RESEARCH ARTICLE



# Two hymenopteran egg sac associates of the tent-web orbweaving spider, Cyrtophora citricola (Forskål, 1775) (Araneae, Araneidae)

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# Abstract

We report the discovery of two wasp species emerging from egg sacs of the spider *Cyrtophora citricola* (Forskål 1775) collected from mainland Spain and the Canary Islands. We identify one as *Philolema palanichamyi* (Narendran 1984) (Hymenoptera, Eurytomidae) and the other as a member of the *Pediobius pyrgo* (Walker 1839) species group (Hymenoptera, Eulophidae). This is the first report of *Philolema* in Europe, and the first documentation of hymenopteran egg predators of *C. citricola*. The latter finding is particularly relevant, given the multiple invasive populations of *C. citricola* in the Americas and the Caribbean, where neither egg sac predation nor parasitism is known to occur. We describe rates of emergence by *Ph. palanichamyi* from spider egg sacs collected from the southern coast of Spain and estimate sex ratios and body size variation among males and females. We also re-describe *Ph. palanichamyi* based on the female holotype and male paratype specimens.

# Keywords

colonial spider, Eulophidae, Eurytomidae, natural enemy, orbweaver

# Introduction

*Cyrtophora citricola* (Forskål, 1775) is a widespread tent-web spider historically occurring in Mediterranean Europe, Asia, the Middle East, and across Africa (Forskål 1775; Kullmann 1958, 1959). Its distribution has burgeoned across the globe recently, with reported introductions in Colombia (Floréz-Daza 1996; Dossman et al. 1997; Levi 1997), the Dominican Republic (Alayón-García et al. 2001; Serra 2005), USA (Mannion et al. 2002), Cuba (Alayón-García 2003), Brazil (Álvares and De Maria 2004), Haiti (Starr 2005), Costa Rica (Víquez 2007), Jamaica (Crews et al. 2015), and other Caribbean islands (Armas 2010; Sewlal and Starr 2011).

The ecological impact of *C. citricola* in its new invasive ranges remains largely unknown; its colonial, group-living behavior results in wide-spanning networks of individual capture webs (Leborgne et al. 1998) that can swathe large areas of the trees and other plants where they build webs (Edwards 2006). This is why these spiders have been termed nuisances in Colombian coffee plantations and Dominican citrus trees, as well as general backyard pests in Florida (Cárdenas-Murillo et al. 1997; Serra 2005; Edwards 2006). Given ongoing range expansions of *C. citricola* in some of its invasive ranges (Sánchez-Ruiz and Teruel 2006; Martín-Castejón and Sánchez-Ruiz 2010), knowledge of natural enemies from its native range is of particular interest.

In this study, we report two hymenopteran species reared from *C. citricola* egg sacs collected from their native Spanish range. We reared the egg predator *Philolema palanichamyi* (Narendran, 1984) from egg sacs collected from the Iberian Peninsula as well as Tenerife in the Canary Islands. We only found *Pediobius* sp., a member of the *pyrgo* (Walker, 1839) species group, in Tenerife egg sacs. It is a suspected hyperparasitoid; these parasitize primary parasitoids. *Philolema palanichamyi* is one of several related species (formerly *latrodecti* species group of *Eurytoma*) that uses spider eggs as a larval host, whereas other species of *Philolema* attack insects as primary or secondary parasitoids (Noyes 2018).

The discovery of a *Philolema* species in Spain with *C. citricola* as a host is interesting for several reasons. Firstly, *Philolema* has not been documented in Europe, having previously been recorded with an Afrotropical, Neotropical, and Oriental distribution (van Noort 2019). Secondly, *Ph. palanichamyi* was originally recorded as an egg predator of *Cyrtophora cicatrosa* (Stoliczka, 1869) in India but has never been reported with *C. citricola* (Narendran 1994). In fact, only one other parasitoid of *C. citricola* has been previously reported: *Eurytoma cyrtophorae* Zerova from Yemen (Zerova et al. 2008). It is clear from the illustrations and description that *E. cyrtophorae* belongs in *Philolema*, and is perhaps synonymous with *Ph. palanichamyi*. We were unable to compare our specimens with the type specimen of *E. cyrtophorae*, as it was unavailable for examination.

Members of the *Pe. pyrgo* species group are most often primary parasitoids of Lepidoptera or hyperparasitoids through primary hymenopteran parasitoids. Spider associations have been previously documented in this genus: Jamali et al. (2018) described *Pe. hebbalensis* Jamali, Zeya & Veenakumari, 2018 from an unidentified spider egg sac in India and Schoeninger et al. (2015) report *Pe. pyrgo* as a primary predator in egg sacs of *Latrodectus geometricus* Koch, 1841 from Brazil. A series in the Smithsonian

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National Museum of Natural History (**USNM**) is labeled as reared from the egg sacs of *Latrodectus "scomotricus*". This specific epithet does not apply to any described *Latrodectus* species and we suspect that this is a transcription error of "*geometricus*", which is known from Florida (Pearson 1936).

Here, we describe our observations of *Ph. palanichamyi* emerging from *C. citricola* egg sacs in the wild and present preliminary data collected on the prevalence of egg parasitism, mean parasitoid emergence, and mean spiderling emergence in parasitized egg sacs from wild-collected egg sacs. We also report on the morphological variation within both sexes, provide mean body size measurements, and describe sex ratio variation. In laboratory settings we test whether wasps can infect *C. citricola* egg sacs without intermediate hosts. Importantly, we also re-describe the female holotype and for the first time a male paratype of *Ph. palanichamyi*.

# Materials and methods

We hand-collected *C. citricola* egg sacs between 30 May and 16 June 2016 and between 4 October and 1 November 2018 on the Iberian Peninsula. These areas experience hot summer Mediterranean (Cádiz and Málaga provinces) and cold semi-arid steppe (Murcia and Valencia provinces) Köppen climates of south and east coastal Spain. We further collected egg sacs from Tenerife, Canary Islands, between 29 May and 16 June 2018, in mountainous habitats of the north and dry, scrub habitats of the south (Figs 1, 2). In all areas, we primarily found *C. citricola* colonies in sun-exposed habitats with non-native succulents, such as *Opuntia* spp. (Cactaceae), *Austrocylindropuntia* spp. (Cactaceae), and *Agave* spp. (Asparagaceae) (Chuang and Leppanen 2018). These were most commonly identified to *Opuntia ficus indica* L. (Mill.), *Austrocylindropuntia subulata* (Muehlenpf.) Backeb., and *Agave americana* L. (Deltoro, personal communication) and found in dry, grass-dominated habitats by roadsides or cultivated on rural private property (Figs 3–5).

In the field, we transferred egg sacs to 59.1 mL clear polypropylene containers with clear polyethylene lids. These were then transported to the University of Tennessee (Knoxville, Tennessee, USA) and stored in the laboratory at 21.0–23.5 °C and a 14 (light): 10 (dark) hour photoperiod. We misted the egg sacs weekly with water for up to eight weeks after the collection period. The wasps were confined to cups as they emerged, and we killed them by freezing and made post-mortem wasp counts and egg sac dissections afterwards.

We estimated *Ph. palanichamyi* variation in body size and sex ratio from those individuals reared from 11 egg sacs. In two cases, two egg sacs were conjoined and the wasps emerged from each of the pairs into their one shared container. The remaining seven egg sacs were kept in seven separate containers. Hence, we used nine batches of wasps, seven originating from single egg sacs and two from pairs of egg sacs. After we froze them, wasps and egg sacs were allowed to air-dry. We dissected egg sacs by teasing the looser silk-domed surface from the firmer silk flat 'floor'. All of the wasps that had emerged, or were still inside the egg sac, were sexed and measured provided that



**Figures 1–2.** Points indicate where *Ph. palanichamyi* was found in **I** Tenerife, Canary Islands as well as **2** along the southern coast of Spain. *C. citricola* is known from the shaded regions (Cardoso and Morano 2010). *Pe. pyrgo* was only found at Tenerife locations.



**Figures 3–5.** Representative *C. citricola* colonies in mainland Spain and the Canary Islands where *Ph. palanichamyi* and *Pe. pyrgo* were observed or collected.

they were in suitable condition. Wasps without a gaster or that had failed to emerge completely from the pupal case were not measured. We measured the distance from the front of the pronotum to the tip of the metasoma to estimate body size using a squared graticule in a microscope eyepiece at uniform magnification. The head was omitted from the body length measurement because it had become detached from a number of specimens. In total, 665 wasps were sexed and 576 were measured.

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To test whether *Ph. palanichamyi* reared in the laboratory could infect *C. citricola* egg sacs, we placed a freshly laid egg sac (produced under laboratory conditions) in a container with multiple adult male and female wasps. After seven days, we removed the egg sac and placed it in a separate container at room temperature  $(20\pm 2 \text{ °C})$  for four weeks after which we carefully opened the egg sac to reveal its contents.

For specimen preparation, we preserved the wasps in 80% ethanol and dehydrated them through increasing concentrations of ethanol before transferring them to hexamethyldisilazane (HMDS) (Heraty 1998) for point-mounting. We used a Leica 205c stereomicroscope with 10X oculars and a Leica LED ring light source for pointmounted specimen observation.

We took scanning electron microscope (SEM) images with a Hitachi TM3000 (Tungsten source). We adhered body parts of disarticulated specimens to a 12.7 × 3.2 mm Leica/Cambridge aluminum SEM stub by a carbon adhesive tab (Electron Microscopy Sciences, #77825-12). We used a Cressington Scientific 108 Auto to sputter coat stub-mounted specimens with gold-palladium from multiple angles to ensure complete coverage (~20–30 nm coating). To capture the habitus image of the holotype and recently reared female we used a Macropod Pro 3D system (Canon 6D Mark II body) with a Canon EF 70-200 mm telephoto with affixed 10× objective lens (Macroscopic Solutions, LLC). Our image series were merged into a single in-focus, composite image with the program Zerene Stacker (ver. 1.04). Post-imaging processing was completed with built-in editing tools in Zerene Stacker, Photoshop CS4 and InDesign CS5.

Specimens from the Smithsonian Institution National Museum of Natural History and borrowed holotypes of *Ph. palanichamyi* and *Ph. lankana* Narendran, 1994 were compared with our reared specimens by MG. RA provided independent confirmation of chalcidoid identity. We used keys in Bouček (1965) and Cao et al. (2017) for determining *Pediobius* and deposited specimens reared as part of this study in the National Museum of Natural History, Washington, DC.

The hymenopteran terminology we use for surface sculpture follows Harris (1979) and for morphology follows Gibson et al. (1997). Fu is used as an abbreviation for funicular segment, Gt for gastral tergum, and Gs for gastral sternum. We took several measurements, including the following: body length, in lateral view from the anterior projection of the face to the tip of the metasoma; head width through an imaginary line connecting the farthest lateral projection of the eyes; head height through an imaginary line from the vertex to the clypeal margin bisecting both the median ocellus and the distance between the toruli; malar space, in lateral view between the ventral margin of the eye and lateral margin of the oral fossa; posterior ocellar line (POL), the shortest distance between the posterior ocelli; ocular ocellar line (OOL), the shortest distance between the lateral margin of the posterior ocellus and the eve orbit; posterior ocellar diameter (POD), the longest diameter of the posterior ocellus; marginal vein, the length coincident with the leading forewing edge to the base of the stigmal vein; stigmal vein, the length between its base on the marginal vein and its apex; and postmarginal vein, the length from the base of the stigmal vein to its apex on the leading forewing edge. We measured the mesosomal sclerites and metasomal terga dorsally

along the midline. **LS** stands for multiporous plate sensilla; wing venation abbreviations are **MV** (marginal), **PMV** (postmarginal), and **STG** (stigmal).

We use the following abbreviations for collections: **USNM** (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA) and **BMNH** (The Natural History Museum, London, England).

# Results

# Field and laboratory observations *Philolema palanichamyi* emergence rates

In the field, we observed *Ph. palanichamyi* emerging from *C. citricola* egg sacs on 11 June 2016 in Málaga, Andalusia. Owing to the central location of the egg sacs within a web, dozens of these wasps were immediately snared in the surrounding web or consumed by nearby *C. citricola* colony members.

*Philolema palanichamyi* emerged from 43 of 103 groups of 1-5 (mean =  $1.86 \pm 0.11$ ) conjoined egg sacs collected in Cádiz (21 of 37 groups), Málaga (14 of 21 groups), Murcia (8 of 34 groups), but not in Valencia (0 of 11 groups) (Figs 1, 2; Table 1). A mean of  $56.7 \pm 11.4$  (median = 43) wasps emerged from 18 singly confined egg sacs. A mean of  $38.6 \pm 22.2$  (median = 13) spiderlings emerged in 7 of 18 singly confined egg sacs with wasps and  $102.4 \pm 21.0$  (median = 117) in 18 of 34 egg sacs without wasps.

### Philolema palanichamyi body sizes and sex ratios

We sexed 665 adults from 11 egg sacs. Between 5 and 151 wasps emerged from single egg sacs (median = 59; mean = 67.29). Wasps emerged from up to eight exit holes made without an observed preference in either the domed surface or the silken floor of the egg sac. The sex ratio varied greatly among egg sacs from 5.0% to 75.0% males (median = 20.0%; mean 24.6% males) (Table 2). Of the 576 adults measured, females were slightly larger than males, although both male and female body sizes varied greatly both within and among egg sacs (Fig. 6): mean female body size was 1.68 mm

Ph. palanichamyi parasitism of C. citricola egg sacs by location									
Location	# Egg sac	Parasitism rate (%)							
	With wasps	Total							
Cádiz	21	37	56.8						
Málaga	14	21	66.7						
Murcia	8	33	24.2						
Valencia	0	12	0						
Total	43	103	41.7						

**Table 1.** Parasitism rate by *Ph. palanichamyi* for each of four collection locations. We describe parasitism rate per string of egg sacs ranging from 1–5 egg sacs per female.

ID	# Egg sacs	# Females	# Males	# Wasps	Sex ratio (% males)	Female av. length	Male av. length
1	1	4	1	5	20	2.16	1.98
2	1	5	15	20	75	1.71	1.69
3	1	21	1	22	4.5	1.82	1.56
4	1	55	4	59	6.8	1.71	1.29
5	1	60	20	80	25	1.97	1.8
6	2	50	33	83	39.8	1.47	1.14
7	2	84	26	110	23.6	1.6	1.35
8	1	109	25	134	18.7	1.52	1.35
9	1	119	32	151	21.2	1.75	1.57

Table 2. Female and male average lengths and sex ratios for each group of measured egg sacs.



**Figure 6.** Histograms showing the distribution of body sizes of females (green) and males (grey; any overlap between males and females is greyish green) from 9 batches of wasp emergences: 7 batches from single egg sacs and 2 batches from pairs of egg sacs.

(median = 1.69 mm; range: 0.84-2.41 mm) while mean male body size was 1.47 mm (median = 1.44 mm; range: 0.80-2.07 mm). It should be noted that 'body size' is less than the total body length because the head was not included in the measurement.

A variable primary sex ratio (i.e., that of deposited eggs) that favors females is usual in Chalcidoidea, enabled by their haplodiploidy. Several factors can influence this ratio in favor of an increased proportion of males (unfertilized eggs), including smaller hosts, less quality hosts, or more numerous hosts at increased density (Godfray 1994). None of these effects are apparent in our limited sample. Older female wasps may suffer a diminishing supply of stored sperm so that increasing numbers of unfertilized eggs are laid. This is one possible explanation for the brood that was 75% male. It is also possible that smaller males may be favored when larval food is limited.

# Parasitism under laboratory conditions

When we opened the egg sac presented in the laboratory to adult *Ph. palanichamyi*, several live wasp larvae were visible, confirming the association of *Ph. palanichamyi* with *C. citricola*. Furthermore, each larva appeared considerably larger than a single spider egg, suggesting that a single larva might feed on multiple eggs within the egg sac. This and the lower ratio of wasps to spiderlings found in parasitized compared with unparasitized egg sacs also suggests that this species is an egg predator, not an egg parasitoid. The first wasp offspring eclosed from its pupa seven weeks after the fresh egg sac was introduced to adult wasps.

# Taxonomy

# *Philolema palanichamyi* Narendran Figs 7–16

**Re-description.** Based on female holotype (Fig. 7; Fig. 8: female from specimens reared during this study, not used in re-description). Length 2.2 mm. Body black except the following: scape, pedicel, apex femur, apex and base tibia (yellowish brown to brown), tarsus (golden), flagellum, mid coxa, femur, and tibia (brown). Ovipositor sheaths brown. Wings hyaline, setation pale. Venation golden.

*Head* (Fig. 9).  $1.43 \times$  as broad as long, broader than mesosoma. Eye  $1.08 \times$  as long as malar space. POL  $2.0 \times$  as large as OOL; the latter  $1.88 \times$  as large as POD. Malar space  $0.93 \times$  as long as width of oral fossa and equal to height of eyes. Distance between toruli  $1.0 \times$  their own diameter. Adscrobal area subequal in width to acarinate antennal scrobes. Lower face striate, clypeus emarginate. Malar space with groove in dorsal half below eye, continuous with striation. Gena striate to umbilicate.

Antenna (Fig. 10). Scape linear,  $3.0 \times$  as long as broad. Pedicel short,  $1.29 \times$  as long as broad. Funicle 6-segmented, setae decumbent. Fu<sub>1</sub>  $1.30 \times$  as long as broad, Fu<sub>2</sub> just longer than broad, Fu<sub>3-5</sub> quadrate. Funiculars with single row LS. Clava 2-segmented,  $1.80 \times$  as long as broad; bearing also the same pattern of LS as for the funiculars; segments fused, bearing two rows of LS.

*Mesosoma* (Figs 11–13). 1.71× as long as broad. Pronotal collar 3.60× as broad as long; mesoscutum 1.25× as broad as long; mesoscutellum 1.33× as long as broad. Mesosoma dorsally umbilicate, interstices coriaceous. Notauli shallow, crenulate.



Figures 7–8. Philolema palanichamyi 7 female holotype 8 female habitus. Scale bars: 1 mm.

Puncturation of mesoscutellum somewhat sparser than that of mid lobe of mesoscutum; mesoscutellum overhanging postscutellum. Axillar grooves crenulate, shallow. Postscutellum punctured mesally. Propodeum sloping at an angle of about 80° with main axis of mesosoma, evidently convex from side to side, with incomplete areolate stripe mesally delimited submedian ridges, on either side irregularly areolate; setation fine, erect and proclinate between the spiracles, dense very long and reclinate laterally; spiracle elliptic at posterior margin of metanotum. Tegula umbilicate. Prepectus with lateral panel glabrous. Mesopectus anteriorly depressed as scrobes to receive forecoxae,



Figures 9–16. *Philolema palanichamyi* 9 female, anterior head 10 female antenna 11 female dorsal mesosoma 12 female mesosoma, lateral 13 female ventral mesosoma 14 female fore wing 15 female petiole 16 male antenna.

mesodiscrimen produced as beak-like prominence (Fig. 13, red arrow), scrobes glabrous anteriorly becoming coriaceous, adscrobal area umbilicate dorsally, coriaceous below; mesepisternum with femoral scrobe finely and densely reticulate; mesepimeron reticulate-carinate on ventral 1/3, with longitudinal carinae in dorsal 2/3. Metepimer-on umbilicate, setae dense and long. Mesotrochantinal plate entirely sclerotized, its anterior carinate margin emarginate at mesofurcal pit. Metepisternum with lateral lobes anterad metacoxal foramina that overhang metafurcal pits.

*Legs* (Fig. 13). Procoxa depressed anteriorly with diagonal carina delimiting depression (Fig. 13, blue arrow). Mesocoxa without lamella. Metacoxa bare dorsobasally, mostly coriaceous to finely reticulate.

*Forewing* (Fig. 14). Setation fine and pale making wing appear sparsely setose, MV:PMV:STG as 20:25:19. Stigma with line of four sensilla placodea; parastigma bearing 3 adjacent sensilla placodea forming a triangle. Cubital and basal folds setose; basal cell with 3–6 irregularly distributed setae.

*Petiole* (Fig. 15). In dorsal view just longer than broad, carinate anteriorly and produced anterolaterally as angulate processes, surface rugulose.

**Gaster.** Smooth dorsally, very faintly alutaceous laterally, just shorter than mesosoma, Gt4 longest tergum, ~2.0× as long as  $Gt_3$ .  $Gt_1$  and  $Gt_2$  asetose,  $Gt_3$  and  $Gt_4$ with a few setae dorsolaterally [some appear to have been abraded],  $Gt_5$  and  $Gt_6$  and syntergum more densely setose.

**Male** (Figs 16–18). Similar to female in color and sculpture, differing in form of antenna and metasoma as below.

Antenna (Fig. 16). Scape 2.11× as long as broad, with smooth surface on protuberant ventral plaque bearing pores (visible under high magnification only) on apical two thirds. Pedicel 1.50× as long as broad. Funicle 5-segmented with funiculars progressively shortening, each funicular with two whorls of long setae and a single row of LS visible;  $F_{1-3}$  asymmetric themselves progressively shortening;  $Fu_{4-5}$  symmetric, subquadrate. Clava 2-segmented, 2.30× as long as broad.

**Petiole** (Figs 17, 18). 1.66× as long as broad, slightly carinate anteriorly, its surface reticulate.

*Gaster.* Smooth dorsally, very faintly alutaceous laterally, somewhat shorter than mesosoma, Gt4 longest tergum, ~1.25× as long as Gt3. Gt1 and Gt2 asetose, Gt3 and Gt4 with a few setae dorsolaterally [some appear to have been abraded], Gt5 and Gt6 and syntergum more densely setose.

*Variation.* Specimens vary greatly in size from approximately 0.8–2.5 mm total length. Prominence and extent of morphological characters diminishes with decreasing body size; for example, general body sculpture is less apparent in small specimens.

**Material examined. Holotype**,  $\Im$ : INDIA: Timadu, Palani, 1992, coll. Palanichamy, Host *Cyrtophora cicatrosa* (spider); **holotype**, *Desantisca palanichamyi*  $\Im$ , sp. nov., det. Narendran 1983; B.M. TYPE HYM 5.3060; NHMUK013455729 (BMNH). **Paratype**,  $\Im$ : INDIA: Timadu, Palani, 1992, coll. Palanichamy, Host *Cyrtophora cicatrosa* (spider); **paratype**; *Desantisca palanichamyi*  $\Im$  sp. nov., det. Narendran 1983 (USNM). **Other material**, SPAIN: Murcia: Murcia, 5.VI.2016, 37.9176N -1.20633W, A. Chuang, Lot #593 (88  $\Im$   $\Im$ , 15  $\Im$   $\Im$ ), Lot#971-16 (6  $\Im$   $\Im$ ); Málaga: Mál-



**Figures 17–19.** *Philolema palanichamyi* **17** male petiole, lateral **18** male petiole, dorsal **19** Dissected *C. citricola* egg sac, blue arrow = dried, consumed spider eggs, red arrow = dead *Ph. palanichamyi*.

aga, 8.VI.2016, 36.73705N -4.40486W, A. Chuang, Lot #609-1 (108  $\bigcirc \bigcirc$ , 25  $\bigcirc \bigcirc$ ), Lot#956-16 (7  $\bigcirc \bigcirc$ ), Lot #615 (16  $\bigcirc \bigcirc$ , 3  $\bigcirc \bigcirc$ ); Cádiz: Cádiz, 13.VI.2016, 36.31301N -5.8865W, A. Chuang, Lot #627-1 (27  $\bigcirc \bigcirc$ , 4  $\bigcirc \bigcirc$ ); Cádiz, 16.VI.2016, 36.29552N -6.0748W, A. Chuang, Lot #637-4 (14  $\bigcirc \bigcirc$ , 2  $\bigcirc \bigcirc$ ), Lot#926-1 (8  $\bigcirc \bigcirc$ , 1  $\bigcirc$ ); Tenerife: 28.07608N -16.6483W, Lot#899-A (15  $\bigcirc \bigcirc$ , 4  $\bigcirc$ ). All deposited in USNM.

**Recognition.** This species can be distinguished from the widespread *Philolema latrodecti* Fullaway, 1953 by the suberect flagellar setation (females only, adpressed in *Ph. palanichamyi*) and smaller ventral plaque (males only, less than half the depth seen in *Ph. palanichamyi*). Usually, the sculpture of the tegula is much more distinct in *Philolema latrodecti*. Also, *Ph. palanichamyi* is known only from the eggs of *Cyrtophora* spp. while *Ph. latrodecti* is known only from the eggs of *Latrodectus* spp.

Biology. Egg predator (Fig. 17) of Cyrtophora spp. (Araneae, Araneidae).

# Pediobius species group pyrgo

We examined 4  $\bigcirc$  and 3  $\bigcirc$  of a *Pediobius* species reared from *C. citricola* egg sacs collected by AC on Tenerife in the Canary Islands in May 2018. These specimens closely resemble material identified as *Pe. pyrgo* (Walker) from England and elsewhere in Europe reared from lepidopteran hosts as primary or very often secondary parasitoids. Only small and probably insignificant differences could be found. It seems best to regard the *Pediobius* material reared from *Cyrtophora* egg sacs collected in Tenerife as probably *Pe. pyrgo* until more material is available for morphological and molecular analyses.

As currently understood, *Pe. pyrgo* has been reported from an unusually broad range of primary hosts (Noyes 2018) that it attacks directly or as a facultative hyperparasitoid. Lepidoptera are the most frequently recorded hosts, but Dermaptera, Diptera, and Hymenoptera have also been reported. Larvae or pupae from thirteen families of Lepidoptera, including leaf-miners, web spinners, case-bearers, as well as exposed feeders are known as hosts. However, in many instances it is their parasitoids that are attacked by *Pe. pyrgo*, especially ichneumonoid Hymenoptera but also other chalcidoids (Eulophidae, Pteromalidae).

*Pediobius pyrgo* is a solitary or slightly gregarious endoparasitoid of larvae and pupae, and it has been described as a koinobiont larva/pupal parasitoid of *Leucoptera* (Lep., Lyonetiidae) (Mey 1993). It is widespread in the Palaearctic and Oriental regions with a few New World records from North, South, and Central America. Schoeninger et al. (2015) record *Pe. pyrgo* as associated with *Latrodectus* eggs sacs in South America, and *Pe. brachycerus* (Thomson) and a few other species of *Pediobius* are known to be associated with spider egg sacs (Bouček 1965; Noyes 2018), but these do not belong to the species group *pyrgo*.

# Discussion

Although egg parasitism or predation in spiders has received little attention, case studies suggest it may be common in native ranges (e.g., van Wingerden 1973; Rollard 1985; van Baarlen et al. 1994; Dinter 1996; Sacher 2001; Finch 2005; Leborgne and Pasquet 2005; Krehenwinkel et al. 2016; Wawer and Kostro-Ambroziak 2016), resulting in high mortality rates in parasitized egg sacs (Finch 2005; Krehenwinkel et al. 2016; Wawer and Kostro-Ambroziak 2016). Smith (1982) documented fewer offspring of the orbweaver spider *Philoponella oweni* (Chamberlin 1924) owing to parasitism by the pteromalid wasp *Arachnopteromalus dasys* Gordh, 1976. Hesse reported that *Philolema arachnovora* (Hesse 1942) appears to be an egg predator of *Latrodectus indistinctus* Pickard-Cambridge, 1904, consuming more than one egg per wasp larva. This was based on dissections of parasitized egg sacs, similar to our own findings.

Overall, we found that *Ph. palanichamyi* was present in about 40% of egg sacs. While wasp presence did not completely preclude spiderling emergence, it was as-

sociated with about 60% fewer spiderlings. It thus seems likely that *C. citricola* eggs in Spain experience predation pressure from the wasplarvae, an issue that is ripe for more detailed examination. Additionally, it would be useful to understand whether wasp predation rates remain stable across *C. citricola*'s range and breeding season and whether they promote extinction patterns in this spider, as is well-known among spider colonies (Avilés 1997).

The discovery of these two hymenopteran associates of *C. citricola* in its native Spanish range has particularly important implications for the multiple introductions of this spider throughout the Americas and Caribbean. No wasp associates have been reported from any of the non-native populations of *C. citricola*, even though yearly surveys of two expanding populations in Florida from 2014–2017 have been conducted by AC (Chuang, unpublished data). This is notable because in Florida, the range of *C. citricola* overlaps *L. geometricus* and its egg sac parasitoid *Ph. latrodecti*, known only to parasitize the widow spiders *Latrodectrus* spp. (Bibbs and Buss 2012) and with no evidence of a host shift to *C. citricola*. The introduction of *Ph. palanichamyi* may thus have effects on the population dynamics, range expansion, and impacts of non-native *C. citricola* spiders.

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# Supplementary material I

# Table S1. Egg predator and spiderling emergence rates

Authors: Angela Chuang, Michael W. Gates, Lena Grinsted, Richard Askew, Christy Leppanen

Data type: species data

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RESEARCH ARTICLE



# Two new species of the genus Cyanopenthe Nikitsky, 1998 (Coleoptera, Tetratomidae) from southwest China

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# Abstract

The genus *Cyanopenthe* Nikitsky, 1998 is first recorded from mainland China. Two new species, *C. granulata* **sp. nov.** and *C. hirtiscutellara* **sp. nov.**, are described and illustrated. This genus is redefined, and an updated key to the known species is presented.

# Keywords

polypore fungus beetles, taxonomy, Xizang, Yunnan

# Introduction

The family Tetratomidae Billberg, 1820 within the superfamily Tenebrionoidea Latreille, 1802 consists of approximately 150 extant species belonging to 13 genera of five subfamilies (Nikitsky 1998, 2004, 2005, 2008, 2016; Pollock 2012; Hsiao et al. 2015; Saitô and Konvička 2017) and six fossil species belonging to six genera of two subfamilies (Nikitsky 1977; Alekseev 2014; Soriano et al. 2014; Cai et al. 2016; Yu et al. 2016; Hsiao et al. 2018). Among them, 21 extant species of eight genera in five subfamilies are recorded from China, primarily in the southwest and southeast (Nikitsky 1998, 2004, 2005, 2008, 2016; Hsiao et al. 2015; Yoshitomi and Yamasako 2016).

The genus *Cyanopenthe* Nikitsky, 1998 belongs to the subfamily Penthinae Lacordaire, 1859 and contains only four described species in the world (Champion 1916; Ni-

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kitsky 1998; Hsiao et al. 2015). Penthe metallica Champion, 1916 was described based on single female without locality data. Another two female specimens were discovered from northern India and Bhutan (Nikitsky 2005; Hsiao et al. 2015). Subsequently, a revision of the family Tetratomidae was contributed by Nikitsky (1998). In this work, a new genus, Cyanopenthe Nikitsky, 1998, was established and compared with the genus Penthe Newman, 1838; Penthe metallica Champion, 1916 was designated as the type species of this new genus, and one new species, C. thailandcia Nikitsky, 1998, was described. The latter was similarly based on a single female from northwestern Thailand with only a line drawing habitus of the holotype. A line drawing habitus of the holotype of C. metallica (Champion, 1916) was also provided by Nikitsky (1998). In 2005, a detailed key to the *Cyanopenthe* species was given by Nikitsky with corresponding figures that including the ovipositor of the holotype of C. thailandica Nikitsky, 1998. In a recent work by Hsiao et al. (2015), two new species, C. taiwana Hsiao et al., 2015 and C. leei Hsiao et al., 2015, were described based on both sexes with color habitus from Taiwan of China, as well as the female ovipositor and the male genitalia; the female ovipositor of C. metallica (Champion, 1916) was also presented for the first time, along with a supplementary description, and a key to all species of the genus was provided.

# Materials and methods

The specimens were examined and dissected under a Nikon SMZ800 microscope. Photographs of adult habitus were taken with a Canon EOS 5D Mark III connected to a Canon MP-E 65 mm macro lens. Photographs of other morphological details were taken using a Leica M205A stereomicroscope. Adobe Photoshop 7.0 software was used in image processing. The aedeagus and ovipositor were detached from the body with insect needles, then glued to separate cards and pinned under the specimens. Specimens examined in this study are deposited in **MHBU** (Museum of Hebei University, Baoding, China) and **IZCAS** (Institute of Zoology, Chinese Academy of Sciences, Beijing, China). A double slash (//) separates data of different labels.

Body length was measured from the anterior margin of the clypeus to elytral apex; the terminology of the ovipositor follows Hsiao et al. (2015); absolute measurements are indicated in millimeters (mm).

# Taxonomy

# Key to species of the genus *Cyanopenthe* Nikitsky, 1998 (modified from Hsiao et al. 2015)

- *C. taiwana* Hsiao et al., 2015
  Elytra and abdomen more elongate; anterolateral margin of pronotum less rounded; lateral margins of parameres of tegmen subparallel to slightly divergent distally; proctiger of ovipositor slightly slender in ventral view .....
- - (Hsiao et al. 2015: fig. 26) ...... *C. metallica* (Champion, 1916)

# Genus Cyanopenthe Nikitsky, 1998

2

- *Cyanopenthe* Nikitsky, 1998: 29; 2005: 20; 2008: 63; Hsiao et al. 2015: 579; Yoshitomi and Yamasako 2016: 30.
- Type species. Penthe metallica Champion, 1916 (by original designation).

**Diagnosis.** Body black, shining, with dark metallic blue or green-blue, covered with dense and black erect pubescence. Head small, dorsal surface with narrow, longi-

tudinal median depression. Eyes lateral, large and protruding. Antennae long, antennomeres VIII–XI ( $\delta$ ) or VII–XI ( $\mathfrak{Q}$ ) strongly broadened into a pectinate club. Pronotum transverse, disc weakly convex, flattened laterally with pair of large impressions near base. Prosternal process strongly broadened posteriorly and somewhat roundly truncate apically, slightly exceeding the posterior margin of prothoracic coxae. Scutellum large, triangular or transverse, covered with dense and decumbent yellow to reddish, bronzed pubescence, with or without dark rounded impression at middle. Elytra broadly oval, much wider than pronotum, disc convex, depressed from middle to humeri along lateral margins. Legs slender and long, underside of metafemora with [or maybe without (not mentioned in the previously described species)] dense yellow hairbrush from base to middle in male, metatarsomere I shorter than the remaining tarsomeres combined.

Aedeagus ensiform, parameres slightly shorter than or as long as phallobase. Distal part of parameres divergent in dorsal and ventral view, curved to ventral side in lateral view.

Ovipositor flattened, paraproct elongated, lateral margins subparallel, straight or weakly curved; proctiger semicircular in dorsal view, tapered posteriorly and more or less curved in ventral view.

Distribution. Bhutan, China (Taiwan, Xizang, Yunnan), India, and Thailand.

# Cyanopenthe granulata sp. nov.

http://zoobank.org/09F5F8A2-92C8-4DEC-9118-43B6659B6FB5 Figs 1–17

**Type material. Holotype:** ♂ (MHBU) (Fig. 1), with the following labels: "西藏波密 县加龙坝村 // 30°02'18"N, 95°15'34"E // 2470 m 2018.VIII.23 魏中华" translated into English as "Jialongba Village, Bomê County, Xizang // 30°02'18"N, 95°15'34"E // Elev. 2413 m, 23.VIII.2018, Zhonghua Wei leg". Paratype: 1♀ (IZCAS) (Fig. 2), with the following labels:"西藏察隅县上察隅 // 2000 m 杨树桩 // 2005.VIII.24 吴捷" translated into English as "Shang Zayü Town, Zayü, County, Xizang // Elev. 2000 m, Poplar stump // 24.VIII.2005, Jie Wu leg".

**Diagnosis.** This species is similar to *C. metallica* (Champion, 1916), but can be distinguished by the following characters (based on females): dorsal side of body greenblue; antennomere V nearly as long as VI; densely granulate on pronotum; scutellum bronzed; elytral surface with large punctures nearly in rows; posterior margin of abdominal ventrite V more broadened; paraproct of ovipositor more elongate (1.7 times as long as wide), lateral margins weakly curved.

**Description.** Dorsal side of body royal blue, antennae, femora, tibiae and ventral side of body dark blue, some of sternum and abdomen blue-green. Scutellum bronzed, bordered with distinct blue-violet metallic sheen on elytra. Body with dense and black erect pubescence dorsally as well as ventrally. Scutellum with dense and decumbent orange pubescence. Underside of metafemora densely with yellow hairbrush from base to middle in male.



Figures 1, 2. Habitus of *Cyanopenthe granulata* sp. nov. 1 male 2 female.

**Male** (Figs 1, 3, 5–8, 10–13). *Head* small, length 1.0 mm, width 1.5 mm, densely and finely punctured, dorsal surface with narrowly, longitudinal median depression. Eyes lateral, large and protruding, ratio of eye diameter to interocular space 1.0: 1.9. Maxillary palpomere II elongate-triangular, III suborbiculate, IV obliquely rounded at apex, sides subparallel, surface of extend part somewhat rough and dull, no shin-ing. Antennae (Fig. 3) length 3.8 mm, antennomere I cylindrical, II suborbiculate, III strongly elongate and somewhat clavate, IV–VI clavate, VII somewhat broadened into a pectinate club, approximately as long as projection, VIII–XI strongly broadened into a pectinate club, projections 1.7 times longer than wide; ratio of antennomere lengths as follows: 3.0: 2.0: 6.0: 4.0: 3.2: 2.5: 2.0: 3.2: 3.4: 3.7: 2.8.

*Pronotum* (Fig. 5) transverse, length 1.2 mm, width 2.6 mm, 1.7 times as wide as head. Disc weakly convex, flattened laterally with pair of large impressions extending from base to approximately 1/3 length of pronotum. Surface with dense granules, separated by less than their diameter. Anterior margin slightly sinuate, posterior margin sinuate; lateral margins widest at anterior angles and narrowing posteriorly. Anterior angles rounded, posterior angles rectangular. Prosternal process strongly broadened posteriorly and somewhat roundly truncate apically, slightly exceeding posterior margin of prothoracic coxae. *Scutellum* (Fig. 5) large, triangular, 1.1 times as wide as long; surface densely and finely punctate, without dark rounded impression centrally.

*Elytra* broadly oval, length 6.4 mm, width 4.0 mm, much wider than pronotum. Disc convex, depressed from middle to humeri along lateral margins. Surface with tiny



Figures 3–9. *Cyanopenthe granulata* sp. nov. 3–4 antennae: 3 male 4 female 5 pronotum and scutellum of male 6 metatarsi of male 7 abdomen of male 8–9 abdominal ventrite V: 8 male 9 female.

punctures, and large punctures nearly in rows medially on each elytron. Diameter of punctures in spaces between striae 1.7 times smaller than that of punctures in rows.

*Abdomen* (Figs 7–8) oval, linearly narrowed posteriorly, apex rounded. Surface densely and finely punctured. Ventrites with irregular grooves laterally.

*Legs* slender and long. Length of metafemora 2.5 mm, metatibiae 2.1 mm and metatarsi 2.0 mm. Metatarsomere I shorter than II–IV combined. Length ratio of metatarsomeres (Fig. 6) as follows: 10.0: 3.3: 3.0: 8.0.

*Aedeagus* (Figs 10–13) ensiform, parameres as long as phallobase (0.8 mm), phallobase twice as long as wide. Parameres widest at base, lateral margins subparallel, narrowing evenly towards apex, distal part divergent in middle in dorsal and ventral view, curved to ventral side in lateral view. Median lobe 1.2 times as long as tegmen.



Figures 10–16. *Cyanopenthe granulata* sp. nov. 10–13 aedeagus: 10 aedeagus lateral view 11–13 parameres dorsal, ventral and lateral view. 14–16 ovipositor dorsal, ventral and lateral view.

**Female** (Figs 2, 4, 9, 14–16). Body larger than male, dark metallic green-blue. Head length 1.1 mm, width 1.6 mm; ratio of eye diameter to interocular space 1.0: 2.3. Antennae (Fig. 4) length 4.1 mm, antennomere VII strongly broadened into a pectinate club, more well-developed than that of male, projection 1.6 times longer than length of antennomere, VIII–X 1.7 times as long as respective antennomeres; length ratio of antennomeres as follows: 3.0: 1.8: 6.5: 3.3: 2.8: 2.6: 3.0: 3.2: 4.0: 3.5: 2.9. Pronotum length 1.4 mm, width 3.0 mm. Elytra length 7.6 mm, width 4.2 mm. Abdominal ventrite V (Fig. 9) protuberant, slightly broadened posteriorly than that of male. Underside of metafemora without yellow hairbrush. Length of metafemora 2.8 mm, metatibiae 2.9 mm and metatarsi 2.4 mm. Length ratio of metatarsomeres as follows: 10.0: 4.0: 2.4: 6.6.

