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The hydrobioid freshwater gastropods (Caenogastropoda, Truncatelloidea) of Greece: new records, taxonomic re-assessments using DNA sequence data and an update of the IUCN Red List Categories

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Abstract

Hydrobioid freshwater gastropods were collected from mainland and insular Greece. Several threatened taxa, such as *Graecoanatolica vegorriticola*, *Pseudamnicola negropontina*, *Pseudamnicola pieperi*, *Pseudobithynia eubooensis* and *Pseudoislamia balcanica*, were recorded from new localities. *Trichonia trichonica*, which has been considered extinct from its type locality for the last twenty eight years, was re-discovered, whereas the presence of *Daphniola exigua*, *G. vegorriticola*, *Marstoniopsis graeca*, *P. pieperi* and *Pseudobithynia trichonis* in their type localities was verified. The taxonomic status of *P. negropontina* and the newly discovered populations of *G. vegorriticola* was elucidated using *COI* sequence data. The new data recorded during this survey indicate that the IUCN status of some Greek endemic hydrobioids needs to be updated.

Keywords

Hydrobioids, Rissooidea, Truncatelloidea, Gastropoda, Greece, freshwater, taxonomy, IUCN status, conservation

Introduction

Hydrobioid gastropods include the family Hydrobiidae Troschel, 1857 and several other families of Rissooidea s.l. that resemble Hydrobiidae in general features (Hershler and Ponder 1998). Rissooidea s.l. is one of the largest and most taxonomically challenging gastropod superfamilies (Bouchet and Rocroi 2005). Recently, Criscione and Ponder (2013) using molecular data showed that that there are two major clades encompassing taxa previously included in Rissooidea. These clades are the Rissooidea s.s. and the Truncatelloidea, the latter containing the hydrobioid families (Wilke et al. 2013).

Greek freshwater ecosystems are widely recognized as hotspots of European freshwater biodiversity (e.g. Glöer and Maassen 2009, Glöer et al. 2010, Oikonomou et al. 2012). Hydrobioids are in accordance with this observation since they are highly diverse; 77 species and subspecies belonging to 29 genera have already been recorded in Greek freshwater systems, most of them (i.e. 68.4 % of the species and subspecies and 34.5% of the genera) being endemic for Greece (Bank 2006, Falniowski and Szarowska 2011a, Radea 2011, Szarowska and Falniowski 2011a, Glöer and Georgiev 2012, Georgiev 2013a,b, Radea et al. 2013). Nevertheless, the freshwater gastropod fauna of Greece remains understudied compared to other taxa (i.e. freshwater fishes) due to the complex hydrographic network of its drainages.

Almost all described Greek hydrobioid species are included in the IUCN Red List of Threatened Species (2012) and 55% of them have been classified as threatened. *Graecoanatolica macedonica* Radoman & Stankovic, 1978 is characterized as Extinct, and is followed by twenty four species that have been classified as Critically Endangered, five as Endangered, nine as Vulnerable, three as Near Threatened, five as Least Concern and twenty two as Data Deficient.

During 2012, several localities across Greece were sampled by the authors for hydrobioid freshwater gastropods. The sampling took place following the goals of the research project "Species on the brink of extinction" that was funded by the public benefit foundation "John S. Latsis". The goals of this project was a) to assess and evaluate the population status of 10 freshwater snails species of Greece (9 endemics) which, according to the recent report from the International Union for Conservation of Nature (IUCN), are classified as either Extinct or Critically Endangered, b) to evaluate the status of the wetlands these species are present in and to assess the main anthropogenic regime of threats. In the network of localities sampled for the purposes of the project, we added several more localities hosting water bodies that are frequently reported in the freshwater literature of Greece or are in the vicinity of the primarily targeted localities. Therefore, we do not consider our fieldwork to be exhaustive; rather it is focused on freshwater localities that have been searched before, are frequently reported in the literature, as hosting (or having hosted) threatened species, and we complemented these localities with surrounding ones that could have served as the refuges of the threatened species.

Some of the findings of this survey as well as suggestions for the IUCN status update of some hydrobioids collected, are presented and discussed herein. Additionally, using *COI* sequence data generated from some specimens, we aimed to elucidate the taxonomic status of:

(i) The Greek endemic taxon of the genus *Pseudamnicola* Paulucci, 1878, namely *Pseudamnicola* (*Pseudamnicola*) *macrostoma negropontina* (Clessin, 1878). Based on the slight morphological and anatomical differences between *P.* (*P.*) *macrostoma negropontina* and *P.* (*P.*) *macrostoma macrostoma* (Küster, 1853), Szarowska et al. (2006) claimed that these taxa should be considered as distinct species. This claim was evaluated in the light of the generated sequence data.

(ii) Two new populations of the genus *Graecoanatolica* Radoman, 1973, which were recorded in Sterea Ellada (Voiotia). The morphological and anatomical studies were not conclusive in assigning these populations to the known Greek *Graeconatolica* species, *Graeconatolica vegorriticola* (Schütt, 1962) and *G. macedonica*, or even to a new species of *Graecoanatolica*. Therefore, *COI* sequence data were used to compare the *G. vegorriticola* specimens collected from the type locality of the species, with those of the newly located southern populations.

Overall, the current study offers new distributional data and, in the light of these, is evaluating the conservation status of some hydrobioid species of Greece. Furthermore, using newly generated *COI* sequence data, we are resolving taxonomic uncertainties that could not be elucidated based on morphology alone.

Methods

The freshwater localities of almost all the threatened hydrobioids of Greece were carefully sampled (Fig.1). The snails were hand collected from stones, gravel and dead leaves (Fig. 2). Specimens were placed into vials filled with water and were transported alive to the laboratory. A portion of the specimens collected from each locality was stored in -20°C for molecular analysis, whereas others were preserved un-relaxed in 70% ethanol for morphological and anatomical studies.

Shell characters (shell height and width, apertural height and width) were measured using the micrometer of a stereomicroscope (Stemi 2000-C, Zeiss). Digital pictures were taken using a Canon EOS 1000D camera that was attached to the stereomicroscope. During this procedure, the specimens were submerged into water in order to avoid the malformation of important taxonomic features that can be caused by the long-term tissue preservation buffers.

Details of the specimens used in the molecular analyses are provided in Table 1. The entire individuals were used for total genomic DNA isolation. In total, we extracted DNA from one specimen of *Pseudamnicola macrostoma macrostoma* and sixteen specimens of *Graecoanatolica* from Pella and Voiotia.

DNA was extracted using the Purelink Genomic DNA mini kit (Invitrogen) following the manufacturers' protocol. A fragment of the mitochondrial gene cy-

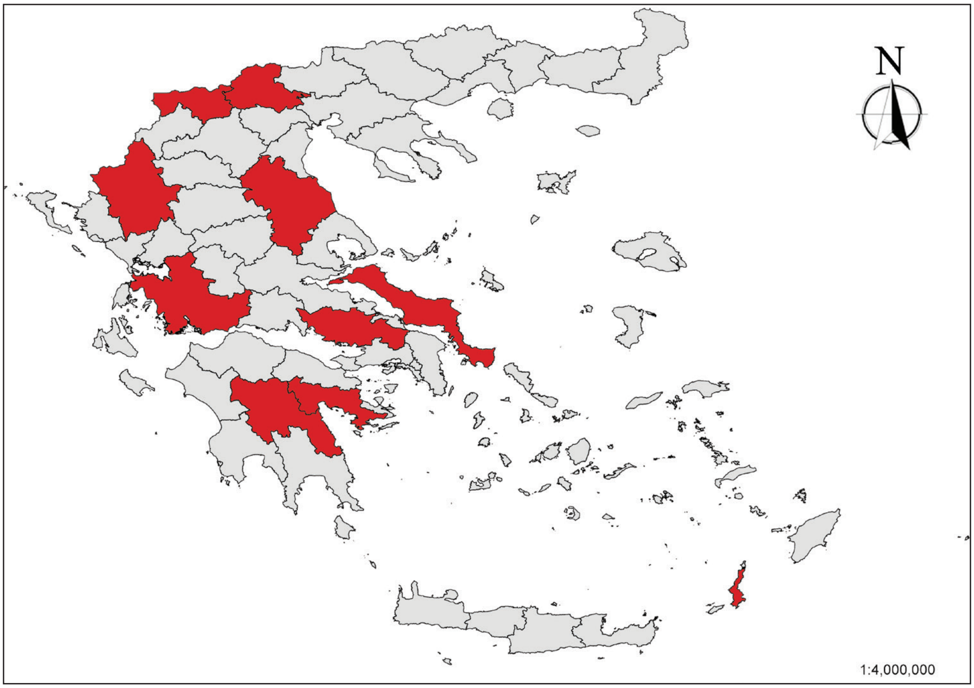

Figure 1. Map showing the administrative units of Greece where "hydrobioid" localities were sampled during the fieldwork of this study.

Table 1. Details on the origin of the specimens used in the *COI* sequence divergence analyses.

