

A sea anemone of many names: a review of the taxonomy and distribution of the invasive actiniarian *Diadumene lineata* (Diadumenidae), with records of its reappearance on the Texas coast

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Abstract

Diadumene lineata (Actiniaria: Diadumenidae) is a prolific invader of coastal environments around the world. First described from Asia, this sea anemone has only been reported once from the western Gulf of Mexico at Port Aransas, Texas. No subsequent sampling has located this species at this locality. The first record of the reappearance of *D. lineata* on the Texas coast from three locations in the Galveston Bay area is provided, and its geographic distribution and taxonomic history reviewed.

Keywords

Galveston Island, Gulf of Mexico, invasive species, salt marsh, Texas coast

Introduction

Diadumene lineata (Verrill, 1869) is perhaps the most widespread actiniarian in the world (Seaton 1985, Fautin et al. 2009a). Believed to be native to Japan or Hong Kong (Verrill 1869, Uchida 1932) where it was first described by the American naturalist A.E. Verrill, it has since been reported from almost every coast. The sea anemone

was discovered in North America at Woods Hole, Massachusetts in 1892 by Verrill's daughter, Lucy. He wrote in his description, "My attention was first called to this species... by my young daughter, Miss Lucy L. Verrill, for whom I have named it" (Verrill 1898). Not realizing that the anemone was the same species he had described from Hong Kong (Sagartia lineata), he named it Sagartia luciae. Parker (1902) extended the known range of *D. lineata* in New England to Rhode Island, and by 1929 it had been collected in Cape Charles, Virginia (Richards 1931). Gosner (1971), in his key to invertebrates from Cape Hattarus to the Bay of Fundy, reported this animal occurred as far south as North Carolina. Calder and Hester (1978) included D. lineata (as H. *luciae*) in a checklist of actiniarians from South Carolina, describing them as "common to abundant." On the Pacific coast, Torrey (1904) described Sagartia davisi from San Pedro, California, now considered a synonym for D. lineata. The species was included in a faunal checklist of California as early as Johnson and Snook (1927), who wrote "Sagartia luciae... has been reported from San Francisco. We have found this similar form to be very common at certain points in San Diego Bay and Mission Bay." Cutress (1949) reported this sea anemone occurred on the Oregon coast and Morris et al. (1980) extended the range to Washington, expanding its occurrence to the entire US Pacific coast. Hand (1964) reported its first occurrence in Britain as 1896; however, if Chrysoela chrysosplenium was originally composed of both itself and D. lineata as suggested by Fautin (2015), it was found in Cornwall as early as 1847. Cornelius et al. (1995) included the species as *H. lineata* in their guide to European marine fauna, and wrote that it occurred on, "all British coasts... widely distributed through Europe and the rest of the world" (Cornelius et al. 1995). Ocaña and Den Hartog (2002) included D. lineata from the Canary Islands off the coast of North Africa, and Zabin et al. (2004) reported the first occurrence of the anemone on the Hawaiian Islands. It was first recorded from India by Parulekar (1968) in a list of Actiniaria of Bombay. Fautin et al. (2009b) added *D. lineata* in their checklist of actiniarians known to be common on the coast of Singapore, and Cha and Song (2001) report the species occurred on Jeju Island of South Korea by 1985. Liu et al. (2003), in a review of the diversity of sea anemones of Lianyun Harbor in the Jiangsu Province of China, included Haliplanella luciae in their species list. Along the southwest Atlantic coast of South America, the anemone was first reported from Rio de Janeiro, Brazil by Belem and Monteiro (1977), and found at the busy Port of Recife by Farrapeira et al. (2007) as part of a study of fouling organisms on ship hulls. In the Argentine Sea, D. lineata was first reported by Excoffon et al. (2004). Diadumene lineata was not reported from the South Pacific coast until 2015, when it was found at Coquimbo, in northern Chile (Häussermann et al. 2015).

There exist only two reports of *D. lineata* in the Gulf of Mexico, most recently by Minasian and Marsical (1979) from northwestern Florida. The earlier record is from Port Aransas, Texas at the University of Texas Marine Research Station (Carlgren and Hedgpeth 1952). Hedgpeth (1954) included *D. lineata* (as *Aiptasiomorpha luciae*) in the checklist of cnidarians of the Gulf of Mexico, citing Carlgren and Hedgpeth (1952) as the only known record from west of Florida. However, *D. lineata* has not been found



Figure 1. *Diadumene lineata* at Sportsman Road on Galveston Island with tentacles fully retracted (**a**), and with tentacles extended (**b**).

at Port Aransas since (Wicksten, pers. obs.). Additionally, to our knowledge no other reports of *D. lineata* exist in the Gulf west of northern Florida.

The small (5–10 mm in diameter), often inconspicuous sea anemone is dark green or brown with orange, yellow, white, or green vertical stripes (Ruppert and Fox 1988) and resembles a gelatinous peppermint (Figure 1). *Diadumene lineata* occurs in dense numbers on rock jetties, pilings, oyster reefs, and in salt marshes where it has been reported to associate with *Spartina alterniflora* (Molina et al. 2009). *Diadumene lineata*'s incredible potential for invasion has been attributed to asexual reproduction via longitudinal fission or pedal laceration (Ting and Geller 2000) and the tendency for larvae to adhere to boat hulls (Gollasch and Riemann-Zürneck 1996). Johnson and Snook (1927) note that when submerged in a bucket of seawater, "[the sea anemone] will move to the bottom or sides... and begin dividing without delay." Additionally, *D. lineata* can survive and reproduce in a broad range of conditions. The species can tolerate salinities ranging from over 35 ppt down to 5 ppt, or even lower at cold temperatures, and can withstand temperatures from 0 °C to at least 27.5 °C (Shick 1976). It can also withstand moderate levels of hypoxia (Jewett et al. 2005). The ability to survive in such diverse and sometimes harsh conditions has contributed to *D. lineata*'s ability to spread and survive.

Materials and methods

To date, *D. lineata* has been discovered at three separate locations in the Galveston Bay area (Figure 2). *D. lineata* was first discovered on Galveston Island in November 2016 on a jetty at East Beach (29°19.9'N, 94°43.5'W) (Sutherland, personal communication). Another population was discovered on a rock jetty that supports a *Crassostrea virginica* Gmelin reef on Bolivar Peninsula on March 14, 2017 (29°22.2'N, 94°45.0'W). Five individuals were gently scraped from the oyster shells and preserved in 70% ethanol. A third population was discovered on a bed of oyster shells near a salt marsh off Sportsman Road on Galveston Island on July 7, 2017 (29°15.2'N, 94°55.1'W) (Figure 3).



Figure 2. Map showing the Texas Coast with Port Aransas and Galveston marked. Inset shows the three locations around Galveston Bay where *D. lineata* populations were found: East Beach (29°19.9'N; 94°43.5'W), Bolivar jetty (29°22.2'N; 94°45.0'W), and Sportsman Road (29°15.2'N; 94°55.1'W).

Individuals from the Sportsman Road population were collected and photographed with a Moticam 10 microscope camera, and subsequently stored in 70% ethanol. These individuals were small, less than 5 mm in diameter, and were located by removing several oysters and bringing them back to the laboratory for observation under a dissecting microscope. Additional specimens have been collected and stored in formalin.

As the anemones were dark green with the characteristic orange or white stripes, they were readily diagnosed as *D. lineata*. This is one of the few species of anemones that is unmistakable by color pattern (Fautin et al. 2009a).



Figure 3. A view of the Sportsman Road site. The circled area indicates where anemones were sampled from in this study; anemones have also previously been located on the jetty in the background (Wicksten, pers. obs.).

Taxonomic review of Diadumene lineata

Hand (1964) wrote concerning *D. lineata*, "This common and well known species has a very discouraging synonymy..." A list of all synonymized names from the World Register of Marine Species (WoRMS) includes various names (Fautin 2015; Table 1). The taxonomic history of *D. lineata* has been plagued with confusion since the beginning.

Verrill (1871) described *Sagartia lineata*, a species he stated to be "common on stones and pebbles among gravel" that had been collected by Dr. William Stimpson on the Hong Kong harbor. McMurrich (1887) soon after described *S. pustulata* from Beaumont, North Carolina, which is included as a synonym for both *D. lineata* and *Actinothoe pustulata* (Carlgren 1949; Hand 1955; Fautin 2015). Later, Verrill (1898), unsuspecting that *S. lineata* could occur in both Hong Kong and Woods Hole, erected *Sagartia luciae*. As stated in the Introduction, Torrey (1904) described what he thought a new species of *Sagartia, S. davisi*, from California. Stephenson (1925) suggested reorganizing *S. luciae* to the genus *Diadumene* after McMurrich (1921) presented anatomical evidence suggesting the species did not belong in the genus *Sagartia* (for example, McMurrich notes six pairs of mesentery and the lack of a sphincter).

McMurrich (1921) considered *S. luciae* to be synonymous with *S. chrysosplenium* (Cocks, 1847). Johnston (1847), citing an unpublished description by W.P. Cocks, described a small anemone that was "bright pea-green to dark holly-leaf tint, stripped or dotted with bright yellow" that occurred in St. Ives, Cornwall. Cocks himself would later include the anemone, which he called *Actinia chrysosplenium*, in two later works from Falmouth (Cocks 1850, 1851). Goess (1855) moved the anemone to the newly

Taxonomic identification	Taxonomic identification Locality	
Actinia chrysosplenium	St. Ives, Cornwall	Johnston 1847
Actinea chrysoplinum*	Falmouth, Cornwall	Cocks 1850
Actinea chrysosplenum*	Falmouth, Cornwall	Cocks 1851
Bunodes chrysosplenium	Britain	Gosse 1855
Sagartia chrysosplenium	Cornwall	Gosse 1860
Chrysoela chrysosplenium	Cornwall	Gosse 1860
Sagartia lineata	Hong Kong	Verrill 1869
Sagartia chrysosplenium*	Britain	Pennington 1885
Sagartia pustulata	North Carolina	McMurrich 1887
Sagartia luciae	Woods Hole, Massachusetts	Verrill 1898
Sagartia davisi	San Pedro, California	Torrey 1904
Diadumene luciae	Britain	Stephenson 1925
Aiptasiomorpha luciae	Oregon	Cutress 1949
Haliplanella luciae	California	Hand 1955
Haliplanela luciae*	France	Dominique et al. 1985
Diadumene lineata	Wells-next-the-sea, Norfolk	Williams 1980
Haliplanella lucia*	Korean Strait	Song 1992
Haliphlanella luciae*	N/A	Hand and Uhlinger 1994
Haliplanella lineata	Europe	Cornelius et al. 1995
Haliplannella luciae*	N/A Grosholz and Ruiz 1996	
Haliplanella liciae*	China	Pei 1998

Table 1. Synonyms, misidentifications, and their localities for D. lineata (after Fautin 2015).

*Indicates misspellings, not unique taxonomic identifications



Figure 4. Synonymy flowchart, including misidentifications (i.e., *Chrysoela chrysosplenium* and *Acti-nothoe pustulata*). Synonyms based on misspellings have been omitted.

erected genus *Bunodes* as he at the time believed the surface was "warty" after examining plates that appeared in Johnston (1847). However, Gosse (1860), quoting a correspondence with W.P. Cocks, stated, "...when I examined the body of *chrysosplenium* with a lens of two inches' focus... in appearance resembled a piece of smooth Indianrubber... not the slightest trace of tubercles apparent," leading Gosse to reclassify the anemone to the genus *Sagartia*. Even in this assignment Gosse was uncertain, and suggested placing the species in a new genus, which he called *Chrysoela*, meaning "that which is studded with golden nails" (Gosse 1860).

While McMurrich (1921) considered *S. luciae* and *S. chrysosplenium* (=*Chrysoela chrysosplenium*) to be synonymous, Stephenson (1925) argued that the two species were distinct, finally fully separating *S.lineata-luciae* from *C. chrysosplenium*, a separation that exists to this day. Hand (1955), in a review of the anemone's synonymy, concluded that the relationship between *D. lineata* and *C. chrysosplenium* could not be determined as an actiniarian matching Cocks' original description had not been sampled. Fautin (2015) includes *C. chrysosplenium* both as a synonym for *D. lineata* and as a separate species.

McMurrich (1921) offered the first suggestion that *S. lineata* and *S. luciae* were the same species. Calgren (1949) listed *D. lineata* as *Aiptasiomorpha luciae*, which would be adopted by Cutress (1949) in the description of anemones on the Oregon coast and Carlgren and Hedgpeth (1952) in the record of *D. lineata* at Port Aransas. Hand (1955) combined *Aiptasiomorpha* and *Diadumene*, arguing that the only distinguishing feature was the presence of catch tentacles in the latter, but not all individuals of the Diadumenidae possessed them. She further erected the family Haliplanellidae with a single genus *Haliplanella* on the basis of a novel combination of nematocysts in the acontia of basitrichs, microbasic p-mastigophores, and microbasic amastigophores, and removed *D. luciae* to this new genus. However, the genus *Haliplanella* had previously been established by Treadwell (1943) for a group of polychaetes, thus rendering it invalid. The invalid name *Haliplanella luciae* still appears in many modern texts (Calder and Hester 1978; Morris et al. 1980; Ruppert and Fox 1988; Ruppert et al. 2004).

Hand (1989) conceded the anemone to the genus established by Stephenson (1925), and recognizing Verrill's original description the name *Diadumene lineata* was established. Despite this, Fautin and Hand (1977) and Fautin et al. (2009a) petitioned the International Commission of Zoological Nomenclature (ICZN) to preserve the genus *Haliplanella* by suppression of Treadwell (1943), and were rendered an affirmative opinion by the commission (ICZN 2013). Fautin et al. (2009a) acknowledged the wide acceptance of Verrill's original description of *S. luciae* to be a synonym for *S. lineata* (Seaton 1985), and proposed the proper name to instead be *Haliplanella lineata*. Given the commission's opinion that the genus name *Haliplanella* be accepted, writing that it is, "conserved for a widespread sea anemone," (i.e. *D. lineata*), confusion as to the generic position of *D. lineata* remains. Several recent publications mention the name *Haliplanella lineata* (e.g. Goulletquer et al. 2002; Molnar et al. 2008), but at present, the name *Diadumene lineata* is considered valid (Cairns et al, 2002; Fautin 2015). A flowchart is presented here depicting taxonomic reorganizations of *D. lineata*, including all known synonyms and misidentifications, excluding misspellings (Figure 4).



Figure 5. A map showing name usage by location for the synonyms of *D. lineata*, not including misspelled names. Inset shows Britain and the northern coast of Europe where there is a very high density of references (adapted from Fautin 2015).

A map of *D. lineata*'s range indicates the first occurrences at each respective location (Figure 5). We have adopted the binomen *Diadumene lineata* here on the basis that the most recent studies of the sea anemone have used this name (Ting and Geller 2000; Cairns et al. 2002; Molina et al. 2009; Podbielski et al. 2016). The shifts in usage of *D. lineata* synonyms in publications through time are presented in a histogram (Figure 6).

To our knowledge, no comprehensive molecular study exists suggesting that *D. lineata* is a species complex instead of a single, worldwide species. However, *D. lineata* populations do harbor greater genetic diversity than had been previously thought (Ting and Geller 2000).



Figure 6. Histogram showing the shift in name usage over time. In the time period 2000-2011, *Diad-umene lineata* showed the greatest increase in usage. Data compiled from Fautin (2015).

Associated species

Organisms found in association with *D. lineata* were those typical of Texas coast oyster reefs, including corophiid amphipods, an array of polychaete worms, nemerteans, cheilostomate bryozoans, xanthid crabs, the anomurans *Clibanarius vittatus* Dana and *Petrolisthes armatus* Gibbes, and the solitary ascidian, *Molgula* sp.

Discussion

While D. lineata has been reported as cosmopolitan (Cornelius et al. 1995, Fautin and Daly 2009), its occurrence in the western Gulf of Mexico has been uncertain since Carlgren and Hedgpeth (1952). All references to D. lineata as being present in the western Gulf rely on this single citation (Fautin and Daly 2009). While we do not dispute that the anemone appeared in Port Aransas in 1952, it is likely to have not become established, suffering local extinction. This is not uncommon for D. lineata invasions (Ruppert and Fox 1988). As this anemone is small, the possibility exists that it has been overlooked; however, we consider this unlikely as regular sampling trips to Port Aransas have been carried out since 1990 as part of the Texas A&M University Marine Biology field trip, which includes transects of various rock jetties, and have not located it. After Carlgren and Hedgpeth (1952) there is no record of any search for the anemone in Port Aransas until the 1980s (Wicksten, personal observation). Therefore, it is impossible to know when in that time period the anemone disappeared. Without this information we are unable to establish an exact cause of extinction. Possible causes include unusually high summer temperatures in one or more years heating the shallow waters the anemones inhabit above their survival range, or an inability to establish a stable population due to the relatively small amount of foreign shipping at Port Aransas introducing few individuals.

Therefore, based on the assumed disappearance of the Port Aransas population, the current report represents a reappearance of *D. lineata* on the Texas coast, and the first established population in the western Gulf of Mexico to the best of our knowledge. Other locations, including Christmas Bay (29°53.4'N, 95°6.9'W) and the Fin and Feather Reef on Redfish Bay (27°53.4'N, 97°6.9'W), have been examined in the past, but no sea anemones were found (Wicksten, personal observation). Based on the three separate observations of the Galveston Bay *D. lineata* populations, which occurred across a range of locations and dates, we are confident that *D. lineata* is well established in the bay. Salt marshes near the Sportsman Road jetty were examined for any association between *D. lineata* and *S. alterniflora* as reported by Molina et al. (2009), but no sea anemones were found. This could reflect that the association between *D. lineata* and *S. alterniflora* is limited to the Bahia Blanca estuary, or simply the difficulty of locating such tiny animals in the dense muddy marsh without the use of cores and laboratory observation.

It is unsurprising that *D. lineata* became established in Galveston Bay of all locations in the western Gulf. An enormous amount of cargo passes through Galveston Bay every year, as passage through the Bay is required to reach not only the Port of Galveston, but also the much larger ports of Houston and Texas City. In 2015 the American Association of Port Authorities ranked the ports of Houston and Texas City 1st and 15th respectively in terms of the total tons of annual foreign imports (American Association of Port Authorities 2015). The port of Galveston itself includes regular shipping lines for vessels that dock in areas known to have been invaded by *D. lineata*. For example, American Roll-On Roll-Off Carrier (ARC) includes a trade route that connects Galveston to Southampton, roughly 150 km from Cornwall where the species has been located previously. Höegh Autoliners, another Galveston port regular, ports extensively through Southeast Asia where *D. lineata* is believed to originate. Past studies have found this species adhering to boat hulls and suggested this as a likely explanation for its worldwide invasion (e.g., Gollasch and Riemann-Zürneck 1996; Farrapeira et al. 2007).

The generic position of *D. lineata* still appears in dispute, as the taxonomic review above indicated, while the specific name *D. lineata* seems accepted due to precedent (Fautin et al. 2009a; Fautin 2015). The 2013 ICZN decision to suppress Treadwell (1943) does not appear to resolve the generic position; however, the tide seems to have shifted in favor of *Diadumene* as the proper genus (Ting and Geller 2000; Molina et al. 2009; Fautin 2013; Podbielski et al. 2016).

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SHORT COMMUNICATION



Aroui minusetosus, a new species of Scopelocheiridae from Korea (Crustacea, Amphipoda, Lysianassoidea)

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Abstract

A new species, *Aroui minusetosus* **sp. n.**, is recorded from Korean waters with detailed description and illustrations. A new key to all known *Aroui* is provided. The Korean material of this scopelocheirid is readily assigned to the genus *Aroui* by the presence of long and distally barbed setae on the outer plate of maxilla 2. This new species is distinguished from congeners by ventrally smooth coxae 1-3 and a setose posteroventral margin of coxa 4, the subchelate gnathopod 2 having a row of four robust setae on its posterior margin (including an elongate single locking seta), and the setation of all appendages which is less dense than in other species. This is the first record of scopelocheirid amphipods from Korean waters.

Keywords

Aroui minusetosus sp. n., amphipod, Korea, lysianassoid, Scopelocheiridae, taxonomy

Introduction

The family Scopelocheiridae was established by Lowry and Stoddart (1997) based on the synapomorphy of a strongly shortened dactylus of gnathopod 1 which is covered with various distal setae from the propodus. Simultaneously, they recognized two subgroups in this family according to the differences of the mandibular molar shape: the *Scopelocheirus* group, which has a columnar molar bearing a small triturative surface and the *Paracallisoma* group, in which the molar is a non-setose triangular flap or is absent (Lowry and Stoddart 1997). Kilgallen and Lowry (2015) reviewed the worldwide species of

Scopelocheiridae and the two subgroups mentioned by Lowry and Stoddart (1997) were ranked as subfamily level: Scopelocheirinae and Paracallisominae. The Scopelocheirinae is a small group including only eight species of three genera (Kilgallen and Lowry 2015). The genus *Aroui* Chevreux, 1911 included in the Scopelocheirinae is characterized from other Scopelocheiridae genera by bearing unusually long and distally barbed setae on the outer plate of maxilla 2. There are only four valid species worldwide: *Aroui americana* Lowry & Stoddart, 1997 from the Gulf of Mexico and Argentina; *Aroui hamatopodus* Lowry & Stoddart, 1989 from Australia; *Aroui onagawae* (Takekawa & Ishimaru, 2000) from Japan; and *Aroui setosus* Chevreux, 1911 from the Mediterranean Sea (Chevreux 1911, Lowry and Stoddart 1997, 1989, Takekawa and Ishimaru 2000, Kilgallen and Lowry 2015). However, hitherto the Scopelocheiridae has not been recorded from Korean waters. In this study, a new scopelocheirid lysianassoid, *Aroui minusetosus* sp. n., is reported with detailed description and illustrations, and a key to all known species of the genus *Aroui* is provided.

Materials and methods

The collected specimens of lysianassids were initially fixed in 80% ethyl alcohol in the field and then preserved in 95% ethyl alcohol after sorting in the laboratory. Specimens were stained with lignin pink before dissection. Their appendages were dissected in petri dishes or excavated microscopic slides filled with mixed solution of glycerolethanol using dissecting forceps and needles under a stereomicroscope (Leica M205), and mounted onto temporary slides using glycerol. To prepare illustrations, pencil drawings were made under a light microscope (Leica DMLB) with the aid of a drawing tube. These were then scanned, digitally inked, and arranged on digital plates using the methods described by Coleman (2003, 2009). Definition of the term for 'seta' and its types follows that of Watling (1989). Examined specimens were deposited in the collection of the National Institute of Biological Resources (**NIBR**) of Korea.

Systematic account

Order Amphipoda Latreille, 1816 Superfamily Lysianassoidea Dana, 1849

Family Scopelocheiridae Lowry & Stoddart, 1997

Korean Name: Teol-son-gin-pal-yeop-sae-u-gwa, new

Subfamily Scopelocheirinae Kilgallen & Lowry, 2015

Korean Name: Teol-son-gin-pal-yeop-sae-u-a-gwa, new

Genus Aroui Chevreux, 1911

Korean Name: Teol-son-gin-pal-yeop-sae-u-sok, new

Aroui minusetosus sp. n. http://zoobank.org/0DE2B629-ED1F-46EB-883C-EFDF8196AC78 Figs 1–4 Korean Name: Teol-son-gin-pal-yeop-sae-u, new

Type locality. Somaemul Island (34°37.656'N, 128°32.467'E, depth 52 m), Gyeong-sangnam-do, South Korea.

Material examined. Holotype: adult male, 4.3 mm, NIBRIV0000806536. Paratype: one male, 3.0 mm, NIBRIV0000807161; all dissected appendages and remain bodies of type specimens were preserved in 95% ethanol; collected from the type locality at 12 May 2012, by grab sampling. These specimens were provided by Prof. H.-Y. Soh.

Etymology. The composite epithet of the specific name of *minusetosus* is a combination of the Latin *minus* and *setosus*, referring to having less setose appendages.

Diagnosis. Head eyes ovoid, ommatidia large. Antennae calceoli absent. Mandible with columnar molar process, elevated, triturative surface weakly developed. Maxilla 1 inner plate with plumose setae along medial margin and apex; outer plate with toothed setae apically in 7/4 arrangement; palp article 3 swollen distally, with dentate setae apically. Maxilla 2 inner plate longer than outer plate; outer plate with marginal and submarginal rows composed of barbed and simple setae apically (all setae extremely elongate). Coxae 1-3 not densely setose ventrally; coxa 4 setose posteroventrally. Gnathopod 1 scopelocheirin form; coxa 1 subtriangular; propodus slightly longer than carpus, with rows of long setae forming tuft distally, palm absent; dactylus extremely reduced, anchored at posterodistal corner. Gnathopod 2 propodus subrectangular, with four robust setae posterodistally (distal locking seta extremely elongate), palm nearly transverse, with small protrusion. Pereopods 3-4 moderately developed. Pereopod 5 coxa anterior lobe slightly expanded downward than posterior lobe; basis shorter than coxa, wider than long, anterior margin distal 2/3 length with many elongate robust setae marginally and minute setae submarginally, posterior lobe largely expanded; ischium and merus lined with many simple and robust setae anteriorly; merus posterior margin expanded, with slender setae on distal 2/3 length, posterodistal corner produced (reaching 1/3 length of merus) with robust seta. Pereopod 6 longer and slender than pereopod 5; merus about half as long as basis, expanded posteriorly; carpus rectangular, $1.2 \times as$ long as merus; propodus linear, $1.1 \times as$ long as carpus, with simple long setae on distal half of posterior margin. Percopod 7 slightly longer and stouter than pereopod 6; basis longer than that of pereopod 6; merus posterior lobe weaker, but setae stouter than those of percopod 6; carpus and propodus stouter than those of percopod 6. Epimeron 1 anteroventral corner angulate with one robust seta, posterior margin round and with small notches. Epimera 2-3 expanded and with facial setae

anteroventrally, posterior margins lined with small notches. Urosomite 1 with deep dorsal depression and mid-dorsal carina. Uropods 1–2 peduncles longer than rami, with robust setae on lateral and medial margins; outer rami with lateral robust setae only. Uropod 3 shorter than uropod 2; outer ramus bi-articulate; inner ramus not reaching distal end of proximal article of outer ramus in position. Telson cleft about 70%, each lobe with deep apical notch bearing one pair of robust and sensory seta, with one robust seta and one pair of sensory setae dorsolaterally.

Description of holotype male. *Head.* Lateral cephalic lobes expanded anteriorly, round; eyes ovoid, ommatidia large (Fig. 1B).

Antenna 1 (Fig. 1C, D) $0.6 \times$ as long as antenna 2, as long as head to pereonite 1 combined; peduncular article 1 swollen anteriorly; accessory flagellum 3-articulate; flagellum article 1 distinctly elongate; calceoli absent.

Antenna 2 (Fig. 1E) $0.3 \times$ as long as body; peduncular articles moderately developed; flagellum 21-articulate; calceoli absent.

Upper lip (Fig. 1F) epistome concave, separated from upper lip; upper lip slightly produced, rounded.

Lower lip (Fig. 1G) with developed mandibular processes

Mandible (Fig. 1H–J) incisor smooth but bearing blunt denticles on both sides; lacinia mobilis present on left mandible only, stemmed, expanded distally, with irregularly cusped blade; three small raker setae present on both mandibles, patch setules between raker setae and molar processes absent; molar process columnar, elevated, with triturative, rather smooth surface; lateral setigerous crest absent; palp attached midway, 3-articulate; article 2 longest, swollen anteriorly, with an oblique row of ten setae distally; article 3 weakly falcate, $0.7 \times$ as long as article 2, with twelve setae from middle of inner margin to apex.

Maxilla 1 (Fig. 2A, B) inner plate narrowing distally, not short, setose, with nine plumose setae along medial margin and apex; outer plate with eleven toothed setae apically in 7/4 arrangement and with several setae submarginally; palp bi-articulate, distal article swollen distally, apical margin oblique, with six mono-dentate short setae, one multi-dentate elongate seta, and one plumose seta.

Maxilla 2 (Fig. 2C) each plate broad; inner plate $1.3 \times as$ long as outer plate, with two rows of simple and plumose setae along distal half of medial and apical margins; outer plate with one marginal and one submarginal rows composed of barbed and simple setae apically (all setae extremely elongate).

Maxilliped (Fig. 2D) inner plate with one mediodistal row of plumose setae, apex with three nodular setae; outer plate well developed, subovoid, apex reaching the middle of palp article 3, lined with 13 nodular setae, eleven plumose setae, and two pairs of simple setae along mediodistal margin; palp 4-articulate, article 2 $2.0 \times$ as long as article 1, article 4 $0.7 \times$ as long as article 3, with one short apical seta.

Pereon. Gnathopod 1 (Fig. 2E–G) scopelocheirin form; coxa 1 large, subtriangular, expanded distally, round ventrally; basis $0.9 \times$ as long as coxa, anterior margin straight, lined with short setae, posterior margin slightly expanded; ischium $0.4 \times$ as long as basis; carpus elongate, $0.7 \times$ as long as basis; propodus subrectangular, $1.1 \times$ as long



Figure 1. Aroui minusetosus sp. n., holotype male, NIBRIV0000806536, 4.3 mm. **A** habitus **B** head **C** antenna 1, lateral **D** antenna 1, medial **E** antenna 2 **F** upper lip, lateral **G** lower lip **H** left mandible **I**, **J** right mandible. Scale bars: 0.1 mm (**F–J**), 0.2 mm (**B–E**), 0.5 mm (**A**).



Figure 2. *Aroui minusetosus* sp. n., holotype male, NIBRIV0000806536, 4.3 mm. **A** maxilla 1 **B** inner plate of maxilla 1 **C** maxilla 2 **D** maxilliped **E** gnathopod 1 **F** coxa 1 **G** palm and dactylus of gnathopod 1 **H** gnathopod 2 **I** coxa 2 **J** palm and dactylus of gnathopod 2. Scale bars 0.05 mm (**G**, **J**), 0.1 mm (**A–D**), 0.2 mm (**E**, **F**, **H**, **I**).

as carpus, slightly curved, with one lateral and two medial rows of long setae forming tuft distally, palm absent; dactylus extremely reduced, anchored at posterodistal corner.

Gnathopod 2 (Fig. 2H–J) slender, subchelate; coxa 2 subrectangular, $2.9 \times$ as long as wide, slightly curved posteroventrally, with two small notches posteroventrally; basis $0.9 \times$ as long as coxa, slightly widened distally; ischium elongate, slightly dilated posterodistally, $0.4 \times$ as long as basis; merus $0.6 \times$ as long as ischium, round posteriorly, with many short setae and one cluster of long setae; carpus $0.6 \times$ as long as basis, anterior margin swollen, with several clusters of short setae, longest seta at anterodistal corner reaching distal end of propodus, with two rows of plumose setae posterodistally; propodus subrectangular, $0.6 \times$ as long as carpus, with four robust setae posterodistally (distal locking seta extremely elongate), palm nearly transverse, with small protrusion posterodistally; dactylus falcate, slightly beyond palm.

Pereopod 3 (Fig. 3A) stout; coxa subrectangular, $3.0 \times$ as long as wide, slightly curved and with two small notches posteroventrally; basis $0.7 \times$ as long as coxa, sub-trapezoidal, somewhat expanded posterodistally; ischium elongate, $0.4 \times$ as long as basis, anterior lobe weak; merus expanded anteriorly, anterodistal corner weakly produced; carpus $0.7 \times$ as long as merus, somewhat expanded distally, with simple and robust setae on posterior margin; propodus $1.5 \times$ as long as carpus, lined with robust setae on posterior margin, with one pair of locking setae posterodistally; dactylus falcate, $0.4 \times$ as long as propodus, unguis developed.

Pereopod 4 (Fig. 3B) coxa deeper than wide, expanded posteroventrally; other articles nearly similar to those of pereopod 3.

Pereopod 5 (Fig. 3C) coxa large, subquadrate, slightly wider than long, weakly bilobate and anterior lobe slightly expanded downward than posterior lobe, posteroventral margin oblique, lined with minute setae, with three minute notches each bearing seta; basis subovoid, smaller than coxa, wider than long, anterior margin round, with many elongate robust setae marginally and minute setae submarginally on distal 2/3 length, posterior lobe largely expanded, posterodistal end reaching 1/4 length of merus, margin weakly crenulate and lined with minute setae, with one row of four setae medially; ischium and merus lined with many simple and robust setae anteriorly; merus posterior margin expanded, with five slender setae distally on 2/3 length, posterodistal corner produced (reaching 1/3 length of merus) with one robust seta; carpus dilated posterodistally, 0.8 × as long as merus, anterior margin crenulate and lined with robust setae; propodus 1.3 × as long as carpus, with single and paired robust setae on anterior margin; dactylus falcate, 0.4 × as long as propodus, unguis developed.

Pereopod 6 (Fig. 3D) longer and more slender than pereopod 5; coxa subrectangular, smaller than that of pereopod 5, bilobate, anterior lobe smaller than posterior lobe, expanded downward, with three plumose setae anteriorly, posterior lobe weakly crenulate, with three plumose setae posteroventrally and minute setae on posterior margin; basis ovoid, 1.7 × as long as wide, anterior margin round proximally and remains nearly straight bearing short robust setae, with one cluster of elongate and short robust setae at anterodistal corner, posterior margin well expanded, smooth, weakly crenulate, posterodistal end reaching 1/4 length of merus; merus half as long as basis,



Figure 3. *Aroui minusetosus* sp. n., holotype male, NIBRIV0000806536, 4.3 mm. **A** pereopod 3 **B** pereopod 4 **C** pereopod 5 **D** pereopod 6 **E** pereopod 7. Scale bars 0.2 mm (**A–E**).

expanded posteriorly; carpus rectangular, $1.2 \times as$ long as merus; propodus linear, $1.1 \times as$ long as carpus, with single and paired robust setae on anterior margin and simple long setae on distal half of posterior margin; dactylus falcate, $0.4 \times as$ long as propodus, unguis developed.

Pereopod 7 (Fig. 3E) $1.1 \times as long as and stouter than pereopod 6; coxa unilobate, with two plumose setae anteriorly, dilated and weakly crenulate posteroventrally; basis larger than that of pereopod 6, anterior margin slightly concave distally at 2/3 length, posterior lobe well developed, margin weakly crenulate, with two plumose setae proximally, somewhat flattened and angulate distally; merus posterior lobe weaker, but setae stouter than those of pereopod 6; carpus and propodus stouter than those of pereopod 6; dactylus falcate, 0.4 × as long as propodus, unguis developed.$

Pleon. Epimeron 1 not produced but angulate bearing one robust seta anteroventrally, posterior margin round and with three small notches. Epimera 2–3 expanded, with facial setae anteroventrally, posterior margins lined with small notches. Urosomite 1 with deep dorsal depression and mid-dorsal carina (Fig. 4A).

Uropod 1 (Fig. 4B) longest; peduncle $1.4 \times as$ long as outer ramus, with seven robust setae on dorsolateral margin and five elongate robust setae on dorsomedial margin; outer ramus with five lateral robust setae only; inner ramus $0.9 \times as$ long as outer ramus, with two medial and four lateral robust setae.