*Ovipositor* (Figs 14–16) flattened, length 1.8 mm, paraproct elongated, 1.7 times as long as wide, lateral margins weakly curved and subparallel; proctiger semicircular in dorsal view, tapered posteriorly in ventral view.

# Distribution. China: Xizang.

**Etymology.** This species is named from the Latin *granulus*, referring to the densely granulose pronotum.

**Bionomics.** The holotype was found on a dead wood with fungi of Polyporaceae in the forest (Fig. 24). The paratype was found on a stump of poplar.

**Remarks.** The variation of color in male and female could be caused by fading or differences between male and female individuals; we are not sure. The aedeagus of the holotype and the ovipositor of the paratype are somewhat damaged.

# Cyanopenthe hirtiscutellara sp. nov.

http://zoobank.org/F6D0FF83-7ADA-4D6C-BFEB-4C5FA0AF06F2 Figs 17–23

**Type material. Holotype:** ♀ (MHBU) (Fig. 17), with the following labels: "2009. VI.2 // 云南独龙江钦郎当 // 1500 m 朱笑愚" translated into English as "2.VI.2009 // Qinlangdang Village, Drungjiang Township, Gongshan County, Yunnan // Elev. 1500 m, Xiaoyu Zhu leg".

**Diagnosis.** This species is closely related to *C. granulata* sp. nov. and *C. metallica* (Champion, 1916), but can be distinguished by the following characters (based on female): dorsal side of body blue-violet; pronotum densely granulate, except coarsely punctured in anterior half of disc; scutellum yellow; lateral margins of paraproct of ovipositor nearly straight, proctiger almost as long as gonocoxites, gonostylus with long setae.

**Description.** Dorsal side of body blue-violet, antennae, femora, tibiae and ventral side of body dark blue, some individuals with sternum and abdomen blue. Scutellum yellow, around scutellum with distinct dark-blue metallic sheen on elytra. Body with dense and black erect pubescence, dorsally and ventrally. Scutellum with dense and decumbent yellow pubescence.

**Female**. *Head* small, length 1.0 mm, width 1.6 mm, densely and finely punctured, dorsal surface with narrowly, longitudinal median depression. Eyes lateral, large and protruding, ratio of eye diameter to interocular space 1.0: 2.0. Maxillary palpomere II elongate-triangular, III suborbiculate, IV obliquely rounded at apex, sides subparallel, surface of extend part somewhat rough and dull, no shining. Antennae (Fig. 20) length 4.0 mm, antennomere I cylindrical, II suborbiculate, III strongly elongate and somewhat clavate, IV–VI clavate; projection of VII about 1.3 times length of the antennomere, VIII 1.7 times longer than width, IX and X 1.6 times longer than width; apices of projections rounded, 1.2 times longer than width; ratio of antennomere lengths as follows: 3.0: 2.0: 7.0: 3.3: 3.3: 2.6: 3.4: 3.3: 3.8: 4.0: 4.4.



Figures 17–23. Cyanopenthe hirtiscutellara sp. nov. 17 Habitus of Cyanopenthe hirtiscutellara sp. nov. 18 pronotum and scutellum 19 abdominal ventrite V 20 antennae 21–23 ovipositor dorsal, ventral and lateral view.

*Pronotum* (Fig. 18) transverse, length 1.3 mm, width 2.8 mm, 1.7 times as wide as head. Disc weakly convex, flattened laterally with a pair of large impressions extending from base to approximately 1/3 length of pronotum. Surface densely granulate, except coarsely punctured in anterior half of disc. Anterior margin slightly sinuate, poste-



Figure 24. Habitat of *Cyanopenthe hirtiscutellara* sp. nov. Jialongba Village, Bomê County, Xizang.

Table	. Diagnostic	characters s	separating ty	pe species	and two	new species	(based o	on femal	es).
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	C. metallica	C. granulata sp. nov.	C. hirtiscutellara sp. nov.				
Color of dorsal side	Blue	Green-blue	Blue-violet				
Antennomere	V evidently longer than VI	V nearly as long as VI	V evidently longer than VI				
Pronotum	Densely and coarsely punctured	Densely granulate	More densely granulate, except coarsely punctured in anterior half of pronotal disc				
Color of scutellum	Bronzed or reddish bronzed	Bronzed	Yellow				
Punctures of elytral suface	Irregular	Large punctures nearly in rows	Irregular				
Posterior margin of abdominal ventrite V	More narrow	More broad	More narrow				
Paraproct	1.4 times as long as wide	1.7 times as long as wide	1.4 times as long as wide				
Proctiger	Longer than gonocoxites	Almost as long as gonocoxites	Almost as long as gonocoxites				
Gonostylus	Without setae	Lost in dissection	With long setae				
Distribution	Northern India and Bhutan	China (Xizang)	China (Yunnan)				

rior margin sinuate; lateral margins widest at anterior angles and narrowing posteriorly. Anterior angles rounded, posterior angles rectangular. Prosternal process strongly broadened posteriorly and somewhat roundly truncate apically, slightly exceeding posterior margin of prothoracic coxae. *Scutellum* (Fig. 18) large, triangular, 1.1 times as wide as long; surface densely and finely punctured. *Elytra* broadly oval, length 7.4 mm, width 5.0 mm, much wider than pronotum. Disc convex, depressed from middle to humeri along lateral margins. Surface with tiny punctures and irregular large punctures.

*Abdomen* (Fig. 19) oval, linearly narrowed posteriorly, apex rounded. Surface densely and finely punctured. Ventrites with irregular grooves laterally.

Legs slender and long. Length of metafemora 2.7 mm, metatibiae 2.6 mm.

*Ovipositor* (Figs 21–23) flattened, length 1.7 mm, paraproct 1.4 times as long as wide, lateral margins straight; proctiger almost as long as gonocoxites, proctiger semicircular in dorsal view, tapered posteriorly in ventral view; gonostylus with long setae.

Distribution. China: Yunnan.

**Etymology.** This species is named from the Latin *hirtus* and *scutella*, in reference to the dense decumbent pubescence on the scutellum.

# Discussion

As far as we know, *Cyanopenthe* species inhabit moist and warm forest habitats, and feed on fungi of Polyporaceae at night in small aggregations or alone; all known species occur in Bhutan, China (Taiwan, Xizang, Yunnan), northern India and northwestern Thailand of Southeast Asia. We believe that more species may be discovered in the Himalayas, Myanmar, Laos, Vietnam and Southern China in the future.

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CHECKLIST



# Drainage basin checklists and dichotomous keys for inland fishes of Texas

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### Abstract

Species checklists and dichotomous keys are valuable tools that provide many services for ecological studies and management through tracking native and non-native species through time. We developed nine drainage basin checklists and dichotomous keys for 196 inland fishes of Texas, consisting of 171 native fishes and 25 non-native fishes. Our checklists were updated from previous checklists and revised using reports of new established native and non-native fishes in Texas, reports of new fish occurrences among drainages, and changes in species taxonomic nomenclature. We provided the first dichotomous keys for major drainage basins in Texas. Among the 171 native inland fishes, 6 species are considered extinct or extirpated, 13 species are listed as threatened or endangered by U.S. Fish and Wildlife Service, and 59 species are listed as Species of Greatest Conservation Need (SGCN) by the state of Texas. Red River drainage basin was the most speciose with 120 fishes. Rio Grande & Pecos drainage basin had the greatest number of threatened or endangered fishes (N = 7) and the greatest number of SGCN fishes (N = 28). We revised drainage basin occurrences for 77 species. Drainage basin checklists and dichotomous keys provide finer resolution of species distributions within the geopolitical boundaries of Texas and can reduce probability of errors in fish identification errors by removing species not occurring within a natural boundary.

#### **Keywords**

Texas, fish, checklist, dichotomous key, key, identification, occurrence, drainage

# Introduction

Species checklists consolidate biodiversity records using standardized taxonomic nomenclature and updated species occurrences within pre-defined boundaries (Fleishman et al. 2006; Martellos and Nimis 2015). Benefits of checklists include use in ecological studies and natural resources management, such as assessments of global patterns in species richness (Gaston 2000), identification of biodiversity hotspots (Kent et al. 2002), occurrences for species distribution models (Caicco et al. 1995), and expansion and contraction of native and non-native species (Lee et al. 2008; Magurran et al. 2010). Often coupled with checklists, dichotomous keys facilitate species identification using a series of distinguishing characteristics (Griffing 2011). Dichotomous keys usually are created for taxa within geopolitical boundaries (e.g., Hubbs et al. 2008); however, geopolitical boundaries often are arbitrary to species distributions (Forman 2014). Recent development and use of dichotomous keys along natural boundaries, such as drainage basin (Worsham et al. 2016), provide finer resolution on species distributions and reduce probability of identification errors by removing species not occurring within a natural boundary.

Within Texas, Evermann and Kendall (1894) published the first checklist of freshwater fishes. A revised checklist was published by Baughman (1950a, 1950b), using standardized taxonomic nomenclature provided by Jordan et al. (1930). Jurgens and Hubbs (1953) were the first to publish a checklist using standardized taxonomic nomenclature provided by American Fisheries Society Committee on Names of Fishes (Chute et al. 1948). This checklist was periodically revised by Hubbs (i.e., Hubbs 1957, 1958, 1961, 1972, 1976, 1982). Knapp (1953) published a checklist and the first dichotomous key for freshwater fishes of Texas. Texas drainage basin checklists were published for western Gulf Slope drainage basins (Conner and Suttkus 1986), Mississippi River drainage basins (Cross et al. 1986), and Rio Grande drainage basin (Smith and Miller 1986). Statewide checklist and dichotomous key were revised by Hubbs et al. (1991) and Hubbs et al. (2008).

Revisions of checklists for freshwater fishes of Texas were necessary through time to accommodate additions of previously unreported species, multiple species described from a single species, and non-native species introductions (Hanks and McCoid 1988; Eisenhour 2004; Gallaway et al. 2008) and to accommodate removal of introduced fishes that did not establish populations (Howells 2001). In addition, species distributions were updated to document range expansions (e.g., *Percina carbonaria*, Hubbs et al. 2008), range contractions (e.g., *Ictalurus lupus*, Kelsch and Hendricks 1990), and name changes (e.g., *Micropterus treculi* to *Micropterus treculii*) using standardized taxonomic nomenclature (e.g., Nelson et al. 2004). Since Hubbs et al. (2008), American Fisheries Society and American Society of Ichthyologists and Herpetologists (AFS-ASIH) Committee of Names of Fishes published a revised common and scientific names list (Page et al. 2013), new native species were reported within Texas (e.g., Craig et al. 2015), a fish name was synonymized (Echelle et al. 2013), introduced species became established (e.g., Cohen et al. 2014), and species ranges expanded (e.g., Dautreuil et al. 2016) and contracted (e.g., Craig et al. 2017).

Purposes of this paper were to develop drainage basin checklists and dichotomous keys for Texas freshwater fishes. As with previous revisions, we updated the statewide checklist and dichotomous key with new species, removal of species, and range changes. However, our checklists and dichotomous keys differ markedly from previous revisions. We identified fishes as inland, rather than freshwater, and divided the geopolitical boundary into natural boundaries using major drainage basins. Texas is particularly well suited for drainage basin checklists and keys because majority of the drainage basins became independent of one another during the early Holocene (i.e., river termini in Gulf of Mexico bays), generally restricting freshwater fish movement among drainage basins. As such, fishes are rarely homogenously distributed among all drainage basins, with 41% of fishes restricted to one or two drainage basins (Conner and Suttkus 1986; Hubbs et al. 2008).

# Materials and methods

Development of a freshwater fish checklist is a challenge within natural or geopolitical boundaries having fresh and marine environments (Ross 2001; Moyle 2002). Inclusions of marine fishes on a freshwater fish checklist are subjective (Ross 2001). Knapp (1953) included marine fishes if observed in waters with salinities < 2 ppt. Hubbs et al. (1991) included marine fishes if found in "low salinity habitats". Using salinity as an objective measure is limiting. Several fishes found in upper reaches of the Canadian River, Red River, Brazos River, Colorado River, and Pecos River inhabit saline waters with salinities exceeding 50 ppt at times (Echelle et al. 1972), so excluding fishes based on salinity tolerances would exclude several species not known to inhabit marine or estuarine environments. Avoiding salinity as a measure, we used the term "inland" instead of "freshwater" to represent fishes found in Texas rivers generally upstream from transitory freshwater-saltwater boundaries. We accepted fishes as inland if they hatch, feed, and reproduce within inland waters (i.e., all water bodies upstream of river termini). We also accepted two forms of marine fishes as inland fishes: diadromous fishes (i.e., Anguilla rostrata, Agonostomus monticola, and Trinectes maculatus) and fishes with reported self-sustaining populations within inland waters (e.g., Syngnathus scovelli, Martin et al. 2013). Our acceptance of fishes as inland oversimplifies the complex and dynamic relationship of fish communities within estuarine systems of the Gulf of Mexico (Gelwick et al. 2001); therefore, our inland fish checklists underestimate the number of fishes encountered in estuarine systems.

Drainage basins were defined as major independent rivers that flow directly into the Gulf of Mexico (i.e., Sabine & Neches, Trinity & San Jacinto, Brazos, Colorado & Lavaca, Guadalupe & San Antonio, Nueces, and Rio Grande & Pecos) or beyond Texas borders (i.e., Canadian and Red) (Figure 1). Drainage basin checklists were developed using specific (Conner and Suttkus 1986; Cross et al. 1986; Smith and Miller 1986) and generalized (Hubbs et al. 2008) drainage basin checklists. Checklists were consolidated and updated based on drainage basin distribution records for each



**Figure 1.** Map of Texas with major drainage basins outlined and labeled. Also included are major cities to serve as reference points.

species using Texas Natural History Collections database (Hendrickson and Cohen 2015), published consolidated species accounts (e.g., Lee et al. 1980), and published individual species range accounts (e.g., Wilde and Bonner 2000). We only included species from previous checklists if species were recognized by Page et al. (2013) to minimize taxonomic inflation (Isaac et al. 2004). New species were added to checklists and keys based on published accounts of self-sustaining populations (Ameiurus nebulosus; Craig et al. 2015). A species was designated as native if it occurs within at least one Texas drainage basin without human aid. Transient border species (i.e., Pimephales notatus, Lee and Shute 1980; Hiodon tergisus, Gilbert 1980; Cyprinella panarcys, Pinion et al. 2018) with occurrences in boundary waters of Texas were excluded because of uncertainty in self-sustaining populations. At least 80 non-native fishes have been introduced into Texas drainage basins; however, the majority did not establish selfsustaining populations (Howells 2001). Non-native fishes were included in drainage basin checklists if we had evidence (i.e., publications, personal communications) of self-sustaining populations or regular stocking (e.g., Ctenopharyngodon idella). Fishes considered extinct (IUCN 2018) were included in the checklist but excluded from keys because of low likelihood of encounter.

Each drainage basin dichotomous key consists of family and species keys. We developed novel distinguishing characteristics for family and species keys along with modifying and using characteristics from original species descriptions (e.g., Eisenhour 2004) and existing keys (e.g., Robison and Buchanan 1988; Sublette et al. 1990; Boschung and Mayden 2004; Thomas et al. 2007; Hubbs et al. 2008). Distinguishing characteristics were comprised of external and internal morphologies, meristics, and color patterns of adult fishes. Each couplet lists the most pronounced distinguishing characteristic first, followed by additional, generally less pronounced, distinguishing characteristics.

# **Results and discussion**

The composite drainage basin checklist included 196 inland fishes, representing 79 genera and 30 families (Table 1). Dichotomous keys were developed for nine drainage basins (Suppl. material 1). The number of inland fishes, based on our definition herein, reported in previous checklists ranged from 93 (Evermann and Kendall 1894) to 191 (Hubbs et al. 2008). Hubbs et al. (2008) and our composite drainage basin checklist were the most similar but with differences. Our checklist included three fishes reported in Texas after 2008: native Ameiurus nebulosus (Craig et al. 2015), non-native Xiphophorus variatus (Cohen et al. 2014), and non-native Hypophthalmichthys nobilis (T. Bister, Texas Parks and Wildlife Inland Fisheries, personal communication 10 March 2019). Fishes included by Hubbs et al. (2008) and excluded from our checklist were *Cyprinella* sp., Cycleptus sp., and Ictalurus sp., because Page et al. (2013) did not recognize these three putative species. Also based on Page et al. (2013), fish names were changed for three species: Herichthys cyanoguttatus, Erimyzon claviformis, and Menidia audens. One species (i.e., Gambusia clarkhubbsi) was included by Hubbs et al. (2008) and Page et al. (2013) but excluded from our checklist, because G. clarkhubbsi was later determined to be a junior synonym for Gambusia krumholzi (Echelle et al. 2013). Gambusia krumholzi replaced G. clarkhubbsi in our checklist. We excluded 8 non-native fishes reported by Hubbs et al. (2008), each lacking evidence of self-sustaining populations: Scardinius erythrophthalmus, Agamyxis pectinifrons, Platydoras armatulus, Pterygoplichthys multiradiatus, Esox lucius, Perca flavescens, Sander canadensis, and Tilapia zillii. Our checklist includes updated distributions of several fishes from previous checklists. Our checklist has 77 fishes with different drainage basin distributions compared to the drainage basin checklists of Conner and Suttkus (1986), Cross et al. (1986), and Smith and Miller (1986). Although interpreted from generalized descriptions, we determined our checklist has different drainage basin distributions of at least 46 fishes compared to Hubbs et al. (2008). Differences in distributions of fishes are largely due to the generalized nature of Hubbs et al. (2008) descriptions, but also include range expansions and contractions.

Our composite drainage basin checklist has 171 native and 25 non-native inland fishes. Among native species, three fishes (i.e., *Notropis orca, Gambusia amistadensis*, and *Gambusia georgei*) are considered extinct, and three fishes (i.e., *Notropis simus, Oncorhynchus clarkii*, and *Gambusia senilis*) are considered extirpated (Hubbs et al. 2008).

**Table 1.** Fishes in Texas inland waters. Presence is denoted by "X". All scientific and common names were from Page et al. (2013). Asterisk next to scientific name denotes species that were not included in the dichotomous keys due to low likelihood of encounter. "Native" denotes species is native to any Texas drainage basin. "Ext/exp" denotes species is extinct or extirpated from Texas. "USFWS" denotes species that are federally listed as Threatened or Endangered Species by United States Fish and Wildlife Service. "SGCN" denotes species that are state listed as Species of Greatest Conservation Need.

	Family	Species	Common Name	Native	Ext/exp	USFWS	SGCN	Canadian	Red	Sabine & Neches	Trinity & San Jacinto	Brazos	Colorado & Lavaca	Guadalupe & San Antonio	Nueces	Rio Grande & Pecos
1	Petromyzontidae	Ichthvomvzon castaneus	Chestnut Lamprev	X					Х	X						
2		Ichthyomyzon gagei	Southern Brook Lamprey	Х					Х	Х	Х					
3	Acipenseridae	Scaphirhynchus platorynchus	Shovelnose Sturgeon	Х			Х		Х							
4	Polyodontidae	Polyodon spathula	Paddlefish	Х			Х		Х	Х	Х					
5	Lepisosteidae	Atractosteus spatula	Alligator Gar	Х			Х		Х	Х	Х	Х	Х	Х	Х	Х
6	-	Lepisosteus oculatus	Spotted Gar	Х					Х	Х	Х	Х	Х	Х	Х	Х
7		Lepisosteus osseus	Longnose Gar	Х					Х	Х	Х	Х	Х	Х	Х	Х
8		Lepisosteus platostomus	Shortnose Gar	Х					Х							
9	Amiidae	Amia calva	Bowfin	Х					Х	Х	Х	Х	Х			
10	Hiodontidae	Hiodon alosoides	Goldeye	Х			Х		Х							
11	Anguillidae	Anguilla rostrata	American Eel	Х			Х		Х	Х	Х	Х	Х	Х	Х	Х
12	Clupeidae	Dorosoma cepedianum	Gizzard Shad	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
13		Dorosoma petenense	Threadfin Shad	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
14	Cyprinidae	Campostoma anomalum	Central Stoneroller	Х				Х	Х		Х	Х	Х	Х	Х	Х
15		Campostoma ornatum	Mexican Stoneroller	Х			Х									Х
16		Carassius auratus	Goldfish						Х	Х	Х	Х	Х	Х	Х	Х
17		Ctenopharyngodon idella	Grass Carp					Х	Х	Х	Х	Х	Х	Х	Х	Х
18		Cyprinella lepida	Plateau Shiner	Х			Х							Х	Х	
19		Cyprinella lutrensis	Red Shiner	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
20		Cyprinella proserpina	Proserpine Shiner	Х			Х									Х
21		Cyprinella venusta	Blacktail Shiner	Х					Х	Х	Х	Х	Х	Х	Х	Х
22		Cyprinus carpio	Common carp					Х	Х	Х	Х	Х	Х	Х	Х	Х
23		Dionda argentosa	Manantial Roundnose Minnow	Х			Х									Х
24		Dionda diaboli	Devils River Minnow	Х		Х	Х									Х
25		Dionda episcopa	Roundnose Minnow	Х			Х									Х
26		Dionda nigrotaeniata	Guadalupe Roundnose Minnow	Х			Х						Х	Х		
27		Dionda serena	Nueces Roundnose Minnow	Х			Х								Х	
28		Gila pandora	Rio Grande Chub	Х			Х									Х
29		Hybognathus amarus	Rio Grande Silvery Minnow	Х		Х	Х									Х
30		Hybognathus hayi	Cypress Minnow	Х					Х	Х						
31		Hybognathus nuchalis	Mississippi Silvery Minnow	Х					Х	Х	Х	Х				
32		Hybognathus placitus	Plains Minnow	Х				Х	Х			Х	Х			
33		Hybopsis amnis	Pallid Shiner	Х						Х	Х	Х	Х	Х		
34		Hypophthalmichthys nobilis	Bighead Carp						Х							
35		Luxilus chrysocephalus	Striped Shiner	Х					Х							
36		Lythrurus fumeus	Ribbon Shiner	Х					Х	Х	Х	Х	Х	Х		
37		Lythrurus umbratilis	Redfin Shiner	Х					Х	Х	Х					
	Family	Species	Common Name	Native	Ext/exp	USFWS	SGCN	Canadian	Red	Sabine & Neches	Trinity & San Jacinto	Brazos	Colorado & Lavaca	Guadalupe & San Antonio	Nueces	Rio Grande & Pecos
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38	Cyprinidae	Macrhybopsis aestivalis	Speckled Chub	Х			Х									Х
39	21	Macrhybopsis australis	Prairie Chub	Х			Х		Х							
40		Macrhybopsis hyostoma	Shoal Chub	X					X	х	х	Х	х			
41		Macrhybopsis marconis	Burrhead Chub	X									X	Х		
42		Macrhybopsis storeriana	Silver Chub	Х			Х		Х			Х				
43		Macrhybopsis tetranema	Peppered Chub	X			X	х								
44		Notemigonus crysoleucas	Golden Shiner	X				X	х	х	х	Х	х	Х	Х	х
45		Notropis amabilis	Texas Shiner	Х			Х						Х	Х	Х	Х
46		Notropis atherinoides	Emerald Shiner	X				х	х	х	х					
47		Notropis atrocaudalis	Blackspot Shiner	X			х		X	X	X	Х				
48		Notropis bairdi	Red River Shiner	X			X		X							
49		Natrapis blennius	River Shiner	x				x	x							
50		Notropis bravtani	Tamaulinas Shiner	x			x	~								x
51		Notropis buccula	Smalleve Shiner	X		x	X					x	x			Λ
52		Notropis buchanani	Chost Shiner	x					v	x	v	v	x	v	v	v
52		Notropis chabbaaus	Ironcolor Shinor	v			v		v	v	v	Λ	Λ	v	Λ	Λ
5/		Notropis chihudhud	Chihuahua Shiner	X			X		л	л	л			л		x
55		Notropis cininaunau	Arkansas River Shiner	X		v	X	v								Λ
56		Notropis girurai	Pio Crando Shinor	л V		л	л V	л								v
57		Notropis jemezanas	Taillight Shinor	v v			л V		v							л
59		Notropis macaatas	Phantom Shinor	v	v		Λ		Λ							v
50		Notropis on when shus	Sharppace Shiper	v	Λ	v	v					v	v			Λ
60		Notropis oxymynthus	Chub Shinor	v v		л	л V		v		v	л V	л			
61		Notropis potieri Notropis cabinas	Sabina Shinar	v			v		Λ	v	v	Λ				
62		N-turt is shown and:	Sabine Sinner	л v			л v		v	л V	л v	v	v			
62		Notropis snumarai	Bluntnoso Shinor	v v	v	v	л V		л	л	л	л	л			v
64		Notropis simus	Sand Shinar	л V	л	л	л	v	v		v	v	v	v	v	л V
64		Notropis stramineus	Ward Shiner	л V				л	A V	v	A V	л V	л v	A V	A V	л
65		Notropis texanus	Mining Shiner	A V					A V	A V	A V	A V	A V	A V	A V	
66		Notropis voluceuus	Nimic Shiner	A V					A V	A V	A V	A V	A V	A V	A V	
6/		Opsopoeodus emilide	Pugnose Minnow	X				v	X	X	X	Х	X	Х	А	
68		Phendcobius mirabilis	Suckermouth Minnow	X				X	X	X	X	v	X	v	v	v
70		Pimephales prometas	Patnead Minnow	A V				A V	A V	A V	A V	A V	A V	A V	A V	A V
70		Pimephales vigilax	Fluinead Minnow	A V				A V	А	А	А	л	А	л	л	А
71		Platygobio gracilis	Planead Chub	A V			v	А	v							
72		Pleronotropis muoosi	bluenead Shiner	A V			A V		л							v
73		Krinichinys cataractae		A V			л		v	v	v	v				л
74	Committee	Centie des centies	Diver Communities	л v				v	A V	A V	A V	л v	v	v	v	v
76	Catostomidae	Carpiones carpio	Plue Sucker	л v			v	л	A V	A V	A V	л V	л v	л V	A V	л v
70		Cyclepius elongalus	Western Carols	л v			A V		A V	A V	A V	л V	л	л	л	л
		Erimyzon cuavijormis	Chubsucker				л		л 	л	л 	л 				
78		Erimyzon sucetta	Lake Chubsucker	Х					Х	Х	Х	Х		Х		
79		Ictiobus bubalus	Smallmouth Buffalo	Х					Х	Х	Х	Х	Х	Х	Х	Х
80		Ictiobus cyprinellus	Bigmouth Buffalo	Х					Х	Х		<b>T</b> -				*-
81		Ictiobus niger	Black Buffalo	Х					Х	Х		Х	Х			Х
82		Minytrema melanops	Spotted Sucker	Х					Х	Х	Х	Х	Х			
83		Moxostoma austrinum	Mexican Redhorse	Х			Х									Х
84		Moxostoma congestum	Gray Redhorse	Х								Х	Х	Х	Х	Х
85		Moxostoma erythrurum	Golden Redhorse	Х					Х							
86		Moxostoma poecilurum	Blacktail Redhorse	Х						Х	Х					_
87	Characidae	Astyanax mexicanus	Mexican Tetra	Х					Х		Х	Х	Х	Х	Х	Х

	Family	Species	Common Name	Native	Ext/exp	USFWS	SGCN	Canadian	Red	Sabine & Neches	Trinity & San Jacinto	Brazos	Colorado & Lavaca	Guadalupe & San Antonio	Nueces	Rio Grande & Pecos
88	Ictaluridae	Ameiurus melas	Black Bullhead	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
89		Ameiurus natalis	Yellow Bullhead	х				Х	х	х	х	х	Х	Х	Х	Х
90		Ameiurus nehulosus	Brown Bullhead	x					x							
01		Interarius neo aiosus	Plue Cetfel	v					v	v	v	v	v	v	v	v
91		Iciaiurus jurcaius		A V			v		л	л	л	л	A V	A V	л V	A V
92		ictaiurus iupus	Headwater Catrish	A			л		37		37	37	A	A	A	A
93		Ictalurus punctatus	Channel Catfish	Х				х	Х	Х	Х	Х	Х	Х	х	Х
94		Noturus gyrinus	Tadpole Madtom	Х					Х	Х	Х	Х	Х	Х	Х	Х
95		Noturus nocturnus	Freckled Madtom	Х					Х	Х	Х	Х				
96		Pylodictis olivaris	Flathead Catfish	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
97		Satan eurystomus	Widemouth Blindcat	Х			Х							Х		
98		Trogloglanis pattersoni	Toothless Blindcat	Х			Х							Х		
99	Loricariidae	Hypostomus plecostomus	Suckermouth Catfish											Х		Х
100		Pterygoplichthys anisitsi	Southern Sailfin Catfish								Х			Х		
101		Pterygoplichthys disjunctivus	Vermiculated Sailfin Catfish								Х			Х		Х
102	Salmonidae	Oncorhynchus clarkii	Cutthroat Trout	Х	Х		Х									Х
103		Oncorhynchus mykiss	Rainbow Trout					Х	Х	Х	Х	Х	Х	Х	Х	Х
104	Esocidae	Esox americanus	Redfin Pickerel	Х					Х	Х	Х	Х				
105		Esox niger	Chain Pickerel	х					х	х						
106	Aphredoderidae	Aphredoderus savanus	Pirate Perch	x					x	x	x	x	x			
107	Mugilidaa	Mugil caphaluc	Stringd Mullet	v					v	v	v	v	v	v	v	v
107	wugindae		March Miller	л V					л	N V	л V	л V	л V	л V	л v	л v
108		Agonostomus monticola	Mountain Mullet	X					37	X	X	X	Х	Х	Х	Х
109	Atherinopsidae	Labidesthes sicculus	Brook Silverside	Х					Х	Х	X	х				
110		Membras martinica	Rough Silverside								Х			Х		Х
111		Menidia audens	Mississippi Silverside	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
112	Fundulidae	Fundulus blairae	Western Starhead Topminnow	Х					Х	Х	Х	Х				
113		Fundulus chrysotus	Golden Topminnow	Х					Х	Х	Х	Х	Х	Х		
114		Fundulus grandis	Gulf Killifish						Х			Х	Х			Х
115		Fundulus kansae	Northern Plains Killifish	Х				Х								
116		Fundulus notatus	Blackstripe Topminnow	Х					Х	Х	Х	Х	Х	Х	Х	
117		Fundulus olivaceus	Blackspotted Topminnow	Х					Х	Х	Х	Х				
118		Fundulus zebrinus	Plains Killifish	Х					Х		Х	Х	Х			Х
119		Lucania goodei	Bluefin Killifish											Х		
120		Lucania parva	Rainwater Killifish	Х									Х	Х		Х
121	Cyprinodontidae	Cyprinodon bovinus	Leon Springs Pupfish	Х		Х	Х									Х
122		Cyprinodon elegans	Comanche Springs Pupfish	Х		Х	Х									Х
123		Cyprinodon eximius	Conchos Pupfish	Х			Х									Х
124		Cyprinodon pecosensis	Pecos Pupfish	Х			Х									Х
125		Cyprinodon rubrofluviatilis	Red River Pupfish	Х			Х	Х	Х			Х	Х			
126		Cyprinodon variegatus	Sheepshead Minnow								Х	Х	Х	Х		Х
127	Poeciliidae	Gambusia affinis	Western Mosquitofish	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
128		Gambusia amistadensis*	Amistad Gambusia	Х	Х											Х
129		Gamhusia oaioei	Big Bend Gambusia	X		х	х									X
130		Gamhusia geiseri	Largespring Gambusia	x									х	х		x
131		Gamhusia georgei*	San Marcos Gambusia	x	x	x								X		
122		Gambucia batavaah:	Clear Creek Cambusia	v	Λ	v	v						v	л		
1.92		Juniousu DEVETULISIT	Great Greek Gambusha	л		л	л						Λ			

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	Family	Species	Common Name	Native	Ext/exp	USFWS	SGCN	Canadian	Red	Sabine & Neches	Trinity & San Jacinto	Brazos	Colorado & Lavaca	Guadalupe & San Antonio	Nueces	Rio Grande & Pecos
133	Poeciliidae	Gambusia krumholzi	Spotfin Gambusia	Х												Х
134		Gambusia nobilis	Pecos Gambusia	х		Х	Х									Х
135		Gambusia senilis	Blotched Gambusia	X	х		X									X
136		Gamhusia speciosa	Tex-Mex Gambusia	x												x
137		Heterandria formosa	Least Killifish	x						х						
138		Poecilia formosa	Amazon Molly	~										x	x	x
130		Poecilia latitina	Sailfin Molly							x	v	v	v	X	X	X
1/0		Doscilia raticulata	Cuppy							л	л	л	л	л V	л	л
140		Visheshema kellenii	Guppy											л v		
141		Xipnopnorus neuerii	Green Swordtall										v	А		
142	C	Aipnopnorus variatus	C ICD: C I	v									А	v		
143	Syngnathidae	Syngnathus scovelli	Gulf Pipefish	X					37	37	37		37	X	37	
144	Moronidae	Morone chrysops	White Bass	X				Х	X	X	X	X	Х	Х	Х	Х
145		Morone mississippiensis	Yellow Bass	Х					X	X	X	X				
146		Morone saxatilis	Striped Bass						Х	Х	Х	Х	Х	Х	Х	Х
147	Centrarchidae	Ambloplites rupestris	Rock Bass											Х		
148		Centrarchus macropterus	Flier	Х					Х	Х	Х					
149		Lepomis auritus	Redbreast Sunfish						Х	Х	Х	Х	Х	Х	Х	Х
150		Lepomis cyanellus	Green Sunfish	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
151		Lepomis gulosus	Warmouth	Х					Х	Х	Х	Х	Х	Х	Х	Х
152		Lepomis humilis	Orangespotted Sunfish	Х				Х	Х	Х	Х	Х	Х	Х	Х	
153		Lepomis macrochirus	Bluegill	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
154		Lepomis marginatus	Dollar Sunfish	Х					Х	Х	Х	Х				
155		Lepomis megalotis	Longear Sunfish	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
156		Lepomis microlophus	Redear Sunfish	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
157		Lepomis miniatus	Redspotted Sunfish	Х					Х	Х	Х	Х	Х	Х	Х	Х
158		Lepomis symmetricus	Bantam Sunfish	Х					Х	Х	Х	Х	Х			
159		Micropterus dolomieu	Smallmouth Bass					Х	Х			Х	Х	Х	Х	Х
160		Micropterus punctulatus	Spotted Bass	Х					Х	Х	Х	Х	Х	Х		
161		Micropterus salmoides	Largemouth Bass	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
162		Micropterus treculii	Guadalupe Bass	Х			Х					Х	Х	Х	Х	
163		Pomoxis annularis	White Crappie	Х				Х	Х	Х	Х	Х	Х	Х	Х	Х
164		Pomoxis nigromaculatus	Black Crappie	Х					Х	Х	Х	Х	Х	Х	Х	Х
165	Percidae	Ammocrvpta clara	Western Sand Darter	х			Х		Х	Х						
166		Ammocrypta vivax	Scaly Sand Darter	х					х	х	х					
167		Etheostoma artesiae	Redspot Darter	х					Х	Х						
168		Etheostoma asprigene	Mud Darter	х					х	х						
169		Etheostoma chlorosoma	Bluntnose Darter	x					x	x	х	х	х	х		
170		Etheostoma fonticola	Fountain Darter	x		x	x							x		
171		Etheostoma fusiforme	Swamp Darter	x					v	x						
172		Ethoostoma jusijoime	Slough Darter	v					v	v	v	v	v	v	v	v
172		Ethoostoma grache	Pio Crando Dartor	л V			v		л	л	л	л	л	л	л	л V
175		Etheostoma granami	Hadaania Darter	л v			л		v	v						л
174		Elneosioma mistrio	Hariequin Darter	A V					л	л			v	v	v	
1/5		Etheostoma lepiaum	Greenthroat Darter	A V					v	v	v	v	A V	А	л	
1/6		Etheostoma parvipinne	Goldstripe Darter	X					X	X	X	Х	X			
1/7		Etheostoma proeliare	Cypress Darter	X					X	Х	Х		Х			
1/8		Etheostoma radiosum	Orangebelly Darter	X			Х		X		* 7	* 7	* 7	* *		
1/9		Etheostoma spectabile	Orangethroat Darter	X					Х		Х	Х	Х	X		
180		Percina apristis	Guadalupe Darter	Х			Х							Х		
181		Percina caprodes	Logperch	Х					Х							
182		Percina carbonaria	Texas Logperch	Х							Х	Х	Х	Х	Х	
183		Percina macrolepida	Bigscale Logperch	Х					Х	Х	Х	Х	Х	Х		Х
184		Percina maculata	Blackside Darter	Х			Х		Х	Х	Х					

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185	Percidae	Percina phoxocephala	Slenderhead Darter	Х					Х							
186		Percina sciera	Dusky Darter	Х					Х	Х	Х	Х	Х			
187		Percina shumardi	River Darter	Х					Х	Х				Х		
188		Sander vitreus	Walleye					Х								Х
189	Sciaenidae	Aplodinotus grunniens	Freshwater Drum	Х					Х	Х	Х	Х	Х	Х	Х	Х
190	Elassomatidae	Elassoma zonatum	Banded Pygmy Sunfish	Х					Х	Х	Х	Х				
191	Cichlidae	Herichthys cyanoguttatus	Rio Grande Cichlid	Х							Х	Х	Х	Х	Х	Х
192		Oreochromis aureus	Blue Tilapia								Х	Х	Х	Х	Х	Х
193		Oreochromis mossambicus	Mozambique Tilapia											Х	Х	Х
194	Gobiidae	Awaous banana	River Goby	Х												Х
195		Gobiosoma bosc	Naked Goby										Х			Х
196	Achiridae	Trinectes maculatus	Hogchoker	Х						Х	Х	Х	Х	Х	Х	
			Total	171	6	13	59	37	120	101	102	96	94	94	66	95

**Table 2.** Non-native fishes established in Texas and their continent of origin with respective citation.Presence denoted by "X".

Family	Species	Common Name	Marine	North America	Asia	Africa	South America	Europe	Citation
Cyprinidae	Carassius auratus	Goldfish			Х				Hubbs et al. 2008
	Ctenopharyngodon idella	Grass Carp			Х				Guillory and Gasaway 1978
	Cyprinus carpio	Common carp						Х	Allen 1980
	Hypophthalmichthys nobilis	Bighead Carp			Х				Kolar et al. 2007
Loricariidae	Hypostomus plecostomus	Suckermouth Catfish					Х		Hubbs et al. 2008
	Pterygoplichthys anisitsi	Southern Sailfin Catfish					Х		Nico and Martin 2001
	Pterygoplichthys disjunctivus	Vermiculated Sailfin Catfish					Х		Nico and Martin 2001
Salmonidae	Oncorhynchus mykiss	Rainbow Trout		Х					Hubbs et al. 1991
Atherinopsidae	Membras martinica	Rough Silverside	Х						Hubbs et al. 1991
Fundulidae	Fundulus grandis	Gulf Killifish	Х						Hubbs et al. 1991
	Lucania goodei	Bluefin Killifish		Х					Gallaway et al. 2008
Cyprinodontidae	Cyprinodon variegatus	Sheepshead Minnow	Х						Hubbs et al. 1991
Poeciliidae	Poecilia formosa	Amazon Molly	Х						Hubbs et al. 1991
	Poecilia latipinna	Sailfin Molly	Х						Hubbs et al. 1991
	Poecilia reticulata	Guppy					Х		Hubbs et al. 2008
	Xiphophorus hellerii	Green Swordtail		Х					Hubbs et al. 2008
	Xiphophorus variatus	Variable Platyfish		Х					Cohen et al. 2014
Moronidae	Morone saxatilis	Striped Bass	Х						Hubbs et al. 1991
Centrarchidae	Ambloplites rupestris	Rock Bass		Х					Hubbs et al. 1991
	Lepomis auritus	Redbreast Sunfish		Х					Hubbs et al. 1991
	Micropterus dolomieu	Smallmouth Bass		Х					Hubbs et al. 1991
Percidae	Sander vitreus	Walleye		Х					Hubbs et al. 1991
Cichlidae	Oreochromis aureus	Blue Tilapia				Х			Hubbs et al. 2008
	Oreochromis mossambicus	Mozambique Tilapia				Х			Hubbs et al. 2008
Gobiidae	Gobiosoma bosc	Naked Goby	Х						T. Bonner, unpublished data

Thirteen fishes are listed as threatened and endangered by U.S. Fish and Wildlife Service (USFWS), and 59 fishes are listed as Species of Greatest Conservation Need (SGCN, Texas Parks and Wildlife 2012). Number of native fishes by drainage basin ranged from 32 in the Canadian to 111 in the Red. Rio Grande & Pecos had the greatest number of USFWS threatened and endangered fishes (N = 7) and SGCN fishes (N = 28). Number of non-native fishes by drainage basin ranged from five in the Canadian to 20 in the Guadalupe & San Antonio. Origins of non-native fishes are from marine waters of Texas and from inland waters of North America and other continents (Table 2). Based on published accounts, non-native fishes were introduced for human consumption and sport (Nico and Fuller 1999), bait bucket releases (Howells 2001), vegetation control (Guillory and Gasaway 1978), accidental aquaculture releases (Howells 2001), and aquarium releases (Cohen et al. 2014).

