156
- Gray L, Meza-Lázaro R, Poe S, Nieto Montes de Oca A (2016) A new species of semiaquatic Anolis (Squamata: Dactyloidae) from Oaxaca and Veracruz, Mexico. Herpetological Journal 26: 253–262.
- Hammer O, Harper DAT, Ryan PD (2001) Past: Paleontological Statistics software package for education and data analysis. Paleontologica Electronica 4: 1–9.
- Hedges SB, Conn CE (2012) A new skink fauna from Caribbean islands (Squamata, Mabuyidae, Mabuyinae). Zootaxa 3288: 1–244. https://doi.org/10.11646/zootaxa.3288.1.1
- Hérnandez IU, Ellis EA, Gallo CA (2013) Aplicación de teledetección y sistemas de información geográfica para el análisis de deforestación y deterioro de las selvas tropicales en la región Uxpanapa, Veracruz. GeoFocus 13: 1–24.
- Hernández-Ordóñez O, Arroyo-Rodríguez V, González-Hernández A, Russildi G, Luna-Reyes R, Martínez-Ramos M, Reynoso VH (2015) Range extensions of amphibians and reptiles in the southeastern part of the Lacandona rainforest, Mexico. Revista Mexicana de Biodiversidad 86: 457–468. https://doi.org/10.1016/j.rmb.2015.04.005
- IUCN [International Union for Conservation of Nature]. (2021) The IUCN Red List of Threatened Species. Version 2021-1. http://www.iucnredlist.org [accessed on June 25, 2021]
- Iverson JB, Le M, Ingram C (2013) Molecular phylogenetics of the mud and musk turtle family Kinosternidae. Molecular Phylogenetics and Evolution 69: 929–939. https://doi. org/10.1016/j.ympev.2013.06.011
- Jadin RC, Blair C, Orlofske SA, Jowers MJ, Rivas GA, Vitt LJ, Ray JM, Smith EN, Murphy JC (2020) Not withering on the evolutionary vine: systematic revision of the Brown Vine Snake (Reptilia: Squamata: *Oxybelis*) from its northern distribution. Organisms Diversity & Evolution 20: 723–746. https://doi.org/10.1007/s13127-020-00461-0
- Johnson JD, Mata-Silva V, Ramírez-Bautista A (2010) The herpetofauna of southeastern Mexico: Biogeography and conservation. In: Wilson LD, Townsend JH, Johnson J (Eds) Conservation of Mesoamerican amphibians and reptiles. Eagle Mountain Publishing, Utah, 322–369.
- Johnson JD, Wilson LD, Bryson Jr RW, Köhler G, Nicholson K, Schuett GW, Townsend JH (2018) Herpetofaunal List for Mesoamerica. Mesoamerican Herpetology. http://www. mesoamericanherpetology.com/taxonomic-list.html [accessed on March 5, 2018]
- Köhler G (2010) A revision of the Central American species related to *Anolis pentaprion* with the resurrection of *A. beckeri* and the description of a new species (Squamata: Polychrotidae). Zootaxa 2354: 1–18. https://doi.org/10.11646/zootaxa.2354.1.1
- Köhler G, Townsend JA, Petersen CB (2016) A taxonomic revision of the Norops tropidonotus complex (Squamata, Dactyloidea), with resurrection of N. spilorhipis (Álvarez del Toro and Smith, 1956) and the description of two new species. Mesoamerican Herpetology 3: 8–41.
- Köhler G, Trejo-Pérez RG, Petersen CB, Méndez de la Cruz F (2014) A revision of the Mexican Anolis (Reptilia, Squamata, Dactyloidae) from the Pacific versant west of the Isthmus de Tehuantepec in the states of Oaxaca, Guerrero, and Puebla, with the description of six new species. Zootaxa 3862: 1–210. https://doi.org/10.11646/zootaxa.3862.1.1

- Lee JC (1996) The Amphibians and Reptiles of the Yucatan Peninsula. Cornell University Press, Ithaca, 512 pp.
- Linkem CW, Diesmos AC, Brown RF (2011) Molecular systematics of the Philippine forest skinks (Squamata: Scincidae: *Sphenomorphus*): testing morphological hypotheses of interspecific relationship. Zoological Journal of the Linnean Society 163: 1217–1243. https:// doi.org/10.1111/j.1096-3642.2011.00747.x
- Lira-Torres I, Galindo-Leal C, Briones-Salas M (2012) Mamíferos de la Selva Zoque, México: riqueza, uso y conservación. Revista de Biología Tropical 60: 781–797. https://doi. org/10.15517/rbt.v60i2.3999
- López-Luna M (2017) Estado actual de la diversidad y poblaciones de los anfibios y reptiles de Los Tuxtlas. In: Reynoso VH, Coates RI, Vázquez-Cruz ML (Eds) Avances y Perspectivas en la Investigación de los Bosques Tropicales y sus Alrededores: la Región de Los Tuxtlas. Instituto de Biología, Universidad Nacional Autónoma de México, Mexico, 347–369.
- Luna-Reyes R, Cundapí-Pérez C, Pérez-López PE, López-Villafuerte A, Rodríguez-Reyes MÁ, Luna-Sánchez JA (2017) Riqueza y diversidad de anfibios y reptiles en Nuevo San Juan Chamula y Veinte Casas, Reserva de la Biosfera Selva El Ocote, Chiapas. In: Ruiz-Montoya L, Álvarez-Gordillo G, Ramírez-Marcial N, Cruz-Salazar B (Eds) Vulnerabilidad Social y Biológica Ante el Cambio Climático en la Reserva de la Biosfera Selva El Ocote. El Colegio de la Frontera Sur, Chiapas, 355–393.
- Lynch FJ, Wake DB (1989) Two new species of *Pseudoeurycea* (Amphibia: Caudata) from Oaxaca, Mexico. Contributions in Science, Natural History Museum of Los Angeles County 411: 11–22.
- Magurran AE (2004) Measuring Biological Diversity. Blackwell Publishing, Oxford, 256 pp.
- McCranie JR, Hedges SB (2016) Molecular phylogeny and taxonomy of the *Epictia goudotii* species complex (Serpentes: Leptotyphlopidae: Epictinae) in Middle America and northern South America. PeerJ 4: e1551. https://doi.org/10.7717/peerj.1551
- McCranie JR, Matthews AJ, Hedges SB (2020) A morphological and molecular revision of lizards of the genus *Marisora* Hedges & Conn (Squamata: Mabuyidae) from Central America and Mexico, with descriptions of four new species. Zootaxa 4763: 301–353. https://doi. org/10.11646/zootaxa.4763.3.1
- Meza-Lázaro RN, Nieto-Montes de Oca A (2015) Long forsaken species diversity in the Middle American lizard *Holcosus undulatus*. Zoological Journal of the Linnean Society 175: 189–210. https://doi.org/10.1111/zoj.12264
- Miller K, Chang E, Johnson N (2001) Defining Common Ground for the Mesoamerican Biological Corridor. World Resources Institute, Washington, District of Columbia, 45 pp.
- Monterrubio-Solís C, Newing HS (2013) Challenges in ICCA Governance: The case of El Cordon del Retén in San Miguel Chimalapa, Oaxaca. In: Porter-Bolland L, Ruiz-Mallén I, Camacho-Benavides C, McCandless SR (Eds) Community Action for Conservation Mexican Experiences. Springer, New York, 63–82. https://doi.org/10.1007/978-1-4614-7956-7\_5
- Morrone JJ (2005) Hacia una síntesis biogeográfica de México. Revista Mexicana de Biodiversidad 76: 207–252. https://doi.org/10.22201/ib.20078706e.2005.002.303
- Muñoz-Alonso LA, Nieblas-Camacho J, Chau-Cortez MA, González-Navarro AB, López-Pérez J, Pérez-López J (2017) Diversidad de anfibios y reptiles en la Reserva de la Biosfera Selva El Ocote: su vulnerabilidad ante la fragmentación y el cambio climático. In: Ruiz-Montoya

L, Álvarez-Gordillo G, Ramírez-Marcial N, Cruz-Salazar B (Eds) Vulnerabilidad social y biológica ante el cambio climático en la Reserva de la Biosfera Selva El Ocote. El Colegio de la Frontera Sur, Chiapas, 395–447.

- Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858. https://doi.org/10.1038/35002501
- Navarro-Sigüenza AG, Meave del Castillo JA (1998) Inventario de la biodiversidad de vertebrados terrestres de los Chimalapas, Oaxaca. Informe final SNIB-CONABIO, proyecto No. B002. Universidad Nacional Autónoma de México. Facultad de Ciencias. México, D.F.
- Nieto-Montes de Oca A, Barley AJ, Meza-Lázaro RN, García-Vázquez UO, Zamora-Abrego JG, Thomson RC, Leaché AD (2017) Phylogenomics and species delimitation in the knob-scaled lizards of the genus *Xenosaurus* (Squamata: Xenosauridae) using ddRADseq data reveal a substantial underestimation of diversity. Molecular Phylogenetics and Evolution 106: 241–253. https://doi.org/10.1016/j.ympev.2016.09.001
- Nuñez-Orantes O, Muñoz-Alonso A (2000) Inventario Herpetofaunístico de la reserva de la biosfera La Sepultura, Chiapas, México. Informe final SNIB-CONABIO, proyecto No. L003. Secretaría de Medio Ambiente Vivienda e Historia Natural. México, D.F.
- Ortiz-Pérez MA, Hernández-Santana JR, Figueroa JM (2004) Reconocimiento fisiográfico y geomorfológico. In: García-Mendoza AJ, Ordóñez MJ, Briones-Salas MA (Eds) Biodiversidad de Oaxaca. Instituto de Biología, UNAM, Fondo Oaxaqueño para la Conservación de la Naturaleza, World Wildlife Fund, Mexico, 43–54.
- Pérez-Farrera1 MA, Espinosa-Jiménez JA, López A, Gómez-Domínguez H, Gordillo-Ruiz MC (2016) Flora y vegetación de la Selva Zoque de Chiapas. In: Semahn (Ed.) Ecoregión Zoque: Retos y Oportunidades Ante el Cambio Climático. Secretaria de Medio Ambiente e Historia Natural, Chiapas, 52–76.
- Peterson AT, Navarro-Sigüenza A, Hernández-Baños BE, Escalona-Segura G, Rebón-Gallardo F, Rodríguez-Ayala E, Figueroa-Esquivel EM, Cabrera-García L (2003) The Chimalapas Region, Oaxaca, Mexico: a high-priority region for bird conservation in Mesoamerica. Bird Conservation International 13: 227–253. https://doi.org/10.1017/S0959270903003186
- Porras LW, Wilson LD, Schuett GW, Relserer RS (2013) A taxonomic reevaluation and conservation assessment of the common cantil, *Agkistrodon bilineatus* (Squamata: Viperidae): a race against time. Amphibian and Reptile Conservation 7: 48–73.
- Pulsford SA, Driscoll DA, Barton PS, Lindenmayer DB (2017) Remnant vegetation, plantings and fences are beneficial for reptiles in agricultural landscapes. Journal of Applied Ecology 54: 1710–1719. https://doi.org/10.1111/1365-2664.12923
- Ramírez-Reyes T, Flores-Villela O, Piñero D, Lathrop A, Murphy RW (2021) Genomic assessment of the *Phyllodactylus tuberculosus* complex (Reptilia: Phyllodactylidae) in America. Zoologica Scripta. https://doi.org/10.1111/zsc.12492
- Reyes-Velasco J, Adams RH, Boissinot S, Parkinson CL, Campbell JA, Castoe TA, Smith EN (2020) Genome-wide SNPs clarify lineage diversity confused by coloration in coralsnakes of the *Micrurus diastema* species complex (Serpentes: Elapidae). Molecular Phylogenetics and Evolution 147: e106770. https://doi.org/10.1016/j.ympev.2020.106770
- Reynoso VH, Paredes-León R, González-Hernández A (2011) Anfibios y reptiles del estado de Chiapas con algunos comentarios sobre los reportes y estudios de diversidad herpeto-
faunística en la región. In: Álvarez F (Ed.) Chiapas: Estudios sobre su diversidad biológica. Universidad Nacional Autónoma de México, Mexico, 459–509.

- Rodríguez-Gómez F, Gutiérrez-Rodríguez C, Ornelas JF (2013) Genetic, phenotypic and ecological divergence with gene flow at the Isthmus of Tehuantepec: The case of the azurecrowned hummingbird (*Amazilia cyanocephala*). Journal of Biogeography 40: 1360–1373. https://doi.org/10.1111/jbi.12093
- Ruane S, Bryson Jr RW, Pyron RA, Burbrink FT (2014) Coalescent species delimitation in milksnakes (Genus *Lampropeltis*) and impacts on phylogenetic comparative analyses. Systematic Biology 63: 231–250. https://doi.org/10.1093/sysbio/syt099
- Scarpetta S, Gray L, Nieto Montes de Oca A, Castañeda M, Herrel A, Losos JB, Luna-Reyes R, Jímenez-Lang N, Poe S (2015) Morphology and ecology of the Mexican cave anole *Anolis alvarezdeltoroi*. Mesoamerican Herpetology 2: 261–270.
- SEMARNAT [Secretaría de Medio Ambiente y Recursos Naturales] (2001) Programa de manejo Reserva de la Biosfera Selva El Ocote. SEMARNAT, CONANP, Chiapas, México, 144 pp.
- SEMARNAT [Secretaría de Medio Ambiente y Recursos Naturales] (2010) Norma Oficial Mexicana NOM-059. Protección ambiental de especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. Proyecto de Modificación. Agosto 2018. México D.F., México. Diario Oficial de la Federación.
- Semlitsch RD, Bodie JR (2003) Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. Conservation Biology 17: 1219–1228. https:// doi.org/10.1046/j.1523-1739.2003.02177.x
- Smith GC, Lewis T, Hogan LD (2015) Fauna community trends during early restoration of alluvial open forest/Woodland ecosystems on former agricultural land. Restoration Ecology 23: 787–799. https://doi.org/10.1111/rec.12269
- Taylor EH (1951) A new Veracrucian salamander. The University of Kansas Science Bulletin 34: 189–193.
- Uetz P, Freed P, Hošek J (2018) The Reptile Database. http://www.reptile-database.org [accessed on July 28, 2018]
- Vidal-Zepeda R (1990) Mapa de precipitación media anual, escala 1: 4000000. En Precipitación. Tomo II, Sección IV, 4.6. Atlas Nacional de México (1990–1992). Instituto de Geografía, UNAM, Mexico.
- Wallach V (2016) Morphological review and taxonomic status of the *Epictia phenops* species group of Mesoamerica, with description of six new species and discussion of South American *Epictia albifrons*, *E. goudotii*, and *E. tenella* (Serpentes: Leptotyphlopidae: Epictinae). Mesoamerican Herpetology 3: 215–374.
- Wendt T (1987) Las selvas de Uxpanapa, Veracruz-Oaxaca, México: Evidencia de refugios florísticos cenozoicos. Anales del Instituto de Biología, Serie Botánica 58: 29–54.
- Wilson LD, Mata-Silva V, Johnson JD (2013) A conservation reassessment of the reptiles of Mexico based on the EVS measure. Amphibian and Reptile Conservation 7: 1–47.
- Wüster W, Ferguson JE, Quijada-Mascareñas JA, Pook CE, Graca-Salomao M, Thorpe RS (2005) Tracing an invasion: landbridges, refugia, and the phylogeography of the Neotropical rattlesnake (Serpentes: Viperidae: *Crotalus durissus*). Molecular Ecology 14: 1095– 1108. https://doi.org/10.1111/j.1365-294X.2005.02471.x

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Abbreviations: a = species recorded during our fieldwork. LS = La Sepultura, LT = Los Tuxtlas, MF = Mayan Forest, SZ = Selva Zoque. NA = Species not considered in the EVS system. Exotic species recorded in the evaluated regions: Anolis cristatellus (Calakmul), Anolis sagrei (Selva Zoque, Los Tuxtlas, Calakmul, Sian Ka'an and Mayan Forest), Gehyra mutilata (Selva Zoque), Hemidactylus frenatus (Selva Zoque, Las Choapas, Lacandona, Los Tuxtlas, Calakmul and Sian Ka'an), Hemidactylus List of native reptile species recorded in the Selva Zoque region and seven other regions in northern Mesoamerica, their distribution range and conservation status. turcicus (Los Tuxtlas, La Sepultura) and Indotyphlops braminus (Las Choapas, Los Tuxtlas).

Species	Distribu- tion range	Selva Zoque	Las Choapas	Lacan- dona	Los Tuxtlas	La Sep- ultura	Calak- mul	Sian Ka'an	Mayan Forest	NOM- 059	IUCN RED LIST	EVS	Category of vulner- ability according to the EVS
CLASS REPTILIA													
Order Squamata													
Suborder Lacertilia													
Anguidae													
<i>Abronia bogerti</i> Tihen	SZ	1	0	0	0	0	0	0	0	Р	Data Deficient	18	High
Abronia chiszari Smith & Smith	LT	0	0	0	1	0	0	0	0	Р	Endangered	17	High
<i>Abronia morenica</i> Clause, Luna-Reyes & Nieto-Montes de Oca	LS	0	0	0	0	1	0	0	0		Not evaluated	NA	NA
<i>Abronia ornelasi</i> Campbell	SZ	1	0	0	0	0	0	0	0	Р	Data Deficient	18	High
<i>Abronia ramirezi</i> Campbell	LS	0	0	0	0	1	0	0	0		Data Deficient	18	High
<i>Abronia reidi</i> Werler & Shannon	LT	0	0	0	1	0	0	0	0	Р	Data Deficient	18	High
Celestus enneagrammus (Cope)	MAMx	1	0	0	0	0	0	0	0	$\mathbf{Pr}$	Least Concern	14	High
Celestus ingridae (Werler & Campbell)	LT	0	0	0	1	0	0	0	0		Data Deficient	17	High
Celestus rozellae (Smith) a	MA	1	0	1	0	0	0	0	1	$\Pr$	Least Concern	13	Medium
Gerrhonotus liocephalus Wiegmann	MAMx	1	0	0	1	1	0	0	0	$\mathbf{Pr}$	Least Concern	9	Low
Corytophanidae													
Basiliscus vittatus Wiegmann <sup>a</sup>	ΜD	1	1	1	1	1	1	1	1		Least Concern	7	Low
Corytophanes cristatus (Merrem)	ΜD	0	0	1	0	0	1	1	1	$\mathbf{Pr}$	Least Concern	11	Medium
Corytophanes hernandesii (Wiegmann) <sup>a</sup>	MA	1	1	1	1	1	1	0	1	$\mathbf{Pr}$	Least Concern	13	Medium
Laemanctus longipes Wiegmann	MA	1	0	1	1	0	1	0	1	$\Pr$	Least Concern	6	Low
Laemanctus serratus Cope	MA	0	0	0	1	0	1	1	0	$\mathbf{Pr}$	Least Concern	8	Low
Dactyloidae													
Anolis alvarezdeltoroi Nieto-Montes de Oca ª	MAMx	1	0	0	0	0	0	0	0		Data Deficient	17	High
Anolis barkeri (Schmidt)	MAMx	1	1	0	1	0	0	0	0	$\mathbf{Pr}$	Vulnerable	15	High
Anolis beckeri Boulenger <sup>a</sup>	MA	1	-	1	1	0	1	0	1		Least Concern	12	Medium
Anolis biporcatus (Wiegmann) <sup>a</sup>	ΜD	1	1	1	1	0	1	1	1	$\Pr$	Not evaluated	10	Medium
Anolis boulengerianus Thominot	MAMx	1	0	0	0	0	0	0	0	$\mathbf{Pr}$	Data Deficient	16	High
Anolis capito Peters	MA	1	0	1	0	0	0	0	1		Least Concern	13	Medium