Species	Number of specimens	Nomos	Exact locality	Habitat	Latitude, Longitude	Sample code	*COI* Accession Numbers
Graecoanatolica vegorriticola	6	Voiotia	Orchomenos, Pigi Chariton	Spring	38°29'41"N, 22°58'23"E	GraVeg_Cha	KF758767-72
Graecoanatolica vegorriticola	2	Voiotia	Livadia, Spring of Krya	Spring	38°25'49"N, 22°52'22"E	GraVeg_Kr1	KF758773-74
Graecoanatolica vegorriticola	8	Pella	Pella, Vegorritis Lake	Lake	40°44'38"N, 21°49'07"E	GraVeg_Veg	KF758775-82
Pseudamnicola macrostoma macrostoma	1	Attiki	Marathon, Kato Souli	Stream	38°09'28"N, 24°00'19"E	PsdMac_KaSou	KF758783
Pseudamnicola macrostoma negropontina	1	Evvoia	Marmaris	-		-	EF061915 (Szarowska et al. 2006) †

†According to the GenBank registration records the locality of origin of EF061915 is Kato Souli. However, this must be an erroneous record since in the reference (Szarowska et al. 2006) associated with this accession number, it is clearly stated that the specimen of *P. macrostoma negropontina* originated from Marmaris (Marmari) in Evvoia Island.

tochrome oxidase subunit I (*COI*) was amplified from each specimen using the universal primers LCO1490 and HCO2198 (Folmer et al. 1994). PCR was performed in a 25 μl volume, in which 1–2 μl of template DNA was mixed with 0.2 mM

Figure 2. Sampling localities. **A** Ag. Sophia, Aitoloakarnania **B** Olympos, Karpathos **C** Stoupaioi, Evvoia **D** Peraia, Lake Vegorritis **E** Megali Vrysi, Argolida. The arrows point the exact site where the specimens were found.

dNTPs, 0.4 mM of each primer and 0.5 units of Taq polymerase. The concentration of the $MgCl_2$ was 3.5 mM. Thermocycling was performed in a MyCycler (Biorad) thermocycler. The cycle program comprised an initial denaturation step at 95 °C for 3 min, followed by 40 cycles of 15 s at 95 °C, 1 min at 42 °C, and 1.5 min at 72 °C. The cycling was ended with 10 min sequence extension at 72 °C. Automated sequencing of both strands of the PCR amplicons was performed in a PE-ABI3740 automated sequencer (using Big-Dye terminator chemistry).

The primers in the sequencing reactions were the same as in the PCR amplifications. Sequences generated for this study were deposited in GenBank under the accession numbers provided in Table 1.

Sequence alignment and genetic data analysis

The newly generated sequences were viewed and edited using CodonCode Aligner v. 2.06 (Genecodes Corporation). The authenticity of the mtDNA sequences and the homology with the targeted mitochondrial gene were evaluated by a BLAST search in the NCBI genetic database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). All sequences alignments were performed with CodonCode Aligner v. 2.06 by implementing the Clustal algorithm.

For deciphering whether the two *Pseudamnicola* taxa represent two different species or if they are subspecies of a single species, we estimated the sequence divergence separating them by using the Kimura 2-parameter (K-2p) model (Kimura 1980) of nucleotide substitution as implemented in MEGA5 (Tamura et al. 2011). The estimated *COI* sequence divergence of the two *Pseudamnicola* taxa (one produced for this study and one retrieved from GenBank, see Table 1) was compared to that separating well defined *Pseudamnicola* species. The sequence data for these species were also retrieved from GenBank.

In the case of the newly discovered *Graeconatolica* populations from southern Greece, based on the K-2p model, we estimated the *COI* sequence divergence of these specimens from the *G. vegorriticola* specimens sampled from the type locality of the species.

An alphabetical sequence of families, genera and species applies to the hydrobioid list below. Abbreviations used: Nom.= Nomos (administrative unit).

Results

Family Amnicolidae Tryon, 1863
Genus *Marstoniopsis* van Regteren Altena, 1936

Marstoniopsis graeca (Radoman, 1978)

http://species-id.net/wiki/Marstoniopsis_graeca

New records. Nom. PELLAS: Lake Vegorritis, stony bank close to Peraia, ca 515 m asl, 40°44'38"N, 21°49'07"E, 15.xi.2012, Radea and Parmakelis.

Remarks. *Marstoniopsis graeca* was described from Lake Vegorritis as *Parabythinella graeca* Radoman, 1978 and, according to Radoman (1983), the stony east bank of the lake, to the north of the village Farangi, is the type locality of this taxon. Szarowska (2006) refers that gross anatomy and histology of the two taxa of *Parabythinella, P. graeca* and *P. macedonica* Hadžišče, 1963*,* do not differ from the one described for *Marstoniopsis*. The two taxa are most probably no more than two subspecies of *M. macedonica. M. graeca* is known only from Lake Vegorritis and Schütt (1985) considers that it is a common taxon for the area. Szarowska (2006) collected numerous specimens from Lake Vegorritis during the period 2003–2005. During our study, we found only one individual north of the locality where the species was discovered by Radoman (1978).

Family Bythinellidae Kobelt, 1878
Genus *Bythinella* Moquin-Tandon, 1856

Bythinella cf. *charpentieri* (Roth, 1855)

New records. Nom. EVVOIAS: pool with *Nasturtium* sp. and *Platanus orientalis*, 3.8 km NE of Paradeisi to Ag. Dimitrios, ca 335 m asl, 38°05'26"N, 24°23'57"E, 25.xi.2012, Radea and Constantinidis; spring close to the road towards Ag. Dimitrios beach, ca 120 m asl, 38°08'26"N, 24°27'01"E, 25.xi.2012, Radea and Constantinidis; spring close to the road from Ag. Dimitrios to Kalianoi, ca 295 m asl, 38°07'21"N, 24°26'30"E, 25.xi.2012, Radea and Constantinidis; cistern, ca 0.7 km NNW of Kalianoi, ca 205 m asl, 38°07'22"N, 24°29'26"E, 26.xi.2012, Radea and Constantinidis; spring in Myloi village, ca 205 m asl, 38°01'56"N, 24°26'09"E, 26.xi.2012, Radea and Constantinidis; spring close to Stoupaioi, ca 255 m asl, 38°07'21"N, 24°18'51"E, 26.xi.2012, Radea and Constantinidis; stream with *Nasturtium* sp. and *Helosciadium* sp., on coastal flats 3.2 km NW of Marmari, ca 0 m asl, 38°04'16"N, 24°18'06"E, 26.xi.2012, Radea and Constantinidis.

Nom. VOIOTIAS: Krya spring in Livadia, ca 240 m asl, 38°25'49"N, 22°52'22"E, 17.iv.2012, Radea and Constantinidis; spring Pigi Chariton close to Orchomenos, ca 170 m asl, 38°29'41"N, 22°58'23"E, 17.iv.2012, Radea and Constantinidis.

Remarks. The populations of *Bythinella* found in Evvoia and Voiotia have morphological and anatomical similarity to *Bythinella charpentieri*, which is the only known species of the genus inhabiting Attiki, Evvoia and Parnassos Mt. (Falniowski and Szarowska 2011b). In particular, the shape and the morphometry of the tubular gland of the penis, which is an important feature to distinguish *Bythinella* spp. (Glöer 2013), are identical to those of *B. charpentieri*. Therefore, we consider that the populations from Evvoia and Voiotia belong to *B. charpentieri*. However, molecular data would be very useful to elucidate completely their taxonomic status since Falniowski et al. (2009), Falniowski and Szarowska (2011b) and Falniowski et al. (2012) have demonstrated that there are many cryptic species in *Bythinella*, due to the morphostatic evolution, and, consequently, it is difficult to distinguish species without molecular analysis.

Family Bithyniidae Gray, 1857
Genus *Pseudobithynia* Glöer & Pesic, 2006

Pseudobithynia euboeensis Glöer, Falniowski & Pesic, 2010

http://species-id.net/wiki/Pseudobithynia_euboeensis
Figure 3

New records. Nom. EVVOIAS: stream with dense vegetation composed mainly by *Nasturtium* sp. and *Helosciadium* sp., on coastal flats 3.2 km NW of Marmari, ca 0 m asl, 38°04'16"N, 24°18'06"E, 26.xi.2012, Radea and Constantinidis.