A limitation of the drainage basin checklist and dichotomous keys is that documentation of species by drainage is incomplete. As such, our drainage basin checklists and dichotomous keys should be viewed as living documents and will need periodic updates. While using a drainage basin key, we caution users that the key only includes species known to occur within a basin, and the drainage basin might include more species. If an unknown specimen does not seem to key to a species, we recommend using a key from an adjacent drainage basin. Periodic updates of checklists for Texas inland fishes will come from previously unreported species, nonnative species introductions, extirpations of introduced and native fishes, and multiple species described from a single species through genetic analyses. Sources of this information will be dependent on publications and ichthyological records, such as Texas Natural History Collections (Hendrickson and Cohen 2015). In addition to publications and ichthyological records, an emerging tool for documenting species occurrences is the use of citizen science through web-based applications (e.g., iNaturalist, http://www.inaturalist.org). We plan to publish revised checklists and keys following the next release of revised common and scientific names list by the AFS-ASIH Committee of Names of Fishes.

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## Supplementary material I

### Drainage Basin Keys

Authors: Cody Andrew Craig, Timothy Hallman Bonner

Data type: Microsoft Word file

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RESEARCH ARTICLE



# First record of the genus Oodera Westwood, 1874 (Hymenoptera, Pteromalidae, Cleonyminae, Ooderini) from the Arabian Peninsula, with the description of four new species

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# Abstract

The genus *Oodera* Westwood, 1874 (Hymenoptera, Pteromalidae, Cleonyminae) is recorded for the first time for the Arabian Peninsula, from the Kingdom of Saudi Arabia and the Sultanate of Oman. The present study is based on specimens reared from xylophagous beetle larvae of the family Buprestidae (Co-leoptera) infesting dead *Acacia* trees from Al-Dakhiliyah and Dhofar governorates in Oman and Al-Baha, Asir and Riyadh regions in Saudi Arabia. Four new species, *Oodera arabica* **sp. nov.**, *O. omanensis* **sp. nov.**, *O. rapuzzii* **sp. nov.**, and *O. similis* **sp. nov.** are described, illustrated and compared with closely related *Oodera* species. An illustrated key and the xylophagous host records of the species are also provided.

# Keywords

Acacia, Oman, parasitic wasp, Saudi Arabia, systematics, xylophagous hosts

# Introduction

*Oodera* Westwood, 1874 (Hymenoptera, Pteromalidae, Cleonyminae) is a relatively small genus comprising currently twenty valid species (Werner and Peters 2018). It includes different-sized species (3.6–17 mm) (Werner and Peters 2018). Based on Holt et al.

(2013), they are distributed in the Oriental region (eight species), Afrotropical region (six species), Palaearctic region (six species) and Nearctic region (one species) (Noyes 2018; Werner and Peters 2018). However, the relatively recent report of *O. formosa* (Giraud) from the United States of America results from an accidental introduction from Europe (Werner and Peters 2018). Almost all *Oodera* species are reported as parasitoids of xylophagous beetle larvae of the families Buprestidae and Curculionidae (Coleoptera, Scolytinae) (Bouček 1958; Bouček and Rasplus 1991; Yang 1996; Gibson 2003; Werner and Peters 2018). Other details about their biology are still unknown (Werner and Peters 2018).

The phylogenetic status of *Oodera* has remained in dispute for a long time (Gibson 1989). It has been proposed as forming a link or a bridge between Cleonyminae (Pteromalidae) and Eupelminae (Eupelmidae) (Bouček 1958, 1988; Graham 1969) and has been classified and keyed in Eupelmidae rather than Cleonyminae by some authors (Ashmead 1904; Nikol'skaya 1952; Graham 1969). It was transferred from Eupelmidae to Pteromalidae by Bouček (1958) who established the monotypic tribe Ooderini for the genus in the subfamily Cleonyminae, family Pteromalidae (Heraty et al. 2013). The presence of a peculiar system of spines and spine-like setae along the ventral margin of the profemur, and the absence of a flexible transscutal articulation both support the monophyly of *Oodera* (Gibson 2003). On the other hand, the very distinctive structures and modifications of its middle legs (thickened mesotibial spur; presence of mesotarsal pegs; the presence of a membranous area anterior to each mesocoxa) might support the hypothesis of it being a sister of Eupelmidae or some part of it (Gibson 1989).

The first comprehensive work dealing with the taxonomy of the genus *Oodera* was that of Werner and Peters (2018), who revised the world species based on the morphological examination of 115 specimens. They reported twenty valid species for the genus, of which ten species were described as new to science. An illustrated key to all species, re-description of the other valid ones, with taxonomic treatments to some of them, were also given.

In the present study, *Oodera* is recorded for the first time for the fauna of the Arabian Peninsula, from Oman and Saudi Arabia. Specimens were reared from the dead wood of *Acacia* sp. trees. Four new species are described and illustrated. An illustrated key and the xylophagous hosts of species are also provided.

## Material and methods

The present study is based on 25 specimens reared from dead wood of *Acacia* sp. trees collected from Al-Dakhiliyah and Dhofar governorates in Oman and Al-Baha, Asir and Riyadh regions in Saudi Arabia, as follows:

Oman, Al-Dakhiliyah: Al-Hamra (23°10'26"N, 57°08'49"E, alt. 825 m). Oman, Dhofar: Mirbat (17°11'09"N, 54°59'31"E, alt. 500 m); Rawiyya (17°20'45"N, 54°03'57"E, alt. 650 m).

- Saudi Arabia, Al-Baha: Shada Al-Ala Natural Reserve (19°51'40"N, 41°18'16"E, alt. 1248 m); The Ain Village (19°55'47"N, 41°26'38"E, alt. 760 m); Wadi Tourabah (20°11'36"N, 41°17'50"E, alt. 1830 m); Wadi Shoqab (20°40'27"N, 41°15'02"E, alt. 1440 m); Wadi Yabah (19°16'32"N, 41°48'33"E, alt. 440 m).
- Saudi Arabia, Asir: Wadi Sabian (28 km S. Muhayil) (18°17'55"N, 42°07'41"E, alt. 809 m).
- Saudi Arabia, Riyadh: Wadi Al Hesiyah (40 km NW Riyadh) (24°55'22"N, 46°12'15"E, alt. 790 m).

The collected specimens were pinned directly for further study. Identification of the new species was made with the help of Werner and Peters' key (2018). Abbreviations used for measurements are based on Werner and Peters (2018), as follows: bdy.l = body length; cor.l = corona length; cor.w = corona width; F1, F2, F3 = first, second, third flagellomeres; **hea.h** = head height (frontal view); **hea.l** = maximum length of head (lateral view); **hea.w** = maximum width of head (frontal view); **eye.h** = height of eye (lateral view); **msp.l** = malar space; **eye.d** = shortest distance between eyes; **POL** = shortest distance between posterior ocelli (dorsal view); **OOL** = shortest distance between posterior ocellus and eye (dorsal view); **no.l** = pronotum length; **no.w** = pronotum maximum width; **msc.l** = mesoscutum length; **msc.w** = mesoscutum maximum width (= mesonotum width); msn.l = mesonotum length; sct.l = mesoscutellum length; sct.w = mesoscutellum width; **ppd.l** = propodeal length; **fm1.l** = profemur length; **fm1.w** = profemur width; **mav.l** = marginal vein length; **pmv.l** = postmarginal vein length; **mts.l** = metasomal length; **mts.w** = metasomal width; **ovp.l** = ovipositor length. Description format, characters definition, and ranges of measured ratios follow Werner and Peters (2018) to facilitate comparison. Body-sculpture terminology follows Harris (1979).

Photographic images were taken using a Canon EOS 70D camera attached to a Leica MZ 125 stereomicroscope. Individual source images were then stacked using HeliconFocus v6.22 (HeliconSoft Ltd) extended depth of field software. Further image processing was done using the software Adobe Photoshop CS5.1 (ver. 12.1x 32) and Adobe Photoshop Lightroom 5.2. Morphological measurements of the different parts were made with the help of a Zeiss Stemi 2000-C stereomicroscope with an ocular micrometer (100 lines per mm). Body part measurements were taken with the same magnification (20× eyepiece, 2.5× objective) for calculating different body ratios accurately and facilitate comparison. The detailed description for each species under study is based on the holotype specimen; for the diagnosis, all specimens under study were measured, and the minimum and maximum values are used.

The distribution of the prospected sites is plotted using ArcGIS 10.4. (Fig. 1). The type specimens of the new species are deposited in King Saud University Museum of Arthropods (**KSMA**), Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia.



Figure 1. Distributional map of collection localities of Oodera species in the Arabian Peninsula.

# Systematic account

## Oodera Westwood, 1874

 Oodera Westwood, 1874. Thesaurus Entomologicus Oxoniensis: 145. Type species: Oodera gracilis Westwood; subsequent designation by Ashmead (1904: 288).
Stellophora Risbec, 1951. Mem. Inst. Er. Afr. Noire 63: 239. Type species: Stellophora magnifica Risbec by monotypy. Synonymized by Bouček (1958: 375).

**Diagnosis.** Individuals of *Oodera* are diagnosed by the following combination of character states: head with deep scrobes in the form of an inverted V; parascrobal area of the head crested (= corona of Werner and Peters 2018); mesosoma dorsally flattened, with pronotum (no.) usually longer than wide, pentagonal (rounded in few cases), without differentiated collar, widened anteriorly and narrowed towards mesoscutum; mesoscutum (msc.) with star-like grooves, arranged radially from almost one point, notauli sulcate, V-shaped, extended to anterior margin of mesoscutellum; axillae conspicuously large, triangular, greatly advanced anterior to mesoscutellum; mesoscutellum (sct.) longitudinally ridged dorsally, with smoother coraceous apex; profemur (fm1.) distinctly enlarged, oval-shaped, with a row of oblique strong black bristles and a comb of peculiar pegs along its outer ventral margin; protibia (tb1.) curved, strongly carinate along its dorsal and ventral margins; mesocoxae with small membranous area anterior to each one; postmarginal vein (pmv.) of forewing slightly longer or slightly

shorter than marginal vein (mav.); metasomal petiole very short, membranous medioventrally; ovipositor (ovp.) sheaths varying in length among the different species, from shorter than, to distinctly longer than metasomal length (mts.l) (Bouček 1958, 1988; Gibson 1989, 2003; Bouček and Rasplus 1991; Werner and Peters 2018).

# Key to Oodera species of the Arabian Peninsula (male of O. arabica is unknown)

- 1 Body medium-sized (7.0-7.2 mm); antenna with scape, pedicel and basal half of F1 red, rest of flagellum black (Fig. 3A); pronotum 1.20-1.25× as wide as long, with broadest part at midlength (Figs 8A, 9A); mesoscutellum dull, black, with faint purple tint, and entirely lineate (median lines straight) (Figs 8A, 9E); apical segment of maxillary palp relatively long, angled baso-ventrally, lined ventrally with dense short whitish spines together with scattered long setae (Fig. 7A); metasoma 1.92× as long as wide (Fig. 2A); stigmal vein slender, straight, with stigma slightly roundly swollen apically (Fig. 10A)...... Oodera arabica sp. nov. Body small-sized (4.5-6.5 mm); antenna with only scape or part of it red, rest of antenna black (Fig. 3B-D); pronotum as wide as long, with broadest part before or behind midlength (Figs 8B-D, 9B-D); mesoscutellum shiny, metallic green or coppery, and lineate on anterior three-fourths or slightly more, but at least finely areolate before frenal line (median lines converging) (Figs 8B-D, 9F-H); apical segment of maxillary palp distinctly shorter, smoothly rounded baso-ventrally, without such short spines along its ventral margin (Fig. 7B-D); metasoma 2.12–2.45× as long as wide (Fig. 2B–D); stigmal vein relatively thick, curved, with smoothly quadrate stigma (Fig. 10B-D) ...... 2 Scape with basal two-thirds red and apical third black (Fig. 5C); horizontal crests 2 of corona distinctly prominent (high) and widely spaced (Figs 5G, 6C); prono-

- 3 Forewing partly infumate (Fig. 10B); pronotum narrow anteriorly, with broadest part behind midlength (Fig. 9B); face bluish to purplish (Fig. 5B, F); mesoscutellum with posterior half purplish (Fig. 9F); propodeum medium (ppd.l/msc.l

0.13-0.15); ovipositor rather long (16-17× as long as metasoma length); volsella of male genitalia with four teeth (Fig. 11D) ...... *Oodera omanensis* sp. nov.

## Oodera arabica Gadallah & Soliman, sp. nov.

http://zoobank.org/6D4D5A30-8081-4B52-988B-6AEC6BB41156 Figs 2A, 3A, 4A, 5(A, E), 6A, 7A, 8A, 9(A, E), 10A

**Material examined. Holotype**  $\bigcirc$ : SAUDI ARABIA, Al-Baha (Al-Mikhwah, Shada Al-Ala Natural Reserve), 29.iii.2017, leg. D. Baiocchi, e.l. *Acacia* [KSMA]. **Paratype** 1 $\bigcirc$ : SAUDI ARABIA, Al-Baha (Al-Mikhwah, The Ain Village), 13.iv.2016, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA].

**Diagnosis** (female) (N = 2). See Table 1.

Description. Female (holotype): Body length 7.2 mm (excluding the ovipositor).

**Colour.** Head black with strong coppery and green luster on face and faint green tint on gena (Figs 5A, E, 6A); scape, pedicel and basal half of FI red, rest of antenna black (Fig. 3A); maxillary and labial palpi dark brown to black (Fig. 7A). Mesosomal dorsum black with extremely faint purple and blue-green luster on pronotum, anterior part of axilla, mesoscutellum and propodeum (Figs 8A, 9A, E); mesosomal venter and coxae blue, midcoxa blackish (Fig. 4A); protrochanter and profemur black, the latter with slight blue-green tint on outer side (Fig. 3A); meso- and metatrochanters, tibiae and tarsi red, tarsi lighter (Figs 3A, 4A). Metasoma black, Gt2–5 with patches of green and slight coppery luster laterally (Figs 2A, 3A, 4A). Wings hyaline, veins yellow to light brown (Fig. 10A).

*Head.*  $1.6 \times$  as wide as long, hea.w  $4.5 \times$  eye.d (Fig. 5A); face setiferous foveatereticulate, setae lanceolate, white and short (Fig. 5A); msp.l  $0.37 \times$  head height (Fig. 5E); corona  $0.6 \times$  as long as eye.h (Fig. 5A); POL  $1.37 \times$  OOL (Fig. 6A); scape  $3.23 \times$  as long as pedicel; clava  $0.15 \times$  as long as funicle; flagellum  $1.2 \times$  as long as hea.w; F1  $0.6 \times$ as long as F2; F2  $1.12 \times$  as long as F3.

*Mesosoma.* Pronotum pentagonal, 0.48× as long as mesonotum (Fig. 8A); mesonotum 1.38× as long as mesoscutum (Fig. 8A); mesoscutum 0.9× as long as wide (Fig. 8A); mesoscutellum 0.38× as long as mesoscutum (Fig. 8A); profemur 1.4× as long as protibia.

*Forewing* (Fig. 10A). Forewing  $2.87 \times$  as long as wide; costal cell  $0.35 \times$  as long as forewing; marginal vein  $0.19 \times$  as long as forewing; marginal vein  $3.36 \times$  as long as stigmal vein; postmarginal vein  $3.86 \times$  as long as stigmal vein.

*Metasoma* (Fig. 2A, 3A, 4A). mts.l./mts.w = 1.92.

Male. Unknown.

Host record. Anthaxia (Haplanthaxia) kneuckeri zabranskyi Bílý, 1995 (Buprestidae).



Figure 2. Dorsal habitus, Holotype A *Oodera arabica* sp. nov. B *Oodera omanensis* sp. nov. C *Oodera rapuzzii* sp. nov. D *Oodera similis* sp. nov.



Figure 3. Lateral habitus, Holotype A *Oodera arabica* sp. nov. B *Oodera omanensis* sp. nov. C *Oodera rapuzzii* sp. nov. D *Oodera similis* sp. nov.

Distribution. Saudi Arabia (Al-Baha region).

**Remarks.** The new species resembles the Afrotropical species *O. mkomaziensis* Werner & Peters (Tanzania) and *O. namibiensis* Werner & Peters (Namibia) in having body medium-sized (7.0–7.2 mm in length), head and mesosoma uniformly black with tinges of dark green and coppery or purple, eye large (eye.h/hea.h 0.72–0.74), corona thick (cor.l/cor.w 3.08–3.60), with structure interrupted and propodeum medium (ppd.l/msc.l 0.15). It differs from *O. mkomaziensis* in the following: body robust, msn.l/msc.w 1.25–1.29 (in *mkomaziensis* slender, msn.l/msc.w 1.5); head rounded, 1.34–1.38× as high as long (oval, 1.56× as high as long in *mkomaziensis*); pronotum

Character	O. arabica sp. nov. $(N = 2)$	O. omanensis sp. nov. $(N = 4)$	O. rapuzzii sp. nov. $(N = 16)$	O. similis sp. nov. $(N = 3)$
Bdy.l (in mm)	Medium-sized (7.0-7.2)	Small-sized (5.5-6.3)	Small-sized (4.5-5.6)	Small-sized (6.3-6.5)
Body shape (msn.l/msc.w)	Robust (1.25-1.29) (Fig. 8A)	Robust (1.3-1.34) (Fig. 8B)	Robust to slender (1.33-1.45) (Fig. 8C)	Robust (1.28-1.30) (Fig. 8D)
Head shape in lateral view (hea.h/hea.l)	Rounded (1.34–1.38)	Rounded (1.40–1.44)	Rounded (1.35–1.36)	Rounded (1.37–1.38)
Eye size (eye.h/hea.h)	Large (0.72-0.74)	Large (0.70–0.75)	Large (0.70–0.72)	Large (about 0.75)
Corona shape	Thick (3.08-3.60), with interrupted	Thick (3.33-3.50), with interrupted	Thick (3.25-4.0), with interrupted structure	Thick (3.11-3.40), with interrupted
(cor.l/cor.w), its structure	structure (Fig. 6A)	structure (Fig. 6B)	(Fig. 6C)	structure (Fig. 6D)
Length and shape of apical	Relatively long, distinctly angulate baso-	Relatively short, evenly rounded (not	Relatively short, evenly rounded (not	Relatively short, evenly rounded (not
segment of maxillary palp	ventrally, lined ventrally with dense short whitish spines together with scattered long setae (Fig. 7A)	angulate) baso-ventrally, without such short spines along its ventral margin (Fig. 7B)	angulate) baso-ventrally, without such short spines along its ventral margin (Fig. 7C)	angulate) baso-ventrally, without such short spines along its ventral margin (Fig. 7D)
Pronotum length (according to its width), its anterior margin	Wider than long $(1.20-1.25\times)$ , with anterior margin truncate (Fig. 9A)	As long as wide, with anterior margin truncate (Fig. 9B)	As long as wide, anterior margin rounded (Fig. 9C)	As long as wide, anterior margin truncate (Fig. 9D)
Pronotum broadest part	At midlength (Fig. 9A)	Behind midlength (Fig. 9B)	Slightly behind midlength (Fig. 9C)	Slightly before midlength (Fig. 9D)
Mesoscutellum shape (sct.l/	Normal (0.60–0.62), anterior margin hardly	Normal (0.62-0.64), anterior margin hardly	Normal to slender $(0.65 - 0.75)$ , anterior	Normal (0.60-0.72), anterior margin hardly
sct.w), its anterior margin	convex (Fig. 9E)	convex (Fig. 9F)	margin hardly convex (Hig. 9പ)	convex (Fig. 9H)
Mesoscutellum sculpture	Entirely lineate (median lines straight) (Fig. 9E)	Almost completely lineate, finely areolate slightly before frenal line (median lines converging) (Fig. 9F)	Lineate on anterior two-thirds and finely areolate on posterior third (median lines converging) (Fig. 9G)	Almost completely lineate, finely areolate slightly before frenal line (median lines converging) (Fig. 9H)
Propodeum size ppd.l/msc.l	Medium (about 0.15) (Fig. 8A)	Medium (0.13-0.15) (Fig. 8B)	Medium (0.13–0.14) (Fig. 8C)	Large (0.17–0.21) (Fig. 8D)
Profemur shape (fm1.l/fm1.w)	Robust to medium (1.90–2.08)	Robust to medium (1.92–2.00)	Robust (1.95–2.00)	Robust (1.95–2.00)
Forewing	Hyaline (Fig. 10A)	Partly slightly infumate (Fig. 10B)	Hyaline (Fig. 10C)	Hyaline (Fig. 10D)
Marginal vein length mav.l/pmv.l	Short to medium (0.87–0.92) (Fig. 10A)	Medium (0.92–1.00) (Fig. 10B)	Medium (0.95–1.00) (Fig. 10C)	Medium (0.95-1.00) (Fig. 10D)
Metasoma length mts.l/ bdy.l	Short (0.42-0.43) (Fig. 2A)	Short (about 0.43) (Fig. 2B)	Short to medium (0.44-0.47) (Fig. 2C)	Short (0.42-0.45) (Fig. 3D)
Ovipositor length ovp.l/ mts.l	Short (1.00-0.13) (Fig. 2A).	Rather long (0.16-0.17) (Fig. 2B)	Rather long (0.16–0.19) (Fig. 2C)	Short (0.13-0.14) (Fig. 3D)
Volsella teeth (male genitalia)	unknown	Four (Fig.11D)	Four (Fig.11E)	Five (Fig. 11F)

Table 1. Diagnosis of *Oodera* species in the Arabian Peninsula.



Figure 4. Ventral habitus, Holotype A *Oodera arabica* sp. nov. B *Oodera omanensis* sp. nov. C *Oodera rapuzzii* sp. nov. D *Oodera similis* sp. nov.



Figure 5. Frontal and lateral view of head, Holotype A, E Oodera arabica sp. nov. B, F Oodera omanensis sp. nov. C, G Oodera rapuzzii sp. nov. D, H Oodera similis sp. nov.

pentagonal (oval in *mkomaziensis*); mesoscutellum entirely lineate (in *mkomaziensis* lineate in anterior two-thirds, finely areolate in posterior third); metasoma short,  $0.42-0.43\times$  as long as body (in *mkomaziensis* longer,  $0.52\times$  as long as body). The new species also differs from *O. namibiensis* in the following: forewing hyaline (partly infumate in *namibiensis*); antennal scape and pedicel and basal half of F1 are red, rest of antenna black (scape yellow, darkened apically, rest of antenna black in *namibiensis*); metasoma short,  $0.42-0.43\times$  as long as body length (in *namibiensis* medium to long,  $0.49-0.55\times$  as long as body length).

**Etymology.** Named in reference to the country of Saudi Arabia, where the type specimen was collected.

# *Oodera omanensis* Soliman & Gadallah, sp. nov. http://zoobank.org/3F7B9623-168C-40E3-BA69-F66E66830648 Figs 2B, 3B, 4B, 5(B, F), 6B, 7B, 8B, 9(B, F), 10B, 11(A, D)

**Material examined. Holotype**  $\bigcirc$ : OMAN, Dhofar (Rawiyya), 16.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA]; **Paratypes:** 2 $\bigcirc$  & 1 $\bigcirc$ : OMAN, Dhofar (Mirbat), 15–18.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA].

**Diagnosis.** Both sexes (N = 4). See Table 1.

Description. Female (holotype): Body length 6.3 mm (excluding the ovipositor).



Figure 6. Dorsal view of head, Holotype A *Oodera arabica* sp. nov. B *Oodera omanensis* sp. nov. C *Oodera rapuzzii* sp. nov. D *Oodera similis* sp. nov. (coronal structure indicated).

**Colour.** Face purple with bluish reflections (Fig. 5B), corona and scrobal depression black with slight bluish tint, gena and occiput dark green (Figs 5B, F, 6B); scape redbrown, with slight black tint on apical fourth, rest of antenna, mandible, maxillary and labial palpi dark brown to black (Figs 3B, 5B, 7B). Mesosomal dorsum black with purple luster on pronotum, anterior third of mesoscutal median lobe, anterior part of axilla and on propodeum (Figs 8B, 9B, F); mesoscutellum purple, with slight green and blue luster anteriorly (Fig. 9F); mesosomal venter black, with blue and green luster on prosternum and mesopleuron respectively (Fig. 4B); coxae black with strong purple luster on procoxa and green on metacoxa respectively (Fig. 4B); protrochanter black, meso- and metatrochanters, tibiae and tarsi red-brown (Figs 3B, 4B); profemur black with purple tint on outer side (Fig. 3B). Metasoma black, tergites with patches of blue laterally (Figs 2B, 3B, 4B). Forewing partly slightly infumate, veins dark brown (Fig. 10B).

*Head.*  $1.7 \times$  as wide as long, hea.w  $4.5 \times$  eye.d (Fig. 5B); face setiferous foveatereticulate, setae lanceolate, white and short (Fig. 5B); msp.l  $0.43 \times$  head height (Fig. 5F); corona  $0.5 \times$  as long as eye.h (Fig. 5B); POL  $1.4 \times$  OOL (Fig. 6B); scape  $3 \times$  as long as pedicel; clava  $0.11 \times$  as long as funicle; flagellum  $1.25 \times$  as long as hea.w; F1  $0.8 \times$  as long as F2; F2 hardly longer than F3.

**Mesosoma.** Pronotum pentagonal,  $0.53 \times$  as long as mesonotum (Fig. 8B); mesonotum  $1.4 \times$  as long as mesoscutum (Fig. 8B); mesoscutum  $0.95 \times$  as long as wide (Fig. 8B); mesoscutellum  $0.4 \times$  long as mesoscutum (Fig. 8B); propodeum with costate ridge in front of the setose area (Fig. 9F); profemur  $1.4 \times$  as long as protibia.



Figure 7. Maxillary palp, Holotype A *Oodera arabica* sp. nov. B *Oodera omanensis* sp. nov. C *Oodera rapuzzii* sp. nov. D *Oodera similis* sp. nov. (apical segment of maxillary palp indicated).

*Forewing* (Fig. 10B). Forewing with dense and long setae,  $2.8 \times as$  long as wide; costal cell  $0.37 \times as$  long as forewing; marginal vein  $0.2 \times as$  long as forewing; marginal vein  $4 \times as$  long as stigmal vein; postmarginal vein  $4.3 \times as$  long as stigmal vein.

*Metasoma* (Figs 2B, 3B, 4B). mts.l/mts.w = 2.16.

**Male.** Similar to female. **Genitalia** (Fig. 11A, D): narrowly rounded above; volsella with four outwardly curved, sharp teeth.

**Host record.** *Anthaxia* (*Haplanthaxia*) *abdita* Bílý, 1982, *A.* (*H.*) *kneuckeri zabranskyi* Bílý, 1995 (Buprestidae).

Distribution. Oman (Dhofar governorate).

**Remarks.** The new species, *O. omanensis*, closely resembles *O. circularicollis* Werner & Peters, *O. formosa* (Giraud), and *O. niehuisorum* Werner & Peters, but differs from them in the following:

**O.** omanensis vs. **O.** circularicollis (Morocco). Eye large,  $0.70-0.75 \times$  as high as head (small,  $0.54-0.56 \times$  as high as head, in *circularicollis*); POL  $1.4 \times$  OOL (as long as OOL in *circularicollis*); pronotum as long as wide, with anterior margin (collar) truncate ( $0.91 \times$  as long as wide, with collar virtually round in *circularicollis*); mesoscutellum almost completely lineate, finely areolate slightly before frenal line (lineate in anterior two-thirds, rugulose in posterior third in *circularicollis*); marginal vein  $4 \times$  as long as stigmal vein ( $2.5-3.53 \times$  as long as stigmal vein in *circularicollis*).

*O. omanensis* vs. *O. formosa* (Southern and Central Europe, Russia, Eastern United States, Eastern Canada). Head and mesosoma blue and purplish (dark green and coppery in *formosa*); scape of antenna red-brown, with black tint apically (yellow, darkening apically in *formosa*); head width 4.5× eye distance (3.00–3.78× eye distance in *formosa*); eye 0.75× as height as head (0.55–0.68× as height as head in *formosa*); corona with structure interrupted



**Figure 8.** Dorsal aspect of mesosoma, Holotype **A** *Oodera arabica* sp. nov. **B** *Oodera omanensis* sp. nov. **C** *Oodera rapuzzii* sp. nov. **D** *Oodera similis* sp. nov.



Figure 9. A-D Dorsal aspect of pronotum, Holotype A *Oodera arabica* sp. nov. B *Oodera omanensis* sp. nov. C *Oodera rapuzzii* sp. nov. D *Oodera similis* sp. nov. E-H Mesoscutellum, metanotum and propodeum E *Oodera arabica* sp. nov. F *Oodera omanensis* sp. nov. G *Oodera rapuzzii* sp. nov. H *Oodera similis* sp. nov. (anterior margin of pronotum indicated in A-D, propodeal lateral ridge indicated in G, H).

(with structure continuous in *formosa*); pronotum with broadest part behind midlength (with broadest part at midlength in *formosa*); mesoscutellum almost completely lineate, finely areolate slightly before frenal line (lineate in anterior half to anterior two-thirds, rugulose in posterior half or third in *formosa*); profemur robust to medium, 1.92–2.00× as long as wide (usually medium to elongated, 1.94–2.33× as long as wide, in *formosa*).

**O.** omanensis vs. **O.** niehuisorum (Egypt and Israel). Forewing partly slightly infumate (hyaline in *niehuisorum*); corona thick, 3.33–3.50× as long as wide, with structure interrupted (usually medium, 3.8–6.0× as long as wide, with structure continuous in *niehuisorum*); pronotum with broadest part behind midlength (broadest part before midlength in *niehuisorum*); mesoscutellum almost completely lineate, finely areolate slightly before frenal line (densely lineate in anterior half to anterior two-thirds, areolate in posterior half or third in *niehuisorum*); marginal vein medium, 0.92–1.00× as long as postmarginal vein (short, 0.78–0.89× as long as postmarginal vein in *niehuisorum*).

*O. omanensis* sp. nov. also resembles the new species *O. similis*, but differs from it in the following combination of characters: forewing partly infumate, with dark brown to black veins (hyaline in *O. similis*, with pale brown veins); head with bluish to purplish luster (green and coppery in *O. similis*); mesoscutellum with green basal half, violet posteriorly (entirely green in *O. similis*); pronotum distinctly narrow anteriorly, with dense lanceolate whitish setae longitudinally along the middle area, with broadest part behind midlength (distinctly broad anteriorly, with fewer setae along the middle area longitudinally, with broadest part before midlength in *O. similis*); propodeum medium, ppd.l/msc.l 0.13–15 (large, ppd.l/msc.l 0.17–0.21, in *O. similis*); volsella of male genitalia with four sharp teeth, aedeagus with parallel outer sides (with five teeth, aedeagus with strongly convex outer margins in *O. similis*).

**Etymology.** Named in reference to the country of Oman, where the type specimen was collected.



Figure 10. Forewing, Holotype A *Oodera arabica* sp. nov. B *Oodera omanensis* sp. nov. C *Oodera rapuzzii* sp. nov. D *Oodera similis* sp. nov.



Figure 11. Dorsal aspect and tip of male genitalia, Paratype **A**, **D** *Oodera omanensis* sp. nov. **B**, **E** *Oodera rapuzzii* sp. nov. **C**, **F** *Oodera similis* sp. nov. (numbers 1–5 indicate number of teeth on volsellae).

# Oodora rapuzzii Soliman & Gadallah, sp. nov.

http://zoobank.org/BAC4F734-2978-458C-A25D-D69975B2BC5D Figs 2C, 3C, 4C, 5(C, G), 6C, 7C, 8C, 9(C, G), 10C, 11(B, E)

**Material examined. Holotype**  $\bigcirc$ : SAUDI ARABIA, Al-Baha (Wadi Tourabah), 12.iv.2016, leg. P. Rapuzzi, e.l. *Acacia* [KSMA]. **Paratypes:**  $3 \bigcirc \& 2 \circlearrowright$ : SAUDI ARA-BIA, Al-Baha (Al-Mikhwah, The Ain Village), 13.iv.2016, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $3 \circlearrowright$ : SAUDI ARABIA, Al-Baha (Wadi Shoqab), 12.iv.2016, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : SAUDI ARABIA, Al-Baha (Wadi Yabah), 15.iv.2016, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : SAUDI ARABIA, Al-Baha (Wadi Yabah), 15.iv.2016, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : SAUDI ARABIA, Al-Baha (Wadi Yabah), 15.iv.2016, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : SAUDI ARABIA, Al-Baha (Wadi Yabah), 15.iv.2016, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \diamondsuit$ : SAUDI ARABIA, Al-Baha (Wadi Yabah), 15.iv.2016, leg. D. Baiocchi, e.l. Acacia sp. [KSMA];  $2 \circlearrowright \& 2 \circlearrowright$ : OMAN, Dhofar (Rawiyya), 16.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : OMAN, Dhofar (Mirbat), 15–18.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : SAUDI ARABIA, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : OMAN, Dhofar (Mirbat), 15–18.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : SAUDI ARABIA, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : OMAN, Dhofar (Mirbat), 15–18.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : SAUDI ARABIA, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA];  $1 \circlearrowright$ : OMAN, Dhofar (Mirbat), 15–18.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA].

**Diagnosis.** Both sexes (N = 16). See Table 1.

**Description.** Female (holotype): Body length 5.6mm (excluding the ovipositor).

**Colour.** Head black on corona, scrobal depression and occiput, coppery on face (lower face with green luster), blue on gena (Figs 5C, G, 6C); scape red-brown on basal two-thirds, rest of antenna, maxillary and labial palpi dark brown to black (Figs 3C, 5C, 7C). Mesosomal dorsum black with blue luster on pronotal disc, green on anterior half of mesoscutum, green and purple on anterior part of axilla and on all mesoscutel-lum, blue and purple on propodeum (Figs 8C, 9G); mesosomal venter with strong blue, green and purple luster (Fig. 4C); coxae black with strong purple luster on ventral side of procoxa (Fig. 4C); profemur black with green tint on outer face (Fig. 3C); trochaters, meso- and metafemora brown, tarsi yellow-brown (Fig. 4C). Metasoma black, with patches of green-purple on lateral sides of Gt2–4 (Figs 2C, 3C, 4C). Wings hyaline, veins brown (Fig. 10C).

*Head.* 1.66× as wide as long (Fig. 5C); hea.w 4.56× eye.d (Fig. 5C); face setiferous foveate-reticulate, setae lanceolate, white and short (Fig. 5C); msp.l 0.43× head height (Fig. 5G); corona 0.6× as long as eye.h (Fig. 5C); POL 1.33× OOL (Fig. 6C); scape 3× as long as pedicel; clava 0.15× as long as funicle; flagellum 1.45× as long as head width; F1 0.68× as long as F2; F2 hardly longer than F3, 1.05×.

**Mesosoma.** Pronotum pentagonal,  $0.5 \times$  as long as mesonotum (Fig. 8C); mesonotum  $1.5 \times$  as long as mesoscutum (Fig. 8C); mesoscutum as long as wide (Fig. 8C); mesoscutellum  $0.44 \times$  as long as mesoscutum (Fig. 8C); propodeum laterally with a smooth ridge in front of the setose area (Fig. 9G); profemur  $1.29 \times$  as long as protibia; pronotal disc and posterior part of mesoscutum with stout short white setae.

*Forewing* (Fig. 10C). Forewing  $2.84 \times$  as long as wide; costal cell  $0.35 \times$  as long as forewing; marginal vein  $0.21 \times$  as long as forewing; marginal vein  $3.62 \times$  as long as stigmal vein; postmarginal vein  $3.79 \times$  as long as stigmal vein.

*Metasoma* (Fig. 2C, 3C, 4C). mts.l/mts.w = 2.45.

**Male.** Similar to female but slightly darker in colour. **Genitalia** (Fig. 11B, E) widely rounded above; volsella with four outwardly directed, sharp teeth, the innermost one is very short compared with the others.

Host record. Anthaxia (Haplanthaxia) abdita Bílý, 1982, A. (H.) cf. angustipennis (Klug, 1829), A. (H.) kneuckeri zabranskyi Bílý, 1995, A. (H.) marginifera dhofarica Bílý, 2003, A. (H.) wittmeri Bílý, 1979; Chalcogenia halperini arabica Bílý, 2008 (Buprestidae).

Distribution. Oman (Dhofar governorate); Saudi Arabia (Al-Baha and Asir regions).

**Remarks.** The new species closely resembles *O. formosa* (Giraud), but differs from it in the following combination of characters: wing hyaline (partly infumate in *O. formosa*); corona thick,  $3.25-4.00\times$  as long as wide, with structure interrupted (thick to medium, 3.20-6.67, structure continuous in *O. formosa*); metasomal length short to medium,  $0.44-0.47\times$  as long as body (short to long, 0.43-0.55 in *O. formosa*); ovipositor length rather long,  $0.16-0.19\times$  as long as metasoma (usually short, 0.09-0.17 in *O. formosa*); head and mesosoma with strong blue colour in some parts (never with blue evident in *O. formosa*).

**Etymology.** This species is named in honour of Pierpaolo Rapuzzi, who participated in the breeding of this species from the dead wood of *Acacia* sp.

## Oodera similis Gadallah & Soliman, sp. nov.

http://zoobank.org/95C405C2-217D-430F-8C70-5D27412D49D8 Figs 2D, 3D, 4D, 5(D, H), 6D, 7D, 8D, 9(D, H), 10D, 11(C, F)

**Material examined. Holotype**  $\Im$ : SAUDI ARABIA, Riyadh (Wadi Al Hesiyah, 40 km NW Riyadh), 30.iv.2017, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA]; **Paratypes:** 1 $\Im$  & 1 $\Im$ , OMAN Al-Dakhiliyah (Al-Hamra), 21.i.2018, leg. D. Baiocchi, e.l. *Acacia* sp. [KSMA].

**Diagnosis.** Both sexes (N = 3). See Table 1.

Description. Female (holotype): Body length 6.3 mm (excluding the ovipositor).

*Colour.* Head black with slight blue-green tint on corona, scrobal depression and occiput (Figs 5D, 6D), become coppery with green luster on face, and blue on gena (Figs 5D, H); scape red-brown, rest of antenna, maxillary and labial palpi dark brown to black (Figs 3D, 7D). Mesosomal dorsum black with green and purple luster on pronotum, anterior third of mesoscutal median lobe, anterior part of axilla and on propodeum (Figs 8D, 9D, H); mesoscutellum metallic green, with slight coppery luster anteriorly (Fig. 9H); mesosomal venter blue-green, with purple luster on mesopleuron (Fig. 4D); coxae black with strong green luster on ventral side, mesocoxa mostly black (Fig. 4D); trochanters, tibiae and tarsi red-brown, protrochanter darker (Figs 3D, 4D); profemur black with purple-green tint on outer side (Fig. 3D). Metasoma black, tergites with patches of blue laterally (Fig. 2D, 3D, 4D). Wings hyaline, veins brown (Fig. 10D).

*Head.*  $1.7 \times$  as wide as long, hea.w  $4.2 \times$  eye.d (Fig. 5D); face setiferous foveate-reticulate, setae lanceolate, white and short (Fig. 5D); msp.l  $0.4 \times$  head height (Fig. 5H); corona  $0.5 \times$  as long as eye.h (Fig. 5D); POL  $1.7 \times$  OOL (Fig. 6D); scape  $3.5 \times$  as long as pedicel; clava  $0.13 \times$  as long as funicle; flagellum  $1.3 \times$  as long as hea.w; F1  $0.75 \times$  as long as F2; F2 hardly longer than F3,  $1.05 \times$ .

**Mesosoma.** Pronotum pentagonal,  $0.5 \times$  as long as mesonotum (Fig. 8D); mesonotum  $1.5 \times$  as long as mesoscutum (Fig. 8D); mesoscutum  $0.9 \times$  as long as wide (Fig. 8D); mesoscutellum  $0.5 \times$  as long as mesoscutum (Fig. 8D); propodeum with costate ridge in front of the setose area (Fig. 9H); profemur  $1.3 \times$  as long as protibia.