Uropod 2 (Fig. 4C) $0.7 \times as$ long as uropod 1; peduncle with two robust setae medially (distal seta stoutest) and five robust setae laterally on each dorsal margin; outer ramus $1.3 \times as$ long as peduncle, with five lateral robust setae only; inner ramus $1.1 \times as$ long as outer ramus, with four lateral and three medial robust setae.

Uropod 3 (Fig. 4D) $0.8 \times as$ long as uropod 2; peduncle $0.7 \times as$ long as outer ramus; each ramus with plumose setae on medial margin; outer ramus bi-articulate, distal article $0.2 \times as$ long as proximal article; inner ramus $0.8 \times as$ long as outer ramus, not reaching distal end of proximal article of outer ramus in position.

Telson (Fig. 4E) longer than broad, cleft about 70%, each lobe with deep apical notch bearing one robust and one sensory seta apically, with one robust seta and one pair of sensory setae dorsolaterally.

Remarks. The subfamily Scopelocheirinae is a small group of Scopelocheiridae, composed of only eight species in three genera, united by the synapomorphy of the narrow columnar mandibular molar different from other lysianassoids (Kilgallen and Lowry 2015). In their review, the diagnosis of *Aroui* was restricted concerning the sharing of the unusual long, distally barbed setae on the outer plate of maxilla 2 (Kilgallen and Lowry 2015). The Korean scopelocheirid specimens in this study also show these synapomorphic characters and they are easily assigned to the genus *Aroui*. This genus has only four valid species: *A. americana* Lowry & Stoddart, 1997; *A. hamatopodus* Lowry & Stoddart, 1997; *A onagawae* (Takekawa & Ishimaru, 2000); and *A. setosus* Chevreux, 1911. *Aroui minusetosus* sp. n. is readily distinguished from *A. americana*, *A. hamatopodus* and *A. setosus* in that only coxa 4 has setose margin posteroventrally (vs. all coxae 1–4 of *A. americana*, *A. hamatopodus* and *A. setosus* are densely setose



Figure 4. Aroui minusetosus sp. n., holotype male, NIBRIV0000806536, 4.3 mm. **A** pleon, lateral **B** uropod 1 **C** uropod 2 **D** uropod 3 **E** telson. Scale bars 0.1 mm (**E**), 0.2 mm (**B**–**D**), 0.5 mm (**A**).

ventrally). The new species is quite similar to *A. onagawae* from Japan having nonsetose coxae 1–3, but show a difference in coxa 4: in *A. minusetosus*, it is densely setose with long setae posteroventrally, but only with a moderate setation posteriorly in *A. onagawae*. On the other hand, this new species has a less dense setation on the whole body compared to the original description of *A. onagawae* (Takekawa and Ishimaru 2000). At first, we considered the much weaker setation related to the developmental stage, but the Korean specimens show an elongated antenna 2 that indicates maturity in male, and the body length of the new species is longer than the type specimen of *A. onagawae* (Takekawa and Ishimaru 2000). Additionally, the new species shows some differences from *A. onagawae* as follows: 1) the eyes are ovoid and their ommatidia are

Characters	A. americana	ricana A. hamatopodus A. onagawae		A. setosus	A. minusetosus
Eyes	poorly developed	well developed	well developed	well developed	well developed
shape	ovoid	ovoid	pyriform	ovoid	ovoid
size of each ommatid	large	moderate moderat		moderate	large
Antenna 1					
flagellum	9-articlulate	9-articlulate	13-articlulate	10-articlulate	8-articlulate
Gnathopod 1	-	~	~		
length ratio of carpus and propodus	1.0 : 1.2	1.0 :1.1	1.0 :1.1	1.0 :1.0	1.0 :1.1
dactylus	covered with sensory setae	covered with sensory setae	more reduced, without sensory setae	covered with sensory setae	more reduced, without sensory setae
Gnathopod 2					
palm	minutely chelate	minutely chelate	minutely chelate	minutely chelate	subchelate
defining setae	paired	paired	paired	absent	single
Coxae 1–4					
coxa 1 anterior margin	convex	convex	straight	concave	convex
ventral margin	densely setose	densely setose densely setose		densely setose	not setose in coxae 1–3 (coxa 4 setose posteroventrally)
Pereopods 3–7					
setation	moderate	moderate	densely setose	moderate	moderate
pereopod 5 basis medial row of plumose setae	present	absent	absent	absent	absent
pereopod 6 propodus hooked setae on posterior margin	present	present	present	absent	absent
Uropod 1 number of setae (medial + lateral)		1	1		
peduncle	5 + 6	7 + 6	14 + 22	many setae (?)	5 + 7
inner ramus	0 + 3	3 + 4	9 + 11	?	2 + 4
outer ramus	1 + 1	0 + 5	0 + 10	?	0 + 5
Uropod 2 number of setae (medial + lateral)					
peduncle	5 + 3	3 + 8	10 + 15	many setae (?)	2 + 5
inner ramus	2 + 3	5 + 5	12 + 12	3 + 2	3 + 4
outer ramus	0 + 3	0 + 4	0 + 9	0 + 2	0 + 4
Telson					
dorsal robust setae	one pair	absent	three pairs	absent	one pair
Reference	Lowry and Stoddart (1997)	Lowry and Stoddart (1989)	Takekawa and Ishimaru (2000)	Lowry and Stoddart (1989)	In this study

Table 1. Morphological differences between worldwide Aroui species.

larger in *A. minusetosus* sp. n. (vs. pyriform and smaller ommatidia in *A. onagawae*); 2) antenna 1 flagellum is composed of eight articles in *A. minusetosus* sp. n. (vs. 13 articles in *A. onagawae*); 3) gnathopod 2 is subchelate with a small protrusion on the palm in *A. minusetosus* sp. n. (vs. minutely chelate and without protrusion in *A. onagawae*); 4) gnathopod 2 propodus has a row of four robust setae posterodistally (distal locking seta is elongate and not paired) in *A. minusetosus* sp. n. (vs. having a pair of locking setae in *A. onagawae*); and 5) both telson each lobes have a robust seta dorsally in *A. minusetosus* sp. n. (vs. three dorsal robust setae in *A. onagawae*) (see the Table 1, Takekawa and Ishimaru 2000). This is the first record of a scopelocheirid lysianassoid from Korean waters.

Key to worldwide species of the genus Aroui Chevreux, 1911

1	Coxae 1–4 ventrally setose
_	Coxae 1-3 ventrally smooth, coxa 4 weakly setose posteriorly or posteroven-
	trally
2	Pereopod 5 basis with an well-developed row of many plumose setae on
	medial surface A. americana Lowery & Stoddart, 1989
_	Pereopod 5 basis with one cluster of several simple setae medially
3	Coxa 1 anterior margin convex A. hamatopodus Lowry & Stoddart, 1997
_	Coxa 1 anterior margin concave
4	All appendages densely setose; gnathopod 2 minutely chelate
_	All appendages less setose; gnathopod 2 subchelate A. minusetosus sp. n.

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RESEARCH ARTICLE



Revision of the family Carabodidae (Acari, Oribatida) XII. Yoshiobodes camerunensis sp. n. and Rugocepheus costaricensis sp. n.

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Abstract

Yoshiobodes camerunensis **sp. n.**, collected in Cameroon, is the first species of this genus reported from the Afrotropical region. Diagnostic characters include lamellae terminating in a bridge and not in lamellar tips; cup-shaped bothridia, bothridial ring present; rostral setae cochleariform, smooth; lamellar setae slightly lanceolate, barbate; fifteen pairs of notogastral setae; c_3 lanceolate, rounded end, with longitudinal shallow grooves; other notogastral setae curved lanceolate-cochleariform. *Rugocepheus costaricensis* **sp. n.** is the third species of the genus to be described, and the first collected outside the African region. Prodorsum presents a Y-shaped structure; elevated interlamellar process, superior flat zone; lamellae lacking lamellar tips; fourteen pairs of notogastral setae; four notogastral furrows, and an unpaired elevated central area devoid of setae. Both species are described and illustrated based on adult specimens, studied by means of optical and SEM microscopy.

Keywords

Carabodidae, Rugocepheus, Yoshiobodes

Introduction

Extensive collection materials of the family Carabodidae sampled in Africa (Cameroon, Kenya, Zimbabwe, Rwanda, South Africa, Madagascar, Gabon, Comoros, Republic of the Congo, Democratic Republic of the Congo, Nigeria, Ghana); South and Central America (Argentina, Chile, Brazil, Paraguay, Uruguay, Bolivia, Peru, Ecuador, Costa Rica, Martinique, Honduras, Guadeloupe, Trinidad-Tobago), and Asia (Vietnam, China, Cambodia, Sri Lanka) are housed in the Museum national d'Histoire naturelles, Paris (**MNHN**), the Museum d'Histoire naturelles Geneva (**MHNG**) and in the senior author's personal collection. Studies of this material have been ongoing, in parallel to the redescriptions of type material of the various genera started in 2013.

The taxonomy of the genus *Yoshiobodes* is complex. This genus comprises 12 species and is divided into three subgenera: *Yoshiobodes*, which includes eight species with Pantropical (excluding Ethiopic) and Subtropical (Holarctic Southern) distribution; *Berndobodes* with two species from Borneo, and *Dongnaibodes* with two species from Vietnam (Subias 2017). According to Reeves (1997), the type species is *Yoshiobodes irmayi* (Balogh & Mahunka, 1969), with Neotropical distribution, and the comparison of *Y. irmayi* collected from North America and from St. Lucia, West Indies, revealed that they are conspecific.

This genus is very difficult to study using optical microscopy due to their small size, cuticular microsculpture, cerotegumental layer, particular topography, and setal particularities. The complimentary use of Scanning Electron Microscopy (SEM) is fundamental to understanding and clarifying several aspects of this fascinating group of Carabodidae. The contribution by Reeves (1997) is remarkable, and the redescription of the type species *Y. irmayi* is given here for the first time including both adults and immatures, as well as SEM micrographs. *Yoshiobodes camerunensis* sp. n. is the first species of this genus found in the Afrotropical region.

The second species, *Rugocepheus costaricensis* sp. n. is described from Costa Rica. Two species of this genus are known previously from Africa, namely *Rugocepheus formosus* Mahunka, 2009 and *Rugocepheus joffrevillei* Fernandez, Theron & Rollard 2013, both from Madagascar.

Materials and methods

The techniques used in the light and scanning electron microscopic investigations of the examined specimens follow those proposed by Fernandez et al. (2013).

The SEM observations were made using Scanning Electron Microscope FEI-Quanta Feg 250, with 10 Kv and working distant (WD) variable.

Measurements taken: total length (from tip of rostrum to posterior edge of notogaster); width (widest part of notogaster) in micrometers (μ m). Leg setation studies making use of standard, polarized and phase contrast microscopes are provisional, due to the fact that only adult specimens were available for study. Setal formulae of the legs include the number of solenidia (in parentheses); tarsal setal formulae include the famulus (ϵ).

Morphological terminology

Morphological terms and abbreviations used are those developed by F. Grandjean (1928–1974) (cf. Travé and Vachon 1975; Norton and Behan-Pelletier 2009 (in Krantz and Walter 2009); Fernandez et al. 2013). For the setal types those of Evans (1992); ornamentation of cuticular surfaces Murley (1951) (in Evans 1992 *op. cit.*: 9) were used.

New taxa descriptions

Yoshiobodes camerunensis sp. n.

http://zoobank.org/55FC7386-4819-4BF0-9F21-32AFABB393ED Figures 1–35, Table 1

Etymology. The specific epithet is derived from Cameroon, country of origin of the type material.

Material examined. Holotype. Adult female "CAM 73/3. Mt.Kala (près de Yaoundé). 800–850 m, terreau troncs pourris et litière. IV–V. 1973. Leg. G. TERRON". Material deposited in the collection of MNHG, Switzerland, preserved in 70% ethanol. **Paratypes**. 2 adult females "CAM 73/3. Mt. Kala (près de Yaoundé). 800–850 m, terreau troncs pourris et litière. IV–V. 1973. Leg. G. TERRON". Material deposited in the collection of MNHG, Switzerland, preserved in 70% ethanol.

Diagnosis. Setation. Rostral setae cochleariform, smooth; lamellar setae slightly lanceolate, barbate, covered by cerotegumental layer; notogastral c_1 , c_2 , da, dm, dp, lm, lp curved lanceolate-cochleriform; c_3 lanceolate, rounded end with longitudinal shallow grooves; subcapitular h, epimeral, genital, anal, adanal setae spiniform. Prodorsum. Shallow lamellar furrow present; lamellae terminate in bridge not lamellar tips. Bothridia cup-shaped, with bothridial ring. Barbed fan-shaped sensillus. Notogaster: fifteen pairs of setae. Genital opening on elevated zone; deep anterior furrow in front of genital opening.

Leg	Femur	Genu	Tibia	Tarsus
Ι	(l). d, dv	(l), v'	(l),(l)	$(pv), s, (a), (u), (p), (tc), (ft), \varepsilon, it$ "
		σ	$\Psi_{I_1} \Psi_2$	$\omega_{I_{\mu}}\omega_{2}$
II	(l), d, dv	(l),d	(l), v'	(pv), s, (a), (tc), (u), (p), (ft),(it)
		σ	φ	ωω
III	l,v	d,v,l'	ľ	(pv), s, (a), (tc), (u), (p), (ft),(it)
		σ	φ	
IV	d,ev	d, l'	(v)	(pv),(u),(p),ft",s,(a),(tc)
			σ	

Table 1. Leg setae and solenidia of Yoshiobodes camerunensis sp. n.



Figures 1–2. *Yoshiobodes camerunensis* sp. n. Adult, with cerotegumental layer. SEM micrographs. **I** lateral view **2** dorsal with slight lateral tilt. Scale bars: 50 µm (**1**, **2**).

Description. Measurements. SEM: length: 301 μ m (296–312). Width: 130 μ m (127–152) (three specimens). Light microscopy: 311 μ m (301–323) × 142 μ m (138–148) (two specimens) All specimens female.

Colour: Specimens without cerotegument, light brown, slightly shiny when observed in reflected light.

Cerotegument: Entire body, femora and genua of legs covered by thin layer of between $0.1-0.5 \mu m$ presenting as a polygonal network (Figures 7, 17, 19, 26, 30, 31 indicated by arrow).

Cuticular microsculpture. Prodorsum. Posterior zone of *e.i.p* and *p.p.d* round to ovoid depressions (Figure 2). Polyhedral depressions (Figs 6, 15) on anterior zone *e.i.p*



Figures 3–5. *Yoshiobodes camerunensis* sp. n. Adult, Optical Microscopy. **3** dorsal view **4** ventral view **5** lateral view. Scale bars: 45 μm (**3**, **4**); 80 μm (**5**).

near *in* setae, extending to near *ro* setae. Remainder of prodorsum with small protuberances (Figures 12, 18). Bothridial zone with large protuberances (Figure 9). Ovoid to irregular depressions of varying size (Figures 6, 15) on lateral zone *Tu*, *s.tu.d* and *Pd I*. Notogaster. Aligned, rectangular to polyhedral protuberances (Figures 6, 16, 20): ante-



Figures 6–13. *Yoshiobodes camerunensis* sp. n. Adult with cerotegumental layer, SEM. **6** frontal view **7** c_3 setae **8** *in* setae **9** *si* and sensillar furrow (*s.fu*) **10** *lam* lateral with *le* setae **11** *lam*, lateral with *le* setae **12** rostral setae **13** *lam* dorsolateral with *le* setae. Scale bars: 50 µm (**6**); 2 µm (**7**); 10 µm (**8**); 10 µm (**9**); 5 µm (**10**); 10 µm (**11**); 5 µm (**12**); 2 µm (**13**).

rior zone between c_1 , c_2 setae and *d.sj*; behind c_1 , c_2 setae and laterally towards *s.c*, ovoid protuberances forming a polyhedral network with 5–7 protuberances (Figures 2, 6, 14, 20, 28). Aligned ridges with small protruberances (Figures 2, 28) in zone between *s.c*


Figures 14–20. *Yoshiobodes camerunensis* sp. n. Adult, with cerotegumental layer, SEM. **14** lateral view, zone of humeral apophysis **15** anterior prodorsum region **16** cerotegument and cuticular zone: anterior notogaster **17** notogastral setae, frontal view **18** cuticular microsculpture **19** cuticular depressions **20** frontal view, notogaster. Scale bars: 10 µm (**14**); 20 µm (**15**); 10 µm (**16**); 10 µm (**17**); 2 µm (**18**); 2 µm (**19**); 20 µm (**20**).

and *b.ng*. Ventral zone. Subcapitular zone between *a* and *h* setae with small protuberances similar to Figure 18 (Figures 21, 27, 32). Round depressions (Figure 19). on posterior zone of subcapitulum (Figure 27). Irregular depressions (Figure 25) on epimeral zone (Figure 27). Posterior to genital opening and aggenital, anal and adanal zones, ovoid protuberances forming a polyhedral network (Figure 21). Legs. Large ovoid to round depressions present on basal zone of all femora.

Setation. Seta in lanceolate, barbate, slightly curving (Figure 8), length 26 µm (22–31); ro setae cochleariform, smooth (Figures 12, 15, 35), 10 µm (8–13); le setae slightly lanceolate, barbate, covered by cerotegumental layer (Figures 10, 11, 13), 16 µm (11–21). Notogastral setae c_1 , c_2 , da, dm, dp, lm, lp, lanceolate-cochleariform, curved, more or less same length (Figures 1, 2, 17, 20, 28), 15 µm (17–22); c_3 setae lanceolate, round end with longitudinal shallow grooves (Figures 1, 7), 5.30 µm (4–7); la, h_3 , p_1 , p_2 , p_3 , h_1 , h_2 lanceolate, round end with longitudinal shallow grooves (Figures 1, 7), 5.30 µm (14–7); la, h_3 , p_1 , p_2 , p_3 , h_1 , h_2 lanceolate, round end with longitudinal shallow grooves (Figure 1), 11 µm (10–13). Subcapitular setae (Figures 21, 27, 31, 32) a sigmoid, 9.5 µm (11–8); m inclined L-shaped, 20 µm (23–17); spiniform: h 2.5 µm (18–3.5); epimeral setae (Figures 25, 34), 0.7 µm (0.4–1.7); ge (Figures 21, 23, 26), 7 µm (10–6); ag (Figure 21), 7 µm (5–10); an (Figure 22), 7 µm (12–4). Adanal setae curved lanceolate-cochleariform (Figure 21) ad_1, ad_2, ad_3 15 µm (17–13).

Prodorsum. Very complex, described from different angles in order to properly interpret the structure. Lateral view (Figure 1) and slightly posterolateral inclination (Figure 2): elevated interlamellar process (e.i.p) at the same level as elevated zone of notogaster; forward directing *in* setae situated in a depressed zone (Figure 8); posterior prodorsal depression (p.p.d) clearly visible in inclined lateral view (Figure 2). Cuticular microsculpture and shallow lamellar furrow (l.l.f) well visible (Figure 2). Lamellar zone (Figures 10, 11, 13): positioning of *le* setae and their particular shape clearly observed; lamellae in anterior zone lacking lamellar tips, anterior zone is connected by a bridge (Figure 11 indicated by arrow). Anterior lateral view (Figure 15): cuticular microsculpture of *s.tu.d* zone and between *Pd I* and *Tu* is clearly visible; the zone between seta *ro* is more or less smooth. Bothridia: cup-shaped, with smooth ovoid bothridial ring, incomplete, with bothridial tooth (Figures 2, 14). Sensillus (*si*) fan-shaped, barbed, directing upwards (Figures 1, 2, 6, 9, 14).

Frontal view (Figures 6, 12): triangular, with depressed central zone (Figure 6, indicated by thick arrow). Both sides of *e.i.p* rounded, with depressed zone where *in* setae are located. Cuticular microsculpture easily observed on depressed central zone of *e.i.p*; *l.l.f* clearly visible; *le* setae hardly discernible; *ro* setae well visible; rostral margin smooth. *Tu* (Figure 6) expanded laterally towards the prodorsal margin, extending to the level of *Pd I*.

Notogaster. Shape: oval in dorsal view; *d.sj* narrow, well-delimited, curving slightly backwards (Figures 2, 3); anterior lateral zone: humeral apophysis (*h.ap*) extending forward, overlapping posterior bothridial zone (Figures 2, 3, 5 14); notogastral anterior depression (*n.a.d*) absent. Frontal view: convex in central zone up to cicumgastric furrow (*s.c*); flat from *s.c* to *b.ng*, slightly tilted downward (Figures 2, 6, 20); *s.c* clearly delimited (Figures 6, 20), running laterally between c_2 *lm*, lp, h_2 , h_1 , dp and h_3 , p_3 , p_2 , p_1 . Setae c_3 and la present on *h.ap*, with conspicuous depressed zone (*s.fu*) lodging the sensillus after leg folding (protection mechanism) (Figure 14) (Fernandez et al. 2013); c_3 setae placed beneath the *s.fu*, while la are placed behind. Cuticular microsculpture:



Figures 21–27. *Yoshiobodes camerunensis* sp. n. Adult, with cerotegumental layer, SEM. **21**ventral view **22** anal plate, ventral view **23** genital plate, ventral view **24** palp **25** epimeral microsculpture with epimeral setae **26** anal setae **27** subcapitulum. Scale bars: 100 μ m (**21**); 20 μ m (**22, 23**); 10 μ m (**26**); 5 μ m (**24**); 2 μ m (**25**); 10 μ m (**27**).

rectangular to polyhedral protuberances are clearly delimited in the anterior zone between *d.sj* and setae c_1 , c_2 and inwards to *s.c* (Figures 16, 20). Setae c_1 , *da*, and *dm* are more or less aligned; while c_2 *lm*, *lp*, h_2 , and h_1 , are arranged in an arc (Figures 6, 20). Lateral view: convex (Figure 1, 5), setae *la*, h_3 , p_3 , p_2 , p_1 situated between *s.c* and *b.ng*; setae c_3 are situated further down from this setal alignment (Figures 1, 14). Fifteen pairs of setae: c_1 , c_2 , c_3 , *da*, *dm*, *dp*, *la*, *lm*, *lp*, h_1 , h_2 , h_3 , p_1 , p_2 , p_3 ; only lyrifissures *im* and *gla* clearly visible between *lm* and *lp* setae (Figure 5). Clearly visible *s.c* in lateral posterior zone (Figure 28); cuticular microsculpture below *s.c* and *bng*, clearly discernible (Figure 28).

Lateral region (Figures 1, 5, 14). A thorough study of the lateral aspect was imperative for observation and interpretation of several structures. Conical *e.i.p* inclining slightly upwards (Figures 1, 5); *lam* clearly discernible (Figures 1, 5, 10, 11, 13, 15); *le* inserted on *lam*, behind level of *ro* setae (Figure 15); no lamelar tips present; *le* setae inserted some distance from where the apical part of *lam* reaches the rest of prodorsum; this zone forming a bridge where *le* setae can be concealed (Figures 10, 15); large, laterally expanded Tu at same level as Pd I (Figure 15); Tu with upward curving margin; several depressions (Figure 15) visible on Tu and zone between Tu and PdI, with variation in shape and depth (Figure 15 indicated by arrows); *s.tu.d* deeply concave; anterior tutorial depression (*a.tu.d*) (Figure 5) and other small depressions present (Figure 15); Pd I: large extended lamina, rounded apex; immediately behind Pd I apex, conspicuous round to polyhedral cuticular depression (Figure 1 indicated by dashed arrow);in posterior zone of Pd I, near Pd II, short deep longitudinal grooves separated from each other by longitudinal depressions (Figure 1, indicated by arrow). Pd II: small lamina, rounded apex; dis a triangular protuberance (Figure 34). Many circular to ovoid depressions (*dep*), delimited by cuticular thickenings, occurring behind, on top of and on lower part coxa IV up to genital opening (Figures 1, 5, 21).

Ventral region. Cuticular microsculpture obviously different on epimeral, aggenital, and adanal zones (See Cuticular microsculpture). Subcapitular setae *a*, *m*, *h* (Figures 21, 27, 31, 32, 34) differing in shape and length (see Setation); setae *h* similar to epimeral, genital, aggenital setae (Figures 22, 23, 25, 33), all spiniform; epimeral setae shorter than others, difficult to observe.

Epimeres well defined by furrows, easily discernible both in animals with cerotegumental layer (Figure 21) and without (Figure 4). Epimeral borders clearly visible (Figure 4); epimeral chaetotaxy 3-1-3-3, but variations exist due to some setae not being clearly visible, in asymmetric position, or lost; apodemes 1, 2, sj and 3 clearly visible (Figure 4); epimera 1, 2; 3 and 4 fused. Genital opening on elevated zone (Figure 21); surrounded anteriorly by a semicircular cuticular thickening (*c.th*) (Figure 21) extending to posterior zone, but not completely surrounding genital opening; depressed zone between cuticular thickening and elevated zone of genital opening; deep anterior furrow (*a.g.f.*) (Figures 4, 21) in front of genital opening, this depressed zone extends to the outside of *c.th*. Four pairs of genital setae in single line (Figures 21, 23). Posterolateral aggenital setae, genital opening far from *ad*₃ setae; very different in shape and size (Figure 21). Three pairs of adanal setae. Anal plate sharply tipped (Figure 22); lyrifissure *iad* situated laterally, hardly discernible (Figure 4). Many circular to ovoid depressions (*dep*), behind coxa IV.

Legs. Setal formulae (trochanter to tarsus) (Table 1) Legs. I: 1-4-3(1)-4(2)-15(2); II: 1-4-3(1)-3(1)-15(2); III: 2-3-1(1)-2(1)-15; IV: 1-2-2-2(1)-12 (trochanter to tarsus).



Figures 28–35. *Yoshiobodes camerunensis* sp. n. Adult with cerotegumental layer, SEM. **28** notogaster, posterolateral **29** subcapitulum, lateral **30** cuticular microsculpture covered by cerotegumental layer, dorsal zone **31** subcapitular *h* setae **32** subcapitular setae *m* **33** anal setae **34** discidium and epimeral setae **35** rostral setae, lateral view. Scale bars 20 μm (**28**); 10 μm (**29, 34**); 5μm (**32, 33**); 2 μm (**30, 31**); 1.5 μm (**35**).

Remarks. The positioning of the *le* setae during activation of the protection mechanism is interesting: these setae are shielded under the lamellae, but are also further protected by the cerotegumental layer (Fig. 10). Protected by the external margin of *Lam* (figure

1), and concealed in the deepest zone of the *s.tu.d*, Legs I are difficult to study. *Yoshiobodes camerunensis* is the first species of this genus from the Afrotropical region. *Y. irmayi* (Balogh & Mahunka, 1969), redescribed by Reeves 1997, is close to *Yoshiobodes camerunensis* sp. n. Principal similarities: presence of *p.p.d* on prodorsum; rectilinear microsculpture between *d.sj;* microsculpture c_1 , c_2 setae and behind setae c_1 , c_2 ; number of notogastral setae; shape of notogastral setae; shape of *in* setae. Principal differences: prodorsal cuticular microsculture, shape of prodorsum; characteristics of *l.l.f*; shape and characteristics of *le* setae; microsculpture of epimeral zone; structure *s.fu*.

Rugocepheus costaricensis sp. n.

http://zoobank.org/97AA08B8-332F-4C20-8631-E7EDE2CEC4E6 Figures 36–61, Table 2

Etymology. The specific epithet is derived from Costa Rica, country of origin of the type material.

Material examined. Holotype. Adult female "CCR 0978 Tu 11 Costa Rica Turrialba foret naturelle du catie alt. 560 m. Triage d'humus coté est surface nid d'Atta au pied de *Castilla elastica* 1.IX. 1978. Leg. P.WERNER" Deposited in the Ccllection of the MHNG, Switzerland, preserved in 70% ethanol. **Paratypes.** 2 adult females, same locality and date of holotype, deposited in the Ccllection of the MHNG, Switzerland, preserved in 70% ethanol.

Diagnosis (adult female). Body and legs entirely covered by simple porous cerotegumental layer. Integumental microsculpture over entire body: irregular, small tuberculate. Prodorsum. More or less triangular in dorsal view; deep low lamellar furrow delineating Y-shaped structure; elevated interlamellar process with superior flat zone; CSO present; rostrum beak-shaped; tutorium and Pedotecta I expanded laterally; small triangular discidium; supratutorial depression deep, with several rounded depressions. Lamellae without lamellar tip, forming bridge concealing le setae. Smooth ring-shaped bothridium; bothridial tooth present; sensillus barbate. Fourteen pairs of notogastral setae c_1 , c_2 , da, dm, dp, la, lm, lp, h_1 , h_2 , h_3 , p_4 , p_5 , p_4 . Four notogastral furrows present: paired central longitudinal furrow; one lateral unpaired semicircular furrow; another unpaired semicircular furrow delimiting an unpaired elevated central area (devoid of setae); a pair of elevated areas (with c_1 , da, dm, dp); unpaired semicircular elevated area (with c2, la, lm, lp, h2, h2). Anterior genital furrow clearly observed; epimere 4 borders elevated; genital plate situated in elevated zone surrounded by furrow. Epimeral chaetotaxy 3-1-3-3; long epimeral setae. Four pairs of genital setae in a single line; crescent-shaped structure anterior to anal plate; anal plate with small sharp tip; aggenital and adanal setae more or less similar in length. Subcapitulum diarthric, three pairs of highly different setae *a*, *m*, *h*. Mentum complex.

Description. *Measurements.* SEM: females 501 μm (489–515) × 270 μm (267–286). Light microscopy: females 506 μm (490–518) ×282 μm (276–301).

Colour. Specimens without cerotegument: females light brown to brown.

Legs	Femur	Genu	Tibia	Tarse	Claw
Ι	v (l);	(v) d	(l),v	(pv); s; (a); (u); (p); (it); (tc); (ft); ε	1
		σ	$\varphi_1 \varphi_2$	$\omega_{L}\omega_{2}$	
II	(l) d	v l	v (v);	d Ad; (pv); s; (a); (u); (p); (it); (tc); (ft).	1
		σ	φ	$\omega_L \omega_2$	
III	l;	d	v (l)	(v) ft; (tc); (it); (p); (u); (a); s; (pv).	1
		σ	φ		
IV	d	v d	(l).	v ft; (tc); (p); (u); (a); s; (pv	1
			φ		

Table 2. Leg setae and solenidia of Rugocepheus costaricensis sp. n.

Cerotegument. Simple layer ($\pm 0.7 \ \mu m$) (Figures 47, 49, 50); uniformly covering entire body and legs. Slightly irregular surface (Figure 50). Large number of pores observable on the surface, porous (0.4–0.7 μm) diameter (Figures 44, 46, 47, 50).

Integument. Microsculpture simple, covering entire body: irregular, small tuberculate (Figure 49); tubercules (1–2.5 µm). Only lateral anterior lamellar zone presenting different microsculpture: round to ovoid depressions (Figures 38, 39, 43).

Setation. Setae in lanceolate (resembling leaf of *Salix* spp.), length 30 μ m (28–34) (Figure 37); *ro* setae lanceolate, 13 μ m (11–14) (Figure 44). Setae *le* lanceolate, slightly curved, basally and medially serrate, 23 μ m (21–25) (Figure 45). Notogastral setae; c_{i} , c_{2} , *da*, *dm*, *dp*, *la*, *lm*, *lp*, h_{i} , h_{2} aciculiform, 51 μ m (41–61) (Figure 46); h_{3} , p_{i} , p_{2} , p_{3} , 25 μ m (23–27) (Figures 36, 46). Simple: ag, 20 μ m (17–22) (Figure 61); ad 20 μ m (17–22) (Figure 61); ge 17 μ m (15–19) (Figure 60); epimeral 18 μ m (15–21) (Figure 53). Spiniform: *an* 10 μ m (11–8) (Figure 55); *m* 3.5 μ m (3–4) (Figure 57); Setae *a* setiform, 7 μ m (5–9) (Figure 56); *h* setae L-shaped, barbate, 19 μ m (18–21) (Figure 58).

Prodorsum. Very complex. For proper understanding of structures, descriptions from various angles/views are included. Dorsal view (Figure 36). More or less triangular with lateral polyhedral expansion at level of *bo* and *in* setal level insertion; anterior expansion of *Tu* (Figure 36) clearly visible. Deep *l.l.f* delineates Y-shaped structure (*Ys*); posterior of *Ys* with depressed rounded zone (*p.Ys*) extending laterally in *p.p.d* *; *ro* setae and *CSO* clearly visible. Beak-shaped rostrum; sensillus barbate.

Frontal view (Figure 39). More or less triangular; *e.i.p* elevated with flat superior medial zone (Figure 38); conspicuous *l.l.f* running to posterior zone of *e.i.p*; from *ro* setal zone, *l.l.f* delineates a Y-shaped structure. On posterior of *e.i.p* the *l.l.f* delimiting a large ear shaped structure where *in* setae are situated. Lamellae (*lam*), running laterally, internal margin delimited by *l.l.f*; *le* setae on the anterior zone of *lam*; *le* setae inserted behind *ro* setal insertion level; small transversal depression posterior to *ro* setae (Figure 38 indicated by thick arrow); *CSO* present anterior to *ro* setal insertion. Rostral zone extended to rounded beak-shape with several transversal semicircular furrows (Figure 38 indicated by dashed arrow).

Lateral inclined view (Figures 38, 43). Elevated *e.i.p* with flat superior zone; *lam* clearly delimited by conspicuous *l.l.f*; particular cuticular microsculpture of round to



Figures 36–39. *Rugocepheus costaricensis* sp. n. Adult (with cerotegument), SEM. **36** dorsal view **37** *in* setae **38** fontal inclined view **39** frontal view. Scale bars: 100 μm (**36**); 5 μm (**37**); 50 μm (**38**); 100 μm (**39**).

ovoid depressions externally to *lam*. Elevated ear-shaped structure where *in* setae are situated; *ro* setae, *CSO*, and beak-shaped rostral zone, easily observed. *Tu* expanded laterally and anteriorly; *Pd I*: large expanded ovoid structure; several depressions (*p.tu.d*, *p.tu.d*₁) between *Tu* and *Pd I*; *s.tu.d* a conspicuous depression, running parallel between *lam* and *Tu*, with internal round depression (*a.tu.d*); *le* setae inserted on anterior zone of *lam*; *lam* zone anterior to *le* insertion, lacking lamellar tip, forming a bridge concealing *le* setae. Bothridium cup-shaped, smooth bothridial ring, incomplete, with bothridial tooth.



Figures 40–42. *Rugocepheus costaricensis* sp. n. Adult (with cerotegument) optical microscopy. 40 dorsal view 41 ventral view 42 lateral view.

Notogaster (Figure 36). Oval, with fourteen pairs of setae: c_1 , c_2 , da, dm, dp, la, lm, lp, h_1 , h_2 , h_3 , p_1 , p_2 , p_3 . Four furrows present: paired central longitudinal (*c.fu*) furrows; one lateral unpaired semicircular furrow (*l.fu*), and one unpaired semicircular (*s.c*) furrow; an unpaired elevated central area (*i.e.a*) is defined by paired *c.fu*.