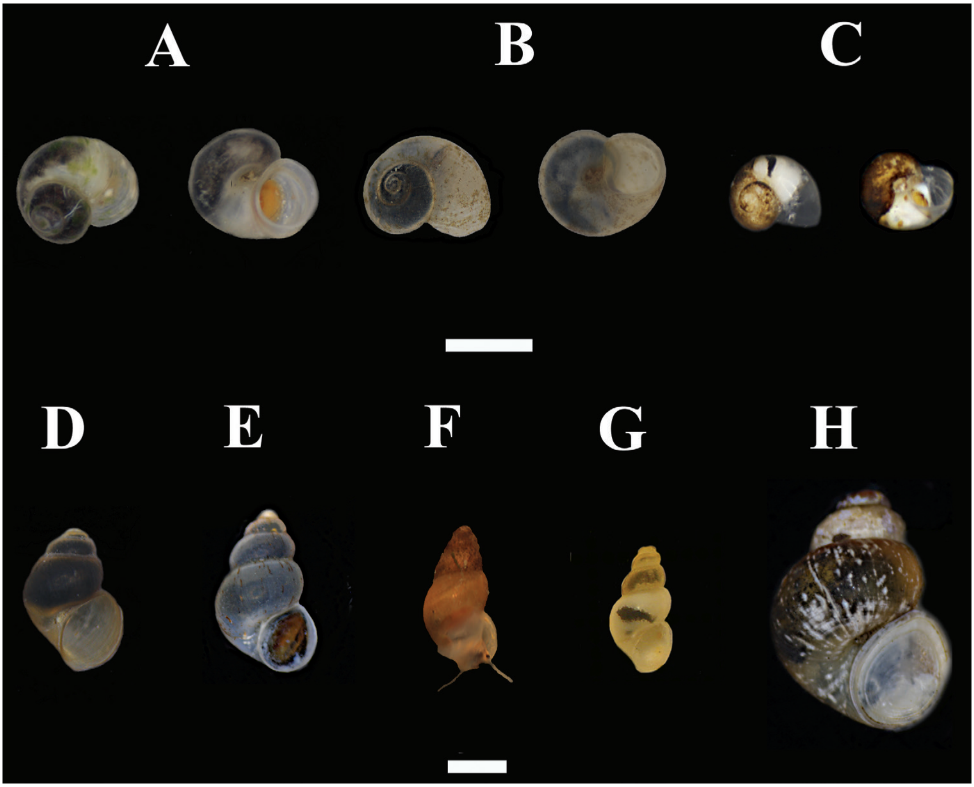

Figure 3. Hydrobioids collected during the survey in mainland and insular Greece. **A** *Daphniola exigua* (dorsal and ventral view) **B** *Isimerope semele* (dorsal and ventral view, Megali Vrysi) **C** *Pseudoislamia balcanica* (dorsal and ventral view, Ag. Sophia) **D** *Pseudamnicola pieperi* (Olympos) **E** *Radomaniola* cf. *curta* (spring of Louros river) **F** *Radomaniola* cf. *curta* (Ag. Sophia spring) **G** *Trichonia trichonica* **H** *Pseudobithynia eubooensis*. Scale bar 1 mm.

Remarks. *Pseudobithynia euboeensis* was collected in 1985 for the first time and it was described by Glöer et al. (2010) from a damp meadow with some small water bodies, formed by the water running from a spring, at the seaside close to Marmari. The above authors report that the type locality was no longer extant in 2003 because all the water from the spring was used for irrigation.

So far, the species was known only from its type locality. The new locality is probably close to the type locality and is likely influenced by touristic activity during summer. Several specimens of *P. euboeensis* were found either on plant material or under stones.

Pseudobithynia trichonis **Glöer, Albrecht & Wilke, 2007**
http://species-id.net/wiki/Pseudobithynia_trichonis

New records. Nom. AITOLOAKARNANIAS: Lake Trichonis, NE rocky and stony shore close to Loutra Myrtias, ca 15 m asl, 38°33'34"N, 21°37'33"E, 09.iii.2012, Radea, Charou, Papadogiannis, Parmakelis.

Remarks. The species was described by Glöer et al. (2007) from rocks covered by microalgae, at 1m depth in the NE bank of Lake Trichonis. Our specimens were rather scattered and were found north of the locality referred by the latter authors, under stones at 10–30 cm depth. According to Albrecht et al. (2009), Lake Trichonis undergoes several human-induced environmental changes with water level loss and eutrophication being the most serious threats for gastropods living in the littoral zone.

Family Hydrobiidae Troschel, 1857
Daphniola Radoman, 1973

Daphniola exigua (A. Schmidt, 1856)
http://species-id.net/wiki/Daphniola_exigua
Figure 3

Remarks. A large population of *Daphniola exigua* was found in Ag. Paraskevi spring, Tempi valley, Nom. Larissas where anthropogenic activity is high. Ag. Paraskevi spring is one of the two known localities where the species is distributed (Schütt 1980, Falniowski et al. 2007). The other locality is Daphni spring, close to Ag. Paraskevi (Radoman 1973, 1983, Falniowski et al. 2007). Radoman (1973) described the genus *Daphniola* from the latter spring, the type species being *D. graeca*. Falniowski et al. (2007), using morphological and *COI* sequence data, showed that *D. exigua* and *D. graeca* are conspecific both belonging to *D. exigua*.

Graecoanatolica Radoman, 1973

Graecoanatolica vegorriticola (Schütt, 1962)
http://species-id.net/wiki/Graecoanatolica_vegorriticola
Figure 4

New records. Nom. VOIOTIAS: Krya spring in Livadia, ca 240 m asl, 38°25'49"N, 22°52'22"E, 17.iv.2012, Radea and Constantinidis; spring Pigi Chariton close to Orchomenos, ca 170 m asl, 38°29'41"N, 22°58'23"E, 17.iv.2012, Radea and Constantinidis.

Nom. PELLAS: Lake Vegorritis, stony bank close to Peraia, ca 515 m asl, 40°44'38"N, 21°49'07"E, 15.xi.2012, Radea and Parmakelis.

Remarks. *Graecoanatolica vegorriticola* was initially described as *Hydrobia vegorriticola* by Schütt (1962) from Lake Vegorritis and, according to Radoman (1983), the small island nearby the north bank of the lake, not far from Arnissa town, is the type locality of this species. The species was known from Lake Vegorritis and Lake Petron (Schütt 1962). Reischütz and Stummer (1990) and Hemmen and Reischütz (1996) report the presence of this species in the waterfalls of Edessa town, Central Macedonia in 1979, 1987 and 1995.

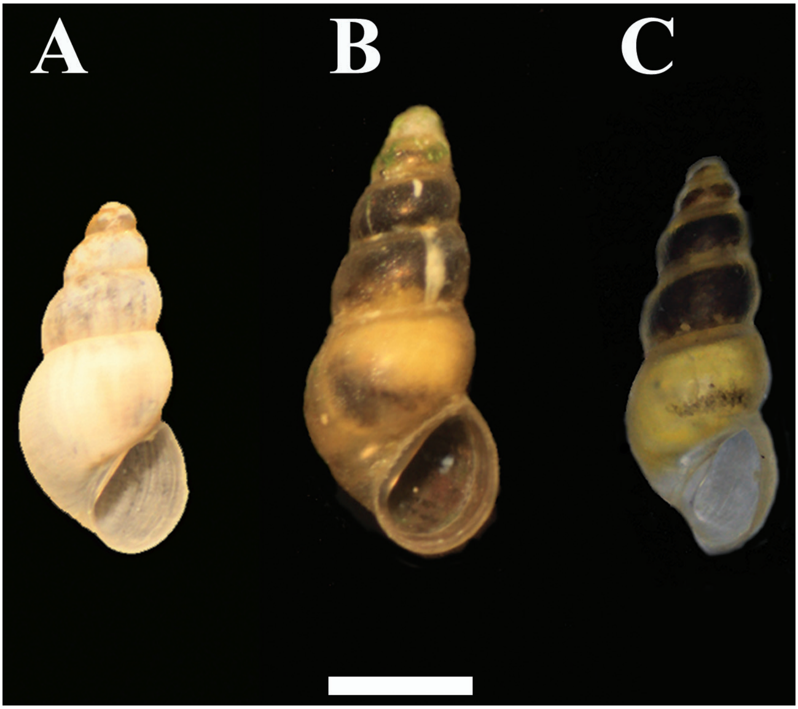

Figure 4. *Graecoanatolica vegorriticola* **A** Krya spring, Voiotia **B** Pigi Chariton, Voiotia **C** Lake Vegorritis, Pella. Scale bar 1 mm.

According to Schütt (1985), the abundance of *G. vegorriticola* used to be very high in the stony bank of Lake Vegorritis. Later on, Szarowska (2006) reported that only empty shells were found in Vegorritis in 2003. Moreover, Albrecht et al. (2006) found neither alive nor empty shells in 2005.

We found a low abundance population of *G. vegorriticola* on the banks of Lake Vegorritis south of Arnissa town. Furthermore, two high abundance populations of *Graecoanatolica* cf. *vegorriticola* with many mature individuals were discovered in Livadia and Orchomenos, Central Greece, ca 270 km away from the type locality of *G. vegorriticola*.

COI sequence data generated from specimens that have been collected from Lake Vegorritis, Livadia and Orchomenos were used to elucidate the taxonomic status of the populations inhabiting the latter two localities. In total, we obtained eight *COI* sequences of *G. vegorriticola* specimens from Lake Vegorritis, two sequences of *Graecoanatolica* from Livadia and six sequences from Orchomenos (Table 1). The *COI* sequence divergence separating specimens from these populations and those of *G. vegorriticola* from Lake Vegorritis is 1.7%. The two newly discovered localities are heavily influenced by tourism and agriculture.