*Forewing* (Fig. 10D). Forewing 2.75× as long as wide; costal cell 0.4× as long as forewing; marginal vein 0.2× as long as forewing; marginal vein 3.5× as long as stigmal vein; postmarginal vein 3.66× as long as stigmal vein.

*Metasoma* (Fig. 2D, 3D, 4D). mts.l/mts.w = 2.12.

**Male.** Similar to female except for second and third metasomal sternites with blue reflection. **Genitalia** (Fig. 11C, F). Narrowly rounded above; volsella with five sharp, outwardly curved teeth, of which the innermost is minute.

Host record. Anthaxia (Haplanthaxia) abdita Bílý, 1982, A. (H.) kneuckeri zabranskyi Bílý, 1995 (Buprestidae).

Distribution. Oman (Al-Dakhiliyah governorate); Saudi Arabia (Riyadh region).

**Remarks.** This species resembles the Oriental species *O. srilankiensis* Werner & Peters 2018 (Sri Lanka) in having the body robust; antennal scape red-brown, rest

of antenna black; flagellum about  $1.3 \times$  as long as head width; pronotum about  $0.5 \times$  as long as mesonotum; pronotum pentagonal, with broadest part before midlength; propodeum large; forewing hyaline; marginal vein medium; ovipositor distinctly shorter than metasoma (less than  $0.25 \times$  metasomal length). However, it differs from *O. srilankiensis* in the following combination of characters: body size larger, 6.30-6.5 mm in length (4.00-5.75 mm in *srilankiensis*); head and mesosoma black with metallic green, blue, purple and coppery in different parts (dark blue to blue-green in *srilankiensis*); head  $1.70 \times$  as wide as long ( $1.28-1.48 \times$  in *srilankiensis*); head width  $4.20 \times$  eye distance ( $3.44-3.85 \times$  in *srilankiensis*); corona  $3.10-3.40 \times$  as long as wide, with structure interrupted ( $3.70-4.75 \times$  as long as wide, with structure continuous in *srilankiensis*); mesoscutellum almost entirely lineate, finely areolate slightly before frenal line (meoscutellum lineate in anterior third to half, rugulose in posterior half or two-thirds in *srilankiensis*); profemur robust,  $1.95-2.00 \times$  as long as wide (usually medium to elongated,  $1.98-2.33 \times$  as long as wide, in *srilankiensis*).

The new species resembles also the Palaearctic species, *O. niehuisorum* Werner & Peters, 2018 in having the small body size; wings hyaline; eye large; metasoma short; pronotum pentagonal, with broadest part before midlength. However, it differs from *O. niehuisorum* in the following combination of characters: head with some blue (never with blue in *niehuisorum*); antenna with scape red-brown, rest dark brown to black (scape and pedicel (except apex of pedicel) yellow, rest dark brown to black in *niehuisorum*); corona thick,  $3.10-3.40\times$  as long as wide, with structure interrupted (medium,  $3.80-6.00\times$  as long as wide, with structure continuous); mesoscutellum normal, sct.l/ sct.w 0.60-0.72 (normal to slender in *niehuisorum*, 0.12-2.15); mesoscutellum completely lineate, slightly finely areolate before frenal line (densely lineate in anterior half to anterior two-thirds, and areolate on posterior half or third in *niehuisorum*); profemur robust, fm1.l/fm1.w 1.95-2.00 (robust to medium in *niehuisorum*, 0.78-0.89); ovipositor short, ovp.l/mts.l 0.13-0.14 (short to rather long in *niehuisorum*, 0.14-0.18).

**Etymology.** From the Latin, refers to the similarity of this species with *O. srilankiensis* Werner & Peters.

#### Discussion

In the present study, four new species of the genus *Oodera* reared from dead *Acacia* trees are collected from different regions of Oman and Saudi Arabia (new locality record), with the help of beetle specialists. The study is based on morphological data of 25 specimens (13 females and 12 males). An illustrated key to Arabian species and detailed description of the new species are provided, in addition to analysis with similar valid species. Intraspecific variation is found to be slight as the number of the collected specimens is relatively small because of the rarity of this genus.

The current study is the second contribution to the study of this beautiful and interesting group of Chalcidoidea, covering a new area (Arabia) that was not considered in previous studies (example Werner and Peters 2018). Four new species are added to the world fauna thus increasing the total number to 24 species.

The world species of *Oodera* was first revised by Werner and Peters (2018), who recognized 20 valid species from which 10 are described as new species. Full descriptions of the new species, and re-description of formerly known ones are given, together with an illustrated key to world species. Few taxonomic changes are also discussed.

The main observation emerging from our study, is the strong correlation of *Oodera* fauna with the intermediate biogeographical situation of the study area. Almost, all of the studied specimens were collected from southwestern and southeastern parts of Arabia, that are exclusively Afrotropical (Larsen 1984; Burckhardt and Mifsud 1998), only one specimen is collected from Riyadh (Palaearctic). A hypothesis that is supported by Werner and Peters (2018) who concluded that *Oodera* species seemingly prefer warmer to temperate regions.

However, because of the biodiversity richness of Arabia, due to its rich floristic diversity, more species of this genus are expected to occur. Therefore, further collections and studies are still needed to clarify the distribution of this genus in other parts of this area.

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RESEARCH ARTICLE



# First checklist of mayflies (Insecta, Ephemeroptera) from Kosovo

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## Abstract

This research provides the first systematic contribution to the mayfly (Ephemeroptera) Fauna of Kosovo. This investigation was conducted from March to November in 2017 and 2018; 32 sites were sampled covering the different freshwater ecosystems of the country. The first checklist of mayflies of Kosovo is provided. During this survey, we found 48 species belonging to 20 genera and nine families. The highest number of species belongs to the following two families, Heptageniidae (24) and Baetidae (9). This investigation is a contribution to the knowledge about taxonomy, biogeography, and ecology of mayflies of the Balkan Peninsula by giving new data on species composition and distribution range in Kosovo.

# Keywords

Aquatic insects, Balkan Peninsula, freshwater fauna, Kosovo, taxonomy

# Introduction

Mayflies (Ephemeroptera) are an ancient insect lineage dating back over 300 million years and are believed to be the most primitive group of extant winged insects (Grimaldi and Engel 2005, Bauernfeind and Soldán 2012). Mayflies are merolimnic insects: the larval stage is strictly aquatic, while the imaginal stage is extremely brief and on the wing. Mayflies are able to colonise every kind of freshwater habitat but are mainly diversified in lotic habitats (streams and rivers). They are distributed worldwide with the

highest diversity in tropical areas; the order encompasses approximately 3500 species, 450 genera and 42 families (Barber-James et al. 2008, Sartori and Brittain 2015). According to the literature (Bauernfeind and Soldán 2012), 369 species are recorded for Europe and North Africa. Mayflies are considered as keystone species and their presence is believed to be an important environmental indicator of oligotrophic to mesotrophic (i.e., low to moderately productive) conditions in running waters (Barbour et al. 1999, Bauernfeind and Moog 2000). High sensitivity of mayfly taxa to oxygen depletion, acidification, and various contaminants including metals, ammonia, and other chemicals was demonstrated in both observational and experimental studies (Hubbard and Peters 1978, Resh and Jackson 1993, Moog et al. 1997). Various Biological Indices including mayflies to assess water quality have been developed over the years (Lenat 1988, Metcalfe 1989, Kerans and Karr 1994). Subsequently, most of the biological water quality assessment methods for streams include Ephemeroptera, for example, the EPT (Ephemeroptera + Plecoptera + Trichoptera) taxa richness (Lenat and Penrose 1996).

Faunistics and taxonomy of mayflies in the Balkans is still in progress and the level of knowledge varies between different countries. Neighbouring mayfly fauna is relatively well known, mostly thanks to studies in Croatia (Vilenica et al. 2015), N. Macedonia (Ikonomov 1961a, 1961b, 1962, 1963, 1964), Serbia (Petrovic et al. 2014, Slovenia (Zabric and Sartori 1997), Bosnia and Hercegovina (Bauernfeind and Soldán 2012), Bulgaria (Vidinova 2003, Vidinova and Janeva 2000, Vidinova and Russev 1997, Vidinova et al. 2006), and Hungary (Kovács and Bauernfeind 2003). By contrast, fewer than ten species are currently known from Albania (Kovács and Murányi 2013) reflecting insufficient research effort in this country.

Kosovo is a small landlocked country in the centre of Balkan Peninsula and is divided into two ecoregions: Dinaric Western Balkan (ER5) and Hellenic Western Dinaric (ER6) (Illies 1978). In hydrographical terms, Kosovo is divided into four river basins: Drini i Bardhë, Ibri, Morava e Binçës, and Lepenci which flow into three sea basins: Black Sea, Adriatic Sea, and Aegean Sea (Fig. 1). Kosovo has a total area of 10,908 km<sup>2</sup> with an altitude range from 265 m to 2656 m. The mountains of Kosovo belong to the Dinarides range with two major mountain massifs, Sharr and Bjeshkët e Nemuna.

Published data on mayflies from Kosovo are scarce. However, as a part of former Yugoslavia, there are some records published by different authors: Puthz 1974 (*Baetis alpinus, Baetis rhodani, Epeorus assimilis* (wrongly identified as *Epeorus sylvicola* (Pictet, 1865)), *Ecdyonurus insignis, Rhithrogena germanica*, and *Ephemerella krieghoffi* (Ulmer, 1920) (now considered as a junior synonym of *Ephemerella mucronata*); Studemann et al. 1989 (*Ephemerella ikonomovi* species now assigned to *Quatica*); Hefti and Tomka 1988 (*Ecdyonurus subalpinus*). Other papers only explored mayflies as part of the general macroinvertebrate assemblage, which is essential for the implementation of bioassessment tools for the local stream systems (Shukriu 1979, Gashi 1993, 2005, Zhushi-Etemi 2005, Kuqi 2006, Ibrahimi 2007). Consequently, little data was available about the mayfly fauna of Kosovo and no major collections have been housed so far. The distribution range of the different species throughout the territory remains unknown. Therefore, in this paper, we include the first checklist of species and the distribution of mayfly species in Kosovo.

# Materials and methods

## Sampling and laboratory methods

Most of the studied material for this research was collected during the two-year sampling period from March to November 2017/2018. Mayflies were sampled every month at 21 sites while at the remainder of sites, sampling was usually performed only once during this time. Specimens were collected in freshwater habitats (mainly lotic and some lentic) in over 32 sites throughout Kosovo's territory (Fig. 1).

The detailed list of the 32 sampling site names with number codes (site ID), altitude, latitude and longitude are presented in Table 1. Mayfly larvae were collected using a hand D-net or picked manually from rocks and pebbles, while imagos were caught with a hand net and light traps, then preserved in 96 % ethanol. Collected specimens were studied under a Leica M205 and Olympus stereomicroscope.

Mayflies were found at all 32 sampled localities (Fig. 1). The majority of specimens were collected at the larval stage, but a small number of adults were caught in the field also. The identification of the mayflies to species level has been performed by using the books by Bauernfeind and Humpesch (2001), Eiseler (2005), Bauernfeind and Soldán (2012), Elliott et al. (1988), and Soldán and Landa (1999); when necessary, morphological characters were checked in the original descriptions (Braasch 1984, Jacob 1974, Jacob and Braasch 1984, 1986, Puthz 1971, Ikonomov 1961a, and Demoulin 1958). Nomenclature and family assignment follow Bauernfeind and Soldán (2012), except for the family Ephemerellidae for which we follow Jacobus and McCafferty (2008) and Baetidae for which we consider *Alainites* and *Procloeon* as valid genera. As part of the species is still incompletely described or one of the two stages remains unknown, the association of larval and adult stages is sometimes challenging in mayflies. Without rearing in the field, the use molecular data such as mitochondrial gene (the animal "barcode") will provide an alternative for association of ontogenetic stages. For these reasons, identification of some species remains difficult; they are therefore referred to as cf. or as sp. All material examined are housed in the Museum of Zoology, Lausanne, Switzerland, and Laboratory of Faculty of Natural Sciences, Pristina, Kosovo. Authorships of the species are indicated in Table 3; they are not mentioned in the main text, except for species not occurring in Kosovo.

# Results

The current research produced the first comprehensive contribution of mayfly taxa inhabiting Kosovo's freshwater habitats. In 32 sampling stations, a total of 7564 individuals of mayfly larvae and adults was collected in rivers, streams and some lentic habitats. Sampling sites included a wide range of elevation with lowest L7 at 345 m and highest L10 at 1664 m (Table 1).

Based on the analysed data, in total, 48 species distributed into 20 genera and nine families were recorded (Table 2). The most diversified family was Heptageniidae (four genera and 24 species), followed by Baetidae (four genera and nine species). The



Figure 1. Map of Kosovo indicating the collection sites.

following families had only one species: Ameletidae, Oligoneuriidae, and Potamanthidae. The most diverse genera were *Ecdyonurus* with 13 species, *Rhithrogena* with seven species, and *Baetis* with five species. Seven species were recorded at only one site: *Potamanthus luteus, Electrogena* cf. *mazedonica, Procloeon* cf. *pulchrum, Ecdyonurus* cf. *siveci, Ephemera vulgata, Caenis horaria* and *Caenis* cf. *strugaensis*.

The most frequently encountered species was *Baetis rhodani* which was recorded from 21 of the 32 sites. *Baetis alpinus* and *Ephemera* cf. *parnassiana* were found at 14 and seven different sites, respectively (Table 3.). Three species were recorded as adults only at one site: *Ecdyonurus graecus* (site L20), *Ecdyonurus* cf. *puma* (site L16) and *Paraleptophlebia submarginata* (site L13). The remaining species occurred at between two to six sites. Taxa richness per locality varied from two to 17 species.
Code	Sampling site	Altitude	Latitude	Longitude	Ecoregion	Sea basin	Habitat
L1	Radavcë	1170 m	42°44.14'N	20°18.51'E	ER5 (Dinaric)	Adriatic	Lotic
L2	Çakorr	1242 m	42°41.31'N	20°04.38'E	ER5 (Dinaric)	Adriatic	Lotic
L3	Mirusha	370 m	42°31.25'N	20°34.50'E	ER5 (Dinaric)	Adriatic	Lotic
L4	L. Deçanit-Manastiri	903 m	42°54.71'N	20°26.66'E	ER5 (Dinaric)	Adriatic	Lotic
L5	L. Erenik-Botusha	874 m	42°30.00'N	20°14.47'E	ER5 (Dinaric)	Adriatic	Lotic
L6	L. Erenik-Devë	567 m	42°28.42'N	20°16.53'E	ER5 (Dinaric)	Adriatic	Lotic
L7	L. Erenik-Travë	345 m	42°22.32'N	20°24.15'E	ER5 (Dinaric)	Adriatic	Lotic
L8	L. Prizren-Vlashnje	364 m	42°10.02'N	20°31.05'E	ER6 (Hellenic)	Adriatic	Lotic
L9	Prizren-Reçan	532 m	42°17.03'N	21°21.74'E	ER6 (Hellenic)	Adriatic	Lotic
L10	Prevall	1664 m	42°16.10'N	20°95.33'E	ER6 (Hellenic)	Adriatic	Lotic
L11	Piran (L.Toplluha)	394 m	42°28.81'N	20°67.17'E	ER6 (Hellenic)	Adriatic	Lotic
L12	Blinaja	721 m	42°51.85'N	20°97.88'E	ER6 (Hellenic)	Black	Lentic
L13	Shtërpcë (Brod)	692 m	42°16.26'N	21°07.73'E	ER6 (Hellenic)	Aegean	Lotic
L14	Nerodime E. Jezercë	810 m	42°21.22'N	21°01.14'E	ER6 (Hellenic)	Aegean	Lotic
L15	Viti	520 m	42°30.62'N	21°36.20'E	ER6 (Hellenic)	Black	Lotic
L16	Mbi Zhegër	660 m	42°29.51'N	21°54.58'E	ER6 (Hellenic)	Black	Lotic
L17	Stanqiq	800 m	42°25.50'N	21°55.02'E	ER6 (Hellenic)	Black	Lotic
L18	Lugu i Kopilaqës	1175 m	42°24.60'N	21°43.11'E	ER6 (Hellenic)	Black	Lotic
L19	Sanakov	625 m	42°25.90'N	21°34.33'E	ER6 (Hellenic)	Black	Lotic
L20	Letnicë	662 m	42°28.72'N	21°45.73'E	ER6 (Hellenic)	Black	Lotic
L21	Slivovë	646 m	42°36.70'N	21°18.19'E	ER6 (Hellenic)	Black	Lotic
L22	Binçë (Debelldeh +Buzovik)	566 m	42°29.48'N	21°37.17'E	ER6 (Hellenic)	Black	Lotic
L23	Mramor	635 m	42°37.84'N	21°16.47'E	ER5 (Dinaric)	Black	Lotic
L24	Keçekoll	754 m	42°43.45'N	21°18.50'E	ER5 (Dinaric)	Black	Lotic
L25	Makovcë	626 m	42°41.92'N	21°14.23'E	ER5 (Dinaric)	Black	Lotic
L26	Dermjak	606 m	42°17.22'N	21°31.57'E	ER6 (Hellenic)	Black	Lotic
L27	Stanishor	622 m	42°36.70'N	21°18.19'E	ER6 (Hellenic)	Black	Lotic
L28	Shushtë-Kabash	525 m	42°28.11'N	21°35.91'E	ER6 (Hellenic)	Black	Lotic
L29	Mjak	735 m	42°25.36'N	21°34.76'E	ER6 (Hellenic)	Black	Lotic
L30	Korbiliq	730 m	42°22.98'N	21°33.58'E	ER6 (Hellenic)	Black	Lotic
L31	Ibri-Leposaviç	544 m	42°59.66'N	20°48.97'E	ER5 (Dinaric)	Black	Lotic
L32	Ligatina e Hencit	545 m	42°51.85'N	20°97.88'E	ER5 (Dinaric)	Black	Lentic

 Table I. Sampling site characteristics.

 Table 2. Kosovo Ephemeroptera composition.

Family	Number of genera	% Genus	Number of species	% Species
Ameletidae	1	5.00	1	2.08
Baetidae	4	20.00	9	18.75
Oligoneuriidae	1	5.00	1	2.08
Heptageniidae	4	20.00	24	50.00
Leptophlebiidae	3	15.00	3	6.25
Potamanthidae	1	5.00	1	2.08
Ephemerellidae	4	20.00	4	8.33
Ephemeridae	1	5.00	2	4.16
Caenidae	1	5.00	3	6.25
	20	100	48	100

Ephemeroptera taxa		Adriatic Sea basin	Black Sea basin	Aegean Sea basin
Family: Ameletidae McCafferty, 1991				
Genus: Metreletus Demoulin, 1951				
1. Metreletus balcanicus (Ulmer, 1920)	NR		L12, L24	
Family: Baetidae Leach, 1815				
Genus: Baetis Leach, 1815				
2. Baetis rhodani (Pictet, 1843)		L3-L11	L12, L15, L16, L17, L20, L21, L23, L24, L25, L28, L29, L30	
3. Baetis alpinus (Pictet, 1843)		L1, L2, L3, L4, L5, L6, L7, L9, L10	L28, L29, L30	L13, L14
4. Baetis buceratus Eaton, 1870	NR	L3	L16, L21, L23	
5. Baetis melanonyx (Pictet, 1843)	NR	L4, L5, L6, L9		L13, L14
6. Baetis pentaphlebodes Ujhelyi, 1966	NR		L12, L21, L32	
Genus: Alainites Waltz & McCafferty, 19	84			
7. Alainites muticus (Linnaeus, 1758)	NR	L1, L5, L6	L32	
Genus: Cloeon Leach, 1815				
8. Cloeon dipterum (Linnaeus, 1761)	NR	L11	L12, L32	
9. Cloeon cf. dipterum (Linnaeus, 1761)	NR		L12, L32	
Genus: Procloeon Bengtsson, 1915				
10. Procloeon cf. pulchrum (Eaton, 1885)	NR		L12	
Family: Oligoneuriidae Ulmer, 1914				
Genus: Oligoneuriella Ulmer, 1924				
11. Oligoneurella rhenana (Imhoff, 1852)	NR	L6, L7	L21	
Family: Heptageniidae Needham, 1901				
Genus: <i>Epeorus</i> Eaton, 1881				
12. Epeorus assimilis Eaton, 1885		L6, L9	L25, L28	
13. Epeorus vougoslavicus (Šamal, 1935)	NR	L2, L9		
Genus: Ecdvonurus Eaton, 1871				L
14. Ecdvonurus graecus Braasch, 1984	NR	L1	L20, L30	
15. Ecdyonurus cf. epeorides Demoulin, 1955	NR	L3	L21, L22, L26, L27	
16. <i>Ecdyonurus</i> cf. <i>puma</i> Jacob & Braasch, 1986	NR		L16, L17	
17. <i>Ecdyonurus macani</i> Thomas & Sowa, 1970	NR	L1	L12, L21	
18. Ecdyonurus starmachi Sowa, 1971	NR	L3	L12, L19, L21, L22	
19. <i>Ecdyonurus vitoshensis</i> Jacob & Braasch, 1984	NR		L18, L24, L31	
20. Ecdyonurus venosus (Fabricius, 1775)	NR	L1, L3	L15, L21, L20, L24	
21. Ecdyonurus submontanus Landa, 1969	NR	L3, L5	L22	
22. Ecdyonurus cf. krueperi (Stein, 1863)	NR		L21, L22	
23. <i>Ecdyonurus</i> cf. <i>siveci</i> Hefti, Tomka & Zurwerra, 1986	NR	L2		
24. <i>Ecdyonurus</i> sp.			L12, L21	
25. Ecdyonurus insignis (Eaton, 1870)		_	_	_
26. Ecdyonurus subalpinus (Klapálek, 1907)		_	_	_

**Table 3.** Kosovo mayfly fauna: first checklist of species with distribution. Key: NR: new records for Kosovo; ▲: data from literature only (Puthz 1974, Hefti and Tomka 1988).

Ephemeroptera taxa		Adriatic Sea basin	Black Sea basin	Aegean Sea basin
Genus: Rhithrogena Eaton, 1881				
27. Rhithrogena braaschi Jacob, 1974	NR	L2, L9	L21	
28. Rhithrogena gr. sowai Puthz, 1972	NR	L9	L21	
29. <i>Rhithrogena</i> cf. <i>bulgarica</i> Braasch, Soldán & Sowa, 1985	NR	L9	L16, L25, L28, L29, L30	
30. Rhithrogena gr. hercynia Landa, 1969	NR	L9	L25	
31. <i>Rhithrogena</i> gr. <i>semicolorata</i> (Curtis, 1834)	NR	L9, L10	L25	
32. Rhithrogena gr. diaphana Navàs, 1917	NR	L9	L15, L21, L24	
33. Rhithrogena germanica Eaton, 1885		_	_	_
Genus: <i>Electrogena</i> Zurwerra & Tomka,	1985			
34. <i>Electrogena</i> cf. <i>mazedonica</i> (Ikonomov, 1954)	NR		L12	
35. Electrogena sp.		L11	L12	
Family: Leptophlebiidae (Banks, 1900)		1		
Genus: Habrophlebia Eaton, 1881				
36. <i>Habrophlebia eldae</i> Jacob & Sartori, 1984	NR		L12, L24	
Genus: Habroleptoides Schoenemund, 19	929			
37. <i>Habroleptoides confusa</i> Sartori & Jacob, 1986	NR	L5, L6	L21, L23, L25	
Genus: Paraleptophlebia Lestage, 1917		1		
38. Paraleptophlebia submarginata (Stephens, 1836)	NR		L13, L15, L20, L21, L28	
Family:Potamanthidae Albarda, 1888		1		
Genus: Potamanthus Pictet, 1843				
39. Potamanthus luteus (Linnaeus, 1767)	NR		L20	
Family: Ephemerellidae Klapálek, 1909		1		
Genus: Torleya Lestage, 1917				
40. Torleya mayor (Klapalek, 1905)	NR	L11	L21, L25, L28	
Genus: Serratella Edmunds, 1959		1		
41. Serratella ignita (Poda, 1761)	NR	L3, L5, L6, L8, L11	L21	
Genus: Quatica Jacobus & McCafferty, 2	008	1		
42. Quatica ikonomovi (Puthz, 1971)		L8, L9	L12, L15	
Genus: Ephemerella Walsh, 1863		·		
43. Ephemerella mucronata (Bengtsson, 1909)	NR	L11	L32	
Family: Ephemeridae Latreille, 1810				
Genus: Ephemera Linnaeus, 1758				
44. Ephemera cf. parnassiana Demoulin, 1958	NR		L12, L17, L20, L21, L23, L24, L29	
45. Ephemera vulgata Linnaeus, 1758	NR		L12	
Family: Caenidae Newman, 1853				
Genus: Caenis Stephens, 1836				
46. Caenis macrura (Stephens, 1835)	NR	L11	L12, L32	
47. Caenis horaria (Linnaeus, 1758)	NR		L12	
48. Caenis cf. strugaensis Ikonomov, 1961	NR		L12	

The highest taxa richness was found along sampling site L21-Slivovë (17 species) and L12-Blinajë (16 species) and the lowest species richness (two species) was observed at site L8. Of the total mayfly species (48) for the three Sea Basins, 42 species were discovered for the Black Sea Basin, 29 species for the Adriatic Sea Basin, and only two species in the Aegean Sea Basin.

## Discussion

Due to the absence of consistent data and research on mayfly fauna as well as of their habitat preferences in Kosovo, this study provides the first global contribution to the mayflies of Kosovo with 48 recorded mayfly taxa. However, out of 48 species, three were previously recorded and not found in the present study: by Puthz (1974) two species (*Ecdyonurus subalpinus*) (Table 3). Most of the species collected during this investigation belong to the Western-Palearctic group with 23 followed by the Balkan group with seven species, the Holarctic group with six species, the South Europe group with six species, the Palearctic group with four species, and the Holomediterranean group with two species.

In comparison with the neighbouring countries and with consideration of their surface areas, the recorded Ephemeroptera diversity in Kosovo could be characterised as intermediate. The highest number of species was listed for Bulgaria with 102 taxa (Vidinova 2003), Serbia with 85 taxa (Petrovic et al. 2014, Croatia with 79 taxa (Vilenica et al. 2015) followed by Slovenia with 75 taxa (Zabric and Sartori 1997), N. Macedonia with 63 taxa (Smith and Smith 2003), and Bosnia and Hercegovina with 51 taxa (Bauernfeind and Soldán 2012). In this research, collecting was carried out mainly in running waters; therefore, lentic species are less diversified. Nonetheless, it was discovered that most of the Kosovo mayfly species are associated with rivers and streams. Some species of the genera Ecdyonurus and Rhithrogena are still considered as cf. and gr. (Table 3) because of the uncertainty of identifications as well as species unknown or poorly known at only one stage. Metreletus balcanicus, a rare European mayfly species, was recorded in two sites (L12 and L24) with low numbers of individuals; consequently, it could be considered a rare species in Kosovo too. According to the Fauna Europaea database, the species is present in Bulgaria, Czech Republic, Germany, Luxemburg, French Mainland, Hungary, Poland, and the European part of Turkey (de Jong et al. 2014). Balkan endemics (Electrogena cf. mazedonica, Rhithrogena braaschi, and Ephemera cf. parnassiana) were also recorded. Regarding the species Electrogena cf. mazedonica, it is a rare Balkan endemic with records in Macedonia (Ikonomov 1964) and provisional records from the northern border of Greece (Bauernfeind 2003). Our findings show it is a rare species also in Kosovo with records in only one sampling station (L12). Rhithrogena braaschi has probably a Pontic origin, recorded so far from the Balkans: Bulgaria (e.g., Vidinova et al. 2006) and Greece (Bauernfeind 2003). In our collections, it occurred in three localities: L2, L9, and L21. Ephemera cf. parnassiana

is a very rare Balkan endemic species present only in Greece (Bauernfeind 2003) and Croatia (Vilenica et al. 2015); nonetheless, in Kosovo, it had a wider distribution with a large number of individuals.

During our research, the highest number of species (17) were recorded from sample site L21-Slivovë and 16 species from the L12-Blinajë. On the other hand, high elevation sites (L2, L10, and L18) had the lowest number of mayfly species (two) as well as one sample site L8 at a low elevation with only two species. Sample sites L12 and L21 were high in species diversity because they were covered with macrophytic vegetation and different substrates in a clean habitat with altitudes of approximately 700 m. On the other hand, L8 was low on species diversity because it is affected by pollution from outside the large town and is well subjected to long-term anthropogenic stress from discharged urban sewage. The majority of Kosovo mayflies belong to the south European, central European, and Mediterranean faunas. For each species, their geographical distribution is presented as well as the sample site at which it was collected (Tables 1, 3).

The new records include some morphologically interesting taxa and difficult complex of species (*Cloeon* gr. *dipterum*, *Rhithrogena* gr. *sowai*, and *Ecdyonurus* gr. *venosus*). As two of the most similar mayfly assemblages of the neighbouring countries (N. Macedonia, Serbia) have several taxa that could also inhabit Kosovo habitats (e.g., *Baetis vardarensis* Ikonomov, 1962, *Baetis liebenauae* Keffermüller, 1974, *Cloeon simile* Eaton, 1870), but were not yet recorded, due to the lack of regular sampling in all seasons, future research should include seasonal sampling of a higher number of sites and habitat types. Further study is required at new sampling sites to determine the distribution of seven species recorded only at a single sampling site (*Potamanthus luteus, Electrogena* cf. *mazedonica, Procloeon* cf. *pulchrum, Ecdyonurus* cf. *siveci, Ephemera vulgata, Caenis horaria*, and *Caenis* cf. *strugaensis*).

Mayflies are generally diverse in lotic ecosystems as the majority of species prefer well-oxygenated habitats (Merritt et al. 2008). Consequently, the highest species diversities in this study were recorded along rivers and streams. The richest genera were *Ecdyonurus* (13 species), *Rhithrogena* (seven species), and *Baetis* (five species), which are known to be very prevalent in running waters of the northern hemisphere (Bauernfeind and Soldán 2012). *Baetis rhodani* was the most commonly encountered taxon in Kosovo and occurred in 66 % of the sampled sites. This species was found at a variety of lotic habitats including rivers and streams. The elevation range of this species in Kosovo extended from 400 to 1000 m. The wide occurrence of this species among our sampled sites is most likely due to its very broad ecological range (Bauernfeind and Soldán 2012). However, several habitats have been poorly investigated, such as those at high altitudes above 1800 m.

The present study is a significant contribution to the understanding of the mayfly fauna in Kosovo and the Balkan Peninsula, with the country's first checklist along with some rare species records. Therefore, the current study adds to a stronger knowledge of Kosovo's mayfly fauna and may promote the development of regional biological water quality indicators. Some interesting taxa with restricted European and local distributions were recorded (e.g., *Rhithrogena* cf. *bulgarica*, *Metreletus balcanicus*, and *Epeorus yugoslavicus*). Considering these species were collected in a limited number of sites in this study, they could be considered as rare. Future studies on the conservation status and ecological features of these species are necessary.

## Conclusion

As there was essentially no systematic research on mayfly fauna (species diversity and distribution) in Kosovo, this research is the first contribution toward mayfly inventory of this part of the Balkan Peninsula based on larvae and adult specimen collections. Kosovo's mayfly fauna comprises 9 families, 20 genera, and 48 species. Out of 48 mayfly taxa, 45 species are new records from Kosovo. The present research gives the record of Kosovo mayflies which belong to the West Palearctic, Central South European, Balkan, and Mediterranean species. Of 48 taxa, approximately half of the species were present in both Ecoregions (ER5 and ER6). Nonetheless, several habitats have been poorly investigated, such as high altitude habitats (above 1800 m). Further, emphasis on lentic habitats will be made as taxa such as *Caenis* spp. and *Cloeon* spp. are still under-sampled. Therefore, this research constitutes a first contribution to mayfly fauna of Kosovo and is far from complete.

The updating of this first mayfly checklist is highly expected with new investigations. Furthermore, the recorded diversity of Ephemeroptera in Kosovo could be defined as intermediate compared to neighbouring countries, taking into account their surface areas. The highest diversity was observed in submontane regions, while the lowest was detected in rivers and the majority of species collected in this research belong to grazers–scrapers and gatherers–collectors. The future challenges will be to identify the cryptic species within a difficult complex of species (*Cloeon* gr. *dipterum*, *Rhithrogena* gr. *sowai*, and *Ecdyonurus* gr. *venosus*) with careful taxonomical examination and the use of DNA barcoding.

This first checklist of mayflies and their distribution are intended to serve as a foundation and stimulation for further research since the records of many species and their distribution patterns within Kosovo can surely be amended in the future. Moreover, given the high diversity of freshwater habitats within four river basins and the scarce research on mayflies, finding species new for the country (or even new to science) are highly expected.

Finally, further research could clarify the ecological preferenda of each species and their degree of vulnerability in Kosovo to offer an essential tool for running water management and river quality assessments. New knowledge about the Ephemeroptera diversity and distribution in Kosovo will be highly beneficial for further investigation and biomonitoring of the environmental changes in freshwater habitats including the evaluation of other anthropogenic impacts.

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CHECKLIST



# An updated checklist of the extant Western Palaearctic Dryininae (Hymenoptera, Dryinidae)

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# Abstract

A checklist of 20 extant species of Dryininae (Hymenoptera, Dryinidae) from the Western Palaearctic subregion is presented.

# Keywords

Checklist, distribution, Chrysidoidea

# Introduction

Pincer wasps (Hymenoptera, Dryinidae) are parasitoids and often also predators of Auchenorrhyncha (Hemiptera) (Olmi 1984). The family includes 50 genera and 16 subfamilies (Olmi and Xu 2015; Tribull 2015). In the Palaearctic region, the subfamily Dryininae is represented by two extant genera, *Dryinus* Latreille, 1804 and *Pseudodryinus* Olmi, 1991. *Pseudodryinus* is known only from the Eastern Palaearctic subregion and *Dryinus* from both Palaearctic subregions, Eastern and Western (Olmi and Xu 2015).

A review of the Western Palaearctic Dryininae (Hymenoptera, Dryinidae) was published by Olmi (1984), and he listed a total of nine species. However, in the last 25 years many additional papers on the Western Palaearctic fauna have been published, so that the number of species has increased to 20, and the need to develop a new checklist of Western Palaearctic Dryininae became evident. The objective of this checklist is to ease further studies on Palaearctic dryinids.

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## Material and methods

The present paper treats all extant Dryininae (fossil species are excluded) present in the Western Palaearctic subregion, i.e., according to Vigna Taglianti et al. (1992, 1999), the part of the Palaearctic region situated in Europe and Asia west to the Ural Mountains and Caspian Sea, from the Azores and Canary Islands to Iran (included). The borders are not always obvious and natural. The eastern boundary runs along the Ural Mountains and the eastern bank of Caspian Sea, reaching Iran. The Russian region situated immediately east of Ural Mountains in parts of Kazakhstan, Turkmenistan, and Iran should be considered transition country to the Eastern Palaearctic subregion, whereas a large part of the Arabian Peninsula should be considered a transition area to the Afrotropical region. All these transition areas are considered in this checklist. The knowledge of the dryinids living in the Western Palaearctic subregion is broadly insufficient, so that this checklist will need to be updated in the future following further research.

Distributional data of Dryininae in the Western Palaearctic region were compiled analysing all the available publications, in addition to many unpublished records obtained by identifying material belonging to various institutions.

All the localities cited in this checklist, except that from Belarus cited by Shlyakhtyenok (2013) (see *Dryinus collaris* (Linnaeus)), were checked by the authors by examining personally all the specimens. The examined specimens are deposited in the following collections:

AEC	Christoph Saure's collection, Berlin, Germany.		
AMNH	American Museum of Natural History, New York, USA.		
ASM	Alexander Shlyakhtenok's collection, Minsk, Belarus.		
BNC	Benoît Nusillard's collection, Montboucher sur Jabron , France.		
BWC	Bogdan Wiśniowski's collection, Ojców National Park, Ojców, Poland.		
CAS	California Academy of Sciences, San Francisco, California, USA.		
CIRAD	Centre International de Recherche Agricole pour le Développement,		
	Montpellier, France.		
CNC	Canadian National Collection of Insects (CNCI), Ottawa, Canada.		
DEI	Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany.		
DEUW	Department of Entomology, University of Wageningen, the Netherlands.		
DISAFA	Dipartimento di Scienze agrarie, forestali e alimentari, University of To-		
	rino, Grugliasco, Torino, Italy.		
DPPZ	Department of Plant Protection, College of Agriculture, University of		
	Zabol, Iran.		
ENSAM	École National Supérieure Agronomique, Montpellier, France.		
FBW	Forstliche Versuchs- und Forschungsanstalt Baden-Württemberg,		
	Freiburg, Germany.		
FSAE	Faculté des Sciences Agronomiques de l'État, Gembloux, Belgium.		
GLPC	Gianluca Parise's collection, Carignano, Torino, Italy.		
GNC	Göran Nilsson's collection, c/o Department of Zoophysiology, Uppsala		
	University, Uppsala, Sweden.		

GPC	Guido Pagliano's collection, Torino, Italy.
HMO	Hope Museum, Oxford, England, United Kingdom.
HTS	Hubert Tussac's collection, Cahors, Lot, France (now c/o Museum
	d'Histoire naturelle, Genève, Switzerland).
IGC	Ilia Gjonov's collection, Sofia, Bulgaria.
IRSN	Institut Royal de Sciences Naturelles de Belgique, Bruxelles, Belgium.
JBZC	Javier Blasco-Zumeta's collection, Pina de Ebro, Zaragoza, Spain.
JTBC	John T. Burn's collection, Sacriston, England, United Kingdom.
LOHC	Lars Ove Hansen's collection, Drammen, Norway.
MBC	Manuel Baena's collection, Cordoba, Spain.
MCNTN	Museo de Ciencias Naturales, Santa Cruz, Tenerife, Canary Islands,
	Spain.
MCSNG	Museo Civico di Storia Naturale "Giacomo Doria" di Genova, Italy.
MCSNV	Museo Civico di Storia Naturale, Verona, Italy.
MHNG	Muséum d'Histoire Naturelle, Genève, Switzerland.
MLUHW	Martin-Luther-Universität, Halle-Wittenberg, Germany.
MNCNM	Museo Nacional de Ciencias Naturales, Madrid, Spain.
MNHN	Muséum National d'Histoire Naturelle, Paris, France.
MMB	Moravian Museum, Brno, Czech Republic.
MOLC	Massimo Olmi's collection, Viterbo, Italy.
MSC	Massimiliano Spinola's collection, c/o Museo Regionale di Scienze Natu-
	rali, Torino, Italy.
MSCS	Martin Schwarz's collections, c/o Institut für Zoologie, Salzburg,
	Austria.
MSNTC	Museo di Storia naturale e del Territorio, Università di Pisa, Calci, Italy.
MZUN	Museo di Zoologia dell'Università, Napoli, Italy.
NHMUK	Natural History Museum, London, United Kingdom.
NMNH	National Museum of Natural History, Budapest, Hungary.
NMPC	National Museum (Natural History), Praha, Czech Republic.
NMW	Naturhistorischen Museum, Wien, Austria.
OLL	Oberösterreichisches Landesmuseum, Linz, Austria.
PNL	Pierre-Nicolas Libert's collection, Somme-Leuze, Belgium.
RNHL	Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands.
SVC	Simo Väänänen's collection, Vantaa, Finland.
SZC	Pier Luigi Scaramozzino's collection, Pisa, Italy.
USNM	National Museum of Natural History, Washington, DC, USA.
VVC	Veli Vikberg's collection, Turenki, Finland.
WHC	Paul Whitehead's collection, Moor Leys, England, United Kingdom.
YUIC	Yeungnam University Insect Collection, Department of Biology, Yeung-
	nam University, Kyongsan, South Korea.
ZIL	Zoological Institute, Lund, Sweden.
ZMK	Zoologisk Museum, Copenhagen, Denmark.
ZMM	Zoological Museum of Moscow University, Moscow, Russia.
ZMUH	Zoological Museum of the University, Helsinki, Finland.

## Checklist of the extant Western Palaearctic Dryininae Haliday, 1833

#### Genus Dryinus Latreille, 1804

#### 1. Dryinus albrechti (Olmi)

Richardsidryinus albrechti Olmi 1984: 909. Dryinus albrechti (Olmi): Olmi 1999: 192.