Figures 43–52. *Rugocepheus costaricensis* sp. n. Adult, (with cerotegument) SEM. **43** lateral view **44** rostral zone **45** *le* setae; **46** notogastral setae **47** notogastral cerotegumental layer, ventral view **48** anterior prodorsal zone **49** notogastral cerotegumental layer, ventrolateral view **50** cerotegumental layer **51** bothridial zone **52** ventrolateral inclined zone. Scale bars: 100 μ m (**43**); 5 μ m (**44**); 10 μ m (**45**); 5 μ m (**46**); 10 μ m (**47**); 20 μ m (**48**);10 μ m (**49**); 2 μ m (**50**); 20 μ m (**51**); 50 μ m (**52**).

A pair of elevated areas (*p.e.a*) defined by *c.fu* and *l.fu*; an unpaired semicircular elevated area (*i.s.e.a*) defined by *l.fu* and *s.c.* The *i.e.a* is devoid of setae; *p.e.a* with c_1 , *da*, *dm*, *dp*; *i.s.e.a* with c_2 , *la*, *lm lp*, h_1 , h_2 . Setae h_3 , p_1 , p_2 , p_3 situated between *s.c* and



Figures 53–61. *Rugocepheus costaricensis* sp. n. Adult, (with cerotegument) SEM. **53** ventral view **54** ad_2 setal zone **55** anal zone **56** *a* subcapitular setae **58** *h* subcapitular setae **57** subcapitulum **59** epimeral *3b* setae **60** genital zone **61** aggenital, adanal setae. Scale bars: 100 µm (**53**); 5 µm (**54**); 20 µm (**55**); 2 µm (**56**); 5 µm (**57**); 5 µm (**58**); 5µm (**59**); 20 µm (**60**); 10 µm (**61**).

b.ng. Setae c_1 , c_2 , *da*, *dm*, *dp*, *la*, *lm*, *dp*, h_1 , h_2 situated on dorsal protuberances (*d.pr*), while h_3 , p_3 , p_2 , p_1 are inserted on lateral thickenings (Figure 43); lyrifissure *im* and *gla* clearly visible (Figure 40).

Lateral region (Figures 43, 48). *Lam* (Figure 48) with elevated zone bearing *in* setae; towards anterior of *le* setae, lacking lamellar tip, forming a bridge, permitting concealment of setae; *s.tu.d* a deep depression; *tu* clearly delimited by prominent thickening; *a.tu.d.*, *p.tu.d*₁, and *p.tu.d*₂ between *tu* and *Pd I*. Rostrum beak-like. Inferior curved margin of lamella continuous with inferior bothridial part; both structures related to *s.tu.d*, permitting concealment of tarsus, tibia and dorsal area of genu and femur of leg I during leg-folding (protection mechanism). *Pd I*: large curved extended lamina. *Pd II*: small rectangular to polyhedral lamina. Humeral apophysis (*h.ap*): large polyhedral structure, conspicuous oblique posterior furrow on surface (*s.fu*); anterior *h.ap*. zone overlapping posterior part of bothridial zone. Discidium (*dis*): small triangular structure. Several large ovoid depressions behind acetabulum IV and posterolateral to genital and anal openings.

Ventral region. Epimeral zone more or less smooth with large elevations and depressions. Paraxial zone of epimera 1 and 2 with longitudinal furrow; large paraxial depression behind *bo.sj*. Epimere 4 posterior border elevated. Anterior genital furrow (*a.g.f.*) well visible (Figure 53); genital plate situated on elevated zone surrounded by furrow (Figure 60). Epimeral chaetotaxy 3-1-3-3 (Figure 53); long epimeral setae (Figure 59). Four pairs of genital setae in a single line (Figure 60). Crescent-shaped structure anterior to anal plate (Figure 55 indicated by large dot); anal plate with small sharp tip. Aggenital and adanal setae more or less similar in length (Figures 54, 61). Subcapitulum diarthric (Figure 57); three pairs of highly differing setae *a*, *m*, *h* (Figure 57). Mentum complex.

Legs (Table 2). I(1-3-3-4-16-1) (1-2-2); II(1-4-2-3-16-1) (1-1-2); III(2-3-1-2-14-1) (1-1-0); IV(1-2-2-2-12-1) (legs similar to other species, therefore not illustrated).

Remarks. Rugocepheus costaricensis sp. n. displays important differences to Rugocepheus joffrevillei Fernandez, Theron & Rollard, 2013 and R. formosus Mahunka, 2009. Principal differences: beak-shaped rostrum; distribution of furrows and elevated areas on dorsal zone of notogaster, central elevated area without setae; ventral zone with discidium differing in shape; genital and anal zone very different.

Discussion. Using SEM allows significant progress in detailed descriptions, as the small body size, morphological characteristics, and complex topology makes *Yoshiobodes* a difficult genus to study. This complexity is compounded by brief, somewhat cryptic original descriptions and illustrations. Reeves (1997), contributed much to our understanding of this genus, specifically due to studies of both adults and immatures. Reeves also originally pointed out the following characters with reference to the adult prodorsum of *Yoshiobodes*: "Dorsosejugal depression deep, slit-like, widest medially" (page 316) (in our series of papers on the revision of the family Carabodidae, this depression is designated as the "posterior prodorsal depression (p.p.d)" Fernandez et al. 2013), but this structure was not noted again until this present paper. The analysis by Reeves of the work done by Bellido (1978) is noteworthy as he analyses the depression observed on the prodorsum in protonymphs, deutonymphs and tritonymphs of *Carabodes*. Reeves (1997) indicates: "The scalloped edged depression on the prodorsum of protonymphs, deutonymphs appears similar to the foreate sclerite found in immatures described by Bellido (1978) of *Carabodes willmanni* Bernini, 1975."

The most recent generic diagnosis by Ermilov et al. 2014 is based on data from Mahunka (1986) and additions by authors, but the type specimen, *Y. irmayi* (Balogh & Mahunka, 1969) does not seem to have been studied. SEM and optical microscopy studies by Reeves (1997) on adults as well as ontogenetic studies, were also not discussed. Reeves 1997 indicated that, on comparison, "a specimen of *Y. irmayi* from St. Lucia (on loan from the Hungarian Natural History Museum) to North American material showed them to be conspecific".

For the purpose of this present paper, *Yoshioiodes* is considered only on the basis of Balogh and Mahunka (1969) (*Carabodes irmayi*) and Reeves (1997). We await further studies on type specimens of the following subgenera: *Yoshiobodes* (*Yoshiobodes*) Mahunka, 1986, type species *Carabodes irmayi* Balogh & Mahunka, 1969; *Yoshiobodes* (*Berndobodes*) Mahunka, 1986 type species, *Berndobodes spiculifer* Mahunka, 1986; *Yoshiobodes* (*Dongnaiobodes*) subgen. n. type species *Yoshiobodes hexasetosus* Ermilov, Shtanchaeva, Subías & Anichkin, 2014. As part of the ongoing revision of the Family Carabodidae (started in 2013), we have studied the type material of *Berndobodes spiculifer* Mahunka, 1986, and further information on this genus will be included in an upcoming revisionary paper.

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RESEARCH ARTICLE



DNA barcoding and morphological analysis for rapid identification of most economically important crop-infesting Sunn pests belonging to *Eurygaster* Laporte, 1833 (Hemiptera, Scutelleridae)

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Abstract

The genus *Eurygaster* Laporte, 1833 includes ten species five of which inhabit the European part of Russia. The harmful species of the genus is *E. integriceps. Eurygaster* species identification based on the morphological traits is very difficult, while that of the species at the egg or larval stages is extremely difficult or impossible. *Eurygaster integriceps, E. maura*, and *E. testudinaria* differ only slightly between each other morphologically, *E. maura* and *E. testudinaria* being almost indiscernible. DNA barcoding based on COI sequences have shown that *E. integriceps* differs significantly from these closely related species, which enables its rapid and accurate identification. Based on COI nucleotide sequences, three species of Sunn pests, *E. maura, E. testudinarius, E. dilaticollis*, could not be differentiated from each other through DNA barcoding. The difference in the DNA sequences between the COI gene of *E. integriceps* and COI genes of *E. maura* and *E. testudinarius* was more than 4%. In the present study DNA barcoding of two *Eurygaster* species was performed for the first time on *E. integriceps*, the most dangerous pest in the genus, and *E. dilaticollis* that only inhabits natural ecosystems. The PCR-RFLP method was developed in this work for the rapid identification of *E. integriceps*.

Keywords

DNA barcoding, Eurygaster, morphological analysis, PCR-RFLP, rapid identification, Sunn pests

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Introduction

The genus *Eurygaster* Laporte, 1833 includes ten species, eight of which have been found in Europe and six in Russia (Göllner-Scheiding 2006). Five *Eurygaster* species inhabit the European part of Russia; four of them are grain crop pests: *E. integriceps* (Puton, 1881), *E. maura* (Linnaeus, 1758), a nominative subspecies of *E. testudinaria* (Geoffroy, 1785), and a nominative subspecies of *E. austriaca* (Schrank, 1776). These species, in particular *E. integriceps* and *E. maura*, reproduce in high numbers on grain crops and considerably reduce crop productivity. Thus, an infestation of Sunn pests (*E. integriceps, E. maura*, and *E. testudinaria*) might result in a 20–30% yield loss for barley and a 50–90% yield loss for wheat (Gul et al. 2006). Furthermore, it greatly reduces the baking quality of the flour due to gluten degradation by proteolytic enzymes (Darkoh et al. 2010, Konarev et al. 2013).

Eurygaster integriceps is the most damaging bread wheat and durum wheat pest in western and central Asia and Eastern Europe (Radjabi 1994, Gul et al. 2006). It is wide-spread in south-eastern Europe, central Asia, and the Middle East (Fig. 1). The range of *E. maura* covers central and southern Europe (including European Russia), Caucasus, Turkey, North Africa (Algeria, Morocco, Tunisia), and central Asia (Fig. 2). *Eurygaster tes-tudinaria* is a transpalaearctic species (Fig. 3). *Eurygaster dilaticollis* is distributed in central and southern Europe (including the middle and southern territories of the European part of Russia), Turkey, central Asia, western and eastern Siberia (Göllner-Scheiding 2006) (Fig. 4). *Eurygaster dilaticollis* Dohrn, 1860 inhabits pastures and natural steppe ecosystems and feeds on grass sap. The extent of crop damage by this species has not been evaluated yet. The range of *E. austriaca* covers central and southern Europe, Caucasus, Turkey, North Africa, and central Asia (Kazakhstan). This species is rare in Eastern Europe.

The species representation and the numbers of Sunn pests constantly changes following changes in climatic conditions, structure of sown areas, and crop cultivation technologies (Critchley 1998). Global climatic changes in the future can expand the habitat of the most dangerous species, *Eurygaster integriceps* (Aljaryian et al. 2015). This creates a need for a rapid and accurate identification of *Eurygaster* species (particularly *Eurygaster integriceps*) infesting crops for the early detection of the pest in a new territories and the use of preventive measures. Until now, such identification has been based mostly on analyses of external morphological features, including male and female genitalia. This requires long-term making of microscopic preparations and study of many specimens in the samples. Moreover, specimens collected from the same area almost always contain representatives of 2–3 *Eurygaster* species, and the insignificant external morphological differences between *E. integriceps, E. maura, E. dilaticollis*, and *E. testudinaria* prevent their accurate identification (unpublished data).

Recently, molecular genetic methods, in particular DNA barcoding and phylogenetic analysis, have become very popular for revealing the taxonomic affiliation of organisms. DNA barcoding has proven itself as a valuable tool for identifying organisms (Hebert et al. 2003a, Ferri et al. 2009). It includes the amplification and sequencing of a gene fragment and its comparison with the corresponding sequences in existing databases, such



Figure 1. Range of *Eurygaster integriceps* (Puton, 1881) (after Göllner-Scheiding 2006 and Vinokurov et al. 2010).



Figure 2. Range of *Eurygaster maura* (Linnaeus, 1758) (after Göllner-Scheiding 2006 and Vinokurov et al. 2010).

as Boldsystems (http://www.boldsystems.org)and GenBank (https://www.ncbi.nlm.nih. gov/genbank). The gene commonly used for barcoding is mitochondrial cytochrome c oxidase subunit I (COI) for animals (Hebert et al. 2003b). DNA barcoding might allow



Figure 3. Range of *Eurygaster testudinaria* (Geoffray, 1758) (after Göllner-Scheiding 2006 and Vinokurov et al. 2010).



Figure 4. Range of *Eurygaster dilaticollis* Dohrn, 1860 (after Göllner-Scheiding 2006 and Vinokurov et al. 2010).

rapid identification of crop pests, which will provide the basis for differential treatment of crops. It should be noted that DNA barcoding of *E. maura* and *E. testudinaria* was carried out earlier (Park 2011).

A significant advantage of molecular methods is the possibility of identifying pests at different stages (egg or larval), i.e., when morphological identification is extremely difficult or impossible. Molecular identification might be useful for the early detection of pests on cereal crops, since the larvae of *E. integriceps* during stages I–III are difficult or impossible to distinguish from other species of the same genus.

Morphological features of *Eurygaster* species were investigated in this study. The variations in the nucleotide sequence of the COI gene of *Eurygaster* species were identified. DNA barcoding of two *Eurygaster* species has been performed for the first time on the most dangerous grain crop Sunn pests *E. integriceps* and *E. dilaticollis*, which inhabits natural steppe ecosystems. We have developed a method for the rapid identification (PCR-RFLP) of the pest *E. integriceps* based on COI sequences.

Materials and methods

Insect resources

Specimens for morphological and molecular genetic studies were collected by the authors in 2013–2015 in three regions of Russia. Specimens of E. integriceps, E. maura, and *E. testudinaria* were collected from the environments of Voronezh city (N51°40', E39°12'; altitude, 150–160 m); Specimens of *E. dilaticollis* were collected in the Teberda State Nature Reserve, north-west Caucasus (43°27'N, 41°45'E; alt., 1350-1600 m) and in the southern Ural State Reserve, southern Urals, (54°11'N, 57°37'E; alt., 285-300 m). Because of the absence of E. austriaca in our collections from cereal crops and natural ecosystems at these points in the European part of Russia during the study period, and the absence of this species as a cereal pest in the vast territory of the European part of Russia, DNA barcoding of this species has not been made by us. The collected specimens from the four species of *Eurygaster* species were stored at the Voronezh State University. Insects were collected in areas containing cereals and wild grasses with an insect collecting net. The bugs that were caught were placed individually in test tubes with 96% ethanol, labeled, and transported on the same day to the laboratory. Prior to analyses the samples were stored at - 20 °C to slow the degradation of DNA. The morphological features of *Eurygaster* species were studied using a collection of more than 800 Eurygaster specimens from different regions of Eurasia stored at the Zoological Institute of the Russian Academy of Sciences (St. Petersburg).

Morphological analysis

Specimen preparation and morphological studies were performed using an MBS-10 binocular light microscope. Photographs of the specimens were taken with a Leica DFC495 camera mounted on a Leica M205C binocular microscope. Image processing and analyses were performed using the Leica Application Suite v4.5 software. Drawings of genitalia of male *Eurygaster* species were made using a RA-6 drawing apparatus after genitalia isolation and treatment with 4% KOH (Golub et al. 2012). Morphological identification was carried out according to the previously developed identification keys (Kiritshenko et al. 1951b, Stichel 1959-1962, Kerzhner and Jaczewski 1964, Golub 1980).

DNA extraction and barcoding

DNA was isolated from the legs of the specimens with a ZR Tissue & Insect DNA MicroPrep kit (Zymo Research, USA). Voucher specimens are stored in the department of Ecology and Systematics of Invertebrates of Voronezh State University. Polymerase chain reaction was performed with an Eppendorf MasterCycler Personal cycler. Each PCR reaction mixture contained 2.5 µl of 10x reaction buffer (Evrogen, Russia), 1 µl of 10 mM dNTPs, 1 µl of 10 µM forward primer, 1 µl of 10 µM reverse primer, 3 µl of 25 mM Mg2⁺, 1 µg of template DNA, 2.5 units of thermostable Taq DNA polymerase (Evrogen, Russia), and deionized water (up to 25 µl). The PCR regime included initial denaturation at 94 °C for 3 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, elongation at 72 °C for 45 s; and final elongation at 72°C for 10 min. The primers used were: forward LepF1 5'-ATTCAACCAATCATAAAGATATTGG (Hebert 2004, Wilson 2012), reverse LepR1 5'-TAAACTTCTGGATGTCCAAAAAATCA (Hebert 2004, Wilson 2012). Also, we used EurG-f 5'-GAATATGAGCCGGAATAGTAGGA and EurG-r 5'-ATGTGTTGAAGTTACGGTCA primers, developed by us. PCR products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized with a TCP-20LM transilluminator at 312 nm. The size of the PCR products was determined using 100+ DNA length standards (Evrogen, Russia).

PCR products were purified from the agarose gel with a commercially available Cleanup Standard kit (Evrogen, Russia) and sequenced with an Applied Biosystems 3500 genetic analyzer using the BigDye Terminator v3.1 Cycle Sequencing Kit. DNA barcoding primers (LepF1, LepR1, EurG-r and EurG-f) were used for sequencing. Sequence alignment was performed with the Clustal Omega tool (http://www.ebi.ac.uk/ Tools/msa/clustalo/). Sequences were translated into amino acid sequences to verify that it was free of stop codons and gaps with EMBOSS Transeq (http://www.ebi.ac.uk/ Tools/st/emboss_transeq/). Phylogenetic analysis was carried out using Mega 6 (Center for Evolutionary Medicine and Informatics, USA) software. The sequences were truncated to 479 bp. Pairwise genetic distances between specimens were calculated using the Kimura 2 Parameter (K2P) model (Kimura 1980). The K2P model provides a substitution framework with free parameters for both transitions and transversions, accounting for the likely higher substitution rate of transitions in mitochondrial DNA. The gene tree reconstruction was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates with pairwise deletion of gaps/missing data and inclusion of all substitutions (transitions and transversions)) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths

in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 35 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% of alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 479 positions in the final dataset. Gene tree reconstruction was conducted in MEGA6 (Tamura et al. 2013). *Odontotarsus purpureolineatus* (Rossi, 1790) (Hemiptera: Scutelleridae) was chosen as outgroup. Estimates of evolutionary divergence between groups were conducted using the Kimura 2-parameter model (Saitou and Nei 1987).

Design of primers and probes

Primer and probe design for the fast identification of *Eurygaster* species was performed according to the most appropriate of the following factors: 1. primer length between 18 bp and 30 bp; 2. no distinct hairpin structure and dimers; 3. GC% from 20% to 80% for primers and probes; 4. the minimum G/C content at the 3 'end of the primers; 5. minimum identical nucleotides together in probes; 6. the 5'-end of probes must not be G; 7. PCR-product size: from 50 bp to 200 bp; 8. the annealing temperature of the probes must be at least 5 °C above the annealing temperature of the primers; 9. several SNPs (for *Eurygaster integriceps* and other species of the same genus) at the DNA-probe hybridization site.

PCR-RFLP

Analysis of suitable restriction enzymes for species differentiation was performed using theoretical diagrams of DNA digestion by enzymes, available from http://www.sibenzyme.com/products/restrictases. The PCR product was obtained with the forward (EurG-f 5'-GAATATGAGCCGGAATAGTAGGG) and reverse (EurG-r 5'-ATGT-GTTGAAGTTACGGTCA) primers that were designed according to the sequencing data. PCR products (10 μ l) were digested in the reaction mixture containing 1.5 μ l of 10X reaction buffer and 10 U of restriction endonuclease Bst2UI, AhlI and PsiI (Sibenzym, Russia) in a total volume of 15 μ l. The mixture was incubated for 2 h at 37 °C, and the enzyme was then inactivated at 75 °C for 15 min. The digestion products were visualized by electrophoresis with bromide ethidium in 2% agarose gel.

Ethics statement

The collection of *Eurygaster* pest species from the territory of Teberda State Nature Reserve (north-west Caucasus) was carried out under the agreement regarding the col-

laboration of scientific research between Voronezh State University and Teberda State Nature Reserve. The collection of *Eurygaster* pest species from the territory of Southern Ural State Reserve (southern Urals) was carried out under the agreement regarding the scientific research collaboration between Voronezh State University and Southern Ural State Reserve. These agreements include the procedures for harvesting, collection, analysis, and publishing of the obtained results for different taxonomic groups of insects, including the pests. The collection of *Eurygaster* pest species from the suburbs of Voronezh city was carried out at the "Venevitinovo", biological station, which is a structural part of Voronezh State University, in accordance with internal university bioethical rules.

Results

Specimens

184 samples of various species of bugs were collected during this study. Morphological and molecular analysis (DNA barcoding and PCR-RFLP) were performed with adult specimens that were not damaged during collection (Table 1).

Morphological studies

The morphological features of the *Eurygaster* species proposed earlier by different authors, including the co-author of the present work were used (Batzakis 1972, Golub 1980, Kerzhner and Jaczewski 1964, Vinogradova 1959), with the addition of the main morphometric features of the three most dangerous cereals pests in eastern European Russia, *E. integriceps, E. maura*, and *E. testudinaria* (Table 3). The main morphological differences between these species are shown in Table 2 and Figs 5–7.

Eurygaster austriaca significantly differs from the above-mentioned three species: the frontal part of its head clypeus is covered by jugal plates (Fig. 7A). *Eurygaster dila-ticollis* differs from other species by a short pronotum that is not much than the head (Fig. 5D).

Morphometric parameters on the base of measurements of both sexes in the samples of three cereals pests from the Voronezh Region are given in Table 3.

Morphometric parameters on the base of measurements of specimens of both sexes in the samples of three cereals pests from the Voronezh Region are given in Table 3.

DNA barcoding

DNA isolated from collected Sunn pest specimens was used for COI gene amplification. It was found that the universal primers LepF, LepF2_t1 and MHemF, commonly

z	Species	Locality, coordinates	Data of collection	Primers for DNA	Voucher number	GenBank reference, product length
1.1				LepF1/LepR1	VSU_003	KR105371.1 658 bp
1.2				LepF1/LepR1	VSU_010	KU760764.1 658 bp
1.3			June 2015	EurG-f/LepR1	VSU_Int_1	KX708594.1 576 bp
1.4		-		EurG-f/LepR1	VSU_Int_2	KX708595.1 576 bp
1.5	- - -	Voronezh city, 51°40'N, 39°12'E		EurG-f/LepR1	VSU_Int_3	KX708596.1 576 bp
1.6	E. integriceps	altitude, 120–160 m		EurG-f/LepR1	VSU_Int_4	KX708597.1 576 bp
1.7				EurG-f/LepR1	VSU_Int_5	KX708598.1 576 bp
1.8			June 2010	EurG-f/LepR1	VSU_Int_6	KX708599.1 576 bp
1.9				EurG-f/EurG-r	VSU_Int_7	KX708600.1 505 bp
1.10-1.20		Voronezh region	June 2016	None	VSU_Int_8 to VSU_Int_18	Verified morphological and PCR- RFLP
2.1		Voronezh city, 51°40'N, 39°12'E altitude, 150–160 m	June 2015	LepF1/LepR1	VSU_008	KU760762.1 658 bp
2.2	E. maura	1	June 2016	EurG-f/LepR1	VSU_Mau_2	KX708603.1 588 bp
2.3–2.12		voronezh region	June 2016	None	VSU_ Mau_3 to VSU_ Mau_12	Verified morphological and PCR- RFLP

specimens.
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Table I. C

N	Species	Locality, coordinates	Data of collection	Primers for DNA barcoding	Voucher number	GenBank reference, product length
3.1			June 2015		VSU_007	KU760761.1 658 bp
3.2				EurG-f/EurG-r	VSU_Tes_1	KX708605.1 504 bp
3.3		Voronezh city, 51°40'N, 39°12'E	71001	EurG-f/EurG-r	VSU_Tes_2	KX708606.1 505 bp
3.4	E. testudinaria	auuuuc, 170–100 III	0107 aun	EurG-f/LepR1	VSU_Tes_3	KX708607.1 573 bp
3.5				EurG-f/LepR1	VSU_Tes_4	KX708608.1 588 bp
36 215		Toursel and the	1 2016	Mana	VSU_Tes_5	Verified morphological and PCR-
(1.0-0.0			June 2010	INOILE	VSU_Tes_14	RFLP
4.1		North-West 105 Caucasus, 43°27'N, 41°45'E; alt., 1350–1600 m	June 2015	EurG-f/LepR1	VSU_009	KU760763.1 613 bp
4.2	E. dilaticollis	Southern Ural,		EurG-f/EurG-r	VSU_Dil_1	KX708601.1 502 bp
4.3-4.12		24^{-11} N $7/5/5$; E att., $285-300$ m	June 2014	None	VSU_Dil_2 to VSU_Dil_11	Verified morphological and PCR- RFLP

		ľ	Morphologica	l features		
Species	Pronotum lateral margins/ lateral angles	Presence of medial keel on scutellum/ tubercles near scutellum anterior angles	Apices of jugal plates	Female median genital plates	Number of sclerotized hooks of aedeagus	Body length, mm
E. integriceps (Fig. 5A)	Slightly convex/ rounded, not salient laterally of the base of hemelytra	Yes/yes	In the plane of clypeus apex or in- significantly above it	Almost reaching lateral margins of abdominal segment VII	4	9.8–13.0
<i>E. maura</i> (Fig. 5B)	Straight or slightly concave/ rounded, not or barely noticeable salient laterally of the base of hemelytra	No/no	In the plane of clypeus apex or in- significantly above it	Reaching or almost reaching lateral margins of abdominal segment VII	2	8.0–11.5
E. testudinaria (Fig. 5C)	Straight or slightly concave/ acuminate, slightly salient laterally of the base of hemelytra	No or barely expressed/no	Distinctly or insignifi- cantly above the plane of clypeus apex	Distinctly not reach- ing or almost reach- ing lateral margins of abdominal segment VII	4	8.0–10.5
<i>E. dilaticollis</i> (Fig. 5D)	Slightly convex, rounded/ barely noticeable salient	Yes/no	In the plane of clypeus apex	Not reaching lateral margins of abdominal segment VII	6	8.0–10.5

Table 2. Morphological features of E. integriceps, E. maura, E. dilaticollis, and E. testudinaria.

used for the identification of insects (Wilson 2012), had a very low specificity toward the isolated DNA of these insects.

658 bp length DNA sequences (Folmer region) obtained with LepF1/LepR1 primers were registered in the GenBank database under the numbers presented in Table 1. The sequences are also registered in the Bold System database with the following Barcode Index Numbers (BINs) assigned: *E. integriceps* – BOLD:AAZ6788; *E. maura* – BOLD:AAZ3231; *E. testudinaria* – BOLD:AAZ3231; *E. dilaticollis* – BOLD:AAZ3231.

Analysis of the nucleotide sequences of COI genes from the three main pests of crops in Eastern Europe, *E. integriceps*, *E. maura*, and *E. testudinaria*, has shown that the difference between the COI gene of *E. integriceps* and that of the two other species was more than 4%.

We failed to amplify the COI gene from *E. dilaticollis* when using either LepF1/ LepR1 primer pair or any of the other primer pairs commonly used for COI amplification (LCO/HCO, LCO_t1/HCO_t1, MLepF1/MLepR1, as well as combinations of these primers). The only two primer pairs that successfully produced the required PCR product were EurG-f /EurG-r and EurG-f /LepR1; however, the amplicon length in this case was shorter than 613 bp. Its nucleotide sequence was the same as those from *E. maura* and *E. testudinaria*. DNA barcoding of *E. dilaticollis* was performed for the first time.



Figure 5. Species of the genus *Eurygaster* Laporte, general view: **A** *E. integriceps* (Puton) **B** *E. maura* L. **C** *E. testudinaria* (Geoffroy) **D** *E. dilaticollis* Dohrn. Specimens **A–C** were collected in the Voronezh Region; specimen D was from the Teberda Nature Reserve, Caucasus.

Species	5	Body length; limits; average, mm	Body width; limits; average, mm	Pronotum length; limits; average, mm	Pronotum width, limits; average, mm	Body length / body width; limits; average	Pronotum width / pro- notum length; limits; average
Eurygaster	33	9.80–12.00; 10.76±0.132	6.80–7.30; 7.04±0.030	3.00–3.30; 3.13±0.018	6.70–6.90; 6.72±0.018	1.42–1.69; 1.53±0.019	2.00–2.33; 2.17±0.020
integriceps	 \$\$	11.90–13.00; 12.30±0.090	6.90–7.60; 7.24±0.042	3.30–3.60 3.46±0.018	6.60–7.00; 6.82±0.024	1.62–1.80; 1.70±0.014	1.91–2.06; 1.97±0.009
Eurygaster maura	33	8.30–10.20; 8.93±0.132	5.90–6.50; 6.21±0.036	2.40-3.00; 2.64±0.036	5.50–5.80; 5.66±0,018	1.31–1.57; 1.44±0.016	1.09–2.28 2.14±0.026
	 \$\$	8.90–11.50; 10.00±0,156	6.30–6.70; 6.47±0,030	2.70-3.10; 2.96±0.024	5.70–6.70; 5.87±0.060	1.43–1.74; 1.55±0.018	1.87–2.31; 1.98±0.026
Eurygaster testudinaria	33	8.70–9.80; 9.24±0.066	5.60–6.00; 5.92±0.036	2.70-3.10; 2.86±0.024	5.30–5.60; 5.40±0.018	1.47–1.64; 1.56±0.010	1.80–1.96; 1.89±0.010
	φç	9.50–10.50; 9.99±0.060	6.00–6.70; 6.47±0.042	2.60-3.10; 2.85±0,030	5.70–6.30 6.02±0.042	1.49–1.58; 1.54±0.006	2.00–2.18; 2.1±0.011

Table 3. Morphometric data of E. integriceps, E. maura, and E. testudinaria from Voronezh region.



Figure 6. Head, anterior view (**A**, **B**) and female median genital plates (**C**, **D**) of *E. maura* L. (**A**, **C**) and *E. testudinaria* (Geoffroy) (**B**, **D**).



Figure 7. Structural details of *Eurygaster* Laporte species (a, clypeus; b, jugal plate): A *E. austriaca* (Schrank), head, dorsal view B *E. integriceps* (Puton), dorsal view C *E. integriceps*, aedeagus D *E. maura* L., aedeagus E *E. testudinaria* (Geoffroy), aedeagus (after Golub, 1980, with changes).

A Neighbor-joining (NJ) tree was shown to be a useful clustering method for large datasets (Yang and Rannala 2012, Tamura et al. 2004). We have reconstructed a phylogenetic tree that reflects genetic distances between *Eurygaster* species using Kimura 2-parameter algorithm and the COI gene sequences of *Eurygaster* species obtained by us as well as all *Eurygaster* species sequences available in the GenBank database (Fig. 8).

The genetic distance between the *E. integriceps* species and the group species that includes the 3 species (*E. maura*, *E. testudinaria* and *E. dilaticollis*) was 0.049. The genetic distance between the *E. integriceps* species and *E. austriaca* was 0.121. The within-group mean distance for *E. integriceps* was 0.007, for *E. maura* 0.001, and for *E. testudinaria* it was 0.002.

Development of a PCR method for the rapid identification of *E. integriceps*

Considering the fact that the COI nucleotide sequence of *E. integriceps* differs significantly from those of *E. maura* and *E. testudinaria*, a method for its rapid identification



Figure 8. Neighbor joining analysis of COI gene sequences from *Eurygaster* species. * - sequences obtained in this work.

has been developed using an analysis of the nucleotide regions of cytochrome oxidase (COI) and two identification methods have been tested: PCR with TaqMan probes and PCR-RFLP (Restriction Fragment Length Polymorphism). Conservative DNA sequences within each species were identified. First, two sets of PCR primers and probes were developed by identifying the SNP-carrying fragments within the COI gene sequence as sites for probe and primer annealing (Table 4).

Species		Primer/probe set	
E. maura E. testudinaria E. dilaticollis	6 . 1	forward primer: MTI-f 5'-AGCAGGTGTTTCCTCAATCTTAG Probe: FAM-ACCCATTGGTATAACACCTGAACGAACCCCA-BHQ1 Reverse primer: MT-r 5'-AGTAATAATGCGGTAATTCCAACTG Product length – 129 bp	
E. integriceps	Set I	forward primer: MTI-f 5'-AGCAGGTGTTTCCTCAATCTTAG Probe: FAM-CGACCCGTTGGTATAACACCTGAACGGATCC-BHQ1 Reverse primer: I-r – 5'-AGTAATAATGCAGTAATTCCAACTG Product length – 129 bp	
E. maura E. testudinaria E. dilaticollis		MT1-f: 5'-ATCAGTTGGAATTACCGCATTATTA Probe: FAM-TACTACTATCATTGCCAGTACTAGCCGGAGC-BHQ1 Reverse primer: MTI1-r – 5'-ATGTGTTGAAGTTACGGTCA Product length – 95 bp	
E. integriceps	- Set 2	I1-f: 5'-ATCAGTTGGAATTACTGCATTATTA Probe: FAM-TGCTACTATCACTACCAGTACTAGCAGGAGC-BHQ1 Reverse primer: MTI1-r: 5'-ATGTGTTGAAGTTACGGTCA Product length – 95 bp	

Table 4. Primer/probe set for species identification.

Table 5. Restriction enzymes for PCR-RFLP and expected lengths of the 585 bp COI fragment cleavage products.

Restriction enzyme	Recognition site	Fragments for <i>E. integriceps</i> , bp	Fragments for <i>E. maura</i> / <i>E. testudinaria</i> / <i>E. dilaticollis</i> , bp
Bst2UI	CCWGG	364, 221	585
PsiI	TTATAA	435, 150	435, 91, 59
AhlI	ACTAGT	317, 268	317, 175, 93

Despite optimization of PCR conditions (temperature, DNA template concentration, primer/probe concentrations), we failed to achieve 100% species-specific identification for either *E. integriceps* or *E. maural E. testudinaria*. Overall, out of nine PCR reactions, non-specific primer and probe annealing (i.e. annealing of primers and probe specific for one of *Eurygaster* species on DNA of other species) was observed in two reactions.

Another method for the express identification of *E. integriceps* is PCR-RFLP. Preliminarily, COI nucleotide sequences were analyzed from various *Eurygaster* species for the presence of restriction enzyme sites that would be different in these species and produce cleavage products suitable for electrophoretic analysis in agarose gel. The possibility of using more than 100 restriction enzymes was examined and three restriction enzymes were chosen. The reaction products for these enzymes are well separated in agarose gel and have specific patterns for the *E. maural E. testudinarial E. dilaticollis* and *E. integriceps* considering intraspecific variability. The selected restriction enzymes are shown in Table 5.