During the field survey, we devoted significant effort in finding and collecting alive specimens of the other Balkan species of this genus, *Graecoanatolica macedonica*, in the Greek part of Lake Dojran. Unfortunately, only empty shells (some of the specimens looking to have recently died) were retrieved.

Isimerope Radea & Parmakelis, 2013

Isimerope semele Radea & Parmakelis, 2013

http://species-id.net/wiki/Isimerope_semele
Figure 3

Remarks. *Isimerope semele* was found in three localities of Peloponnisos, two in Nom. ARGOLIDAS (Megali Vrysi and "Second Spring") and one in Nom. ARKADIAS (Elissonas River, Piana) (Radea et al. 2013). A fourth population of *I. semele* was recorded by Falniowski and Szarowska (2011a) close to the population in Arkadia and reported by these authors as *Graecoarganiella* sp. The abundance of the known populations of *I. semele* seems to be very low (Falniowski and Szarowska 2011a, Radea et al. 2013).

Pseudamnicola Paulucci, 1878

Pseudamnicola negropontina (Clessin, 1878)

http://species-id.net/wiki/Pseudamnicola_negropontina
Figure 5

New records. Nom. EVVOIAS: stream with dense vegetation composed mainly by *Nasturtium* sp. and *Helosciadium* sp., on coastal flats 3.2 km NW of Marmari, ca 0 m asl, 38°04'16"N, 24°18'06"E, 26.xi.2012, Radea and Constantinidis.

Remarks. According to Schütt (1980) this taxon is a subspecies of *Pseudamnicola macrostoma* (Küster, 1853), i.e. *Pseudamnicola macrostoma negropontina* (Clessin, 1878), and it was described from Chalkis in Central Evvoia. Specimens of this taxon were also collected in 1985 and 2003 from an artificial pond in Marmari (Szarowska et al. 2006).

The latter authors consider *P. macrostoma negropontina* as a distinct species, *P. negropontina*. The molecular analysis that we performed after finding fresh specimens of *P. macrostoma* in Kato Souli, Attiki, showed that the *COI* sequence divergence separating the two taxa is 5.8% (K-2p model).

Pseudamnicola (*Pseudamnicola*) *pieperi* Schütt, 1980

http://species-id.net/wiki/Pseudamnicola_pieperi
Figure 3

New records. KARPATHOS island: spring close to Olympos, ca 260 m asl, 35°44'00"N, 27°10'01"E, 30.xi.2012, Radea, Bazos, Contantinidis; spring close to Prasteio, ca 220 m asl, 35°43'01"N, 27°11'00"E, 01.xii.2012, Radea, Bazos, Contantinidis; spring, Vananta, ca 0 m asl, 35°46'00"N, 27°12'01"E, 02.xii.2012, Radea, Bazos, Contantinidis; spring close to Spoa, ca 295 m asl, 35°38'01"N, 27°08'01"E, 03.xii.2012, Radea, Bazos, Contantinidis; spring close to Pyles, ca 305 m asl, 35°31'01"N, 27°07'01"E, 03.xii.2012,

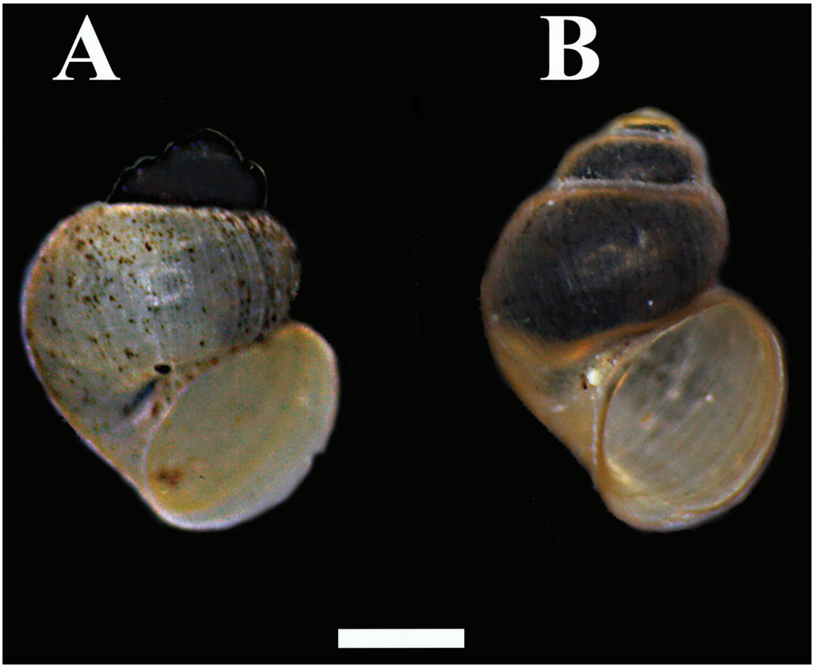

Figure 5. A *Pseudamnicola negropontina* **B** *Pseudamnicola macrostoma*. The first whorls of the shells of *P. negropontina* were heavily encrusted with epibionts. Scale bar 1 mm.

Radea, Bazos, Contantinidis; stream crossing the secondary road leading to Ag. Nikolaos temple, ca 740 m asl, 35°34'36"N, 27°09'39"E, 15.iv.2013, Radea and Constantinidis; discharge from the pumps of a water intake built on the spring close to Ag. Nikolaos temple, ca 705 m asl, 35°34'26"N, 27°09'57"E 15.iv.2013, Radea and Constantinidis.

Remarks. This species was collected for the first time by Pieper in 1977 from Aperi in Karpathos Island and described later by Schütt (1980). The type locality was the only known locality where *P. pieperi* occurred.

In 2012, we found several specimens of *P. pieperi* in the type locality. The seven new localities, where the species was found, are located in the central and northern part of the island. In the majority of the new localities, the population abundance was medium to high.

Genus *Pseudoislamia* Radoman, 1979

Pseudoislamia balcanica Radoman, 1979

http://species-id.net/wiki/Pseudoislamia_balcanica
Figure 3

New records. Nom. AITOLOAKARNANIAS: Lake Trichonis, N shore close to Dougri, ca 15 m asl, 38°36'01"N, 21°34'10"E, two specimens on leaves and stems of *Myri-*

ophyllum sp., depth 2–4 m, 09.iii.2012, Radea, Charou, Papadogiannis, Parmakelis; spring close to Ag. Sophia, 3 km NW from Thermos, ca 305 m asl, 38°34'59"N, 21°38'56"E, three mature and some immature specimens on stones, 10.iii.2012, Radea, Charou, Papadogiannis, Parmakelis.

Remarks. *Pseudoislamia balcanica* is an endemic species previously known only from its type locality, the NE rocky banks of Lake Trichonis near Myrtia (Radoman 1983). Szarowska (2006) considers this species to be extinct. In 2007 and 2008, two small populations were found near Myrtia by Albrecht et al. (2009). Our sampling revealed that although *P. balcanica* forms small sized populations, it is more widespread than previously thought and thrives both in lentic and lotic waters.

Genus *Radomaniola* Szarowska, 2006

Radomaniola cf. *curta* (Küster, 1852)
Figure 3

New records. Nom. AITOLOAKARNANIAS: Spring close to Ag. Sophia, 3 km NW from Thermos, ca 305 m asl, 38°34'59"N, 21°38'56"E, 10.iii.2012, Radea, Charou, Papadogiannis, Parmakelis. Nom. IOANNINON: Lake Toumpa, ca 650 m asl, 39°43'31"N, 20°44'53"E, 29.iv.2012, Parmakelis and Triantis; springs of Louros river, ca 285 m asl, 39°25'56"N, 20°50'25"E, 30.iv.2012, Parmakelis and Triantis; spring in Chani Terovo, ca 205 m asl, 39°23'48"N, 20°50'54"E, 30.iv.2012, Parmakelis and Triantis; springs in Ag. Georgios, ca 105 m asl, 39°16'09"N, 20°51'01"E, 30.iv.2012, Parmakelis and Triantis.

Remarks. *Radomaniola* was proposed by Szarowska (2006) as a replacement name for the genus *Orientalina* (Radoman, 1978). According to Radoman (1983), the Balkan species *Orientalina curta* (Küster, 1852) may be divided into a few subspecies on the basis of shell properties and the same author reported the presence of the subspecies *Orientalina curta albanica* Radoman, 1973 from the area of Ioannina. However, the shell dimensions of the specimens we collected from localities of Aitoloakarnania and Ioannina, (shell height: 1.90–3.65 mm, shell width: 1.20–1.95 mm, aperture height: 0.85–1.30 mm, aperture width: 0.70–1.20 mm) are similar to those of *Orientalina curta curta* (Küster, 1852) (shell height: 2.31–3.23 mm, shell width: 1.39–1.93 mm, aperture height: 0.92–1.30 mm, aperture width: 0.84–1.18 mm) reported by Radoman (1983).