EVS

NOM- IUCN RED LIST

Mayan

Los

Selva

Species	Distribu-	Selva	Las	Lacan-	Los	La Sep-	Calak-	Sian	Mayan	-MON	<b>IUCN RED LIST</b>	EVS	Category of vulner-
	tion range	Zoque	Choapas	dona	Tuxtlas	ultura	hul	Ka'an	Forest	059			ability according to the EVS
Anolis compressicaudus Smith & Kerster <sup>a</sup>	MAMx	-	0	0	0	0	0	0	0		Least Concern	15	High
Anolis cuprinus Smith	MAMx	1	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	16	High
Anolis duellmani Fitch & Henderson	LT	0	0	0	1	0	0	0	0	$\mathbf{Pr}$	Data Deficient	17	High
Anolis laeviventris (Wiegmann)	MA	1	0	0	1	1	0	0	1		Not evaluated	6	Low
Anolis lemurinus Cope <sup>a</sup>	MA	1	1	1	1	0	1	1	1		Least Concern	8	Low
Anolis matudai Smith	MA	0	0	0	0	1	0	0	0	A	Least Concern	13	Medium
Anolis parvicirculatus Alvarez del Toro & Smith	SZ	1	0	0	0	0	0	0	0	A	Least Concern	16	High
Anolis petersii Bocourt	MA	1	0	0	1	0	0	0	0		Not evaluated	6	Low
Anolis purpuronectes Gray, Meza-Lázaro, Poe & Nieto- Montes de Oca ª	SZ	1	0	0	0	0	0	0	0		Not evaluated	16	High
Anolis pygmaeus Alvarez del Toro & Smith <sup>a</sup>	MAMx	1	0	0	0	0	0	0	0	$\mathbf{Pr}$	Endangered	16	High
Anolis rodriguezii Bocourt <sup>a</sup>	MA	1	1	1	1	0	1	1	1		Least Concern	10	Medium
Anolis sericeus Hallowell <sup>a</sup>	MA	1	1	0	1	1	1	1	0		Least Concern	8	Low
Anolis spilorhipis (Alvarez del Toro & Smith)	MAMx	1	0	0	0	0	0	0	0		Not evaluated	NA	NA
Anolis tropidonotus Peters	MA	0	1	1	1	0	1	1	1		Least Concern	7	Low
Anolis uniformis Cope	MA	0	0	1	1	0	0	0	1		Least Concern	13	Medium
Anolis unilobatus Köhler & Vesely	MA	1	0	1	0	0	0	0	1		Least Concern	7	Low
Eublepharidae													
Coleonyx elegans Gray <sup>a</sup>	MA	1	0	1	1	1	1	1	1	Υ	Least Concern	6	Low
Gymnophthalmidae													
<i>Gymnophthalmus speciosu</i> s (Hallowell) <b>Helodermatidae</b>	Ш	0	0	0	0		0	0	0	Pr	Least Concern	6	Low
	1.64	-	¢	c	c		c	c	c	~	-	;	1 1
<i>Heloderma horridum</i> (Wiegmann) <b>Iguanidae</b>	MA	-	0	0	0	-	0	0	0	A	Least Concern	11	Medium
Cachrwr defensar (Cone)	MA	C	C	0	0	0	-	-	0	d	Vulnerable	ر د	Hieh
Crenosaura acanthura (Shaw)	MA			0 0		0 0	. 0	- C	0 0	Pr	Least Concern	12	Medium
(tenasaum bectinata (Wiesmann)	MA	-		0		0	0			<	Least Concern	1 12	Hieh
Cronscaura cimilis (Grav)	CI/M	-		. –				,	. –	A	I east Concern	¢	I ow
			, -		, -					- "C	I and Can some	, <u>;</u>	Madim
uguana iguana (Lunnacus) - Mabuyidae	U M	-	-	I	1	1	Þ	0	1	IJ	Least Concern	71	INICOLU
Marisora lineola McCranie, Matthews & Hedges	MA	0	1	1	1	0	1	1	1		Not evaluated	NA	NA
Marisora syntoma McCranie, Matthews & Hedges	MAMx	1	0	0	0	1	0	0	0		Not evaluated	NA	NA
Phyllodactylidae													
Phyllodactylus magnus Taylor	MAMx	1	0	0	0	1	0	1	0		Not evaluated	NA	NA
Thecadactylus rapicauda (Houttuyn)	ШD	0	0	1	0	0	1	1	1	$\Pr$	Least Concern	10	Medium
Phrynosomatidae													
<i>Phrynosoma asio</i> Cope	MA	-	0	0	0	0	0	0	0	$\mathbf{Pr}$	Least Concern	11	Medium

Species	Distribu- tion range	Selva Zoque	Las Choapas	Lacan- dona	Los Tuxtlas	La Sep- ultura	Calak- mul	Sian Ka'an	Mayan Forest	NOM- 059	IUCN RED LIST	EVS	Category of vulner- ability according to the EVS
Sceloporus carinatus Smith	MA	-	0	0	0	1	0	0	0		Least Concern	12	Medium
Sceloporus chrysostictus Cope	MA	0	0	0	0	0	1	1	1		Least Concern	13	Medium
<i>Scela porus cozumelae</i> Jones	MAMx	0	0	0	0	0	0	1	0	$\mathbf{Pr}$	Least Concern	15	High
Sceloporus internasalis Smith & Bumzahem <sup>a</sup>	MA	1	0	0	0	0	0	0	0		Least Concern	11	Medium
Sceloporus lundelli Smith	MA	0	0	0	0	0	1	1	1		Least Concern	14	High
Scelaporus melanorhinus Bocourt	MA	1	0	0	0	1	0	0	0		Least Concern	6	Low
Sceloporus salvini Günther	MAMx	0	0	0	1	0	0	0	0	А	Data Deficient	15	High
Sceloporus serrifer Cope	МD	0	0	1	0	1	0	0	1	А	Least Concern	9	Low
Sceloporus siniferus Cope	MAMx	1	0	0	0	1	0	0	0		Least Concern	11	Medium
Sceloporus smithi Hartweg & Oliver	MAMx	1	0	0	0	0	0	0	0		Least Concern	15	High
Sceloporus teapensis Günther	MA	1	0	1	1	0	0	0	1		Least Concern	13	Medium
Sceloporus variabilis Wiegmann <sup>a</sup>	ΜD	1	1	0	0	1	0	0	0		Least Concern	Ś	Low
Urosaurus bicarinatus (Duméril)	MA	1	0	0	0	1	0	0	0		Least Concern	12	Medium
Scincidae													
Mesoscincus schwartzei (Fischer)	MAMx	0	0	1	0	0	1	1	1		Least Concern	11	Medium
Plestiodon sumichrasti (Cope) <sup>a</sup>	MA	1	0	1	1	0	1	1	1		Least Concern	12	Medium
Scincella assata (Cope)	MA	1	0	0	0	1	0	0	0		Least Concern	7	Low
Scincella cherriei (Cope) <sup>a</sup>	MA	1	1	1	1	0	1	1	1		Least Concern	8	Low
Scincella gemningeri (Cope)	MAMx	1	0	0	1	0	0	0	0		Least Concern	11	Medium
Scincella incerta (Stuart)	MA	0	0	0	0	1	0	0	0		Least Concern	13	Medium
Scincella silvicola (Taylor)	MAMx	1	0	0	0	0	0	0	0	A	Least Concern	12	Medium
Sphaerodactylidae													
Gonatodes albogularis (Duméril & Bibron)	МD	1	0	0	0	0	0	0	0	$\mathbf{Pr}$	Least Concern	11	Medium
Sphaerodactylus glaucus Cope	MA	1	1	1	1	1	1	1	1	$\Pr$	Least Concern	12	Medium
Sphaerodactylus millepunctatus (Hallowell)	MA	0	0	1	0	0	0	0	1		Least Concern	10	Medium
Teiidae													
Aspidoscelis angusticeps (Cope)	MA	0	0	0	0	0	1	1	1		Least Concern	13	Medium
Aspidoscelis deppei (Wiegmann)	MA	1	1	0	1	1	1	0	0		Least Concern	8	Low
Aspidoscelis guttata (Wiegmann)	MAMx	1	0	0	1	1	0	0	0		Least Concern	12	Medium
Aspidoscelis maslini (Fritts)	MA	0	0	0	0	0	0	1	1	А	Least Concern	15	High
Aspidoscelis motaguae (Sackett)	MA	1	0	0	0	0	0	0	0		Least Concern	12	Medium
Holcosus amphigrammus (Smith and Laufe) <sup>a</sup>	MAMx	1	1	0	1	0	0	0	0		Not evaluated	11	Medium
Holcosus chaitzami (Stuart)	MA	0	0	0	0	0	0	0	1		Data Deficient	NA	NA
Holcosus festivus (Lichtenstein & Martens)	ΜD	0	0	1	0	0	0	0	1		Least Concern	11	Medium
Holcosus gaigeae (Smith & Laufe)	MAMx	0	0	0	0	0	1	1	0		Not evaluated	13	Medium
Holcosus hartwegi (Smith)	MA	0	0	1	0	0	0	0	1		Not evaluated	12	Medium

Species	Distribu- tion range	Selva Zoque	Las Choapas	Lacan- dona	Los Tuxtlas	La Sep- ultura	Calak- mul	Sian Ka'an	Mayan Forest	NOM- 059	IUCN RED LIST	EVS	Category of vulner- ability according to the EVS
<i>Holcosus parvus</i> (Barbour & Noble) Xantusiidae	MA	-	0	0	0	-	0	0	0		Not evaluated	13	Medium
Lepidophyma flavimaculatum Duméril	MA	г	П	1	0	0	0	П	-	Pr	Least Concern	8	Low
<i>Lepidophyma lipetzi</i> Smith & Alavarez del Toro	SZ	1	0	0	0	0	0	0	0	Υ	Endangered	16	High
Lepido phyma mayae Bezy	MA	0	0	0	0	0	0	0	1		Near Threatened	NA	NA
Lepidophyma pajapanensis Werler ª	MAMx	1	1	0	1	0	0	0	0	$\Pr$	Least Concern	13	Medium
<i>Lepidophyma smithii</i> Bocourt	MA	1	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	8	Low
<i>Lepidophyma tuxtlae</i> Werler & Shannon <sup>4</sup>	MAMx	1	1	0	1	0	0	0	0	А	Data Deficient	11	Medium
Xenosauridae													
Xenosaurus arboreus (Lynch & Smith)	SZ	1	0	0	0	0	0	0	0		Not evaluated	17	High
<i>Xenosaurus rackhami</i> Stuart	MA	1	0	0	0	0	0	0	0		Not evaluated	11	Medium
Xenosaurus sammartinensis Werler & Shannon	LT	0	0	0	1	0	0	0	0		Not evaluated	17	High
Suborder Serpentes Boidae													
<i>Boa imperator</i> Daudin	WD	1	1	1	1	1	1	1	1		Least Concern	10	Medium
Dipsadidae													
Adelphicos latifasciatum Lynch & Smith	LS	0	0	0	0	1	0	0	0	$\mathbf{Pr}$	Data Deficient	15	High
Adelphicos quadrivirgatum Jan	MA	1	0	1	0	1	0	0	1		Least Concern	10	Medium
Adelphicos visonimum (Cope)	MA	0	1	0	1	0	0	0	0		Least Concern	12	Medium
Amastridium sapperi (Werner) <sup>a</sup>	MA	1	0	1	1	0	0	0	1		Least Concern	10	Medium
Cenaspis aenigma Campbell, Smith & Hall	SZ	1	0	0	0	0	0	0	0		Not evaluated	16	High
Chersodromus australis Canseco-Márquez, Ramírez-	SZ	1	0	0	0	0	0	0	0		Not evaluated	16	High
González & Campbell													
Clelia clelia (Daudin)	Ш	0	0	0	0	0	0	0	1		Least Concern	80	Low
Clelia scytalina (Cope) <sup>a</sup>	MA	1	1	1	1	1	0	0	0		Least Concern	13	Medium
Coniophanes bipunctatus (Günther)	MA	0	1	1	1	1	0	0	1		Least Concern	10	Medium
Coniophanes fissidens (Günther) <sup>a</sup>	ΜD	1	1	1	1	1	0	0	1		Least Concern	7	Low
Coniophanes imperialis (Baird & Girard) <sup>2</sup>	ШD	1	1	1	1	0	1	1	1		Least Concern	8	Low
Coniophanes piceivittis Cope	MA	1	0	0	1	1	0	0	0		Least Concern	7	Low
<i>Coniophanes quinquevittatus</i> (Duméril, Bibron & Duméril)	MA	0	0	1	1	0	0	0	1		Least Concern	13	Medium
<i>Coniophanes schmidti</i> Bailey	MA	0	0	1	0	0	1	1	1		Least Concern	13	Medium
Conophis lineatus (Duméril, Bibron & Duméril)	MA	0	0	0	1	0	0	0	1		Least Concern	6	Low
Conophis monui7 Pérez-Higareda, López-Luna & Smith	LT	0	0	0	1	0	0	0	0		Data Deficient	17	High
Conophis vittatus Peters	MA	0	0	0	0	1	0	0	0		Least Concern	11	Medium
Dipsas brevifacies (Cope)	MA	0	0	0	0	0	1	1	0	$\mathbf{Pr}$	Least Concern	15	High

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opecies	Distribu- tion range	Selva Zoque	Las Choapas	dona	Los Tuxtlas	La Sep- ultura	calak- mul	oian Ka'an	Mayan Forest	059	10CN KED FIST	Eva	Category of vulner- ability according to the EVS
Emulius flavitorques (Cope)	ШD	0	0	0	0	1	0	0	0		Least Concern	5	Low
Geophis carinosus Stuart	MA	1	0	1	1	0	0	0	0		Least Concern	8	Low
Geophis juliai Pérez-Higareda, Smith & López-Luna	LT	0	0	0	1	0	0	0	0		Vulnerable	13	Medium
Geophis laticinctus Smith & Williams	MAMx	1	0	0	0	0	0	0	0	$\mathbf{Pr}$	Least Concern	11	Medium
<i>Geophis</i> sp. nov. <sup>2</sup>	SZ	1	0	0	0	0	0	0	0		Not evaluated	16	High
Imantodes cenchoa (Linnaeus) <sup>a</sup>	ШD	1	1	1	1	0	1	1	1	$\mathbf{Pr}$	Least Concern	9	Low
Imantodes gemmistratus (Cope)	ΜD	1	0	1	1	1	1	0	1	$\mathbf{Pr}$	Least Concern	9	Low
Imantodes tenuissimus (Cope)	MAMx	0	0	0	0	0	1	0	0	$\mathbf{Pr}$	Least Concern	13	Medium
<i>Leptodeira annulata</i> (Linnaeus)	ΜD	1	1	0	1	1	0	0	0	$\Pr$	Least Concern	9	Low
Leptodeira frenata (Cope)	MA	0	1	1	1	0	1	1	1		Least Concern	12	Medium
Leptodeira maculata (Hallowell)	MA	0	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	7	Low
<i>Leptodeira nigrofasciata</i> Günther	MA	1	0	0	0	0	0	0	0		Least Concern	8	Low
Leptodeira polysticta (Günther) <sup>2</sup>	MA	1	1	0	1	0	0	0	1		Not evaluated	9	Low
Leptodeira septentrionalis (Kennicott)	ΜD	0	0	1	0	1	1	0	0		Least Concern	8	Low
Manolepis putnami (Jan)	MAMx	1	0	0	0	1	0	0	0		Least Concern	13	Medium
Ninia diademata Baird & Girard <sup>a</sup>	MA	1	1	1	1	0	1	0	1		Least Concern	6	Low
Ninia sebae (Duméril, Bibron & Duméril) <sup>2</sup>	MA	1	1	1	1	0	1	1	1		Least Concern	2	Low
Oxyrhopus petolarius (Linnaeus) <sup>a</sup>	ШD	1	1	1	1	0	0	0	1		Least Concern	14	High
Pliocercus elapoides Cope	ШD	1	0	1	1	1	1	1	1		Least Concern	10	Medium
Rhadinaea decorata (Günther) <sup>a</sup>	ШD	1	1	1	1	0	0	0	1		Least Concern	6	Low
Rhadinaea macdougalli Smith & Langebartel	SZ	1	0	0	0	0	0	0	0	$\mathbf{Pr}$	Data Deficient	12	Medium
Rhadinella anachoreta (Smith & Campbell)	MA	0	0	0	0	0	0	0	1		Least Concern	NA	NA
Rhadinella godmani (Günther)	MA	1	0	0	0	0	0	0	0		Least Concern	10	Medium
Sibon dimidiatus (Günther) <sup>a</sup>	MA	1	0	1	1	0	0	0	1		Least Concern	10	Medium
Sibon linearis Pérez-Higareda, López-Luna & Smith	LT	0	0	0	1	0	0	0	0		Data Deficient	16	High
Sibon nebulatus (Linnaeus)	ΜD	1	0	1	1	0	1	0	1		Least Concern	2	Low
Sibon sanniolus (Cope)	MA	0	0	0	0	0	1	1	1		Least Concern	12	Medium
Tretanorhinus nigroluteus Cope	MA	0	0	1	1	0	0	1	1		Least Concern	10	Medium
Tropidodipsas fasciata Günther	ШD	1	0	0	1	1	1	0	0		Least Concern	13	Medium
Tropidodipsas fischeri (Boulenger)	MA	0	0	0	0	0	0	0	0		Least Concern	11	Medium
Tropidodipsas sartorii Cope	MA	1	1	1	1	0	1	1	1	$\mathbf{Pr}$	Least Concern	6	Low
Xenodon rabdocephalus (Wied-Neuwied)	ШD	1	1	1	1	0	1	0	1		Least Concern	13	Medium
Colubridae													
Coluber constructor Linnaeus	ШD	1	0	0	0	0	0	0	1	V	Least Concern	10	Medium
Dendrophidion rufiterminorum Cadle & Savage	MA	0	0	0	0	0	0	0	1		Not evaluated	NA	NA
Dendrophidion vinitor Smith <sup>a</sup>	MA	1	0	0	1	0	0	0	1		Least Concern	13	Medium

Species	Distribu- tion range	Selva Zoque	Las Choapas	Lacan- dona	Los Tuxtlas	La Sep- ultura	Calak- mul	Sian Ka'an	Mayan Forest	NOM- 059	IUCN RED LIST	EVS	Category of vulner- ability according to the EVS
Drymarchon melanurus (Duméril, Bibron & Duméril) <sup>a</sup>	MD	-	-	-	-	-	-	-	-		Least Concern	9	Low
Drymobius chloroticus (Cope)	MA	1	0	0	1	1	0	0	0		Least Concern	8	Low
Drymobius margaritiferus (Schlegel)	Ш	1	1	1	1	1	1	1	1		Least Concern	9	Low
Ficimia publia Cope	MA	1	-	1	1	0	1	1	1		Least Concern	6	Low
Ficimia variegata (Günther)	MAMx	0	0	0	1	0	0	0	0		Data Deficient	14	High
Masticophis mentovarius (Duméril, Bibron & Duméril)	Ш	1	-	1	1	1	0	0	1	А	Least Concern	9	Low
Mastigodryas melanolomus (Cope) a	Ш	1	-	1	1	1	-	1	1		Least Concern	9	Low
Lampropeltis abnorma (Bocourt)	MA	1	1	1	1	1	1	0	1		Least Concern	6	Low
Leptophis abaetulla (Linnaeus) <sup>a</sup>	WD	1	0	1	1	0	-	1	1	А	Least Concern	10	Medium
<i>Leptophis diplotropis</i> (Günther)	MAMx	0	0	0	0	1	0	0	0	A	Least Concern	14	High
Leptophis mexicanus Duméril, Bibron & Duméril	WD	1	1	1	1	1	1	1	1	A	Least Concern	9	Low
Oxybelis potosiensis (Taylor)	MAMx	1	0	1	1	1	1	1	1		Not evaluated	NA	NA
Oxybelis fulgidus (Daudin)	Ш	1	0	1	1	1	1	0	1		Least Concern	6	Low
Phrynonax poecilonotus (Günther)	MA	1	0	1	1	0	1	1	1		Least Concern	10	Medium
Pituophis lineaticollis (Cope)	MA	1	0	0	0	1	0	0	0		Least Concern	8	Low
Pseudelaphe flavirufa (Cope)	MA	1	0	1	1	1	1	0	1		Least Concern	10	Medium
Salvadora lemniscata (Cope)	MA	1	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	15	High
Senticolis triaspis (Cope)	ШD	1	0	1	1	1	1	0	1		Least Concern	9	Low
Spilotes pullatus (Linnaeus) <sup>a</sup>	WD	1	0	1	1	1	1	1	1		Least Concern	9	Low
Stenorrhina degenhardtii (Berthold) <sup>a</sup>	ΜD	1	0	1	1	0	0	0	1		Least Concern	6	Low
Stenorrhina freminvillei (Duméril, Bibron & Duméril)	MA	1	0	1	0	1	0	0	1		Least Concern	7	Low
Symphimus leucostomum Cope	MAMx	0	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	14	High
Symphimus mayae (Gaige)	MA	0	0	0	0	0	1	1	0	$\mathbf{Pr}$	Least Concern	14	High
Tantilla briggsi Savitzky & Smith	SZ	1	0	0	0	0	0	0	0	A	Data Deficient	16	High
Tantilla cuniculator Smith	MA	0	0	0	0	0	0	0	1	$\mathbf{Pr}$	Least Concern	NA	NA
Tantilla jani (Günther)	MA	0	0	0	0	1	0	0	0		Vulnerable	12	Medium
Tantilla moesta (Günther)	MA	0	0	0	0	0	0	0	1		Least Concern	NA	NA
Tantilla rubra Cope	MA	1	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	5	Low
Tantilla schistosa (Bocourt)	MA	0	0	1	1	0	0	0	1		Least Concern	8	Low
Tantilla slavensi Pérez-Higareda, Smith & Smith	LT	0	0	0	1	0	0	0	0	$\mathbf{Pr}$	Data Deficient	14	High
Tantilla tecta Campbell & Smith	MF	0	0	0	0	0	0	0	1		Data Deficient	NA	NA
Tantillita brevissima (Taylor)	MA	0	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	6	Low
Tantillita canula (Cope)	MA	0	0	0	0	0	1	0	1		Least Concern	12	Medium
Tantillita lintoni (Smith) ª	MA	1	0	1	1	0	1	0	1	$\mathbf{Pr}$	Least Concern	12	Medium
Trimorphodon biscutatus (Duméril, Bibron & Duméril)	MA	1	0	0	1	1	0	0	0	A	Not evaluated	4	Low
Natricidae													
Nerodia rhombifer (Hallowell)	ШD	0	0	1	1	0	0	0	0		Least Concern	10	Medium