To obtain a PCR fragment for restriction analysis forward (EurG-f 5'-GAATAT-GAGCCGGAATAGTAGGG) and reverse (EurG-r 5'-ATGTGTTGAAGTTACG-GTCA) primers were used that yielded a 585-bp PCR product. The primers LepF1/LepR1 could not be used in this case because of the low specificity of the LepF1 primer



Figure 9. Restriction fragments of COI PCR products (restriction enzyme, species): 1 AhlI, E. maura or E. testudinaria 2 AhlI, E. integriceps 3 PsiI, E. maura or E. testudinaria 4 PsiI, E. integriceps 5 Bst2UI, E. maura or E. testudinaria 6 Bst2UI, E. integriceps; M, 100 bp DNA ladder.

for *Eurygaster* species. Cleavage of the obtained PCR product resulted in DNA fragments of predicted sizes for all tested species (Fig. 9).

Eight specimens from each *Eurygaster* species were analyzed by this method and any of the restriction enzymes could be successfully used for identification of *E. integriceps*.

Discussion

The differences in the sequences of COI gene from *E. integriceps* and other closely related species largely correlate with the morphological differences between these species (Table 1). The body of *E. integriceps* is, on average, larger with slightly rounded lateral edges of the pronotum (Fig. 5). The observed higher intraspecific variation of the COI nucleotide sequence in *E. integriceps* is possibly associated with its significant migratory activity during the periods of preparation for the winter diapause and the exit from it. Such migrations can occur over large distances (up to dozens of kilometers) and can result in mating between organisms from different populations after wintering (Critchley 1998). This might contribute considerably to the exchange of genes between populations.

The similarity between COI nucleotide sequences of *E. maura* and *E. testudinaria* correlates with the high levels of morphological similarity between these species (Table 1). The high variability of external features (especially morphological characteristics of the head, which can often be present in both species) does not allow for the definite identification of specimens from either species. *Eurygaster maura* and *E. testudinaria* can be distinguished based on the number of sclerotized hooks inside the aedeagus. This difference in the fine structure of male genitalia is a result of evolutionary processes aimed at preventing interspecific hybridization. However, in practical terms, species identification based on the internal structure on the aedeagus is difficult at best, if populations are mixed, it is the only way to identify the species. It should be noted that the variability of external morphological characteristics within each of the

three main harmful species is high enough to separate them (Table 3). Therefore, for accurate determination of species it is necessary to examine the external features of a series of specimens as well as the characteristics of the genitalia. Accurate morphological identification of the adults of *Eurygaster* species is possible; however, it requires a large number of *Eurygaster* specimens without admixture of another species.

It appears that resolution of the classic DNA barcoding is not sufficient for distinguishing some species with small differences between the two species such as structure of genitalia. Indeed, it is known that DNA barcoding is not always capable of differentiating between closely related species (Whitworth 2007, Will and Rubinoff 2004, Meyer and Paulay 2005). Although it is important to search for other molecular genetic markers for definite identification of *E. maura* and *E. testudinaria*, differentiation between these two species is currently not relevant, since the deleterious effect of both species in southern and Eastern Europe and Asia is much lower compared to that of *E. integriceps*.

The obtained tree has two clearly distant branches. The first one includes five Palaearctic species, *E. integriceps, E. maura, E. testudinaria, E. dilaticollis.* The second branch includes one Nearctic species, *E. amerinda* Bliven, 1956. The genetic distance between these two groups clearly reflects continental disjunction and autochthonous morphogenetic processes that took place within the same genus on two different continents during the Cenozoic. Within the Palaearctic group, a subgroup including *E. maura, E. testudinaria*, and *E. dilaticollis* are genetically similar to each other. *Eurygaster maura* and *E. testudinaria* are not always distinguishable. *Eurygaster integriceps* belongs to a separate phylogenetic branch that is closer to the first three species than *E. austriaca* (data not present on tree). The latter is the most distant species, both genetically and morphologically, from the analyzed Palearctic species (Table 1, Figs 5–7). High intraspecific variability was shown for *E. integriceps*. This is consistent with the previous data on the high intraspecific variability postulated in some species of the order Hemiptera (Raupach et al. 2014).

Under the conditions in Eastern Europe and especially the vast territory of southern Russia, Ukraine, central Asia, *E. integriceps* is the most xerophilous and thermophilic species of *Eurygaster* (Critchley 1998). During the emergence of larvae in the early growing season, populations may be represented by several species of this genus and are not easily differentiated. However, the prevalence of *E. integriceps* species is likely to increase much more rapidly than that of other species. In this regard, in order to predict the size of the main *E. integriceps* pest population and prepare the proper treatment with pesticides (earlier treatment with pesticides is needed when *E. integriceps* is identified), monitoring their development and proliferation is necessary. Analyzing the proliferation and the activity of other pest species of the genus *Eurygaster* would not be so important, due to their much lower abundance and less damaging habits. The advantages of the developed PCR-RFLP method for the express identification of *E. integriceps* are its reproducibility, simplicity, and low cost of analysis. It should be noted that this is only a preliminary result and requires tests in populations of Sunn pests from other areas. The early detection of *E. integriceps* in crops as their primary pest is important in connection with the potential expansion of its habitat, due to global climate change (Aljaryian et al. 2015). Rapid detection of this pest in the new territories will prevent additional loss of yield and, to a certain extent, slow down its invasion and expansion into other areas. A platform for the identification of the pest *Eurygaster integriceps* based on PCR-RFLP that was developed in this study will allow the express detection of the presence of the pest in new areas and avoid false positives results.

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RESEARCH ARTICLE



A new species of *Trachymyrmex* (Hymenoptera, Formicidae) fungus-growing ant from the Sierra Madre Oriental of northeastern Mexico

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Abstract

Here we describe a new species of *Trachymyrmex*, *T. pakawa* **sp. n.**, from the Gran Sierra Plegada range of the Sierra Madre Oriental, in the states of Coahuila and Nuevo Leon, northeastern Mexico. *Trachymyrmex pakawa* is a large-sized species compared to other North American *Trachymyrmex*. Its geographic distribution includes the piedmont of the Gran Sierra Plegada at La Estanzuela, Monterrey, as well as peripheral mountains segregated from the Sierra Madre Oriental (Cerro de las Mitras, Sierra de Zapalinamé, Cañon de San Lorenzo, Cerro de las Letras). The preferred habitats of *T. pakawa* include oak-pine forest at La Estanzuela, xeric oak forest at Zapalinamé and mesic Chihuahuan desert scrub with sotol (*Dasylirion*) at other sites. All localities are on slopes, on very rocky, shallow lithosols overlaying large boulders. This species nests under and between large boulders and rocks. It has not been observed on alluvial or better developed, deeper soils, and it is absent from sites with human activity (urban, disturbed, and landscaped areas). It is closely related to and morphologically similar to *Trachymyrmex smithi*. The known distribution ranges of *T. pakawa* and *T. smithi* almost overlap in Saltillo, Coahuila state. The main character that distinguishes the new species from *T. smithi* is longer antennal scapes in *T. pakawa*; also, different nesting

habits (rocky slopes vs. alluvial sites or deep sand in *T. smithi*), and geographic distribution. Phylogenetic analysis of DNA sequences from the mitochondrial marker *cytochrome c oxidase subunit I* (*COI*) and the first intron of the F1 copy of the nuclear protein-coding gene Elongation Factor $1-\alpha$ (*EF1-\alpha-F1*) confirm a sister-species relationship between *T. pakawa* and *T. smithi*. Bayesian coalescent analyses indicate a divergence time of about 8.00 million years before present (95% confidence interval: 4.8–11.5 mya) between *T. pakawa* and *T. smithi*. The divergence of the lineages of *T. pakawa* and *T. smithi* could have been driven by the Pliocene-Holocene desertification of southwestern North America. This process resulted in isolated mesic refugia and forests in the Madrean ranges and piedmonts of northeastern Mexico (the current habitat of *T. pakawa*) while *T. smithi* adapted to the deeper, often sandy soils on the drier desert plains of Coahuila and Chihuahua states in Mexico, and New Mexico and Texas in the USA. Within the Nearctic species of the *Trachymyrmex septentrionalis* species group, *T. pakawa* is the species that is closest (by geographical distribution) to Neotropical species of *Trachymyrmex* like *T. saussurei*.

Keywords

Insecta, Attina, Myrmicinae, symbiosis

Introduction

Trachymyrmex Forel is a New World genus of fungus-growing ants closely related to the genera *Atta* and *Acromyrmex*, the well-known leaf-cutting ants (Schultz and Brady 2008). The three genera share similar life-history traits, but *Trachymyrmex* is less derived than the leaf-cutting ants. One major ecological difference between *Trachymyrmex* and the leaf-cutting ants is that *Trachymyrmex* species generally are functional saprotrophs and decomposers; workers usually forage for insect feces, inflorescences, and fallen leaflets as substrate for their symbiotic fungus (Johnson et al. 2006, Sanchez-Peña 2010), while leaf-cutting ants use mainly green, fresh leaves and fresh flowers, harvested from plants, as substrates for cultivation (Weber 1972).

The genus *Trachymyrmex* is considered to have originated in tropical South America (Weber 1972, Mueller and Rabeling 2008); however, there has been at least one large species radiation of the genus in temperate North America, probably related to the Great American Interchange (Bagley and Johnson 2014) or even before the closure of the isthmus of Panama (Philip Ward, pers. comm.). This radiation resulted in about 12 known species that have successfully colonized diverse ecosystems including dry, warm-temperate habitats in the Sonoran and Chihuahuan deserts, and the oak and pine forests of the southeastern and eastern USA, reaching New York state (Weber 1972, Rabeling et al. 2007).

Phylogenetic relationships among the North American species of *Trachymyrmex* and their phylogenetic relationships with the more numerous South American taxa are not clear. Based on analyses of morphological and molecular data for North American species, Rabeling et al. (2007) acknowledged the possible paraphyletic nature of this taxon assemblage. Based on morphological characters and a multilocus phylogenetic analysis, herein we describe a new species of the genus *Trachymyrmex* from limestone-derived montane landscapes in the semiarid Nearctic of northeastern Mexico.

Materials and methods

Abbreviation of depositories

ASU-SIBR	Social Insect Bio Repository, Arizona State University, Tempe, Arizona, USA
CASC	California Academy of Sciences, San Francisco, California, USA
UNAM	Colleción Nacional de Insectos, Instituto de Biología, UNAM, Ciudad
	de México, México
UAAAN	Departamento de Parasitología, Universidad Autónoma Agraria Antonio
	Narro, Saltillo, México
USNM	National Museum of Natural History, Smithsonian Institution, Wash-
	ington, DC, USA

Field research

Specimens (workers and queens) were manually collected during daylight at three localities in the Mexican states of Nuevo Leon and Coahuila (Table 1), specifically at the eastern and western edges of the northern Gran Sierra Plegada: south and west of the city of Monterrey, Nuevo Leon, and in the municipality of Saltillo, Coahuila. One nest was excavated at Lomas de Lourdes, Saltillo, Coahuila, on September 3, 2014. Approximately 200 workers, one queen, and a substantial amount of fungus garden were collected.

The geographic distribution map (Figure 1) and Table 5 provide detailed information about the localities where *T. pakawa* was collected. A single queen (dealate female) was collected on the soil surface at La Estanzuela, Nuevo Leon, and its measurements are also reported herein.

Morphological analysis

Morphological characters from field-collected specimens were examined with a SMZ-168TL Motic stereoscope (Motic Inc., Hong Kong) at 50x magnification and greater. Measurements of Head Length (HL), Head Width (HW), Scape Length (SL) and Mesosomal Length (ML) (all in mm) as defined by Rabeling et al. (2007) were obtained using a Mitutoyo Digimatic 500-195-30 digital caliper with a resolution of 0.01 mm under the stereoscope. Cephalic Index (CI) = HW*100/HL and Scape Index (SI) = SL*100/HW were also calculated (Rabeling et al. 2007). Means were calculated from a total of 22 individual workers.

Photographs were taken with a Leica MZ16 APO dissecting microscope (Leica Microsystems, Wetzlar, Germany) using a ProgRes 3008 digital camera mounted on the microscope. Images were taken with the PictureFrame 2.3 software package (Optronics, Goleta, CA). Sequential images were stacked to final 3D form with



Figure 1. Geographical distribution of *Trachymyrmex pakawa* n. sp. and its closest relative, *Trachymyrmex smithi*, in the southwestern USA and northern Mexico (from Rabeling et al. 2007 and this study). Red stars: collection records of *T. pakawa*. Blue circles: reported collection points of *T. smithi*. Yellow area, Chihuahuan desert. Green area, Sierra Madre Oriental mountain range.

Helicon Focus software (Helicon Soft, Ltd., Kharkov, Ukraine). Minor corrections (color, background, etc.) were made using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA USA).

Molecular methods

Initially (in 2010) total genomic DNA was extracted from 1 to 3 worker ants of *T. pakawa* and *T. smithi* following the lithium chloride method (Huang et al. 2000), and *cytochrome oxidase subunit I (COI)* was amplified for initial bioinformatic analysis (below). Subsequently, for both *COI* and Elongation Factor $1-\alpha$ (*EF1-\alpha-F1*) analysis, total DNA was extracted from single worker ants of both species (n = 3 each) using the Pure LinkTM Genomic DNA Mini Kit (Invitrogen Carlsbad, CA USA. Identical *COI* sequences were obtained with both methods (see below). Specimens used for analysis were collected at the localities indicated in Table 1.

PCR amplification of the COI gene fragment (marker) was carried out (Jacobson et al. 2006) in a volume of 25 ml consisting of 2.5 ml of 10× PCR buffer, 0.75 ml of 50 mM MgCl., 1.25 ml of 2.5 mM dNTPs, 1.0 ml of genomic ant DNA, 1.3 ml of 13.6 mM CI-J2195 primer, 1.0 ml of 15.2 mM Jerry Garcia-CI primer (Jacobson et al. 2006), 0.3 ml of TaqDNA polymerase, 5U/ml (all reagents from Invitrogen, Carlsbad CA USA), and 16.9 ml of MilliQ water. PCR amplifications were performed at 95 °C (1 min), 35 cycles of 94 °C (1 min each), 43 °C (1 min), 70 °C (2 min), and 70 °C (2 min). PCR amplification of the nuclear gene fragment of the first intron of the $EF1-\alpha$ -F1 was carried out using the primer pairs U52.1 and L53 as described by Rabeling et al. (2007), in a volume of 25 ml consisting of 2.5 ml of 10X PCR buffer, 0.75 ml of 50 mM MgCl₂, 0.25 ml of 20 mM dNTP's, 1.0 ml of genomic ant DNA, 0.5 mM of U52.1 and L53 respectively, 0.3 ml of Taq DNA polymerase (5U/ml) (all reagents from Invitrogen, Carlsbad CA USA), and 19.2 ml of MilliQ water. PCR amplifications were performed at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 30 sec, annealing at 51 °C for 45 sec, extension at 72 °C for 90 sec, and a final extension of 72 °C for 10 min. Amplification products were visualized on 1.2% agarose gel stained with ethidium bromide.

Nucleotide sequencing and purification of PCR fragments were performed at Macrogen Corporation USA (Rockville, MD) using CI-J2195, Jerry Garcia-CI, U52.1, and L53 primers for sequencing.

Phylogenetic Analysis

The raw sequences were edited in CodonCode aligner (CodonCode Corporation). We included in our dataset previously published sequences; see Table 1 for a complete list of accession numbers of sequences used and the novel ones obtained in this study. Sequences were aligned using CLUSTALW implemented in Mega 6.0 (Tamura et al. 2013). Our final concatenated alignment comprises the partial sequences of the *COI* marker (313 bp) and *EF1*- α (715 bp) marker for a total of 1028 aligned sites and 131 parsimony-informative sites.

We submitted four data blocks to PartitionFinder v1.1.1 (Lanfear et al. 2012) as follows: first, second, and third positions of COI and the intron region of EF1-a-F1. Based on the PartitionFinder analysis under the Bayesian information criterion (BIC) and the "all" search algorithm, our dataset was divided into two partitions: 1) First

Section	GenBank, Acces	sion number	Logality	
species	COI	<i>EF1-α-F1</i> intron	Locality	
T. pakawa_NL1	MF669548	MF678563	Cerro de las Mitras, Monterrey	
T. pakawa_NL2	MF669549	MF678564	Estanzuela, Monterrey	
T. pakawa_NL3	MF669550	Not obtained	Estanzuela, Monterrey	
T. pakawa_COAH1	MF669551	MF678565	Lomas de Lourdes, Saltillo	
T. pakawa_COAH2	MF669552	Not obtained	Lomas de Lourdes, Saltillo	
T. smithi T1	EF539726.1	EF539778.1	Brewster, Texas	
T. smithi T61	EF539727.1	EF539779.1	La Rosa, Coah. Mexico	
T. smithi T62	EF539728.1	EF539780.1	La Rosa, Coah. Mexico	
T. smithi T73	EF539729.1	EF539781.1	La Rosa, Coah. Mexico	
T. smithi T87	EF539730.1	EF539782.1	El Paso, Texas	
T. smithi T88	EF539731.1	EF539779.1	Doña Ana, New Mexico	
T. smithi T89	EF539732.1	EF539784.1	Doña Ana, New Mexico	
T. arizonensis	EF539739.1	EF539791.1	Cochise Co., Arizona	
T. carinatus	EF539754.1	EF539806.1	Cochise Co., Arizona	
T. desertorum	EF539748.1	EF539800.1	Gila Co., Arizona	
T. jamaicensis	DQ353390.1	EF539829.1	Voucher RA0247, MCZ*	
T. nogalensis	EF539759.1	EF539811.1	Cochise Co., Arizona	
T. pomonae	EF539785.1	EF539779.1	Cochise Co., Arizona	
T. septentrionalis	EU561588.1	EF539813.1	Prattville, Alabama	
T. turrifex	EU561529.1	EF539815.1	Austin, Texas	

Table 1. *Trachymyrmex* species, accession numbers of *COI* and intron of $EF1\alpha$ -F1 fragment sequences used in the Bayesian phylogenetic and haplotype network analyses, and collection localities of *T. pakawa* and *T. smithi* specimens analyzed.

* Museum of Comparative Zoology.

and second positions of COI and $EF1-\alpha$ and 2) third positions of COI. For the first partition the best model was General Time Reversible with invariant sites and a gamma distribution (GTR+I+G) and for the second partition the best model was GTR+G. We conducted a Bayesian phylogenetic analysis employing MrBayes v3.2.5 (Ronquist et al. 2012), with nucmodel= 4by4, nruns = 2, nchains = 4, and sampled freq = 100. Nodes that had posterior probabilities greater than 0.95, were considered well supported. Additionally, a parsimony method to produce a median joining network was used to visualize genetic distance and the geographic associations among *T. pakawa* and *T. smithi* haplotypes using PopArt v.4.6 (http://popart.otago.ac.nz).

Divergence dates between *T. pakawa* and *T. smithi* were estimated using the mitochondrial data with the Bayesian program BEAST v1.4.8 (Drummond and Rambaut, 2007) following the procedure implemented by Seal et al. (2015) for timing the evolutionary events in *T. septentrionalis*. We applied a lognormal relaxed clock, a Yule process tree prior, a UPGMA starting tree, and a TN93 substitution model (Topaliv2.5). The clock model was estimated from a normal distribution with a mean of 0.075 substitutions/sites/lineages/myr. Three independent experiments were performed for 20 million generations and sampled every 2000 generations, with 10% of the trees discarded (burn-in). The output file was evaluated with TRACER v1.4.8 and the effective sample sizes exceeded 200 (Drummond and Rambaut 2007).

The evolutionary distances were computed using the *p*-distance method and represent the proportion of nucleotide changes calculated in MEGA v6 (Tamura et al. 2013). Standard errors of mean *p*-distances among taxa were calculated using 1000 bootstrap replicates.

Results

Trachymyrmex pakawa Sanchez-Peña, Chacón-Cardosa, Canales-Del Castillo & Reséndez-Pérez, sp. n.

http://zoobank.org/9D80EDC0-EF65-4EED-8CA8-D413F120997C (Figures 2–4)

Type material investigated. Holotype worker: MEXICO, Saltillo, Coahuila; Lomas de Lourdes, 25.365181°N, 100.983217°W, 15.viii.2012, dry oak forest, ex ground (S. R. Sanchez-Peña). Collection code: UAN446. Specimen code: USNMENT01125073 (USNM).

Paratypes. Additional specimens (workers) with the same collection information as the holotype deposited at ASU-SIBR, CASC, UAAAN, UNAM and USNM.

Additional material. Additional material: MEXICO, one worker. Cerro de las Mitras, Monterrey, Nuevo León, 25.704834°N, -100.397027°W, 24.viii.2008. Foragers on montane chaparral (S. R. Sanchez-Peña) (UCDC, Philip Ward).

Diagnosis (worker). Similar to *Trachymyrmex smithi* but antennal scapes clearly longer, resulting in Scape Index values of 93–109 (Figure 2); head not as clearly cordate, nor broader than long; color dark reddish brown or rarely light ferruginous red. See Measurements in Table 2.

Description. Head trapezoidal, weakly cordate (Figure 2); HW= 1.13, HL= 1.1 (mm) (holotype), nearly square or slightly rectangular, very slightly broader than long; head widest at midpoint between the eye and the posterior corner, and tapering anteriorly. Posterior margin of head is only moderately concave, slightly notched.

Vertex of head and gaster moderately tuberculate, more markedly spinulose than *T. smithi*; tubercles thin and resembling spines, base of tubercles not confluent as in *T. smithi*. Tubercles on preoccipital lobes almost as long as preoccipital spines. Supraocular projections absent. Discal area of mandibles finely striated.

In full-face view, the frontal lobes are small, broadly triangular, usually asymmetrical, with anterior margin longer than posterior (Figure 2).

The margin of the frontal lobes is sub-triangular, smooth, and not crenulated; the base of the frontal lobes lacks projections. Anterior and posterior margin of frontal lobes straight.

Base of antennal scapes lacking lobe. Anterior surface of antennal scapes smooth or weakly microtuberculate. Antennal scapes long, surpassing the posterior corner of head by more than twice their maximum diameter.



Figure 2. Full face (top row), lateral (middle row) and dorsal (bottom row) views of *Trachymyrmex pakawa* and *Trachymyrmex smithi*. Left column, *T. pakawa*. Right column, *T. smithi*. Scale bars: top, 0.5 mm; middle and bottom, 1.0 mm.



Figure 3. Nest entrance of *T. pakawa*. Cerro de las Letras, Saltillo, Coahuila.

Frontal and preocular carina ending separately. In full-face view, frontal carina extends almost to posterior corners, but weakening before reaching vertex. Preocular carina well developed, crossing nearly half distance between eye and frontal carina, curving mesad towards, but not reaching, the frontal carina; frontal carina faintly reaching posterior cephalic margin, forming weakly developed, closed antennal depressions ("scrobes") without apical tubercles.

Tubercles of gaster and mesosoma small, tubercular setae are weakly to strongly recurved; tubercles on sides of mesosoma minuscule and sparse (Figure 2). Pilosity of gaster and femora consists of hairs only, lacking fine pubescence. Texture of body surface rough, sandpaper-like. In T. *pakawa* the-color is dull reddish to (almost always) dark reddish to dark brown, but unlike *T. smithi*, no blackish specimens have been observed. Dorsal projections of mesosoma are multituberculate swellings, tooth- or spine-like (Figure 2). Two median pronotal projections are present, appearing as ridges or multituberculate swellings. The inferior pronotal corner has a rounded, blunt, weakly developed, projecting tooth. Anterior median promesonotal tubercles short, vertical, tooth-like in frontal or posterior view. The anterior mesonotal projections are microscopically multituberculate or multidentate swellings, especially in Saltillo collections; they are nearly as long as pronotal lateral projections in La Estanzuela (Monterrey) but notably longer than pronotal lateral ones in material from Saltillo. Posterior mesonotal projections present, shaped as a ridge or multituberculate tooth or tumulus. Pilosity of mesopleura consisting of approxi-

	Head Length (HL)	Head Width (HW)	Cephalic Index (CI) (HW*100/HL)	Scape Length (SL)	Scape Index (SI)(SL* 100/HW)	Mesosomal Length (ML)
WORKER	0.975-1.23	1.0-1.375	97.5–116	1.05-1.26	93.5-109.5	1.35-1.71
T. pakawa	$\bar{x} = 1.080$	$\bar{x} = 1.115$	$\bar{x} = 103.25$	$\bar{x} = 1.146$	$\bar{x} = 103.00$	$\bar{x} = 1.548$
WORKER T. smithi	0.94–1.25	1.0–1.375	100-111	0.86–1.19	84–89	1.25–1.69

Table 2. Dimensions (mm) of morphological features of the worker caste of *Trachymyrmex pakawa* (n = 22) and *Trachymyrmex smithi*

Table 3. Dimensions (mm) of morphological features of the queen caste of *Trachymyrmex pakawa* (n = 1) and *Trachymyrmex smithi*

	HW	HL	CI= HW*100/HL	SL	SI = SL*100/HW	ML
QUEEN T. pakawa	1.28	1.235	103.6	0.99	77.3	1.89
QUEEN T. smithi	1.35	1.2	113–114	1.05–1.1	78–79	1.9–2.0

mately twelve short, pale (not reddish) thin curved hairs, about half as long as hairs on mesosomal projections. Projections on inferior and superior margin of mesopleura absent.

The propodeal teeth are strongly divergent, spine-like, and longer than distance separating their bases; the teeth are longer than any promesonotal projections, and longer than projections of basal face. The petiolar node has one pair of teeth in specimens from La Estanzuela, and two (rather clearly) defined pairs of teeth in specimens from Saltillo. Petiolar node from above is as long as broad. Postpetiole from above is distinctly wider than long; the posterior border of postpetiole is notably excised.

Etymology. From the name of an ancient, vanished Native American tribe that used to live in the same general area of arid northeastern Mexico, where *Trachymyrmex pakawa* is known to occur.

Distribution. From warm-temperate forest and scrubland habitats at the northern Sierra Madre Oriental range in the Mexican states of Coahuila and Nuevo León: more specifically, in the northern Gran Sierra Plegada range and mountains between the cities of Monterrey and Saltillo. This species has also been collected in the mountains in the municipality of Iturbide, Nuevo Leon, near coordinates 24.721111, -99.896389, about 100 km to the south of Monterrey.

Results and discussion: Molecular analysis

We identified two *T. pakawa* haplotypes for each marker (*COI* and *the EF1-\alpha-F1*) (Figure 4). A phylogenetic tree was constructed using Bayesian analyses (Figure 4A) of previously published *Trachymyrmex* sequences and sequences of *T. smithi* and *T. pakawa* obtained for this work (Table 1). The *T. smithi* specimens from Ojinaga, Chi-

huahua, Mexico, yielded *COI* and *EF1-\alpha-F1* sequences 100% and 99.99% identical to *T. smithi* Genbank accessions EF539730 and EF539779.1, respectively, from New Mexico, USA; these (New Mexico) and additional *T. smithi* Genbank sequences for both markers were used in our molecular analysis (Table 1). Our phylogenetic tree (Fig. 9A) is consistent with the unrooted tree of Rabeling et al. (2007). From the analyses we inferred that *T. pakawa* is a member of the *septentrionalis* species group, and that *T. pakawa* and *T. smithi* are sister species. The *T. pakawa -T. smithi* clade is most closely related to the clade consisting of *T. septentrionalis* and *T. pomonae*. These four species are the sister group of a clade consisting of *T. arizonensis*, *T. carinatus*, *T. desertorum*, and *T. nogalensis* (Figure 4); the monophyletic group including these eight species belongs to the *septentrionalis* group (Rabeling et al. 2007). The remaining two species, *T. jamaiciensis* and *T. turrifex*, are more distantly related to the members of the *septentrionalis* group and belong to the *jamaicensis* and *urichii* groups.

Haplotype network maps indicate the genetic distance between *T. smithi* and *T. pakawa* (Figure 4B). For *COI*, there is a difference of > 40 base pairs (bp) between the two species. For the more slowly evolving nuclear *EF1*- α marker the nucleotide difference between the two species is 4 bp.

In our pooled analysis of North American *Trachymyrmex* taxa (Rabeling et al. 2007), the average genetic distance between pairs of species for the *COI* marker without *T. pakawa* is 0.139 (SE = 0.012), whereas the average genetic distance for *COI* between *T. pakawa* and the remaining taxa is comparable: 0.132 (SE = 0.014). The distance between *T. pakawa* and *T. smithi* (0.12) is larger than the distance between *T. smithi*–*T. arizonensis* (0.11) and *T. smithi*–*T. carinatus* (0.10). These data support the hypothesis that *T. pakawa* is genetically distinct and reproductively isolated from other *Trachymyrmex* species.

For the EF1- α marker, the same patterns are present (although overall distances across all ant taxa considered are much smaller), with the distance between *T. pakawa* and *T. smithi* (0.002) larger than the distance between *T. smithi*-*T. pomonae* (0.000) (Table 4). But besides the genetic distances, unique insertions and deletions for *T. pakawa* (not shared with the other species) were observed in *EF1*- α .

The pooled analysis for the concatenated *COI-EF1*-a sequences indicated that the average genetic distance between pairs of *Trachymyrmex* species without *T. pakawa* is 0.082 (SE = 0.006), whereas the average distance between *T. pakawa* and the remaining taxa is comparable: 0.066 (SE = 0.005). Also the distance between *T. pakawa* and *T. smithi* (0.04) is similar to the distance between *T. smithi* and other species (0.046–0.054), thus supporting the new species status of *T. pakawa* (Table 4).

In general, the larger genetic distances among these North American *Trachymyrmex* species and clades are always between [*T. turrifex* or *T. jamaiciensis*] and the remaining taxa including *T. pakawa*.

Both phylogenetic and network analyses showed the same relationships among *T. smithi* and *T. pakawa*. No shared haplotypes were found between *T. smithi* and *T. pakawa* (Figure 4B). For *COI*, there is a difference of 34 base pairs (bp), whereas for *EF1-* α the difference is 3 bp (Figure 4B). Considering the small number of specimens analyzed

		COI -EF1- <i>α</i> -F1								
		1	2	3	4	5	6	7	8	9
1	T. turrifex									
2	T. jamaicensis	0.09								
3	T. pomonae	0.12	0.12							
4	T. arizonensis	0.13	0.11	0.05						
5	T. desertorum	0.14	0.13	0.06	0.06					
6	T. carinatus	0.13	0.13	0.05	0.05	0.04				
7	T. nogalensis	0.13	0.13	0.05	0.05	0.04	0.03			
8	T. septentrionalis	0.12	0.12	0.04	0.05	0.07	0.06	0.05		
9	T. smithi	0.13	0.12	0.05	0.05	0.06	0.05	0.05	0.05	
10	T. pakawa	0.13	0.12	0.04	0.06	0.06	0.05	0.05	0.04	0.04
						COI				
	T. turrifex									
	T. jamaicensis	0.19								
	T. pomonae	0.17	0.14							
	T. arizonensis	0.19	0.12	0.11						
	T. desertorum	0.22	0.15	0.14	0.12					
	T. carinatus	0.17	0.15	0.11	0.11	0.11				
	T. nogalensis	0.19	0.16	0.12	0.11	0.11	0.09			
	T. septentrionalis	0.17	0.16	0.10	0.11	0.15	0.13	0.11		
	T. smithi	0.20	0.15	0.13	0.11	0.13	0.10	0.12	0.15	
	T. pakawa	0.19	0.16	0.12	0.14	0.13	0.11	0.11	0.12	0.12
						EF1-α-F	1			
	T. turrifex									
	T. jamaicensis	0.04								
	T. pomonae	0.09	0.10							
	T. arizonensis	0.10	0.11	0.02						
	T. desertorum	0.11	0.11	0.02	0.03					
	T. carinatus	0.10	0.11	0.02	0.02	0.01				
	T. nogalensis	0.10	0.11	0.02	0.02	0.01	0.01			
	T. septentrionalis	0.10	0.10	0.01	0.02	0.03	0.03	0.02		
	T. smithi	0.09	0.10	0.00	0.02	0.02	0.02	0.02	0.01	
	T. pakawa	0.10	0.10	0.00	0.02	0.02	0.02	0.02	0.01	0.002

Table 4. Genetic distances (for *COI* and *EF1-\alpha-F1* marker sequences) between North American species of *Trachymyrmex* including *T. pakawa*. The three datasets are: A) concatenated sequences, *COI* and *EF1-\alpha-F1*; B) *COI*; C) *EF1-\alpha-F1*.

(five for *COI* and three for *EF1*-a) there is possibly significant genetic variation within *T. pakawa*, since two haplotypes were found for each marker (Figure 4B). Comprehensive analyses are required to elucidate the population structure of *T. pakawa*.

Bayesian coalescent analyses indicate a divergence time of about 8.00 million years before present (95% confidence interval: 4.8–11.5 mya) between *T. pakawa* and *T. smithi*. The divergence of the lineages of *T. pakawa* and *T. smithi* could have been driven by the Pliocene-Holocene desertification of southwestern North America.



Figure 4. A Bayesian phylogenetics estimates and **B** haplotype networks of *COI* and *EF1-a-F1* sequences of North American *Trachymyrmex* species. The phylogenetics inference was calculated with *COI* and *EF1-a-F1*; clade support is indicated above branch posterior probability by Bayesian inference. Due to the incomplete taxon sampling the tree was midpoint rooted. In the minimum-spanning haplotype networks for *COI* and *EF1-a-F1*, each circle represents a haplotype, with size proportional to the haplotype's frequency in the population. Numbers along branches represent substitutions.

General Discussion and Comments

Among the Nearctic *T. septentrionalis* species group, this species is the closest (by geographical distribution) to Neotropical species, including the related *Trachymyrmex* saussurei. We initially identified these ants as specimens of *T. smithi* (Buren 1944). However, the main differences between *T. pakawa* and *T. smithi* include differences in morphology, geographical distribution, ecology, and habitat preference. Morphologically, the antennal scapes of *T. pakawa* are distinctly longer that those of *T. smithi* throughout its range (Figure 2) resulting in Scape Index values of 93–109, vs. 84–89 in *T. smithi* (Rabeling et al. 2007). This is the most obvious differential trait. Also, the head is not as clearly cordate as in *T. smithi*. The color of workers is dark reddish brown, or rarely light ferruginous red, as opposed to deep dark brown, almost black, in *T. smithi* (Figure 2). In general, *T. pakawa* has a more spinulose appearance than *T. smithi* (Figure 2).

Standard measurements of the queen caste are also provided from one specimen collected at La Estanzuela, Monterrey, NL (Table 3). Morphological measurements for this caste are very similar between *T. pakawa* and *T. smithi* (Table 3).

Trachymyrmex pakawa is one of the largest species in size for this genus in North America. Color is almost always dark- brown; only one collection, from La Estanzuela, included rusty workers; but these could have been callow (newly emerged) foragers.

The specimens from La Estanzuela had the longest scapes (0.96-1.26 mm; n = 14, compared to Lomas de Lourdes, Saltillo: 0.98-1.10 mm, n = 6) and Mitras (1.05-1.00 mm; n = 6)

1.11, n = 2); however these differences could be influenced by small sample size. In 14% of *T. pakawa* workers, HL is larger than HW (i.e., the head is longer than wide).