Recently, Falniowski et al. (2012) analyzed molecular data from populations of the *Radomaniola/Grossuana* group collected from several localities of Greece and other countries of Balkan Peninsula. Two of the Greek localities were "spring in the city centre of Thermos, NE of Trichonida Lake" and "springs of Louros". In the Bayesian tree based on *COI* sequences provided by Falniowski et al. (2012), the specimens from these localities belong to the genus *Radomaniola* s. stricto. The above authors refer to the specimens from Thermos as "*Trichonia kephalovrissonia*" in the Bayesian tree. This fact indicates that *Trichonia kephalovrissonia* Radoman, 1973 [Schütt (1980) considers this taxon as a synonym of *Semisalsa steindachneri* (Westerlund, 1902) (=*Heleobia* (*Semisalsa*) *steindachneri* (Westerlund, 1902)] should be assigned to *Radomaniola kephalovrissonia* (Radoman, 1973). In the Bayesian tree mentioned above, the specimens from Louros seem to be close to *Radomaniola montana* (Radoman, 1973) and rather far from *Radomaniola curta curta* (Küster, 1852) and other *R. curta* (Küster, 1852) subspecies.

Despite the fact that our sampling localities (Fig. 1) are situated very close to those of Falniowski et al. (2012), we consider that all specimens collected from Aitoloakarnania and Ioannina belong to *Radomaniola curta* as long as only their morphological and anatomical characters have been examined and no molecular data are available.

Genus *Trichonia* Radoman, 1973

Trichonia trichonica Radoman, 1973

http://species-id.net/wiki/Trichonia_trichonica
Figure 3

New records. Nom. AITOLOAKARNANIAS: Lake Trichonis, N shore close to Dougri, ca 15 m asl, 38°36'01"N, 21°34'10"E, three specimens on leaves and stems of *Myriophyllum* sp. and *Potamogeton* sp., depth 2-4 m, 09.iii.2012, Radea, Charou, Papadogiannis, Parmakelis.

Remarks. *T. trichonica* was described by Radoman (1973) from Lake Trichonis, by the NE rocky bank near Myrtia but Schütt (1980) states that the species had previously been found in the sub-littoral zone of the stony southern shore of this lake. It seems that the species was found alive and collected from Lake Trichonis for the last time in 1985 (Szarowska 2006). Albrecht et al. (2006, 2009) referred that they did not find alive specimens of this species in Lake Trichonis in 2005, 2007 and 2008. However, a few specimens of *T. trichonica* were discovered close to the new locality by Radea in 2009.

Frogley and Preece (2007) found that *T. trichonica* lives on stones and aquatic vegetation at the mouth of Krya's spring discharging at the northern bank of another ancient Greek lake, Lake Pamvotis, Ioannina, Ipirus. The fieldwork of the latter authors was carried out in 1994, 1998 and 2005. Recently, Szarowska and Falniowski (2011b) reported that in 2003 there was no trace of the spring on the northern bank of Lake Pamvotis. During our sampling in Lake Pamvotis, we were not able to relocate the species and, additionally, we ascertained that the Krya's spring was destroyed since a water intake was built on it.

Discussion

Among other findings of this study, we found *Graecoanatolica vegorriticola* in two new localities quite distant from all the known localities of this taxon. In our effort to properly assign these populations to the species they belong to, we realized that the morphological and anatomical studies were not conclusive. To overcome this issue, *COI* sequence data were used to compare the *G. vegorriticola* specimens collected from the type locality of the species, with those of the newly located southern populations. The level of *COI* sequence divergence (1.7%) between the population from the type locality and those from the new localities is well within the range separating conspecific populations of Hydrobiidae genera, e.g. *Pyrgulopsis* 0–3.44% (Hurt 2004), *Grossuana* 3.4% (Szarowska et al. 2007), *Daphniola* 1.3–2.7% (Falniowski et al. 2007), *Isimerope* 3% (Radea et al. 2013) and other truncatelloidean genera e.g. *Austropyrgus* 3–5% (Perez et al. 2005). Consequently, the above populations are conspecific and belong to *G. vegorriticola*. The genus *Graecoanatolica* is distributed in the Balkans (two species) and in Turkey [eight species (Radoman 1983, 1985, Kebapçi et al. 2012)]. Radoman (1985) refers that the disjunct distribution of the genus could be an evidence of a paleohydrogeographical link between Anatolia and the Balkans. However, this connection could not be verified by molecular and anatomical data as the Balkan species were reported to have gone extinct (Kebapçi et al. 2012). The discovery of fresh specimens of *G. vegorriticola* in three localities of Greece eliminates this obstacle, and the alledged paleohydrogeographical link hypothesis can now be evaluated, provided that sequence data from the Turkish species become available.

Due to insufficient morphological and anatomical differentiation, the nominal subspecies *Pseudamnicola macrostoma macrostoma* (Küster, 1853) and *P. macrostoma negropontina* cannot be discriminated. However, Szarowska et al. (2006) supported that these taxa should be considered as distinct species. This claim was evaluated in the light of the generated sequence data from the newly collected specimens. The sequence divergence separating *P. macrostoma macrostoma* and *P. macrostoma negropontina* is 5.8% and this level of sequence divergence falls within the range 3.7–7.0% [Szarowska and Falniowski (2011), table 5 and p. 126] that separates Greek *Pseudamnicola* species. Therefore, it can be claimed that based on the *COI* sequence divergence levels, additional data exist to support the view that, despite the morphological and anatomical similarity between the two taxa, a species status should be assigned to *P. macrostoma negropontina*, as Szarowska et al. (2006) suggested.

During the field survey undertaken for this study, several threatened taxa, such as *Graecoanatolica vegorriticola*, *Pseudamnicola negropontina*, *Pseudamnicola pieperi*, *Pseudobithynia eubooensis* and *Pseudoislamia balcanica*, were recorded from new localities. *Trichonia trichonica*, which has been considered extinct from its type locality for the last twenty eight years (Albrecht et al. 2006), was re-discovered, whereas the presence of *Daphniola exigua*, *G. vegorriticola*, *Marstoniopsis graeca*, *P. pieperi* and *Pseudobithynia trichonis* in their type localities was verified. These findings combined with the recent discovery of the new endemic genus and species, *Isimerope semele* (Radea et al. 2013), besides confirming the crucial role of the Greek freshwater systems in shaping Europe's freshwater biodiversity (Glöer and Maassen 2009, Glöer et al. 2010), they also highlight two major issues, a) the Greek freshwater systems have yet a lot to offer to this diversity if comprehensively studied, and b) the IUCN status of some Greek endemic hydrobioids needs to be updated. Towards the latter issue we support that the new data derived from this survey allow us to propose transfers between categories for some species included in the IUCN Red List Threatened Species (2012) ver.3.1 (Table 2). These transfer proposals are based on the newly acquired knowledge regarding the distributional ranges of the species (*G. vegorriticola*, *P. balcanica* and *T. trichonica*) as well as to the elucidation of the standing taxonomic confusion of certain species i.e. *newly split* (*P. negropontina*) and *newly described* (*I. semele*). We followed the IUCN guidelines regarding the definition of the term "location": "a geographically or ecologically distinct area in which a single event (e.g. pollution) will soon affect all individuals of the taxon present" (IUCN 2011).

Graecoanatolica vegorriticola. Our findings indicate that none of the criteria of the category Critically Endangered are met since the extent of occurrence (EOO) and the area of occupancy (AOO) become >100 km^2 and >10 km^2, respectively. Therefore, this species may be down-listed to the category Endangered if the criteria of the category Critically Endangered continue to not be met for the next five years. Additionally, (a) and b(iii) are met because the number of locations is ≤ 5 and a continuing decline is observed in the quality of the habitat, respectively; c(iv) is also met because extreme fluctuation in the number of mature individuals has been recorded (Hauffe et al. 2011).

Pseudoislamia balcanica. The discovery of this species in a new locality increases the extent of its occurrence (EOO), which becomes >100 km^2. Therefore, *P. balcanica* may be down- listed to the category Endangered if it continues to thrive in other locality(ies), apart from Lake Trichonis, at least for the next five years. Additionally, (a) and b(iii) are met because the number of locations is ≤ 5 and a continuing decline is observed in the quality of the habitat, respectively.

Trichonia trichonica. We re-discovered this species in Lake Trichonis. On the contrary, no single specimen or even empty shells were recorded in Krya's spring (Lake Pamvotis) due to the water intake built on it. Therefore, the criterion B1, the extent of occurrence (EOO) <100 km^2, is met for *T. trichonica*. Additionally, (a) and b(iii) are met because the number of locations is 1 and a continuing decline is observed in the quality of the habitat, respectively.

Pseudamnicola negropontina has not yet been evaluated because it is now elevated to species level (*newly split*). The area of occupancy of this species is < 10 km^2 and, consequently, the criterion B2 is met. Additionally, (a) and b(ii,iii) are met because the number of locations is 1 and a continuing decline is observed in the area of occupancy and the quality of the habitat, respectively.

Isimerope semele has not yet been evaluated because it is a newly described species (*newly described*). The extent of occurrence of this species is 100 km^2<EOO<5000 km^2 and therefore the criterion B1 for the category Endangered is met. Additionally, (a) and b(iii) are met because the current distribution of the species is severely fragmented and a continuing decline is observed in the quality of the habitat, respectively.