Species	Distribu-	Selva	Las	Lacan-	Los	La Sep-	Calak-	Sian	Mayan	-MON	IUCN RED LIST	EVS	Category of vulner-
	tion range	Zoque	Choapas	dona	Tuxtlas	ultura	hul	Ka'an	Forest	059			ability according to the EVS
Thannophis cyrtopsis (Kennicott)	МD	-	0	0	0	0	0	0	0	A	Least Concern	~	Low
Thamnophis marcianus (Baird & Girard)	ШD	0	0	1	0	0	1	0	0	A	Least Concern	10	Medium
Thamnophis proximus (Say)	WD	0	1	0	1	1	0	1	1	A	Least Concern	7	Low
Sybinophiidae													
Scaphiodontophis annulatus (Duméril, Bibron & Du- méril) ª	Ш	1	0	1	1	1	0	0	1		Least Concern	11	Medium
Elapidae													
<i>Micrurus apiatus</i> (Jan)	MAMx	0	0	1	0	0	1	1	1		Not evaluated	NA	NA
Micrurus broumi Schmidt & Smith	MA	1	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	8	Low
Micrurus diastema (Duméril, Bibron & Duméril) <sup>a</sup>	MA	1	1	0	1	0	0	0	0	$\mathbf{Pr}$	Least Concern	8	Low
<i>Micrurus elegans</i> Jan	MA	1	1	1	1	0	0	0	0	$\Pr$	Least Concern	13	Medium
Micrurus limbatus Fraser	LT	0	0	0	1	0	0	0	0	$\mathbf{Pr}$	Least Concern	17	High
Leptotyphlopidae													
Epictia phenops (Cope)	MA	1	0	0	0	1	0	0	0		Not evaluated	4	Low
<i>Epictia resetari</i> Wallach	MAMx	0	0	0	1	0	0	0	0		Not evaluated	11	Medium
Loxocemidae													
Loxocemus bicolor Cope	MA	1	0	0	0	1	0	0	0	$\mathbf{Pr}$	Least Concern	10	Medium
Typhlopidae													
Amerotyphlops microstomus (Cope)	MA	0	0	0	0	0	1	0	1		Least Concern	12	Medium
Amerotyphlops tenuis (Salvin)	MA	1	0	0	1	0	0	0	0		Least Concern	11	Medium
Viperidae													
Agkistrodon bilineatus Günther	MA	0	0	0	0	1	1	0	1	$\mathbf{Pr}$	Near Threatened	11	Medium
Bothriechis bicolor (Bocourt)	MA	0	0	0	0	1	0	0	0	A	Least Concern	14	High
Bothriechis rowleyi (Bogert)	MAMx	1	0	0	0	0	0	0	0	$\Pr$	Vulnerable	16	High
Bothriechis schlegelii (Berthold)	WD	1	0	1	0	0	0	0	1		Least Concern	12	Medium
Bothrops asper (Garman) <sup>a</sup>	ШD	1	1	1	1	1	1	1	1		Not evaluated	12	Medium
Cerrophidion godmani (Günther)	MA	1	0	0	0	0	0	0	0		Least Concern	11	Medium
<i>Crotalus ehecatl</i> Carbajal-Márquez, Cedeño-Vázquez, Martínez-Arce, Neri-Castro & Machkour-M'Rabet	MAMx	1	1	0	0	1	0	0	0		Not evaluated	NA	NA
Grotalus mietlanteeuhtli Carbajal-Márquez, Cedeño- Vázquez, Martínez-Arce, Neri-Castro & Machkour- M'Rabet	LT	0	0	0	1	0	0	0	0		Not evaluated	NA	NA
Crotalus simus Latreille	MA	0	0	1	0	0	0	0	0		Least Concern	11	Medium
Crotalus tzabcan Klauber	MAMx	0	0	0	0	0	1	1	1		Least Concern	NA	NA
Metlapilcoatlus mexicanus (Duméril, Bibron & Duméril)	MA	0	0	1	0	0	0	0	1		Least Concern	12	Medium
Metlapileoatlus olmec (Pérez-Higareda, Smith & Julia- Zertuche) <sup>a</sup>	MA	1	0	0	1	0	0	0	0	V	Least Concern	15	High

Species	Distribu- tion range	Selva Zoque	Las Choapas	Lacan- dona	Los Tuxtlas	La Sep- ultura	Calak- mul	Sian Ka'an	Mayan Forest	NOM- 059	IUCN RED LIST	EVS	Category of vulner- ability according to the EVS
Porthidium dunni (Hartweg & Oliver)	MAMx	-	0	0	0	-	0	0	0	A	Least Concern	16	High
Porthidium nasutum (Bocourt)	WD	0	0	1	0	0	0	0	1	$\mathbf{Pr}$	Least Concern	14	High
Porthidium yucatanicum (Smith)	MA	0	0	0	0	0	0	1	0	$\mathbf{Pr}$	Least Concern	17	High
Order Testudines													
Suborder Cryptodira													
Cheloniidae													
Caretta caretta (Linnaeus)	MD	0	0	0	1	0	0	1	0	Ъ	Vulnerable	NA	NA
Chelonia mydas (Linnaeus)	MD	0	0	0	1	0	0	1	0	Ъ	Endangered	NA	NA
Eretmochelys imbricata (Linnaeus)	WD	0	0	0	1	0	0	1	0	Ъ	Critically Endangered	NA	NA
Lepidochelys kempii Garmin	Ш	0	0	0	1	0	0	0	0	Р	Critically Endangered	NA	NA
Chelydridae													
Chelydra rosignonii (Bocourt)	MA	0	1	1	1	0	0	0	1	$\mathbf{Pr}$	Vulnerable	17	High
Dermatemydidae													
Dermatemys mawii Gray	MA	1	0	1	1	0	0	0	1	Р	Critically Endangered	17	High
Dermochelyidae													
Dermochelys coriacea (Vandelli)	ШD	0	0	0	1	0	0	1	0	Ъ	Vulnerable	NA	NA
Emydidae													
Terrapene carolina (Linnaeus)	ШD	0	0	0	0	0	1	0	0	$\mathbf{Pr}$	Vulnerable	10	Medium
Trachemys venusta (Gray)	MD	1	1	1	1	0	1	1	1	$\mathbf{Pr}$	Not evaluated	13	Medium
Geoemydidae													
Rhinoclemmys areolata (Duméril, Bibron & Duméril)	MA	0	1	1	1	0	1	1	1	A	Near Threatened	13	Medium
Rhinoclemmys pulcherrima (Gray)	MA	0	0	0	0	1	0	0	0	A	Not evaluated	8	Low
Rhinoclemmys rubida (Cope)	MAMx	1	0	0	0	1	0	0	0	$\mathbf{P}_{\mathbf{r}}$	Near Threatened	14	High
Kinosternidae													
Claudius angustatus Cope	MA	0	1	0	1	0	1	0	1	Р	Near Threatened	14	High
Kinosternon acutum Gray <sup>a</sup>	MA	1	1	1	1	0	1	0	1	$\mathbf{P}_{\mathbf{\Gamma}}$	Near Threatened	11	Medium
<i>Kinosternon creaseri</i> Hartweg	MAMx	0	0	0	0	0	1	1	0		Least Concern	15	High
Kinosternon leucostomum (Duméril, Bibron & Duméril)	WD	1	1	1	1	0	1	1	1	$\mathbf{Pr}$	Not evaluated	10	Medium
Kinosternon scorpioides (Linnaeus) <sup>a</sup>	WD	1	0	0	1	1	1	1	1	$\mathbf{Pr}$	Not evaluated	10	Medium
Staurotypus triporcatus (Wiegmann) Order Crocodylia	MA	1	1	1	1	0	1	1	1	A	Near Threatened	14	High
Suborder Eusuchia													
Crocodylidae													
<i>Crocodylus acutus</i> (Cuvier) <sup>a</sup>	ШD	1	0	1	0	0	0	1	1	$\mathbf{Pr}$	Vulnerable	14	High
Crocodylus moreletii (Duméril & Bibron)	MD	-	-			0			-	Pr	Least Concern	13	Medium

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DATA PAPER



# Sea cucumbers (Echinodermata, Holothuroidea) from the JR275 expedition to the eastern Weddell Sea, Antarctica

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Academic editor: Yves Samyn   Received 12 October 2020   Accepted 11 May 2021   P	Published 4 August 2021

**Citation:** Mackenzie M, O'Loughlin PM, Griffiths H, Van de Putte A (2021) Sea cucumbers (Echinodermata, Holothuroidea) from the JR275 expedition to the eastern Weddell Sea, Antarctica. ZooKeys 1054: 155–172. https://doi.org/10.3897/zooKeys.1054.59584

#### Abstract

Thirty-seven holothuroid species, including six potentially new, are reported from the eastern Weddell Sea in Antarctica. Information regarding sea cucumbers in this dataset is based on Agassiz Trawl (AGT) samples collected during the British Antarctic Survey cruise JR275 on the RRS *James Clark Ross* in the austral summer of 2012. Species presence by site and an appendix of holothuroid identifications with registrations are included as supplementary material. Species occurrence in the Weddell Sea is updated to include new holothuroids from this expedition.

#### Keywords

Antarctic, benthic, biodiversity, dataset, holothuroid, Southern Ocean

## Introduction

The British Antarctic Survey (BAS) JR275 research cruise on the RRS *James Clark Ross* visited the Weddell Sea from February to March in 2012 as part of a core EvolHist (Evolutionary History of the Polar Regions) project. Prior to this expedition, the south-eastern Weddell Sea had been a relatively under sampled area on the Antarctic continental shelf, according to a gap analysis carried out by Griffiths et al. (2014). The eastern Weddell Sea is characterized by perennial sea ice cover and very large icebergs. The Filchner Trough is known to be an area responsible for generating the oxygen and nutrient-rich Antarctic Bottom Water (AABW), which helps drive oceanic circulation. By sampling benthic animals from the eastern Weddell Sea continental margin and slope and the deepened shelf basins of the Filchner Trough, the expedition aimed to collect specimens and associated data to investigate patterns of biodiversity and feed into biogeography and phylogeography studies of this important region of the Southern Ocean. Recording current biodiversity in the region is becoming increasingly urgent with the drastic decline in summer sea ice in the Weddell Sea over the last 5 years likely to have major implications for the marine ecosystem (Turner et al. 2020). Echinoids from JR275 were reported by Saucède et al. (2015) and the Asteroidea are included by Moreau et al. (2018).

This dataset reports holothuroid species occurrences and richness for individual Agassiz Trawls (AGTs) during the JR275 expedition and is provided for comparison with and updating of known lists of Weddell Sea and other Antarctic holothuroids. O'Loughlin et al. (2010) provided a comprehensive overview of Antarctic sea cucumber species, listing 187 species (including 51 still undescribed at the time of that publication) along with cryptic species and synonymies indicated by mtDNA sequence data. O'Loughlin et al. (2010) also reported 37 known species from shelf and slope depths in the Weddell Sea; here we add 11 new known species to this list for comparative depths (Table 5). The Weddell Sea is reported to be one of the most species-rich regions for holothuroids in Antarctica (O'Loughlin et al. 2010). Subsequent papers have continued to expand our knowledge of the previously undescribed Antarctic holothuroid fauna including papers by O'Loughlin and VandenSpiegel (2010) on apodids, O'Loughlin and Whitfield (2010) on psolids, O'Loughlin et al. (2013) on new holothuroids from Admiralty Bay, Davey and Whitfield (2013) on additional psolid fauna, O'Loughlin et al. (2014) on new Antarctic holothuroids and taxonomic reviews of some genera, Bohn and Hess (2014) on Echinopsolus and revisions within Psolidae and Cucumariidae, O'Loughlin et al. (2015a) on a new species of apodid from this JR275 expedition, and O'Loughlin et al. (2015b) on sea cucumbers of the Kerguelen Plateau. Gutt et al. (2014) also provided the original dataset for earlier voyages by German research vessel Polarstern to the Weddell Sea, including the holothuroid identifications and distributions published by Gutt (1990, 1991a, b) and subsequently referenced by O'Loughlin (2002), before further examination and revision by O'Loughlin et al. (2010). The dataset itself has since been updated by Piepenburg (2019). O'Loughlin et al. (2016) also gave an overview of Antarctic holothuroids collected during the historic Discovery expeditions. This current paper continues to build on our knowledge of Antarctic holothuroids.

This is a contribution to the SCAR (Scientific Committee on Antarctic Research) AntEco (State of the Antarctic Ecosystem) Programme.

# **Design description**

The dataset was published through the AntOBIS which is the Antarctic Marine Node of the international OBIS and GBIF, as a contribution to the EU-Lifewatch ERIC (https://www.lifewatch.eu/). Regarding the dataset, the Integrated Publishing Toolkit of the SCAR Antarctic Biodiversity Portal was used (http://ipt.biodiversity.aq/), following the Darwin Core event core. The dataset was uploaded in the AntOBIS (Antarctic Ocean Biogeographic Information System) database, and the taxonomy was matched against the Register of Antarctic Marine Species, using the Taxon Match tool (http://www.marinespecies.org/rams/aphia.php?p=match). The dataset meets the Darwin Core requirements and was designed around this event-core schema.

## **Material and methods**

Collecting equipment used on JR275 included an Agassiz trawl (AGT) and an Epibenthic Sledge (EBS) fitted with camera. This paper reports on the holothuroid specimens collected at 51 of the AGT sampling sites in the eastern Weddell Sea at depths of between ~400 and ~2,000 m, and a single test location at ~280 m depth off the South Orkney Islands (Fig. 1, Table 1). Weddell Sea deployments were mostly conducted along two transects, one running from south to north along the edge of the Filchner Trough and one running from west to east out of the Filchner Trough onto the shallower shelf. Over-deepened basins close to the Brunt Ice shelf were also sampled. At each site, three replicate AGT individual stations were taken and where the substrate was suitable a single EBS trawl was also conducted. EBS samples have not been examined for holothuroids at this stage and are not discussed further here, but this material is currently stored in the BAS collections in Cambridge, UK. The AGT used an inner mesh size of 1 cm, had a mouth width of 2 m, and was trawled at 1 knot for between 2 and 10 minutes depending on depth, substrate, and condition of animals in the initial catch. The deployment protocol was standardized and is outlined in full in the JR275 cruise report, available from the British Oceanographic Data Centre (https://www. bodc.ac.uk/resources/inventories/cruise\_inventory/report/10598/).

Live or early preservation images of specimens were taken on board the RRS *James Clark Ross* by Camille Moreau (Institut Universitaire Europeen de la Mer, Brest, France) with assistance from Melanie Mackenzie (Museums Victoria), James Rudd (BAS), and Douglas Hamilton (University of East Anglia) using a digital SLR Nikon D3X with 60 mm lens and flash rigging and Nikon D700 with 60 mm lens.

Preliminary identification of holothuroid material was carried out during the cruise by Melanie Mackenzie. DNA tissue samples (chiefly tentacle or tube foot subsamples) were taken by Melanie Mackenzie with assistance from Stuart MacMillan (BAS) for BOLD barcoding. A total of 190 specimens from 50 AGT stations (~15% of all specimens) were sampled during the cruise. DNA extractions of some Elpidiidae holothuroid specimens were conducted onboard by Jennifer Jackson (BAS) with



Figure 1. Sample locations for JR275 AGT holothuroid records.

assistance from Melanie Mackenzie using QIAGEN DNeasy Blood & Tissue molecular kit. Tissues samples of *Peniagone* specimens were also sent to Pamela Brannock at Auburn University and Rollins College in USA and a sample of *Protelpidia* was sent

Deployment	Start latitude	End latitude	Start	End longitude	Minimum	Maximum	Date
number			longitude	-	depth (m)	depth (m)	
8*	-60.68	-60.68	-44.01	-44.01	279.04	281.57	11/2/2012
20	-77.36	-77.36	-35.37	-35.36	654.34	654.35	19/2/2012
21	-77.35	-77.35	-35.35	-35.34	648.18	652.80	19/2/2012
22	-77.35	-77.35	-35.33	-35.32	650.78	654.20	19/2/2012
25	-76.33	-76.33	-32.90	-32.90	778.81	781.73	20/2/2012
26	-76.32	-76.32	-32.88	-32.88	780.30	789.24	20/2/2012
27	-76.32	-76.31	-32.87	-32.87	779.51	781.36	20/2/2012
29	-76.20	-76.20	-31.86	-31.86	575.95	578.97	20/2/2012
30	-76.20	-76.19	-31.84	-31.84	575.99	578.94	20/2/2012
31	-76.19	-76.19	-31.82	-31.82	564.11	573.00	20/2/2012
33	-76.02	-76.02	-27.00	-26.99	605.21	610.00	21/2/2012
34	-76.02	-76.02	-26.98	-26.97	608.00	613.00	21/2/2012
35	-76.02	-76.02	-26.96	-26.96	607.00	613.01	21/2/2012
38	-76.17	-76.17	-27.80	-27.80	544.89	561.00	21/2/2012
39	-76.17	-76.17	-27.80	-27.80	549.28	555.26	21/2/2012
43	-75.76	-75.76	-30.45	-30.45	427.94	430.00	22/2/2012
44	-75.77	-75.77	-30.46	-30.46	429.39	436.80	22/2/2012
47	-75.74	-75.74	-31.24	-31.24	578.94	584.88	22/2/2012
48	-75.75	-75.75	-31.25	-31.25	584.83	590.75	22/2/2012
49	-75.75	-75.75	-31.26	-31.27	583.36	584.94	22/2/2012
52	-75.24	-75.24	-30.25	-30.25	418.73	419.21	23/2/2012
53	-75.25	-75.25	-30.25	-30.25	417.39	417.78	23/2/2012
54	-75.25	-75.25	-30.26	-30.26	418.70	419.11	23/2/2012
55	-75.26	-75.26	-30.26	-30.27	418.38	418.61	23/2/2012
58	-75.26	-75.26	-31.13	-31.13	604.29	607.13	23/2/2012
59	-75.27	-75.27	-31.14	-31.15	607.10	610.24	23/2/2012
60	-75.27	-75.27	-31.16	-31.17	614.30	616.52	23/2/2012
63	-75.09	-75.09	-32.22	-32.22	609.48	612.28	24/2/2012
64	-75.09	-75.09	-32.22	-32.22	610.62	611.83	24/2/2012
68	-75.18	-75.18	-31.87	-31.87	655.78	676.11	24/2/2012
69	-75.18	-75.18	-31.87	-31.87	654.87	657.46	24/2/2012
70	-75.17	-75.18	-31.87	-31.87	654.65	691.31	24/2/2012
75	-74.37	-74.37	-28.11	-28.10	2052.26	2053.91	26/2/2012
76	-74.38	-74.38	-28.07	-28.06	2056.14	2058.19	26/2/2012
77	-74.39	-74.39	-28.16	-28.15	2006.54	2011.16	26/2/2012
81	-74.51	-74.51	-28.75	-28.74	1558.28	1570.08	28/2/2012
82	-74.50	-74.49	-28.74	-28.74	1580.27	1595.46	28/2/2012
85	-74.67	-74.68	-29.42	-29.43	586.74	604.49	29/2/2012
86	-74.68	-74.68	-29.45	-29.45	573.42	580.99	29/2/2012
88	-74.67	-74.67	-29.43	-29.43	592.71	602.27	29/2/2012
91	-74.71	-74.71	-29.51	-29.51	401.67	410.00	29/2/2012
92	-74.70	-74.70	-29.50	-29.50	427.17	428.55	29/2/2012
93	-74.70	-74.70	-29.50	-29.50	439.76	450.09	29/2/2012
96	-74.63	-74.63	-29.05	-29.04	1018.91	1028.48	1/3/2012
97	-74.63	-74.63	-29.02	-29.02	985.75	1010.63	1/3/2012
98	-74.64	-74.64	-29.00	-28.99	941.94	971.14	1/3/2012
101	-75.24	-75.24	-29.00	-29.01	391.66	398.30	4/3/2012
102	-75.25	-75.25	-29.02	-29.02	392.77	396.83	4/3/2012
103	-75.25	-75.25	-29.03	-29.03	390.17	392.20	4/3/2012
106	-75.24	-75.24	-27.85	-27.85	413.67	415.71	4/3/2012
107	-75.24	-75.24	-27.86	-27.87	414.23	415.15	4/3/2012
108	-75.24	-75.24	-27.88	-27.88	417.56	424.41	4/3/2012

Table 1. AGT sampling stations where holothuroids were collected on JR275.