Morphologically *T. pakawa* is sharply different from the nearly sympatric *T. tur-rifex* (which has subparallel preocular and frontal carinae, shorter anntenal scapes, and is lighter in color) and very clearly larger than *T. septentrionalis* (Rabeling et al. 2007), the southern distribution limit of which is about 300 km to the north, near San Antonio, Texas.

Unlike *T. nogalensis*, *T. pakawa* possesses well-developed, long, frontal carinae that extend to the posterior corner of the head; it thus lacks the short, distinctive antennal depressions (so-called "scrobes" in Rabeling et al. 2007) formed by the frontal and preocular carinae. *T. pakawa* has shorter antennal scapes and longer propodeal spines than *T. nogalensis*. It also lacks the proximal narrowing or "waist" of the scape, and the lobe just distal to the narrowing, present in *T. nogalensis*.

The antennal scape in *T. carinatus* is considerably longer than in *T. pakawa*, (SI 117–152 in *T. carinatus* vs. 93.5–109.5, mean = 103.00 (n=22) in *T. pakawa*). In *T. carinatus*, the body is only moderately tuberculate and the color is yellowish brown. *T. pakawa* also lacks the sharp carinae on the vertex of workers, present in *T. carinatus* and indicated by an arrow in figure 3B of Rabeling et al. (2007), and described by Mackay and Mackay (1997).

In *T. pakawa*, the propodeal teeth are long, longer than the space separating their bases. In other species of the *T. septentrionalis* group (i. e. *T. carinatus* and *T. septentrionalis*) the propodeal spines are as long or shorter than the distance between their bases.

T. pakawa and *T. smithi* differ also in their general habitat and nesting preference: *T. pakawa* nests occur in very rugged, sloping rocky terrain (Figure 3), similar to the nests of *T. nogalensis* (Rabeling et al. 2007), whereas *T. smithi* nests are often in deep sandy soils, on open flat areas or gently sloping bajadas (alluvial fans) (Cole 1952, Rabeling et al. 2007, SRSP unpublished observations).

The nests of *T. pakawa* are very inconspicuous compared to the nests of *T. smithi* (Figure 3). Colonies of the latter species can be rather populous, the largest of the species of *Trachymyrmex* that occur in the United States with more than a thousand workers (Rabeling et al. 2007, SRSP unpublished observations), while (from the observation of one excavated nest) we estimate 200–300 workers for mature nests of *T. pakawa* in favorable habitats. *Trachymyrmex pakawa* appears to be restricted to temperate, often semiarid forest and piedmont desert scrub on the mountain ranges and slopes of the calcareous northern Sierra Madre Oriental of northeastern Mexico, while *T. smithi* inhabits many localities within the Chihuahuan desert, from Coahuila to New Mexico (Mackay and Mackay 2002, Rabeling et al. 2007). Most records of *T. smithi* are from igneous sandy soils at the western Chihuahuan desert (Figure 1; Rabeling et al. 2007).

Trachymyrmex pakawa nesting habits, in the spaces between and under large or huge rocks on the mountain slopes, are similar to those of the fungus-growing ant *Cyphomyrmex wheeleri* Forel (Wheeler 1907) that is sympatric with the new species at several locations of Saltillo (SRSP unpublished observations).

Modifications to existing identification keys

To include *T. pakawa* we modified the taxonomic identification key to North American *Trachymyrmex* workers (Rabeling et al. 2007) as follows:

3 Frontal carinae relatively short, not extending towards the posterior corner of the head. Preocular carinae curving strongly to meet the frontal carinae, forming short, distinctive depressions or "scrobes" that end slightly behind the level of the eye (figure [10B] in Rabeling et al. 2007). Antennal scape long (SI 117-152), narrowing abruptly as it approaches the antennal insertion, a small but conspicuous lobe is present just distal to the narrowing (figures 10 and 11 in Rabeling et al. 2007)...... T. nogalensis Frontal carinae long, extending well past the eye towards the posterior corners of the head. In side view, preocular carinae not joining the frontal carinae (rarely touching the carinae in *T. carinatus*). Antennal scapes short (SI 84–89) or long (SI 94–113), not as long as in T. nogalensis. Scape gradually narrowing as it approaches the antennal insertion, lobe as described above absent (figures Combining the following: In full-face view, frontal lobes prominent, shaped 4 uniquely as in figure 1B in Rabeling et al. 2007; the posterior margin of the lobe forming a broad notch where it meets the frontal carinae. Antennal scapes long (worker SI 103–113; queen SI 96–105). Propodeal spines shorter than the distance separating their bases. First gastric tergite strongly tuberculate (figure 1 in Rabeling et al. 2007)......T. arizonensis In full-face view, frontal lobes simple, not shaped as above, but rounded or triangular (figure 3B in Rabeling et al. 2007). Antennal scapes short (worker SI 84-89 (105 in Rabeling et al. 2007) or long (SI> 93). Propodeal spines longer or shorter than the distance separating their bases. Gastric tubercles 5 Relatively large species (HW 1.0-1.38). In larger workers, head square or clearly broader than long, square or slightly broader than long in smaller workers; in full-face view head often appearing cordate in larger workers (figure 17B in Rabeling et al. 2007); antennal scape short, (SI 84-89) or long (SI 93.5 - 109.5). Propodeal spines longer than the distance separating their bases. Color dark reddish brown, fuscous or black (figure 17 in Rabeling et al. 2007); from typical flat, open Chihuahuan desert habitats, or rocky habitats of the northern Sierra Madre Oriental6 Smaller species (HW 0.78-1.12). Head shape variable, usually more or less square, sometimes broader than long. Posterior border weakly to moderately emarginate, but head never appearing cordate in full-face view-even in larger workers (figure [3B] in Rabeling et al. 2007). Propodeal spines variable in length, often as long as or shorter than the distance separating their bases. Color never black, usually not dark reddish brown, commonly brownish yellow to medium reddish-brown.....7

Known species range

Specimens of *Trachymyrmex pakawa* were collected in the Mexican states of Nuevo Leon and Coahuila. The known species range extends between the opposite edges (west and east) of the Gran Sierra Plegada section of the northern Sierra Madre Oriental mountain range. The more geographically distant populations observed and sampled at the edges of this mountain range, are separated by about 80 km on a straight line (see coordinates, La Estanzuela and Cerro de las Letras). In between these known extreme distribution points, several populations have been detected (Table 5).

Habitats

This species inhabits very rocky soils, on moderate to very steep slopes, usually between and under large limestone boulders and rocks with a very thin (10–20 cm) cover of

Locality	Altitude	Google Earth coordinates
Estanzuela 1, Monterrey, Nuevo Leon	600 m	25.540425°N, -100.272864°W
Estanzuela 2, Monterrey	700 m	25.536932°N, -100.276289°W
Cerro de las Mitras 1 (Pico Apache), Monterrey	900 m	25.704834°N, -100.397027°W
Cerro de las Mitras 2 (Pico Apache) Monterrey	1300 m	25.704166°N, -100.400833°W
UAAAN reforestation edge, Saltillo, Coahuila	1650 m	25.351378°N, -101.041464°W
Cerro de las Letras, Saltillo	1800 m	25.358855°N, -101.049164°W
Lomas de Lourdes spring, Saltillo	1750 m	25.359984°N, -100.980606°W
Lomas de Lourdes dry creek, Saltillo	1700 m	25.365181°N, -100.983217°W
Cañon de San Lorenzo, Saltillo	1900 m	25.330996°N, -100.986779°W

Table 5. Collection localities for Trachymyrmex pakawa.

lithosol (limestone derived). This seems the main common aspect to most habitats where *T. pakawa* has been found. The sites are rather diverse montane habitats of the southern Nearctic: gallery forests, oak and oak-pine forests, and xerophilous Chihua-huan scrub on slopes ("submontane scrub") (Table 4).

With the possible exception of the Lomas de Lourdes (Saltillo) population, all these localities are within stands of lechuguilla (*Agave lechuguilla*), or within 300 m or less from rocky outcrops where this plant is present. Even the gallery forest at La Estanzuela is within a few hundred meters from lechuguilla stands. This underscores the xerophilous nature of *T. pakawa*.

Description of localities where T. pakawa has been collected

Coahuila state

1. We collected T. pakawa in August of 2012–2015, above the Lomas de Lourdes area of Saltillo, on the slopes of the Sierra de Zapalinamé, a small isolated mountain range separated from the main Sierra Madre range by narrow, arid valleys. 25.35998°N, -100.98060°W. Altitude is 1750 m. The ant was also collected at 25.365181°N, -100.983217°W, at an altitude of 1700. Vegetation at these sites is a xerophilous forest of small oaks (4–5 m tall) (Quercus laeta and the endemic Q. saltillensis), mountain mahogany (Cercocarpus spp.), antelope bush (Purshia plicata), weeping juniper (Juniperus *flaccida*), and madrone (Arbutus xalapensis). The canopy is rather dense; the ants were observed mainly on exposed slopes. Annual precipitation here is 400 mm, with abundant fog spells. On this western (rain shadow) side of the Sierra Madre Oriental, the soil is granular, and usually covered with a thick, but usually dry, litter layer of oak leaves. The ants live in the more mesic creeks and microhabitats of the range (NW slope) in an area with a few very small, intermittent springs, indicating a shallow water table. As elsewhere for T. pakawa, this population is not abundant; only five colonies and one nest entrance have been observed despite active searching over several years. The ants nest in very shallow lithosol covering a rocky layer, or among large limestone boulders (outcrops); the nests are possibly at least one meter deep in order to reach moisture pockets in spaces underneath the boulder layer. The tridimensional structure of aquifers is complex on these slopes, and nesting location might be influenced by the water table.

2. Cañon de San Lorenzo, Sierra de Zapalinamé. 3 August 2013. 25.330996°N, -100.986779°W; altitude is 1900 m. This site is at about 5 km (south) from the previous location. Foraging workers were collected on a sun-exposed limestone peak (western slope) covered with xerophilous scrub (*A. lechuguilla*), sotol (*Dasylirion* sp.), and *Yucca filifera*; above and at short distance from a mesic creek with Arizona cypress, (*Cupressus arizonica*). The foragers were walking among boulders. The site is a generally arid landscape with very small, scattered springs.

3. Reforestation edge, Universidad Autónoma Agraria Antonio Narro (UAAAN) fields, located at 5 km from the Cañon de San Lorenzo population. 10 October 2013. 25.351378°N, -101.041464°W. Altitude is 1650 m. The habitat is Chihuahuan desert; the observed nest was under low scrub of *Acacia greggii* (uña de gato, cat's claw) at the edge of an area reforested with exotic pines (*Pinus halepensis*).

4. Cerro de las Letras, west of UAAAN campus. 10 September 2012. 25.35885°N, -101.04916°W; altitude is 1750 m. This site is on the steep eastern slope of a hill; the vegetation consists of Chihuahuan desert scrub with sotol, cacti (*Opuntia* spp.), *Tecoma stans* (yellow bells) and *Yucca* sp. The site is right above the creosote bush (*Larrea tridentata*) life zone. At this location (and probably others), *T. pakawa* is sympatric with the cryptic, highly xerophilous fungus-growing ant, *Cyphomyrmex wheeleri* (SRSP unpublished observations). One nest entrance of *Trachymyrmex pakawa* was also observed here, on a very small flat area on rocky outcrop; it had a small pile (a 4 cm fan) of excavated, lighter soil particles on one side of the entrance (Figure 3).

Nuevo Leon state

5. La Estanzuela creek (a state park of Nuevo Leon), on the southern edge of the city of Monterrey. 8 August 2009. 25.54042°N,-100.27286°W, 600 m altitude. This location is along an intermittent stream that flows down from the Sierra Madre into the Rio La Silla, which is part of the Rio Grande Basin. This site is on the eastern slope (not rain shadow side) of the Sierra. The locality is a warm-temperate gallery forest of sycamore (*Platanus mexicana*), Monterrey oak, *Quercus polymorpha*, and other *Quercus* species. *Trachymyrmex pakawa* forages at a few meters from the pebbly riverbed, on loam-clay reddish soil of variable depth with interspersed large rocks. The surrounding habitat within a short distance (sometimes 200 m) from the stream is clearly xerophilus: on sun-exposed boulders, there is a thorny leguminous scrub with *Agave lechugilla, Acacia* spp., *Caesalpinia mexicana, Cordia boissieri*, and Cactaceae. Annual precipitation is 500–700 mm; annual temperatures range between 5–40° C. We collected *T. pakawa* here repeatedly from 2007–2013.

6. Upland and up the stream at La Estanzuela, at the previous location. 15 August 2012. 25.536932°N, -100.276289°W; 700 m altitude. This site is an ecotone of oak forest (*Quercus rysophylla*, *Quercus polymorpha*)-pine forest (*Pinus pseudostrobus*), over a limestone cliff. A very shallow, reddish litosol between boulders covers the bedrock. A nest entrance was observed there on deeper soil on a slope. The entrance was an inconspicuous hole (2–3 mm diameter) on a flat, sloped area on loamy soil. No accumulations of soil or detritus were observed at this nest entrance, probably due to the slope and rain.

7. Pico Apache (a peak at Cerro de las Mitras mountain) Monterrey, Nuevo León. 24 July 2008. 25.704834°N, -100.397027°W; 950 m altitude. This site is on the NE slope of the mountain, in Chihuahuan desert scrub/"submontane" scrub, with presence of *Agave lechuguilla* and sotol (*Dasylirion* sp.) scrub among boulders.

8. Pico Apache, Cerro de las Mitras, Monterrey, Nuevo León. 24 July 2008. 25.704166°N, -100.400833°W; 1300 m altitude. The vegetation at this site is scrub with sotol, *Agave bracteosa* (squid agave), *A. lechuguilla, Opuntia stricta, Calia secun-diflora* (mescalbean or frijolillo), and short oaks, up to 2.5 m tall (*Quercus* sp.). Both locations at Cerro de las Mitras are moister than the surrounding plains.

Ecological observations

Unlike *T. smithi*, its closest relative, *T. pakawa* has very inconspicuous nest entrances that are rather hard to find (Figure 3). There is no mound, crater, or turret as in other attines like *Trachymyrmex turrifex* or *Mycetosoritis hartmanni* (Wheeler 1907). *Trachymyrmex pakawa* workers usually forage and arrive at the nest entrance singly; loose trails of four and six ants were observed late in the afternoon on only two occasions. At any time only about four ants can be seen in the general vicinity of the nest. Foragers are very shy and, when disturbed, they retreat inside the nest or feign death and drop to the ground if disturbed at some distance from the nest. This makes them difficult to detect in the field because they are easily scared. Apparently only a very small fraction of the nest's population leaves the nest at any time to forage, at least during the day. It is not known if nocturnal foraging is significant. Regarding worker numbers in colonies, at least 250 workers emerged from the partially excavated nest by the spring at Lomas de Lourdes. The observed nest chamber was at about 50 cm deep in this nest.

The reddish detritus accumulations (exhausted fungal substrate) typical of several higher attines (Weber 1972, Sanchez-Peña 2005) are piled at 10–60 cm from the entrance hole and are conspicuous when present. The detritus accumulations of *T. pakawa* are flat, about 6 cm across. On slopes the detritus does not accumulate.

Trachymyrmex pakawa is a rather cryptic ant. At all locations it is never abundant and foraging workers seem to avoid open flat spaces and clearings, instead walking inconspicuously between vegetation and rocks. At La Estanzuela, Nuevo Leon, workers were noticeably more common at highest elevations (650 m), in the oak-pine forest, at the most mesic conditions observed for this ant.

Soil type (or lack of) might be a major, common factor regarding the distribution of *T. pakawa*. Excepting the lower altitude site at La Estanzuela, rocky, very shallow soils prevail at all locations, with large buried rocks, and always on slopes. Precipitation varies from 300 (UAAAN) to 600 mm (Estanzuela). However, drizzle and fog are frequent at Lourdes and UAAAN, and contribute an unmeasured amount of effective moisture.

This ant nests in the more mesic microhabitats (permanent and intermittent springs, creeks, or under and between large rocky outcrops) in a generally arid mountain range. The extant populations could be relicts of a more widespread distribution that contracted due to the post-glacial desertification process reducing the extension of woodlands and originating the Chihuahuan desert about 8000 years ago (Elias et al. 1995).

Although it is spread over an area covering a few hundred square kilometers, *T. pa-kawa* appears to have a discontinuous distribution in its range, and it is not abundant or common.

Trachymyrmex pakawa is not found in disturbed habitats. No population seems to have colonized agricultural areas (irrigated or dry land), or urban/landscaped areas in the neighboring cities (Monterrey and Saltillo) unlike other *Trachymyrmex* species. In this respect, this species differs markedly from geographically close North American species of *Trachymyrmex* that colonize (sometimes abundantly) gardens, yards, and

landscaped areas in towns and cities, like the following: *T. smithi*, in northern Chihuahua (Ojinaga) and adjacent Presidio, Texas (SRSP unpublished observations); *T. septentrionalis*, in Austin, Texas (SRSP unpublished observations); and *T. turrifex*, in gardens in Pesqueria, Nuevo Leon, and in Matamoros, Tamaulipas, Mexico (Lower Rio Grande Valley); in this last case at more than 10 nest entrances/m² (Sanchez-Peña 2010; see Rabeling et al. 2007). These apparently anthropo-tolerant *Trachymyrmex* populations live in deep, sandy, light-colored soils, unlike the thin, rocky, shallow limestone-based lithosols and steep alluvial soils near Monterrey and Saltillo colonized by *T. pakawa*. This spatial separation from humans might result from the strong observed preference of *T. pakawa* for rocky slopes and its avoidance of flat areas with deep soils.

Some of the collected specimens at La Estanzuela were conspicuously lighter than the ants from Saltillo and other sites; these are dark brown and initially they were confused with the even darker-colored *T. smithi*. Subsequent collections at La Estanzuela were more uniformly dark. It is possible that the lighter workers had emerged recently (callow workers). However, presumably callow workers from an excavated nest at Saltillo, covered with actinomycete growth (Cole 1952, Rabeling et al. 2007) possessed uniformly dark cuticle under the actinomycete layer. Recently emerged (callow) workers of *Trachymyrmex* spp. are commonly covered with growth of (potentially symbiotic) actinomycetes that give them a "sugary" appearance (Rabeling et al. 2007). The taxonomic relevance of the color differences described above is unknown.

Additional differences between *T. smithi* and *T. pakawa* include: in *T. smithi*, nests are often readily visible on flat soils in clearings, with conspicuous detritus piles nearby; nests can have a few hundred ants/nest; *T. smithi* is also somewhat aggressive and, when the nest or workers are disturbed, workers display aggressive behavior, and sometimes exit the nest resolutely, in small numbers, in an aggressive way (SRSP unpublished observations) unlike the very timid *T. pakawa*.

The discovery of *T. pakawa* indicates that the temperate Madrean ecoregion of the Sierra Madre Oriental might harbor additional interesting, undescribed arthropod taxa. This underscores the relevance of analysis and urgent conservation plans for these regions and biomes.

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RESEARCH ARTICLE



Omoglymmius (s. str.) wukong sp. n., a new species from Xizang, China (Coleoptera, Rhysodidae, Omoglymmiini)

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Abstract

Omoglymmius (s. str.) wukong **sp. n.** (Coleoptera: Rhysodidae: Omoglymmiini) is described from Xizang, China. Relevant morphological characters of the new species are illustrated with colour plates, and known distribution of the subgenus *Omoglymmius* in the Himalayan region is mapped.

Keywords

China, new species, Omoglymmiini, Omoglymmius, Rhysodidae, taxonomy

Introduction

Omoglymmius Ganglbauer, 1891 is the most speciose genus of Rhysodidae (Coleoptera), almost cosmopolitan, but absent from Madagascar, New Zealand, and South America. Bell and Bell (1982) excellently revised *Omoglymmius* and established eleven subgenera to classify the congeneric species. The nominotypical subgenus is the largest with 97 species (Lorenz 2005, Bell and Bell 2009, Hovorka 2015). However, in the fauna of East Asia, only two species in the subgenus *Omoglymmius* had been recorded before this study, namely *O. (s. str.) sakuraii* (Nakane, 1973) (China (Taiwan), Japan,

Vietnam) and O. (s. str.) laticeps Bell, 1977 (Bhutan, India). In this paper, a new species, O. (s. str.) wukong sp. n., is described and illustrated from Xizang Autonomous Region, China. The new species is compared to the two related species, with some selected and important morphological characters presented in a table.

Materials and methods

Specimens were relaxed and softened in a hot saturated solution of potassium hydroxide for 4 minutes (for mounted dry specimens) or 8 minutes (for alcohol-preserved specimens), and then transferred to distilled water to rinse the residual potassium hydroxide off and stop any further bleaching. The softened specimens were placed in glycerine and dissected to observe morphological details. After examination, the body parts were mounted on a glass slide with Euparal Mounting Medium for future studies. Habitus photographs were taken using a Canon macro photo lens MP-E 65mm on a Canon 550D. Observations, photographs, and measurements of morphological details were performed using a Zeiss Axio Zoom.V16 motorized stereo zoom microscope with a Zeiss AxioCam MRc 5. Photographs in Figure 6 were taken with an Olympus BX53 microscope with an Olympus DP73 camera. The final deep focus images were created with Zerene Stacker 1.04 stacking software. Adobe Photoshop CS6 was used for final processing. Precise label data are cited, while authors' remarks and addenda are placed in square brackets; separate label lines are indicated by a slash (/), and separate labels are indicated by a double slash (//). Measurements are averages taken from five specimens. The morphological terminology follows Bell and Bell (1978, 1982). Rhysodid beetles are treated as an independent family, following the publications of Bell (2003), Bousquet (2012), and Makarov (2008).

The material examined for this study is deposited in the following collections and museums (with names of curators in parentheses):

BITS	Bin Insect Taxonomy Studio, Beijing, China (B. Liu)
COHP	Collection of Oldřich Hovorka, Prague, Czech Republic
NHMB	Naturhistorisches Museum, Basel, Switzerland (M. Borer)
NMEG	Naturkundemuseum, Erfurt, Germany (M. Hartmann)
NMPC	Národní museum, Prague, Czech Republic (M. Fikáček, J. Hájek)

Measurement criteria in millimetres (mm) are used as follows:

Antennal length	length between the antennal base and the apex.
Body length	length between the mandibular apex (mandibles closed) and the
	elytral apex.
Elytral length	length between the basal border of elytra and the apex along suture.
Elytral width	widest part of both elytra combined.
Eye length	length of a single compound eye in lateral view.

Eye width	width of a single compound eye in lateral view.
Head length	length between the anterior apex of clypeus and the posterior margin
	of temporal lobe along the midline.
Head width	widest part of head (including compound eyes).
Pronotal length	length of the pronotum along the midline.
Pronotal width	widest part of pronotum.

Results

Genus Omoglymmius Ganglbauer, 1891

Vernacular name: 雕条脊甲属

Subgenus Omoglymmius s. str.

Vernacular name: 雕条脊甲指名亚属

Omoglymmius (s. str.) wukong sp. n.

http://zoobank.org/22673184-1DD7-4237-8BDF-C4C270BC6985 Figs 2, 3, 4, 5, 6D-F Vernacular name: 悟空雕条脊甲

Material examined. Holotype: 3, CHINA: Xizang, / Chayu County, / Shangchayu Town [上察隅镇], / 16.VIII.2015, / Lu Qiu leg. (NMPC). **Paratypes:** 6332, same data as holotype (233 in BITS, 2331, in COHP, 13 in NHMB, 13 in NMEG and 12 in NMPC); 33312, same data as holotype except: 2000 m, fallen wood, / 24.VIII.2005 (BITS); 12, same data as holotype except: 2000 m, *Populus* stump, / 24.VIII.2005 (BITS).

Diagnosis. Head with orbital groove extended before or near the middle of eye, following 1–2 separate coarse dorsal punctures far away from posterior margin of temporal lobe (Figs 2A, C; red arrow in 3A). Pronotal sides gently curved (Figs 2A, C; 3E); (pronotal length)/(pronotal width) = 1.1–1.2 (Figs 2A, C; 3E); outer carina with a distinct oblique microgroove at about basal 1/4 of medial margin (Figs 2A, C; 3E); inner carina impunctate, gradually narrowed in apical part, and weakly undulated at medial margin (Figs 2A, C; 3E); median groove much narrowed in middle part (Figs 2A, C; 3E); marginal groove narrower (Figs 2A, C; 3E); propleuron smooth, almost impunctate except sporadic coarse punctures near margins (Fig. 2B, D); prosternum with sparse coarse punctures and distinct precoxal carinae (Fig. 2B, D). Elytra with stria punctures relatively small (Figs 2A, C; 3F); stria IV with one seta at about basal 2/9, one seta at about apical 2/7 of its length and one seta subapically (Fig. 2A, C). Metasternum with only a few coarse punctures sparsely located along the mid-



Figure I. Habitus of *Omoglymmius (s. str.)* spp. from East Asia. **A–D** *O. (s. str.) sakuraii* (Nakane, 1973) (Vietnam: Tam Dao **A–B** $\stackrel{{}_{\sim}}{\bigcirc}$ **C–D** $\stackrel{{}_{\sim}}{\bigcirc}$) **E–H** *O. (s. str.) laticeps* Bell, 1977 (**E–F** Bhutan: Thimphu $\stackrel{{}_{\sim}}{\bigcirc}$ **G–H** holotype $\stackrel{{}_{\sim}}{\bigcirc}$). (**A, C, E, G** dorsal view **B, D, F, H** ventral view).

line; more coarse punctures closely arranged almost into a row near lateral margins; remainder of disc smooth; a shallow median pit present posteriorly (Figs 2B, D; 3G). Aedeagus with right paramere simply curved at outer margin and expanded in apical part (Fig. 6E). Female profemur without tooth on ventral side (Fig. 2D).



Figure 2. Habitus of *Omoglymmius* (*s. str.*) *wukong* sp. n. (**A–B** holotype $\stackrel{\wedge}{\circ}$ **C–D** paratype $\stackrel{\circ}{\circ}$). (**A, C** dorsal view **B, D** ventral view).

Description. *Male.* Medium size, body $6.5-7.0 \text{ mm} \log (6.7 \text{ mm} \text{ in holotype})$. Length (mm) of different body parts: head (1.0–1.1), pronotum (1.5–1.7), antenna (1.7–1.8), elytra (3.8–4.1); width (mm): head (0.9–1.0), pronotum (1.2–1.3), elytra (1.5–1.6).

Habitus (Fig. 2A–B) elongate, rather narrow, lustrous. Body colour mostly blackish brown to black; antennae and legs somewhat reddish brown; mouthparts reddish brown to yellowish brown.

Head (Fig. 3A–C) broad, as wide as long. Median lobe short, broad, subtruncate at tip. Frontal space short, nearly V-shaped, margins only shallowly sinuate. Temporal lobes longer than wide; medial angles rounded, contiguous; posteriomedial margin evenly rounded into posteriolateral margin; occipital angle scarcely evident; orbital groove impressed, extended before or near the middle of eye, following one or two separate coarse dorsal punctures far away from posterior margin of temporal lobe (red arrow in Fig. 3A); remainder of temporal lobe smooth except micropunctures; temporal setae absent; postorbital tubercle minute, not pilose, appearing as a slight convexity in lateral view. Eye entire, curvilinearly triangular, length/width = 1.1. Mentum surface coarsely and continuously punctate, with many setae. Antenna (Fig. 3D) without stylet; antennomeres V–X with minor setae in form of subapical rings; basal setae absent; all antennomeres impunctate.



Figure 3. *Omoglymmius (s. str.) wukong* sp. n. (paratype, ♂). **A–C** head **D** antenna **E** pronotum **F**, elytral apex **G** metasternum & abdomen. (**A**, **E**, **F** dorsal view **B** left lateral view **C**, **D**, **G** ventral view).

Pronotum (Fig. 3E) subelliptical, distinctly narrowed anteriorly and posteriorly, widest at about basal 4/9, length/width = 1.1–1.2. Sides gently curved, hardly sinuate before hind angle; hind angles broadly rounded. Carinae subequal at middle; outer carina with base distinctly narrowed, with medial margin sinuate before base and with a distinct oblique microgroove at about basal 1/4 of its length; inner carina distinctly narrowed in basal part, gradually narrowed in apical part, and weakly undulated at



Figure 4. *Omoglymmius (s. str.) wukong* sp. n. (paratype, \mathcal{O}). **A** fore leg **B** middle leg **C** hind leg. (**A–C** ventral view).

medial margin; both pairs of carinae impunctate except micropunctures. Median and paramedian grooves narrow; median groove much narrower in middle part, opening both anteriorly and posteriorly. Pronotal setae absent. Pronotal hypomeron with many small punctures. Propleuron smooth, almost impunctate except sporadic coarse punctures near margins. Prosternum with sparse coarse punctures; precoxal carinae distinct, sinuate.

Elytra (Figs 2A; 3F) elongate, narrow, length/width = 2.2–2.3. Striae impressed, coarsely punctate, punctures relatively small and deep; intervals only slightly convex; stria IV with one seta at about basal 2/9, one seta at about apical 2/7 of its length and one seta subapically; subapical striole with one seta; stria VII with four setae near apex (some specimens with one seta behind the insertion level of hind leg). Metathoracic wings fully developed.

Protibia (Fig. 4A) nearly cylindrical, not swollen at middle; profemur with a large and somewhat rounded tooth at medial position of ventral side. Mesotibia (Fig. 4A) with one curved spur and one minute calcar. Metatibia (Fig. 4C) with one straight spur and one calcar small, subtriangular, obtusely rounded at apex.

Ventral surfaces of *pterothorax* and *abdomen* (Figs 2A; 3G) obviously much smoother than in the related *Omoglymmius* (s. str.) sakuraii and O. (s. str.) laticeps. Metaster-



Figure 5. *Omoglymmius* (*s. str.*) *wukong* sp. n. (paratype, ♂). **A–C** aedeagus **D** median lobe **E** genital ring. (**A** right lateral view **B**, **E** ventral view **C** left lateral view **D** dorsoapical view).

num with only a few coarse punctures sparsely located along the midline; more coarse punctures closely arranged almost into a row near lateral margins; remainder of disc smooth; a shallow median pit present posteriorly. Each abdominal sternum with coarse punctures arranged into two or three irregular transverse rows; sternum IV with deep, round lateral pits; sternum V without visible pits; sternum VI with two setae near apical margin.



Figure 6. Aedeagi of *Omoglymmius (s. str.)* spp. **A–C** *Omoglymmius (s. str.) laticeps* Bell, 1977 (Bhutan: Thimphu) **D–F** *Omoglymmius (s. str.) wukong* sp. n. (paratype, ♂) **A, D** left parameres **B, E** right parameres **C, F** median lobes. (**A, D** dorsal view **B, C, E, F** right lateral view).

Genital ring (Fig. 5E) subquadrate, with long handle, nearly parallel-sided, and rounded at tip.

Aedeagus (Fig. 5A–C) with median lobe thick, tubular; opening of apical orifice (Fig. 5D) large, subelliptical; left paramere (Fig. 6D) broad, subelliptical; right paramere (Fig. 6E) small, simply curved at outer margin, expanded in apical part. Endophallus as shown in Fig. 6F.

Female. Similar to male in general appearance, but distinguished by the following characteristics (Fig. 2C–D): mentum surface with fewer setae, less coarsely punctate; profemur without tooth on ventral side; meso- and metatibiae without calcars; ab-dominal sternum IV with lateral pits distinctly larger.

Etymology. The specific epithet is from the name of "Sun Wukong", also known as the Monkey King, a mythological figure who features in a body of legends, which can be traced back to the period of the Song dynasty.

Distribution. China (Xizang) (Fig. 7).

Remarks. This new species is probably closely allied to the two known species of the subgenus *Omoglymmius* from East Asia, *O. (s. str.) sakuraii* (Nakane) and *O. (s. str.) laticeps* Bell. They resemble each other in general appearance, but detailed comparison of selected morphological characters of importance show their differences (Table 1).

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Table

		O. wukong sp. n.	O. sakuraii (Nakane)	O. laticeps Bell
Body lengt	h (mm)	6.5-7.0	2.8–5.2	6.1–7.1
		extended before or near the middle of eye, fol-	extended before the middle of eye, follow-	extended after the middle of eve. following 2–4
Head	Orbital groove	lowing 1–2 separate coarse dorsal punctures far	ing 2–4 separate coarse dorsal punctures	separate coarse dorsal punctures near posterior
	0	away from posterior margin of temporal lobe (Figs 2A, C; red arrow in 3A)	tar away from posterior margin of temporal lobe (Fig. 1A, C)	margin of temporal lobe (Fig. 1E, G)
	Pronotal sides	gently curved (Figs 2A, C; 3E)	more parallel-sided (Fig. 1A, C)	gently curved (Fig. 1E, G)
	(Pronotal length)/ (pronotal width)	1.1–1.2 (Figs 2A, C; 3E)	1.3 (Fig. 1A, C)	1.2 (Fig. 1E, G)
	Outer carina	with a distinct oblique microgroove at about basal 1/4 of medial margin (Figs 2A, C; 3E)	without microgroove (Fig. 1A, C)	without microgroove (Fig. 1E, G)
		impunctate, gradually narrowed in apical	impunctate, gradually narrowed in apical	with 2–3 punctures near base, abruptly nar-
Prothorax	Inner carina	part, and weakly undulated at medial margin	part, and strongly undulated at medial	rowed in apical part, and weakly undulated at
		(Figs 2A, C; 3E)	margin (Fig. 1A, C)	medial margin (Fig. 1E, G)
	Median groove	much narrowed in middle part (Figs 2A, C; 3E)	narrow in middle part (Fig. 1A, C)	wide in middle part (Fig. 1E, G)
	Marginal groove	narrower (Figs 2A, C; 3E)	narrower (Fig. 1A, C)	wider (Fig. 1E, G)
	Pronleuron	smooth, almost impunctate except sporadic	with many coarse punctures on disc (Fig.	with many coarse munctures on disc (Fig. 1F H)
	TOMAN	coarse punctures near margins (Fig. 2B, D)	1B, D)	will many course punctures on this (118, 11, 11)
	Prosternum	with sparse coarse punctures (Fig. 2B, D)	with dense coarse punctures (Fig. 1B, D)	with dense coarse punctures (Fig. 1F, H)
	Strial punctures	relatively small (Figs 2A, C; 3F)	relatively large (Fig. 1A, C)	relatively large (Fig. 1E, G)
	1	with one seta at about basal 2/9, one seta at	with one seta at about basal $1/5$, one	the second standard of the second structure second s
Layua	Stria IV	about apread 2/7 of its ferigur and one seta subapically	seta at middle of its length and one seta	with one seta at about apreal 2/7 of its ferigut and one seta subapically (Fig. 1E, G)
		(Fig. 2A, C)	subapically (rig. 177, C)	
		with only a faw coarse munching connealy lo-	with more coarse punctures located along	with more coarse punctures located along the
		with oild a rew coarse punctures sparsely to-	the midline; more coarse punctures closely	midline; more coarse punctures closely ar-
Matator	-	cated atolig the minutifie, more coarse punctures	arranged almost into a row near lateral mar-	ranged almost into a row near lateral margins;
Metasternu		closely arranged almost into a row near lateral	gins; remainder of disc also with a certain	remainder of disc also with a certain number
		margins; remainder of disc smooth; a shallow	number of coarse punctures; a deep median	of coarse punctures; a deep median pit present
		median pit present posterioriy (rigs $2D, D; 3G$)	pit present posteriorly (Fig. 1B, D)	posteriorly (Fig. 1F, H)
Aedeamic	richt naramere	simply curved at outer margin and more	umouzun	undulate at outer margin and less expanded in
cingennati	ugui paramere	expanded in apical part (Fig. 6E)	TIMOTRITI	apical part (Fig. 6B)
Female pro	femur	without tooth on ventral side (Fig. 2D)	without tooth on ventral side (Fig. 1C–D)	with a small tooth on ventral side (Fig. 1H)



Figure 7. Distribution of Omoglymmius (s. str.) species from the Himalayan region.