During the field survey we ascertained that many of the "hydrobioid" localities in Greece are heavily influenced by various human activities such as tourism, agriculture,

Table 2. Scale of endemism of the hydrobioids collected and the suggested transfers between IUCN Red List Categories (with bold our sampling localities).

	Scale of Endemism	**IUCN Red List Category (2012) ver. 3.1**	**Transfers**
Family Amnicolidae			
Marstoniopsis graeca	$E_{\textbf{PELLA (LAKE VEGORRITIS)}}$	Critically Endangered B1ab(i,iii)	-
Family Bythinellidae			
Bythinella charpentieri *Bythinella* cf. *charpentieri*	$E_{\text{ATTIKI+EVVOIA+PARNASSOS Mt.}}$ **EVVOIA+VOIOTIA**	Least Concern	-
Family Bithyniidae			
Pseudobithynia euboeensis	$E_{\textbf{EVVOIA (SPRING)}}$	Critically Endangered B2ab(iii)	-
Pseudobithynia trichonis	$E_{\textbf{AITOLOAKARNANIA (LAKE TRICHONIS}\text{+LAKE LYSIMACHEIA)}}$	Endangered B1ab(iii)	-
Family Hydrobiidae			
Daphniola exigua	$E_{\textbf{THESSALIA (AG. PARASKEVI SPRING+}\text{DAPHNI SPRING)}}$	Endangered B2ab(iii)	-
Graecoanatolica vegorriticola	$E_{\textbf{PELLA (LAKE VEGORRITIS}\text{+LAKE PETRON)}\textbf{+VOIOTIA (SPRINGS)}}$	Critically Endangered B1ab(i,iii,iv) c(iv)+2ab(i,iii,iv)c(iv)	Endangered B1ab(iii)c(iv)
Isimerope semele	$E_{\textbf{ARGOLIDA (SPRINGS)+ARKADIA (RIVER+}\text{SPRING)}}$	Not Evaluated	Endangered B1ab(iii)
Pseudamnicola negropontina	$E_{\textbf{EVVOIA (SPRING)}}$	Not Evaluated	Critically Endangered B2ab(ii, iii)
Pseudamnicola pieperi	$E_{\textbf{KARPATHOS (SPRINGS)}}$	Vulnerable D2	-
Pseudoislamia balcanica	$E_{\textbf{AITOLOAKARNANIA (LAKE TRICHONIS+SPRING)}}$	Critically Endangered B1ab(iii)	Endangered B1ab(iii)
Radomaniola curta *Radomaniola* cf. *curta*	$E_{\text{ALBANIA+GREECE}}$ **AITOLOAKARNANIA+IOANNINA**	Least Concern	-
Trichonia trichonica	$E_{\textbf{AITOLOAKARNANIA (LAKE TRICHONIS)}}$	Critically Endangered B2ab(i,iii)	Critically Endangered B1ab(iii)

livestock, industry, housing development and forestry. Thus, a decline or even loss of local freshwater gastropods is expected and, in some cases, it has already been reported (Ryan and Griffiths 2001, Szarowska and Falniowski 2004, Albrecht et al. 2006, Regnier et al. 2009).

Against the loss of hydrobioids due to the declining number of suitable habitats, a taxonomically accurate record of taxa, especially for those thriving in springs and spring brooks (crenobionts), will contribute significantly in assigning high conservation priorities (Arconada and Ramos 2001, Haase 2003). Without serious and effective intervention it is only a matter of time before the vast majority of the unprotected hydrobioids we recorded in this study become extinct.

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Taxonomic revision of Madagascan *Rhantus* (Coleoptera, Dytiscidae, Colymbetinae) with an emphasis on Manjakatompo as a conservation priority

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Abstract

We review the diving-beetle genus *Rhantus* Dejean of Madagascar (Coleoptera, Dytiscidae, Colymbetinae) based on museum collection holdings and recently collected expedition material. Both morphology and DNA is used to test species boundaries, in particular whether newly collected material from the Tsaratanana mountains in the north represent a new species or are conspecific with *Rhantus manjakatompo* Pederzani and Rocchi 2009, described based on a single male specimen from the central Ankaratra mountains. DNA of the holotype of *R. manjakatompo* was successfully extracted in a non-destructive way and sequenced. The general mixed Yule coalescent model applied to an ultrametric tree constructed from mitochondrial cytochrome c oxidase subunit I (*COI*) sequence data delimited three species. Morphological characters supported the same species unambiguously. We therefore recognise three species of *Rhantus* to occur in Madagascar: *R. latus* (Fairmaire, 1869), *R. bouvieri* Régimbart, 1900 and *R. manjakatompo* Pederzani and Rocchi, 2009. All three species are endemic to Madagascar and restricted to the highlands of the island. *Rhantus stenonychus* Régimbart, 1895, **syn. n.,** is considered a junior synonym of *R. latus*. We designate lectotypes for *R. bouvieri* and *R. goudoti* Sharp, 1882, the latter a junior synonym of *R. latus*. We provide descriptions, a determination key, SEM-images of fine pronotal and elytral structures, distribution maps, habitus photos, and illustrations of male genitalia and pro- and mesotarsal claws. We discuss the role of the Manjakatompo forest as a refugium for Madagascan *Rhantus* diversity and other endemics of the montane central high plateau.

Keywords

Diving beetles, Madagascar, GMYC, species delimitation, refugium, lectotype designation, new synonymy

Introduction

Rhantus Dejean is a large cosmopolitan genus of medium-sized aquatic diving beetles. Several studies have pointed out that the genus as presently defined is clearly paraphyletic and will likely be split in the future (Balke et al. 2007). *Rhantus* has a track record of colonizing oceanic islands and forming island endemics (Balke et al. 2007, 2009), which indicates capacity for infrequent long-distance dispersal. It has colonized several islands in the Pacific with island-endemics on Fiji (Balke et al. 2007), New Caledonia (Balke et al. 2010), Galapagos (Peck and Balke 1993) and Hawaii (Balke 1993) to name a few. The Afrotropical region harbours only a modest part of the global *Rhantus* diversity (Guignot 1961). Balke (1992a) revised the *Rhantus* species of the Mascarene Islands outside of Madagascar and concluded that previous records were erroneous and that three species were endemic for this area, each to one of the three islands Mauritius, La Reunion and Ile Rodrigues. The Mascarene Islands are of rather recent volcanic origin (8-15 mya) and are known to have been on the receiving end of flora and fauna from Madagascar. Madagascar itself is a large island with ecosystems spanning from rainforests to semi deserts. The level of endemism is extremely high which has granted Madagascar a top placement among biodiversity hotspots (Myers et al. 2000). But the fauna is also known for its microendemic patterns – species distributions are often restricted to smaller geographical areas, almost like “islands within the island”. Some colonizations have led to rich allopatric species radiations, but the seemingly low species diversity of *Rhantus* perhaps indicates that their relatively high dispersal capacity has prevented the genus from a significant radiation.

The first *Rhantus* species described from Madagascar was *Rhantus latus* (Fairmaire 1869, in the genus *Colymbetes* Clairville). Since then four additional species have been described from the island, but the validity of some of these are questionable (Balke 1992b, Pederzani and Rocchi 2009) and no modern revision exist. In addition, apart from *R. latus*, Madagascan *Rhantus* are very poorly represented in collections and are seemingly rare. Recent expeditions by the Swedish Museum of Natural History in collaboration with the Entomology department of the University of Antananarivo, have unearthed significant new material of Madagascan *Rhantus*, especially from Manjakatompo forestry station in the Ankaratra mountain massif. The new material both enables the assessment of intraspecific character variation and the extraction and sequencing of DNA data to test species limits. The purpose of this study is to revise the Madagascan *Rhantus* species based on this material, type material and other museum holdings, notably from the collections at the Museé National d’Historie Naturelle in Paris. In particular, the discovery in 2004 of what seemed to be a new undescribed *Rhantus* species from the Tsaratanana mountain massif in the north of Madagascar warranted a revisonary treatment of the group. In 2009 however, Pederzani and Rocchi (2009) described a new *Rhantus* species from the Ankaratra mountains in central Madagascar based on a single male specimen. The description showed both clear similarities but also some differences to the new species discovered in 2004 and it was hypothesized that DNA data and explicit species delimitation tests could aid in resolving this taxonomic question. Results of all these studies are summarized in the presented paper.

Material and methods

All specimens examined in this study are registered in the NHRS collection objects database (interface via www.naturarv.se) but are deposited in the following collections and referred to by the abbreviations:

BMNH	Museum of Natural History, London, Great Britain;
MNHN	Muséum National d'Histoire Naturelle, Paris, France;
NMW	Naturhistorisches Museum Wien, Austria;
NHRS	Swedish Museum of Natural History, Stockholm, Sweden;
DEUA	Departement d'Entomologie, Université d'Antananarivo, Antananarivo, Madagascar.