SPAIN: Canary Islands: Fuerteventura, Las Peñitas (MNCNM) (Olmi 1984); Lanzarote,
El Risco de Famara (AMNH) (Olmi 1984); Tenerife, Orotava (ZMUH) (Olmi 1984).
Distribution: Spain.

#### 2. Dryinus balearicus Olmi

Dryinus balearicus Olmi 1987: 418; Olmi 1999: 209.

**SPAIN: Balearic Islands**: Ibiza, 5 km N San José (NHMUK, AMNH) (Olmi 1987). **Continental Spain**: Huesca Prov., near Torla, Fanlo (NHMUK). **TUNISIA**: Barrage Mellègue (MOLC).

Distribution: Spain, Tunisia.

#### 3. Dryinus berlandi (Bernard)

Chelothelius berlandi Bernard 1935: 41; Olmi 1984: 609. Dryinus berlandi (Bernard): Olmi 1999: 202.

**FRANCE**: Var, Fréjus, Saint-Raphaël beach (MNHN) (Bernard 1935). **MOROC-CO**: along Road P 39, 69 km Melilla, Dar Driouch (MBC) (Olmi 1999). **TUNISIA**: 10 km N of Jendouba (OLL).

Distribution: France, Morocco, Tunisia.

### 4. Dryinus canariensis (Ceballos)

*Paradryinus canariensis* Ceballos 1927: 101. *Dryinus canariensis* (Ceballos): Olmi 1984: 734; 1999: 184.

**SPAIN: Canary Islands**: Gomera, San Sebastian, Barranco de Marchar (MNCNM) (Olmi 1984); Gomera, Chejelipes (NHMUK); Hierro, Frontera (MCNTN); La Palma, Montaña Brena (OLL); Tenerife, Barranco Santos (AMNH, MCNTN, MNCNM) (Olmi 1984); Tenerife, Desembocadura del Barranco de Tejina (AMNH); Tenerife, La Cuesta (MNCNM) (Ceballos 1927); Tenerife, Tahodio (MNCNM) (Olmi1984);

Tenerife, Médano, Los Calderones (MCNTN, MNCNM) (Olmi 1984); Tenerife, Médano (MNCNM); Tenerife, Arico, Montaña Atalaya (MNCNM); Tenerife, Bajamar (MNCNM); Tenerife, Las Mercedes (MNCNM); Tenerife, Carretera de San Andres, Jagua (AMNH); Tenerife, Raguo Negro (OLL). **EGYPT**: Sinai, Saint Catherine area (MOLC). **GREECE**: Rhodes Island, ridge N of Psinthos (NHMUK).

Distribution: Egypt, Greece, Spain.

### 5. Dryinus collaris (Linnaeus)

Sphex collaris Linnaeus 1767: 946.
Dryinus formicarius Latreille 1805: 228 (synonymized by Fitton et al. 1978).
Campylonyx ampuliciformis Westwood 1835: 52 (synonymized by Olmi 1984).
Lestodryinus formicarius (Latreille): Kieffer 1914a: 20.
Lestodryinus corsicae Kieffer 1914a: 21 (synonymized by Olmi 1984).
Dryinus (Lestodryinus) formicarius Latreille: Haupt 1932: 15.
Dryinus collaris (Linnaeus): Olmi 1999: 185; Olmi and Xu 2015: 133.

AUSTRIA: Niederösterreich, Piesting (NMW); Oberösterreich, Hinteraigen, E Aibach/Donau, 48°24'N, 13°57'E (OLL); Salzburg, Werfen (RNHL) (Olmi 1984); Steiermark, O-Steiermark, E Weiz, Hoferberg (OLL); Wien, Dornbach (MNHN) (Olmi 1984). BELARUS: Polyese Radiacyonno-ekologichesky zapovednik, Dronki (ASM) (Shlyakhtyenok 2013). BELGIUM: Brabant, Forêt de Soignes (IRSN); Liège, Flémalle-Haute aux Roches (IRSN); Namur, Ave-et-Auffe, Thérimont (IRSN); Namur, Somal (PNL). CROATIA: Istra, Opatija (NMNH) (Olmi 1984); Krapina (NMNH) (Olmi 1984). FRANCE: Alpes de Haute-Provence, Digne (MNHN) (Olmi 1984); Corse (ENSAM) (Olmi 1999); Gard, Bez-et-Esparon (CIRAD) (Tussac and Olmi 1998); Haute-Garonne, Clermont-le-Fort (HTS) (Tussac and Olmi 1998); Haute-Garonne, Toulouse, L'Isle-Jourdain (FSAE); Haute-Loire, Le Puy (MNHN) (Olmi 1984); Haute-Savoie, Bossy-Frangy (MHNG); Hérault, Saint-Gély-du-Fesc (MNHN) (Olmi 1984); Hérault, Mons-la-Triviale, Gorges d'Héric (CIRAD) (Tussac and Olmi 1998); Hérault, Saint-Guilhem-le-Désert (MNHN) (Olmi 1984); Landes, Mont-de-Marsan (MNHN) (Olmi 1984); Pyrénées-Orientales, Forêt de Boucheville (DEUW); Rhône, Lyon (MNHN) (Westwood 1835); Saône-et-Loire, Les Gerraux (MNHN) (Olmi 1984); Var, Hyères (MNHN) (Olmi 1984); Var, Toulon (MNHN) (Olmi 1984); Var, Sainte-Baume (MNHN); Vaucluse, near Bédoin (NHMUK). GERMANY: Baden-Württemberg, Baden, Freiburg i. B., Bechtaler Wald, 48°12'N, 07°42'E (FBW); Baden-Württemberg, Freiburg im Breisgau, Mooswald-Nord (AEC); Nordrhein - Westfalen, Aix-la-Chapelle (= Aachen) (Kieffer and Marshall 1905). HUNGARY: Borsod-Abaúj-Zemplén county, Cserépfalu, Hór-völgy (NMNH, AMNH) (Szöllösi-Tóth and György 2009). ITALY: Campania, Napoli Prov., Napoli (Kieffer and Marshall 1905); Emilia Romagna, Bologna Prov., Gaibola (AMNH) (Olmi 1984); Liguria, Genova Prov., S. Olcese (NHMUK) (Olmi 1984); Piemonte, Cuneo Prov., Valmala, along Comba di Valmala, Ponte Parasacco (IRSN); Piemonte,

Torino Prov., Rosta (AMNH) (Olmi 1984); Piemonte, Torino Prov., Strambino (DIS-AFA) (Olmi 1999); Puglia, Taranto Prov., Mottola, S. Basilio (Móczár 1965); Sicilia, Catania Prov., Bronte, Maletto, Mt. Etna, Contrada Paviglione (AMNH) (Olmi 1999); Toscana, Lucca Prov., Lucca (Kieffer and Marshall 1905); Toscana, Lucca Prov., Lido di Camaiore (MOLC) (Olmi 2005b); Trentino Alto Adige, Bolzano Prov., Bolzano (Schmiedeknecht 1907). MONTENEGRO: Herceg Novi (= Castelnuovo di Cattaro) (DEI); Zelenika (NMNH) (Olmi 1984). POLAND: East bank of Oder River, 10 km N of Cedynia, Bielinek (= Bellinchen) (Haupt 1932, as Dryinus (Lestodryinus) formicarius). SLOVAKIA: SW Slovakia, Little Carpathians (Malé Karpaty), near confluence Danube and Morava Rivers, Devínska Kobyla Hill (Lukás 1998). SPAIN: Balearic Islands: Mallorca, Porto Cristo (RNHL). Continental Spain: Alicante Prov., Sierra de Altana (RNHL). SWITZERLAND: Genève, Peney (MHNG) (Olmi 1984); Genève (MSC) (Olmi 1984); Genève, Bois de Collex (MHNG); Genève, Place des Nations (NMNH); Valais, Châteauneuf (MHNG); Ticino, Gandria (NMNH, AMNH) (Olmi 1999). THE NETHERLANDS: Lexmond (RNHL); Neercanne, Cannerbos (RNHL) (De Rond 2004). TURKMENISTAN (Ponomarenko 1978). UNITED KINGDOM: England: Berkshire, High Standinghill Wood, Windsor Forest (NHMUK); Middlesex, Ruislip, Victoria Road (NHMUK); Surrey, Banstead Downs (NHMUK); Surrey, Shere (Capron 1885); Surrey, Reigate, 30 Park Lane East (only photographed, not collected); West Kent, Cobham (Richards 1939); West Kent, Eltham (JTBC); Worcestershire, Malvern Hills (WHC) (Whitehead 2010).

**Distribution**: Austria, Belarus, Belgium, Croatia, France, Germany, Hungary, Italy, Montenegro, Poland, Slovakia, Spain, Switzerland, the Netherlands, United Kingdom, in addition to Turkmenistan (transition country to Eastern Palaearctic subregion).

### 6. Dryinus corsicus Marshall

Dryinus corsicus Marshall 1874: 207; Olmi and Xu 2015: 133. Mesodryinus corsicus (Marshall): Kieffer 1907: 10. Mesodryinus escorialensis Ceballos 1927: 102 (synonymized by Olmi 1984). Richardsidryinus corsicus (Marshall): Móczár 1965: 377.

**CYPRUS**: Limassol (NHMUK) (Olmi 1984). **FRANCE**: Alpes-Maritimes, Breil-sur-Roya, Col des Termes (CIRAD); Aude, Brouilla (BNC); Bouches-du-Rhône, Aix-en-Provence (NHMUK) (Olmi 1984); Corse, Ajaccio, Campoloro (NMNH) (Olmi 1984); Drôme, Montségur-sur-Lauzon (HTS); Drôme, Mévouillon (BNC); Drôme, S.te Jalle (RNHL); Drôme, Séderon, Col de l'Homme mort (AMNH); Drôme, Col de Macuègne (NHMUK); Gironde, Barsac (HTS) (Tussac and Olmi 1998); Hérault, Cazevieille (MNHN) (Tussac and Olmi 1998); Hérault, Montpellier (HTS) (Tussac and Olmi 1998); Hérault, Grabels (HTS); Hérault, La Figarède (MNHN) (Olmi 1984); Hérault, St. Gély-du-Fesc (MNHN) (Olmi 1984); Hérault, Balliarguet CSIRO, 43°41.12'N, 03°62.24'E (CNC); Haute-Garonne, Castelmaurou (HTS) (Tussac and Olmi 1998); Lot, Cahors (HTS) (Tussac and Olmi 1998); Lot, Le Montat (HTS); Var, near St. Zacharie (NHMUK); Vaucluse, Sérignan (MNHN) (Olmi 1984); Vaucluse, Lagarde d'Apt, Mt St Pierre (BNC). **GREECE**: Olympia, Ilia (NHMUK) (Olmi 1984); Peloponisos, Monemvasia (ZMK). **HUNGARY**: Somogy county, Kaposvár (NMNH) (Olmi 1984). **ITALY**: Calabria, Crotone Prov., Sila, along road from Pagliarelle to Mt. Gariglione, about 9.7 km from Pagliarelle, 39°07.382'N, 16°41.553'E (MOLC); Friuli Venezia Giulia, Trieste Prov., Villa Opicina (DEI) (Olmi 1984); Emilia Romagna, Forlì Prov. Campigna Forest (MCSNV) (Olmi 1999); Toscana, Pisa Prov., Lajatico, 43°27.86'N, 10°40.73'E (MOLC) (Olmi 2005b). **KAZAKHSTAN**: Tchimkent obl., Karatau Ridge near Suzak (ZMM) (Ponomarenko and Olmi 2006). **SPAIN**: Barcelona, Palamos (SZC) (Olmi 1984); Murcia, near Manzarron (NHMUK) (Olmi 1984); Murcia, Sierra de Espuña, near Totana (NHMUK) (Olmi 1984); Madrid, El Escorial (MNCNM) (Ceballos 1927); Granada, Cubillas (AMNH, NHMUK) (Olmi 1984); Granada, Nerja (NHMUK); Castellon, Benicasim (NHMUK); 10 km from Abejar, Soria (RNHL); Alicante, Jávea (HTS); Zaragoza, Pina de Ebro, Monegros (HTS, JBZC) (Olmi et al. 1998).

**Distribution**: Cyprus, France, Greece, Hungary, Italy, Spain, in addition to Kazakhstan (transition country to Eastern Palaearctic subregion).

# 7. Dryinus dayi (Olmi)

Mesodryinus dayi Olmi 1984: 1003. Dryinus dayi (Olmi): Olmi 1999: 204.

**GREECE**: Thessalia, Kalambaka (NHMUK) (Olmi 1984). **Distribution**: Greece.

#### 8. Dryinus delvarei Olmi

Dryinus delvarei Olmi 1998: 72.

ALBANIA: Mirditë District, Salitë (MOLC). ITALY: Toscana, Arezzo Province, Upacchi, 43°30'N, 11°59'E (MSCS); Toscana, Grosseto Prov., Maremma Natural Park, 42°38.44'N, 11°04.42'E (MSNTC) (Olmi 2005b). TURKEY: 18 km NW Korkuteli (AMNH) (Olmi 1998).

**Distribution**. Albania, Italy, Turkey.

### 9. Dryinus gharaeii Olmi

Dryinus gharaeii Olmi 2005a: 207; Olmi and Xu 2015: 144.

IRAN: Ilam Province, Chogasabz Region, Ilam (MOLC) (Olmi 2005a). Distribution. Iran.

# 10. Dryinus gryps (Reinhard)

Chelothelius gryps Reinhard 1863: 410. Dryinus gryps (Reinhard): Dalla Torre 1898: 544; Olmi 1995: 5.

**FRANCE**: Bouches-du-Rhône, Fonscolombe (NHMUK); Drôme, Montségur-sur-Lauzon (BNC); Gard, Ussel-Goudargues (AMNH) (Olmi 1984); Hérault, Montagnac, Mas de Linares (HTS) (Tussac and Olmi 1998); Lot, Cahors (HTS); Southern France (MNHN) (Olmi 1984). **ITALY**: Sicilia, Siracusa Province, Lentini (AMNH) (Olmi 1999); Toscana, Livorno Province, near Piombino, Salivoli, 42°56.79'N, 10°30.20'E (MOLC) (Olmi 2005b); Toscana, Pisa Province, Monteverdi Marittimo, 43°09.59'N, 10°43.24'E (MOLC) (Olmi 2005b); Trentino-Alto Adige, Bolzano (Reinhard 1863). **SPAIN**: Zaragoza, Pina de Ebro, Los Monegros (HTS) (Olmi et al. 1998); Madrid, El Pardo (MNCNM); Cataluña, Tarragona, El Perello (HTS). **TURKEY**: Konya, Meram (OLL).

Distribution. France, Italy, Spain, Turkey.

# 11. Dryinus ibericus (Olmi)

Alphadryinus ibericus Olmi 1990: 137. Dryinus ibericus (Olmi): Olmi 1999: 208.

**SPAIN**: Murcia, Albacete Prov., near Molinicos, El Pardal (MNHN) (Olmi 1990); Granada, Sierra de Cazorla, Vacillo (HTS) (Olmi 1999).

Distribution. Spain.

# 12. Dryinus maroccanus (Olmi)

Richardsidryinus maroccanus Olmi 1984: 910. Dryinus maroccanus (Olmi): Tussac and Olmi 1998: 488; Olmi 1999: 193.

ALGERIA: Oran (MHNG, MOLC) (Olmi 1984). FRANCE: Alpes-Maritimes, Valbonne (BNC); Hérault, Cazevieille (CIRAD) (Olmi 1999). MOROCCO: Tangeri (MHNG) (Olmi 1984). SPAIN: Madrid, El Pardo, El Goloso (AMNH) (Olmi 1999). Distribution. Algeria, France, Morocco, Spain.

# 13. Dryinus niger Kieffer

Dryinus niger Kieffer 1904: 352; Olmi 1999: 206.

*Mesodryinus niger* (Kieffer): Kieffer and Marshall 1906: 497; Olmi 1984: 1005. *Mesodryinus brittanicus* Richards 1939: 228 (synonymized by Richards 1953).

ALBANIA: Arras, 10 km NW Peshkopi (OLL) (Olmi 1994). CYPRUS: Cherkes (NHMUK) (Olmi 1984). CZECH REPUBLIC: Central Bohemia, Celakovice, Lipovka (NMPC) (Macek 2007); Oriental Bohemia, Zelezné hory PLA, Zlatá louka National Reserve (NMPC) (Macek 2007). DENMARK: South Jutland, Sotrup (ZMK) (Olmi 1994). FINLAND: Satakunta, Eurajoki (Väänänen and Vikberg 2007) (SVC, VVC). FRANCE: Haute-Garonne, Castelmaurou (AMNH, MNHN) (Tussac and Olmi 1998); Lot, Lavercantière (HTS) (Tussac and Olmi 1998); Lot, Cahors (HTS); Vaucluse, Mont Ventoux, Malaucène (MNHN). GERMANY: Rheinland-Pfalz, Gönnersdorf (Cölln and Sorg 2001). GREECE: Peloponnesus, Monemvasia (ZMK). ITA-LY: Campania, Salerno Prov., Vallo della Lucania (MCSNG) (Kieffer 1904); Liguria, Genova (MCSNG) (Olmi 1984); Piemonte, Cuneo Prov., Valdieri (AMNH) (Olmi 1999); Piemonte, Vercelli Prov., Piode, Alpe Meggiana (MOLC). NORWAY: Inner Telemark, Notodden, Lisleherad (LOHC) (Hansen and Olmi 1996; Olmi 1994). SLOVAKIA: Southern Slovakia, Senec, Cierna voda river (NMPC) (Macek 2007). SWEDEN: Småland (ZIL) (Olmi 1994); Värmland, Ekshärad (CNC) (Olmi 1984); Västmanland, Kärrbo, Solbacken (GNC) (Olmi 1994). THE NETHERLANDS: Zuid Holland, Lexmond (De Rond 2004); Gelderland, Kesteren, Lienden (De Rond 2004). UNITED KINGDOM: England: Dorset, Glanvilles Wootton, f# holotype of M. brittanicus (HMO) (Richards 1939); Northants, Ayno, 21-25.VI.1945, R.B. Benson leg., 1f# (NHMUK) (Olmi 1984); Oxfordshire, Otmoor, 19.VII.1961, M.W.R. de V. Graham leg., 1f# (NHMUK).

**Distribution**. Albania, Cyprus, Czech Republic, Denmark, Finland, France, Germany, Greece, Italy, Norway, Slovakia, Sweden, The Netherlands, United Kingdom.

### 14. Dryinus sanderi Olmi

Dryinus sanderi Olmi 1984: 731; Olmi 1999: 211. Alphadryinus sanderi (Olmi): Olmi 1991: 284.

**BULGARIA**: Melnik (AMNH) (Olmi 1984); Sandanski (= Liljanovo) (MMB); Upper Thracian Plain, Besapari hills, Novo selo vill., 42.0974N, 24.4690E (IGC) (Lapeva-Gjonova et al. 2018). **CYPRUS**: 10 km W Cape Gréko, Ayia Napa (ZMK). **FRANCE**: Alpes-Maritimes, Moulinet, Sentier Col de Turini-Faysset, 43°58.51'N, 07°24.40'E (CIRAD); Drôme, Séderon, Col de l'Homme mort (AMNH) (Olmi 1999); Hérault, Grabels (HTS) (Tussac and Olmi 1998). **ITALY**: Piemonte, Torino Prov., Susa, Giaglione (AMNH) (Olmi 1999). **RUSSIA**: **European Russia**: Orenburg District, Orsk (Ponomarenko 1992: as *Richardsidryinus albrechti* Olmi).

Distribution. Bulgaria, Cyprus, France, Italy, Russia.

# 15. Dryinus tamaricicola Rakhshani & Olmi

Dryinus tamaricicola Rakhshani and Olmi in Derafshan et al. 2016: 412.

**IRAN**: Sistan and Baluchestan Prov., Zabol County, Zabol (MOLC) (Derafhsan et al. 2016).

Distribution. Iran.

## 16. Dryinus tarraconensis Marshall

Dryinus tarraconensis Marshall 1868: 204; Olmi 1984: 742.

*Dryinus szepligetii* Kieffer in Kieffer and Marshall 1905: 77 (synonymized by Olmi 1984).

Plastodryinus szepligetii (Kieffer): Kieffer and Marshall 1906: 496.

Lestodryinus tarraconensis (Marshall): Kieffer 1914a: 21.

Lestodryinus gregori Hoffer 1936: 164 (synonymized by Móczár 1965).

Lestodryinus bidens Haupt 1937 (synonymized by Olmi 1984).

Dryinus szepligetii Nec Kieffer: Ponomarenko 1981: 879.

BULGARIA: Damianitsa, 8 km S of Sandanski (CAS); Sandanski (= Liljanovo) (MMB); Slnoev Brjag (OLL); Nessebar (AMNH); Mt. Strandzha, Izgrev village, 42°08.41'N, 27°48.37'E (IGC). CROATIA: Dalmatia, Novi (NMNH) (Olmi 1984). CZECH REPUBLIC: South Moravia, Pouzdranyi (Hoffer 1936; Móczár 1965); Moravia, Kobylí (OLL); Moravia, S of Brno, Bratcice, 49°03'N 16°31'E (OLL); Moravia, Havraniky, Znojmo, 48°49'N, 15°59'E (OLL). FRANCE: Alpes de Haute-Provence, Simiane-la-Rotonde (HTS); Aude, Salles d'Aude (NMNH); Bouches-du-Rhône, Fonscolombe (NHMUK); Gironde, Barsac (HTS) (Tussac and Olmi 1998); Loiret, Orléans (MNHN) (Olmi 1984); Lot, Cahors (HTS) (Tussac and Olmi 1998); Haute-Garonne, Castelmaurou (HTS) (Tussac and Olmi 1998); Hérault, Grabels (HTS); Hérault, Montpellier (HTS) (Tussac and Olmi 1998); Hérault, Selagou Lake (NHMUK); Vaucluse, near St. Didier, Grange Neuve (NHMUK); Vaucluse, Les Constants, near Bédoin (NHMUK). GERMANY: Baden-Württemberg, Mühlacker-Mühlhausen (De Rond, pers. comm.; see Olmi and De Rond 2001). GREECE: Corfu Island, Kato Karakiana (JTBC) (Burn 2011); Rhodes Island, Kremasti Hills (NHMUK) (Olmi 1984); Rhodes Island, Ixia (NHMUK); Chalkidiki Peninsula, Amoliani Island (MCSNG). HUNGARY: Veszprém county, Balatonkenese (NMNH) (Olmi 1984); Crkvenica (AMNH) (Olmi 1984); Nógrád county, Ipolytarnóc (NMNH) (Szöllösi-Tóth and György 2009); Kiskunság National Park, Bugac puszta (AMNH). IRAN: Kerman Prov., Bam County, Sangemes, 28°56'33.44"N, 58°07'52.36"E (DPPZ) (Derafshan et al. 2016); Kermanshah Prov., Kermanshah, Moghoye, 28°57'24.18"N, 58°06'34.90"E (DPPZ) (Derafshan et al. 2016). IRAQ:

Baghdad (OLL, USNM) (Olmi 1984). ITALY: Abruzzi, Pescara Prov., Mt. Maiella (MZUN) (Olmi 1984); Calabria, Crotone Prov., N of Petilia, near road to Pagliarelle, 39°07.333'N, 16°47.036'E (MOLC); Emilia Romagna, Parma Prov., Parma (MZUN) (Olmi 1984); Lazio, Viterbo Prov., Capodimonte (AMNH) (Olmi 1999); Lazio, Viterbo Prov., Sutri (MOLC); Liguria, La Spezia Prov., Vernazza, 44°08.36'N, 09°41.73'E (MOLC); Liguria, Savona Prov., Pietra Ligure (GPC) (Olmi 1984); Piemonte, Alessandria Prov., Gavi (MCSNG) (Olmi 1984); Piemonte, Alessandria Prov., Montaldo di Cerrina (GLPC); Piemonte, Cuneo Prov., Valdieri, Juniperus phoenicea Reserve (AMNH) (Olmi 1999); Cuneo Prov., S. Benedetto Belbo (AMNH) (Olmi 1984); Torino Prov., Carignano (Guglielmino et al. 2015); Puglia, Lecce Prov., S. Maria di Leuca Cape (MNHN) (Olmi 1999); Puglia, Taranto Prov., Castellaneta, Bosco dei Terzi, 40°41'26,6"N, 16°57'22,9"E (MOLC); Sardegna, Sassari Prov., Luras (MOLC) (Olmi 2005b); Sicilia, Caltanissetta Prov., S. Cataldo (AMNH) (Olmi 1984); Sicilia, Catania Prov., M. Etna, Bronte, Maletto, Contrada Paviglione (AMNH) (Olmi 1999); Sicilia, Messina Prov., Messina (ZMK) (Olmi 1984); Toscana, Grosseto Prov., Natural Park of Maremma, 42°38.44'N, 11°04.42'E (MOLC) (Olmi 2005b); Toscana, Livorno Prov., Venturina (MOLC) (Olmi 2005b); Toscana, Livorno Prov., Capraia Island (MOLC) (Olmi 2005b); Toscana, Pisa Prov., San Rossore, 43°41'N 10°39'E (MOLC) (Olmi 2005b); Toscana, Pisa Prov., Cipollini (MOLC) (Olmi 2005b); Toscana, Pisa Prov., Monteverdi Marittimo, 43°09.59'N, 10°43.24'E (MOLC) (Olmi 2005b); Toscana, Pisa Prov., Lajatico, 43°27.86'N, 10°40.73'E (MOLC) (Olmi 2005b); Toscana, Pistoia Prov., Montecatini Terme (AMNH) (Olmi 1984); Umbria, Perugia Prov., Perugia (MCSNG) (Olmi 1984); Valle d'Aosta, Aosta Prov., Sarre (NHMUK) (Olmi 1984). MONTENEGRO: Sutomore (NMNH) (Olmi 1984); near Kotor, Krasici (NHMUK). MOROCCO: High Atlas, 25 km N of Taroudant, Sebt Tafraoute (NHMUK) (new record). POLAND: East bank of Oder River, 10 km N of Cedynia, Bielinek (= Bellinchen) (MLUHW, MNHN) (Haupt 1937); Wyzyna, Malopolska, Rzezusnia k/Golczy (BWC). ROMANIA: Transilvania, Nagyenyed (NMNH). RUSSIA: European Russia: Volgograd District (Ponomarenko 1978); Far East: Primorskij Kraj, Evseyevka, 15 km SE of Spassk (Ponomarenko 1992). SLO-VAKIA: South Slovakia, Stiavnické, Hronsky Benadik (MMB). SOUTH KOREA: GB, Gyeongsan-si, Dae-dong, Yeungnam-Univ., 35°58'N 128°47'E (YUIC). SPAIN: Huesca, Torla (NMNH) (Marshall 1868); Madrid, El Escorial (NHMUK) (Olmi 1984); Madrid, El Pardo, El Goloso (MNCNM); Zaragosa, Pina de Ebro, Monegros (HTS) (Olmi et al. 1998); Castellon, Benicasim (NHMUK) (Olmi 1984); Tarragona, Salou (AMNH, NHMUK) (Olmi 1984); Granada, Sierra Nevada (AMNH, CNC); Alicante, Calpe (HTS); Almeria, Carboneras (RNHL); Soria, Ucero (RNHL). TA-JIKISTAN: Kulyab obl., 20 km ENE Pyandzh (ZMM) (Ponomarenko and Olmi 2006). TURKEY: Urfa, Halfeti (RNHL); Mugla, Köycegiz (RNHL); Hakkari, SW of Yüksekova, Varegös, Sat Dag (RNHL); Pamphylia, W of Alanya (ZMK).

**Distribution**. Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Iran, Iraq, Italy, Montenegro, Morocco, Poland, Romania, Russia (incl.

Far East), Slovakia, Spain, Turkey, in addition to South Korea and Tajikistan (Eastern Palaearctic subregion).

## 17. Dryinus tigarae Olmi

Dryinus tigarae Olmi 2008: 365.

**UNITED ARAB EMIRATES**: Abu Dhabi, Sweihan District, Al Ain (CNC) (Olmi 2008).

Distribution. United Arab Emirates.

#### 18. Dryinus turcicus Olmi

Dryinus turcicus Olmi 1991: 259; 1999: 199.

**TURKEY**: Hakkari District, Hakkari (RNHL) (Olmi 1991). **Distribution**. Turkey.

#### 19. Dryinus tussaci Olmi

Dryinus tussaci Olmi 1991: 260.

**FRANCE:** Var, Vidauban (MNHN). **ITALY**: Sardegna, Sassari Prov., Berchidda, 40°47.99'N, 09°08.87'E (MOLC) (Olmi 2005b); Toscana, Grosseto Prov., Natural Park of Maremma, 42°38.17'N, 11°04.26'E (MOLC) (Olmi 2005b); Toscana, Pisa Prov., Monteverdi Marittimo, 43°09.59'N, 10°43.24'E (MOLC) (Olmi 2005b). **MO-ROCCO**: about 20 km N Agadir, Tarhazoute (MNHN) (Olmi 1991). **SPAIN**: Jaen, Alcaudete, Sierra del Ahillo (AMNH, MBC) (Olmi 1999).

Distribution. France, Italy, Morocco, Spain.

#### 20. Dryinus yemenensis Olmi and Van Harten

Dryinus yemenensis Olmi and van Harten 2006: 327.

**OMAN**: Dhofar, Salalah East, Dahariz, 17°01.02'N, 54°09.32'E (MOLC). **YEMEN**: Al Lahima (MOLC) (Olmi and van Harten 2006); 12 km NW of Manakhah (MOLC) (Olmi and van Harten 2006); Al-Kowd (MOLC) (Olmi and van Harten 2006).

Distribution. Oman, Yemen.

## Discussion

Dryininae of the Western Palaearctic subregion are insufficiently known from many points of view. The 20 listed species are known mainly on the basis of only one sex (Derafshan et al. 2016; Olmi 1999, 2008; Olmi and van Harten 2006; Olmi and Xu 2015). In fact, only females are known in 11 species (*D. berlandi, dayi, delvarei, gharaeii, gryps, ibericus, maroccanus, tigarae, turcicus, tussaci, yemenensis*). Both opposite sexes are known in six species (*D. balearicus, collaris, corsicus, niger, sanderi, tarraconensis*). In three species, the male was associated to the female tentatively, i.e. the association is doubtful (*D. albrechti, canariensis, tamaricicola*). This situation depends on the large morphological differences between female and male, so that the association of the opposite sexes is impossible, if it is not supported by rearings or DNA analysis. However, very few researchers rear dryinids or study their DNA.

The knowledge is insufficient also in the association of the species to their hosts. *Dryinus* species are parasitoids of Fulgoromorpha (Guglielmino et al. 2013). However, in the Western Palaearctic subregion, the hosts are known only in six species (*D. balearicus, collaris, corsicus, niger, sanderi, tarraconensis*). Also in this case, the situation depends on the scarcity of rearings.

From the biogeographical point of view, according to the categories presented by Vigna Taglianti et al. (1992, 1999, the chorotypes of the 20 species listed in the Western Palaearctic subregion are the following: *D. albrechti* (endemic, Macaronesian); *D. balearicus* (Western Mediterranean); *D. berlandi* (Western Mediterranean); *D. canariensis* (Mediterranean-Macaronesian); *D. collaris* (Turan-European); *D. corsicus* (Turan-European); *D. dayi* (endemic, Greece); *D. delvarei* (Eastern Mediterranean); *D. gharaeii* (endemic, Iran); *D.gryps* (Southern European); *D. ibericus* (endemic, Spain); *D. maroccanus* (Western Mediterranean); *D. niger* (European); *D. sanderi* (Turan-European); *D. tamaricicola* (endemic, Iran); *D. tarraconensis* (Asian-European); *D. tigarae* (endemic, United Arab Emirates); *D. turcicus* (endemic, Turkey); *D. tussaci* (Western Mediterranean); *D. yemenensis* (endemic, Yemen, Oman). Eight species of the above list are considered endemic provisionally, because dryinids are understudied, so their geographic distribution could be larger.

Olmi and Xu (2015) listed 10 species of *Dryinus* and one species of *Pseudodryinus* from the Eastern Palaearctic subregion. So the dryinid population of the two subregions has about the same numerical strength. However, the composition is different. Few species are present also in the Western Palaearctic subregion, i.e. *D. collaris*, *D. corsicus*, and *D. tarraconensis*.

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RESEARCH ARTICLE



# New species in the sponge genus Tsitsikamma (Poecilosclerida, Latrunculiidae) from South Africa

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### Abstract

The genus *Tsitsikamma* Samaai & Kelly, 2002 is to date exclusively reported from South Africa. Three species are known from the southern coast: *Tsitsikamma favus* Samaai & Kelly, 2002, from the Garden Route National Park Tsitsikamma Marine Protected Area (MPA) and Algoa Bay; *T. pedunculata* Samaai, Gibbons, Kelly and Davies-Coleman, 2003, collected from Cape Recife in St. Francis Bay, and *T. scurra* Samaai, Gibbons, Kelly and Davies-Coleman, 2003, collected from a wreck site in a small bay west of Hout Bay on the west coast of South Africa. Here two new species are described: *Tsitsikamma michaeli* Parker-Nance, **sp. nov.**, a small green purse-like species, collected from Algoa Bay, and *Tsitsikamma nguni* Parker-Nance, **sp. nov.**, from The Garden Route National Park, Tsitsikamma MPA. Additional morphological characteristics, spicule morphology, and distribution records are provided for *T. favus* and *T. pe*-*dunculata* from Algoa Bay. The phylogenetic relationship of these five *Tsitsikamma* species is investigated.

### Keywords

Algoa Bay, marine sponge, Western Indian Ocean, 28S rRNA

# Introduction

The family Latrunculiidae Topsent 1922 consists of seven genera: *Bomba* Kelly, Reiswig & Samaai, 2016; *Cyclacanthia* Samaai, Govender & Kelly, 2004; *Latrunclava* Kelly, Reiswig & Samaai, 2016; *Latrunculia* du Bocage, 1869; *Sceptrella* Schmidt, 1870; *Strongylodesma* Lévi, 1969; and *Tsitsikamma* Samaai & Kelly, 2002. The genus *Latrunculia* incorporates three sub-genera: *Biannulata* Samaai et al., 2006; *Latrunculia* du Bocage, 1869 (Samaai et al. 2003, 2006, 2012); *Uniannulata* Kelly, Reiswig & Samaai, 2016 (Kelly et al. 2016). Genera *Latrunculia, Strongylodesma, Cyclacanthia*, and *Tsitsikamma* include known South African species, the latter two are thus far, autochthonous to South Africa. The genus *Tsitsikamma*, named after the type locality Tsitsikamma, a Marine Protected Area (MPA) part of The Garden Route National Park, includes three known species; *Tsitsikamma favus* Samaai & Kelly, 2002, collected from the Tsitsikamma MPA and Algoa Bay, *T. pedunculata* Samaai, Gibbons, Kelly & Davies-Coleman, 2003, from St. Francis Bay, Western Indian Ocean, and *T. scurra* Samaai, Gibbons, Kelly & Davies-Coleman, 2003, collected west of Hout Bay on the Atlantic side of the Cape Peninsula.

Tsitsikamma are similar in their general morphology to other Latrunculiidae, with fistular oscula and areolate porefields distributed over the sponge surface. However, within Latrunculiidae, Tsitsikamma species are notably tough and leathery due to the reinforced densely spiculous nature of the ectosome, and firmness is added where species are internally reinforced with dense spiculose tracts dividing the interior into discrete chambers, visible to the unaided eye (Samaai and Kelly 2002, Samaai et al. 2003). The main skeletal component of both the thick tracks within the choanosome, prominent in T. favus (Samaai and Kelly 2002) and T. scurra, the reinforced stalk in T. pedunculata (Samaai et al. 2003) and the delicate compressible choanosome, are anisostyles, often polytylote (Samaai and Kelly 2002, Samaai et al. 2003). It is, however, the shape of the isochiadiscorhabd microscleres that is characteristic of the genus. Spines develop simultaneously on the end of a straight thin protoisochiarhabd shaft followed, when present, by median spines. The spines develop into truncate tubercles with rounded acanthose ends on a stout shaft (Samaai and Kelly 2002). A medium whorl is present in T. favus and T. scurra but lacking on the very short microscleres of T. pedunculata (Samaai and Kelly 2002, Samaai et al. 2003).

The genus has attracted much interest due to the production of cytotoxic pyrroloiminoquinone alkaloids including tsitsikammamines and brominated discorhabdins (Hooper et al. 1996, Beukes 2000, Antunes et al. 2004, 2005). The tsitsikammamines, were once thought to be unique to *T. favus*, and considered taxonomic markers differentiating this species from others in the genus and family (Samaai and Kelly 2002, Samaai et al. 2003). However, tsitsikammamines have since been reported from Australian *Zyzzya fuliginosa* (Davis et. al 2012) and Antarctic *Latrunculia* (*Latrunculia*) *biformis* (Li et al. 2018). Recent research reporting makaluvamines for the first time in a *Tsitsikamma* species also discovered the existence of two distinct *T. favus* chemotypes, the one producing predominantly discorhabdins and tsitsikammamines while the second produces makaluvamines (Kalinski et al. 2019). The source of the bioactive properties of these sponges has been hypothesized to be microbial in origin with a close relationship between the sponges and their microbial symbionts (Walmsley et al. 2012, Matcher et al. 2017). *Tsitsikamma favus* is the first sponge reported to have *Betaproteobacteria* and *Spirochetes* as the dominant microbial taxon (Walmsley et al. 2012), which are conserved within the microbiomes of six species in three genera within the Latrunculiidae (Matcher et al. 2017).

In this study examination of the morphological features of multiple specimens suggests the grouping of *Tsitsikamma* species into two morphological forms. The first resembles *T. favus*, with a thick encrusting or hemispherical growth form, large attachment area and a choanosome structurally reinforced by dense spiculose tracts (Samaai and Kelly 2002) together with *T. scurra* and *T. nguni* sp. nov. The second morphological group has a reinforced peduncle that supports a rounded body without any reinforcing tracts subdividing the delicate interior (Samaai et al. 2003) and is represented by *T. pedunculata* and *T. michaeli* sp. nov. We provide additional morphological characteristics and new information on the geographical distribution of known species, describe two new species and investigate the integrity of two morphological groups considered using 28S rRNA gene sequence analysis.

# Materials and methods

Samples were collected by SCUBA or Remotely Operated Vehicle equipped with a collection arm and deployed from the coastal Research Vessel uKwabelana. Specimens were collected from Tsitsikamma Marine Protected Area and Algoa Bay within the Agulhas Ecoregion from depths of 18-40 m. All sponges were preserved in 70% ethanol or frozen at -20 °C. Photographic records were collected in situ, of freshly collected and preserved specimens, where possible. The majority of the samples, type specimens and reference material are lodged with the South African Institute for Aquatic Biodiversity (SAIAB) a National Research Foundation (NRF) National Collection facility (for further information please visit www.saiab.ac.za) and have the prefix SAIAB. Additional samples collected by the Coral Reef Research Foundation (CRRF) on behalf of the United States National Cancer Institute shallow-water collection programme, are now held at the California Academy of Sciences. Specimens belonging to collections held at South African Museum (SAM), Cape Town, the British Natural History Museum (NHMUK), London, and California Academy of Sciences Invertebrate Zoology Collection (CASIZ), San Francisco, are as such indicated by the abbreviation as prefix to the sample number. Voucher specimens of all newly collected specimens will be sent to the South African Museum. All specimens listed in this publication were collected by Shirley Parker-Nance except where otherwise indicated.

Chiadiscorhabd microsclere morphology changes as the spicules develop (Samaai and Kelly 2002) and vary intra- and interspecifically within the genus. Measurements of the spicule shaft length and width, the apical whorl and manubrium diameter and the total length of the spicules were made to quantify differences. The largest of 40 megascleres and 20 microscleres presented in 20 images taken from permanent pre-

pared slides were used to define size attributes. A distinction was made between the smaller apical whorls and a larger manubrium, and both are provided for the micro-scleres measured.

Sponge DNA was extracted either according to the method described by Walmsley et al. (2012) or using the ZR Soil Microbe DNA MiniPrep kit (Zymo Research, Cat. No. D6001). Partial 28S rRNA gene sequence was PCR amplified as in Waterworth et al. (2017) using primer pairs (SP18cF: 5'-GACCCGTCTTGAAACACGA-3'and SP18dR: 5'-ACACACTCCTTAGCGGA-3). The PCR products were either cloned into the pGEM-T Easy vector (Promega) or sequenced directly by Sanger sequencing. Primer pairs (The LCO1490: 5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and (HCO2198:5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') were used to amplify COI gene fragment as per Walmsley et al. (2012). Seventeen 28S rRNA sequences (629 bp) and two COI sequences (658 bp), supplemented by relevant sequence obtained from GenBank, were aligned using ClustalW and the phylogenetic trees constructed using the Neighbour-Joining method with 500 bootstrap replicates in MEGA X (Kumar et al. 2018).