Omoglymmius (s. str.) sakuraii (Nakane, 1973)

Figs 1A-D Vernacular name: 樱井雕条脊甲

Nakane 1973: 5 (*Rhysodes* (*Omoglymmius*); type locality: Hatsuno, Amami-Ōshima, Japan); Nakane 1978: 130 (*Omoglymmius*; redescription); Bell and Bell 1978: 75 (*Omoglymmius* (*s. str.*); taxonomic combination); Bell and Bell 1982: 207 (*Omoglymmius* (*sensu stricto*); redescription); Bell and Bell 1987: 685 (*Omoglymmius* (*s. str.*); distribution; remarks; key); Bell and Bell 2000: 74 (*Omoglymmius* (*s. str.*); distribution).

Material examined. 1∂1♀, N Vietnam, 1985 / Tam dao, 3.–11.6. / 900–1400 m / J. Jelínek lgt. // Omoglymmius / (*s. str.*) / sakuraii (Nakane, 1973) / det. O. Hovorka, 1994 (NMPC).

Distribution. China (Taiwan), Japan, Vietnam. **Diagnosis.** See Table 1 under *Omoglymmius (s. str.) wukong* sp. n. above.

Omoglymmius (s. str.) laticeps Bell, 1977

Figs 1E-H, 6A-C Vernacular name: 侧头雕条脊甲

Bell 1977: 157 (*Omoglymmius*; type locality: BHUTAN: Nobding, 41 kilometers east of Wangdi Phodrang, elevation 2800 meters; NHMB); Bell and Bell 1978: 75 (*Omoglymmius (s. str.*); taxonomic combination); Bell and Bell 1982: 206

(*Omoglymmius* (s. str.); redescription); Bell and Bell 2009: 51 (*Omoglymmius*); (*Omoglymmius*); description of male; distribution).

Material examined. Type material. Holotype: \bigcirc , [BHUTAN:] Nobding 41 km O / Wangdi Ph. [Phodrang] 2800 m // Natl. –Hist. Museum / Basel – Bhutan / Expedition 1972 // Omoglymmius / laticeps / det. R. T. Bell // \bigcirc // (NHMB).

Additional material. 1 Å, BHUTAN, W / Thimphu env. / 2500 m NN / 01–18. VII.1988 / leg. C. Holzschuh // Omoglymmius / *s. str.* laticeps (Bell) Å / det. R. T. Bell // collection / Naturkunde- / museum Erfurt (NMEG); 1 \bigcirc , BHUTAN, W, distr. / Thimphu, E Dochu La / Menshunang, 2400 m / NN, 07.VII.1988 / leg. C. Holzschuh // Omoglymmius / *s. str.* laticeps (Bell) \bigcirc / det. R. T. Bell // collection / Naturkunde- / museum Erfurt (NMEG).

Distribution. Bhutan, India (Fig. 7).

Diagnosis. See Table 1 under Omoglymmius (s. str.) wukong sp. n. above.

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RESEARCH ARTICLE



Pseudaspidimerus palatus, a new species of the genus Pseudaspidimerus Kapur, 1948 from the Malay Peninsula (Coleoptera, Coccinellidae)

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Abstract

A new species of the genus *Pseudaspidimerus* Kapur, 1948 (Coleoptera: Coccinellidae), *Pseudaspidimerus palatus* Huo & Wang, **sp. n.** from the Malay Peninsula is described with illustrations and a distribution map. The genus *Pseudaspidimerus* is recorded for the first time from Malaysia and Singapore.

Keywords

Aspidimerini, lady beetles, new record, Malaysia, Singapore

Introduction

Pseudaspidimerus Kapur is a small genus of the tribe Aspidimerini Weise, 1900, with only ten species described until now (Poorani 2001; Huo et al. 2014). Species of this genus mostly prey on aphids, mealybugs, scale insects, and whiteflies, and are important natural enemies of economically important pests of various crops (Poorani 2001; Basu and Patro 2007; Megha et al. 2015). Kapur (1948) established this genus based on its parallel prosternal carinae forming a rectangular area (Fig 2b) and its robust

penis (Fig. 1g), which are the most important generic characters. During the course of our study of borrowed specimens from different repositories, a new species of this genus was discovered from the Malay Peninsula. This is the first record of the genus *Pseudaspidimerus* from Malaysia and Singapore.

Materials and methods

All studied materials were borrowed from the following museums:

HNHM Hungarian Natural History Museum, Budapest, Hungary;MIZ Museum and Institute of Zoology PAS, Warsaw, Poland;NMPC Natural History Museum, Prague, Czech Republic.

Type specimens are deposited in the above museums, except two paratypes kept in South China Agricultural University (**SCAU**).

Dry specimens were softened in 70°C water for 12 hours; the abdomen was detached and cleared in warm 10% KOH solution for 1–2 hours. Genitalia of both sexes were dissected, rinsed with distilled water, transferred to glycerol, and examined on slides. Genitalia images were photographed with digital cameras (Axiocam 506 color) connected to the microscope (ZEISS Imager M2). The software ZEN 2.3 was used to capture genitalia images.

External morphology was observed with a stereomicroscope (SteREO Discovery V20, Zeiss). Images were photographed with digital cameras (AxioCam HRc) connected to the microscope. Measurements were made using the measurement tools of the software AxioVision Rel. 4.8. The following abbreviations are used in the description:

- TL total length, from apical margin of clypeus to apex of elytra;
- TW total width, across both elytra at widest part;
- TH total height, through the highest point of elytra to metaventrite;
- **HW** head width, including eyes;
- PL pronotal length, from the middle of anterior margin to the base of pronotum;
- **PW** pronotal width at widest part;
- EL elytral length, along the suture, from the apex to the base including the scutellum;
- **EW** elytral width, across both elytra at widest part;
- **ID** interocular distance, nearest distance between eyes.

Scanning electron micrographs were made using HITACHI S-3400N in the Electron Microscopy Laboratory of the MIZ. The distribution map was downloaded from a free map website (http://alabamamaps.ua.edu) and all images were cleaned up and laid out in plates with Adobe Photoshop CS5. Morphological terms follow Ślipiński and Tomaszewska (2010).

Taxonomy

Pseudaspidimerus palatus Huo & Wang, sp. n.

http://zoobank.org/7961DD3D-2423-42E1-A7E5-35C53C0A4612 Figures 1–3

Types. Holotype: 13, "Thailand, Ranong prov. Ban Na env., 22-26.III.1996, 9°34'N, 98°42'E, K Majer leg" (NMPC); **Paratypes** (5): 29, "S Thailand, Betong Gunung Cang dun vill., Yala dist., 25.3.-22.4.1993, J. Horák leg" (NMPC, SCAU); 23, "Singapore, 29 Dr. Baum" (MIZ, SCAU); 19, "Malacca Biró/ Kwala-Lumpur/" (HNHM).

Diagnosis. Elytra black with apical part yellowish brown, maculae oblique expending from apical 1/3 of suture to lateral 1/2 length of lateral margin; Penis extremely robust, arcuate, swollen anteriorly with small inner branch, narrowest at middle; Penis guide, in lateral view, widest at base, gradually narrowing to pointed apex. In ventral view, shovel shaped, only a little longer than broad, slightly narrowing to basal 1/3, thence gradually narrowing to small rounded apex.

Description. TL: 1.91–2.07 mm, EL: 1.43–1.49 mm, TW: 1.56–1.66 mm, TH: 1.00–1.06 mm, PL: 0.64–0.73 mm, PW: 1.13–1.25 mm, HW: 0.76–0.81 mm, ID: 0.40–0.43 mm, TL/TW: 1.22–1.25, PL/PW: 0.57–0.58, EL/EW: 0.90–0.92, HW/ PW: 0.65–0.67, PW/EW: 0.72–0.75, ID/HW: 0.53.

Body oblong oval, densely covered with short, silvery white pubescence (Fig. 1a). Head yellow in male (Fig. 1b) and black in female. Pronotum black with anterior margin narrowly reddish brown and ante-lateral corners with a small triangular yellow spot. Elytra black with apical part yellowish brown, maculae oblique expending from apical 1/3 of suture to lateral 1/2 length of lateral margin. Underside dark brown to black except mouthpart, prothoracic hypomeron, and legs yellow. Mentum dark brown. Posterior margin of ventrite 6 strongly emarginate in male and broadly rounded in female (Fig. 1d–e). Punctures on frons fine and dense, 0.5–1.0 diameters apart; on elytra and pronotum sparse, 1.0–2.0 diameters apart; on metaventrite fine and sparse on middle part, coarse and densely distributed laterally (Fig. 2a). Ventral surface with short, dense, silvery pubescence.

Male genitalia. Penis extremely robust, arcuate, swollen anteriorly with small inner branch, narrowest at middle (Fig. 1g). Tegminal strut slightly shorter than main part of tegmen. Parameres slightly longer than phallobase length and a little shorter than penis guide, apex with long sparse setae (Fig. 1h). Penis guide, in lateral view, widest at base, gradually narrowing to pointed apex. In ventral view, shovel shaped, only a little longer than broad, slightly narrowing to basal 1/3, thence gradually narrowing to small rounded apex (Fig. 1i).

Female genitalia. Coxites fairly broad, 0.5 times as long as wide, with a projection on basal end, apical and outer margin with dense, long setae. Spermatheca curved, C-shaped, ramus and nodulus not clearly differentiated (Fig. 1f).

Distribution. Thailand (Ranong, Yala), Malaysia (Kuala Lumpur), Singapore.



Figure 1. *Pseudaspidimerus palatus* Huo & Wang sp. n. **a** dorsal view **b** frontal view **c** lateral view **d** female abdomen **e** male abdomen **f** female genitalia **g** penis **h** lateral view of tegmen **i** ventral view of tegmen.

Remarks. In general appearance, this species is similar to a variation of *P. mauliki* (Huo et al. 2014, Fig. 2e) with elytral apical third with an oblique yellowish brown spot laterally extending up to nearly the half length of elytra. The penis of male genitalia is also very similar to that of *P. mauliki* (Huo et al. 2014, Fig. 2j): penis extremely robust, arcuate,



Figure 2. Pseudaspidimerus palatus Huo et Wang sp. n. a ventral view b prosternum and mouth part.



Figure 3. Distribution map of Pseudaspidimerus palatus Huo & Wang, sp. n.

swollen anteriorly with a small inner branch, narrowest at middle. The only difference is the ventral view of penis guide. In *P. mauliki*, penis guide is about 1.2 times as long as broad at base, widest at basal third and then gradually narrowing to a fairly broad apex, which is weakly emarginate at middle (Huo et al. 2014, Fig. 2k). In *P. palatus*, penis guide is shovel

shaped, only a little longer than broad, widest at basal third, gradually narrowed thereafter to a rounded apex. The shape of the penis guide is also close to that of *P. lambai* Kapur, 1967 and *P. flaviceps* (Walker, 1859). In *P. lambai* and *P. flaviceps*, penis guide is more slender in lateral view and longer in ventral view, and about two times as long as broad.

Etymology. The specific name is derived from the Latin noun "pala" and postfix "-atus", referring to that its penis guide looks like a shovel in ventral view.

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Supplementary material I

Table of localities

Authors: Lizhi Huo, Wenjing Li, Xingmin Wang

Data type: occurence

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CHECKLIST



A species checklist of the subgenus Culicoides (Avaritia) in China, with a description of a new species (Diptera, Ceratopogonidae)

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Abstract

A checklist of the subgenus *Culicoides (Avaritia* Fox) (Diptera: Ceratopogonidae: *Culicoides*) in China, currently including 57 species, is provided. Their full citations, more detailed locations of the type locality, and distribution of each species by province, and/or state of each species are also provided. *Culicoides (Avaritia) fenggangensis* Liu & Hou, **sp. n.** is described and illustrated, based on both male and female specimens from China. The new species is compared with its similar congeners, *C. (A.) comparis* Liu & Yu, 2005 and *C. (A.) dentiformis* McDonald & Lu, 1972.

Keywords

Biting midges, Ceratopogonidae, checklist, Culicoides, subgenus Avaritia

Introduction

Biting midges of the genus *Culicoides* Latreille (Diptera, Ceratopogonidae) are found everywhere in the world and females are the smallest of insect vectors (Mellor et al. 2000). Some species of *Culicoides* spread disease in humans and livestock as vectors of arboviruses, such as bluetongue virus (BTV), Schmallenberg virus (SBV), and others (Borkent

2005), which leads to direct economic costs for agriculture (Gibbens 2012; Velthuis et al. 2010). In China, Bluetongue has been recorded from several provinces in south, such as Yunnan, Hubei, Anhui, Sichuan, Shanxi and so on (Zhang et al. 2015), and the vector *Culicoides* of BTV mainly distributed in the south region of north latitude 40° (Zhang et al. 2014). Therefore, members of this genus have great international significance and have attracted more scientific attention in recent years. The genus *Culicoides*, currently with a total number of species of 1415 in the world, 1368 extant species and 47 fossil species (Borkent, 2016), has 348 species in China. Many of the important vectors are in the subgenus *Culicoides* (*Avaritia* Fox). The purpose of this paper is to provide a checklist of this subgenus in China, and describe and illustrate *Culicoides* (*A.) fenggangensis* sp. n.

Materials and methods

The specimens were collected with light traps near households in the mountains of Fenggang County of Guizhou Province. For microscopic observation, specimens were preserved in 100% ethanol and then slide-mounted in Canada balsam following the technique described by Yu et al. (2005). Diagnostic features were microphotographed using an Imaging System of Upright Research Microscope adapted to a microscope (Nikon Eclipse Ni-E) and a Digital System of Large depth-of-field 3D Digital Microscope (Keyence VHX-1000C), and Photoshop CS4 was used to obtain the final images. Morphological terms are from the chapter on Ceratopogonidae by Yu et al. (2005). Terms of structures specific to *Culicoides* follow those described by Santarém et al. (2014) and Han et al. (2017). Measurements of wings, flagellar segments, palpus, and legs are given in millimeters, and measurements of spermathecae are given in microns. Meristic information is presented as ranges of values, followed by mean and sample size. The type specimens are deposited in Insect Collection of Zunyi Medical University, Guizhou Province, China.

Taxonomy

Checklist of the subgenus Avaritia in China in alphabetical order

Subgenus Avaritia Fox, 1955 Avaritia Fox, 1955: 218. Type species. Ceratopogon obsoletus Meigen, 1818.

Culicoides (Avaritia) abchazicus Dzhafarov, 1964

Culicoides abchazicus Dzhafarov, 1964: 263; Liu et al. 2011: 385. Type Locality: Georgia. **Distribution.** China (Liaoning); Georgia.

Culicoides (Avaritia) actoni Smith, 1929

Culicoides actoni Smith, 1929: 255; McDonald and Lu 1972: 411; Lee 1988: 29; Yu et al. 2005: 907. Type locality: India.

Culicoides okumensis Arnaud, 1956: 119. Type locality: Japan.

Culicoides imperceptus Das Gupta, 1962a: 538. Type locality: India.

Distribution. China (Heilongjiang, Jiangsu, Anhui, Fujian, Taiwan, Shandong, Hubei, Guangdong, Guangxi, Hainan, Sichuan, Yunnan, Tibet, Shaanxi, Hunan); India, Indonesia, Malaysia, Philippines, Vietnam, Japan, Thailand.

Culicoides (Avaritia) albifascia Tokunaga, 1937

Culicoides albifascia Tokunaga, 1937: 319; Lee 1978: 28; Yu et al. 2005: 908. Type locality: China: Taiwan.

Distribution. China (Heilongjiang, Tibet, Sichuan, Yunnan, Taiwan).

Culicoides (Avaritia) bawanglingensis Yu, Wang & Chen, 2012

Culicoides bawanglingensis Yu, Wang & Chen, in Wang et al., 2012: 283. Type Locality: China: Hainan, Bawangling.

Distribution. China (Hainan).

Culicoides (Avaritia) brevipalpis Delfinado, 1961

Culicoides brevipalpis Delfinado, 1961: 654; Yu et al. 2005: 910. Type locality: Philippines. **Distribution.** China (Taiwan, Guangdong, Hainan, Yunnan); Australia, Indonesia, Malaysia, Japan, Sri Lanka, Thailand, Philippines.

Culicoides (Avaritia) brevitarsis Kieffer, 1917

Culicoides brevitarsis Kieffer, 1917: 187; Yu et al. 2005: 911. Type locality: Australia. *Culicoides robertsi* Lee & Reye, 1953: 386. Type locality: Australia. *Culicoides radicitus* Delfinado, 1961: 657. Type locality: Philippines. *Culicoides superfulvus* Das Gupta, 1962b: 253. Type locality: India. **Distribution.** China (Anhui, Taiwan, Hainan); Australia, Philippines, India, Laos, Malaysia.

Culicoides (Avaritia) bubalus Delfinado, 1961

Culicoides bubalus Delfinado, 1961: 658; Yu et al. 2005: 913. Type locality: The Philippines.

Distribution. China (Taiwan), Philippines.

Culicoides (Avaritia) chiopterus (Meigen), 1830

Ceratopogon chiopterus Meigen, 1830: 263. Type locality: Europe. Ceratopogon amoenus Winnertz, 1852: 35. Type locality: Germany. Monohelea similis Goetghebuer, 1927: 203. Type locality: Belgium. Culicoides dobyi Callot & Kremer, 1969: 610. Type locality: France. Distribution. China (Hebei, Inner Mongolia, Liaoning, Jilin, Heilongjiang, Tibet); Germany, France, Belgium, Britain, Russia.

Culicoides (Avaritia) clavipalpis Mukerji, 1931

Culicoides clavipalpis Mukerji, 1931: 1052; McDonald and Lu 1972: 403; Yu et al. 2005: 916. Type locality: India.

Culicoides candidus Sen & Das Gupta, 1959: 620. Type locality: India.

Distribution. China (Jiangsu, Fujian, Shandong, Hainan, Sichuan); India, Indonesia, Laos, Malaysia, Philippines, Thailand.

Culicoides (Avaritia) comparis Liu & Yu, 2005

Culicoides comparis Liu & Yu, in Yu et al., 2005: 917. Type locality: China: Tibet, Nielamu.

Distribution. China (Tibet).

Culicoides (Avaritia) conaensis Liu & Yu, 1990

Culicoides conaensis Liu & Yu, 1990: 19; Yu et al. 2005: 919. Type locality: China: Tibet, Cuona.

Distribution. China (Tibet).

Culicoides (Avaritia) dentiformis McDonald & Lu, 1972

Culicoides dentiformis McDonald & Lu, 1972: 403; Yu et al. 2005: 921. Type locality: China: Taiwan.

Distribution. China (Taiwan).

Culicoides (Avaritia) elongatus Chu & Liu, 1978

Culicoides elongatus Chu & Liu, 1978: 83; Yu et al. 2005: 922. Type locality: China: Yunnan, Mengla.

Distribution. China (Fujian, Yunnan).

Culicoides (Avaritia) fenggangensis Liu & Hou, sp. n.

Culicoides fenggangensis Liu & Hou, sp. n., this paper. Type locality: China: Guizhou, Fenggang.

Distribution. China (Guizhou).

Culicoides (Avaritia) filicinus Gornostaeva & Gachegova, 1972

Culicoides filicinus Gornostaeva & Gachegova, 1972: 522; Yu et al. 2005: 924. Type locality: Russia.

Distribution. China (Tibet, Heilongjiang); Russia.

Culicoides (Avaritia) gaponus Yu, 1982

Culicoides gaponus Yu, 1982: 202; Yu et al. 2005: 925. Type locality: China: Hainan, Diaoluoshan.

Distribution. China (Hainan).

Culicoides (Avaritia) holcus Lee, 1980

Culicoides holcus Lee, 1980: 85; Yu et al. 2005: 927. Type locality: China: Yunnan. **Distribution.** China (Yunnan).

Culicoides (Avaritia) hui Wirth & Hubert, 1961

Culicoides hui Wirth & Hubert, 1961: 16; Yu et al. 2005: 928. Type locality: China: Taiwan. **Distribution.** China (Taiwan, Guangdong, Hainan, Yunnan, Hunan); Indonesia, Laos, Malaysia.

Culicoides (Avaritia) imicola Kieffer, 1913

Culicoides imicola Kieffer, 1913: 11; Yu et al. 2005: 930. Type locality: Kenya. *Culicoides pallidipennis* Carter, Ingram & Macfie, 1920: 265. Type locality: Ghana. *Culicoides iraqensis* Khalaf, 1957: 343. Type locality: Iraq. *Culicoides minutus* Sen & Das Gupta, 1959: 622. Type locality: India. *Culicoides pseudoturgidus* Das Gupta, 1962a: 537. Type locality: India. **Distribution.** China (Hainan); Wide spread in Africa, the Middle and Far East, India, Laos, Sri Lanka, Vietnam.

Culicoides (Avaritia) incertus Yu & Zhang, 1988

Culicoides incertus Yu & Zhang, in Yu, 1988: 136; Yu et al. 2005: 931. Type locality: China: Tibet.

Distribution. China (Tibet).

Culicoides (Avaritia) innoxius Sen & Das Gupta, 1959

Culicoides innoxius Sen & Das Gupta, 1959: 626; Yu et al. 2005: 933. Type locality: India.

Distribution. China (Hainan); India, Cambodia, Indonesia, Laos, Malaysia, Sri Lanka, Thailand.

Culicoides (Avaritia) insignipennis Macfie, 1937

Culicoides insignipennis Macfie, 1937b: 469; Lee 1988: 65; Yu et al. 2005: 935. Type locality: Malaysia.

Distribution. China (Fujian, Taiwan, Yunnan, Hunan); Malaysia, Brunei, Indonesia, Laos, Philippines, Singapore, Thailand.

Culicoides (Avaritia) iphthimus Zhou & Lee, 1984

Culicoides iphthimus Zhou & Lee, 1984: 295; Yu et al. 2005: 936. Type locality: China: Chongqing.

Distribution. China (Chongqing).

Culicoides (Avaritia) jacobsoni Macfie, 1934

Culicoides jacobsoni Macfie, 1934: 215; Yu 1982: 48. Yu et al. 2005: 938. Type locality: Indonesia.

Culicoides buckleyi Macfie, 1937a: 117. Type locality: Malaysia.

Culicoides kitaokai Tokunaga, 1955: 6. Type locality: Japan.

Culicoides unisetiferus Tokunaga, 1959: 236. Type locality: Papua New Guinea.

Distribution. China (Fujian, Taiwan, Guangdong, Guangxi, Hainan, Yunnan, Tibet, Hunan); Indonesia, Malaysia, Japan, New Guinea, Philippines, Thailand.

Culicoides (Avaritia) kepongensis Lee, 1988

Culicoides kepongensis Lee, 1988: 69; Wirth and Hubert 1989: 346; Yu et al. 2005: 940. Type locality: China; Malaysia.

Distribution. China (Fujian, Taiwan); Malaysia, Laos, Thailand.

Culicoides (Avaritia) kinabaluensis Wirth & Hubert, 1989

Culicoides kinabaluensis Wirth & Hubert, 1989: 211; Yu et al. 2005: 941. Type locality: Indonesia.

Distribution. China (Hainan).

Culicoides (Avaritia) lansangensis Howarth, 1985

Culicoides lansangensis Howarth, 1985: 58; Liu et al. 1996: 38; Yu et al. 2005: 942. Type locality: Laos.

Distribution. China (Guangdong, Hainan, Hunan).

Culicoides (Avaritia) lengi Yu & Liu, 1990

Culicoides lengi Yu & Liu, 1990: 10; Yu et al. 2005: 944. Type locality: China: Guangdong, Shixing.

Distribution. China (Guangdong).

Culicoides (Avaritia) liui Wirth & Hubert, 1961

Culicoides liui Wirth & Hubert, 1961: 20; Yu et al. 2005: 946. Type locality: China: Taiwan.

Distribution. China (Taiwan, Yunnan); Indonesia, Laos, Malaysia, Philippines, Thailand.

Culicoides (Avaritia) longirostris Qu & Wang, 1994

Culicoides longirostris Qu & Wang, 1994: 486; Yu et al. 2005: 947. Type locality: China: Tibet.

Distribution. China (Tibet).

Culicoides (Avaritia) malayae Macfie, 1937

Culicoides malayae Macfie, 1937b: 471; Yu 1982: 190; Yu et al. 2005: 949. Type locality: Malaysia.

Distribution. China (Fujian, Taiwan, Guangdong, Guangxi, Hainan, Yunnan); Indonesia, Malaysia, Philippines, Thailand.

Culicoides (Avaritia) mamaensis Lee, 1979

Culicoides mamaensis Lee, 1979: 101; Yu et al. 2005: 951. Type locality: China: Tibet, Cuona.

Distribution. China (Sichuan, Tibet).

Culicoides (Avaritia) motoensis Lee, 1978

Culicoides motoensis Lee, 1978: 75; Yu et al. 2005: 952. Type locality: China: Tibet, Motuo.

Distribution. China (Tibet).

Culicoides (Avaritia) nielamensis Liu & Deng, 2000

Culicoides nielamensis Liu & Deng, 2000: 246; Yu et al. 2005: 954. Type locality: China: Tibet, Nielamu.

Distribution. China (Tibet).

Culicoides (Avaritia) nigritus Fei & Lee, 1984

Culicoides nigritus Fei & Lee, 1984: 345; Yu et al. 2005: 955. Type locality: China: Inner Mongolia, Linxi.

Distribution. China (Inner Mongolia).

Culicoides (Avaritia) nujiangensis Liu, 1990

Culicoides nujiangensis Liu, 1990: 59; Yu et al. 2005: 956. Type locality: China: Yunnan, Nujiang.

Distribution. China (Yunnan).

Culicoides (Avaritia) obsoletus (Meigen), 1818

Ceratopogon obsoletus Meigen, 1818: 76. Type locality: Europe. Ceratopogon varius Winnertz, 1852: 35. Type locality: Germany. *Ceratopogon yezoensis* Matsumura, 1911: 60. Type locality: Russia. Culicoides obscuripes Santos Abreu, 1918: 297. Type locality: Spain. Culicoides lacteinervis Kieffer, 1919a: 47. Type locality: Slovak Republic, Ukraine. Culicoides rivicola Kieffer, 1921: 56. Type locality: Germany. Culicoides clavatus Kieffer, 1921: 56. Type locality: Germany. Culicoides heterocerus Kieffer, 1921: 57. Type locality: Germany. *Culicoides pegobius* Kieffer, 1922: 235. Type locality: Germany. Culicoides kabyliensis Kieffer, 1922: 505. Type locality: Algeria. Culicoides concitus Kieffer, 1922: 71. Type locality: Germany. Culicoides intermedius Okada, 1941: 22. Type locality: Japan. Culicoides sintrensis Cambournac, 1956: 591. Type locality: Portugal. *Culicoides seimi* Shevchenko, 1967: 173. Type locality: Ukraine. Distribution. China (Shanxi, Inner Mongolia, Liaoning, Jilin, Heilongjiang, Fujian, Shandong, Sichuan, Chongqing, Yunnan, Xinjiang, Gansu, Tibet); wide distribution in Palaearctic Region, Britain, Germany, Russia, Canary Islands, Algeria, Japan, Portugal, Slovakia, Ukraine.

Culicoides (Avaritia) orestes Wirth & Hubert, 1989

Culicoides orestes Wirth & Hubert, 1989: 222; Yu et al. 2005: 959. Type locality: Malaysia.

Distribution. China (Hainan); Malaysia.

Culicoides (Avaritia) orientalis Macfie, 1932

Culicoides orientalis Macfie, 1932: 490; Lee 1978: 83; Yu et al. 2005: 961. Type locality: Malaysia.

Culicoides nayabazari Das Gupta, 1963: 35. Type locality: India.

Distribution. China (Fujian, Taiwan, Hainan, Sichuan, Yunnan, Tibet, Hunan); Malaysia, India, Indonesia, Philippines, Thailand, Vietnam, the Solomon Islands.

Culicoides (Avaritia) palauensis Tokunaga, 1959

Culicoides palauensis Tokunaga, in Tokunaga & Murachi, 1959: 348; Yu 1982: 57; Yu et al. 2005: 963. Type locality: USA.

Distribution. China (Guangdong, Hainan, Yunnan); Oceania.

Culicoides (Avaritia) pastus Kitaoka, 1980

Culicoides pastus Kitaoka, 1980: 11; Lee 1988: 91; Yu et al. 2005: 964. Type locality: Japan.

Distribution. China (Sichuan, Yunnan); Japan.

Culicoides (Avaritia) pelius Liu & Yu, 1990

Culicoides pelius Liu & Yu, 1990: 23; Liu and Deng 2000: 245; Yu et al. 2005: 966. Type locality: China: Tibet, Cuona. **Distribution.** China (Tibet).

Culicoides (Avaritia) peregrinus Kieffer, 1910

Culicoides peregrinus Kieffer, 1910: 191; Yu et al. 2005: 967. Type locality: India.
Culicoides judicandus Bezzi, 1916: 8. Type locality: Philippines.
Culicoides esmoneti Salm, 1917b: 136. Type locality: Indonesia.
Culicoides philippinensis Kieffer, 1921: 564. Type locality: Philippines.
Culicoides assamensis Smith & Swaminath, 1932: 183. Type locality: India.
Culicoides quadratus Tokunaga, 1951: 108. Type locality: Indonesia.
Distribution. China (Hebei, Inner Mongolia, Liaoning, Jiangsu, Fujian, Taiwan, Jiangxi, Henan, Guangdong, Guangxi, Hainan); India, Philippines, Indonesia.

Culicoides (Avaritia) qionghaiensis Yu & Liu, 1990

Culicoides qionghaiensis Yu & Liu, 1990: 4; Yu et al. 2005: 969. Type locality: China: Sichuan, Xichang.

Distribution. China (Sichuan).

Culicoides (Avaritia) ruiliensis Lee, 1980

Culicoides ruiliensis Lee, 1980: 86; Yu et al. 2005: 971. Type locality: China: Yunnan. **Distribution.** China (Yunnan).

Culicoides (Avaritia) scoticus Downes & Kettle, 1952

Culicoides scoticus Downes & Kettle, 1952: 65; Yu et al. 2005: 972. Type locality: Great Britain.

Distribution. China (Tibet); Great Britain, France.

Culicoides (Avaritia) sinanoensis Tokunaga, 1937

Culicoides sinanoensis Tokunaga, 1937: 331; Yu et al. 2005: 973. Type locality: Japan. *Culicoides obsoletiformis* Amosova, 1957: 233. Type locality: Russia. **Distribution.** China (Liaoning, Jilin, Heilongjiang, Yunnan, Shaanxi); Japan, Russia.

Culicoides (Avaritia) suiyangensis Hou, Han, Lv & Jiang, 2014

Culicoides suiyangensis Hou, Han, Lv & Jiang, 2014: 98. Type Locality: China: Guizhou, Suiyang.

Distribution. China (Guizhou).

Culicoides (Avaritia) sumatrae Macfie, 1934

Culicoides sumatrae Macfie, 1934: 190; Yu et al. 2005: 975. Type locality: Malaysia.
Culicoides amamiensis Tokunaga, 1937: 325. Type locality: Japan.
Culicoides kagiensis Tokunaga, 1937: 327. Type locality: Taiwan.
Culicoides ohmorii Takahashi, 1958: 113. Type locality: Japan.
Culicoides assimilis Delfinado, 1961: 660. Type locality: Philippines.
Distribution. China (Fujian, Taiwan, Guangdong, Guangxi, Hainan, Yunnan, Tibet);
Malaysia, Japan, Philippines.

Culicoides (Avaritia) suzukii Kitaoka, 1973

Culicoides suzukii Kitaoka, 1973: 212; Lee 1988: 107; Yu et al. 2005: 977. Type locality: Japan.

Distribution. China (Taiwan, Yunnan); Japan.

Culicoides (Avaritia) tainanus Kieffer, 1916

Culicoides tainanus Kieffer, 1916: 114; Yu et al. 2005: 979. Type locality: China: Taiwan. Ceratopogon maculatus Shiraki, 1913: 294. Type locality: China: Taiwan. Culicoides kii Tokunaga, 1937: 284. Type locality: Japan. Culicoides sigaensis Tokunaga, 1937: 322. Type locality: Japan. Culicoides kyotoensis Tokunaga, 1937: 329. Type locality: Japan. Culicoides suborientalis Tokunaga, 1951: 106. Type locality: Indonesia. Distribution. China (Fujian, Hainan, Yunnan, Shaanxi, Shandong); Indonesia, Japan, Laos, Malaysia, Philippines, Thailand, Vietnam.

Culicoides (Avaritia) tibetensis Chu, 1977

Culicoides tibetensis Chu, 1977: 102; Yu et al. 2005: 980. Type locality: China: Tibet. **Distribution.** China (Sichuan, Tibet).

Culicoides (Avaritia) trimaculatus McDonald & Lu, 1972

Culicoides trimaculatus McDonald & Lu, 1972: 415; Yu et al. 2005: 982. Type locality: China: Taiwan.

Distribution. China (Taiwan).

Culicoides (Avaritia) wadai Kitaoka, 1980

Culicoides wadai Kitaoka, 1980: 14; Yu et al. 2005: 984. Type locality: China: Taiwan. **Distribution.** China (Fujian, Guangxi, Taiwan).

Culicoides (Avaritia) wandashanensis Wang & Liu, 1999

Culicoides wandashanensis Wang & Liu, 1999: 328; Yu et al. 2005: 985. Type locality: China: Heilongjiang, Raohe.

Distribution. China (Heilongjiang).

Culicoides (Avaritia) yamii Lien, Lin & Weng, 1998

Culicoides yamii Lien, Lin & Weng, 1998: 57; Yu et al. 2005: 987. Type locality: China: Taiwan, Lanyu. **Distribution.** China (Taiwan).

Culicoides (Avaritia) yuchihensis Lien, Lin & Weng, 1998

Culicoides yuchihensis Lien, Lin & Weng, 1998: 58; Yu et al. 2005: 988. Type locality: China: Taiwan.

Distribution. China (Taiwan).

Culicoides (Avaritia) fenggangensis Liu & Hou, sp. n. http://zoobank.org/52A53C15-0F18-4040-B83C-B678BD2E9A66 Figs 1–3

Diagnosis. Male: only species of *Culicoides* in China with the following combination of features: the 3^{rd} segment of the palpus is slender, PR 3.11; the apex of 9^{th} tergite has lateral processes; parameres with apical portion elongate, bent abruptly; aedeagus nearly triangular, with a long ovoid process at its apex. Female: only species of *Culicoides* in China with the following combination of features: cell m₂ with four sparsely distributed pale spots; the 3^{rd} segment of the palpus is slender, PR 3.20–3.75.