Measurements were taken on specimens in a horizontal position. The following measurements were taken: **ML**, maximum length from head to tip of elytra; **MW**, maximum width; **LP**, length of pronotum medially; **WPB**, pronotal width at base; **LE**, length of elytra from tip of scutellum to apex. The measurements were taken using an Olympus SZX12 stereomicroscope with an Infinity X camera and a calibrated ruler tool in the software DeltaPix Insight 2.0. Environmental scanning electron microscopy was done using a FEI/Philips XL30 FEG ESEM at the Institute for Surface Chemistry, Stockholm, Sweden. The images were generated at 350× magnification, with a gaseous secondary electron detector in low vacuum mode; the accelerating voltage of the electron beam was 17 kV. In opposite to a backscattered detector, a gaseous secondary electron detector depicts depressions of the surface brightly.

Molecular data

DNA was extracted from legs of ethanol-preserved material collected after 2004 using Qiagen DNeasy 96 Tissue kit (see Table 1 for specimen information). For the dry-mounted holotype of *R. manjakatompo*, (collected 2001) the QIAamp® DNA Micro kit was used, following the protocol for animal tissue with incubation at 56°C overnight. A single hindleg was carefully removed and after incubation re-glued to the body. Two fragments of the gene cytochrome c oxidase subunit I (*COI*) were

Table 1. Data on specimens sequenced for COI and Genbank accession numbers. First accession number is for the 3' end (patdyt-jerry) and second accession number is for the 5' end (lco-hco) of COI.

Cat. ID	Species	Locality	Date	Collector	Mus.	GB Acc. No.
BMNH-743391	Rhantus bouvieri	Andringitra	9.V.2006	Bergsten et al.	NHRS	KF548613, KF548639
BMNH-743392	Rhantus bouvieri	Andringitra	9.V.2006	Bergsten et al.	BMNH	KF548614, KF548640
BMNH-829990	Rhantus latus	Ambalavao, 15km SW of			BMNH	KF548615, KF548641
NHRS-JLKB000000089	Rhantus latus	Ambohijanahary	19.XII.2009	Bergsten et al.	NHRS	KF548616, KF548642
NHRS-JLKB000000090	Rhantus latus	Ambohijanahary	19.XII.2009	Bergsten et al.	NHRS	KF548617, KF548643
BMNH-741961	Rhantus latus	Ambositra, 34km S of	06.V.2006	Bergsten et al.	BMNH	KF548618, KF548644
BMNH-829991	Rhantus latus	Andasibe	04.I.2007	Isambert et al.	BMNH	KF548619, KF548645
BMNH-742090	Rhantus latus	Andringitra	9.V.2006	Bergsten et al.	BMNH	KF548620, KF548646
BMNH-729860	Rhantus latus	Col des Tapia	8.XII.2004	Balke et al.	BMNH	KF548621, KF548647
BMNH-729861	Rhantus latus	Col des Tapia	8.XII.2004	Balke et al.	BMNH	KF548622, KF548648
BMNH-729862	Rhantus latus	Col des Tapia	8.XII.2004	Balke et al.	BMNH	KF548623, KF548649
BMNH-729863	Rhantus latus	Col des Tapia	8.XII.2004	Balke et al.	BMNH	KF548624, KF548650
BMNH-729864	Rhantus latus	Col des Tapia	8.XII.2004	Balke et al.	BMNH	KF548625, KF548651
BMNH-829992	Rhantus latus	Col des Tapia	8.XII.2004	Balke et al.	BMNH	KF548626, KF548652
BMNH-741810	Rhantus latus	Isalo	11.V.2006	Bergsten et al.	BMNH	KF548627, KF548653
BMNH-742359	Rhantus latus	Sendrisoa	7.V.2006	Bergsten et al.	BMNH	KF548628, KF548654
BMNH-741979	Rhantus latus	Zombitse	14.V.2006	Bergsten et al.	BMNH	KF548629, KF548655
BMNH-742639	Rhantus latus	Zombitse	15.V.2006	Bergsten et al.	BMNH	KF548630, KF548656
BMNH-742640	Rhantus latus	Zombitse	15.V.2006	Bergsten et al.	BMNH	KF548631, KF548657
BMNH-742641	Rhantus latus	Zombitse	15.V.2006	Bergsten et al.	BMNH	KF548632, KF548658
BMNH-729851	Rhantus latus				BMNH	KF548633, KF548659
NHRS-JLKB000030412	Rhantus manjakatompo	Manjakatompo	8.X.2001	Gerecke & Goldschmidt	NMW	KF548634, na
BMNH-672725	Rhantus manjakatompo	Tsaratanana	20–24. XII.2004	Lees & Ranaivosolo	BMNH	KF548635, KF548660
BMNH-672726	Rhantus manjakatompo	Tsaratanana	20–24. XII.2004	Lees & Ranaivosolo	BMNH	KF548636, KF548661
BMNH-672730	Rhantus manjakatompo	Tsaratanana	20–24. XII.2004	Lees & Ranaivosolo	NHRS	KF548637, KF548662
BMNH-672731	Rhantus manjakatompo	Tsaratanana	20–24. XII.2004	Lees & Ranaivosolo	NHRS	KF548638, KF548663

Table 2. Primers used for the PCR to amplify two fragments of COI.

Primer	Direction	Sequence (5'-3')
PatDyt[1]	Reverse	TCATTGCACTAATCTGCCATATTAG
Jerry[2]	Forward	CAACATTTATTTTGATTTTTTGG
LCO[3]	Forward	GGTCAACAAATCATAAAGATATTGG
HCO[3]	Reverse	TAAACTTCAGGGTGACCAAAAAATCA

[1] Isambert et al. (2011)

[2] Simon et al. (1994)

[3] Folmer et al. (1994)

sequenced for analysis. Primers used for amplification and sequencing were derived from several sources (Table 2).

DNA fragments were PCR amplified using “Ready-to-go” PCR Beads from Pharmacia Biotech and Phire Hot Start II PCR Master mix following the manufacture’s standard protocols. The thermal cycling profile for “Ready-to-go” PCR was 95°C for 5 min, followed by 40 cycles of 95°C for 30 s, 50°C for 30 s, 72°C for 1 min and finally 72°C for 8 min. PCR cycling for Phire Hot Start PCR was 98°C for initial denaturation 30 s, followed by 40 cycles of 98°C for 5 s, 53°C for 5 s, 72°C for 15 s, 72°C for 1 min. Product yield, specificity of amplification and contamination were investigated using agarose gel electrophoresis. PCR products were purified using ExoFAP Cleanup mix and cycle sequenced using the same primers used to amplify. For sequencing reactions the ABI BigDye Terminator kit ver. 3.1 was used. Sequencing products were purified using the DyeEx 96 kit and fragments were analysed on an ABI377xl analyser from Applied Biosystems. Gene regions were sequenced in both directions. The contigs were assembled from the forward and reverse reads and primers were trimmed in Sequencher 5.0 (Gene Codes Corporation 2011). All sequences are deposited in Genbank under the accession numbers KF548613 - KF548663 (Table 1).

Analyses

Sequence data for 26 specimens were aligned in ClustalX 2.1 (Larkin et al. 2007) using default settings. The resulting combined matrix based on the two gene fragments was gap-free and had a total length of 1486 base pairs. An ultrametric tree was obtained from Bayesian MCMC analysis conducted in MrBayes 3.2 (Ronquist et al. 2012). We used a GTR+I+Γ model jointly for all codon positions but allowed each position a separate rate multiplier. Branch lengths were estimated under an autocorrelated relaxed clock model (TK02). Two separate MCMC analyses were run for 200000 generations with chains sampled every 100 generations, and a burn-in of 10% was chosen after visual examination of the –lnL plotted against generations and the convergence statistics provided by the program. A species delimitation test was conducted using the General Mixed Yule-Coalescent (GMYC) approach (Pons et al. 2006) and the *splits* package (Ezard et al. 2009), implemented in R 2.14.0 (R Development Core Team 2011). The single threshold method was used on the tree to find the maximum likelihood solution of the transition point between coalescent and interspecific branching patterns on the tree. Genetic distances were calculated under the Kimura-2-parameter model using Mega 5.0 (Tamura et al. 2011).

Results

GMYC species delimitation

The GMYC model was significantly better than a single coalescence model with a likelihood ratio test (lgL GMYC: 158.9, lgL single coalescence: 149.4, p = 0.0003), and divided the terminals into tree separately coalescing units: *R. manjakatompo*, *R. latus* and *R. bouvieri* (Figure 1). No other solution had a log-likelihood value within 2 units (an approximate confidence interval) of the maximum likelihood solution. The holotype of *R. manjakatompo* (NHRS-JLKB000030412) from Ankaratra mountains was nested within the single coalescing unit represented by the four specimens from the Tsaratanana mountains (Figure 1). The genetic distance between the holotype from Ankaratra and the specimens from Tsaratanana was 0.008-0.011, which was within the range among the Tsratanana specimens alone (0-0.012). The within-species genetic variation was 0.015 for *R. bouvieri* and 0-0.019 (mean = 0.009) for *R. latus*. The distances between the three species were almost an order of magnitude greater: 0.11-0.13 between *R. bouvieri* and *R. latus*, 0.17 between *R. bouvieri* and R. *manjakatompo* and 0.16-0.17 between *R. latus* and *R. manjakatompo*.