### Taxonomy

Class Demospongiae Sollas, 1885 Order Poecilosclerida Topsent, 1928 Family Latrunculiidae Topsent, 1922 Genus *Tsitsikamma* Samaai & Kelly, 2002

## Genus Tsitsikamma Samaai & Kelly, 2002

*Tsitsikamma favus* Samaai & Kelly, 2002 *Tsitsikamma pedunculata* Samaai, Gibbons, Kelly & Davies-Coleman, 2003 *Tsitsikamma scurra* Gibbons, Kelly & Davies-Coleman, 2003 *Tsitsikamma michaeli* Parker-Nance, sp. nov. *Tsitsikamma nguni* Parker-Nance, sp. nov.

**Diagnosis.** Hemispherical, thick encrusting or pedunculate Latrunculiidae with a smooth, in some species generously folded, surface with cylindrical or volcano-shaped oscula and prominent areolate porefields. The ectosome is resident and leathery, the colour varies between species from pinkish to dark liver brown, dark turquoise or green in life. Megascleres are anisostyles with isochiadiscorhabd microscleres. The microscleres are present in an irregular palisade layer on the surface ectosome and line the internal tracts (from Samaai and Kelly 2002, Samaai et al. 2003).

Type species. Tsitsikamma favus Samaai & Kelly, 2002

**Remarks.** The diagnostic character that unites species of *Tsitsikamma* is the possession of isochiadiscorhabd microscleres. Isochiadiscorhabd or isochia(acantho)dis-

corhabds have a short straight smooth shaft bearing an apex whorl and manubrium and when present median whorls. These whorls consist of singular or grouped conicocylindrical tubercles, radiating from the shaft, with the distal end acanthose. These differ from microscleres present in other Latrunculiidae such as the acanthose isospinodiscorhabds with stout straight shaft, with similar terminal whorls and discrete conical spines unevenly distributed along it in *Cyclacanthia*; microscleres with disk-like whorls of spines that are different in shape and size, such as the anisodiscorhabds found in *Latrunculia*; or isoconicodiscorhabds or 'sceptres' with stout straight shaft and undifferentiated terminal whorls found in *Sceptrella* (Samaai and Kelly 2002, Samaai et al. 2006, Kelly et al. 2016). The ontogeny of the microscleres further set the genera within this family apart, as the protorhabd projections develop simultaneously in *Tsitsikamma*, *Cyclacanthia*, and *Sceptrella* but not so in *Latrunculia* (Samaai and Kelly

Interestingly, *Tsitsikamma* species occur in two very different growth forms. In two of the species, *T. favus* and *T. scurra*, the interior of the sponge is partitioned by reinforced dense spiculose tracks through the delicate choanosome. The third species, *T. pedunculata*, has a spicule dense stalk that supports a spherical pouch without the characteristic spicule tracts penetrating into the choanosome. The description of an additional two *Tsitsikamma* species, presented in this work, support this separation further as one has internal tracts and the other is purse-shaped.

### Tsitsikamma favus Samaai & Kelly, 2002

Figure 1a-p

2002, Samaai et al. 2004).

*Tsitsikamma favus* Samaai & Kelly, 2002: 718, fig. 6A–G. Samaai, Gibbons, Kelly & Davies-Coleman, 2003: 19.

**Type locality.** Western Cape Province, Garden Route National Park, Tsitsikamma, Rheeders Reef, South Africa.

**Type material.** *Holotype* – NHMUK 1997.7.3.2: Rheeders Reef; Tsitsikamma MPA, Eastern Cape Province, Garden Route National Park, -34.166667, 23.90000, 22 m, collector Philip Coetzee, 1995 (Samaai and Kelly 2002).

**Material examined.** SAIAB 141112: The Knoll, Tsitsikamma MPA, Garden Route National Park, Eastern Cape Province, -34.02555, 23.90708, 18 m depth, collected by Colin Buxton, 2 May 1993, three specimens; SAIAB 207166, SAIAB 207167: Rheeders Reef, Tsitsikamma MPA, Garden Route National Park, Western Cape Province, -33.84548, 25.81663, 25–30 m depth, 25 May 1994, collected by John Allen and Steve Brower, nine specimens; SAIAB 141356: Rheeders Reef, Tsitsikamma MPA, Garden Route National Park, Eastern Cape Province, 22 m depth, 18 March 1995 collected by Rob Palmer, Brad Carté and Philip Coetzee, two specimens (material collected at same locality and time as type material); SAIAB 207168: Rheeders Reef, Tsitsikamma MPA, Garden Route National Park, Eastern Cape Prov-



**Figure 1.** *Tsitsikamma favus* **a** in situ SAIAB 207193 **b** collected specimens SAIAB 207193 **c** collected SAF1995-001 **d** section through preserved specimen SAIAB 141356 **e** isochiadiscorhabds arrangement on the surface of the ectosome **f** section of ectosome with underlying choanosome SAIAB 141356 **g**, **h** thin sinuous style **i** large sinuous centrally thickened style **j** occasionally tylote styles **k** rare short thick strongyles **I–o** isochiadiscorhabds SAIAB 207218 SEM **p** acanthose tubercles SAIAB 207217. Scale bars 5 cm (**b**, **c**); 1 cm (**d**); 1 mm (**f**); 100 μm (**k**); 20 μm (**I–p**).

ince, 30 m depth, 25 May 1994, collected by John Allen and Steve Brower; SAIAB 207172 and SAIAB 207174: RIY Bank, Algoa Bay, Eastern Cape Province, 28 m depth, 23 February 1999, collected by Coral Reef Research Foundation, Koror, Palau (CRRF); SAIAB 207175: Whitesands Reef, Algoa Bay, Eastern Cape Province, 20 m depth, 18 May 2001; SAIAB 103531: Whitesands Reef, Algoa Bay, Eastern Cape Province, -33.99980, 25.70842, 15 m depth, 20 March 2002, collected by Scripps; SAIAB 207176, SAIAB 207221, SAIAB 207222, SAIAB 207223, SAIAB 207224, SAIAB 207225, SAIAB 207226, and SAIAB 207227: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84297, 25.81647, 25-30 m depth, 15 May 2009; SAIAB 207177: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.81663, 30 m depth, May 2009; SAIAB 207217 and SAIAB 207218: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.31663, 25-33 m depth, 10 October 2010; SAIAB 207179, SAIAB 207180, SAIAB 207184, SAIAB 207185, SAIAB 207186, SAIAB 207187, and SAIAB 207188: RIY Banks, Algoa Bay, Eastern Cape Province, -33.98868, 25.86553, 25-30 m depth, 14 December 2012; SAIAB 207189: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.316633, August 2014, 22-30 m depth, 10 specimens; SAIAB 207190 and SAIAB 207228: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.31663, 30 m depth, 6 September 2015; SAIAB 207192: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.3166315, 20 m depth, 2 June 2016, collected by Thomas Bornman, Shaun Deyzel, and Shirley Parker-Nance, several specimens; SAIAB 207193: Shark Alley, Bell Buoy Reef, Algoa Bay, Eastern Cape Province, -33.98248, 25.69430, 9–10 m depth, 5 June 2016.

Additional material. CASIZ 300636: White Sands Reef, Algoa Bay, Eastern Cape Province, -33.99537, 25.70790, 14 m, 14 February 1999, collected by Coral Reef Research Foundation, Koror, Palau CRRF, identified by Michelle Kelly, National Institute of Water and Atmosphere, Auckland (NIWA); CASIZ 300535: Table Top Reef, Algoa Bay, Eastern Cape Province, -33.98067, 25.69367, 16 m, 4 October 1998, collected by CRRF, identified by Michelle Kelly, NIWA; CASIZ 301054: Grootbank Reef, Plettenberg Bay, Western Cape Province, -34.00765, 23.49647, 10–13 m, 22 March 2000, collected by CRRF, identified by Michelle Kelly, NIWA.

**Diagnosis** (emended from Samaai and Kelly 2002). Large, firm, dark brown hemi-spherical to thick encrusting sponges, up to 15 cm high and 20 cm in diameter, sessile with a large area of attachment. Surface smooth and firm although undulant presenting a folded or bumpy appearance in some specimens (Fig. 1a–c), only slightly to moderately compressible, resilient and leathery. Surface with large single to multichambered cylindrical lance-shaped oscula, and pedunculate cauliform areolate porefields, colour in life is light to dark brown or liver brown.

*Skeleton*. The ectosome is composed of a thick dense feltwork of anisostyles with a single layer of erect isochiadiscorhabds arranged perpendicular to the underlying megascleres (Fig.1e). The ectosome is generally thinner than the dense spiculose tracts that penetrate and divide the soft choanosome into honeycomb-like chambers (Table 1, Fig. 1d, f).

*Spicules.* Megascleres. Slightly sinuous anisostyles, hastate, mucronate or blunt, occasionally tylote form the main structural components with two categories pre-

Samaai and Kelly (2002)	Material examined (n = 14)
900	660 (430–1120) (n = 20)
1000-17000	1200 (740–1780) (n = 20)
5800	5108 (3611-8450) (n = 20)
(i) 621 (537–700) x 14 (14)	(i) 570 (420–788) x 14 (9–19)
(ii) 530 (480–566) x 9.6 (9.6) (n = 20)	(ii) 598 (449–907) x 10 (3–16)
	(n = 520)
	494 (139–751) x 13 (8–21) (n = 42)
48 (41–60) x 9 (7.2–9.6)	53 (40–68) x 9 (6–14)
(n = 20)	Additional measurements
	shaft length 43 (29-59)
	apex whorl diameter 24 (17–34)
	manubrium diameter 26 (19–37)
	(n = 280)
	Samaai and Kelly (2002) 900 1000–17000 5800 (i) 621 (537–700) x 14 (14) (ii) 530 (480–566) x 9.6 (9.6) (n = 20) 48 (41–60) x 9 (7.2–9.6) (n = 20)

Table 1. Skeletal and spicule dimensions (µm) for Tsitsikamma favus.

sent; (i) long slightly curved and thickened centrally and (ii) shorter, thinner slightly curved centrally (Table 1, Fig. 1g–j). Short thick anisostrongyles, may also be present (Fig. 1k). **Microscleres.** Isochiadiscorhabd, with three whorls of conico-cylindrical tubercles terminally acanthose (Fig. 1p), line the tracts and are found abundantly throughout the choanosome (Fig. 1l). In addition to the three whorled microscleres, as described by Samaai and Kelly (2002) for the type material, are chiadiscorhabds with up to five complete whorls as well as many intermediate forms (Fig. 1m–o). Typically, the manubrium and the apical whorl differ slightly in diameter (Table 1) and tubercles projections arranged pairwise or in groups of three respectively (Fig. 1l–p). Isochiadiscorhabds with the terminal tubercles arranged in such a way to give a flattened appearance are also present (basal whorl in Fig. 1o). Oocytes were present in specimens (after Samaai and Kelly 2002).

**Distribution.** Plettenberg Bay, Tsitsikamma Marine Protected Area and Algoa Bay. **Substrate, depth range, and ecology.** Collected from rocky benthic reef, 9–33 m deep, occurring singly or in clumps of two or three, in abundance on both shallow reef

systems such as Bell Buoy on the top of medium profile reef and at Evans Peak on the sides of high steep profile reef. Note that for some of the older collections the GPS position of the collection site is not available or inaccurate; for clarity Rheeders Reef is an inshore reef system within the Tsitsikamma MPA situated east of Storms River Mouth and the Knoll between -34.025730, 23.906138 and -34.032780, 23.960138 inshore and -34.044530, 23.906138 and -34.04453, 23.96013 off shore.

**Remarks.** Examined material compares well with the type description given by Samaai and Kelly (2002) including the shape of the oscula, distribution of the pedunculate cauliform areolate porefields, arrangement, and distribution and size of megascleres and microscleres (Table 1). The structure of the chiadiscorhabds corresponds with the type description Samaai and Kelly (2002); however, some sponges exhibited various ratios of typical microscleres with three whorls, as per the type description (Samaai and Kelly 2002), to microscleres with tubercles not arranged in or missing from or present between complete whorls (Fig. 1m–o). These variations were suggested but not discussed in the type description (see Samaai and Kelly 2002: fig. 6J, central
two images; Samaai et al. 2004: fig. 2B, central image) which suggests that spicules of this nature were observed in the type specimen. It is interesting to note that *T. favus* specimens, even some collected no more than 10 cm apart and although clearly *T. favus* with respect to 28S rRNA sequence analysis (as shown by sequence identity or a maximum of one nucleotide difference), differ in the firmness or compressibility of the individual sponge. Closer inspection of the spicules showed an increased occurrence of misshaped or irregular microscleres and a distinct chromatographic profile in these *T. favus* sponges (Kalinski et al. 2019).

Live or freshly collected specimens are dark brown, olive or dark green in colour and may be heavily encrusted with soft corals, hydroids, ascidians and other encrusting sponges with the oscula and porefields protruding through the surface epibionts. As freshly collected specimens are preserved, the extract dyes the preservative (70% ethanol) a deep brown colour which intensifies as the tissue lightens; long exposure to the stained preservative darkens the tissue again. Successive preservative changes (long-term curated specimens) remove the pigment and the specimens are beige in colour. Frozen material may be dark slate green to tan externally, and the tracks are prominently tan and the choanosome dark brown.

An estimation of divergence between sequences, intraspecific genetic diversity of *T. favus* included in this study, was found to be 0.16 % for the 28S rRNA gene sequence and 0–0.18 % for COI (Walmsley et al. 2012; Walmsley 2013). Interspecific diversity between *T. favus*, *T. nguni*, and *T. scurra* at 28S was 0.16 % (Suppl. material 1: Table S1).

Examination of specimens collected from Tsitsikamma in 1993 showed that one sample contained two distinct species, the one clearly *T. favus* the other a new species included below (SAIAB 207216: The Knoll, Tsitsikamma MPA Garden Route National Park, Eastern Cape Province, 18 m, 2 May 1993, collected by Colin Buxton).

# *Tsitsikamma pedunculata* Samaai, Gibbons, Kelly & Davies-Coleman, 2003 Figure 2a–l

Tsitsikamma pedunculata Samaai, Gibbons, Kelly and Davies-Coleman, 2003: 19.

**Type locality.** *Holotype* – NHMUK 2003.1.10.2 (CASIZ 300661): Thunderbolt Reef off Cape Recife, St. Francis Bay, Eastern Cape Province, -34.05233, 25.68933, 40 m depth, 25 February 1999, collected by P.L. Colin, CRRF (after Samaai et al. 2003).

**Material examined.** SAIAB 207194: St. Francis Bay, 5 November 2002, specific collection site unknown; SAIAB 207195, SAIAB 207196: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84418, 25.81522, 34–38 m depth, 30 October 2015, collected by Ryan Palmer and Shirley-Parker-Nance, ROV from the coastal Research Vessel uKwabelana; SAIAB 207197, SAIAB 207198, SAIAB 207199, SAIAB 207200: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.81663, 30–34 m depth, 12 November 2015, collected by Ryan Palmer and Shirley-Parker-Nance, ROV from the coastal Research Vessel uKwabelana





**Figure 2.** *Tsitsikamma pedunculata* **a**, **b** in situ **c** collected specimens showing vascular stalk and covered with sponge epibiont Mycale (Mycale) sp. SAIAB 207196 **d** section of ectosome with underlying choanosome SAIAB 207166 **e** outer section of ectosome with chiadiscorhabds in a dense layer externally SAIAB 207166 **f** section through the stalk showing lumen of vascular-interior SAIAB 207166 **g**, **h** various sinuous styles **i**, **j** collection of SEM images of chiadiscorhabds **k**, **l** light microscope image of chiadiscorhabds. Scale bars 6 cm (**b**); 1 cm (**d**); 100 μm (**g**, **h**); 20 μm (**i**–**l**).

**Diagnosis** (emended from Samaai et al. 2003). Characteristic dirty pink, pinkbrown pedunculate species with well-defined, ball-shaped head, up to 7 cm in diameter, on a narrow stalk, 1–3 cm wide and up to 7 cm long (Fig. 2a, b). Living sponges appear dirty pink although this is often obscured by epibionts, especially the yellow encrusting *Mycale* (*Mycale*) sponge also found growing on other members of this genus (Fig. 2a–c). Freshly collected material is a dusty pink to pink-brown to dark purple while preserved material has an olive green, cream to tan colour (Fig. 2c). Small wellspaced cone-shaped oscula 1.5–2 mm high and 1.5–3 mm in diameter are present over the upper part of the head gradually replaced by small to bigger elevated circular fungiform areolate porefields, 1–4.5 mm high and 2–7.5 mm in diameter, toward the base where the stalk is attached (Fig. 2a). In preserved specimens the oscula retain their shape but the upper border of the porefields contracts inwards giving it a button like appearance. A tough, resistant leathery ectosome surrounds a much softer choanosome. The sponge is resilient, but compressible. Salmon pink to pinkish brown between the oscula and dark pink between the areolate porefields.

*Skeleton.* Microscleres are abundant throughout the choanosome and form an irregular palisade of oblique or erect microscleres over the dense feltwork of tangential and paratangential styles together forming the ectosome (Table 2, Fig. 2e) The resistant ectosome encapsulate soft choanosome with delicate tracts (Samaai et al. 2003) (Fig. 2d). The stalk consists of densely arranged spicules and has longitudinal cavities filled with soft choanosome tissue distributed regularly along the axis of the reinforced stalk (Fig. 2c, f).

*Spicules.* Megascleres consist of two size classes of styles; (i) slightly sinuous, robust centrally thickened, acerate, conical, hastate or somewhat blunt even mucronate styles, and (ii) thin conspicuously sinuous and sometimes conspicuously centrally thickened styles (Table 2, Fig. 2g, h). Microscleres. Isochiadiscorhabds with only two whorls of cylindrical, conical tubercles acanthose on apex, arranged on the ends of a short shaft (Samaai et al. 2003). The large manubrium is easily distinguishable from the conspicuously smaller apex with terminally acanthose tubercles arranged in a pincushion-like way to form the apex whorl of the microsclere (Table 2, Fig. 2i–l).

Distribution. Algoa Bay and St. Francis Bay

**Substrate, depth range and ecology.** Abundant on deep reef systems between 34–40 m. All specimens collected were attached to rock on the sides of medium profile reef

	Samaai et al. (2003)	Examined material
Ectosome	1300	818 (200–1800) (n = 11)
Styles	(i) 684 (591–728) x 16	(i) 636 (541–788) x 15 (12–17)
	(ii) 536 (500–555) x 11	(ii) 673 (562–798) x 11 (4–15)
	(n = 20)	(n = 160)
Isochiadiscorhabds	29 (27–30) x 7	29 (26–34) x 7 (5–9)
	(n = 20)	Additional measurements
		shaft length 19 (16–24)
		apex whorl diameter 19 (12–24)
		manubrium diameter 23 (19–27)
		(n = 80)

Table 2. Skeletal and spicule dimensions (µm) for Tsitsikamma pedunculata.

adjacent to sandy gullies. A thin delicate light yellow *Mycale* (*Mycale*) species is commonly found growing on the globular head surface around the oscula and porefields.

**Remarks.** The shape of the sponge, the long peduncle, round head, colour and the shape of the microscleres set this species well apart from any other species in this genus.

No intraspecific genetic diversity was found for the 28S rRNA gene sequence of specimens of *T. pedunculata* included in this study. An interspecific genetic diversity of 0.32–0.65 % for the 28S rRNA gene sequence was found between *T. pedunculata* and *T. favus* (Suppl. material 1: Table S1).

# *Tsitsikamma scurra* Samaai, Gibbons, Kelly & Davies-Coleman, 2003 Figure 3a–k

Tsitsikamma scurra Samaai, Gibbons, Kelly and Davies-Coleman, 2003: 20.

**Type locality.** *Holotype* – NHMUK 2003.1.10.3 (CASIZ 301103): Hout Bay. Western Cape Province, -34.03600, 18.30567, 28 m depth, 31 March 2000, near the wreck of British "The Maori", collected by P.L. Colin; Paratype – SAM H-4971: Hout Bay. Western Cape Province, -34.03600, 18.30567, 28 m depth, 25 January 2003, near the wreck of British "The Maori, collected by Lynden West of the Scripps Institute of Oceanography (after Samaai et al. 2003).

**Material examined.** SAIAB 207201, SAIAB 207229: west of Hout Bay Western Cape Province, -34.03600, 18.30567, 28 m depth, 25 January 2003, near the wreck of British "The Maori, collected by Lynden West of the Scripps Institute of Oceanography.

**Diagnosis** (emended from Samaai et al. 2003). Sponge massive, semispherical to thick encrusting and lime green in life, compressible with a tough sandpapery ectosome. Samaai et al. (2003) noted the surface crowded with large hollow strap-like oscula with the apex slightly expanded and fungiform areolate porefields, with the overall skeleton dominated by an ectosomal envelope of tangential megascleres, extending up into the large oscular tubes (Fig. 3a, b). In the preserved specimen small pear-shaped oscula (2–5.5 mm high and 1–1.5 mm in diameter) and long narrow stalked areolate porefields (Fig. 3a–d).

*Skeleton.* The ectosome is thin with a fine sandpapery feel that seems to continue and fold within the interior of large specimens to form smaller subunits or internal chambers (Table 3, Fig. 3c–e). The choanosome is soft and may contain varying amounts of sand, shell and other foreign material (Fig. 3d).

*Spicules.* Megascleres consist of slightly curved styles, conspicuously thickened centrally sometimes bend basally and thinner styles, slightly curved centrally (Table 3, Fig. 3f–h). Microscleres. Isochiadiscorhabds with three whorls of conico-cylindrical tubercles, the apex of each is acanthose. The median whorl is polar and situated closer to the apex whorl than to the slightly larger manubrium (Table 3), this polarity may be less pronounced in larger microscleres (Fig. 3i). The acanthose tubercles arranged in pairs in the apex whorl and manubrium (Fig. 3i–k). Microscleres are abundant throughout the choanosome (after Samaai et al. 2003).



**Figure 3.** *Tsitsikamma scurra* **a**, **b** freshly collected CASIZ 301103 and SAIAB 207201 **c** preserved specimens SAIAB 207229 **d** preserved specimens SAIAB 207201 **e** section of ectosome with underlying choanosome SAIAB 207201 **f**, **g** robust centrally thickened sinuous style SAIAB 207201 **h** thin slightly centrally thickened sinuous style SAIAB 207201 **i**, **j** light microscope image of chiadiscorhabds SAIAB 207201 **k** light microscope image of chiadiscorhabds SAIAB 207201 **k** light microscope image of chiadiscorhabds SAIAB 2072029. Scale bars 2.5 cm (**c**); 5 cm (**d**); 1 mm (**e**); 100 μm (**f–h**); 20 μm (**i–k**).

	Samaai et al. (2003)	Examined material
Ectosome	230–540	530 (380–880) (n = 10)
Internal chambers		150 (100–230) (n = 6)
Styles	(i) 829 (774–882) x 24	(i) 702 (480–884) x 19 (14–27)
	(ii) 669 (585–738) x 17	(ii) 692 (518–821) x 10 (5–15)
	(n = 20)	(n = 80)
Isochiadiscorhabds	41 (38–45) x 8	43 (41–48) x 8 (6–10)
		Additional measurements
		shaft length 37 (19–41)
		apex whorl diameter 20 (19–22) manubrium diameter 22 (19–25)
		(n = 40)

**Table 3.** Skeletal and spicule dimensions (µm) for *Tsitsikamma scurra*.

**Distribution.** West of Hout Bay, a local area known as Maori Bay along the Western Cape Province coast.

**Remarks.** The specimens examined compared well with the description given by Samaai et al. (2003) except that the colour in life of the type specimen was described as lime green and colour photographs of the freshly collected specimen indicate a brownish colouration (Fig. 3a). Preserved specimens are a medium to dark brown colour in ethanol (Fig. 3c, d). *Tsitsikamma scurra* differs from all other known *Tsitsikamma* species in the folded globular thick encrusting growth structure (Fig. 3d) with thin sandpaper-like ectosome (Table 3). Epifauna may be present on the sponge surface and the interior may contain a substantial amount of sand particles and shell fragments.

We obtained 28S rRNA gene sequences for only one *T. scurra* specimen. The interspecific diversity of the 28S rRNA gene sequence for *T. scurra* and other *Tsitsikamma* did not support clear genetic identity, with between 0.16–0.32 % at 28S for *T. favus* and 0.32 % for *T. pedunculata*.

## Tsitsikamma michaeli Parker-Nance, sp. nov.

http://zoobank.org/140E1C34-5012-4FCB-B89C-458F34D51A9A Figure 4a–n

**Type material.** *Holotype* – SAIAB 207202 Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.81663, 30–34 m depth, 12 November 2015.

*Paratype* – TIC2009-009, Evans Peak, Algoa Bay, Eastern Cape Province, -33.84297, 25.81647, 25–30 m depth, 15 May 2009.

Material examined. SAIAB 207204, SAIAB 207205: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.31663, 25–33 m depth, 10 October 2010; SAIAB 207206, SAIAB 207207, SAIAB 207208, SAIAB 207209: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84418, 25.81522, 30–34 m depth, 30 October 2015; SAIAB 207210: Evans Peak, Algoa Bay, Eastern Cape Province, -33.84548, 25.31663, 30 m depth, April 2011; SAIAB 207211: Algoa Bay, Eastern Cape Province, 5 November 2002.

**Description.** Small olive-green, purse shaped sponge up to 5 cm high (2 cm stalk and 3 cm rounded head) or sessile, 5–10 cm in diameter. In some cases, the large



**Figure 4.** *Tsitsikamma michaeli* sp. nov. **a–c** in situ **d** collected specimens with yellow encrusting *Mycale* (*Mycale*) sp. short stalk visible SAIAB 207204 **e** section through the ectosome with underlying choanosome SAIAB 207220 **f** robust centrally thickened sinuous style SAIAB 207202 **g** thin slightly ventrally thickened sinuous style SAIAB 207208 **h** short slightly sinuous strongyles **i–l** SEM images of chiadiscorhabds SAIAB 207204 **m–n** light microscope images of chiadiscorhabds SAIAB 207204 and SAIAB 207220. Scale bars 6 cm (distance between laser points) (**a–c**); 5 cm (**d**); 1 mm (**e**); 100 μm (**f–h**); 20 μm (**i–n**).

sponge may be loosely subdivided into sections (Fig. 4a–c). Small short tube-shaped oscula, 2.5–4 mm high and 1.8–5.5 mm wide at the base narrows to a point and may be laterally flattened in preserved material. The particularly large stalked cauliform porefields are 3–7 mm high and 3–6.5 mm wide, with the porefields spilling over the supporting stalk (Fig. 4d). The freshly collected sponge is a dark to olive green colour with light cream tipped oscula and darker brown green areolate porefields (Fig. 4d). The interior choanosome is bright green. Preserved specimens are olive to tan in colour.

**Skeleton**. The soft delicate, bright green, interior choanosome is encapsulated by a protected firm resilient green ectosome 1000 (200–1500)  $\mu$ m thick (Fig. 4d, e). The attachment area or short stalk is represented by a thickening of the ectosome. No reinforced tracts are present through the interior.

**Spicules.** Megascleres consists of acerate, hastate or blunt styles that are prominently centrally thickened prominently; (i) 713 (537–935) x 21 (12–30)  $\mu$ m and (ii) long slender styles 622 (439–769) x 9 (4–13)  $\mu$ m, with occasionally short thick anisostrongyles (Fig. 4f–h). Microscleres. Isochiadiscorhabds are 38 (34–44)  $\mu$ m in length with three or four whorls. The shaft is 30 (19–37) x 6 (4–8)  $\mu$ m. The terminal whorls consist of a clearly larger manubrium 19 (14–23)  $\mu$ m and apical whorls 16 (13–21)  $\mu$ m in diameter (Table 4). The acanthose tubercles are arranged in sets of two to four, radiating from the terminal ends (Fig. 4i–n). The sponge is often encrusted by a yellow *Mycale (Mycale)* sponge species also found on the surface of *T. pedunculata*.

**Etymology.** *Tsitsikamma michaeli* sp. nov. is named after Professor Michael T Davies-Coleman, Dean of Science, Department of Medical BioScience, University of the Western Cape in recognition of his outstanding contributions to our knowledge of the diversity of South African marine fauna and their production of bioactive secondary metabolites.

## Distribution. Algoa Bay

**Substrate, depth range, and ecology.** *Tsitsikamma michaeli* sp. nov. is a small species found on similar reef habitat as to *T. pedunculata* in Algoa Bay, sometimes in close proximity, at depths between 33–38 m. It shares the same epibiont *Mycale (Mycale)* species, which grow on the sponge surface between the oscula and porefields.

**Remarks.** The absence of reinforcing spicule-dense tracts through the interior choanosome differentiates this new species from *T. favus* and *T. scurra*. The sac- or purselike shape of the *T. michaeli* sp. nov. and the well-spaced oscula and porefields resemble

	T. pedunculata	T. michaeli sp. nov.
Ectosome	818 (200–1800) (n = 11)	1000 (200–1500) (n = 10)
Megascleres	(i) 536(500–555) x 11*	
(n = 20) *	(ii) 684(591–728) x 16 *	(i) 622(439–769) x 9(4–13)
(n = 160)	(i) 673(562–798) x 11(4–15)	(ii) 713(537–935) x 21(12–30)
	(ii) 636(541–788) x 15(12–17)	
Microscleres		
(n = 20) *	29 (27–30) x 7 *	38 (34–44) x 6 (4–8)
(n = 80)	29 (26–34) x 7 (5–9)	

**Table 4.** Comparison between morphological structures (µm) *T. pedunculata* and *T. michaeli* sp. nov.

\*From Samaai et al. (2003)

those of *T. pedunculata* but the species differs in colour, bright to olive-green compared to the purplish pink to brown of *T. pedunculata*. It does not have a stalk, although the basal attachment area of *T. michaeli* sp. nov. is reinforced by a thickening of the ecto-some (Fig. 4d). The resistant ectosome is of similar thickness for the two species (Table 4). The category (i) megascleres are shorter and thinner in *T michaeli* sp. nov. while the category (ii) styles are longer and more robust than that of *T. pedunculata* (Table 4). The microscleres of these two species have a similarly structured manubrium with tubercle in groups of four or more, but the tubercles are arranged in groups of three in *T. pedunculata* and in pairs in the apex whorl of *T. michaeli* sp. nov. *Tsitsikamma pedunculata* lack the median whorl and the spicule is shorter (Table 4), while *T. michaeli* sp. nov. may have up to two whorls between the apical whorls and manubrium.

There was no intraspecific genetic diversity for the 28S rRNA gene region for *T. michaeli* and no interspecific genetic diversity for *T. michaeli* and *T. pedunculata* was observed in this work (Suppl. material 1: Table S1). There was, however, interspecific genetic diversity of between 0.48–0.65 % between *T. michaeli* and *T. favus* and 0.32 % between *T. michaeli* and *T. scurra*.

#### Tsitsikamma nguni Parker-Nance, sp. nov.

http://zoobank.org/94AAF755-0507-4C05-BC69-4F5EE84E3E27 Figure 5a–l

**Type material.** *Holotype* – SAIAB 207212: Rheeders Reef, Tsitsikamma, Garden Route National Park, Eastern Cape Province, -34.02735, 23.90468, 20–21 m depth, 8 June 2015.

*Paratype* – SAIAB 207213: Rheeders Reef, Tsitsikamma, Garden Route National Park, Eastern Cape Province, -34.02735, 23.90468, 20–21 m depth, 9 June 2015. SAIAB 207214, SAIAB 207215: Rheeders Reef, Tsitsikamma, Garden Route National Park, Eastern Cape Province, -34.02735, 23.90468, 20–21 m depth, 8 June 2015; SAIAB 207216: The Knoll, Tsitsikamma, Garden Route National Park, Eastern Cape Province, -34.02555, 23.90708, 18 m depth, 2 May 1993, collected by Colin Buxton.

**Description.** Large thick encrusting or sessile hemispherical or convex cushions, dark slate-coloured when alive but very dark brown to black in preservative. The sponge is very firm and rigid, 3–6 cm high and 3–10 cm in diameter (Fig. 5a–d). The upper third to half of the sponge surface is dominated by small short, blunt rounded knob-shaped or button-like oscula, 2–5 mm high and 2.5–5 mm wide at the base. The surface surrounding the upper osculate area, the shoulder and upper side of the sponge, has well-spaced small round slightly elevated or sessile porefields. These gradually merge to form larger round porefields that join to form irregular or blotch-shaped structures along the base of the sponge. In general, porefields are 1–4 mm high and 3–14 mm in diameter (Fig. 5a–c).

**Skeleton**. The ectosome is 780 (430–1560)  $\mu$ m thick guarded externally by a prominent palisade of microscleres arranged perpendicularly to the prominent inner style layer (Fig. 5e). The softer choanosome is divided into small uneven circular to



**Figure 5.** *Tsitsikamma nguni* sp. nov.: **a, b** in situ **c** collected specimens **d** preserved specimen SAIAB 207216 **e** section of ectosome with underlying choanosome SAIAB 207214 **f, g** sinuous style, two size classes sometime tylote **h** short thick strongyles **i** collection of chiadiscorhabds and **j, k** acanthose tubercles visible on SEM image of chiadiscorhabds, and l) developing chiadiscorhabds SAIAB 207215. Scale bars 5 cm (**c, d**); 1 mm (**e**); 100  $\mu$ m (**f–h**); 20  $\mu$ m (**i–l**).

oval shaped chambers 6640 (2290–19770)  $\mu$ m in diameter by reinforcing tracts 1410 (530–3200)  $\mu$ m thick (Fig. 5c). Sand particles and shell fragments may be present in the sponge choanosome.

**Spicules.** Megascleres are slightly sinuous or curved, hastate or mucronate styles, in two size categories; (i) thick styles are robust and conspicuously centrally thickened 555 (428–672) x 14 (10–19)  $\mu$ m and (ii) very long thinner styles 561 (449–832) x10 (3–14)  $\mu$ m (Fig. 5f, g). Occasionally short thick strongyles or anisostrongyles are present 463 (287–552) x 14 (7–21)  $\mu$ m (Fig. 5h). Microscleres are isochiadiscorhabds generally with three whorls (Fig. 5j–l), although intermediate forms in which the microscleres have partial whorls of conico-cylindrical tubercles are not uncommon and spicules with two intermediate whorls are also present (Fig. 5i). Chiadiscorhabds are 51 (40–60)  $\mu$ m in total length, with a shaft measuring 42 (34–54) x 9 (6–13)  $\mu$ m. The manubrium is 25 (18–37)  $\mu$ m and the apical whorls 23 (16–32)  $\mu$ m in diameter. Whorls are constructed of acanthose conico-cylindrical tubercles arranged in groups of two to three in the apex whorl and four or more in the manubrium.

**Etymology.** The Nguni cattle breed is unique to southern Africa with characteristic dappled colour and blotchy patterns on the hide, reminiscent of the elaborate blotch-shaped areolate porefields typical of the larger *T. nguni* sp. nov. specimens.

**Distribution.** Tsitsikamma Marine Protected Area, Garden Route National Park, Eastern Cape Province.

**Substrate, depth range, and ecology.** The species is common in the shallow coastal zone within the Tsitsikamma Marine Protected Area on low profile reefs at a depth of 18–21 m.

**Remarks.** Live specimens of *T. nguni* sp. nov. appear a dark slate or very dark grey, almost black in colour. Freshly collected specimens consist of the dark olive-brown to black exterior with dark brown surface structures (Fig. 5a, b). The interior tracts are light olive, cartilaginous with softer withdrawn olive-brown choanosome, which may contain sand and shell fragments (Fig. 5c). Preserved specimens are a uniform dark brown colour staining the preservative (70% ethanol) a deep rich brown to almost black colour (Fig. 5d).

*Tsitsikamma favus* and *T. nguni* sp. nov. differ considerably from *T. scurra* in the texture and thickness of the ectosome, internal tracts and surface structures (Table 5)

**Table 5.** Dimensions (mm) of surface and skeletal structures (data for ectosome and tracts given as thickness, internal honeycomb-shape chamber as mean diameter and ranges, oscula and porefields as mean height and diameter with ranges).

	T. scurra	T. favus	T. nguni sp. nov.
Ectosome	0.5 (0.4–0.9)	0.6 (0.4–1.1)	0.8 (0.4–1.6)
Internal tracts	0.7 (0.7)	1.2 (0.7–1.8)	6.6 (2.3–19.8)
Internal chambers	0.2 (0.1-0.2)	5.1 (3.6-8.5)	1.4 (0.5–3.2)
Oscula height	2.4 (2.0-5.5)	4.4 (2.0-8.0)	3.1 (2.0-4.5)
Oscula diameter	1.1 (1.0–1.5)	4.0 (1.5-8.0)	3.2 (2.5-4.5)
Porefield height	8.3 (7.0–9.0)	5.1 (1.0-10.0)	2.1 (1.0-4.0)
Porefield diameter	3.4 (2.5–5)	3.5 (2.0–14)	7.1 (3.0–14)

as well as the dimensions of the spicules (Table 6). Defining the differences between T. favus and T nguni sp. nov. is more challenging. Most apparent is the surface morphology. The basal part and sides of T. favus sponges are dominated by stalked cauliform porefields, densely crowded and gradually giving way to prominent lance-shaped oscula with a large basal diameter distributed over the upper surface of the sponge, giving the sponge surface an uneven, messy appearance (Samaai and Kelly 2002) (Fig. 1a). In contrast, the lower basal parts and sides of T. nguni sp. nov. is dominated by flat to slightly raised elaborate blotch-shaped porefields which become smaller, more circular in shape and more isolated towards the upper part of the sponge where they are replaced by well-spaced, small button-shaped (in life, see Fig. 5a) or small and pointed (preserved, Fig. 5d) oscula over the upper part of the sponge. Both species have similar partitioning of the choanosome, although T. nguni sp. nov. is notably firmer, has larger more regular chambers with generally thicker spicule tracks and a slightly thicker ectosome (Table 5). The megasclere and microsclere shape and dimension are very similar (Table 6) although the species differ in the number of acanthose conico-cylindrical tubercles grouped together to make up the manubrium, three per group in T. favus (Fig. 1l) and four to six in the new species (Fig. 5j, k).

The general appearance of *T. nguni* sp. nov., shape of the porefields, and smaller size of the oscula, the colour, both in life and preserved, the slightly shorter styles (Table 6), slight difference in the arrangement of the acanthose tubercles of the microsclere manubrium, the slightly thicker ectosome, the more robust interior spicule-dense tracts, and larger chambers (Table 6) all contribute to a species that is distinctly different in appearance from *T. favus*. In freshly collected specimens fixed in 70% ethanol, the preservative extracts some secondary metabolites and pigment from the specimen. *Tsitsikamma nguni* sp. nov. colours the fixative intense dark solid brown almost

Sample	Megascleres	Microscleres (µm)
T. scurra	(i) 702 (480–884) x 19 (14–27)	43 (41–48) x 8 (6–10)
	(ii) 692 (518–821) x 10 (5–15)	Additional measurements
	(n = 80)	shaft length 37 (19–41)
		apex whorl diameter 20 (19–22)
		manubrium diameter 22 (19–25)
		(n = 40)
T. favus	(i) 570 (420–788) x 14 (9–19)	53 (40-68) x 9 (6-14)
	(ii) 598 (449–907) x 10 (3–16)	Additional measurements
	(n = 520)	shaft length 43 (29–59)
		apex whorl diameter 24 (17–34)
		manubrium diameter 26 (19–37)
		(n = 280)
T. nguni sp. nov.	(i) 555 (428–672) x 14 (10–19)	51 (40-60) x 9 (6-13)
	(ii) 561 (449-832) x10 (3-14)	Additional measurements
	(n = 160)	shaft length 42 (34-54)
		apex whorl diameter 23 (16–32)
		manubrium diameter 25 (18–37)
		(n = 80)

**Table 6.** Spicule dimensions ( $\mu$ m) of *T. scurra* (n = 2), *T. favus* (n = 14), and *T. nguni* sp. nov. (n = 4) for material examined. Data in table given as mean total length (range) × shaft width (range).

black colour, this is very different from the lighter brown semi-translucent colouration given to the fixative by *T. favus*.

*Tsitsikamma nguni* was found to show no genetic diversity with respect to the 28S rRNA gene sequence from *T. scurra*, 0.16–32 % from *T. favus*, and 0.32 % from *T. pedunculata* and *T. michaeli* (Suppl. material 1: Table S1).