Description. Female. *Head* (Fig. 2a). Brown. Eyes (Fig. 2b) contiguous, abutting medially for length of 1.5 ommatidia, with interfacetal hairs. Antennal pedicel brown; Lengths of antennal flagellomeres in proportion of 19: 14: 14: 15: 15: 15: 16: 17: 25: 25: 25: 31: 47; AR 1.22–1.28 (1.24, n = 3); sensilla coeloconica on flagellomeres 1, 9–13. 3^{rd} segment of palpus slender, slightly swollen at apical 1/3, with small, rounded sensory pit (Fig. 2c–d); PR 3.20–3.75 (3.45, n = 3). Mandible with 14–18 (16, n = 4) teeth (Fig. 2e); Maxilla with 17 teeth (n = 3); P/H ratio 1.04–1.17 (1.11, n = 3).

Thorax (Fig. 2f). Dark brown. Scutellum without distinct pattern in slide-mounted specimens. Wing (Fig. 1a) with contrasting pattern of pale, dark spots; distal 1/2 of 1st, proximal 1/2 of 2nd radial cell in dark spot; three pale spots near anterior margin: 1st over base of cell r, and r-m crossvein extending from below M, to margin of costa, 2nd over distal of cell r, from dorsal portion of M, to costa, 3rd morphological variation, from dorsal portion of M₁ to just below costa; cell m₁ with two separated pale spots: one spot small, ovoid, another large, triangle, far from distal portion of wing; cell m, with four different shapes, sizes pale spots: 1st proximal to CuA, 2nd between medial and mediocubital forks, 3^{rd} and 4^{th} below M_2 , latter reaching wing margin; cua, with a large, ovoid pale spot abutting wing posterior margin; anal cell with two pale spots: proximal pale spot on Cu, and CuA, distal pale spot near mediocubital fork; wing base with faint pale spot on M; macrotrichia sparsely distributed on distal 1/3 of wing, but not in basal cell; wing length 1.45-1.60 (1.52, n = 3) mm, width 0.68–0.73 (0.70, n = 3) mm; CR 0.55–0.59 (0.57, n = 3). TR and F-T of legs (Fig. 2f) are given as Table 1, metatibial distal bristles (Fig. 2g-h) with 5 or 6 spines, 1st spine is longest.

Table 1. Tarsal ratios (TR) and measurements of leg segments and tarsomeres from femur to tarsomere 5 (F-T) of all legs of *C. fenggangensis* sp. n. (\bigcirc).

Leg	TR	F-T
Foreleg	2.12	85:88:53:25:16:10:11
Midleg	2.31	109:114:60:26:16:10:11
Hindleg	1.73	109:115:57:33:17:11:12

Abdomen. Brown. Two subequal-size ovoid spermathecae (Fig. 2i), measuring $65.0-72.5 \times 50.0-57.5$ (n = 3) µm, $65.0-72.5 \times 50.0$ (n = 2) µm, slender sclerotized



Figure 1. Culicoides (A.) fenggangensis sp. n. Left Wing. a Female b Male.



Figure 2. a–**j** *Culicoides (A.) fenggangensis* sp. n. **a** Head, anterior view **b** Eye contiguous, anterior view **c** Left palpus, anterior view **d** Right palpus, anterior view **e** Mandibular teeth **f** Thorax and legs, lateral view **g** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of first tarsomere, posterior view. **h** Apex of hind tibia and base of hind

necks with 2.5–7.5 (n = 3) μ m; third slender, elongate rudimentary spermatheca, length 20.0–25.0 (n = 3) μ m.

Male. Similar to female with usual sexual differences. Sensilla coeloconica on flagellomeres 1, 11–13; AR 0.87 (n = 1); PR 3.11 (n = 1). Wing with pattern of pale spots as in



Figure 3. *Culicoides (A.) fenggangensis* sp. n. **a** Genitalia and aedeagus (parameres absent), ventral view **b** parameres, ventral view.

Leg	TR	F-T
Foreleg	2.67	95 : 92 : 56: 21 : 15 : 8 : 9
Midleg	1.94	115:112:64:33:16:8:9
Hindleg	1.49	117:117:55:37:19:9:10

Table 2. TR and F-T of all legs of *C. fenggangensis* sp. n. (\mathcal{C}).

Fig. 1b, wing length 1.58 (n = 1), width 0.55 (n = 1); CR 0.57 (n = 1). TR and F-T of legs are given as Table 2. Genitalia (Fig. 2j): 9th tergite squarish, distal portion flat with short, conical processes at apicolateral. Ninth sternite with broad, deep, semicircle caudomedian excavation. Gonocoxite twice as long as broad, sclerotized; gonostylus tapering distally, distal portion curved. Parameres (Fig. 3b) separate, contiguous in midportions; each with moderately long, slender basal arm, swollen at base, stem long, slightly curved near base; apical portion tapered, elongate, abruptly bent without lateral fringe of spicules. Aedeagus (Fig. 3a) nearly triangle, basal arms short, unciform, tapering toward end, basal arch low, extending to 1/5 of total length, distal process 1/6 total length, long ovoid process at apex.

Type material. Holotype female, Chongxin village, Yong'an town, Fenggang county, Zunyi city, Guizhou province, China (28°06'31.49"N, 107°35'57.94"E), 12. IV. 2016, alt. 908m, Qiongqiong Chang col. Paratypes: 2 males and 3 females, same data as holotype.

Distribution. China (Guizhou Province).

Etymology. This species is named in tribute to Fenggang county, where the specimens were collected.

Taxonomic discussion

Culicoides (Avaritia) fenggangensis sp. n. is very similar to *C. (A.) comparis* Liu & Yu, 2005 and *C. (A.) dentiformis* McDonald & Lu, 1972 based on the interfacetal hairs

on the eyes and sensilla coeloconica on the flagellomeres. C. fenggangensis sp. n. can be distinguished from these two congeners by the number and distribution of pale spots on the wing (cell m, with two and three pale spots respectively in C. dentiformis and C. comparis) (Yu et al. 2005), elongate and cylindrical third palpus segment (third palpus segment is swollen in C. comparis and C. dentiformis). Females of C. fenggangensis sp. n. have a different wing size (wing length 1.33 mm and width 0.63 mm in C. comparis and wing length 1.78 mm and width 0.76 mm in C. dentiformis), distribution of the heavy macrotrichia on the wing (respectively distal 4/5 and 1/4 of the wing in C. *comparis* and *C. dentiformis*), they have a more slender 3rd palpus segment compared to the most species of subgenus Avaritia (only 12 species PR > 3.2), PR 3.2-3.75 (respectively PR 2.27 and 2.5 in C. comparis and C. dentiformis), different size of spermathecae (measuring $45.0 \times 40.0 \ \mu\text{m}$ in *C. comparis* and $60.0 \times 45.0 \ \mu\text{m}$ in *C. dentiformis*). Because male of C. comparis is unknown, the new species will only compare with C. dentiformis. Males of C. fenggangensis sp. n. have a different shape and structure of genitalia, with two lateral processes on the distal portion of ninth tergite (without lateral process in C. dentiformis), parameres apical portion tapered and abruptly bent (linear in C. dentiformis), long ovoid process at apex of aedeagus (diamond-shape process in C. dentiformis). Therefore, the distinctive features to separate C. fenggangensis sp. n. from others are cell m, and m, with 2 and 4 pale spots respectively.

The male of *C. fenggangensis* keys to C. dentiformis in Yu et al. (2005) where it may be distinguished by the presence of conical apicolateral processes on tergite 9 which are absent in *C. dentiformis*. The female of *C. fenggangensis* keys to the couplet with *C. dentiformis* and *C. comparis* in Yu et al. (2005) where it may be distinguished by the presence of 4 pale spots in cell m₂ which are absent in *C. dentiformis* and *C. comparis*.

The biogeographical territory of China spans the Palaearctic and Oriental Regions, which results in a rich diversity of biting midge species. The species of the subgenus *Culicoides (Avaritia)* are distributed in most provinces of China, except Qinghai and Ningxia. There are 15 species distributed in the Palaearctic Region, accounting for 26.3% of the total (C. abchazicus, C. actoni, C. albifascia, C. chiopterus, C. comparis, C. incertus, C. longirostris, C. nielamensis, C. nigritus, C. obsoletus, C. peregrinus, C. scoticus, C. sinanoensis, C. tainanus, C. wandashanensis). There are 49 species present in the Oriental Region, accounting for 86.0% of the total (C. actoni, C. albifascia, C. bawanglingensis, C. brevipalpis, C. brevitarsis, C. bubalus, C. clavipalpis, C. conaensis, C. dentiformis, C. elongates, C. filicinus, C. gaponus, C. holcus, C. hui, C. longirostris, C. imicola, C. innoxius, C. insignipennis, C. iphthimus, C. jacobsoni, C. kepongensis, C. kinabaluensis, C. lansangensis, C. lengi, C. liui, C. malayae, C. mamaensis, C. motoensis, C. nujiangensis, C. obsoletus, C. orestes, C. orientalis, C. palauensis, C. pastus, C. pelius, C. peregrinus, C. qionghaiensis, C. ruiliensis, C. sinanoensis, C. suiyangensis, C. sumatrae, C. suzukii, C. tainanus, C. tibetensis, C. trimaculatus, C. wadai, C. yamii, C. yuchihensis, C. fenggangensis). Finally, there are seven species present in both Regions, accounting for 12.3% of the total. This geographical distribution of biting midges in China is consistent with the distribution of other animals (Yan and Yu 1998).

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RESEARCH ARTICLE



A new species of Scinax from the Purus-Madeira interfluve, Brazilian Amazonia (Anura, Hylidae)

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Abstract

A new tree frog species of the genus *Scinax* from the interfluve between the Purus and Madeira rivers, Brazilian Amazonia, is described and illustrated. The new species is diagnosed by medium body size, snout truncate in dorsal view, ulnar and tarsal tubercles absent, nuptial pads poorly developed, skin on dorsum shagreen, dorsum light brown with dark brown spots and markings, white groin with black spots, anterior and posterior surfaces of thighs black, and iris bright orange. The advertisement call consists of a single short note, with 16–18 pulses and dominant frequency at 1572–1594 Hz. Tadpoles are characterized by body ovoid in dorsal view and triangular in lateral view, tail higher than body, oral disc located anteroventrally and laterally emarginated, dorsum of body uniformly grey-brown with dark brown eye-snout stripe in preservative, fins translucent with small to large irregular diffuse dark brown spots.

Keywords

Amazonian rainforest, Amazonas, anuran diversity, Brazil, Rondônia, Scinax onca sp. n.

Introduction

With nearly 70 currently recognized species, the genus *Scinax* Wagler, 1830 represents one of the most species-rich hylid genera in the Neotropics. Nevertheless, an increasing rate of new *Scinax* species recognition in the few last years (e.g., Fouquet et al. 2007, Brusquetti et al. 2014; Sturaro and Peloso 2014; Araujo-Vieira et al. 2015; Araujo-Vieira et al. 2016; Juncá et al. 2015; Ferrão et al. 2016) indicates that our knowledge of the actual species diversity in this genus is still very incomplete. Similarly, despite an intensive research in the last decades (e.g. Faivovich 2002, Faivovich et al. 2005, Duellman et al. 2016) many questions concerning our knowledge of phylogenetic relationships of this and correlated genus remain still an object of discussion.

The *Hyla rubra* species group was first recognized by Leon (1969). Several years later, Fouquette and Delahoussaye (1977) resurrected the generic name *Ololygon* Fitz-inger, 1843 (type species *Hyla strigilata* Spix, 1824) to harbour the members of the *H. rubra* group and delimited five other species groups. However, the correct generic name to these tree frogs is *Scinax* Wagler, 1830 (type species *Hyla aurata* Wied, 1821) as noted by Pombal and Gordo (1991). Consequently, Duellman and Wiens (1992) defined the genus *Scinax* based on external morphology of adults and tadpoles, osteology, and reproductive behaviour.

Faivovich (2002) tested monophyly of species groups traditionally recognized in *Scinax* and defined two main monophyla: the *S. ruber* Clade (comprising members of the *S. rostratus* and *S. ruber* species groups) and the *S. catharinae* Clade (involving members of the *S. catharinae* and *S. perpusillus* species groups). In following comprehensive review of the systematics of Hylidae, Faivovich et al. (2005) confirmed the monophyly of *S. ruber* and *S. catharinae* Clades and recovered *Hyla uruguaya* (Schmidt, 1944) in sister position to the *S. ruber* Clade (*H. uruguaya* was transferred to *Scinax* to avoid paraphyly of the genus). In the phylogeny proposed by Faivovich et al. (2005) the *S. ruber* Clade is composed by *S. rostratus* and *S. uruguayus* species groups plus species unassigned to any group and the *S. catharinae* Clade consists of *S. catharinae* and *S. perpusillus* species groups.

Recently, Duellman et al. (2016) revised the phylogeny of the family Hylidae and proposed three major changes in *Scinax* taxonomy: (i) resurrection of the genus *Ololy-gon* to harbour species of the former *Scinax catherinae* Clade, (ii) introduction of a new genus *Julianus* for members of the former *Scinax uruguayus* species group (sensu Faivovich et al. 2005), and (iii) restriction of the genus *Scinax* to the members of the former *S. ruber* Clade. As noted by Duellman et al. (2016), the separation of *Ololygon* and *Scinax* was evident in the cladistic analyses published by Faivovich (2002) and Faivovich et al. (2005). Moreover, Faivovich (2002) already stated that "*Scinax* could be partitioned at the level of the *catharinae* and *rubra* clades, and certainly there are names available for them. If desired, *Scinax* is available for the *rubra* clade, and the name *Ololygon* could be applied to the *catharinae* clade". In this paper, we follow Duellman et al. (2016) whose analysis is based on presently widest dataset.

The genus *Scinax* has a wide distribution area ranging from Mexico to central Argentina and Uruguay (Frost 2017). At present, 28 *Scinax* species are known to occur in Amazonia (see Sturaro and Peloso 2014, Brusquetti et al. 2014). However, a surprisingly high *Scinax* species diversity was recently revealed in the rainforests covering the area of the Purus-Madeira Interfluve (PMI; Fig. 1), where at least seven confirmed candidate species remain unnamed (Ferrão et al. 2016).

The PMI is crossed by an abandoned Trans-Amazonian highway (BR-319). Current proposals to reconstruct this highway bring a very serious threat for regional forest habitats and their fauna. Recent studies warn that one third of the PMI rainforest will be lost as a consequence of massive logging if this road improvement scheme goes ahead (Maldonado et al. 2012). Habitat loss has been widely reported as the major cause for populations decline and local extinctions for many groups of organisms, including frogs (e.g Soto-Azat et al. 2013). Therefore, current need of conservation of PMI is more urgent than ever before. In this respect, studies of species diversity resulting in descriptions of new species are of particular importance as they bring needed supporting data for wildlife conservation.

Here, we describe a new species of *Scinax* (*Scinax* sp. 3 *sensu* Ferrão et al. 2016) from the middle to southern PMI. The new species is described through external morphology of adults and tadpoles, and advertisement call.

Materials and methods

Adult specimens of the new species were collected in four sampling areas in the PMI (Fig. 1). Two sampling areas are located in the middle portion of the study area, one at the kilometre 350 of the BR-319 highway (5°15'57"S, 61°55'58"W, ca. 59 m a.s.l.: Fig. 1A) and the other at the Floresta Estadual Tapauá Reserve (06°22'37"S, 63°17'19"W, ca. 69 m a.s.l.: Fig. 1B). The remaining two sampling areas are located in the southern region of the PMI, near the left margin of upper Madeira river, about 100 kilometres from the municipality of Porto Velho (9°9'32"S, 64°37'60"W, ca. 105 m a.s.l., Fig. 1C; 9°17'52"S, 64°46'10"W, *ca.* 101 m a.s.l., Fig. 1D).

All adult specimens were collected at night, anesthetised, and killed with topic solution of 10% benzocaine, fixed in 10% formaldehyde solution and stored in 70% ethanol. Tissue samples were obtained from all adult specimens and stored in 96% ethanol at Albertina Lima's laboratory at INPA (Instituto Nacional de Pesquisas da Amazônia), Manaus, Brazil. Measurements were taken to the nearest 0.1 mm with digital calliper under a dissecting microscope. Sex and maturity of specimens were identified by observing secondary sexual characters (vocal sac, vocal slits), and gonads through dissection. The format for the description and diagnostic characters follows Duellman and Wiens (1993) and Duellman et al. (2006). Webbing formulae follow the standards of Savage and Heyer (1967) and Myers and Duellman (1982), while all other terminology is that of Duellman (1970), Heyer et al. (1990) and Napoli (2005). Measurement abbreviations used throughout the text are: SVL (snout–vent length), HL (head length, the straight line distance from the posterior edge of the jaw articulation to the tip of the snout), HW (head width at angle of jaw), IND (internarial dis-



Figure 1. Distribution of *Scinax onca* sp. n. and *Scinax iquitorum* in Brazilian Amazonia. Yellow star: **A** type locality of *S. onca* sp. n., kilometre 350 of the BR-319 Highway, municipality of Beruri, State of Amazonas. Yellow circles: **B** paratype locality of *S. onca* sp. n., Floresta Estadual Tapauá Reserve, municipality of Tapauá, State of Amazonas **C–D** paratype localities of *S. onca* sp. n., municipality of Porto Velho, State of Rondônia. White triangles: **E** record of *S. iquitorum* near southern distribution of *S. onca* sp. n. according Melo-Sampaio and Souza (2015), municipality of Plácido de Castro, State of Acre, Brazil **F** record of *S. iquitorum* according Machado et al. (2015), municipality of Cruzeiro do Sul, State of Acre, Brazil.

tance), EN (eye to nostril distance), ED (horizontal eye diameter), ELW (upper eyelid width), IOD (minimal interorbital distance), TD (horizontal tympanum diameter), HAL (hand length), THL (thigh length) TL (tibia length), TAL (tarsus length), FL (foot length as the distance from the heel to the tip of the fourth toe), Fin3DW (Finger III disk width), Toe4DW (Toe IV disk width). Field notes and colour images were used for descriptions of coloration in life. Collected specimens were deposited in the herpetological section of the Zoological Collections of INPA (INPA-H). Specimens examined for comparative diagnoses are listed in the Appendix 1.

Tadpoles of the new species were collected in a 25m² pond not connected to stream, in the sampling area near the kilometre 350 of the BR-319 highway (5°15'57"S, 61°55'58"W, ca. 59 m a.s.l.: Fig. 1A). Tadpoles were killed with a 5% lidocaine solution diluted in water, and preserved in 5% formalin (tail of one tadpole was stored in 100% ethanol). All tadpoles were deposited in one lot (INPA-H 35411) in the INPA-H collection. The determination of the tadpoles was verified using molecular barcoding (GenBank accession number KU317421; see Ferrão et al. [2016]). Tadpoles were staged according to Gosner (1960). The format for the tadpole description follows Schulze et al. (2015). The description was based on six tadpoles in the Gosner Stage 37. Following morphometric characters were measured according to Lavilla and Scrocchi (1986) and Altig and McDiarmid (1999): total length (TL), body length (BL), tail length (TAL), maximum width of the tail muscle (TMW), maximum height of the tail (MTH), maximum tail muscle height (TMH), interorbital distance (IOD), internarial distance (IND); eye diameter (ED) and eye-nostril distance (END).

The interspecific pairwise genetic distances in the 16S rRNA between the new species and other available *Scinax* species were presented by Ferrão et al. (2016). However, the intraspecific pairwise genetic distances between specimens of the new species remain unknown. Due to that, we calculated uncorrected p and Kimura-2-parameter distances (Kimura 1980) between 16S rRNA sequences from specimens of the two clades of the new species (see Ferrão et al. 2016, Fig. 2) with MEGA 6.06 (Tamura et al. 2013). Sequences of adults and one tadpole from middle PMI clade (KU317415, KU317416, KU317421, KU317422, KU317423, KU317425, KU317426) and of adults from southern PMI clade (KU317417, KU317418, KU317419, KU317420, KU317424, KU317427) were obtained from GenBank. Preliminarily to distance calculations, the sequence set was aligned using the Clustal W algorithm (Thompson et al. 1994) implemented in BioEdit (Hall 1999). Genetic distance is presented in the subsection "Variation".

Advertisement calls of one male (INPA-H 26624) from the Floresta Estadual Tapauá Reverve (middle PMI: Fig. 1B) were recorded in 12 October 2013 using a Sony PCM - D50 digital recorder. Air temperature at the time of recording was not measured. We analysed fifteen calls with the sound analysis software Raven 1.5 (Bioacoustics Research Program 2014). Obtained oscillograms and spectrograms were analysed through Blackman window, 80 Hz of frequency resolution and Fast Fourier Transformation (FFT) of 1024 points. The following call parameters were measured: call duration, inter-call interval, number of pulses per call, dominant frequency, and call repetition rate (number of calls emitted within one minute of vocalization). Terminology of call descriptions follows Köhler et al. (2017).

Institutional abbreviations are as follows:

INPA-H	Collection of amphibians and reptiles of Instituto Nacional de Pesquisas
	da Amazônia, in Manaus, Brazil
KU	University of Kansas, Museum of Natural History, Division of Herpetology,
	Lawrence, Kansas, USA
QCAZ	Museo de Zoología, Pontifica Universidad Católica del Ecuador, Quito,
	Ecuador
RMNH	Rijksmuseum van Natuurlijke Historie, Leiden, The Netherlands
NHMG	Naturhistoriska Museet, Göteborg, Sweden
NMP6V	National Museum, Prague, Czech Republic
ZFMK	Zoologisches Forschungsinstitut und Museum Alexander Koenig, Bonn,
	Germany
	-

Taxonomy

Scinax onca sp. n.

http://zoobank.org/91C1811A-D2FC-4699-8E2D-76D203D0BCC7 Figs 2–3, 5–7, 9–10 Suggested English name: Jaguar Snouted Treefrog.

Scinax iquitorum: Almeida et al. 2015: 142, Appendix II. *Scinax* sp. 3: Ferrão et al. 2016: 7–9, figs 1 & 2B, Supporting table S2–S4.

Holotype (Figs 2–3, 4A–B). INPA-H 34584, an adult male from kilometre 350 of the BR-319 Highway (5°15'57"S, 61°55'58"W, ca. 59 m a.s.l., Fig. 1A), municipality of Beruri, State of Amazonas, Brazil, collected on 15 November 2013 by Miquéias Ferrão and Rafael de Fraga.

Paratypes (Figs 5C–F, 6–7). Sixteen specimens: five adult males (INPA-H 34581, INPA-H 34582, INPA-H 34585, INPA-H 34586, INPA-H 34587) and one adult female (INPA-H 34583), same locality and collecting data as the holotype; one adult male (INPA-H 26624) and one adult female (INPA-H 26625) from the Floresta Estadual Tapauá Reserve (06°22'37"S, 63°17'19"W, ca. 69 m a.s.l., Fig. 1B), municipality of Tapauá, State of Amazonas, Brazil, collected on 12 October 2013 by Alexandre P. Almeida; five adult males (INPA-H 34588, INPA-H 34592, INPA-H 34593, INPA-H 34594, INPA-H 34595) and one adult female (INPA-H 34589) from municipality of Porto Velho (9°9'32"S, 64°37'60"W, ca. 105 m a.s.l., Fig. 1C), State of Rondônia, Brazil, collected on 2 November and 7 February 2014 by Albertina P. Lima; one adult male (INPA-H 34590) and one adult female (INPA-H 34591) from municipality of Porto Velho (9°17'52"S, 64°46'10"W, ca. 101 m a.s.l., Fig. 1D), State of Rondônia, Brazil, collected on 25 February 2010 by Albertina P. Lima.

Referred material. Two: INPA-H 35413 and INPA-H 35414, newly metamorphosed specimens from the kilometre 350 of the BR-319 Highway (5°15'57"S, 61°55'58"W, ca. 59 m a.s.l., Fig. 1A), municipality of Beruri, State of Amazonas, Brazil, collected on 17 January 2014 by Miquéias Ferrão.

Generic placement. We assign the new species to *Scinax* based on general morphological similarity to other members of the genus, cloacal tube of tadpoles positioned above the margin of the lower fin (a synapomorphy of the former *S. ruber* Clade sensu Faivovich [2002], currently *Scinax* sensu Duellman et al. [2016]).

Diagnosis. A medium-sized species of *Scinax* characterized by the following combination of characters: (1) SVL 31.3–34.5 mm (n = 13) in males and 35.5–40.4 mm (n = 4) in females; (2) snout truncate in dorsal view, bluntly rounded in lateral view; (3) tarsal tubercles absent; (4) tubercles on lower jaw and knee absent; (5) skin on dorsum shagreen; (6) dentigerous processes of vomers triangular; (7) in life, ground colour of dorsum light brown with dark brown spots and markings; dorsolateral stripes or X-shaped blotch on dorsum absent; flanks light brown with or without dark brown spots; axillar region and groin white with black irregular spots; anterior and posterior surfaces



Figure 2. Holotype of *Scinax onca* sp. n. Dorsal and ventral view of the preserved holotype of *S. onca* sp. n. INPA-H 34584 from middle Purus-Madeira Interfluve, at the kilometre 350 of the BR-319 highway, municipality of Beruri, State of Amazonas, Brazil. Scale bar 5 mm.



Figure 3. Hand and foot of holotype of *Scinax onca* sp. n. Ventral view of the hand and foot of the preserved holotype of *Scinax onca* sp. n. Scale bar 5 mm.

of thighs black (usually bordered by an irregular white streak); webbing between toes black; belly white to yellow, with round dark brown spots; iris bright orange; (8) advertisement call consisting of a single pulsed note; note duration 102–121 ms; 16–18 pulses/note; dominant frequency 1572–1594 Hz; (9) tadpoles with body triangular in lateral view; labial tooth row formula 2(2)/3(1); labial arm absent.

Comparisons. Until now, the following 28 valid species of Scinax occur in Amazonia (Sturaro and Peloso 2014, Brusquetti et al. 2014, Frost 2017): S. baumgardneri (Rivero, 1961), S. blairi (Fouquette & Pyburn, 1972), S. boesemani (Goin, 1966), S. chiquitanus (De la Riva, 1990), S. cruentommus (Duellman, 1972), S. danae (Duellman, 1986), S. exiguus (Duellman, 1986), S. funereus (Cope, 1874), S. fuscomarginatus (Lutz, 1925), S. fuscovarius (A. Lutz, 1925), S. garbei (Miranda-Ribeiro, 1926), S. ictericus Duellman & Wiens, 1993, S. iquitorum Moravec, Tuanama, Pérez & Lehr, 2009, S. jolyi Lescure & Marty, 2000, S. karenanneae (Pyburn, 1992), S. kennedyi (Pyburn, 1973), S. lindsayi Pyburn, 1992, S. madeirae (Bokermann, 1964), S. nebulosus (Spix, 1824), S. oreites Duellman & Wiens, 1993, S. pedromedinae (Henle, 1991), S. proboscideus (Brongersma, 1933), S. rostratus (Peters, 1863), S. ruber (Laurenti, 1768), S. sateremawe Sturaro & Peloso, 2014, S. villasboasi Brusquetti, Jansen, Barrio-Amorós, Segalla & Haddad, 2014, S. wandae (Pyburn & Fouquette, 1971), and S. x-signatus (Spix, 1824). Members of the genus Julianus occur in Uruguay, extreme southern Brazil, and in northern Corrientes, Argentina (J. uruguayus [Schmidt, 1944]) and in Serra do Cipó, Minas Gerais, Brazil (J. pinimus [Bokermann & Sazima, 1973]). Species of the genus Ololygon are distributed in Atlantic Coastal Forest of eastern Brazil, gallery forests of the Brazilian Cerrado and in Argentina (see Duellman et al. 2016). Among species of *Scinax* distributed in Amazonia, except by the species that occur in open habitats, all other species are endemic to the biome. Regarding the fact that Scinax onca sp. n. is an exclusive forest dweller known from the lowland rainforest of southern part of Central Amazonia we focus the comparison on Amazonian Scinax species, including six confirmed candidate species discovered recently in PMI (Scinax sp. 1–2 and Scinax sp. 4–7 of Ferrão et al. 2016).

Morphologically, *Scinax onca* sp. n. can be distinguished from all other Amazonian *Scinax* species by having bright orange iris and white groin with black spots in life and by the following combinations of characters (characters of other species in parentheses or brackets unless otherwise stated):

The new species differs from *S. baumgardneri*, *S. garbei*, *S. jolyi*, *S. kennedyi*, *S. nebulosus*, *S. pedromedinae*, *S. proboscideus*, and *S. rostratus* by snout truncate in dorsal view and bluntly rounded in lateral view, and by the absence of tubercles on the lower jaw and knee (elongated or pointed snout, and tubercles present on the lower jaw and knee; Duellman 1972, Pyburn 1973, Duellman and Wiens 1992, Lescure and Marty 2000, Lima et al. 2004). In addition, tadpoles of *S. onca* sp. n. differ from those of *S. garbei*, *S. nebulosus*, *S. pedromedinae*, and *S. rostratus* by the absence of labial arm (labial arm present; Duellman 1978, Hero and Mijares-Urrutia 1995, Duellman 2005, Gomes et al. 2014).

The male SVL 31.3–34.5 mm of *S. onca* sp. n. is larger than male SVL of *S. blairi* (27.8–30.1 mm; Fouquette and Pyburn 1972), *S. cruentommus* (24.8–27.7 mm; Duellman 1972), *S. danae* (24.5–27.4 mm; Duellman 1986), *S. exiguus* (18.0–20.8 mm; Duellman 1986), *S. fuscomarginatus* (15.7–26.7 mm; Brusquetti et al. 2014), *S. karenanneae* (SVL 26.6–28.9 mm; Pyburn 1993), *S. lindsayi* (about 24 mm; Pyburn 1992), *S. madeirae* (18.0–23.1 mm; Brusquetti et al. 2014), *S. villasboasi* (16.7–20.0 mm; Brusqu
quetti et al. 2014), *S. wandae* (23.4–26.9 mm; Pyburn and Fouquette 1971), *Scinax* sp. 1 (20.2–22.5 mm, n = 5), *Scinax* sp. 2 (*sensu* Ferrão et al. 2016) (18.1–20.4 mm, n = 15), *Scinax* sp. 4 (*sensu* Ferrão et al. 2016) (23.2 mm), *Scinax* sp. 6 (*sensu* Ferrão et al. 2016) (25.1–26.7 mm, n = 6), and *Scinax* sp. 7 (*sensu* Ferrão et al. 2016) (22.6–25.9 mm, n = 28). The males of *S. onca* sp. n. are smaller than those of *S. fuscovarius* (SVL 41.0–44.0 mm; Cei 1980) and *S. sateremawe* (35.2–38.1 mm; Sturaro and Peloso 2014).

Scinax onca sp. n. can be distinguished from *S. boesemani* by conspicuous dark brown spots on the dorsum (light spots on dorsum) and belly (no spots), black posterior surfaces of thighs (light brown), and black webbing between toes (light brown; Goin 1966). The call of *S. onca* sp. n. differs from that of *S. boesemani* in duration (102–121 ms vs.160–290 ms in *S. boesemani*; Duellman and Pyles 1983).

The new species differs from *S. chiquitanus* in having snout truncate in dorsal view (rounded), head wider than body (narrower), black posterior surfaces of thighs (brown), and in having dark brown spots on the belly (light brown when present; De la Riva 1990). The call of *Scinax onca* sp. n. differs from the call of *S. chiquitanus* in duration (102–121 ms vs.185.3–338.8 ms in *S. chiquitanus*), number of pulses (16–18 vs. 23–42 in *S. chiquitanus*) and dominant frequency (1572–1594 Hz vs. 2100–2261.5 Hz in *S. chiquitanus*; De la Riva et al. 1994, Ferrão et al. 2016).

Scinax onca sp. n. differs from *S. ruber* by the snout truncate in dorsal view (rounded), black posterior surfaces of thighs (brown with yellow or orange mottling), and absence of dorsolateral stripes (tan to yellow dorsolateral stripes present; Duellman and Wiens 1993). There are seven available names in the synonymy of *S. ruber: Hyla conirostris* Peters, 1863 (type locality "Surinam"), *Hyla lateristriga* Spix, 1824 (type locality: Brazil, by implication), *Hyla lineomaculata* Werner, 1899 (type locality "Arima, Trinidad"), *Hyla robersimoni* Donoso-Barros, 1965 "1964" (type locality "Pajonales al sur de Macuro, Penisula de Paria, Venezuela"), *Hyla rubra hübneri* Melin, 1941 (type locality "Taracuá, Rio Uaupes", "São Gabriel, Rio Negro", and "Vicinity of Manaus", all localities in the State of Amazonas, Brazil), *Scytopis alleni* Cope, 1870 (type locality State of Pará, Brazil, by lectotype locality "Nauta", Region Loreto, Peru). According to their original descriptions, all these names are associated with specimens that have yellow blotches on the anterior and posterior surfaces of the thighs, and in some cases undersurfaces of tibiae (Moravec et al. 2009).

From *Scinax x-signatus* (Spix, 1824) the new species can be distinguished by absence of the X-shaped mark (present) and presence of dark brown spots on the dorsum (absent; Lutz 1973).

Scinax onca sp. n. differs from *S. ictericus* by snout truncate in dorsal view (bluntly round), absence of ulnar and tarsal tubercles (tubercles present), and by black posterior surfaces of thighs (light to dark brown; Duellman and Wiens 1993). The call of *Scinax onca* sp. n. differs from the call of *S. ictericus* in duration (102–121 ms vs. 70–90 ms in *S. ictericus*). The tadpoles of the new species differ in having triangular body in lateral view (ovoid; Duellman and Wiens 1993).

The new species can be distinguished from *S. funereus* (Fig. 4A) by its truncate snout in dorsal view (acutely rounded; Duellman 1978), absence of tarsal tubercles (a



Figure 4. Adult specimens of *Scinax funereus* and *S. iquitorum*. **A** Female specimen of *Scinax funereus* (KU221960b) from San Jacinto, Region Loreto, Peru, and **B** male paratype of *Scinax iquitorum* (NMP6V 71267/1) from Puerto Almendras, Region Loreto, Peru. Photograph by W.E. Duellman (**A**) and Jiří Moravec (**B**).

row of low tubercles on outer edge of tarsus; Duellman 1971), shagreen skin (strongly tuberculate; Duellman and Wiens 1993), flanks light brown (yellow; Duellman and Wiens 1993), orange iris (bicolored iris; see Fig. 4A), and black posterior surfaces of thighs (yellow with dark brown spots or pale with discrete dark brown blotches; Duellman and Wiens 1993). Labial tooth row formula 2(2)/3(1) of the tadpoles of *S. onca* sp. n. differs from that of *S. funereus* (2(2)/3; Duellman 1978). There are two available names in the synonymy of *S. funereus*: *Hyla depressiceps* Boulenger, 1882 (type locality "Ecuador") and *Hyla rubra inconspicua* Melin, 1941 (type locality "Roque, Region San Martín, Peru"). According to the original description, *H. depressiceps* differs from the new taxon in having black and whitish marbled limbs. An examination of the holotype of *Hyla rubra inconspicua* shows that it differs by the presence of small tubercles on the head, dorsum and limbs including the tarsal area (see Moravec et al. 2009).