\* Note: Site 8 is in the South Orkneys, all other sites are eastern Weddell Sea.

to Akito Ogawa at the National Museum of Nature and Science in Japan for further genetic analysis. Holothuroid specimens were preserved in 96% undenatured and pre-cooled (at -20 °C) ethanol and subsequently transferred to -20 °C freezer for a minimum of 48 hours with rotation of containers to ensure full preservation of material. Material was sent on to Melanie Mackenzie and Mark O'Loughlin at Museums Victoria, in Australia, for further examination and determination using stereo and compound microscopes. New species of Apodida (as Synaptida) specimens from this collection were described in O'Loughlin et al. (2015a) and are reported in Table 5 and Suppl. material 1: Table S1 alongside determinations of the remaining sea cucumber samples from AGT trawls. Specimens were identified to species level where possible, and to higher taxonomic levels where species-level determinations could not be made. Determinations here are based on morpho-taxonomic methods. Future genetic and morphological work may lead to additions or changes.

## Abbreviations

AntaBIS	Antarctic Biodiversity Information System;
AntEco	State of the Antarctic Ecosystem;
AntOBIS	Antarctic Thematic Node of the Ocean Biogeographic Information System;
BAS	British Antarctic Survey;
EvolHist	Evolutionary History of the Polar Regions (a BAS core project);
NHMUK	British Museum of Natural History (registration number prefix NHMUK);
NMV	Museums Victoria, Australia, used with registration number prefix F.;
SCAR	The Scientific Committee on Antarctic Research.

# **Taxonomic coverage**

**Remarks.** Miller et al. (2017) assessed and revised the phylogeny of extant Holothuroidea. Apodida was highlighted as a sister to the rest of Holothuroidea, the previously paraphyletic Aspidochirotida was split into Molpadida, Dendrochirotida, and Elasipodida (in part) and a new order, Holothuriida, was erected. For this paper, we follow the taxonomic groups put forward by Miller et al. (2017) and currently accepted in the World Register of Marine Species database (WoRMS 2020).

**General taxonomic coverage description.** The present dataset focuses on the class Holothuroidea (Echinodermata). Of the seven orders currently sitting within class Holothuroidea, six are represented, with only Molpadiida not being collected in the AGT catches on this voyage. This dataset looks at over 1200 specimens belonging to 10 families, and at least 23 genera and 31 species.

Phylum: Echinodermata

Class: Holothuroidea

**Orders:** Apodida, Dendrochirotida, Elasipodida, Holothuriida, Molpadida, Persiculida, Synallactida

Families: Chiridotidae, Cucumariidae, Paracucumidae, Psolidae, Elpidiidae, Laetmogonidae, Mesothuriidae, Molpadiodemidae, Pseudostichopodidae, Synallactidae

Genera: Paradota, Sigmodota, Taeniogyrus, Echinopsolus, Heterocucumis, Parathyonidium, Pentactella, Psolicrux, Staurocucumis, Trachythyone, Crucella, Paracucumis, Psolidium, Psolus, Elpidia, Peniagone, Protelpidia, Rhipidothuria, Laetmogone, Mesothuria, Molpadiodemas, Pseudostichopus, Bathyplotes

**Species:** Paradota weddellensis. Sigmodota magdarogera, Sigmodota magnibacula, Taeniogyrus bamberi, Echinopsolus acutus, Echinopsolus mollis, Heterocucumis steineni, Parathyonidium incertum, Psolicrux iuvenilesi, Staurocucumis liouvillei, Trachythyone bouvetensis, Trachythyone cynthiae, Crucella hystrix, Paracucumis turricata, Psolidium gaini, Psolidium tenue, Psolidium whittakeri, Psolus dubiosus, Psolus lockhartae, Elpidia glacialis, Peniagone incerta, Peniagone vignoni, Protelpidia murrayi, Rhipidothuria racovitzai, Laetmogone wyvillethomsoni, Mesothuria bifurcata, Molpadiodemas crinitus, Pseudostichopus spiculiferus, Pseudostichopus peripatus complex, Bathyplotes bongraini, Bathyplotes moseleyi.

**Other:** The following specimens with suffix sp. 1 belong to none of the known species listed in the dataset and will likely be described as new species after further morphological and genetic analyses: Cucumariidae sp. 1 (sp Mov 7265), *Echinopsolus* sp. 1 (sp Mov 7266), *Pentactella* sp. 1 (sp Mov 7267), *Staurocucumis* sp. 1 (sp Mov 7268), *Psolus* sp. 1 (sp Mov 7269), and *Peniagone* sp. 1 (sp Mov 7270).

In Tables 2–4, specimens identified as cf. species or only identified to a higher taxonomic level, i.e. species indeterminate (sp. indet.), genus indeterminate (gen. indet.), and family indeterminate (fam. indet.), are recorded on separate rows.

### Spatial coverage

General spatial coverage: East Weddell Sea, Antarctica. Coordinates: 60.68°S and 77.36°S; 44.01°W and 26.78°W. Temporal coverage: February 12, 2012–March 4, 2012.

#### Natural collections description

Initial collection identifier: British Antarctic Survey.

Collection name: EvolHist JR275 Weddell Sea Holothuroids.

**Final Lodgment Institutions:** British Antarctic Survey (BAS), Natural History Museum UK (NHMUK), Museums Victoria (NMV). Location and Registration Numbers as per Suppl. material 1: Table S1.

Collection identifier: O'Loughlin and Mackenzie.

Specimen preservation method: Ethanol (original fixative 95%).

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Order	Family	Genus	Species	JR275 Station Number / Site	
				8 20 21 22 25 26 27 29 30 31 33 34 35 38 39 43 44	47 48 49
APODIDA	Chiridotidae	Paradota	weddellensis		
		Sigmodota	magdarogera		
		Sigmodota	magnibacula	XX	
		Taeniogyrus	bamberi		
DENDROCHIROTIDA	Cucumariidae	Cucumariidae	sp.1	X	
		Echinopsolus	acutus	XX	
		Echinopsolus	mollis	X	
		Echinopsolus	cf. mollis	X	
		Echinopsolus	sp.1		
		Echinopsolus	sp. indet.		
		Heterocucumis	steineni		
		Parathyonidium	incertum	X	
		Pentactella	sp.1	X	
		Psolicrux	iuvenilesi	XX	
		Psolicrux	sp. indet.		
		Staurocucumis	liouvillei	X X X X X X X X X	×
		Staurocucumis	sp. 1	XX	×
		Trachythyone	bouvetensis	X X X X X X X	Х
		Trachythyone	cynthiae	XXX	
		Cucumariidae	gen. indet.		
	Paracucumidae	Crucella	hystrix	Х	
		Paracucumis	turricata	X	x
	Psolidae	Psolidium	gaini		
		Psolidium	tenue	X X X	
		Psolidium	whittakeri		
		Psolidium	sp. indet.		
		Psolus	dubiosus	X X X X X X	ХХ
		Psolus	cf. dubiosus		
		Psolus	lockhartae		
		Psolus	sp. 1		
		Psolidae	gen. indet.		
		Dendrochirotida	fam. indet.		
ELASIPODIDA	Elpidiidae	Elpidia	glacialis	X X X X X	X
		Peniagone	sp. 1		
		Peniagone	incerta		

Table 2. Continued.																						
Order	Family	Genus	Species							JR27	5 Sta	tion <b>N</b>	Jumb	er / S	ite							1
				8	0 21	22	25	26	27	29	30	31	33	34 3	5 3	8	9 43	44	47	48	<del>6</del> 4	i.
ELASIPODIDA	Elpidiidae	Peniagone	cf. incerta																			
		Peniagone	vignoni		×	×	×	×	×	×	×	×			×	×		×	×		×	
		Protelpidia	murrayi									×							×			
		Rhipidothuria	racovitzai		×	×							×	×	×				×	×		
		Elpidiidae	gen. indet.																			
	Laetmogonidae	Laetmogone	wyvillethomsoni				×	×														
HOLOTHURIIDA	Mesothuriidae	Mesothuria	bifurcata				×	×	×	×	×	×				×		×	×	×	×	1
PERSICULIDA	Molpadiodemidae	Molpadiodemas	crinitus									×										
	Pseudostichopodidiae	Pseudostichopus	spiculiferus	×												×						
		Pseudostichopus	peripatus complex																			
SYNALLACTIDA	Synallactidae	Bathyplotes	bongraini																			
		Bathyplotes	moseleyi				×		×													
		Bathyplotes	sp. indet.				×	×														
		Synallactidae	gen. indet.																			
Table 3. Presence-on	lv matrix of sea cucumber	species in AGT	rawls on IR275	Static	ins 5	2 to	88 (c	vluc	stati	ons	with	holc	thu	roids	incl	ndec	I).					
		-					·	`														
Order	Family	Genus	Species							JR27	5 Sta	tion <b>N</b>	Jumb	er / S	ite							
				52 5	3 54	55	58	59	60	63	64	68	69	20	5 7	9	7 81	82	85	86	88	1
APODIDA	Chiridotidae	Paradota	weddellensis												×	×						
		Sigmodota	magdarogena					×			×											
		Sigmodota	magnibacula																			

Order	Family	Genus	Species	JR275 Station Number / Site
				22 53 54 55 58 59 60 63 64 68 69 70 75 76 77 81 82 85 86 88
APODIDA	Chiridotidae	Paradota	weddellensis	ХХ
		Sigmodota	magdarogera	X X
		Sigmodota	magnibacula	
		Taeniogyrus	bamberi	X
DENDROCHIROTIDA	Cucumariidae	Cucumariidae	sp. 1	X X X
		Echinopsolus	acutus	X
		Echinopsolus	mollis	
		Echinopsolus	cf. mollis	
		Echinopsolus	sp. 1	X
		Echinopsolus	sp. indet.	X
		Heterocucumis	steineni	X X
		Parathyonidium	incertum	
		Pentactella	sp. 1	
		Psolicrux	iwenilesi	XX
		Psolicrux	sp. indet.	

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Order	Family	Genus	Species					IR275 9	station ]	Numbe	:/Site					
			ı	52 53 54	52	58 59	60	63 64	68	69 7(	75	76 7	7 81	82 8	5 86	88
DENDROCHIROTIDA	Cucumariidae	Stawocucumis	liouvillei	×		×	×	×							×	×
		Staurocucumis	sp. 1													
		Trachythyone	bouvetensis				×									
		Trachythyone	cynthiae											×		
		Cucumariidae	gen. indet.	×											×	
1	Paracucumidae	Crucella	hystrix			×										
		Paracucumis	turricata													
1	Psolidae	Psolidium	gaini											×		
		Psolidium	tenue	×		×			×							
		Psolidium	whittakeri													
		Psolidium	sp. indet.													
		Psolus	dubiosus			×								×		
		Psolus	cf. dubiosus					×								
		Psolus	lockhartae								×	X				
		Psolus	sp. 1								×					
		Psolidae	gen. indet.												×	×
1		Dendrochirotida	fam. indet.	×												×
ELASIPODIDA	Elpidiidae	Elpidia	glacialis					×	×							
		Peniagone	sp. 1											×		
		Peniagone	incerta								×	×				
		Peniagone	cf. incerta								×	$\left  \right\rangle$	×			
		Peniagone	vignoni	X X X	×	×			×	×						
		Protelpidia	murrayi					×		×						
		Rhipidothuria	racovitzai													
		Elpidiidae	gen. indet.	x												
1	Laetmogonidae	Laetmogone	wyvillethomsoni													
HOLOTHURIIDA	Mesothuriidae	Mesothuria	bifurcata		×	x x	×	×		×						
PERSICULIDA	Molpadiodemidae	Molpadiodemas	crinitus	Х												
1	Pseudostichopodidiae	Pseudostichopus	spiculiferus						×							
		Pseudostichopus	peripatus complex								×					
SYNALLACTIDA	Synallactidae	Bathyplotes	bongraini													
		Bathyplotes	moseleyi			X							×			
		Bathyplotes	sp. indet.				×									
		Synallactidae	gen. indet.			×										

Order	Family	Genus	Species	91	92	93	96	•7*	98	101 1	02 10	3 106	107	108
APODIDA	Chiridotidae	Paradota	weddellensis											
		Sigmodota	magdarogera											
		Sigmodota	magnibacula											
		Taeniogyrus	bamberi			×								
DENDROCHIROTIDA	Cucumariidae	Cucumariidae	sp. 1											
		Echinopsolus	acutus											
		Echinopsolus	mollis											
		Echinopsolus	cf. mollis											
		Echinopsolus	sp. 1											
		Echinopsolus	sp. indet.											
		Heterocucumis	steineni											
		Parathyonidium	incertum											
		Pentactella	sp. 1											
		Psolicrux	iuvenilesi											
		Psolicrux	sp. indet.	×										
		Staurocucumis	liouvillei				×	×	×			×		×
		Staurocucumis	sp. 1											
		Trachythyone	bouvetensis											
		Trachythyone	cynthiae											
		Cucumariidae	gen. indet.	×	×									
I	Paracucumidae	Crucella	hystrix											
		Paracucumis	turricata								~			
I	Psolidae	Psolidium	gaini											
		Psolidium	tenue					×	×					
		Psolidium	whittakeri				×							
		Psolidium	sp. indet.		×									
		Psolus	dubiosus				×				×			
		Psolus	cf. dubiosus						×					
		Psolus	lockhartae											
		Psolus	sp. 1											
		Psolidae	gen. indet.	×	Х									
1		Dendrochirotida	fam. indet.	×	×	×				×				

Order	Family	Genus	Species	91	92	93	96	97*	98	101 1	02 10	03 10	6 10	7 10	98
ELASIPODIDA	Elpidiidae	Elpidia	glacialis												
		Peniagone	sp. 1												
		Peniagone	incerta												
		Peniagone	cf. incerta												
		Peniagone	vignoni								X	×	X		
		Protelpidia	murrayi												
		Rhipidothuria	racovitzai												
		Elpidiidae	gen. indet.							×					
	Laetmogonidae	Laetmogone	wyvillethomsoni												
HOLOTHURIIDA	Mesothuriidae	Mesothuria	bifurcata								×				~
PERSICULIDA	Molpadiodemidae	Molpadiodemas	crinitus												
	Pseudostichopodidiae	Pseudostichopus	spiculiferus												
		Pseudostichopus	peripatus complex												
SYNALLACTIDA	Synallactidae	Bathyplotes	bongraini								×				
		Bathyplotes	moseleyi				×	×	×						
		Bathyplotes	sp. indet.												
		Synallactidae	gen. indet.												

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Table 4. Continued.

**Remarks.** A diverse holothuroid assemblage was collected, with over 1,200 holothuroids (~13.5 kg) from the Agassiz trawls alone, making these echinoderms one of the most abundant groups collected during the voyage and reinforcing previous records of high holothuroid abundance and diversity in this area. Holothuroids were found in 51 of the possible 55 AGTs, including in the iceberg scoured 'graveyards' of events 91 to 93.

Table 5. Holothuroid species reported from the Weddell Sea to 1180 m (following O'Loughlin et al. 2010).

APODIDA	
Chiridoti	dae
	Paradota weddellensis Gutt, 1990
	Sigmodota magdarogera O'Loughlin in O'Loughlin et al. 2015
	Sigmodota magnibacula (Massin & Hétérier, 2004)
	Taeniogyrus bamberi O'Loughlin in O'Loughlin et al. 2015
	Taeniogyrus contortus (Ludwig, 1875)
DENDROCHIROT	TIDA
Cucumari	iidae
	Cucamba psolidiformis (Vaney, 1908)
	"Cucumaria georgiana (Lampert, 1886) group" (by Gutt 1990)
	Echinopsolus acanthocola Gutt, 1990
	Echinopsolus acutus (Massin, 1992)
	Echinopsolus charcoti (Vaney, 1906)
	Echinopsolus mollis (Ludwig & Heding, 1935)
	Echinopsolus parvipes Massin, 1992
	Echinopsolus splendidus (Gutt, 1990)
	Heterocucumis denticulata (Ekman, 1927)
	Heterocucumis steineni (Ludwig, 1898)
	Parathyonidium incertum Heding in Heding and Panning 1954
	Psolicrux coatsi (Vaney, 1908)
	Psolicrux iuvenilesi O'Loughlin & Manjón-Cabeza, 2009
	Staurocucumis liouvillei (Vaney, 1914)
	Staurocucumis turqueti (Vaney, 1906)
	Trachythyone bouvetensis (Ludwig & Heding, 1935)
	Trachthyone cynthiae O'Loughlin, 2009
	Trachythyone maxima Massin, 1992
	Trachythyone parva (Ludwig, 1875)
Paracucu	midae
	Crucella hystrix Gutt, 1990
	Crucella scotiae (Vaney, 1906)
	Paracucumis turricata (Vaney, 1906)
Psolidae	
	Psolidium gaini Vaney, 1914
	Psolidium pawsoni O'Loughlin & Ahearn, 2008
	Psolidium tenue Mortensen, 1925
	Psolidium whittakeri O'Loughlin & Ahearn, 2008
	Psolus antarcticus (Philippi, 1857)
	Psolus dubiosus Ludwig & Heding, 1935
	Psolus cf. lockhartae (O'Loughlin & Whitfield, 2010)*
ELASIPODIDA	
Elpidiidad	e
	<i>Elpidia glacialis</i> Théel, 1876
	Peniagone incerta (Théel, 1882)
	Peniagone vignoni Hérouard, 1901
	Protelpidia murrayi (Théel, 1879)
-	Rhipidothuria racovitzai Hérouard, 1901
Laetmogo	nidae
HOLOMUNET	Laetmogone wyvillethomsoni Théel, 1879
HOLOTHURIIDA	
Mesothur	iidae

MOLPADIDA Molpadiidae Molpadia musculus Risso, 1826 PERSICULIDA Molpadiodemidae Molpadiodemas crinitus O'Loughlin & Ahearn, 2005 Pseudostichopodidae Pseudostichopus spiculiferus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongnaini Vaney, 1914 Bathyplotes bongnaini Vaney, 1914	Mesothuria bifurcata Hérouard, 1901
Molpadiidae Molpadia musculus Risso, 1826 PERSICULIDA Molpadiodemidae Molpadiodemas crinitus O'Loughlin & Ahearn, 2005 Pseudostichopodidae Pseudostichopus spiculiferus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongnaini Vaney, 1914 Bathyplotes gaurdani (Vaney, 1914)	MOLPADIDA
Molpadia musculus Risso, 1826 PERSICULIDA Molpadiodemidae Molpadiodemas crinitus O'Loughlin & Ahearn, 2005 Pseudostichopodidae Pseudostichopus spiculiferus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gaurdoni (Vaney, 1914)	Molpadiidae
PERSICULIDA Molpadiodemidae Molpadiodemas crinitus O'Loughlin & Ahearn, 2005 Pseudostichopodidae Pseudostichopus peripatus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gourdoni (Vaney, 1914)	Molpadia musculus Risso, 1826
Molpadiodemidae Molpadiodemas crinitus O'Loughlin & Ahearn, 2005 Pseudostichopodidae Pseudostichopus spiculiferus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gourdoni (Vaney, 1914)	PERSICULIDA
Molpadiodemas crinitus O'Loughlin & Ahearn, 2005 Pseudostichopodidae Pseudostichopus spiculiferus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gaurdoni (Vaney, 1914)	Molpadiodemidae
Pseudostichopodidae Pseudostichopus spiculiferus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gaurdani (Vaney, 1914)	Molpadiodemas crinitus O'Loughlin & Ahearn, 2005
Pseudostichopus spiculiferus (O'Loughlin, 2002) Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongnaini Vaney, 1914 Bathyplotes gaurdani (Vaney, 1914)	Pseudostichopodidae
Pseudostichopus peripatus (Sluiter, 1901) complex SYNALLACTIDA Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gourdoni (Vaney, 1914)	Pseudostichopus spiculiferus (O'Loughlin, 2002)
SYNALLACTIDA Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gourdoni (Vaney, 1914)	Pseudostichopus peripatus (Sluiter, 1901) complex
Synallactidae Bathyplotes bongraini Vaney, 1914 Bathyplotes gourdoni (Vaney, 1914)	SYNALLACTIDA
Bathyplotes bongraini Vaney, 1914 Bathyplotes gourdoni (Vaney, 1914)	Synallactidae
Bathyplates gourdoni (Vaney, 1914)	Bathyplotes bongraini Vaney, 1914
Dansy porto gon along ( ) and ( ) and ( )	Bathyplotes gourdoni (Vaney, 1914)
Bathyplotes moseleyi (Théel, 1886)	Bathyplotes moseleyi (Théel, 1886)

\*Note: Psolus cf. lockhartae was found at shallower comparative depths and is noted in the table above.