**Discussion.** The species in *Tsitsikamma* exhibit two morphological growth forms: *T. favus, T. scurra*, and *T. nguni* sp. nov. are thick encrusting to hemispherical sponges with spicule-dense tracts that reinforce the internal choanosome while *T. pedunculata* and *T. michaeli* sp. nov. are purse-shaped species, with or without a prominent stalk. The growth form, surface architecture, colour, skeletal structure, and spicule morphology are important diagnostic characteristics (Samaai and Kelly 2002, Samaai et al. 2003). An identification key for the Latrunculiidae genera and species within *Tsit-sikamma* incorporating important morphological characteristics, skeletal architecture, spicule morphology, and ontogeny has been constructed which incorporates descriptive information from Samaai and Kelly (2002), Samaai et al. (2003), Samaai et al. (2004), Samaai et al. (2009), Samaai et al. (2006), and Kelly et al. (2016) (Fig. 6). This identification key is in agreement with the relationships presented in the 28S rRNA and COI sequence based phylogenetic trees constructed for available sequences (Fig. 7, Table 7).

The phylogenetic analysis presented here of partial 28S rRNA gene sequences and COI sequences is incomplete and although lacking COI sequences for some *Tsitsi*-

Species	Specimen number	28 S rRNA GenBank accession #	COI GenBank accession #
Cyclacanthia bellae	TIC2008-085A	MG820030	
Latrunculia (Biannulata) algoaensis	Walmsley sp06	KC471505.1	KC471497
Latrunculia (Latrunculia) biformis	NIWA36068		LN850207
Latrunculia (Latrunculia) biformis	NIWA37305		LN850209
Latrunculia (Latrunculia) brevis	NIWA29141		LN850236
Latrunculia (Biannulata) lunaviridis	NCI417	KC869489.1	
Mycale (Arenochalia) mirabilis	NCI445	KC869613	
Mycale (Arenochalia) mirabilis	QMB G305553		HE611592
Mycale (Mycale) sp.	SAIAB 207209	KU695578	
Mycale (Mycale) sp.	Walmsley sp08		KC471501
Sceptrella biannulata			KF017195
Tsitsikamma favus	SAIAB 207190	KU695576	
Tsitsikamma favus	SAIAB 207176	KC471502.1	KC471494
Tsitsikamma favus	SAIAB 207221	KC471503.1	KC471495
Tsitsikamma favus	SAIAB 207228	MG203890	
Tsitsikamma favus	Walmsley sp03		KC471496.1
Tsitsikamma favus			JF930154.1
<i>Tsitsikamma michaeli</i> sp. nov.	SAIAB 207202	KC471507.1	
<i>Tsitsikamma michaeli</i> sp. nov.	SAIAB 207207	MG203894	
<i>Tsitsikamma michaeli</i> sp. nov.	SAIAB 207209	KU695577	
Tsitsikamma nguni sp. nov.	SAIAB 207213	KU695575	
Tsitsikamma pedunculata	SAIAB 207195	KU695579	
Tsitsikamma pedunculata	SAIAB 207196	KU695580	
Tsitsikamma pedunculata	SAIAB 207198	MG203896	
Tsitsikamma scurra	SAIAB 207201	MG686549	

**Table 7.** List of species, specimen numbers, and GenBank accession numbers for 28S rRNA gene sequences and COI gene sequences used to construct phylogenetic trees.



**Figure 6.** Identification key for Latrunculiidae incorporating descriptive information contained in Samaai and Kelly (2002), Samaai et al. (2003), Samaai et al. (2004), Samaai et al. (2009), Samaai et al. (2006), and Kelly et al. (2016).

*kamma* representatives, the diagnostic key constructed for morphological characteristics distinguishing members of the Latrunculiidae is not contradicted by the relatedness between taxa presented in these preliminary phylogenetic trees based on DNA sequence comparison. Both suggest that *Tsitsikamma* is closely related to *Cyclacanthia* (Fig. 7A) and *Sceptrella* (Fig. 7B). The separation between *Tsitsikamma* and *Latrunculia* underline the ontogenetic nature of the spicule and resulting microsclere morphology with similar terminal structures such as isochiadiscorhabds in *Tsitsikamma*, isospinodiscorhabds in *Cyclacanthia*, or isoconicodiscorhabds in *Sceptrella*, which are more similar in development than the anisodiscorhabds characteristic of *Latrunculia* [after Samaai and Kelly (2002), Samaai et al. (2003), Samaai et al. (2004), Samaai et al. (2009), Samaai et al. (2006), Kelly et al. (2016)].

The morphological similarity of species in the two morphological groups within *Tsitsikamma* is borne out by the similarity of their 28S rRNA gene sequences as shown (Fig. 7A) and reflected in pair wise distance analysis of the sequences. Interestingly, we observed significant intraspecific genetic diversity in *T. favus* but not in *T. pedunculata* or *T. michaeli* sp. nov. However, interspecific genetic diversity for the 28S rRNA gene did support the morphological species identity overall (see Suppl. material 1: Table S1, Figs 6, 7). This study highlights the limitations of commonly used genetic markers in their current coverage for the resolution of closely related species and the importance of rigorous morphological data for taxonomic classification of the Latrunculiidae sponges. An extended phylogenetic investigation encompassing the full rRNA cistron would improve our understanding of the phylogenetic relationship of not only the higher taxa but also at species level.



**Figure 7.** Tree representation of the results of a molecular phylogenetic analysis by Neighbour-Joining method (MEGA) for **A** 28S rRNA gene sequence and **B** COI gene sequence (numbers below branches indicate bootstrap values for maximum likelihood and scale distance of the branches) for available species in the Latrunculiidae and *Mycale* (*Mycale*) sponge species (commonly found as epibionts).

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Author Contributions: SPN sponge identification description; prepared the first draft of manuscript; RD, SCW, SH, and TW contributed 28S sequence data that was used for the phylogenetic tree; RD edited the manuscript.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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# Supplementary material I

# Table S1. Estimates of evolutionary divergence between sequences

Authors: Shirley Parker-Nance, Storm Hilliar, Samantha Waterworth, Tara Walmsley, Rosemary Dorrington

Data type: molecular data

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# Revision of the genus Ceriantheomorphe (Cnidaria, Anthozoa, Ceriantharia) with description of a new species from the Gulf of Mexico and northwestern Atlantic

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#### Abstract

The present study presents a revision of the genus *Ceriantheomorphe* Carlgren, 1931, including redescriptions of the two presently recognized species, *Ceriantheomorphe ambonensis* (Kwietniewski, 1898) and *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919), **comb. nov.**, and a description of the new species *Ceriantheomorphe adelita* **sp. nov.** 

#### Keywords

Biogeography, cnidarian taxonomy, North America, Pacific Ocean, South America

# Introduction

Ceriantharia is a subclass within the cnidarian class Anthozoa, consisting of species commonly known as tube anemones. This taxon has several taxonomic inconsistencies (Stampar et al. 2016) that are understudied, in part because of sampling difficulties (den Hartog 1977; Stampar et al. 2016). Moreover, ecology, behavior and life cycle in most species are poorly known (Nyholm 1943; Stampar et al. 2015, 2016). Also, most systematic studies are solely based on morphological characters of few specimens (Carlgren 1912; Arai 1965; den Hartog 1977), leading to unreliable terminology (Arai 1965), and contributing to taxonomic uncertainty. A combination of these problems occurs in the genus *Ceriantheomorphe* Carlgren, 1931, which currently includes only two species: *Ceriantheomorphe brasiliensis* sensu Carlgren, 1931, and *Ceriantheomorphe ambonensis* (Kwietniewski 1898) (Carlgren 1931; den Hartog 1977).

The genus *Ceriantheomorphe* was described by Carlgren (1931) through the description of *C. brasiliensis* from southeastern Brazil. In this study, Carlgren also proposed that two species, *Cerianthus ambonensis* Kwietniewski, 1898 described from Ambon, Indonesia and *Cerianthus brasiliensis* Mello-Leitão, 1919 described from Guanabara Bay (Rio de Janeiro, Brazil) should be reassigned to the genus *Ceriantheomorphe*. As well, Carlgren (1931) also pointed out that *Cerianthus brasiliensis* is likely a synonym of *Ceriantheomorphe brasiliensis* sensu Carlgren 1931.

However, assigning *Cerianthus ambonensis* as "*Ceriantheomorphe ambonensis*" would have been premature because the simple description made by Kwietniewski (1898) did not include any mention of deposited type material. Additionally, Carlgren (1931) was not able to observe the holotype of *Cerianthus brasiliensis* described by Mello-Leitão (1919), so his assignment of the species to *Ceriantheomorphe* must be viewed as tentative.

More than two decades after the description of *Ceriantheomorphe*, Carlgren and Hedgpeth (1952) reported *C. brasiliensis* from the Gulf of Mexico. However, the authors suggested that these specimens could possibly be another species due to the disjunct occurrence in relation to South American specimens (Carlgren and Hedgpeth 1952; den Hartog 1977; Spier et al. 2012).

Despite this taxonomic confusion, *Ceriantheomorphe brasiliensis* had been listed as an endangered species in Brazil for over 10 years (MMA 2004). Furthermore, the tubes built by *C. brasiliensis*, and Ceriantharia in general, play an important ecological role in providing suitable alternative substrates to many invertebrate groups (e.g., Bryozoa, Crustacea, Anthozoa) (Tiffon 1987; Kim and Huys 2012; Vieira and Stampar 2014). For example, some species, such as the phoronid *Phoronis australis* Haswell, 1883, are only found in ceriantharian tubes (Stampar et al. 2010). Thus, the survival of *P. australis* may be related to the management of the cerianthid species that houses them in the southern Atlantic.

This study aims to present a taxonomic review of the genus *Ceriantheomorphe* including a redescription of the holotype of *C. brasiliensis*, a redescription of *C. ambonensis*, and the description of a new species from the North Atlantic.

# Material and methods

# Specimens

Twenty specimens of *Ceriantheomorphe* were sampled by SCUBA: sixteen of *C. brasiliensis* from the South Atlantic, three from the North Atlantic, and one, *C. ambonensis*, from the Pacific Ocean (Table 1).

## Morphological studies

The morphology of all specimens was studied through internal anatomy and cnidome studies, both based on criteria adopted by Carlgren (1931), Arai (1965), den Hartog (1977) and Stampar et al. (2015). All specimens were observed separately. Specimens were longitudinally dissected along the ventral side using surgical scalpels, photographed under an Opticam stereomicroscope, using the OPT HD 3.7 software and a general description of each body region was made. The morphological characters were compared between specimens and descriptions available in the relevant literature (Kwietniewski 1898; Carlgren 1931; Spier et al. 2012).

All protomesenteries/directive mesenteries (P) were measured. Five quartets of mesenteries were measured for each specimen. We also divided the metamesenteries

**Table 1.** List of *Ceriantheomorphe* specimens in this study. Abbreviations: ES = Espírito Santo State; RJ = Rio de Janeiro State; SP = São Paulo State; SC = Santa Catarina State; UFRJ Biologia = cnidarian collections of the Department of Zoology, Biology Institute, Universidade Federal do Rio de Janeiro, Brazil; MZSP = Zoology Museum, Universidade de São Paulo, Brazil; MNHN Montevideo = National Museum of Natural History, Montevideo, Uruguay; USNM = United States National Museum, Washington DC, USA.

Species	Country	Locality	Coordinates	Museum code
C. brasiliensis	Brazil	Guanabara Bay-RJ	22°49'6''S, 43°8'45''W	MNRJ 200
		Arraial do Cabo-RJ	23°0'4''S, 42°0'29''W	MZSP 8470
		Araçá Beach-SP	23°48'58''S, 45°24'24''W	MZSP 8471
		Araçá Beach-SP	23°48'58''S, 45°24'24''W	MZSP 8472
		Cagarras Islands-RJ	23°1'55''S, 43°11'58''W	MZSP 8473
		Canasvieiras-SC	27°25'31''S, 48°27'0.2''W	MZSP 8475
		Camburi Beach-ES	20°16'39''S, 40°16'29''W	UFRJ Biologia 0293
		Camburi Beach-ES	20°16'39''S, 40°16'29''W	UFRJ Biologia 0337
		Rio de Janeiro-RJ	-	UFRJ Biologia 2-141
		Urca-RJ	-	UFRJ Biologia 2-086
		Zimbro Beach-SP	23°49'27''S, 45°25'4''W	UFRJ Biologia 2-11
		Sabacu Island-RJ	23°0'43''S, 44°22'7''W	MNRJ 2766
	Uruguay	José Ignacio-Maldonado	35°00'S, 54°24'2''W	MZSP 8474
		La Paloma-Rocha	34°42'3''S, 54°0.5'W	UFRJ-Biologia 2-464 A
		La Paloma-Rocha	34°42'3''S, 54°0.5'W	UFRJ-Biologia 2-464 B
		Punta del Diabo	34°04'S, 53°29'W	MNHN Montevideo I-1168
C. adelita sp. nov.	Mexico	Punta de Almagre-Tamaulipas	-	USNM 50016
	United States of America	Pass A'Loutre-Louisiana	-	USNM 51253
		Port Aransas, Corpus Christi-Texas	-	USNM 50015
C. ambonensis	Indonesia	Jakarta Bay-Jakarta	_	MZSP 8476

(type M and type m) value and betamesenteries (type B and type b) value to calculate the ratio between these mesentery types. We calculated the proportion occupied by protomesenteries in the gastrovascular cavity using the following equation:

F (length of protomesentery) × 100 / E (length of gastrovascular cavity)

The cnidome study was based on the sampling of 30 cnidae capsules for each cnida type from each body region (superior tip of marginal and labial tentacles, actinopharynx region, column, metamesenteries and betamesenteries). Each cnida was classified according to their shape based on different authors (Mariscal 1974; den Hartog 1977; Stampar et al. 2015) and measured using a Nikon Eclipse E200 microscope and MOTIC IMAGES PLUS 2.0 imaging software.

#### Systematic results

Phylum Cnidaria Verrill, 1865 Class Anthozoa Ehrenberg, 1834 Subclass Ceriantharia Perrier, 1893 Suborder Spirularia den Hartog, 1977 Family Cerianthidae Milne-Edwards & Haime, 1851

Genus Ceriantheomorphe Carlgren, 1931

**Diagnosis.** Cerianthidae with fertile mesenteries, except for directives. Two pairs of mesenteries connected to the siphonoglyph. Mesenteries grouped in quartets following M, B, m, b order (after Carlgren 1931; Spier et al. 2012).

Type species. Ceriantheomorphe brasiliensis (Mello-Leitão, 1919).

Valid species. Ceriantheomorphe brasiliensis (Mello-Leitão, 1919) new comb., Ceriantheomorphe ambonensis (Kwietniewski, 1898), Ceriantheomorphe adelita sp. nov.

**Distribution.** Southwestern Atlantic (Brazil and Uruguay), Gulf of Mexico (United States of America and Mexico), Central West Pacific (Java Sea, Indonesia).

# Ceriantheomorphe brasiliensis (Mello-Leitão, 1919), comb. nov.

Fig. 1 A–C

Cerianthus brasiliensis Mello-Leitão, 1919: 38-39.

*Ceriantheomorphe brasiliensis* sensu Carlgren 1931: 2–6; Carlgren 1940: 6, 11–12; Carlgren and Hedgpeth 1952: 148, 169–170; Frey 1970: 309; Molodtsova 2009: 365–367; Stampar et al. 2010: 205–209; Silveira and Morandini 2011: 3; Rodriguez et al. 2011: 52, 54–55; Spier et al. 2012: 1–3; Stampar et al. 2012: 5–6, 9; Stampar et al. 2014a: 2, 5, 8; Stampar et al. 2014b: 344, 347, 351, 353; Stampar and Morandini 2014: 2; Vieira and Stampar 2014: 370; Stampar et al. 2015: 3; González-Muñoz et al. 2016: 5, 9; Stampar et al. 2016: 64, 67, 68.

Ceriantheomorphe brasiliensis (not) - Hedgpeth 1954: 286.



**Figure 1.** Holotype of *Ceriantheomorphe brasiliensis* (MNRJ 200). **A** Specimen inside the tube **B** specimen without the tube **C** dissected specimen. Scale bars: 2 cm.

Material examined (16 specimens). Holotype: MNRJ 200 • adult individual (16.5 cm long), Guanabara Bay, Rio de Janeiro, Brazil (22°49'6"S, 43°8'45"W), Mello-Leitão leg. (Fig. 1 A-C). Paratypes: MZSP 8470 • adult individual (9.3 cm long), Arraial do Cabo (near Farol Island, 18 m depth), Rio de Janeiro, Brazil (23°0'4"S, 42°0'29"W), S.N. Stampar leg. (20/i/2009); MZSP 8471 • adult individual (24 cm long), Araçá Beach (intertidal), São Sebastião, São Paulo, Brazil (23°48'58"S, 45°24'24"W), J.A. Petersen leg. (03/ii/1965); MZSP 8472 • adult individual (16.5 cm long), same locality data as for preceding; MZSP 8473 • juvenile individual, (8.5 cm long), Cagarras Islands (22 m depth), Rio de Janeiro, Brazil (23°1'55"S, 43°11'58"W), S.N. Stampar leg. (15/ iv/2009); MZSP 8474 • adult individual (22.2 cm long), José Ignacio (27 km from the coast, 38 m depth), Maldonado, Uruguay (35°00'S, 54°24'2"W), F. Scarabino leg. (18/ ix/2005); MZSP 8475 • adult individual (14.4 cm long), Canasvieiras Beach, Florianópolis (4 m depth), Santa Catarina, Brazil, (27°25'31"S, 48°27'0.2"W), S.N. Stampar leg. (21/i/2009) (Fig. 2D); UFRJ Biologia 0293 • adult individual (17 cm long), Camburi Beach, Espírito Santo, Brazil (20°16'39"S, 40°16'29"W), (18/viii/1989) (Fig. 2A); UFRJ Biologia 0337 • adult individual (16.5 cm long), same data as for preceding, (17/iv/1990) (Fig. 2B); UFRJ Biologia 2-141 • adult individual (22 cm



**Figure 2.** Dissected specimens of *Ceriantheomorphe brasiliensis* from southwestern Atlantic. **A** Individual UFRJ Biologia 0293 from Camburi (ES) **B** specimen UFRJ Biologia 0337 from Camburi (ES) **C** specimen MNHN Montevideo I-1168 from Punta del Este (Uruguay) **D** individual from Canasvieiras Beach, Santa Catarina.

long), Rio de Janeiro, Brazil, A. Saldanha leg. (1966); UFRJ Biologia 2-086 • damaged adult individual, Urca, Rio de Janeiro, Brazil, (1959); UFRJ Biologia 2-11 • adult individual (10.9 cm long), Zimbro Beach, São Sebastião (4–6 m depth), São Paulo, Brazil, (23°49'27"S, 45°25'4"W), E.Q. Cez leg. (04/ix/1967); UFRJ Biologia 2-464 A • damaged individual, (34 m depth), La Paloma, Uruguay, (34°42'3''S, 54°0.5'W), Conversut I #4557 exped. (17/ix/77); UFRJ Biologia 2-464 • damaged individual, same data as for preceding specimen; MNRJ 2766 B • adult individual (14.5 cm long), Sabacu Island, Angra dos Reis (6 m depth), Rio de Janeiro, Brazil (23°0'43"S, 44°22'7"W), C.C. Ratto leg. (07/xii/1993); MNHN Montevideo I-1168 • adult individual (11 cm long), Rocha (6 km from the coast, in line of Santa Teresa Fortress, 18 m depth), Punta del Diabo, Uruguay (34°04'S, 53°29'W), Navio Hero (3A) exped. (21/vii/1972) (Fig. 2C).



**Figure 3.** Mesenteries arrangement of the holotype of *Ceriantheomorphe brasiliensis* (MNRJ 200). **MT** Marginal tentacles **LT** Labial tentacles **M** and **m** Metamesenteries **B** and **b** Betamesenteries.

Diagnosis. Large cerianthid, 8.5–24 cm long and 1.5–13.8 cm wide. 132–392 marginal tentacles arranged in (1)1123.1123 and 108-384 labial tentacles arranged in (1)1122.1122 or (1)1123.1123. Pharynx occupies about 8–27% of total body length. Five pairs of protomesenteries, of which two pairs connected to the siphonoglyph, (directives and P2). Gastrovascular cavity takes up to 33-72% of total body length. All fertile mesenteries, except for directives. Number of mesenteries about 170-642. Directives mesenteries longer than protomesenteries P3, P5 and metamesenteries m, except by m of the 2<sup>nd</sup> and 3<sup>rd</sup> cycles. Protomesenteries (P2) longer than all mesenteries, extending up to the aboral pore (Fig. 3). Protomesenteries (P3) shorter than protomesenteries (P4) and longer than protomesenteries (P5) and betamesenteries (B), except by B of the 1<sup>st</sup> and 2<sup>nd</sup> cycles. Protomesenteries (P4) longer than directive mesenteries, P5 and metamesenteries (m), except by m of the 2<sup>nd</sup> and 3<sup>rd</sup> cycles. Protomesenteries (P5) shorter than all others protomesenteries and metamesenteries M and m. Ratio of 1.2-3.1% between betamesenteries ( $B \times b$ ) and 1.1–3.1% between metamesenteries ( $M \times m$ ). Directive mesenteries, protomesenteries P3, P4 and P5 occupies about 36.6%, 12.2%, 38.8% and 11.1% of total gastrovascular cavity length, respectively, while protomesenteries P2 extend over 80%. Cnidome composed of spirocysts, microbasic b-mastigophores (six types), atrichous (two types), ptychocysts and holotrichous (Fig. 5A–J, Table 2).

**Distribution.** Southwestern Atlantic-Brazil (from the State of Espírito Santo (20.5°S) to Rio Grande do Sul (33.7°S) State) and Uruguay (34°S). This species was only observed in shallow waters (1–40 m depth).

**Description of holotype.** (MNRJ 200) (Fig. 1A–C). Large ceriantharian, 16.5 cm long and 7.7–10.4 cm wide. 388 marginal tentacles (4.9 cm long in preserved specimen) and 312 labial tentacles (1.7 cm long in preserved specimen), both disposed in four cycles. Marginal tentacles arrangement: (1)1243.1243.1123.1123..., labial ten-

**Table 2.** Measurements of 30 cnida capsules for each cnida type in 6 distinct body regions of *Ceriantheo-morphe brasiliensis* (N = 16). Information inside parentheses indicates cnidae length and width, respectively, and information outside parentheses indicates average of cnidae size.

Body part/cnida type	Ceriantheomorphe brasiliensis	
Marginal tentacles		
Microbasic b-mastigophore type I	65.56 (50.50-80.63) × 13.13 (7.57-18.69)	
Microbasic b-mastigophore type II	38.23 (27.96–48.5) × 4.99 (3.13–6.86)	
Microbasic b-mastigophore type III	31.16 (18.36–43.97) × 3.96 (1.97–5.95)	
Microbasic b-mastigophore type IV	16.55 (10.61–22.49) × 4.10 (2.2–6.01)	
Microbasic b-mastigophore type V	27.87 (18.01-37.73) × 7.02 (1.6-5.42)	
Labial tentacles		
Microbasic b-mastigophore type I	48.75 (36.89–60.61) × 9.11 (5.41–12.82)	
Microbasic b-mastigophore type II	34.93 (25.2–44.66) × 5.12 (3.65–6.6)	
Microbasic b-mastigophore type III	28.27 (17.20-39.35) × 4.03 (1.71-6.35)	
Microbasic b-mastigophore type IV	24.11 (17.25–30.97) × 2.73 (1.64–3.83)	
Microbasic b-mastigophore type V	26.10 (15.03-37.18) × 3.29 (1.79-4.79)	
Pharynx		
Atrichous type I	38.33 (26.15-50.52) × 5.95 (2.68-9.22)	
Microbasic b-mastigophore type I	52.64 (35.56-69.73) × 8.38 (5.43-11.33)	
Microbasic b-mastigophore type II	44.39 (32.10–56.68) × 6.09 (3.28–8.91)	
Microbasic b-mastigophore type III	34.97 (21.86–48.09) × 3.35 (2.13–4.57)	
Microbasic b-mastigophore type V	27.62 (23.37–31.88) × 2.81 (2.19–3.43)	
Column		
Ptychocyst type I	71.99 (56.21-87.77) × 24.41 (13.75-35.08)	
Ptychocyst type II	77.14 (50.15–94.14) × 14.12 (8.86–19.38)	
Atrichous type I	48.85 (30.09–67.61) × 11.09 (4.41–17.78)	
Microbasic b-mastigophore type I	41.33 (26.47–56.2) × 6.25 (3.96–8.54)	
Microbasic b-mastigophore type IV	28.14 (23.83–32.45) × 3.12 (2.48–3.76)	
Microbasic b-mastigophore type V	29.95 (22.51–37.4) × 3.03 (2.08–3.98)	
Holotrichous	50.95 (30.04–71.86) × 14.88 (7.3–22.46)	
Mesenteries M		
Microbasic b-mastigophore type I	51.58 (35.0-68.17) × 10.09 (6.41-13.77)	
Microbasic b-mastigophore type IV	22.25 (10.93–33.58) × 5.76 (2.25–9.28)	
Microbasic b-mastigophore type III	20.03 (13.3–26.77) × 4.90 (2.91–6.9)	
Mesenteries b		
Microbasic b-mastigophore type I	54.65 (39.57–69.74) × 10.44 (7.16–13.73)	
Microbasic b-mastigophore type II	33.69 (24.83-42.56) × 5.01 (3.32-6.7)	
Microbasic b-mastigophore type III	19.97 (12.1–27.85) × 4.17 (1.95–6.4)	
Microbasic b-mastigophore type IV	19.59 (8.62–30.56) × 4.06 (2.24–5.89)	



Figure 4. *Ceriantheomorphe brasiliensis* sectioned at actinopharynx region, showing mesenteries connected to the siphonoglyph. S Siphonoglyph area, 1 and 2. Mesenteries connected to the siphonoglyph.



**Figure 5.** Cnidome of *Ceriantheomorphe brasiliensis*. **A** Microbasic b-mastigophore type I **B** microbasic b-mastigophore type II **C** Microbasic b-mastigophore type III **D** microbasic b-mastigophore type IV **F** microbasic b-mastigophore type VI **G** microbasic b-mastigophore type V **H** ptychocyst type I atrichous type I **J** holotrich **K** ptychocyst type II. Scale bars: 15 μm.

tacles arrangement: (1)1234.1122.1243.1243... Small pharynx, 15% of total body length, well-marked siphonoglyph. Five pairs of protomesenteries, two of which connected to the siphonoglyph. Indistinct hyposulcus and hemisulci. With exception of short directives, all mesenteries are fertile. Long protomesenteries P2 extending up to the aboral pore and longer than metamesenteries all mesenteries. Arrangement of mesenteries is M,B,m,b (Fig. 3). Mesenteric filaments of almost the same length of mesenteries. Craspedonemes only on initial part of gastrovascular cavity. Cnidome composed of spirocysts, microbasic b-mastigophores (two types), atrichous and ptychocyst.

# Ceriantheomorphe ambonensis

Fig. 6A-B

Cerianthus ambonensis Kwietniewski, 1898: 426; Pax 1910: 167; McMurrich 1910: 26–28; Carlgren 1912: 44–47.
Cerianthus sulcatus McMurrich, 1910: 28–30.
Ceriantheomorphe ambonensis – Carlgren 1931: 1.

**Material examined. (MZSP 8476):** • young individual (3.8 cm long) from Jakarta Bay, Indonesia, K. Cassiolato leg. (viii/2011) (Fig. 6A–B).

**Diagnosis.** Small cerianthid, 3.8 cm long and 2.1 cm wide. 48 marginal tentacles and 72 labial tentacles, both disposed in three cycles. Directive marginal and labial tentacles absent. Marginal tentacles arrangement: (0)1123.1121.1213.1213... Labial tentacles arrangement: (0)112.1121.1121.1121... Pharynx occupies about 18% of total body length. Hyposulcus and hemisulci absent. Gastrovascular cavity occupies about 55% of total body length. Three pairs of protomesenteries, all connected to the siphonoglyph (directive mesenteries, P2 and P3). About 96 mesenteries arranged in M,B,m,b (Fig. 7). Directive mesenteries shorter than all other mesenteries. Protomesenteries (P2) longer than all metamesenteries. Ratio of 4% between betamesenter-



**Figure 6.** Specimen of *Ceriantheomorphe ambonensis* (MZSP 8476). **A** Dissected specimen and **B** external morphology.



Figure 7. Mesenteries arrangement of *Ceriantheomorphe ambonensis*. MT Marginal tentacles LT Labial tentacles, M and m. Metamesenteries, B and b. Betamesenteries.

ies (B × b) and 2.2–3.5% between metamesenteries (M × m). Directive mesenteries, protomesenteries P2 and P3, occupy 2.3%, 85.7%, 14.2% of total gastrovascular cavity length, respectively. Cnidome (Fig. 8, Table 3) composed of spirocysts, microbasic b-mastigophores (six types), atrichous (one type), ptychocyst and holotrichous.

Distribution. Indonesia, shallow waters.

**Description of specimen.** Small individual, with 3.8 cm long and 2.1 cm wide. 48 marginal tentacles and 72 labial tentacles, both disposed in three cycles. Marginal tentacles arrangement: (0)1123.112..., labial tentacles arrangement: (0)112.112.112... Small pharynx, occupies 18% of total body length. Hyposulcus and hemisulci absent. Well-marked siphonoglyph with three pairs of mesenteries connected to it (one pair of directive mesenteries and two pairs of protomesenteries). Long protomesenteries (P2) extending to the terminal pore and longer than other mesenteries. Directive mesenteries (M and m) and longer than betamesenteries (B and b). 96 mesenteries arranged in M,B,m,b (Fig. 6). Mesenteric filaments and craspedonemes present on initial portion of the gastrovascular cavity. Gastrovascular cavity occupies approximately 55% of the



**Figure 8.** Cnidome of *Ceriantheomorphe ambonensis*. **A** Microbasic b-mastigophore type I **B** microbasic b-mastigophore type II **C** microbasic b-mastigophore type III **D** microbasic b-mastigophore type IV **E** microbasic b-mastigophore type V **F** microbasic b-mastigophore type VI **G** atrichous **H** holotrich I ptychocyst. Scale bars: 15 μm.

**Table 3.** Measurements of 30 cnida capsules for each cnida type in 6 distinct body regions of *Ceriantheomorphe ambonensis* (N = 1). Information inside parentheses indicates cnidae length and width, respectively, and information outside parentheses indicates average of cnidae size.

Body part/cnida type	Ceriantheomorphe ambonensis	
Marginal tentacles	ê	
Microbasic b-mastigophore type II	36.02 (23.16-48.89) × 6.18 (4.89-7.47)	
Microbasic b-mastigophore type IV	19.54 (14.42–24.66) × 6.18 (4.89–7.47)	
Microbasic b-mastigophore type V	18.90 (16.21–21.60) × 2.56 (2.22–2.90)	
Labial tentacles		
Microbasic b-mastigophore type I	46.84 (42.40–51.28) × 8.05 (6.46–9.65)	
Microbasic b-mastigophore type II	30.31 (26.15-34.47) × 4.58 (3.30-5.87)	
Microbasic b-mastigophore type III	27.68 (24.16–31.20) × 3.54 (2.83–4.25)	
Microbasic b-mastigophore type V	23.52 (18.13–28.92) × 2.82 (2.05–3.59)	
Pharynx		
Atrichous	40.36 (33.48–47.25) × 5.99 (4.81–7.17)	
Microbasic b-mastigophore type I	50.45 (44.63–56.28) × 7.49 (5.92–9.07)	
Microbasic b-mastigophore type II	36.49 (32.28–40.70) × 5.17 (3.58–6.76)	
Microbasic b-mastigophore type III	29.92 (24.42–35.42) × 3.59 (2.48–4.71)	
Column		
Ptychocyst	61.96 (53.31–70.62) × 21.63 (17.22–26.05)	
Atrichous	48.50 (41.69–55.32) × 11.38 (8.74–14.03)	
Microbasic b-mastigophore type I	41.45 (34.51–48.39) × 9.64 (8.74–10.54)	
Holotrichous	55.10 (47.45–62.76) × 14.97 (11.27–18.68)	
Mesenteries M		
Microbasic b-mastigophore type I	49.11 (43.91–54.31) × 9.24 (6.92–11.57)	
Microbasic b-mastigophore type IV	19.03 (16.70–21.37) × 4.99 (3.38–6.61)	
Mesenteries b		
Microbasic b-mastigophore type IV	22.34 (16.34–28.34) × 5.93 (4.10–7.76)	

entire body length. Directive mesenteries and protomesenteries P3 occupy 2.3% and 14.2% of total gastrovascular cavity length, respectively, while protomesenteries P2 occupies 85.7%. Ratio of 2.2–3.5% between metamesenteries ( $M \times m$ ) and 4% between betamesenteries ( $B \times b$ ). Cnidome (Fig. 7) composed of spirocysts, microbasic b- mastigophores (six types), atrichous (one type), ptychocyst and holotrichous.

# Ceriantheomorphe adelita Lopes, Morandini & Stampar, sp. nov.

http://zoobank.org/702BDFDD-870C-43EB-B59A-05A994177D56 Fig. 9A–B

*Ceriantheomorphe brasiliensis* Carlgren, 1931 (in part): 2–6; Carlgren and Hedgpeth 1952: 148, 169–170; Hedgpeth 1954: 286–290; Molodtsova 2009: 365–367; Stampar et al. 2010: 205–209; Spier et al. 2012: 1–3.

**Material examined (3 specimens). Holotype:** USNM 50015 • adult individual, 19 cm long and 5.4–7.3 cm wide, Port Aransas, 32 km South off Corpus Christi, Texas, United States of America, W. Close leg. 07/ix/1947 (Fig. 9B). **Paratypes:** USNM 50016 • damaged individual, Tamaulipas, Punta de Almagre to North of Hut's Bayo,



**Figure 9.** Specimens of *Ceriantheomorphe adelita* sp. nov. **A** Damaged specimen USNM 51253 from Louisiana **B** holotype specimen USNM 50015 from Corpus Christi (USA).

Pelican R/V exped. 17/iii/1949; USNM 51253 • damaged juvenile individual, 5.0– 5.9 cm wide from Pass A'Loutre (22 m depth), Louisiana, United States of America, Oh Johnny R/V exped. 25/vi/1969 (Fig. 9A).

**Diagnosis.** Large cerianthid, 19 cm long and 5.0–7.3 cm wide. 192–352 marginal tentacles (2.4–3.0 cm long in preserved animal) and 144 to 336 labial tentacles (0.5–2.0 cm long in preserved animal), both disposed in four cycles. Marginal tentacles arrangement: (0)1123.1122.1123.1122..., labial tentacles arrangement: (0)1123. 1122... Siphonoglyph well-marked by two protuberant tissues. Three pairs of protomesenteries (directive mesenteries, P2 and P3), all connected to the siphonoglyph. Well distinct hyposulcus and hemisulci absent. Protomesenteries (P3) longer than metamesenteries (m). Ratio from 2.7–5.2% between metamesenteries (M × m) and 3% between betamesenteries (B × b). Directive mesenteries, P2 and P3, extend up to 30.5%, 92.5% and 56.4% of total gastrovascular cavity length, respectively. Cnidome (Fig. 10A–I, Table 4) composed of spirocysts, microbasic b-mastigophores (five types), atrichous (two types) and ptychocyst.

**Etymology.** The specific name "*adelita*" is an allusion to an important group of women that fought during the Mexican Revolution. Occasionally, they adopted the identities of men to join in combat against the enemy.

**Distribution.** Gulf of Mexico (Northern Mexico) to North Atlantic (North Carolina, United States of America), shallow waters.

**Description of holotype.** USNM 50015, adult specimen, 19 cm long and 5.4–7.3 cm wide. 352 marginal tentacles (2.7 cm long in preserved animal) and 336 labial tentacles (2.0 cm long in preserved animal), both disposed in four cycles. Marginal tentacles arrangement: (0)1132.1122.1123.1122.1122..., labial tentacles arrangement (0)1122.1122... Directive tentacle absent. Pharynx occupies about 21% of entire body length, siphonoglyph well-marked by two lateral protuberances. Three



**Figure 10.** Cnidome of *Ceriantheomorphe adelita* sp. nov. holotype. **A** Atrichous **B** ptychocyst type I **C** holotrich **D** ptychocyst type II **E** microbasic b-mastigophore type I **F** microbasic b-mastigophore type III **G** microbasic b-mastigophore type III **H** microbasic b-mastigophore type IV **I** microbasic b-mastigophore type V. Scale bars: 15 µm.

pairs of mesenteries connected to the siphonoglyph. Gastrovascular cavity taking up to 56% of total body length. Mesenteric filaments of almost the same length of mesenteries; with craspedonemes only in the initial part of the gastrovascular cavity. Distinct hyposulcus and hemisulci absent. Fertile mesenteries, except for the directives. About 236 mesenteries arranged in M,B,m,b (Fig. 11). Directive mesenteries longer than betamesenteries (b and B) and metamesenteries (m). Protomesenteries (P2) extend to aboral pore. Protomesenteries (P3) longer than directive mesenteries, betamesenteries (B and b) and metamesenteries (m). Ratio from 2.7–5.2% between metamesenteries (M × m) and 3% between betamesenteries (B × b). Directive mesenteries and P3 extend up to 30.5%, and 56.4% of total gastrovascular cavity length, respectively, while protomesenteries (P2) occupy 92.5%. Cnidome composed of microbasic b-mastigophores (five types), atrichous (two types) and ptychocyst (Fig. 10A–I, Table 4). **Table 4.** Measurements of 30 cnida capsules for each cnida type in 6 distinct body regions of *Ceriantheomorphe adelita* sp. nov. (N = 3). Information inside parentheses indicates cnidae length and width, respectively, and information outside parentheses indicates average of cnidae size.

<i>Ceriantheomorphe adelita</i> sp. nov.
39.19 (34.20–44.18) × 5.04 (4.07–6.01)
25.12 (20.16–30.09) × 3.04 (2.09–3.99)
48.72 (39.22–58.22) × 6.71 (5.24–8.19)
36.32 (28.18–44.46) × 4.77 (3.55–6.00)
41.66 (32.23–51.09) × 5.74 (4.13–7.35)
51.43 (40.10-62.77) × 7.7 (6.25-9.15)
44.2 (35.29–53.11) × 5.13 (3.97–6.29)
36.75 (27.57–45.93) × 3.36 (2.53–4.20)
48.12 (38.88–57.37) × 9.28 (7.38–11.19)
55.42 (50.08–60.77) × 13.92 (9.49–18.35)
41.74 (47.80–35.68) × 6,0 (4.16–7.84)
64.3 (58.8–69.8) × 17.1 (15.0–19.2)
18.77 (23.27–14.27) × 4.24 (2.5–5.99)
38.76 (34.01–43.51) × 4.36 (3.53–5.20)
19.94 (15.46–24.42) × 4.79 (3.98–5.60)
23.37 (19.45–27.29) × 3.12 (2.15–4.10)



**Figure 11.** Mesenteries arrangement of *Ceriantheomorphe adelita* sp. nov. **MT** Marginal tentacles **LT** Labial tentacles **M** and **m** Metamesenteries **B** and **b** Betamesenteries.

#### Comparison between congeners

Both *Ceriantheomorphe brasiliensis* and *C. adelita* sp. nov. have labial and marginal tentacles disposed in four cycles, whereas *C. ambonensis* has its tentacles arranged in three cycles. All three species have distinct labial and marginal tentacles arrangements (Table 5). Labial and marginal directive tentacles are present in *C. brasiliensis* and absent in *C. ambonensis. Ceriantheomorphe brasiliensis* has only two pairs of mesenteries connected to the siphonoglyph (Fig. 4), while *C. adelita* sp. nov. and *C. ambonensis* have three. In *C. brasiliensis*, the directive mesenteries are longer than P3 and P5 unlike *C. adelita* sp. nov. and *C. ambonensis*. Both *C. brasiliensis* and b), while in *C. adelita* sp. nov. have directive mesenteries (B and b), while in *C. ambonensis* the opposite happens (Table 5). Protomesenteries (P3) are longer than metamesenteries (m) in *C. adelita* sp. nov. unlike *C. ambonensis* and *C. brasiliensis*. All three species have distinct proportions between metamesenteries (M × m) and betamesenteries (B × b) disposed along the gastrovascular cavity and a distinct size relation between mesenteries is directive and metamesenteries (m) (Table 5).