The new species differs from *S. iquitorum* (Fig. 4B) by snout truncate in dorsal view (bluntly rounded), dentigerous processes of vomers triangular (transverse), presence of conspicuous dark brown spots on dorsum (small dark brown dots concentrated only on head and in areas of scapular and sacral blotches), light brown flanks with or without dark brown spots (bright yellow flanks with numerous distinct round black spots), and by white long bones of hindlimbs (green; Moravec et al. 2009).

Scinax onca sp. n. differs from *Scinax* sp. 5 (*sensu* Ferrão et al. 2016) by light brown dorsum with dark brown spots (yellowish green with diminutive black spots), dark spots on belly (absent), and anterior and posterior surfaces of thighs black (uniformly yellowish green).

Description of the holotype. Adult male 31.3 mm SVL. Body moderately slender; head wider than body, slightly longer than wide (HL/HW = 1.2, HL = 38.0% of SVL, HW = 32.3% of SVL); snout truncate in dorsal view, bluntly rounded in lateral view; nostrils markedly protuberant, elliptic, directed dorsolaterally; eye-nostril distance 76% of ED; internarial region moderately depressed; canthus rostralis rounded in both dorsal and lateral view; loreal region concave, more concave near to nostril;

interorbital distance longer than upper eye width (IOD/ELW = 1.1), IOD 31% of HW; eye diameter 34% of HW; tympanic annulus distinct, tympanic membrane evident, rounded, 51% of ED; supratympanic fold present, slightly distinct; vocal sack subgular, bilobate; vocal slits extend from lateral base of tongue (slightly behind the half distance from the anterior edge) to the mouth angles; dentigerous processes of vomers triangular, bearing 7/6 (left/right) teeth; choanes rounded; tongue lanceolate.

Arm and forearm slender; axillary membrane absent; pectoral fold present; hand length 29% of SVL; fingers long bearing horizontally expanded discs; diameter of disc on finger III 49% of ED; relative length of fingers I<II<IV<III; palmar tubercle bifid, flat, longer than wide; thenar tubercle elongated; distal subarticular tubercle conical on Finger I, subconical on Finger II, rounded on fingers III–IV; supernumerary tubercles small, slightly distinct; nuptial pad poorly developed, slender, extending from proximal base of thenar tubercle to distal base of distal subarticular tubercle on Finger I; fingers II–IV basally webbed; fingers with narrow lateral fringes, external fringe on Finger IV extends to distal portion of thenar tubercle.

Hind limb long; tibia longer than femur, tibia length 52% of SVL, femur length 47% of SVL; tarsus length 27% of SVL; foot length 44% of SVL; toe discs more rounded than finger discs; diameter of disc on Finger IV 44% of eye ED; relative length of toes I<II<III<V<IV; inner metatarsal tubercle oval and flat; outer metatarsal tubercle rounded, flat, three times smaller than inner metatarsal tubercle; subarticular tubercles subconical on toes I–II, rounded on toes III–V; supernumerary tubercles small, rounded, and flat; webbing on toes I $2-2^+$ II 1^+-2 III $2-1^+$ V; distinct external lateral fringe on Toe V extending to outer metatarsal tubercle; fringe on external margin of Toe I extends to inner metatarsal tubercle; tarsal folds and tarsal tubercles absent; tubercles on heels absent.

Skin on dorsum shagreen, almost granular in supratympanic and anterotympanic region; skin smooth on forelimbs, hind limbs, throat, chest, and vocal sac; skin areolate on belly and ventral surface of thighs.

Measurements of the holotype (in mm). SVL 31.3; HL 11.9; HW 10.9; ED 3.7; EN 3.6; ELW 3.1; IND 2.8; IOD 3.4; TD 1.9; HAL 9.1; Fin3DW 1.8; TL 16.3; THL 14.8; TSL 8.6; FL 13.7; Toe4DW 1.6.

Colouration of the holotype in life (Fig. 5A–B). Ground colour of dorsal surfaces of head, body, and limbs light brown; dorsal pattern consisting of W-shaped interorbital mark on the head, an irregular dark brown spot in scapular region, a Λ -shaped mark in sacral region, and numerous round dark brown spots distributed randomly on the head (including lips) and body; a conspicuous dark brown canthal stripe extends to tip of snout; a dark brown supratympanic stripe extends from corner of eye to anterior region of flanks; three dark brown transverse bars on the forearm, the proximal one extends to arm; three brown transverse bars on the tibia; fingers and toes light brown, distal surfaces of disc cream to tan, proximal surfaces grey; toe webbing black; axillar region white with small dark brown spots; flanks light brown with dark brown spots; groin white with dark brown spots; anterior surfaces of thighs black; posterior surfaces of thighs black, bordered with an irregular white streak; throat and vocal sac yellow-



Figure 5. Colour in life of *Scinax onca* sp. n. Colour variation in life of *Scinax onca* sp. n. from the Purus-Madeira Interfluve, Brazilian Amazonia. **A–B** INPA-H 34584 (holotype), adult male from the kilometre 350 of the BR-319 highway, State of Amazonas **C–D** INPA-H 34591, adult female from municipality of Porto Velho, State of Rondônia **E–F** INPA-H 26625, adult female from the Floresta Estadual Tapauá Reserve, municipality of Tapauá, State of Amazonas. Photographs A–D and F were taken after transport of the specimens to the camp, while the image of E was taken immediately in the field.

ish; chest translucent; belly yellowish laterally, white medially, covered with randomly distributed round dark brown spots; anterior ventral surfaces of thighs greyish with black spots; posterior ventral surfaces of thighs dark grey to black; ventral surfaces of hand and foot black; nuptial pad cream; iris bright orange, without black reticulation, bordered by black externally.

Colouration of the holotype in alcohol (Figs 2–3). Dorsal surfaces of head, body, and limbs brown; throat, belly, and axillar area yellowish; groin white; dark brown dorsal and ventral pattern as in life with exception of inconspicuous transverse bars on thighs.

Variations. Both uncorrected p and K2P distances between specimens from southern and specimens from middle PMI groups range between 0.4 and 1.1%. Both the p and K2P distances between individuals from middle PMI varied from 0% to 0.2% and between individuals from southern PMI varied from 0% to 0.6% (Table 1). Despite the high genetic similarity, it appears that some variation in measurements and coloration is evident between specimens from middle PMI and specimens from southern PMI (the straight distance between the closest localities is ca. 500 km).

The specimens from southern PMI exhibit slightly larger average size (t = -3.1, df = 10.4, p = 0.009) and significantly lower values of nine following male body proportions: HL/SVL (t = 2.3, df = 10.9, p = 0.01), IND/SVL (t = 3.4, df = 10.8, p = 0.005), IOD/SVL (t = 3.2, df = 9.6, p = 0.009), HAL/SVL (t = 6.9, df = 8.5, p < 0.001), THL/SVL (t = 2.8, df = 11, p = 0.01), TL/SVL (t = 3.9, df = 8.8, p = 0.003), TAL/SVL (t = 2.6, df = 10.2, p = 0.02), FL/SVL (t = 5.1, df = 10.3, p = 0.003), and X3FD/SVL (t = 2.9, df = 6.7, p = 0.02). Variation of measurements and body proportions of the type specimens is given in Table 2.

Colour change was observed after (Fig. 5A–D, F) and before (Fig. 5E) human manipulation of the specimens. After manipulation, general colouration of individuals became darker and spots and blotches became more conspicuous. In preservative, individuals from the middle PMI (Fig. 6A–C) had a larger number of dorsal spots and blotches in comparison to specimens from southern PMI (Fig. 6D–F). Regarding ventral coloration in preservative, individuals from the middle PMI (Fig. 7A–C) had a larger number of spots, which were concentrated on the belly. In the south, individuals had smaller ventral spots, and these were concentrated on the throat (Fig. 7D–F).

Vocalization. The advertisement call of *Scinax onca* sp. n. consists of a single short multipulsed note (Fig. 8). Quantitative call parameters are as follows (range followed by mean \pm standard deviation in parentheses): call duration, 102–121 ms (110 \pm 5, n = 15); silent interval between calls 526–1844 ms (1089 \pm 438, n = 15), pulses/call 16–18 (16.8 \pm 0.8, n = 15); dominant frequency 1572–1594 Hz (1573 \pm 6, n = 15). Calls were repeated at an approximate rate of 16 notes per minute.

Tadpole description. The following description is based on six tadpoles (Stage 37) of the lot INPA-H 35411. Total length $34.6-38.3 \text{ mm} (37 \pm 1.5, \text{ n} = 6)$, body length $9.1-10.5 \text{ mm} (9.8 \pm 0.5, \text{ n} = 6)$, and tail length $24.6-28.7 \text{ mm} (27 \pm 1.5, \text{ n} = 5)$. Body ovoid in dorsal view, triangular in lateral view (Fig. 9). Snout rounded in

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	Specimens	1	2	3	4	5	9	7	8	6	10	11	12*	13	14
-	NMP6V 71267/3		0.059	0.063	0.059	0.059	0.057	0.059	0.059	0.059	0.059	0.061	0.059	0.059	0.059
5	INPA-H 34592	0.065		0.004	0.000	0.000	0.002	0.000	0.004	0.004	0.004	0.006	0.004	0.004	0.004
3	INPA-H 34588	0.070	0.004		0.004	0.004	0.006	0.004	0.008	0.008	0.008	0.011	0.008	0.008	0.008
4	INPA-H 34594	0.065	0.000	0.004		0.000	0.002	0.000	0.004	0.004	0.004	0.006	0.004	0.004	0.004
Ś	INPA-H 34593	0.065	0.000	0.004	0.000		0.002	0.000	0.004	0.004	0.004	0.006	0.004	0.004	0.004
9	INPA-H 34595	0.062	0.002	0.006	0.002	0.002		0.002	0.006	0.006	0.006	0.008	0.006	0.006	0.006
	INPA-H 34589	0.065	0.000	0.004	0.000	0.000	0.002		0.004	0.004	0.004	0.006	0.004	0.004	0.004
8	INPA-H 20586	0.065	0.004	0.009	0.004	0.004	0.006	0.004		0.000	0.000	0.002	0.000	0.000	0.000
6	INPA-H 34585	0.065	0.004	0.009	0.004	0.004	0.006	0.004	0.000		0.000	0.002	0.000	0.000	0.000
10	INPA-H 34581	0.065	0.004	0.009	0.004	0.004	0.006	0.004	0.000	0.000		0.002	0.000	0.000	0.000
11	INPA-H 34583	0.068	0.006	0.011	0.006	0.006	0.009	0.006	0.002	0.002	0.002		0.002	0.002	0.002
12	INPA-H 35411*	0.065	0.004	0.009	0.004	0.004	0.006	0.004	0.000	0.000	0.000	0.002		0.000	0.000
13	INPA-H 35413	0.065	0.004	0.009	0.004	0.004	0.006	0.004	0.000	0.000	0.000	0.002	0.000		0.000
14	INPA-H 35414	0.065	0.004	0.009	0.004	0.004	0.006	0.004	0.000	0.000	0.000	0.002	0.000	0.000	



Figure 6. Colour in preservative of dorsum of *Scinax onca* sp. n. Dorsal colour variation of preserved specimens of *Scinax onca* sp. n. Specimens from middle (**A–C**) and southern (**D–F**) Purus-Madeira Interfluve, Brazilian Amazonia. **A** INPA-H 34581, male, SVL 34.3 mm **B** INPA-H 34583, female, SVL 35.5 mm **C** INPA-H 34582 male, SVL 31.5 mm **D** INPA-H 34594, male, SVL 32.6 mm **E** INPA-H 34589, female, SVL 38.9 mm **F** INPA-H 34593, male, SVL 34.5 mm.

dorsal and lateral view, distinct from body. Nostrils large, rounded, positioned and directed dorsally, eye-nostril distance represents 63-88% (74 ± 9, n = 6) of eye diameter. Inter nostril distance represents 62-70% (65 ± 3 , n = 6) of inter orbital distance. Eyes large, positioned and directed laterally, with diameter 15–19 % (17 \pm 1, n = 6) of body length. Spiracle tube single, sinistral, visible from dorsal view, inner wall and ventral right wall of the tube free from the body. Tail higher than body, point of maximum height of tail about half tail length. Tail musculature visible. Dorsal fin emerging nearly in the middle of the body, rising moderately, descending gradually to flagellum. Ventral fin approximately of the same height and shape as the dorsal fin. Cloacal tube positioned above the margin of the lower fin. Oral disc located anteroventrally, emarginated laterally, protuberant when closed (Fig. 10). Upper labium with uniseriate marginal papillae on distal portion and two rows of papillae (with small median gap) close to mouth angle. Lower labium with triseriate marginal papillae close to mouth angle and biseriate papillae on medial portion. Papillae are long, rounded on tip, distributed irregularly. Jaw sheaths moderately robust and serrated, upper jaw M-shaped and lower jaw V-shaped. Labial tooth row formula 2(2)/3(1). The row A-1 nearly the



Figure 7. Colour in preservative of venter of *Scinax onca* sp. n. Ventral colour variation of preserved specimens of *Scinax onca* sp. n. Specimens from middle (**A–C**) and southern (**D–F**) Purus-Madeira Interfluve, Brazilian Amazonia. **A** INPA-H 34583, female, SVL 35.5 mm **B** INPA-H 34582, male, SVL 31.5 mm **C** INPA-H 34581 male, SVL 34.3 mm **D** INPA-H 34588, male, SVL 34.1 mm **E** INPA-H 34593, male, SVL 34.5 mm **F** INPA-H 34589, female, SVL 38.9 mm.

same length of A-2, P-2 slightly longer than P-1, P-3 shorter than P-1 and P-2. The gap in P-1 approximately the same length of the gap in A-2.

In life, dorsal and lateral surfaces of body silvery-green. Fins silvery-green, translucent, having dark grey spots. In preservative, dorsum of body uniformly grey-brown. A dark brown eye-snout stripe and dark brown interorbital blotch present. Fins translucent with small to large irregular diffuse dark brown spots. Tail musculature light brown. Ventral surfaces of the body white, slightly transparent.

Etymology. The specific name *onca* refers to the Brazilian common name for the jaguar *Pantera onca* (Linnaeus, 1758) due the blotchy colour pattern of the new species. Furthermore, the specific name is a reference to frequent encounters of *P. onca* during the fieldwork in the PMI. The name is used as a noun in apposition.

Distribution, ecology, and threat status. *Scinax onca* sp. n. is an exclusive forest dweller, known from two small areas located in the middle section of the PMI (State of Amazonas, Brazil), and two small areas lying in southern part of PMI, close to municipality of Porto Velho (Rondônia, Brazil). The maximum straight distance between the localities is around 500 km (Fig. 1). The middle PMI is covered by tropical lowland

	Middle Purus-M	ladeira interfluve	Southern Purus-N	Madeira interfluve
	Males (n = 7)	Females (n = 2)	Males (n = 6)	Females (n = 2)
SVL	32 ± 1.1 (31.3-34.3)	36.5 ± 1.0 (35.5-37)	33.6 ± 0.7 (32.6-34.5)	39.6 ± 1 (38.9-40.4)
HL	12.1 ± 0.3 (11.8-12.6)	13.1 ± 0 (13.1-13.1)	12.3 ± 0.3 (12-12.7)	13.6 ± 0.1 (13.6-13.7)
HW	11.3 ± 0.4 (10.9–11.9)	12.3 ± 0.3 (12.1-12.6)	11.8 ± 0.3 (11.4-12.1)	13.2 ± 0.1 (13.1–13.3)
ED	3.7 ± 0.3 (3.5-4.2)	3.7 ± 0.3 (3.5-3.9)	3.6 ± 0.2 (3.3-3.9)	$3.9 \pm 0.1 (3.8-4)$
TD	2.1 ± 0.2 (1.9-2.4)	2.3 ± 0.3 (2.1-2.4)	2.2 ± 0.1 (2-2.4)	2.3 ± 0.1 (2.3-2.4)
UEW	3.1 ± 0.2 (2.7-3.2)	3.1 ± 0.1 (3-3.2)	3.2 ± 0.2 (2.9-3.4)	3 ± 0.1 (2.9-3.1)
IOD	3.3 ± 0.2 (3.1-3.7)	3.7 ± 0.3 (3.5-3.9)	3.2 ± 0.2 (3-3.4)	3.8 ± 0.2 (3.7-4)
IND	2.7 ± 0.1 (2.6-2.8)	3.2 ± 0.1 (3.1-3.3)	2.7 ± 0.1 (2.7-2.8)	3 ± 0.1 (2.9-3.1)
TAL	9 ± 0.3 (8.6-9.6)	10 ± 0.3 (9.8-10.2)	9 ± 0.3 (8.7-9.3)	10.5 ± 0 (10.5-10.5)
FL	13.7 ± 0.4 (13.4–14.4)	15.7 ± 0.4 (15.4-16)	13.4 ± 0.4 (12.9–14.1)	16.5 ± 0.4 (16.2–16.7)
HAL	9.5 ± 0.5 (9.1-10.4)	10.9 ± 0 (10.9–10.9)	9.2 ± 0.2 (8.9-9.5)	11.4 ± 0.7 (11–11.9)
3FD	1.8 ± 0.1 (1.7–1.9)	2.2 ± 0 (2.2-2.2)	1.7 ± 0.2 (1.5–1.9)	2 ± 0.2 (1.8-2.2)
4TD	$1.7 \pm 0.1 \ (1.6 - 1.8)$	2 ± 0.1 (1.9-2.1)	$1.6 \pm 0.2 \ (1.4 - 1.8)$	$1.9 \pm 0.2 (1.8 - 2.1)$
END	$3.9 \pm 0.2 (3.6 - 4.2)$	3.9 ± 0.2 (3.6-4.2)	4 ± 0.2 (3.7-4.2)	4.4 ± 0.1 (4.4-4.5)
TL	17 ± 0.5 (16.3–17.6)	19.2 ± 0.6 (18.7–19.6)	$17 \pm 0.4 (16.5 - 17.7)$	19.8 ± 0.5 (18.5–19.2)
THL	15.9 ± 0.6 (14.8–16.5)	18.2 ± 0.7 (17.8–18.7)	15.8 ± 0.7 (14.7-16.9)	18.2 ± 0.7 (17.8–18.7)
HL/SVL	$0.38 \pm 0.01 \ (0.37 - 0.39)$	0.36 ± 0.01 (0.35-0.37)	$0.37 \pm 0.01 \ (0.36 - 0.38)$	0.34 ± 0.01 (0.34–0.35)
HW/SVL	$0.35 \pm 0.01 \ (0.35 - 0.37)$	0.35 ± 0.01 (0.35-0.37)	$0.35 \pm 0.01 \ (0.34 - 0.37)$	0.33 ± 0.01 (0.33-0.34)
ED/SVL	$0.12 \pm 0.01 \ (0.11 - 0.13)$	$0.10 \pm 0.01 \ (0.10 - 0.11)$	0.11 ± 0.01 (0.10-0.12)	$0.10 \pm 0.01 \; (0.09 {-} 0.10)$
TD/SVL	$0.06 \pm 0.01 \ (0.06 - 0.07)$	$0.06 \pm 0.01 \ (0.06 - 0.07)$	$0.06 \pm 0.01 \ (0.06 - 0.07)$	$0.06 \pm 0 \ (0.06 - 0.06)$
UEW/SVL	$0.10 \pm 0.01 \ (0.09 - 0.10)$	$0.09 \pm 0.01 \ (0.08 - 0.09)$	$0.10 \pm 0.01 \ (0.09 - 0.10)$	$0.08 \pm 0.01 \; (0.07 {-} 0.08)$
IOD/SVL	$0.10 \pm 0.01 \ (0.10 - 0.11)$	$0.10 \pm 0.01 \ (0.09 - 0.11)$	$0.09 \pm 0.01 \ (0.09 - 0.10)$	$0.10 \pm 0.01 \; (0.09 {-} 0.10)$
IND/SVL	$0.09 \pm 0.01 \ (0.08 - 0.09)$	$0.09 \pm 0 \ (0.09 - 0.09)$	$0.08 \pm 0 \ (0.08 - 0.08)$	$0.08 \pm 0.01 \; (0.07 {-} 0.08)$
TAL/SVL	0.28 ± 0.01 (0.27-0.30)	$0.28 \pm 0.01 \ (0.27 - 0.28)$	0.27 ± 0.01 (0.26-0.28)	$0.27 \pm 0.01 \ (0.26 - 0.27)$
FL/SVL	0.43 ± 0.01 (0.42–0.45)	0.43 ± 0 (0.43-0.43)	$0.40 \pm 0.01 \ (0.38 - 0.41)$	$0.42 \pm 0.02 \ (0.40 - 0.43)$
HAL/SVL	0.30 ± 0.01 (0.29-0.31)	0.30 ± 0.01 (0.29-0.31)	0.27 ± 0.01 (0.27-0.28)	0.29 ± 0.02 (0.27-0.31)
3FD/SVL	$0.06 \pm 0.01 \ (0.05 - 0.06)$	$0.06 \pm 0 \ (0.06 - 0.06)$	$0.05 \pm 0.01 \ (0.05 - 0.06)$	$0.05 \pm 0.01 \ (0.05 - 0.06)$
4TD/SVL	$0.05 \pm 0 \ (0.05 - 0.05)$	$0.06 \pm 0.01 \ (0.05 - 0.06)$	$0.05 \pm 0.01 \ (0.04 - 0.05)$	$0.05 \pm 0.01 \ (0.04 - 0.05)$
END/SVL	0.12 ± 0.01 (0.12-0.13)	0.12 ± 0 (0.12-0.12)	0.12 ± 0.01 (0.11-0.13)	0.11 ± 0 (0.11-0.11)
TL/SVL	0.53 ± 0.01 (0.51-0.55)	0.53 ± 0 (0.53-0.53)	0.51 ± 0.01 (0.49-0.51)	0.50 ± 0.03 (0.48-0.52)
THL/SVL	$0.50 \pm 0.02 (0.47 - 0.52)$	$0.50 \pm 0.01 (0.50 - 0.51)$	$0.47 \pm 0.01 (0.45 - 0.49)$	$0.47 \pm 0.02 \ (0.46 - 0.49)$

Table 2. Morphometric data (in mm) of *Scinax onca* sp. n. from the Purus-Madeira interfluve, Brazilian Amazonia. Means followed by standard deviation, and ranges in parentheses. For abbreviations, see Materials and methods.

rainforest characterized by closed canopy with emergent trees whereas the southern part has a more open lowland rainforest formation with frequent palm trees.

The new species is an explosive breeder. All specimens were encountered after (or during) heavy rains when aggregated at middle-sized or large temporary forest ponds. The ponds were not connected to streams. The males were calling from shrubs growing in or next to the water. Calling males adopted both horizontal and vertical positions on leaves and shrub trunks ca. 50–200 cm above the ground. Other tree frogs found in sympatry with *S. onca* sp. n. included *Dendropsophus leucophyllatus* (Beireis, 1783), *D. marmoratus* (Laurenti, 1768), *D. minutus* (Peters, 1872), *D. parviceps* (Boulenger,



Figure 8. Advertisement call of *Scinax onca* sp. n. Spectrogram (**A**) and oscillogram (**B**) of an advertisement call of *Scinax onca* sp. n. The specimen (INPA-H 26624, SVL 32.1 mm) was recorded in Floresta Estadual Tapauá Reserve, middle Purus-Madeira Interfluve, Amazonas, Brazil **C** A series with eleven calls. Air temperature not measured.

1882), *D. rhodopeplus* (Boulenger, 1882), *D. sarayacuensis* (Shreve, 1935), *Phyllome-dusa vaillantii* Boulenger, 1882, and *Scinax* sp. 7 (*sensu* Ferrão et al. 2016).

Based on the sparse data available and due to threats, it is suggested that *S. onca* sp. n. be classified as "Data Deficient" according to the IUCN red list criteria (IUCN 2016). It is necessary to stress out, however, that the known range of the new species is seriously threatened by the planned reconstruction of the Trans-Amazonian highway BR-319 connecting Manaus and Porto Velho. This initiative will facilitate human migration from the "Arc of Deforestation" in southern Rondônia to the PMI (Fearnside and Graça 2006). According to recent predictions, this immigration could result in the deforestation of up 5.4 million hectares of mostly undisturbed rainforests between 2012 and 2050 (Maldonado et al. 2012). Three of four known *S. onca* sp. n. localities occur in the area of predicted deforestation. Only the fourth locality lies within the Floresta Tapauá Reserve, which can serve as refuge for this and other species.



Figure 9. Tadpole of *Scinax onca* sp. n. from the middle Purus-Madeira Interfluve (lot INPA-H 35411). Specimen collected at kilometre 350 of the BR-319 highway, municipality of Beruri, State of Amazonas, Brazil. From top to bottom: dorsal, ventral, and lateral views of preserved tadpole in developmental Stage 37. Scale bar 5 mm.



Figure 10. Oral disc of the tadpole of *Scinax onca* sp. n. (lot INPA-H 35411; developmental Stage 37). Left: ventrolateral and right: ventral view. The tadpole was collected in the middle Purus-Madeira Interfluve, at the kilometre 350 of the BR-319 highway, municipality of Beruri, State of Amazonas, Brazil. Scale bar 2 mm.

Discussion

The morphological data presented here show slight differences between members of the middle and southern PMI populations of Scinax onca sp. n. These differences are consistent with previously obtained molecular phylogeny (Ferrão et al. 2016), where the new taxon is structured into two slightly differentiated lineages corresponding to the middle and southern PMI populations. Nevertheless, until more robust evidence of specific distinction between the two populations is available, we decided to use the same specific epithet for the representatives of the both PMI populations. The observed differences may be a result of local adaptation to different environmental conditions, since the populations live under different climate conditions (drier in south) and inhabit different types of lowland forest. Other possibility is that the differences in measurements and coloration represent two examples of peripheral intraspecific variation, as the known populations are separated by ca. 500 km from each other. Till now we were not able to find individuals corresponding to S. onca sp. n. in the central region of the study area. Therefore, more complete sampling within this distributional gap is necessary to obtain a more exact picture of the morphological variation and genetic structure of S. onca sp. n.

Three records of Scinax iquitorum, species most closely related to S. onca sp. n., were recently reported from Brazilian western Amazonia (State of Acre; Machado et al. 2015, Melo-Sampaio and Souza 2015) and from Floresta Estadual Tapauá Reserve (State of Amazonas; Almeida et al. 2015). Two individuals reported by Almeida et al. (2015) were examined in this study. Since their morphology did not agree with the diagnosis of S. iquitorum, but corresponded fairly well with that of Scinax onca sp. n. (sensu Ferrão et al. 2016) from kilometre 350 of the BR-319 highway (type locality of S. onca sp. n., ca. 190 km from Floresta Estadual Tapauá Reserve) we included them into the type series of the new species described here. Individuals of S. iquitorum reported by Melo-Sampaio and Souza (2015) from the eastern corner of the Acre have very similar colour pattern to S. onca sp. n. from southern PMI, and we tentatively associate them with S. onca sp. n. Nevertheless, DNA barcoding as well as thorough morphological and bioacoustic data are necessary for a definitive determination of the specimens reported from eastern Acre. The same applies also for the proper determination of the individuals of S. iquitorum reported by Machado et al. (2015) from the municipality of Cruzeiro do Sul. In the light of contemporary knowledge of the extensive diversity of *Scinax* species in the State of Amazonas, we stress out that occurrence of *S*. iquitorum in the State of Acre should be verified by non-morphological traits.

It is evident that Brazilian States of Acre, Amazonas, and Rondônia house much more diverse fauna of *Scinax* tree frogs than previously thought. Similarly, this region is probably also home of many other, still unnamed, anuran species. Although a number of new species will be described in the near future, a more complete evaluation of the unique anuran diversity of the PMI is a long-term process, which is unlikely to be successfully completed without an effective wide-scale protection of the lowland Amazonian rainforest.

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Appendix I

List of specimens examined for morphological comparisons. Abbreviations: AM, state highway, State of Amazonas, Brazil; BR, Federal highway, Brazil; PDBFF, Biological Dynamics of Forest Fragments Project; UHE, Hydroelectric Power Plant.

- *Scinax* sp. 1: BRAZIL: *AMAZONAS*: Tapauá, BR-319, km 450 (INPA-H 34688, INPA-H 34691, INPA-H 34689, INPA-H 34692, INPA-H 34690, INPA-H 34700).
- Scinax sp. 2: BRAZIL: AMAZONAS: Humaitá, BR-319, km 620 (INPA-H 34651, IN-PA-H 34657, INPA-H 34664, INPA-H 34666, INPA-H 34667, INPA-H 34668, INPA-H 34669, INPA-H 34670, INPA-H 34671, INPA-H 34672, INPA-H 34673, INPA-H 34674, INPA-H 34675, INPA-H 34676, INPA-H 34677, INPA-H 34678).
 Scinax sp. 4: BRAZIL: AMAZONAS: Humaitá, BR-319, km 620 (INPA-H 34693).
- Scinax sp. 5: BRAZIL: AMAZONAS: Tapauá, BR-319, km 450 (INPA-H 34648, INPA-H 34656, INPA-H 34639, INPA-H 34640, INPA-H 34632); Berurí, BR-319, Km 260 (INPA-H 34703, INPA-H 34693, INPA-H 34696); Borba, BR-319, Km 220 (INPA-H 34710).
- Scinax sp. 6: BRAZIL: AMAZONAS: Careiro da Várzea, BR-319, km 34, Ramal do Purupuru (INPA-H 34597); RONDÔNIA: Porto Velho, UHE Santo Antônio (INPA-H 35559, INPA-H 35561, INPA-H 35562, INPA-H 35563, INPA-H 35564, INPA-H 35565, INPA-H 35566, INPA-H 35567, INPA-H 35568).
- Scinax sp. 7: BRAZIL: AMAZONAS: Careiro da Várzea, BR-319, km 100 (INPA-H 34600, INPA-H 34601, INPA-H 34604, INPA-H 34614, INPA-H 34615, INPA-H 34622, INPA-H 34598, INPA-H 34624, INPA-H 34627, INPA-H 34629), km 168 (INPA-H 34602); Borba, BR-319, km 220 (INPA-H 34620); Beruri, BR-319, km 220 (INPA-H 34608), km 360 (INPA-H 34599, INPA-H 34607, INPA-H 34609, INPA-H 34608), km 360 (INPA-H 34599, INPA-H 34607, INPA-H 34609, INPA-H 34611, INPA-H 34612, INPA-H 34617, INPA-H 34618, INPA-H 34621, INPA-H 34625, INPA-H 34626, INPA-H 34628, INPA-H 34630); Manicoré, BR-319, km 400 (INPA-H 34603, INPA-H 34606, INPA-H 34616, INPA-H 34623); Tapauá, BR-319, km 450 (INPA-H 34613, INPA-H 34619, INPA-H 34605, INPA-H 34665).
- Scinax boesemani: SURINAME: PARAMARIBO (SURINAME): near Zanderij (RMNH12601, holotype). BRAZIL: RORAIMA: Caracaraí, Parque Nacional do Viruá (INPA-H 25972, INPA-H 25974).
- *Scinax chiquitanus*: BRAZIL: *RONDÔNIA*: Porto Velho, UHE Santo Antônio, M-14 (INPA-H 35554, INPA-H 35555, INPA-H 35556, INPA-H 35558, INPA-H 35560).

- Scinax cruentommus: ECUADOR: NAPO: Santa Cecilia (KU 126587, holotype); ORELLANA: Parque Nacional Yasuní (QCAZ 8184), Río Napo (QCAZ 43772, QCAZ 44754). BRAZIL: AMAZONAS: Careiro da Várzea, BR-319, km 34, Ramal do Purupuru (INPA-H 34697).
- Scinax funereus: ECUADOR: ORELLANA: Río Napo, Primavera (QCAZ 43799), Tambococha (QCAZ 55280, QCAZ 55283). PERU: LORETO: San Jacinto (KU221960b).
- Scinax fuscomarginatus: BRAZIL: RORAIMA: Boa Vista, Estação Ecológica de Maracá (INPA-H 34662, INPA-H 34634, INPA-H 34646, INPA-H 34661); Caracaraí, Parque Nacional do Viruá (INPA-H 19371, INPA-H 19372, INPA-H 19376, INPA-H 19378, INPA-H 19383, INPA-H 19384).
- *Scinax garbei*: BRAZIL: *RORAIMA*: Caracaraí, Parque Nacional do Viruá (INPA-H 25964, INPA-H 27496).
- *Scinax* cf. *ictericus*: PERU: *MADRE DE DIOS*: Rio Tambopata (ZFMK 39353, ZFMK 39361, ZFMK 39363, ZFMK 39366).
- Scinax iquitorum: PERU: LORETO: ca. 17 km straight SW of Iquitos, (NMP6V 71267/1-3; paratypes).
- *Scinax madeirae*: BRAZIL: *RONDÔNIA*: Alta Floresta, Parque Estadual Corumbiaria (INPA-H 7050, INPA-H 7051).
- Scinax nebulosus: BRAZIL: PARÁ: Alter do Cháo (INPA-H 34647, INPA-H 34653); RONDÔNIA: Costa Marques, Real Forte Príncipe da Beira (INPA-H 34641); RORAIMA: Caracaraí, Parque Nacional do Viruá (INPA-H 27535, INPA-H 27536, INPA-H 27537).
- Scinax onca sp. n. (tadpoles): BRAZIL: AMAZONAS: Beruri, BR-319, km 350 (lot INPA-H 35411).
- *Scinax pedromedinae*: BOLIVIA: *BENI*: 5 km NE of Riberalta (NMP6V 70700); PERU: *UCAYALI*: Masisea (NMP6V 74902/1–3).
- Scinax proboscideus: BRAZIL: AMAZONAS: Manaus, Reserva Colosso do PDBFF (INPA-H 10304); Presidente Figueiredo, Vila Pitinga (INPA-H 1870); PARÁ: Oriximiná, UHE Cachoeira Porteira, Rio Trombetas (INPA-H 304).
- *Scinax sateremawe*: BRAZIL: *AMAZONAS*: Borba, Ramal Novo Horizonte (INPA-H 34695, INPA-H 34708).
- *Scinax ruber* F: BRAZIL: *AMAZONAS*: Borba, BR-319, km 220 (INPA-H 34642, INPA-H 34652); *RONDÔNIA*: Porto Velho, UHE Santo Antônio, (INPA-H 34633, INPA-H 34635, INPA-H 34649, INPA-H 34655).
- *Scinax ruber* PM: BRAZIL: *AMAZONAS*: Careiro da Várzea, AM-354, km 10 (INPA-H 34645, INPA-H 34654, INPA-H 34658, INPA-H 34659).