#### Plus, potential new species at comparative depths:

*Cucumariidae sp.* 1, *Echinopsolus sp.* 1, *Pentactella sp.* 1, *Staurocucumis sp.* 1, *Psolus sp.* 1 and *Peniagone sp.* 1.

**Plus, one known species at greater depth:** (~2000 m) *Psolus lockhartae* (O'Loughlin & Whitfield, 2010).

### Datasets

Dataset description: Biodiversity.aq – Integrated Publishing Toolkit (IPT version 2.4.0).
 Object name: Sea cucumbers (Echinodermata, Holothuroidea) from the JR275 expedition to the Eastern Weddell Sea, Antarctica – Data.

Character encoding: UTF-8.

Format name: Darwin Core Archive format.

#### Format version: 1.0.

**Distribution:** https://ipt.biodiversity.aq/manage/resource.do?r=bas\_jr275\_ holothuroidea. Mackenzie M, O'Loughlin PM, Griffiths H, Van de Putte AP, Van de Putte A (2021) Sea cucumbers (Echinodermata, Holothuroidea) from the JR275 expedition to the Eastern Weddell Sea, Antarctica – Data. SCAR – AntOBIS. Occurrence dataset https://hes32-ctp.trendmicro.com:443/wis/clicktime/ v1/query?url=https%3a%2f%2fdoi.org%2f10.15468%2f64c2ha&umid=2f9 6c605-47bc-4ec1-9c10-8ab8afdd2922&auth=89a422ce48cf9afc268cabe806cc53ea452e36bd-028570941b7b4372c9f0db6ba972ca8c781fba68 accessed via GBIF.org on 2021-05-23. https://www.gbif.org/dataset/fcc25f03-8437-4ea3-859e-67866de5cb80

Publication date of data: [pending]

Language: English

Metadata language: English

Date of metadata creation: Last modified Oct 7, 2020

Hierarchy level: Dataset

## Acknowledgements

We are very grateful to BAS and Katrin Linse for the opportunity extended to Melanie Mackenzie to join this cruise, and for providing the opportunity for our study of the holothuroid material collected. We acknowledge Museums Victoria for generously supporting expedition participation and subsequent processing of specimens. We thank the captain, crew and JR275 scientific team of the RRS *James Clark Ross*. Particular acknowledgment should be given to Camille Moreau (Institut Universitaire Europeen de la Mer, Brest) for his efforts to capture live images of holothuroid specimens, and to rest of the benthic team for collecting and processing of specimens including Jennifer Jackson (who also conducted on-board tissue sampling), Chester Sands, Rachel Downey (BAS), and Adam Reed (University of Southampton and BAS). We thank Andrew Cabrinovic for assistance with registration information for specimens to be lodged at NHMUK. We thank the journal editor for his diligence, and our reviewers for their insightful comments which have improved this manuscript. The publication of this data paper was supported by the AntaBIS, one of Belgium's contributions to EU Lifewatch.

Other than the above, the authors have no funding, competing interests or support to report.

### References

- Bonn JM, Hess M (2014) The Antarctic holothurian genus *Echinopsolus* Gutt, 1990 (Dendrochirotida, Cucumariidae): brood pouches, spermatozoa, spermatozeugmata and taxonomic implications. Zootaxa 3841(4): 573–591. https://doi.org/10.11646/zootaxa.3841.4.7
- Davey N, Whitfield E (2013) The Psolidae of New Zealand and some additions to the Macquarie Ridge fauna (Echinodermata: Holothuroidea: Psolidae). Memoirs of Museum Victoria 70: 51–67. https://doi.org/10.24199/j.mmv.2013.70.05
- Ekman S (1927) Holothurien der deutschen Südpolar–Expedition 1901–1903 aus der Ostantarktis und von den Kerguelen. Deutsche Südpolar–Expedition 19, Zoology 11: 359–419.
- Griffiths HJ, Van de Putte AP, Danis B (2014) Chapter 2.2. Data distribution: patterns and implications. In: De Broyer C, Koubbi P (Eds) Biogeographic Atlas of the Southern Ocean. The Scientific Committee on Antarctic Research, Cambridge, 16–17. https://www.scar. org/library/scar-publications/occasional-publications/3501-biogeographic-atlas-of-thesouthern-ocean-selected-chapters/
- Gutt J (1990) New Antarctic holothurians (Echinodermata). Five new species with four new genera of the order Dendrochirota. Zoologica Scripta 19: 101–117. https://doi. org/10.1111/j.1463-6409.1990.tb00243.x
- Gutt J (1991a) On the distribution and ecology of holothurians in the Weddell Sea (Antarctica). Polar Biology 11: 145–155. https://doi.org/10.1007/BF00240203
- Gutt J (1991b) Are Weddell Sea holothurians typical representatives of the Antarctic benthos? Meeresforschung 33: 312–329.

- Gutt J, Piepenburg D, Voß J (2014) Asteroids, ophiuroids and holothurians from the southeastern Weddell Sea (Southern Ocean). ZooKeys 434: 1–15. https://doi.org/10.3897/ zookeys.434.7622
- Heding SG, Panning A (1954) Phyllophoridae. Eine Bearbeitung der polytentaculaten dendrochiroten Holothurien des Zoologischen Museums in Kopenhagen. Spolia Zoologica Musei Hauniensis 13: 1–209.
- Hérouard E (1901) Note preliminaire sur les Holothuries rapportees par l'Expedition Antarctique Belge. Archives de Zoologie Expérimentale et Générale, Notes (3) ix: xxxix–xlviii. https://www.biodiversitylibrary.org/bibliography/79165
- Lampert K (1886) Die Holothurien von Süd-Georgien. Jahrbuch der Hamburgischen Wissenschaftlichen Anstalten 3: 10–22. [1 pl.]
- Ludwig H 1874 (1875) Beiträge zur Kenntniss der Holothurien. Arbeiten aus dem Zoologisch-Zootomischen Institut in Würzburg 2: 77–120. [pls 6, 7]
- Ludwig H (1898) Holothurien. In: Ergebnisse der Hamburger Magalhaensischen Sammelreise (1892/1893) Herausgegeben Naturhistorischen Museum Hamburg 1: 98 pp. [3 pls]
- Ludwig H, Heding SG (1935) Die Holothurien der deutschen Tiefsee-Expedition. I. Fußlose und dendrochirote Formen. Wissenschaftliche Ergebnisse der Deutschen Tiefsee-Expedition auf dem Dampfer Valdivia 1898–1899 24(2): 123–214.
- Massin C (1992) Three new species of Dendrochirotida (Holothuroidea, Echinodermata) from the Weddell Sea (Antarctica). Bulletin de l'Institut Royal des Sciences Naturelles de Belgique: Biologie 62: 184–187. http://biblio.naturalsciences.be/rbins-publications/bulletinof-the-royal-belgian-institute-of-natural-sciences-biologie/62-1992
- Massin C, Hétérier V (2004) On a new species of apodid, *Taeniogyrus magnibaculus* n. sp. (Echinodermata, Holothuroidea), from Antarctica, living on the spines of cidarid echinoids. Polar Biology 27(7): 441–444. https://doi.org/10.1007/s00300-004-0607-3
- Miller AK, Kerr AM, Paulay G, Reich M, Wilson NG, Carvajal JI, Rouse GW (2017) Molecular phylogeny of extant Holothuroidea (Echinodermata). Molecular Phylogenetics and Evolution 111: 110–131. https://doi.org/10.1016/j.ympev.2017.02.014
- Moreau C, Mah C, Agüera A, Améziane N, Barnes D, Crokaert G, Eléaume M, Griffiths H, Guillaumot C, Hemery LG, Jażdżewska A, Jossart Q, Laptikhovsky V, Linse K, Neill K, Sands C, Saucède T, Schiaparelli S, Siciński J, Vasset N, Danis B (2018) Antarctic and Sub-Antarctic Asteroidea database. ZooKeys 747: 141–156. https://doi.org/10.3897/zookeys.747.22751
- Mortensen T (1925) On a small collection of echinoderms from the Antarctic Sea. Arkiv för Zoologi 17A (31): 1–12.
- O'Loughlin PM (2002) Report on selected species of BANZARE and ANARE Holothuroidea, with reviews of *Meseres* Ludwig and *Heterocucumis* Panning (Echinodermata). Memoirs of Museum Victoria 59(2): 297–325. https://doi.org/10.24199/j.mmv.2002.59.3
- O'Loughlin PM, Ahearn C (2005) A review of pygal-furrowed Synallactidae (Echinodermata: Holothuroidea), with new species from the Antarctic, Atlantic and Pacific oceans. Memoirs of Museum Victoria 62(2): 147–179. https://doi.org/10.24199/j.mmv.2005.62.5
- O'Loughlin PM, Ahearn C (2008) Antarctic and Sub-Antarctic species of *Psolidium* Ludwig (Echinodermata: Holothuroidea: Psolidae). Memoirs of Museum Victoria 65: 23–42. https://doi.org/10.24199/j.mmv.2008.65.2

- O'Loughlin PM (2009) BANZARE holothuroids (Echinodermata: Holothuroidea) Zootaxa 2196: 1–18. https://doi.org/10.11646/zootaxa.2196.1.1
- O'Loughlin PM, Manjón-Cabeza ME, Ruiz FM (2009) Antarctic holothuroids from the Bellingshausen Sea, with descriptions of new species (Echinodermata: Holothuroidea). Zootaxa 2016: 1–16. https://doi.org/10.11646/zootaxa.2016.1.1
- O'Loughlin PM, VandenSpiegel D (2010) A revision of Antarctic and some Indo-Pacific apodid sea cucumbers (Echinodermata: Holothuroidea: Apodida). Memoirs of Museum Victoria 67: 61–95. https://doi.org/10.24199/j.mmv.2010.67.06
- O'Loughlin PM, Whitfield E (2010) New species of *Psolus* Oken from Antarctica (Echinodermata: Holothuroidea: Psolidae). Zootaxa 2528: 61–68. https://doi.org/10.11646/zootaxa.2528.1.4
- O'Loughlin PM, Paulay G, Davey N, Michonneau F (2010) The Antarctic region as a marine biodiversity hotspot for echinoderms: diversity and diversification of sea cucumbers. Deep-Sea Research II 58(2011): 264–275. https://doi.org/10.1016/j.dsr2.2010.10.011
- O'Loughlin PM, Stępień A, Kuźniak M, VandenSpiegel D (2013) A new genus and four new species of sea cucumbers from the South Shetland Islands (Echinodermata: Holothuroidea: Dendrochirotida: Molpadida). Polish Polar Research 34(1): 67–86. https://doi. org/10.2478/popore-2013-0003
- O'Loughlin, PM, Mackenzie M, Paulay G, VandenSpiegel D (2014) Four new species and a new genus of Antarctic sea cucumbers with taxonomic reviews of *Cladodactyla*, *Pseudocnus*, Paracucumidae and *Parathyonidium* (Echinodermata: Holothuroidea: Dendrochirotida). Memoirs of Museum Victoria 72: 31–61. https://doi.org/10.24199/j.mmv.2014.72.04
- O'loughlin, PM, Mackenzie, M, Vandenspiegel D, Griffiths H (2015a) New taeniogyrinid species of sea cucumber from the Weddell Sea (Echinodermata: Holothuroidea: Synaptida). Zootaxa 3995(1): 271–283. https://doi.org/10.11646/zootaxa.3995.1.23
- O'Loughlin PM, Skarbnik-López J, Mackenzie M, VandenSpiegel D (2015b) Sea cucumbers of the Kerguelen Plateau, with descriptions of new genus and species (Echinodermata: Holothuroidea). Memoirs of Museum Victoria 73: 59–93. https://doi.org/10.24199/j.mmv.2015.73.07
- O'Loughlin PM, Tavancheh E, Harding C (2016) The Discovery Expedition sea cucumbers (Echinodermata: Holothuroidea). Memoirs of Museum Victoria 75: 53–70. https://doi. org/10.24199/j.mmv.2016.75.03
- Philippi RA (1857) Vier neue Echinodermen des chilenischen Meeres. Archiv f
  ür Naturgeschichte 23: 130–148. https://www.biodiversitylibrary.org/page/2439774
- Piepenburg D (2019) Asteroids, ophiuroids and holothurians from the South-Eastern Weddell Sea (Southern Ocean). Version 11.2. SCAR – AntOBIS. Occurrence dataset.
- Risso A (1826) Histoire naturelle des principales productions de l'Europe mérudionale et particulièrement de celles des environs de Nice et des Alpes Maritimes. Vol. 5. Levrault, Paris, 289–293. https://doi.org/10.5962/bhl.title.58984
- Saucède T, Griffiths H, Moreau C, Jackson JA, Sands C, Downey R, Reed A, Mackenzie M, Geissler P, Linse K (2015) East Weddell Sea echinoids from the JR275 expedition. Zoo-Keys 504: 1–10. https://doi.org/10.3897/zookeys.504.8860
- Sluiter CP (1901) Neue Holothurien aus der Tief-See des indischen Archipels gesammelt durch die Siboga-Expedition. Tijdschrift der Nederlandsche Dierkindige Vereeniging (2<sup>de</sup> Serie) 7: 1–28. https://www.biodiversitylibrary.org/page/9760040

- Théel H (1876) Expedition polaire suedoise Holothurie nouvelle. Archives de Zoologie Experimental et Générale 5: xi–xiv. https://www.biodiversitylibrary.org/page/45528956
- Théel H (1879) Preliminary report on the Holothuridae of the exploring voyage of H.M.S. "Challenger" under Professor Sir C. Wyville Thomson F.R.S., Part I. Bihang till K. Svenska Vetenskapsakademiens Handlingar 5(19): 1–20. [pls 1, 2] http://www.vliz.be/imisdocs/ publications/ocrd/235911.pdf
- Théel H (1882) Report on the Holothuroidea dredged by H.M.S. Challenger during the years 1873–76. Part I. Scientific Results of H.M.S. Challenger 1873–1876. Zoology IV (13): 1–176. [pls 1–46] http://19thcenturyscience.org/HMSC/HMSC-Reports/Zool-13/htm/doc.html
- Théel H (1886) Report on the Holothurioidea dredged by HMS Challenger during the years 1873– 1876. Part II. Scientific Results of HMS Challenger 1873–1876. Zoology XIV (39): 1–290. [pls 1–16] http://19thcenturyscience.org/HMSC/HMSC-Reports/Zool-39/htm/doc.html
- Turner J, Guarino MV, Arnatt J, Jena B, Marshall GJ, Phillips T, Bajish CC, Clem K, Wang Z, Andersson T, Murphy EJ, Cavanagh R (2020) Recent decrease of summer sea ice in the Weddell Sea, Antarctica. Geophysical Research Letters 47(11): e2020GL087127. https://doi.org/10.1029/2020GL087127
- Vaney MC (1906) Note préliminaire sur les holothuries recueillies par l'expédition Antarctique Française du Dr Charcot. Bulletin du Muséum d'Histoire naturelle, Paris 12 (6): 402–407. https://biodiversitylibrary.org/page/951259
- Vaney MC (1908) Les Holothuries recueillies par l'Expédition antarctique écossaise. Zoologischer Anzeiger 33: 290–299.
- Vaney MC (1914) Holothuries: Deuxième Expédition Antarctique Française 1908–1910. Sciences Naturelles, Documents Scientifiques, Paris, 54 pp.
- WoRMS(2020)Holothuroidea.http://www.marinespecies.org/aphia.php?p=taxdetails&id=123083 [Accessed on 2020-06-17]

## Supplementary material I

#### Table S1

Authors: Melanie Mackenzie, P. Mark O'Loughlin, Huw Griffiths, Anton Van de Putte Data type: table (xlsx. file)

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Link: https://doi.org/10.3897/zookeys.1054.59584.suppl1



# Exploring the use of micro-computed tomography (micro-CT) in the taxonomy of sea cucumbers: a case-study on the gravel sea cucumber Neopentadactyla mixta (Östergren, 1898) (Echinodermata, Holothuroidea, Phyllophoridae)

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Academic editor: D.V. Spiegel   Received 19 April 2021   Accepted 7 June 2021   I	Published 4 August 2021

**Citation:** Samyn Y, Sonet G, d'Udekem d'Acoz C (2021) Exploring the use of micro-computed tomography (micro-CT) in the taxonomy of sea cucumbers: a case-study on the gravel sea cucumber *Neopentadactyla mixta* (Östergren, 1898) (Echinodermata, Holothuroidea, Phyllophoridae). ZooKeys 1054: 173–184. https://doi.org/10.3897/ zookeys.1054.67088

#### Abstract

Sea cucumber taxonomy and systematics has in the past heavily relied on gross external and internal anatomy, ossicle assemblage in different tissues, and molecular characterisation, with coloration, habitat, and geographical and bathymethric distribution also considered important parameters. In the present paper, we made these observations and techniques in detail and complemented them with the novel technique of micro-computed tomography of the calcareous ring. We investigated a single European species, the so-called gravel sea cucumber, *Neopentadactyla mixta* (Östergren, 1898), using recently collected material from the Chausey Islands, Normandy, France. We redescribed the species, illustrated its ossicle assemblage through scanning electron microscopy, and visualised the calcareous ring through stacking photography and through micro-CT scanning. Additionally, a DNA fragment of 955 base pairs of the 18S ribosomal RNA gene was sequenced from one specimen, which showed a high similarity with the only sequence of *N. mixta* publicly available. We completed this integrative study by providing a detailed distribution of the occurrence of *N. mixta* based on published, verifiable accounts.

#### Keywords

Integrative taxonomy, micro-CT, Normandy, scanning electron microscopy

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## Introduction

Recently, during a leisure expedition, Mr Francis Kerckhof of the Royal Belgian Institute of Natural Sciences collected four specimens of sea cucumbers on a beach in the Chausey Archipelago, Normandy, France. Collecting was done during a low, spring tide, between rocks, on coarse/gravelly sand mixed with shell (fragments), which is characteristic of a wave-beaten environment.

The species was identified as *Neopentadactyla mixta* (Östergren, 1898), the gravelsea cucumber, based on ossicles and the structure of the calcareous ring.

Examination of the calcareous ring, one of the key characters differentiating (sub)families in the order Dendrochirotida Grube, 1840, has in the past been made through intrusive and partly destructive dissections. Micro-computed tomography (i.e., micro-CT or  $\mu$ CT) provides an alternative, non-destructive method to decipher the structure of the calcareous ring. The goal of this paper is to evaluate the efficacy of this method.

Improved methods to study calcareous rings are needed in comparative taxonomic research. Smirnov (2012: 806), after a detailed and well-considered reflection of nearly 200 years of holothuroid classification noted: "the morphology of the calcareous ring is very important, although it is very difficult to describe its morphology and even more difficult to characterize features distinguishing the orders". Other authors, e.g., Cherbonnier (1988) and Thandar (1989), also made extensive use of the structure of the calcareous ring to distinguish dendrochirotids at family and subfamily levels. Study of the calcareous ring has generally been accomplished through dissection, except for some sea cucumbers with a translucent body (e.g., *Epitomapta simentalae* Solis-Marin et al., 2019) or those that readily expel their calcareous ring upon collection (e.g., species of the genus *Massinium* Samyn & Thandar, 2003).

In a broad phylogenetic study of Holothuroidea, Miller et al. (2017) noted that some morphological characters in holothuroid systematics are subject to homoplasy. More detailed anatomical studies, such as non-invasive micro-CT scanning of the calcareous ring and also, to a certain extent, other hard substances such as ossicles, allow a more in-depth appraisal of these structures and may offer resolution of apparently homoplastic characters.

Here we combine a traditional description of external and internal morphology and anatomy, with a *de novo* illustration of ossicle assemblages through SEM (the only and last detailed illustrations for *N. mixta* was provided by decades ago by Féral 1979), a genetic characterization through the sequencing of 955 base pairs (bp) of the 18S ribosomal gene that we compared with the only available 18S DNA sequence available for this species (Lacey et al. 2005), and a visualization of the calcareous ring through micro-CT as well as focus-stacked photography. This threefold approach is an attempt to arrive at an innovative approach to describe and classify sea cucumbers in a more modern and integrative way.

### Materials and methods

On 20 April 2019, four specimens of *Neopentadactyla mixta* were collected at extreme low tide on the Plage de la Grande Grève, (48°52.5'N, 1°50.8'W) on the Grande Île of the Chausey Archipelago, France. The specimens were relaxed in a solution of  $\pm 5\%$  MgCl<sub>2</sub>·6H<sub>2</sub>0 prior to fixation with 70–75% ethanol for 1 day, whereafter they were preserved in 70% ethanol denatured with diethyl ether for another 2 days before being placed in 70% buffered diethyl ether ethanol for permanent storage.