# Discussion

# **Taxonomic studies**

As a result of the disjunct distribution of specimens identified as *Ceriantheomorphe brasiliensis* (Mexico+US/Brazil+Uruguay) and the incomplete description of *Cerianthus ambonensis* made by Kwietniewski (1898) that later was proposed to be reassigned to the genus *Ceriantheomorphe* (Carlgren 1931), Den Hartog (1977) pointed out the need for a revision of Ceriantharia with special focus in the genus *Ceriantheomorphe*.

Based on morphological characters and biogeographic perspectives (Table 5), we were able to identify two different morphotypes among specimens assigned as *Cerian*-*theomorphe brasiliensis*. Specimens from the Gulf of Mexico were recognized as an unde-scribed species, formally described here as *Ceriantheomorphe adelita* sp. nov. Some previous studies with *C. brasiliensis* from the South Atlantic have shown that this species has short-lived planula larvae (unpublished data). This trait could prevent long dispersion due to biogeographic barriers, and thus this species may not be capable of reaching the North Atlantic. This is a different pattern from that reported for Isarachnanthus nocturnus for *Isarachnanthus nocturnus*, which is able to disperse over long distances due to the presence of long-lived planktonic cerinula larvae (Stampar et al. 2012, 2015). Nonetheless, the maintenance of *C. brasiliensis* as a single species occurring in both northern and southern hemispheres would require some biogeographic events of which we have no evidence to date. Thus, in addition to the morphology, biogeographical understanding does not support the maintenance of these two populations as a single taxonomic unit.

Carlgren and Hedgpeth (1952) argued that there were no differences between morphological characters in specimens from both areas (North and South Atlantic).

Characters	Ceriantheomorphe brasiliensis	Ceriantheomorphe adelita sp. nov.	Ceriantheomorphe ambonensis
Number of marginal tentacles	132–392	192-352	48**-150*
Number of labial tentacles	108–384	144-336	72**-150*
Tentacular cycles	4	4	3*
Arrangement of marginal tentacles	(1)1123	(?)1122	(0)112**
Arrangement of labial tentacles	(1)1122	(?)1122	(0)112**
Proportion between pharvnx in	8–27%	21%	18%**
relation to body length			
Siphonoglyph	Two pairs of mesenteries connected	Three pairs of mesenteries connected	Three pairs of mesenteries connected**
Proportion of gastrovascular cavity in relation to body length	33–72%	56%	55%**
Ratio between mesenteries	1.2–3.1% (B × b); 1.1–3.1%(m × M)	3% (B × b); 2.7–5.2% (m × M)	4% (B × b); 2.2–3.5% (M × m)**
P1 (directive mesenteries)	Longer than P3, P5, betamesenteries (B and b) and metamesenteries (m), except for m of the 2 <sup>nd</sup> and 3 <sup>nd</sup> cycles. Shorter than P2, P4 and metamesenteries (M).	Longer than betamesenteries (B and b) and metamesenteries (m). Shorter than P2, P3 and metamesenteries (M).	Shorter than mesenteries.**
P2	Longer than mesenteries	Longer than mesenteries	Longer than mesenteries
P3	Longer than P5, betamesenteries (b) and betamesenteries (B), except for B of the 1 <sup>st</sup> and 2 <sup>nd</sup> cycles. Shorter than directive mesenteries, P2, P4 and metamesenteries (M and m).	Longer than directive mesenteries, betamesenteries (B and b) and metamesenteries (m). Shorter than P2 and metamesenteries (M).	Longer than directive mesenteries and betamesenteries (B and b). Shorter than P2 and metamesenteries (M and m).
P4	Longer than directive mesenteries, P3, P5, betamesenteries (B and b) and metamesenteries (m), except for m of the 2 <sup>nd</sup> cycle. Shorter P2 and metamesenteries (M).	Absent	Absent
P5	Longer than betamesenteries (b) and betamesenteries (B), except for B from 1 <sup>st</sup> to 4 <sup>th</sup> cycles. Shorter than directive mesenteries, P2, P3, P4 and metamesenteries (M and m).	Absent	Absent
Proportion of directive mesenteries in the gastrovascular	36.6%	30.5%	2.3%**
cavity			
Proportion of protomesenteries P2 in the gastrovascular cavity	88.8%	92.5%	85.7%
Proportion of protomesenteries P3 in the gastrovascular cavity	12.2%	56.4%	14.2%
Proportion of protomesenteries P4 in the gastrovascular cavity	38.8%	Absent	Absent
Proportion of protomesenteries P5 in the gastrovascular cavity	11.1%	Absent	Absent

Table 5. Comparison of morphological characters between species of the genus Ceriantheomorphe.

\* Data from Kwietniewski (1898)

\*\* Data from personal observation.

We disagree with this assertion as *C. adelita* sp. nov. has several morphological characters that can distinguish it from other congeners. For instance, (1) marginal tentacles' arrangement, ratio between metamesenteries ( $M \times m$ ) and betamesenteries ( $B \times b$ ), as well as the proportion occupied by protomesenteries (directive mesenteries, P2 and P3) in the gastrovascular cavity contrast with those observed in other *Ceriantheomorphe* (Table 5); (2) protomesenteries P3 are found connected to the siphonoglyph while

Characters observed	Kwietniewski (1898)	This study
Specimen size	8.5 cm	3.8 cm
Number of marginal tentacles	About 150	24
Number of labial tentacles	About 150	36
Arrangement of both tentacles	3 cycles	3 cycles
Pharynx region	About 2.5 cm	0.7 cm long and 2.0 cm wide
Hyposulcus and hemisulci	No information	Absent
Gastrovascular cavity	Noinformation	2.1 cm long and 2.0 cm wide
Siphonoglyph	No information	0.7 cm long and 0.3 cm wide / 3 pairs of mesenteries
		connected.
Mesenteries	Numerous	96
Arrangement of mesenteries	No information	M,B,m,b
Cnidome	No information	Spyrocists, microbasic b-mastigophores (six types), atrichous
		(one type), ptychocyst and holotrichous.

**Table 6.** Compilation of morphological data on *Ceriantheomorphe ambonensis*.

the same is not observed in *C. brasiliensis*; (3) directive mesenteries are shorter than P3, unlike *C. brasiliensis*; (4) the number of mesenteries connected to the siphonoglyph is distinct in *C. brasiliensis* and *C. adelita* sp. nov.; (5) directive mesenteries are longer than betamesenteries (B and b), the same, however, is not observed in *C. ambonensis*; (6) directive mesenteries are longer than all metamesenteries (m), distinct from the other two species of the genus; (7) protomesenteries (P3) are longer than all betamesenteries (B), while in *C. brasiliensis* P3 are shorter than betamesenteries (B) of the 1<sup>st</sup> and 2<sup>nd</sup> cycles; (8) P3 are longer than metamesenteries (m), in contrast to those observed in *C. ambonensis* and *C. brasiliensis*; (9) protomesenteries (P4 and P5) are present in *C. brasiliensis*, while absent in the other species.

Some authors have discussed the taxonomic value of mesenteriel organization regarding the assignment and identification of species (Carlgren 1912; Arai 1965; den Hartog 1977). Spier et al. (2012) have reported that *C. brasiliensis* in southern Brazil has two pairs of mesenteries connected to siphonoglyph. In this study, *C. adelita* sp. nov. was found to have three pairs. Nevertheless, our results also showed that the two species of *Ceriantheomorphe* from the Atlantic Ocean have different numbers of mesenteries connected to siphonoglyph, reinforcing the potential taxonomic value of this character.

Our results also demonstrated that the use of ratios (division of the values) between metamesenteries (M × m) and betamesenteries (B × b) for each quartet can be useful to distinguish species of *Ceriantheomorphe*. In specimens of *C. brasiliensis*, the ratio between metamesenteries (M × m) and betamesenteries (B × b) ranged from 1.1 to 3.1% and from 1.2 to 3.1%, respectively. In comparison, the ratios observed in *C. adelita* sp. nov. are from 2.7 to 5.2% between metamesenteries and 3% between betamesenteries, while in *C. ambonensis* they range from 2.2 to 3.5% between metamesenteries and 4% between betamesenteries.

Similar to the ratio between metamesenteries  $(M \times m)$  and betamesenteries  $(B \times b)$ , the proportion of protomesenteries found in the gastrovascular cavity was also
useful to distinguish *Ceriantheomorphe* species in our study. While protomesenteries (P3) in *C. adelita* sp. nov. extend over half of the entire gastrovascular cavity length (56.4%), the ones in *C. brasiliensis* and *C. ambonensis* are much shorter (12.2% and 14.2%, respectively). Furthermore, we found differences between species while comparing protomesenteries length (Table 5). In this way, we suggest that mesenteries have a taxonomic value when used comparatively.

## Geographic distribution of the genus Ceriantheomorphe

Currently, the genus *Ceriantheomorphe* has a wide geographic distribution; one species is restricted to the warm temperate northwest Atlantic (Gulf of Mexico and United States of America), another to the warm temperate southwestern Atlantic (southeast and South of Brazil and Uruguay) and *C. ambonensis* is recorded from tropical Central Indo-Pacific, Sunda Shelf (Indonesia) (Spalding et al. 2007). However, there are no records of the genus *Ceriantheomorphe* from areas between the Atlantic and Indo-Pacific Oceans, which suggests that the genus has a disjunctive distribution, since there is no evidence of any connection between the extant populations of the valid species (Fig. 12).

Disjunctive distribution patterns are exhibited by some marine invertebrates, even those having a free-swimming phase that would benefit wide dispersal, for instance, the bivalve *Macoma balthica* Linnaeus, 1758 (Luttikhuizen et al. 2003) and the tunicate *Ciona intestinalis* Linnaeus, 1767 (Caputi et al. 2007). However, in the current case, in our opinion the disjunctive distribution of the genus *Ceriantheomorphe* is evidence of the need of further studies on the genus, especially focused on taxonomy in some under-investigated areas of the Indo-Pacific Ocean.



Figure 12. Distribution map of the genus Ceriantheomorphe.

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RESEARCH ARTICLE



# Two new species of the genus *Panorpa* (Mecoptera, Panorpidae) from eastern China and a new synonym

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## Abstract

*Panorpa* Linnaeus, 1758 is the largest genus in the scorpionfly family Panorpidae. Herein we describe two new species from eastern China, *Panorpa jinhuaensis* **sp. nov.** from Jinhua, Zhejiang Province and *Panorpa menqiuleii* **sp. nov.** from Yuexi and Huoshan, Anhui Province. *Panorpa wrightae* Cheng, 1957 from Mount Mogan, Zhejiang Province is considered to be a junior subjective synonym of *Panorpa mokansana* Cheng, 1957 from the same locality. *Panorpa mokansana* Cheng, 1957 is redescribed and illustrated in detail. A key to species of *Panorpa* from eastern China is also provided.

## Keywords

Anhui, biodiversity, Oriental region, scorpionfly, taxonomy, Zhejiang

## Introduction

Panorpidae is the largest family in the order Mecoptera (Bicha 2018; Lin et al. 2019), with ca. 500 extant species in eight genera known hitherto (Wang and Hua 2017, 2019; Gao and Hua 2019; Hu et al. 2019). They are commonly called "scorpionflies" due to their enlarged and upcurving male genitalia, which superficially resemble the stinger of scorpions (Dunford and Somma 2008; Byers 2009). The adults usually scavenge dead invertebrates, and occasionally feed on vegetative materials such as nectar and pollen grains (Palmer 2010). They often inhabit high-elevated moist forests, and

are typically observed sitting on the upper surfaces of leaves of herbs or shrubs (Byers and Thornhill 1983; Wang and Hua 2016, 2019; Bicha 2018).

The Holarctic *Panorpa* Linnaeus, 1758 is the largest genus (ca. 260 spp.) in Panorpidae, and has been regarded a paraphyletic group in several studies (Willmann 1989; Misof et al. 2000; Whiting 2002; Ma et al. 2012; Hu et al. 2015; Miao et al. 2019). This genus can be differentiated from the Oriental genera *Leptopanorpa* MacLachlan, 1875 and *Neopanorpa* van der Weele, 1909 mainly by the vein 1A ending at the same level or distal (cf. proximal) to the origin of Rs, and two (cf. one) cross-veins between 1A and 2A in forewings, with *Panorpa bashanicola* Hua, Tao & Hua, 2018 as an exception (Hua et al. 2018).

An unofficial rank, "species group", is adopted in the taxonomy of *Panorpa* by many researchers (Esben-Petersen 1921; Issiki 1933; Carpenter 1938; Cheng 1957b). Nine species groups were proposed by Issiki (1933) for the East Asian *Panorpa*. In eastern China (including Anhui, Fujian, Jiangsu, Jiangsu, Shandong, and Zhejiang Provinces, as well as Shanghai City), 21 species of *Panorpa* belonging to three groups have been documented (Wang and Hua 2017). For example, *Panorpa baohwashana* Cheng, 1957 (Jiangsu) from the *P. amurensis* group, *Panorpa kellogi* Cheng, 1957 (Fujian) from the *P. japonica* group, and *Panorpa obliqua* Carpenter, 1945 (Jiangxi) and *Panorpa implicata* Cheng, 1957 (Fujian) from the *P. wormaldi* group.

In this paper, we illustrate and describe two new species of *Panorpa* from eastern China. They resemble *P. waongkehzengi* Navás, 1935 (Jiangxi) mainly by the non-elongated cylindrical male A6–A7 (abdominal segments VI–VIII), and the twisted posterior arms in the female medigynium, but can be readily differentiated from the latter by the male genitalia. In addition, *Panorpa wrightae* Cheng, 1957 from Mount Mogan is considered to be a junior subjective synonym of *Panorpa mokansana* Cheng, 1957 from the same locality. A key to species of *Panorpa* from eastern China is also provided.

## Material and methods

Adult scorpionflies were caught with collecting nets, and preserved in 95% ethanol or pinned as permanent collections. The specimens examined are deposited in the Entomological Museum, Northwest A&F University, Yangling (**NWAU**) and the Institute of Zoology, Chinese Academy of Sciences, Beijing (**IZAS**). Specimens were observed under a Nikon SMZ 1500 Stereoscopic Zoom microscope. Measurements of right wings were made with a vernier caliper. The lengths of wings were measured from the base to the apex, and widths from the ending of  $M_4$  to the costal margin vertically. Photographs were taken with a Nikon D7000 digital camera except Figure 1B with a Nikon D7100 digital camera. All pictures were further adjusted and assembled with Adobe Photoshop CS4.

Terminology follows Byers (1989), Hua et al. (2018) and Wang and Hua (2019). The following abbreviations and acronyms are applied: A1, first abdominal segment (and so forth for other segments); T1, first tergum (and so forth for other segments); FL, forewing length; FW, forewing width; HL, hindwing length; and HW, hindwing width.

## Taxonomy

#### Panorpa jinhuaensis sp. nov.

http://zoobank.org/35AC533C-04E8-4FD1-B0C2-D43C43461695 Figures 1, 2

**Type material. Holotype**: ♂ (NWAU), CHINA: Zhejiang Province, Jinhua City [ 金华市], southern slope of Mount Jinhua [金华山], Zhizhe (Wise Man) Temple [智 者寺] (29°10'03"N, 119°37'21"E, 104 m), 2.x.2018, leg. Ji-Shen Wang; **Paratypes**: 20♂13♀ (NWAU), same data as for the holotype.

Etymology. The specific name refers to the type locality, Jinhua City.

**Diagnosis.** This new species is superficially similar to *Panorpa waongkehzengi* Navás, 1935 from Jiangxi, but can be readily differentiated from the latter by: in males, 1) apex of epandrium broadly rounded (cf. abruptly narrowed); 2) inner margin of hypovalve straight (cf. with an inner process); 3) paramere long and exceeding apex of gonocoxites (cf. short and not exceeding apex of gonocoxites); 4) apical portion of paramere spiral (cf. straight); 5) parameres crossed subbasally (cf. not crossed); and in females, 6) main plate of medigynium moderately developed (cf. poorly developed).

**Measurements.** Male FL 10.2–10.8 mm, FW 2.6–2.8 mm; HL 9.0–9.5 mm, HW 2.4–2.6 mm. Female FL 11.0–11.8 mm, FW 3.0–3.2 mm; HL 10.0–10.6 mm, HW 2.8–3.0 mm.

**Description-male.** *Head* (Fig. 2C). Vertex, occiput and frons orange-yellow, with lateral margins of occiput slightly darkened. Black spot enclosing ocellar triangle and slightly spreading anteriorly. Compound eyes black, narrower than base of rostrum. Rostrum orange-yellow, stout, sparsely covered with short setae, with its length approximately 2.6 times as long as basal width. Labrum dark yellowish brown. Maxillary palp with basal four segments dark yellowish brown and distal segment black. Scape yellowish brown with distal margin dark brown; pedicel and flagellum black; flagellomeres 34–36.

*Thorax* (Fig. 2D). Pronotum unevenly orange-yellow, with 10–12 stout setae along anterior margin. Meso- and metanotum orange-yellow and sparsely covered with short setae; scutellar arms slightly deepened. Pleura and legs orange-yellow, with distal tarsomere blackish.

*Wings* (Fig. 1D, 2A). Membrane subtranslucent, strongly tinged with yellow and fading toward apex. Markings black. Veins yellowish brown except apical crossveins pale white. Pterostigma orange-yellow and distinct. Forewing apical band broad, usually with 1–3 hyaline windows enclosing crossveins between  $R_3$  and  $M_1$ , and a separated spot at ending of  $M_2$  posteriorly; apical branch of pterostigmal band variable: intact (Fig. 1D) or detached with pterostigmal band and greatly elongated anteriorly (Fig. 2A); basal branch of pterostigmal band intact and slightly broader than apical branch; marginal spot C-shaped; basal band split into two large spots; an additional transverse band extending from ending of 2A to CuA; basal spot shifted posteriorly and along anal margin; an additional small spot anterior to 3A;  $R_2$  bifurcated. Hindwing similar to forewings but bearing relatively reduced markings: basal band split into



**Figure 1**. *Panorpa jinhuaensis* sp. nov. **A** habitat **B** female, lateral view, photo by En Lin **C** male staying on a leaf of *Humulus scandens* **D** male, lateral view **E** female, dorso-lateral view. Red arrow in **A** points to an irrigation canal with dense herbaceous groundcover, where the specimens were caught. **A**, **C–E** taken on October 2, 2018, and **B** taken on April 19, 2018 from Jinhua City.

a large spot along posterior margin, and a small indistinct spot slightly distal to ORs; spots and band proximal to basal band absent.

*Abdomen* (Fig. 2A, D, E). Terga II–V orange-yellow and slightly darkened at lateral margins, sparsely covered with black short setae; corresponding sterna lighter. Notal organ on posterior margin of T3 slightly prolonged posteriorly with truncated apex, bearing dense black setae on hind margin, and covering acute postnotal organ on anterior portion of T4. A6–A8 orange-yellow, cylindrical. A6 as long as A5 and devoid of anal horns. A7 slightly shorter and narrower than A6. A8 nearly as long as A7, slightly enlarged posteriorly with a beveled apex.

Genital bulb (Fig. 2F, G) long oval, mostly orange-yellow except distal third of gonostyli blackish. Epandrium long and broad, evenly tapering toward rounded apex bearing dense long setae. Cerci clavate, orange-yellow in basal half and black in distal half. Hypandrium with short broad stalk and a pair of longer hypovalves; each hypovalve tapering toward apex, and bearing long stout setae along inner margin. Gonocoxites stout, approximately 1.6 times as long as gonostyli; gonostyli slightly curved on outer margin, and with a rounded median tooth and a large bowl-shaped basal process on inner margin. Paramere (Fig. 2H) slender, with greatly expanded stalk basally; connected to aedeagus through curved bridge-like process dorsally; and extending slightly beyond apex of gonocoxites with spiral and acute apex. Two parameres crossed basal



Figure 2. *Panorpa jinhuaensis* sp. nov. A, C–H male B, I, J female. A, B Habitus, dorsal view C head, frontal view D dorsum, dorsal view E abdomen, lateral view F, G genital bulb, ventral and dorsal views, respectively H aedeagal complex, ventral view I subgenital plate, ventral view J medigynium, ventral view. ae, aedeagus; ax, axis; ce, cercus; dpr, dorsal process; ep, epandrium; gcx, gonocoxite; gs, gonostylus; hv, hypovalve; lpr, lateral process; mp, main plate; no, notal organ; pa, posterior arm; pm, paramere; pno, postnotal organ; sth, stalk of hypandrium; stp, stalk of paramere; vv, ventral valve.

to ventral aedeagal valves. Dorsal aedeagal processes greatly elongated posteriorly with slightly enlarged and beveled apex, and bearing a row of short setae along basal third of inner margin; lateral processes short and stout.

**Description-female.** Similar to males except relatively denser wing markings. In fore- and hindwings, pterostigmal band with apical branch intact, scattered into 1–3 small spots anteriorly (Figs 1B, 2B) or slender and extending to anterior margin, forming an H-shaped pattern (Fig. 1E).

*Female genitalia* (Fig. 2I, J). Subgenital plate oval, slightly tapering toward shallowly emarginate apex, and bearing long stout setae marginally. Medigynium with moderately developed main plate; posterior arms slightly shorter than main plate and twisted ventrally in distal half; axis longer than posterior arms, with apodemes extending beyond main plate and slightly divergent anteriorly; posterior apex of axis subtriangular and slightly extending beyond main plate.

Distribution. China, Zhejiang (Jinhua).

**Remarks.** The new species inhabits dense herbaceous ground cover aside an irrigation canal in a suburban field (Fig. 1A) with a surprisingly low elevation of 104 m (most *Panorpa* species in eastern China prefer higher mountainous regions above 600 m). The species is sympatric with another autumnal species, *Panorpa tetrazonia* Navás, 1935, which can be differentiated from the former by its larger body size (FL 12.0–13.0 mm) and brown body color. Apparently, *P. jinhuaensis* sp. nov. represents the dominant species at the locality, because only three males and two females of the latter were collected on the same day (2.x.2018). In addition, a female adult of *P. jinhuaensis* sp. nov. was photographed (Fig. 1B) in spring (19.iv.2018), likely indicating the bivoltinism of this species.

## Panorpa menqiuleii sp. nov.

http://zoobank.org/A479662E-1E4B-4BDD-9DCB-C57950F573C0 Figure 3

**Type material. Holotype**: ♂ (NWAU), CHINA: Anhui Province, Yuexi County [ 岳西县], Yaoluoping [鹞落坪], 15.viii.2013, leg. Qiu-Lei Men; **Paratypes**: 1♂3♀ (NWAU), same data as for the holotype; 2♂5♀ (IZAS), Huoshan County [霍山县], Majiahe [马家河], 800 m, 31.viii.1978, leg. Wan-Cheng Fu.

**Etymology.** The specific name is dedicated to the main collector of the type specimens, Qiu-Lei Men, for his generous help to our present research.

**Diagnosis.** The new species is superficially similar to *Panorpa waongkehzengi* Navás, 1935 from Jiangxi and *P. jinhuaensis* sp. nov. in general appearance, but can be readily differentiated from the latter two by the presence (cf. absence) of a black pattern on the occiput, and a greatly shortened (cf. long) axis in the female medigynium.

**Measurements.** Male FL 10.0–10.2 mm, FW 2.8 mm; HL 9.0–9.2 mm, HW 2.7 mm. Female FL 10.8–11.0 mm, FW 2.9 mm; HL 9.5–10.0 mm, HW 2.8 mm.

**Description-male.** *Head* (Fig. 3C). Vertex and frons yellow. Transverse black pattern on occiput extending to border of compound eyes laterally, and connected to smaller black spot enclosing ocellar triangle through a thin black line anteriorly. Rostrum yellow, sparsely covered with short black setae, with its length approximately 2.6 times as long as basal width. Labrum yellowish brown. Maxillary palp with basal four segments and basal half of distal segment yellow, and apical half of distal segment black. Scape yellow, pedicel yellowish brown, flagellomeres 32–34, mostly black but dark brown in basal two or three.

*Thorax* (Fig. 3A). Pronotum unevenly yellowish brown with two dark-brown transverse stripes, and bearing 10–12 stout setae along anterior margin. Meso- and metanotum light yellowish brown mesally, brown laterally and dark brown at anterior margin, sparsely covered with short setae; scutellar arms slightly deepened. Pleura and legs light yellowish brown, with distal tarsomere blackish.

Wings (Fig. 3A). Membrane subtranslucent, slightly tinged with whitish yellow and fading toward apex. Markings blackish brown, dentate along longitudinal veins.



**Figure 3**. *Panorpa menqiuleii* sp. nov. **A**, **C–G** male **B**, **H**, **I** female. **A**, **B** Habitus, dorsal view **C** head, frontal view **D** abdomen, lateral view **E**, **G** genital bulb, dorsal and ventral views, respectively **F** aedeagal complex, ventral view **H** subgenital plate, ventral view **I** medigynium, ventral view. **ae**, aedeagus; **ax**, axis; **ce**, cercus; **dpr**, dorsal process; **ep**, epandrium; **gcx**, gonocoxite; **gs**, gonostylus; **hv**, hypovalve; **lpr**, lateral process; **mp**, main plate; **no**, notal organ; **pa**, posterior arm; **pm**, paramere; **pno**, postnotal organ; **sth**, stalk of hypandrium; **stp**, stalk of paramere; **vv**, ventral valve.

Veins dark brown except apical crossveins pale. Pterostigma light yellow. Forewing apical band broad; pterostigmal band with apical branch detached and greatly elongated anteriorly, and connected with apical band along costal margin; basal branch bent inward; marginal spot extending from Sc to beyond R<sub>4+5</sub>; basal band complete or split into two large spots; basal spot shifted posteriorly along anal margin; R<sub>2</sub> unfurcated. Hindwing similar to forewing but with relatively reduced markings; basal spot absent.

*Abdomen* (Fig. 3A, D). Terga II–V yellow mesally and strongly darkened laterally, sparsely covered with black short setae; corresponding sterna light yellow. Notal organ on posterior margin of T3 slightly prolonged posteriorly with a rounded apex, and covering acute postnotal organ on anterior portion of T4. A6–A8 yellow, cylindrical. A6 as long as A5, without anal horns. A7 slightly shorter and narrower than A6. A8 slightly shorter and narrower than A7, slightly enlarged posteriorly toward beveled apex.

Genital bulb (Fig. 3E, G) bold oval, mostly yellow except apex of gonostyli blackish. Epandrium broad, oval with abruptly narrowed apex bearing numerous long and dense setae. Cerci clavate, yellow in basal half and black in distal half. Hypandrium with long broad stalk and a pair of shorter hypovalves; each hypovalve slightly tapering toward apex, and bearing long stout setae along inner margin. Gonocoxites stout, approximately 1.6 times as long as gonostyli; gonostyli bearing a rounded median tooth and a large subtrapezoidal basal process on inner margin. Parameres (Fig. 3F) short, slightly curved inward, with greatly expanded stalk basally; not exceeding apex of ventral aedeagal valves; and bearing a row of long spines along inner margin. Ventral aedeagal valves simple and short; dorsal processes constricted neck-like basally and greatly enlarged apically; lateral processes short and stout.

**Description-female.** Similar to males except relatively denser wing markings and darker terga (Fig. 3B).

*Female genitalia* (Fig. 3H, I). Subgenital plate long oval, with a shallow V-shaped terminal emargination, and bearing long stout setae marginally. Medigynium with poorly developed main plate; posterior arms long, twisted ventrally in distal half; axis shorter than posterior arms, with parallel apodemes extending slightly beyond main plate.

Distribution. China, Anhui (Yuexi and Huoshan Counties).

**Remarks.** Two male-unknown species, *Panorpa pusilla* Cheng, 1949 from Shaanxi and *Panorpa pieli* Cheng, 1957 from Jiangxi, are probably related to *P. menqiuleii* sp. nov. by the unbranched R<sub>2</sub> in both fore- and hindwings, and the twisted posterior arms and short axis in female medigynium. The black pattern on the occiput, however, can readily distinguish *P. menqiuleii* sp. nov. from these two species.

#### Panorpa mokansana Cheng, 1957

Figure 4

Panorpa mokansana Cheng, 1957a: 27, figs 1, 2.

Panorpa wrighti Cheng, 1957a: 28, figs 3, 4; P. wrightae nom. corr., Wang & Hua, 2017: 31. syn. nov.

Materials examined. 2♂17♀, CHINA: Zhejiang Province, Deqing County [德清县], Mount Mogan [莫干山], Weird Rock Corner [怪石角] (30°36'34" N, 119°50'58" E, 640 m), 8.x.2018, leg. Ji-Shen Wang.

**Measurements.** Male FL 10.8–12.0 mm, FW 2.9–3.2 mm; HL 9.8–10.7 mm, HW 2.8–3.0 mm. Female FL 12.9–13.5 mm, FW 3.3–3.5 mm; HL 11.9–12.5 mm, HW 3.0–3.3 mm.

**Redescription-male.** *Head* (Fig. 4A, C). Vertex yellow. Large black pattern enclosing ocelli, and extending posteriorly, forming thin black margin aside yellow occiput. Rostrum yellowish brown and deepened toward apex, with its length approximately 3.7 times as long as basal width. Maxillary palp with basal four segments yellowish



Figure 4. *Panorpa mokansana* Cheng, 1957 A, C–H Male B, I–L female. A, B Habitus, dorsal view C head, frontal view D dorsum, dorsal view E abdomen, lateral view F, G genital bulb, ventral and dorsal views, respectively H aedeagal complex, ventral view I, J A8–A11, ventral and lateral views, respectively K, L medigynium, ventral and lateral views, respectively. ae, aedeagus; ax, axis; ce, cercus; dpr, dorsal process; ep, epandrium; gcx, gonocoxite; gs, gonostylus; hv, hypovalve; lpr, lateral process; mdg, medigynium; mp, main plate; no, notal organ; pm, paramere; pno, postnotal organ; sgp, subgenital plate; sth, stalk of hypandrium; stp, stalk of paramere; vv, ventral valve.

brown and distal segment dark brown. Scape yellowish brown; pedicel dark brown; flagellum black with 39–41 flagellomeres.

*Thorax* (Fig. 4A, D). Pronotum unevenly dark brown and bearing 8–10 stout setae along anterior margin. Meso- and metanotum dark brown antero-laterally, with a broad yellow mesal stripe; scutellar arms dark brown; postnota yellow. Pleura and legs yellowish brown.

*Wings* (Fig. 4A). Membrane hyaline, slightly tinged with yellow and fading toward apex; markings black; veins yellowish brown except apical crossveins pale white; pterostigma indistinct. Forewing apical band broad, with a large hyaline window in posterior portion; pterostigmal band complete, with basal branch two times as wide as apical branch; marginal spot thick and nearly extending to anterior border of thyridium; basal band broad, with posterior half two times as wide as anterior half; basal spot large and irregular; R<sub>2</sub> bifurcated. Hindwing similar to forewing, but marginal spot reduced and not reaching C anteriorly; basal band represented by a large spot along hind margin and an indistinct small spot along anterior margin; and lacking a basal spot.

*Abdomen* (Fig. 4A, E). T2–T5 black anteriorly and reddish brown posteriorly; corresponding sterna reddish brown. Notal organ on T3 broad, very short, bearing numerous dense setae posteriorly, and covering acute postnotal organ on T4. A6–A8 reddish brown. A6 with irregular black pattern on lateral surface, approximately two times as long as A5, subcylindrical, slightly tapering from middle toward abruptly beveled apex. A7 with sooty black texture on lateral surface, greatly constricted stalk-like basally, and greatly enlarged towards truncated apex. A8 similar to A7 but less constricted basally, and rounded apically.

Genital bulb (Fig. 4F, G) reddish brown, oval. Epandrium long and broad, with wide V-shaped emargination terminally and forming a pair of stout processes laterally. Cerci long clavate, yellowish brown with slightly deepened apex. Hypandrium with greatly reduced stalk and a pair of slender hypovalves extending to middle of gonocoxites, and each bearing a row of long setae on inner margin of apical half. Gonocoxites stout, bearing a few long setae on ventral apex. Gonostyli longer than half length of gonocoxites, with prominent middle tooth and stout basal process on inner margin. Paramere (Fig. 4H) bifurcated: ventral branch short and stout, curved mesally; dorsal branch long and slender; both branches bearing numerous long spines along posterior margin. Ventral aedeagal valves membranous and inconspicuous; dorsal process broad basally, slender and curved divergently at distal portion; lateral process stout and inconspicuous.

**Description-female.** Similar to males but darker in body color and denser in wing markings (Fig. 4B). T2–T5 dark brown but reddish brown on hind margins; T6–T10 reddish brown. T9 elongated, nearly 1.5 times as long as and wider than T8, with its lateral margins greatly curled ventrad and enclosing lateral margin of subgenital plate (Fig. 4I, J).

*Female genitalia*. Subgenital plate (Fig. 4I, J) long oval with narrow base, broadest at distal fourth, tapering into subtriangular and indistinctly emarginate apex. Medigynium (Fig. 4K, L) with well-developed main plate; a pair of posterior arms slender and parallel, shorter than main plate, and slightly bending dorsad at distal half; axis approximately as long as main plate, not exceeding main plate posteriorly but slightly exceeding the latter anteriorly; apodemes greatly divergent at shortly bifurcated apexes, with anterior portion covered by main plate ventrally.

Distribution. China, Zhejiang: Deqing County (Mount Mogan).

**Remarks.** Panorpa mokansana Cheng, 1957 and Panorpa wrightae Cheng, 1957 were described from the same locality, Mount Mogan, based on a single male (19. ix.1927) and a single female (28.ix.1927), respectively (Cheng 1957a). The holotypes of these two nominal species are deposited in the Collection of California Academy of Sciences, San Francisco, California. According to Cheng's descriptions, the female of *P. wrightae* resembles the male of *P. mokansana* in wing markings but only differs from the latter in the body color (dull brown vs. mostly reddish brown). During our

recent expedition in Zhejiang Province, a number of new materials were collected from the type locality. The males have been readily determined to be *P. mokansana*, and the females conform to Cheng's description and illustration of *P. wrightae*. Because females are essential for an insect species, it is reasonable for us to consider that *P. mokansana* and *P. wrightae* are very likely conspecific. Therefore, *P. wrightae* is treated as a junior subjective synonym of *P. mokansana* herein.

# Key to males of species of Panorpa from eastern China

(Three species are not included because the males are unknown: *P. klapperichi* Tjeder, 1950, *P. implicata* Cheng, 1957 and *P. pieli* Cheng, 1957)

A7 and A8 cylindrical, not constricted basally2
A7 and A8 constricted basally and enlarged toward apex7
A6-A8 much longer than preceding segments; gonostyli approximately as
long as gonocoxites
A6–A8 shorter than or as long as preceding segments; gonostyli much shorter
than gonocoxites
Gonostyli bearing three small protuberances on apical half of inner margin;
basal stalk of hypandrium three times as long as hypovalves (Fujian)
Panorpa kellogi Cheng, 1957
Gonostyli lacking protuberances on inner margin; hypandrium with extreme-
ly reduced basal stalk and split into a pair of hypovalves basally (Jiangsu)
Panorpa baohwashana Cheng, 1957
Wing membrane hyaline; dorsum of body dark brown; paramere bifurcated
(Jiangxi) Panorpa obliqua Carpenter, 1945
Wing membrane tinged with yellow; dorsum of body yellow to yellowish
brown; paramere simple
R, in both fore- and hindwings bifurcated; apex of epandrium broad and
rounded (Zhejiang) Panorpa jinhuaensis sp. nov.
R <sub>2</sub> in both fore- and hindwings simple; apex of epandrium abruptly nar-
rowed6
Occiput yellowish brown; each hypovalve with a small rounded process on
basal portion of inner margin (Jiangxi) Panorpa waongkehzengi Navás, 1935
Occiput with a black pattern; hypovalve straight on inner margin (Anhui)
T6 with an anal horn at apex8
T6 lacking an anal horn9
A7 stalk-like at base and abruptly enlarged toward apex; paramere bifurcated
(Zhejiang) Panorpa anfracta Ju & Zhou, 2003
A7 evenly enlarged toward apex; paramere simple (Zhejiang, Fujian)
Panorpa kiautai Zhou & Wu in Zhou et al., 1993

9	Pterostigmal band in both fore- and hindwings lacking an apical branch 10
_	Pterostigmal band in both fore- and hindwings with an apical branch13
10	Gonostyli with a large concavity on basal half of ventral surface (Jiangxi)
	Panorpa cladocerca Navás, 1935
_	Gonostyli lacking a concavity on ventral surface
11	Gonocoxites with dense stout setae on inner margin (Fujian, Jiangxi)
	Panorpa trifasciata Cheng, 1957
_	Gonocoxites lacking dense stout setae on inner margin
12	Median tooth of gonostyli acute; apex of paramere bulbous (Zhejiang)
	Panorpa cheni Cheng, 1957
_	Median tooth of gonostyli rounded; paramere slender, sword-shaped (Zheji-
	ang) Panorpa choui Zhou & Wu in Zhou et al., 1993
13	Paramere simple
_	Paramere bifurcated
14	Paramere greatly elongated and extending beyond middle of gonostyli (Fu-
	jian)Panorpa flavicorporis Cheng, 1957
_	Paramere short and not exceeding apex of gonocoxites (Fujian)
	Panorpa fukiensis Tjeder, 1950
15	Wing membrane strongly tinged with yellow; two branches of paramere ap-
	proximately equal in length
_	Wing membrane hyaline or slightly tinged with yellow; two branches of para-
	mere distinctly unequal in length
16	Genital bulb long oval; paramere slender, extending beyond apex of gonocox-
	ites (Fujian, Jiangxi, Zhejiang)
_	Genital bulb broad oval; paramere short, not exceeding apex of gonocoxites
	(Anhui, Zhejiang)
17	Paramere with ventral branch two-thirds as long as dorsal branch (Jiangxi)
_	Paramere with ventral branch shorter than half length of dorsal branch 18
18	Aedeagus with dorsal valves finger-like and parallel (Jiangxi, Zheijang)
	Panorba tetrazonia Navás, 1935
_	Aedeagus with dorsal valves slender and greatly diverged apically (Zheijang)
	Panorpa mokansana Cheng, 1957
	1 8, 12

# Discussion

By adding two new species and synonymizing one species, the species number of *Panorpa* from eastern China is updated to 22.

Evidently, *Panorpa jinhuaensis* sp. nov., *Panorpa menqiuleii* sp. nov. and *Panorpa waongkehzengi* Navás, 1935 are more or less related to the northeastern Asiatic *Panorpa amurensis*, *Panorpa japonica* and *Panorpa kongosana* groups by the following characters: in males, 1) cylindrical A6–A8; 2) long stalk of hypandrium; 3) greatly expanded stalk of paramere; and in females, 4) twisted posterior arms and weakly or moderately de-

veloped main plate in medigynium. In addition, basally crossed male parameres occur only in a small number of species in *Panorpa* (all six species in the *P. amurensis* group, ca. nine species out of eleven in the *P. japonica* group, all three species in the *P. kongosana* group, and *P. jinhuaensis* sp. nov.), likely suggesting their close affinities.

Bivoltinism is frequently reported in some species of *Panorpa*. For example, *Panorpa liui* Hua, 1997 from the *Panorpa amurensis* group (Jiang and Hua 2013), *Panorpa japonica* Thunberg, 1784 from the *Panorpa japonica* group (Ogai 1999), *Panorpa qinlingensis* Chou & Ran in Chou et al., 1981 from central China (Cai and Hua 2009), *Panorpa communis* Linnaeus, 1758, *Panorpa nigrirostris* MacLachlan, 1882 and *Panorpa vulgaris* Imhoff & Labram, 1845 from the *P. communis* group (Sauer et al. 2003; Vermeulen et al. 2009; Dvořák and Ghahari 2016), and presumably, *Panorpa nuptialis* Gerstaecker, 1863 from North America (Byers 1963).

*Panorpa jinhuaensis* sp. nov. is likely a bivoltine insect species. The spring generation (Fig. 1B) was observed to fly in late April, and the summer generation (Fig. 1C–E) in early October. Most species of *Panorpa* prefer cool habitats, and often inhabit highelevated mountainous regions in the subtropical zone, especially in southern and eastern China (Wang and Hua 2016, 2017; Hua et al. 2018). In the low elevated habitat (ca. 100 m a.s.l) of *P. jinhuaensis* sp. nov., however, bivoltinism may give the insect an advantage to avoid the hot summer from June to August (29–33 °C in Jinhua City), and thus enables them to breed in the lowlands in the cooler spring and autumn months. Further investigations are needed to reveal its life history.

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