Ossicles from one out of the four specimens were prepared for light and scanning electron microscopy (SEM) by dissolving small pieces of dorsal and ventral body wall, tube feet, papillae, tentacles, and longitudinal muscle in household bleach, carefully rinsed with distilled water (Samyn et al. 2006) and observed through the microscope; no permanent slides were made. For SEM, ossicles were dried and mounted on aluminium stubs, coated with gold in a sputter coater, and observed with a FEI/Philips XL30 ESEM TMP Scanning Electron Microscope.

Micro-CT scanning was done with a RX EasyTom (RX Solutions, Chavanod, France; http://www.rxsolutions.fr), with an aluminium filter at the Royal Belgian Institute of Natural Sciences in Brussels, Belgium. No contrast agent was used on the specimen studied. For the 3D visualisation of the specimen (Fig. 2A) images were generated at a voltage of 88 kV and a current of 272  $\mu$ A, with a set frame rate of 3.25 and 5 average frames per image. This generated 1824 images and a voxel size of 28.63  $\mu$ m. For the calcareous ring *per se* (Fig. 2B, C), images were generated at a voltage of 71 kV and a current of 412  $\mu$ A, with a set frame rate of 4 and 5 average frames per image. This generated 2016 images and a voxel size of 23.90  $\mu$ m. Reconstructions were performed using X-Act software from RX Solutions. Segmentation, visualization, and analysis were performed using Dragonfly software for Windows (Object Research Systems (ORS) Inc., Montreal, Canada, 2020; software available at http://www.theobjects.com/dragonfly). 3D mesh files are available on https://sketchfab.com/3d-models/ be-rbins-hol-1736-neopentadactyla-mixta-b013d76558234a84a4b6907132eff93d.

The stacked photo reconstruction of the calcareous ring and its surrounding anatomical structures was done with a reflex Canon EOS 6D Mark II equipped with a Canon MP-E 65mm macro lens. This set-up was fixed on a Cognisys StackShot Macro Rail which is guided with Helicon Remote software. Photo-stacking on 30 different pictures was done with Zerene Stacker Software.

DNA was extracted from a piece of body wall using the NucleoSpin Tissue Kit following the manufacturer's protocol (Macherey-Nagel, Germany). Even though other DNA fragments are known to provide better resolution for DNA-based species identification, a fragment of 955 bp of the 18S ribosomal RNA gene was targeted because it is currently the only DNA sequence publicly available for *N. mixta*. The 18S ribosomal RNA gene was amplified using a nested polymerase chain reaction (PCR) protocol based on the primers and the conditions described by Lacey et al. (2005). The PCRs were prepared in volumes of 25 µl containing 1 µl of DNA template, 0.03 U/µl of Plati-

num Taq DNA Polymerase (Life Technologies, USA), 1X PCR buffer, 0.2 mM dNTPs, 0.4 µM of each primer, and 1.5 mM MgCl<sub>2</sub>. The first PCR was performed with primers EC-2F (AYCTGGTTGATYYTGCCAG) and WN-1708R (TGATCCATCTGCAG-GTTCACCT) with the following thermal profile: one step at 94 °C for 3 min, then 40 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 120 s, and a final step at 72 °C for 7 min. The second PCR was performed with primers 421-F (AAACGGCTACCACATC-CAAG) and 1482-R (AGGGCATCACAGACCTGTTA), using 1 µl of the product of the first PCR as template DNA and with the following thermal profile: one step at 94 °C for 3 min, then 40 cycles at 94 °C for 30 s, 53 °C for 30 s and 72 °C for 120 s, and a final step at 72 °C for 7 min. PCR products were visualized on a 1.2% agarose gel after electrophoresis at 100V and purified using the ExoSAP procedure (Exonuclease I -Shrimp Alkaline Phosphatase from ThermoFisher, USA). PCR products were sequenced bi-directionally on an ABI automated capillary sequencer using the BigDye v. 3.1 chemistry following the manufacturer's instructions (Life Technologies, USA). DNA chromatograms were checked, trimmed and assembled using CodonCode Aligner v. 8.0.2 (CodonCode Corp., Centerville, Massachusetts). Consensus sequence was compared to public records using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) of the National Center for Biotechnology Information, U.S. National Library of Medicine (NCBI) and submitted to GenBank (accession number MW522513).

#### Results

Phyllophoridae Östergren, 1907 Neopentadactyla Deichmann, 1944

# Neopentadactyla mixta (Östergren, 1898)

Figures 1A–L, 2A–D

Pseudocucumis mixta Östergren, 1898: 104, 105, 135, fig. 3 (p. 109).

Pseudocucumis mixta: Bedford 1898: 843 (discussion); Östergren 1902: 24, note 1;
Östergren 1904: 659; Östergren 1906: 1–24, figs 1–3; Ohshima 1912 54, 59 (discussion); Lieberkind 1929: 14; Massy 1920: 52; Koehler 1921: 168, fig. 124; Mortensen 1924: 236, fig. 116; Koehler 1927: 195, pl. 16, fig 16; Engel 1933: 33–34 (distribution); Cherbonnier 1952: 570, figs 1, 2; Könnecker and Keegan 1973: 157–162 (*in situ* pictures).

Pseudocucumis Cuenoti Koehler & Vaney, 1905: 395, figs 1-6.

Neopentadactyla mixta: Deichmann, 1944: 736; Heding and Panning 1954: 186, fig. 91; Féral 1979: 111, figs A–L; Féral 1980: 42, figs 1, 2; Smith 1983: 301, figs 1–4; Wood 1988: 143, 151 (drawing), 179; Moyse and Tyler 1990: 866 (key), 869, fig. 15.14 (upper left); Hansen and McKenzie 1991: 123 (lectotype and 3 paralectotypes), fig. 126–140; McKenzie 1991: 126, fig. 1; 132, fig. 3a–d; Picton 1993: 78 (colour photograph); McKenzie 1997: 274; Southward and Campbell 2006: 224, fig. 201.

**Status and location of types.** Museum of Evolution, Uppsala University, Sweden (UP-SZTY 2346): lectotype (249a); 2 paralectotypes (249b) (designated by McKenzie in 1990 according to the database of the Upsala Museum). McKenzie (1991) stated that he did not designate a lectotype and paralectotypes. In another publication Hansen and McKenzie (1991: 123) did designate and describe the lectotype ("Typsamlingen Nr. 249a") from the four syntypes as present in the Uppsala Museum's collection; the three remaining syntypes therefore become paralectotypes. Hansen and McKenzie (1991: 123) stated that they have maintained the original division of the four syntypes over two jars (249a and 249b), "each containing two specimens one of which was dissected, the other intact". According to Hansen and McKenzie (1991), the lectotype is thus together with one of the paralectotype in Jar 249a, while the other two paralectotypes are in jar 249b. We did not revisit this type series.

Type locality. W. Norway (most likely Molde, i.e., about 62°45'N, 7°14'E).

**Material examined.** RBINS I.G. 33990, HOL.1736 (4 specimens plus SEM stubs: I.G. 33990/HOL.1736/1-8).

Description (based on material examined). Body elongate, with central part inflated and anterior and posterior ends more narrow. Length of fixed specimens 50-155 mm (measured along the dorsal face); diameter 30-80 mm at mid-body, 21-43 mm anteriorly and 12-25 mm posteriorly. Color in alcohol after a short period of preservation same as color in life: body light beige, with irregular, brownish spots and patches (Fig. 1A, D); patches larger ventrally. Tentacles with their shaft light beige and the branches brownish with beige tips. According to McKenzie (1991), 10 large, five small, and five intermediate sized tentacles can be observed in live specimens. Insufficient relaxation of the specimens at hand made it impossible to observe the exact position of the tentacles in the specimens under study. Tube feet predominantly in the radii, in irregular double series anteriorly and posteriorly and in up to six rows, spreading into the interradii ventrally. Tube feet light beige. Body wall gritty to the touch. Body wall thin mid-body, thicker anteriorly and distally, possibly an artefact of fixation. Longitudinal muscles visible through the body wall where the skin is thinnest. Longitudinal muscles thick, undivided, and attached to the body wall along their entire width. Retractor muscles arise about 1/3 of the body length from anterior end, attaching to the radial section of the long and thin tubular calcareous ring, which itself is about 1/4 of the body length (Fig. 2A). Calcareous ring with five radial and five interradial parts (Fig. 2B). The radial parts are anteriorly notched, with 1–3 larger plates anteriorly and an irregular meshwork of smaller plates posteriorly. The interradial parts are anteriorly pointed, have 3 or 4 larger plates anteriorly and an irregular meshwork of smaller plates posteriorly (Fig. 2B). Calcareous ring embedded in a thin layer of tissue, with the calcified elements connected by connective tissue. (Fig. 2D). No clear posterior ending visible at the end of the comet-shaped, calcareous ring (Fig. 2A). Internal surfaces of especially the radial pieces are guttered (Fig. 2C). Single, very long Polian vesicle; single curled stone canal embedded in the dorsal mesentery (Fig. 2D).

Tentacle shafts with irregular, complex rosettes,  $30-45 \ \mu m$  long and  $15-25 \ \mu m$  wide (Fig. 1E); tentacle tips with straight to curved, terminally perforated rods,  $30-55 \ \mu m$  long and rosettes similar to those of the tentacle shafts (Fig. 1G); introvert with



**Figure 1.** *Neopentadactyla mixta* (Östergren, 1898) **A** focus-stacked view of the dorsal-lateral view of dissected specimen **B** focus-stacked view of the ventral–lateral view of dissected specimen **C** focus-stacked view of the dorsal–lateral view of a non-dissected specimen **D** focus-stacked view of the ventral–lateral view of a non-dissected specimen **E** SEM view of the rosettes from the shaft of a tentacle **F** SEM view of the 2-pillared tables from the introvert **G** SEM view of the rods and rosettes from a tentacle tip **H** SEM view of the 4-pillared tables from the dorsal body wall **I** SEM view of the 4-pillared tables from the ventral body wall **J** SEM view of the plates from the ventral tube feet **K** SEM view of the plates from the ventral tube feet **L** SEM view of half of an end-plate from a ventral tube foot. Scale bars: 1 cm (**A–D**); 50 μm (**E–L**).

2-pillared tables only, disc smooth, 65–80  $\mu$ m in diameter, irregular in outline, perforated by four central holes and a variable number of irregularly peripheral holes; pillars 40–65  $\mu$ m high, with single cross-beam, ending in a narrow, sparsely-spined crown (Fig. 1F); dorsal and ventral body wall with 4-pillared tables, 80–100  $\mu$ m in diameter, smooth rim, irregular in outline, perforated by four central holes and a variable number of peripheral holes arranged in multiple irregular circles; pillars 60–76  $\mu$ m high,



**Figure 2.** *Neopentadactyla mixta* (Östergren, 1898) **A** micro-CT scan visualizing the position of the calcareous ring **B** lateral view with micro-CT imaging of the anterior part of the calcareous ring (AR: most anterior radial piece; AIR: most anterior interradial pieces; SAR: subsequent anterior radial pieces; SAIR: subsequent interradial anterior pieces; Mesh: meshwork of radial and interradial median to distal pieces) **C** oblique view with micro-CT imaging showing a guttered internal side of the calcareous ring **D** focus-stacked view of the calcareous ring and associated structures (T: tentacles; LM: longitudinal muscle with bifurcation point (BfP); PV: Polian vesicle: SC: stone canal). Scale bars: 1 cm (**A–D**).

with single cross-beam, ending in a narrow, spined crown (Fig. 1H, I). Dorsal and ventral tube feet with plates, 64–95  $\mu$ m long and 40–55  $\mu$ m wide; endplate ±200  $\mu$ m in diameter, with uneven sized holes and with some relief (Fig. 1L). Contrary to Féral's (1980) observation, no tables could be found in the tube feet. Longitudinal muscles devoid of ossicles.

**Distribution.** North and West European coasts: Molde, West Norway (Ötsergren 1898); Arcachon, Bréhat Island, Wimereux, Roscoff, Chausey Islands, France (Koehler and Vaney 1905; Koehler 1921; Cabioch 1965; Féral 1979, 1980; this study); Northern British Islands, Faroe Islands (Östergren 1906; Lieberkind 1929); Tatihou Island, Normandy, France (Östergren 1906), Ireland (Massy 1920; Könnecker and Keegan 1973; Féral 1979); Denmark (Mortensen 1924); Swedish and Norwegian waters (Hansen and McKenzie 1991).

**Bathymetric range.** Intertidal (present study) to 200 m depth (Southward and Campbell 2006).

**Ecology.** *Neopentadactyla mixta* is most frequently found in maerl beds and coarse shell gravels with fairly strong tidal streams. It is a gregarious species, which may occur in densities of up to 297 individuals/m<sup>2</sup> (Könnecker and Keegan 1973; McKenzie 1991; Picton 1993). Könnecker and Keegan (1973) reported *N. mixta* to be a rheophilic suspension feeder with diurnal feeding rhythm. Smith and Keegan (1985) demonstrated that *N.* 

*mixta* individuals on the west coast of Ireland stop their suspension feeding from autumn to spring and retire to depths of 30–60 cm in the coarse sediments during that period. As with other phyllophorids (e.g., *Massinium maculosum* Samyn & Thandar, 2003), this species exposes only its tentacle crown and part of its introvert and the tip of its anus.

The sediment from the beach where the studied specimens were collected consisted of coarse, gravelly sand, characteristic for a wave-beaten environment and harboured a very rich and diverse fauna of other burrowing taxa (Bivalvia, Polychaeta, Sipuncula, Nemertea, etc.)

Systematics. The DNA sequence retrieved from GenBank (http://www.ncbi. nlm.nih.gov) most similar to the 18S sequence obtained here was labelled as Neopentadactyla mixta (accession number AY133482). Its similarity with our sequence is 99.16%. This sequence is currently the only DNA sequences available online for this species. The next most similar public DNA records were from Phyrella mookiei Michonneau & Paulay, 2014 (KX856842, 97.18%), a phyllophorid, and Afrocucumis africana (Semper, 1867) (KX856841, 97.18%), a sclerodactylid. The high DNA similarity with N. mixta supports the morphological identification of the specimen studied here as N. mixta, as a species belonging to Phyllophoridae. This DNA-based result is backed up by its ecology, the gross morphology of the specimens, the structure of the calcareous ring, and the ossicle assemblage. However, the high DNA similarity with A. africana is troubling, as Afrocucumis Deichmann, 1944 is characterized by a very different "skeletal structure": the calcareous ring has its radial pieces with two short, unsegmented, projections; the interradial pieces are without posterior projections; and the body wall holds large, 310-400 µm wide lenticular plates (Massin 1996). In N. *mixta*, no lenticular plates are present, and the calcareous ring is a much more complex structure (see the description above).

**Deposition of images.** SEM images of ossicles and a focus-stacked image of the calcareous ring has been put on the Royal Belgian Institute of Natural Sciences "Virtual Collections" website at http://virtualcollections.naturalsciences.be/virtual-collections/ recent-invertebrates/echinodermata#c4=N&b\_start=0.

3D mesh files have been put on https://sketchfab.com/3d-models/be-rbins-hol-1736-neopentadactyla-mixta-b013d76558234a84a4b6907132eff93d.

### Discussion

Neopentadactyla mixta was originally attributed to the monotypic genus Pseudocucumis Ludwig, 1875 by Östergren (1898). However, as correctly noted by Deichmann (1944), Pseudocucumis was established to accommodate Cucumaria acicula Semper, 1867 because of its particular ossicle assemblage (i.a. tables with reduced disc and fused pillars ending in a narrow crown). Cucumaria acicula, however, was referred to the genus Urodemas Selenka, 1867 (now considered a junior synonym of Cladolabes Brandt, 1835) by Deichmann (1944), making Pseudocucumis the junior synonym of Urodemas and Cladolabes. Deichmann (1944) proposed the generic replacement name Neopenta-
*dactyla* Deichmann, 1944 to accommodate *Pseudocucumis mixta*. *Neopentadactyla* has remained monotypic ever since (Heding and Panning 1954).

Heding and Panning (1954) divided the Phyllophoridae into several new subfamilies and put *Neopentadactyla* in Semperiellinae Heding & Panning, 1954. Since then the systematics of the Dendrochirotida has changed. In an attempt to bring order to the higher systematics of dendrochirote holothuroids, Pawson and Fell (1965) proposed two new families (Placothuriidae and Paracucumidae) and retained the Phyllophoridae as one of the valid families in the order. They diagnosed the Phyllophoridae as those dendrochirotid sea cucumbers with a calcareous ring composed of a mosaic of small pieces, unlike Heding and Panning (1954) who diagnosed Phyllophoridae as those dendrochirotids that present more than 10 tentacles, do not present a welldefined ventral sole, and predominantly present tables or derivatives thereof in their body wall. Thandar (1990) complemented Pawson and Fell's (1954) diagnosis with the description of the ossicle assemblage and the number of tentacles.

Micro-CT scans have been extensively used by one of us (CUA) for imaging hard structures in echinoderms, including calcareous rings of Holothuroidea. Results have been variable but sometimes they have revealed structures that could not be otherwise visualized. Micro-CT images have advantages and disadvantages compared to photography. The main advantage of this rather new technique is the non-destructive approach (especially important when studying rare species or type specimens) and its theoretical capacity to separate hard structures from soft tissues. The structures can also be easily rotated in all orientations, allowing not only for snapshots in optimal positions, but also for visualizing features in their original position and this in three dimensions. However, in practice, there is often a gradient of opacity to X-rays between genuine soft tissues and fully ossified structures. Therefore, it is sometimes difficult to decide of an optimal image setting and misleading image artefacts can appear if too much material of medium X-ray density is digitally removed. Problems of this nature were met with N. mixta, without being too serious. Another disadvantage is the usually rather low resolution of the images, where the surface details can be erased by excessive smoothing. Scanning and editing scans are time consuming and sometimes expensive operations, which require the work of an experienced operator. In the case of N. mixta, micro-CT scans provided images which, while not absolutely perfect, allowed for a more detailed analysis and interpretation of the structure of the calcareous ring. However, they proved insufficient for illustrating the ossicle assemblage. Thus, ossicles were imaged using more traditional scanning electron microscopy.

In conclusion, micro-CT scanning is expensive and demands experienced operators with knowledge of the anatomical structures to be visualized. Here, we applied this technique to the calcareous ring, a key taxonomic character within the Dendrochirotida/Phyllophoridae. Imaging of the ossicle assemblage through micro-CT scanning proved insufficient and SEM is here preferred, both to analyse the structure and the dimensions of the ossicle assemblage. Species identification could be verified by comparison with the only other DNA sequence available in BOLD.

## Acknowledgements

We thank Francis Kerckhof of the Royal Belgian Institute of Natural Sciences in Brussels (RBINS) for finding and collecting the specimens here studied. We are further thankful to Laetitia Despontin (RBINS) for the SEM imaging of the ossicle assemblage and for the mounting of the two figures. We also thank Yves Barette (RBINS) for producing figures of one of the specimens using focus-stacking. Finally, we thank the two referees of this paper, Gustav Paulay (Florida Museum of Natural History) and Jonathan Brecko (RBINS) for their constructive criticism on the draft of this paper.

## References

- Bedford FP (1898) Report on the holothurians collected, by M. J. Stanley Gardiner at Funafuti and Rotuma. Proceedings of the Zoological Society of London 68: 834–848. [pls 52, 53.] https://doi.org/10.1111/j.1096-3642.1898.tb03186.x
- Brandt JF (1835) Echinodermata ordo Holothurina. Prodromus Descriptionis Animalium ab H. Mertensio in Orbis Terrarum Circumnavigatione Observatorum. Fasc. I. Petropoli, 42–62. https://www.biodiversitylibrary.org/page/10765514
- Cabioch L (1965) Notes sur la faune marine benthique de Roscoff. Echinodermes. Cahiers de Biologie Marine 6: 265–268.
- Cherbonnier G (1952) Sur la présence à Roscoff de *Pseudocucumis mixta* Ostergren (Holothurie dendrochirote). Bulletin du Muséum national d'Histoire naturelle, Paris (série 2) 24: 570–573.
- Cherbonnier G (1988) Echinodermes: holothurides. Faune de Madagascar 70: 1-292.
- Deichmann E (1944) Urodemas bifurcatum, a new holothurian from South Africa, with a revision of the genus Urodemas Selenka. Annals Magazine Natural History (series 11) 11: 731–737. https://doi.org/10.1080/00222934408527470
- Engel H (1933) Holothuries. Résultats Scientifiques du Voyage aux Indes Orientales Néerlandaises de LL. AA. RR. Le Prince et la Princesse Léopold de Belgique. Mémoires du Musée Royal d'Histoire Naturelle de Belgique, Hors Série 3(13): 3–42.
- Féral JP (1979) Neopentadactyla mixta (Holothurioidea: Phyllophoridae) Spiculation et croissance. In: Jangoux (Ed). Echinoderms: Present and Past, Rotterdam, 111–113. https://doi. org/10.1201/9781003078913-20
- Féral JP (1980) Variation de la spiculation au cours de la croissance chez *Neopentadactyla* mixta (Östergren) 1898. (Holothurioidea, Phyllophoridae). Cahiers de Biologie Marine 21: 41–49.
- Grube AE (1840) Actinien, Echinodermen und Würmer des Adriatischen- und Mittelmeers nach eigenen Sammlungen beschrieben. J.H. Bon, Königsberg, 92 pp. https://www.biodiversitylibrary.org/page/10662919
- Hansen B, McKenzie JD (1991) A taxonomic review of Northern Atlantic species of Thyonidiinae and Semperiellinae (Echinodermata: Holothuroidea: Dendrochirotida). Zoological Journal of the Linnean Society 103(2): 101–127. https://doi.org/10.1111/j.1096-3642.1991.tb00899.x

- Heding SG, Panning A (1954) Phyllophoridae. Eine Bearbeitung der Polytentaculaten Dendrochiroten Holothurien des Zoologische Museum in Kopenhagen. Spolia Zoologica Musei Hauniensis 13: 1–209.
- Koehler R (1921) Echinodermes. Faune de France, Tome 1. Paul Lechevalier, Paris, 10 pp.
- Koehler R (1927) Les Echinodermes des Mers d'Europe, Tome 2. Librairie Octave, Gaston Doin, Paris, 339 pp.
- Koehler R, Vaney C (1905) Description d'une nouvelle holothurie des côtes de France (*Pseudo-cucumis Cuenoti* nov. sp.) Revue Suisse de Zoologie 13: 395–400. https://www.biodiversi-tylibrary.org/page/10091040
- Könnecker G, Keegan BF (1973) In situ behavioural studies on echinoderm aggregations. Helgoländer wissenschaftliche Meeresuntersuchungen 24: 157–162. https://doi.org/10.1007/ BF01609508
- Lacey KM, McCormack GP, Keegan BF, Powell R (2005) Phylogenetic relationships within the class Holothuroidea, inferred from 18S rRNA gene data. Marine Biology 147: 1149–1154. https://doi.org/10.1007/s00227-005-0009-2
- Lieberkind I (1929) Echinodermata. The Zoology of the Faroes 3(1): 1-20.
- Ludwig HL (1875) Beiträge zur Kenntniss der Holothurien. Arbeiten aus dem zoologischzootomischen Institut Würzburg. 2: 77–118. [pls 6, 7.]
- Massy AL (1920) The Holothurioidea of the Coasts of Ireland. The Scientific Proceedings of the Royal Dublin Society 16: 37–62.
- Massin C (1996) Results of the Rumphius Biohistorical Expedition to Ambon (19990). Part
  4. The Holothurioidea (Echinodermata) collected at Amon during the Rhumphius Biohistorical Expedition. Zoologische Verhandelingen 307: 1–53.
- McKenzie DJ (1991) The taxonomy and natural history of north European dendrochirote holothurians. Journal of Natural History 25: 123–171. https://doi. org/10.1080/00222939100770091
- McKenzie DJ (1997) Echinodermata. In: Howson CM, Picton BE (Eds) The Species Directory of the Marine Fauna and Flora of the British Isles and Surrounding Seas. Ulster Museum and the Marine Conservation Society, Belfast and Ross-on-Wye, 268–276.
- Michonneau F, Paulay G (2014) Revision of the genus *Phyrella* (Holothuroidea: Dendrochirotida) with the description of a new species from Guam. Zootaxa 3760(2): 101–140. https://doi.org/10.11646/zootaxa.3760.2.1
- Miller AK, Kerr AM, Paulay G, Reich M, Wilson NG, Carvajal J, Rouse GW (2017) Molecular phylogeny of extant Holothuroidea (Echinodermata). Molecular Phylogeny and Evolution 111: 110–131. https://doi.org/10.1016/j.ympev.2017.02.014
- Mortensen Th (1924) Pighude (Echinodermer). Danmarks Fauna 27. G.E.C. Gads Forlag, København, 207–256.
- Mortensen Th (1927) Handbook of the Echinoderms of the British Isles. Oxford University Press, Oxford, 71 pp. https://www.biodiversitylibrary.org/page/6277293
- Moyse J, Tyler PA (1990) Echinodermata. In: Hayward PJ, Ryland JS (Eds) The Marine Fauna of the British Isles and North-West Europe (Vol. 2). Clarendon Press, Oxford, 839–871.
- Ohshima H (1912) On the system of Phyllophorinae with description of the species found in Japan Annotationes zoologicae japonenses 8(1): 53–96. [pl. 1.]

- Östergren H (1898) Zur Anatomie der Dendrochiroten, nebst Beschreibungen neuer Arten. Zoologischer Anzeiger 21(551): 10–110. [133–36.]
- Östergren H (1902) The Holothuroidea of Northern Norway. Bergens Museum Aarborg 9: 1–34.
- Östergren H (1904) *Pseudocucumis mixta*. II. Mitteilungen aus Museen, Instituten usw. 1 Naturvetenskapliga Studentsällskapet, Uppsala. Zoologische Sektion, Sitzung den 27 März 1903: e659.
- Östergren H (1906) Einige Bemerkungen über die westeuropäischen *Pseudocucumis-* und *Phyllophorus*-Arten. Arkiv för Zoologi 3(16): 1–24.
- Östergren H (1907) Zur Phylogenie und Systematik der Seewalzen Särtryck ur Zoologiska Studier Tillägnade Professor T. Tullberg. Almquist & Wiksells Buchdruckerei-Aktiengesellschaft, Uppsala, 191–215.
- Pawson DL, Fell HB (1965) A revised classification of the dendrochirote holothurians. Brevoria 214: 1–7.
- Picton BE (1993) A Field Guide to the Echinoderms of the British Isles. IMMEL Publications, London, 96 pp.
- Samyn Y, Thandar AS (2003) Massinium, a new genus in the holothuroid family Phyllophoridae (Echinodermata: Holothuroidea: Dendrochirotida) with description of a new southwest Indian Ocean species M. maculosum. Belgian Journal of Zoology 133: 137–144.
- Samyn Y, VandenSpiegel D, Massin C (2006) Taxonomie des holothuries des Comores. Abc Taxa 1: 1–130.
- Selenka (1867) Beiträge zur Anatomie und Systematik der Holothurien. Zeitschrift für wissenschaftliche Zoologie 17(2): 291–374. [pls XVII–XX.]
- Semper C (1867) Holothurien. In: Semper C (Ed.) Reisen im Archipel der Philippinen. Zweiter Theil. Wissenschaftliche Resultate. Erster Band. W. Engelmann, Leipzig, 288 pp. [40 pls] [1867 = pp. 1–70, pls 1–15]
- Southward EC, Campbell AC (2006) Echinoderms. In: Crothers JH, Hayward PJ (Eds) Synopses of the British Fauna (New Series) 56. The Linnean Society of London and The estuarine and Coastal Sciences Association, Shrewsbyry, 272 pp.
- Smirnov AV (2012) System of the class Holothuroidea. Paleontological Journal 46(8): 793–832. https://doi.org/10.1134/S0031030112080126
- Smith TB (1983) Tentacular ultrastructure and feeding behaviour of Neopentadactyla mixta (Holothuroidea: Dendrochirota). Journal of the marine biological Association of the United Kingdom 63(2): 301–311. https://doi.org/10.1017/S0025315400070697
- Solis-Marin FA, Conejeres-Vargas CA, Caballero-Ochoa AA, Arriaga-Ochoa JA (2019) Epitomapta simentalae sp. n., a new species of apodous sea cucumber from the Central Eastern Pacific coast of Mexico (Echinodermata, Holothuroidea, Apodida). ZooKeys 817: 1–9. https://doi.org/10.3897/zookeys.817.29406
- Thandar AS (1989) The sclerodactylid holothurians of southern Africa, with the erection of one new subfamily and two new genera (Echinodermata: Holothuroidea. South African Journal of Zoology 24(4): 290–304. https://doi.org/10.1080/02541858.1989.11448167
- Thandar AS (1990) The phyllophorid holothurians of southern Africa with the erection of a new genus. South African Journal of Zoology 25(4): 207–223. https://doi.org/10.1080/0 2541858.1990.11448215
- Wood E (1988) Sea Life of Britain & Ireland. Marine Conservation Society, IMMEL Publishing, London, 240 pp.

RESEARCH ARTICLE



# Two new species of the genus Saigona Matsumura (Hemiptera, Fulgoromorpha, Dictyopharidae) from China

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Academic editor: Mike Wilson	Received 6 April 2021	Accepted 1	12 July 2021		Published 4 August 2021
http://zoo	bank.org/FD154A70-6839	)-4068-8A69-5	5A0C66ECBC	28	

**Citation:** Zheng Y-L, Bourgoin T, Yang L, Chen X-S, Luo X-Q, Luo G-J (2021) Two new species of the genus *Saigona* Matsumura (Hemiptera, Fulgoromorpha, Dictyopharidae) from China. ZooKeys 1054: 185–200. https://doi.org/10.3897/zooKeys.1054.67004

#### Abstract

Two new species of the genus *Saigona* Matsumura, 1910, *S. baiseensis* Zheng & Chen **sp. nov.** and *S. mac-ulata* Zheng & Chen **sp. nov.**, from China (Guanxi) are described and illustrated. A revised identification key to the 16 species of *Saigona* is provided. 15 species of the genus are known from China only.

#### Keywords

Fulgoroidea, Oriental region, planthopper, taxonomy

## Introduction

The planthoppers of the family Dictyopharidae Spinola, 1839 (Hemiptera, Fulgoromorpha) currently groups 738 species in 160 extant and extinct genera (Bourgoin 2021). They are currently divided into two subfamilies, Dictyopharinae Spinola,

1839 and Orgeriinae Fieber, 1872, and 19 tribes (Muir 1923; Metcalf 1946; Song et al. 2018; Bourgoin 2021). The genus *Saigona* Matsumura, 1910 was first established by Matsumura (1910) based on *Dictyophora* [sic] *ishidae* Matsumura, 1905 from Japan and later classified in Dictyopharinae (Orthopagini) by Emeljanov (1983). The genus has a rather complex taxonomic history, with several genera synonymized with it and 14 described species. The genus *Neoputala* Distant, 1914 was the first synonymized by Liang (2001), followed by *Leprota* Melichar, 1912 and *Piela* Lallemand, 1942 (Liang and Song 2006). Eight species had been recognized in the genus at that time. Subsequently, Zheng and Chen (2011) and Zheng et al. (2014) added one, then four, new species, all from China. In 2011, Emeljanov (2011) synonymized genus *Orodictya* Kirkaldy, 1913 with *Leprota*, the later genus being resurrected from the synonymy of all these changes is provided in Figure 1.

While sorting and identifying recently collected specimens, two new species, *S. baiseensis* sp. nov. and *S. maculata* sp. nov., were discovered from Guangxi province, China, and they are described here. *Saigona* now includes 16 species, with 15 of them endemic to China. A revised identification key to all species is provided, and the rather restricted distribution of this rather prolific genus is briefly discussed.

#### Materials and methods

The morphological terminologies follow Yang and Yeh (1994) for the head and body, Bourgoin et al. (2015) for the wing venation, Bourgoin (1987, 1993), and Yang and Yeh (1994) for male and female genitalia, respectively. Biogeographical realms terminology follows Holt et al. (2013). The specimens examined have been deposited in the Institute of Entomology, Guizhou University, Guiyang, China (**GUGC**). Dry specimens were used for the descriptions and illustrations. Genital segments of the specimens were macerated in boiling solution of 10% NaOH, transferred to preparations of glycerin jelly, and examined under a Leica MZ12.5 stereomicroscope. Photographs of adult habitus were obtained using a Keyence VHX-1000 system. Illustrations were scanned with Canon Cano Scan LiDE 200 and imported into Adobe Photoshop CS6 for labeling and composition of figures.

The following abbreviations are used in the text:

- **BL** body length (from apex of cephalic process to tip of forewings);
- HL head length (from apex of cephalic process to base of eyes);
- **HW** head width (including eyes);
- **FWL** orewing length.

The usual standardized notation is used for the wing venation as follow:

A1 first anal vein; bc, basal cell; MP media posterior;



**Figure 1.** Taxonomic history of the genus *Saigona* Matsumura, 1010 (uploaded from Bourgoin 2021). For each synonymized genus, their protonyms are in blue boxes, and the current valid name of the genus is in green. Red vertical arrows illustrate synonymy and the red cross the *status revivisco* of the genus *Leprota* Melichar, 1912.

CuA cubitus anterior; CuP cubitus posterior; RP radius posterior; Pcupostcubitus;ScPsubcosta posterior.

## Taxonomy

Family Dictyopharidae Spinola, 1839 Subfamily Dictyopharinae Spinola, 1839 Tribe Orthopagini Emeljanov, 1983

## Genus Saigona Matsumura, 1910

**Type species.** *Dictyophora* [sic] *ishidae* Matsumura, 1905 [= *Almana ussuriensis* Lethierry, 1878], by subsequent designation of Melichar (1912: 50).

*Neoputala* Distant, 1914: 412; Metcalf 1946: 78. Type species: *Neoputala lewisi* Distant, 1906 (by monotypy) [not *Neoputala capitata* Distant, 1914, as stated by Liang (2001: 236)], synonymised by Liang (2001: 236).

*Leprota* Melichar, 1912: 91; Metcalf 1946: 74. Type species: *Dictyophora* [sic] *ful-goroides* Walker, 1858, by original designation and monotypy. synonymized by Liang and Song (2006: 28).

*Piela* Lallemand, 1942: 72. Type species: *Piela singularis* Lallemand, 1942, by original designation and monotypy. synonymized by Liang and Song (2006): 28.

*Orodictya* Kirkaldy, 1913: 16. Type species: *Orodictya monticola* Kirkaldy, 1913; by original designation. Synonymized by Emeljanov (2011: 1144).

Leprota Melichar, 1912: 91. Type species: Leprota melichari Fennah, 1963; status revivisco according to Song et al. (2012: 218).

*Saigona* Matsumura, 1910: 110; Melichar 1912: 28, 50; Metcalf 1946: 47; Nast 1972: 84; Chou et al. 1985: 63; Anufriev and Emeljanov 1988: 482; Emeljanov 1993: 70; Liang 2001: 235; S. Matsumura 1941: 163; Liang and Song 2006: 28; Zheng and Chen 2011: 542; Zheng et al. 2014; Song et al. 2016: 350, 2018: 3.

**Diagnosis.** This species can be distinguished from other dictyopharid planthoppers by the combination of the following diagnostic characters: (1) general color ochraceous or fuscous; (2) vertex and most of genae marked with numerous yellowish or pale brown speckles; (3) cephalic process relatively broad and long; (4) vertex with median longitudinal carina obsolete, posterior region obviously higher than pronotum; (5) legs moderately long, fore femur with a short and blunt spine near apex; hind tibiae with 5 or 6 lateral black-tipped spines and 8 apical black-tipped teeth, spinal formula 8/ (9-12)/(9-12); (6) aedeagus with a pair of phallobasal conjunctival processes apically and phallobase sclerotized and pigmented, with two membranous lobes apically.

**Distribution.** China (Fujian, Guangdong, Guangxi, Heilongjiang, Henan, Hubei, Hunan, Jilin, Jiangxi, Shaanxi, Sichuan, Taiwan, Yunnan, Zhejiang, Gansu, Guizhou); Indo-China; Japan (Hokkaido, Honshu); Russia (Primorye, Far East); Korea (South).

## Key to species of the genus Saigona

(Modified from Liang and Song (2006), as updated by Zheng et al. 2014).

1	Vertex with cephalic process short, shorter than pronotum and mesonotum combined
_	Vertex with cephalic process long, longer than or nearly as long as pronotum and mesonotum combined
2	Postclypeus yellowish or yellowish brown
_	Postclypeus fuscous
3	Mesonotum with a yellowish stripe along median longitudinal carina
_	Mesonotum without a yellowish stripe along median longitudinal carina
	(Zheng et al. 2014: fig. 51) S. dicondylica Zheng, Yang & Chen
4	Pygofer short and broad in lateral aspect, posterior margin straight and angu-
	larly excavated at apical 1/4 apex to accommodate anal tube, aedeagus with
	phallobase having apical ventral membranous lobe with numerous fine spines
	at apex (Liang & Song, 2006: fig. 80, fig. 84)S. ussuriensis (Lethierry)
-	Pygofer large and broad in lateral aspect, posterior margin nearly straight,
	and gently excavated at apical 1/3 to accommodate anal tube, aedeagus with
	phallobase having apical dorsal and ventral membranous lobes with numer-
	ous fine spines at apex (Liang and Song 2006: figs 50, 54)
	S. latifasciata Liang & Song
5	Frons with lateral carinae not reaching frontoclypeal suture (Liang and Song
	2006: fig. 58)
-	Frons with lateral carinae almost reaching frontoclypeal suture
6	Aedeagus with phallobasal conjunctival processes spiraled dorsally (Liang and
	Song 2006: fig. 44) S. henanensis Liang & Song
-	Aedeagus with phallobasal conjunctival processes not spiraled dorsally (Zheng
	et al. 2014: fig. 10) S. anisomorpha Zheng, Yang & Chen
7	Cephalic process bulbous apically, with 3 pairs of symmetrical knob-like pro-
	tuberance on lateral regions
-	Cephalic process not bulbous apically, without knob-like protuberance on
	lateral regions
8	Aedeagus with phallobasal conjunctival processes not produced out of phal-
	lobase (Zheng and Chen 2011: fig. 10)S. saccus Zheng & Chen
-	Aedeagus with phallobasal conjunctival processes produced out of phal-
	9
9	Pygoter posterior margin with an elongate, acute process on dorsocaudal mar-
	gin (Liang and Song 2006: fig. 20); phallobase with membranous lobe simple
	round in ventral view (Liang and Song 2006: fig. 23) <i>S. fulgoroides</i> (Walker)
-	Pygoter posterior margin without an alongate process on dorsocaudal mar-
	gin (Fig. 8); phallobase with membranous lobe complex in ventral view
	(Fig. 13) S. baiseensis Zheng & Chen, sp. nov.

10	Tip of cephalic process with a yellow spot11
_	Tip of cephalic process without a yellow spot12
11	Frons with lateral carinae almost reaching frontoclypeal suture (Liang and
	Song 2006: fig. 15)
_	Frons with lateral carinae reaching the eyes, but not frontoclypeal suture,
	pygofer posterior margin with a slightly blunt process dorsally (Fig. 25); ae-
	deagus with phallobasal conjunctival processes unequal in length (Fig. 28)
	S. maculata Zheng & Chen, sp. nov.
12	Mesonotum with very narrow, yellowish stripe along median longitudinal
	carinaS. taiwanella Matsumura
_	Mesonotum with broad, yellowish stripe along median longitudinal carina13
13	Posterior margin of pygofer produced into a large process dorsally (Zheng et
	al. 2014: fig. 40)S. tenuisa Zheng, Yang & Chen
_	Posterior margin of pygofer not produced into a large process dorsally14
14	Aedeagus with phallobase having apical dorsal and ventral membranous lobes
	(Liang and Song 2006: fig. 74)S. sinicola Liang & Song
_	Aedeagus with phallobase having apical ventral membranous lobes
15	Aedeagus with phallobasal conjunctival processes subparallel apically (Zheng
	et al. 2014: fig. 21) S. daozhenensis Zheng, Yang & Chen
_	Aedeagus with phallobasal conjunctival processes diverging apically (Liang
	and Song 2006: fig. 63)

#### Saigona baiseensis Zheng & Chen, sp. nov.

http://zoobank.org/BC9FDBB3-4918-4E0C-B875-D242F91DE8AA Figures 1–20

Type locality. Tianlangping Baise, Guangxi Zhuang Autonomous Region, China.

**Diagnosis.** This species can be distinguished from other *Saigona* species by the combination of the following diagnostic characters: (1) pygofer large and broad in lateral view, posterior margin with a blunt dorsal process; (2) aedeagus with phallobasal conjunctival processes unequal in length; (3) phallobase narrow and long, curved dorsally, with 2 apical membranous dorsal apical lobes (Fig. 12), dorsal round and large; ventral lobes (Fig. 13) small and slender, with another small membranous lobe on it.

**Description.** *Measurement.* ♂, BL: 17.4–18.9 mm; HL: 1.5–1.6 mm; HW: 1.4–1.5 mm; FWL: 11.3–13.2 mm. ♀, BL: 18.9–20.2 mm; HL: 1.6–1.7 mm; HW: 1.4–1.5 mm; FWL: 13.2–14.6 mm.

**Coloration.** General color dark, marked with fuscous and ochraceous (Figs 2–5). Vertex brown with median carina very faint, lateral margins dark. Genae dark, yellow ventroposteriorly near antennae (Fig. 4). Eyes dark brown, lateral ocelli yellowish, antenna brown and areas surrounding ocellus and antenna beneath eye yellowish. Frons dark brown with yellowish speckles (Fig. 3). Postclypeus and anteclypeus pale brown. Pronotum dark with scattered white speckles; mesonotum dark, with broad median longitudinal yellowish



**Figures 2–5.** *Saigona baiseensis* Zheng & Chen sp. nov. **2** male, holotype, dorsal view **3** male, head and thorax, dorsal view **4** male, head, frons and clypeus, lateral view **5** male, head and pronotum, lateral view. Scale bars: 2 mm (**2–5**).

stripe. Ventral thorax and fore femur dark, other areas yellow. Legs ochraceous except coxae which are dark. Forewing venation brown and pterostigma dark. Abdomen dark with scattered white speckles and median longitudinal yellowish stripe. Male genitalia black.

*Head and thorax.* Head (Figs 2–5) longer than pronotum and mesonotum combined (1.45:1). Vertex (Fig. 3) with median carina very faint, only conspicuous at apex and base; lateral carinate margins sinuate. Frons (Fig. 3) with lateral carinae reaching to front of eyes. Pronotum (Figs 2, 3, 5) with median carina distinct, lateral carinae very faint. Mesonotum (Figs 1, 2) tricarinate on disc, lateral carinae curved towards median carinate at the front. Fore wings (Figs 2, 6) hyaline with ScP+R, MP and Cu branched apically, longer than widest part, with length to maximum width ratio of 3.0; stigma distinct, with 3 or 4 cells, veins with numerous fuscous setae. costal margin distinctly expanded into a narrow, sclerotized costal area, without transverse veins; ScP+R branched apically; MP bifurcating into MP<sub>1+2</sub> and MP<sub>3+4</sub> at basal 3/5 and beyond CuA; CuA forked near middle of forewing; 13 apical marginal cells between RP and CuA; Pcu and A<sub>1</sub> fusing at apical 2/5 of clavus. Hindwings (Fig. 7) well developed, legs moderately elongate, fore femora flattened and dilated; hind tibiae with 5 or 6 lateral black-tipped teeth, spinal formula 8/(10-12)/(10-12).

**Male** *genitalia*. Pygofer (Figs 8–10) large and broad in lateral view, posterior margin with a rounded lobe at level of venter of anal tube. Gonostyli (Figs 8, 9) relatively large and broad, apex sharply rounded, protruded posteriorly in lateral view on the



**Figures 6–13.** *Saigona baiseensis* Zheng & Chen, sp. nov. **6** forewing **7** hindwing **8** genitalia, lateral view **9** pygofer and gonostyli, ventral view **10** pygofer and anal tube, dorsal view **11** aedeagus, lateral view **12** aedeagus, ventral view **13** aedeagus, dorsal view. Scale bars: 2 mm (**6–10**), 0.5 mm (**11–13**).



**Figures 14–18.** *Saigona baiseensis* Zheng & Chen, sp. nov. **14** genitalia of female, ventral view **15** genitalia of female, dorsal view **16** anterior connective lamina of gonapophysis VIII (lateral view) **17** gonapophysis IX (ventral view) **18** gonoplacs (lateral view). Scale bars: 1 mm (**14, 15**), 0.5 mm (**16–18**). Gp, gonoplacs; Gx VIII, gonocoxae VIII; GxL, gonocoxae VIII with endogonocoxal lobe; GxP, gonocoxae VIII with endogonocoxal process; PCL, posterior connective lamina of gonapophysis IX; GpL, posterior lobe of the gonoplac; ACL, anterior connective lamina of gonapophyses.

outer surface of the gonostyli (Fig. 8). Aedeagus (Fig. 11) with phallobasal conjunctival processes unequal in length, left one obviously longer than right one; phallobase narrow and long, curved dorsally, with 2 apical membranous dorsal apical lobes (Fig. 12) dorsal round and large; ventral lobes (Fig. 13) small and slender, with another small membranous lobe on it. Segment X large in lateral view (Fig. 8), large, long, ovoid in dorsal view (Fig. 10), ratio of length to width at middle about 1.5.

**Female** *genitalia* (Figs 14, 16) with gonocoxae VIII with GxP membranous and flattened (Fig. 16). Gonopophyses VIII with anterior connective lamina of gonapophyses (ACL) moderately sclerotized with 7 unevenly sized teeth in lateral view. (Fig. 16). Gonopophyses IX (Fig. 17) with posterior connective lamina of gonapophysis IX (PCL) triangular, symmetrical in ventral view, connected at base and separated from 1/3 base. Gonoplacs (Fig. 18) with 2 sclerotized lobes: gonoplacs (Gp), with 3 or 4 long spines at apex, and posterior lobe of the gonoplac (GpL) with membranous structure at top. Segment X in dorsal view relatively round and large, with ratio of length to width at middle about 0.8 (Fig. 15).

**Type material.** *Holotype*  $\mathcal{J}$ , Tianlangping Baise, Guangxi Zhuang Autonomous Region, 24 Apr. 2012, Weicheng Yang. *Paratypes*,  $8\mathcal{J}\mathcal{J}2\mathcal{Q}\mathcal{Q}$ , same to holotype, Weibin Zheng, Zaihua Yang, Jiankun Long.

**Etymology.** This new species is named for the type locality, Baise City, Guangxi, China.

**Distribution.** China (Guangxi).

**Remarks.** This species is similar to *S. fulgoroides* (Walker, 1858) (Liang and Song 2006: figs 20, 23–25) but can be distinguished by the large, broad pygofer (in lateral view) with its posterior margin bearing a somewhat blunt process dorsally (vs sharp, dorsoposteriorly directed process near apex in *S. fulgoroides*); the aedeagus has a conjun

## Saigona maculata Zheng & Chen, sp. nov.

http://zoobank.org/74E6E965-788E-4065-BE18-DB7C5C2A9871 Figures 19–31

**Type locality.** Lang Ping town, Tianlin County, Guangxi Zhuang Autonomous Region, China.

**Diagnosis.** (1) Head moderately long, longer than pronotum and mesonotum combined. Cephalic process relatively long and robust, somewhat upturned; (2) pygofer with posterior margin sinuate in lateral view; (3) aedeagus with phallobasal conjunctival processes unequal in length.

**Description.** *Measurement.*  $\mathcal{J}$ , BL: 15.7 mm; HL: 2.1 mm; HW: 0.8 mm; FWL: 11.8 mm.

**Coloration.** General color dark brown, marked with fuscous and ochraceous speckles (Figs 19–22). Vertex dark brown with a yellowish green spot at top. Genae brown, eyes brown, ocellus yellowish, antenna brown and areas surrounding ocellus and anten-



**Figures 19–22.** *Saigona maculata* Zheng & Chen sp. nov. **19** male, holotype, dorsal view **20** male, head and thorax, dorsal view **21** male, frons and clypeus, ventral view **22** male, head and pronotum, lateral view. Scale bars: 2 mm (**19–22**).

na beneath eye yellowish. Frons yellowish brown. Postclypeus and anteclypeus yellow. Pronotum brown with median carina yellowish; lateral, ventrally curved areas yellowish. Mesonotum fuscous, with a narrow, yellow stripe along median longitudinal carina. Abdomen fuscous, scattered white speckle, with median longitudinal green stripe. Forewing venation brown and stigma dark. Legs ochraceous. Genitalia black.

*Head and thorax.* Head (Figs 19–22) moderately long, longer than pronotum and mesonotum combined (5.1:1). Cephalic process relatively long and robust, somewhat upturned. Vertex (Fig. 20) with median carina very faint, only conspicuous at base, lateral carinate margins curved in front of eyes. Frons (Fig. 21) with lateral carinate reaching to the front of eyes, not to frontoclypeal suture. Pronotum (Figs 19, 20, 22) with median carina distinct, lateral carinae very faint; mesonotum tricarinate on disc, lateral carinae curved towards median carinae at front.

*Fore wings* (Figs 19, 23) hyaline with Sc+R, M and Cu branched apically, longer than widest part, with length to maximum width ratio of 3.0; pterostigma distinct, with 2 cells, veins with numerous fuscous setae. costal margin distinctly expanded into a narrow, sclerotized costal area, without transverse veins; ScP+R branched apically; MP bifurcating  $MP_{1+2}$  and  $MP_{3+4}$  at basal 3/5 and beyond CuA; CuA forked into two branches near middle of forewing 14 apical cells between RP and CuA; Pcu and A<sub>1</sub> fusing in apical 2/5 of clavus. Hindwings (Fig. 24) well developed. Legs moderately elongate, fore femora flattened and dilated; hind tibiae with 5 lateral black-tipped spines and 8 apical black-tipped teeth, spinal formula 8/(9–11)/(10–12).



Figures 23–31. *Saigona maculata* Zheng & Chen sp. nov. 23 forewing 24 hindwing 25 genitalia, lateral view 26 pygofer and anal tube, dorsal view 27 pygofer and gonostyles, ventral view 28 aedeagus process 29 aedeagus, lateral view 30 aedeagus, ventral view 31 aedeagus, dorsal view. Scale bars: 2 mm (22–30), 0.5 mm (28–34).

**Male** *genitalia*. Pygofer (Figs 25–27) in lateral view with posterior margin slightly sinuate. Gonostyli (Figs 25, 27) relatively large, broad in lateral view (Fig. 25), apex sharply rounded, apex sharply rounded, protruded posteriorly in lateral view on the



Figure 32. Geographical distribution of the genus Saigona species.

outer surface of the gonostyli. Aedeagus with phallobasal conjunctival processes unequal in length, left one obviously longer than right one (Fig. 28); phallobase narrow and long, curved dorsally, with 2 apical membranous dorsal apical lobes (Fig. 31) sclerotized on both sides with circular membranous processes in the middle; ventral lobes (Fig. 30) large, membranous fold, with spines at base. Segment X (Figs 25, 26) large, nearly triangular in lateral view; round in dorsal view, ratio of length to width at middle about 1.5:1.

#### Female. unknown.

**Type material.** *Holotype* ♂, Lang Ping town, Tianlin County, Guangxi Zhuang Autonomous Region, 23 Apr. 2012, Zaihua Yang. *Paratypes*, 1♂, same as holotype.

**Etymology.** The name of the new species is derived from the Greek word *maculata* (spotted), in reference to the vertex with a yellowish green spot at the apex.

**Distribution.** China (Guangxi).

**Remarks.** This species is similar to *S. tenuisa* Zheng, Yang & Chen, 2014 but can be distinguished from the latter by the pygofer with the posterior margin slightly sinuate in lateral view and the aedeagus with the phallobasal conjunctival processes unequal in length.

#### **Discussion and conclusions**

Species of *Saigona* are externally similar to those of *Leprota* Melichar, 1912, but *Leprota* can be separated from *Saigona* by the following: 1) body generally rust-brown or rust-red above, without pale speckles in *Leprota* (vs ochraceous or fuscous, with pale

speckles on the vertex and most of the genae in *Saigona*); 2) head covered in numerous irregular transverse wrinkles in *Leprota* (vs not covered irregular transverse wrinkles, head long and broad, distinctly produced into a cephalic process, vertex with lateral margins carinate, sinuate in front of eyes in *Saigona*); 3) forewings elongate, with numerous netted veins on apical 1/5 in *Leprota* (vs relatively short, with sparse netted veins on apical area in *Saigona*); and 4) the fore femora normal in *Leprota* (the fore femora flattened and dilated, with short and blunt spine near apex in *Saigona*) (Song et al. 2012).

The distribution of the genus is quite restricted (Fig. 32), extending from the northeastern Sino-Japanese to north-eastern Oriental realms. *Saigona ussuriensis* is widely distributed in the north but not crossing into the Palearctic realm, and *S. henannensis*, *S. fuscoclypeata*, *S. sinicola* and *S. robusta* are Sino-Japanese. All other species occur in south and eastern continental China and can be considered as Oriental; *S. fulgoroides* and *S. taiwanella* from Taiwan are also in the Oriental group of species. One species, *S. capitata* from South Korea provides the south and western limits of the genus. Absent from the Palearctic and India, and wrongly reported from Indonesia (Sumatra, Borneo) (Song et al. 2012), *Saigona* is almost exclusively a Chinese endemic genus. However, the species diversity observed in this genus suggests that the discovery of additional species in the Indochinese peninsula cannot be excluded.

#### Acknowledgements

This project was supported by the Program of Guizhou Science and Technology Foundation ([2019] 1242) and the National Natural Science Foundation of China (grant no. 32060343 and 31472033); the Science and Technology Support Program of Guizhou Province (grant no. 20201Y129); and the Program of Excellent Innovation Talents, Guizhou Province (grant no. 20154021). I appreciate the help given to me by David Tembong Cham in revising the English of this manuscript.

#### References

- Anufriev GA, Emeljanov AF (1988) Dictyopharidae. In: Lehr PA (Ed.) Key to Insects of Soviet Far East of the USSR, Vol. 2. Homoptera and Heteroptera. Nauka, Leningrad, 480–483. [in Russian]
- Bourgoin T (1987) A new interpretation of the homologies of the Hemiptera male genitalia, illustrated by the Tettigometridae (Hemiptera, Fulgoromorpha). In: Vidano C, Arzone A, Consiglio nazionale delle ricerche (Eds) Proceedings of the 6<sup>th</sup> Auchenorrhyncha meeting Turin, Italy, 7–11 September, 113–120.
- Bourgoin T (1993) Female genitalia in Hemiptera Fulgoromorpha, morphological and phylogenetic data. Annales de la Société Entomologique de France (Nouvelle Série) 29: 225–244.

- Bourgoin T, Wang RR, Asche M, Hoch H, Soulier-Perkins A, Stroiński A, Yap S, Szwedo J (2015) From micropterism to hyperpterism: recognition strategy and standardized homology-driven terminology of the forewing venation patterns in planthoppers (Hemiptera: Fulgoromorpha). Zoomorphology 134(1): 63–77. https://doi.org/10.1007/s00435-014-0243-6
- Bourgoin T (2021) FLOW (Fulgoromorpha Lists On the Web), a world knowledge base dedicated to Fulgoromorpha. Version 8. http://hemiptera-databases.org/flow/ [accessed 2021-4-1]
- Chou I, Lu JS, Huang J, Wang SZ (1985) Economic Insect Fauna of China. Fasc. 36, Homoptera: Fulgoroidea. Science Press, Beijing, 152 pp. [in Chinese with English summary]
- Distant WL (1914) Some additions to the genera and species in the homopterous family Fulgoridae. Annals and Magazine of Natural History 8(13): 409–413. https://doi. org/10.1080/00222931408693503
- Emeljanov AF (1980) Phylogeny and evolution of subfamily Orgeriinae (Homoptera, Dictyopharidae). Tshtenija pamjati Cholodkovskovo 32: 3–96. [in Russian]
- Emeljanov AF (1983) A species of Dictyopharidae from the Cretaceous of the Taimyr Peninsula (Insecta, Homoptera). Paleontologicheskii Zhurnal 3: 79–85. [in Russian]
- Emeljanov AF (1993) Morphological peculiarities of the larvae of the family Dictyopharidae (Homoptera). 1. General characteristic and a key to genera of the Palaearctic fauna. Entomologicheskoe Obozrenie 72(4): 794–812. [In Russian; English translation in Entomological Review 73: 59–78, 1994] https://doi.org/10.2307/25177406
- Emeljanov AF (2008) New genera and species of the family Dictyopharidae (Homoptera), with notes on the systematics of the subfamily Dictyopharinae. Entomologicheskoye Obozreniye 87: 360–396. [In Russian; English translation in Entomological Review 88: 296–328, 2008] https://doi.org/10.1134/S0013873808030056
- Emeljanov AF (2011) Improved tribal delimitation of the subfamily Dictyopharinae and description of new genera and new species (Homoptera, Fulgoroidea, Dictyopharidae). Entomologicheskoe Obozrenie 90: 299–328. [English translation in Entomological Review 91: 1122–1145, 2011] https://doi.org/10.1134/S0013873811090053
- Emeljanov AF, Kuznetsova VG, Nokkala C, Nokkala S (2005) Phylogeny and evolution of the subfamily Orgeriinae (Homoptera, Dictyopharidae). In: Abstracts of the 12<sup>th</sup> International Auchenorrhyncha Congress and the 5<sup>th</sup> International Workshop on Leafhoppers and Planthoppers of Economic Importance. University of California, Berkeley, CA, Symposium Auchenorrhychan Feeding Processes, S-15.
- Holt BG, Lessard JP, Borregaard MK, Fritz SA, Araújo MB, Dimitrov D, Fabre PH, Graham CH, Graves GR, Jønsson KA, Nogués-Bravo D, Wang Z, Whittaker RJ, Fjeldså J, Rahbek C (2013) An update of Wallace's zoogeographic regions of the world. Science 339: 74–78. https://doi.org/10.1126/science.1228282
- Kirkaldy GW (1913) On some new species of leafhoppers. Part 1. Bulletin Hawaiian Sugar Planters' Association Experiment Station, Division of Entomology, Honolulu 12: 7–27.
- Lallemand V (1942) Notes sur quelques espèces recueillies par Le R. Piel (Musée Heude Shanghai) et Le R. P. de Cooman (Hoa Binh, Tonkin). Notes d'Entomologie Chinoise 9: 69–77.
- Liang AP (2001) Taxonomic notes on Oriental and eastern Palaearctic Fulgoroidea (Hemiptera). Journal of the Kansas Entomological Society 73(4): 235–237.

- Liang AP, Song ZS (2006) Revision of the Oriental and eastern Palaearctic planthopper genus *Saigona* Matsumura, 1910 (Hemiptera: Fulgoroidea: Dictyopharidae), with descriptions of five new species. Zootaxa 1333: 25–54. https://doi.org/10.11646/zootaxa.1333.1.2
- Matsumura S (1910) Monographie der Dictyophorinen Japans. Transactions of the Sapporo Natural History Society 3: 99–113.
- Melichar L (1912) Monographie der Dictyophorinen (Homoptera). Abhandlungen der k. k. Zoologisch-Botanischen Gesellschaft in Wien 7(1): 1–221.
- Metcalf ZP (1946) General catalogue of the Hemiptera, Fasc. IV. Fulgoroidea, Part 8 Dictyopharidae. Smith College, Northampton, Massachusetts, 246 pp.
- Muir F (1923) On the classification of the Fulgoroidea (Homoptera). Proceedings of Hawaiian Entomological Society 5: 205–247.
- Muir F (1930) On the classification of the Fulgoroidea. The Annals and Magazine of Nature History 80: 461–473. https://doi.org/10.1080/00222933008673237
- Nast J (1972) Palaearctic Auchenorrhyncha (Homoptera). An annotated check list. Polish Scientific Publishers, Warszawa, 550 pp.
- Song ZS, Deckert J, Liang AP (2012) Revision of the Oriental genus *Leprota* Melichar (Hemiptera, Fulgoromorpha, Dictyopharidae), with description of a new species from northern Borneo, Malaysia. Deutsche Entomologische Zeitschrift 59: 219–226.
- Song ZS, Webb M, O'Brien L, Liang AP (2016) Phylogenetic analysis of the Oriental genera of Orthopagini Emeljanov, 1983 (Hemiptera: Fulgoromorpha: Dictyopharidae: Dictyopharinae), with a systematic revision of the genus Centromeria Stål, 1870. Zoological Journal of the Linnean Society 178: 33–87. https://doi.org/10.1111/zoj.12401
- Song ZS, Bartlett CR, O'Brien L, Liang AP, Bourgoin T (2018) Morphological phylogeny of Dictyopharidae (Hemiptera: Fulgoromorpha). Systematic Entomology 43: 637–658. https://doi.org/10.1111/syen.12293
- Szwedo J (2008) A new tribe of Dictyopharidae planthoppers from Eocene Baltic amber (Hemiptera: Fulgoromorpha: Fulgoroidea), with a brief review of the fossil record of the family. Palaeodiversity 1: 75–85.
- Yang CT, Yeh WB (1994) Nymphs of Fulgoroidea (Homoptera: Auchenorrhyncha) with descriptions of two new species and notes on adults of Dictyopharidae. Chinese Journal of Entomology, Special Publication 8: 1–187.
- Zheng YL, Chen XS (2011) A new species of genus *Saigona* (Hemiptera: Fulgoroidea: Dictyopharidae) from China. Sichuan Journal of Zoology 30(4): 541–543. [in Chinese with English summary]
- Zheng YL, Yang L, Chen XS (2014) Four new species of the genus Saigona Matsumura (Hemiptera, Fulgoromorpha, Dictyopharidae) from China. ZooKeys 462: 27–41. https://doi. org/10.3897/zookeys.462.7500