Taxonomic part

Rhantus manjakatompo Pederzani & Rocchi, 2009

http://species-id.net/wiki/Rhantus_manjakatompo
Figures 2a–b, 3a–c, 4a, 5a–b, 6a–b, 7a–b, g

Rhantus manjakatompo Pederzani & Rocchi, 2009: 88

Type locality. Madagascar, Antananarivo province, Ankaratra mountains, Manjakatompo reserve.

Type material (NMW). Holotype ♂ "Madagascar, Ankaratra (Antananarivo), Reserve Manjakatompo, spring stream exp. SE. N deviation to Analamitana (left affl. MD 107) m 1850 asl, 8.x.2001, 16.0°C, 0.003 mS/cm. Gerecke & Goldschmidt collectors BMNH (E) 2004-46", "Holotype Rhantus manjakatompo Pederzani & Rocchi 2008", "Data in NHRS-JLKB 000030412" "DNA Voucher".

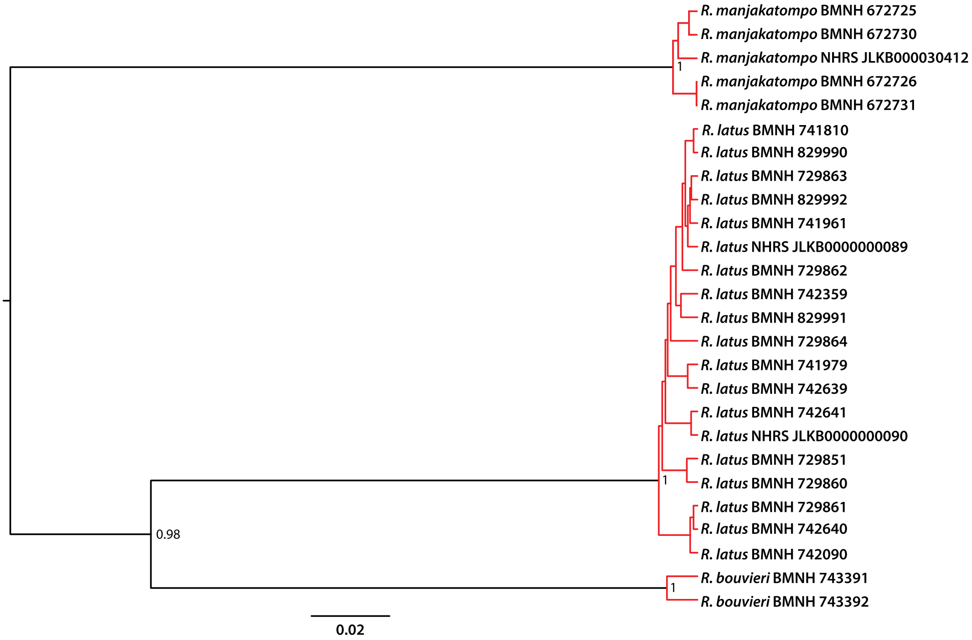

Figure 1. Ultrametric tree obtained from Bayesian analysis of of the two concatenated *COI* gene fragments. Red branches indicate separately coalescing clusters corresponding to species. Node values show posterior probability values and scale bar indicates the expected number of substitutions per site. "Rhantus manjakatompo NHRS–JLKB000030412" is the holotype of *Rhantus manjakatompo*.

Additional material studied (NHRS, BMNH, DEUA, see Appendix): **Sofia region (former provinces: Mahajanga):** 2♂, 2♀ (Cat. No. BMNH-672731, 672730, 672726, 672725): Tsaratanana [Antetykalambazaha Camp], 14.1824S, 48.9448E, 1700m, 20–24.xii.2004, Malaisetrap, Leg. Lees, Ranaivosolo.

Vakinankaratra region (former provinces: Antananarivo): 4spp. (Cat. No. NHRS-JLKB000021018) Manjakatompo [Analamitana] [swamp near stream][MJK12-02], 19.3640S, 47.2991E, 1760m, 22.i.2012, Leg. Ranarilalatiana, Randriamihaja, 1sp. (Cat. No. NHRS-JLKB000021019) Manjakatompo [Tavolatara][swamp near stream][MJK12-08], 19.3491S, 47.2784E, 2050m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 5spp. (Cat. No. NHRS-JLKB000021020) Manjakatompo [Tavolatara][pool near stream][MJK12-09], 19.3491S, 47.2780E, 2050m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 11spp. (Cat. No. NHRS-JLKB000021021) Manjakatompo [Tavolatara][swamp near source][MJK12-10], 19.3496S, 47.2779E, 2050m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 3spp. (Cat. No. NHRS-JLKB000021022) Manjakatompo [Anosiarivo][lake near source][MJK12–13], 19.3449S, 47.3041E, 2070m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 1spp. (Cat. No. NHRS-JLKB000021023) Manjakatompo [Andongolongo][pool near source][MJK12–12], 19.3536S, 47.3006E, 1900m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja.

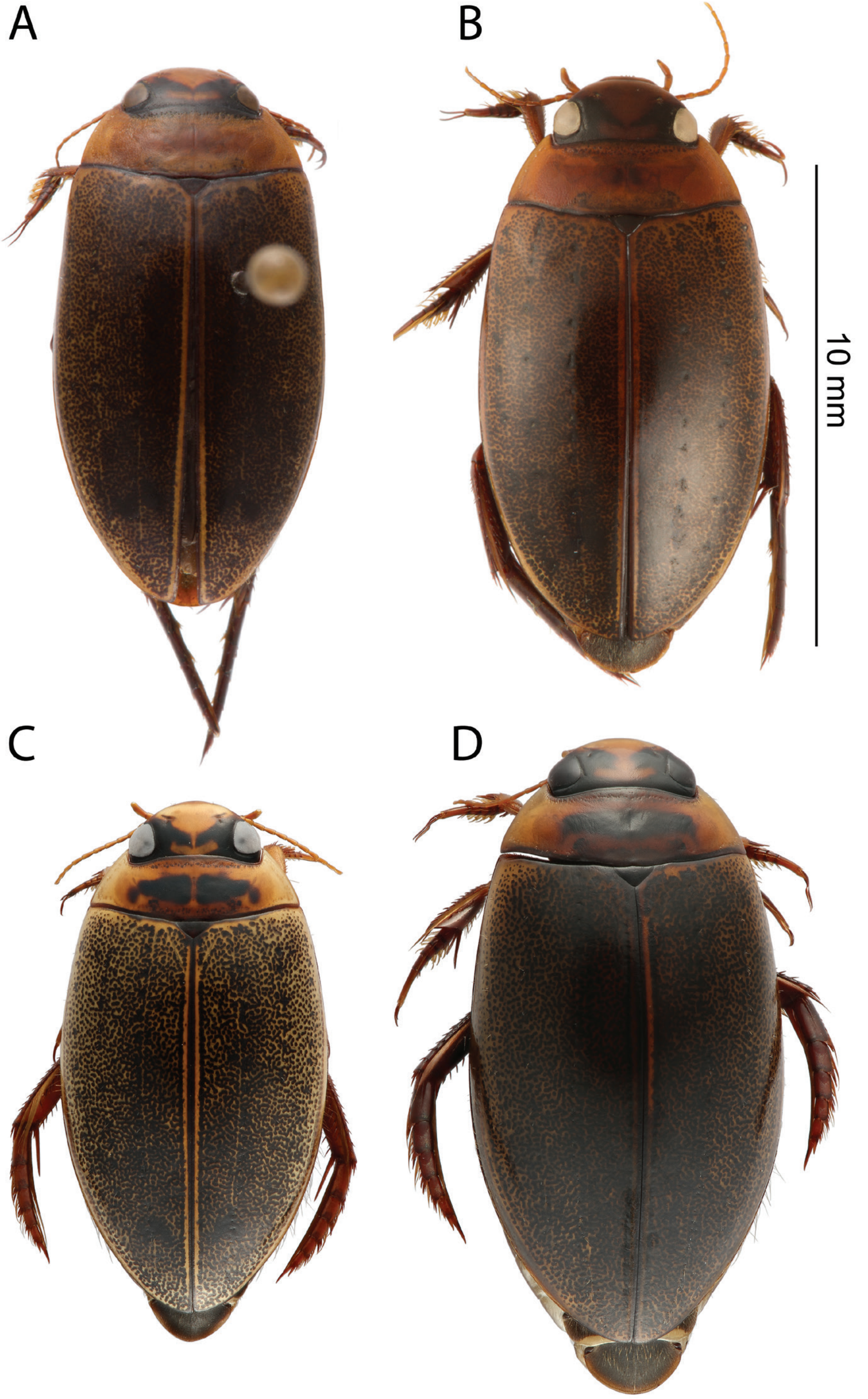

Figure 2. Habitus, dorsal view: **A** *Rhantus manjakatompo* holotype (Ankaratra mountains) **B** *Rhantus manjakatompo* (Tsaratanana mountains) **C** *Rhantus bouvieri* **D** *Rhantus latus.*

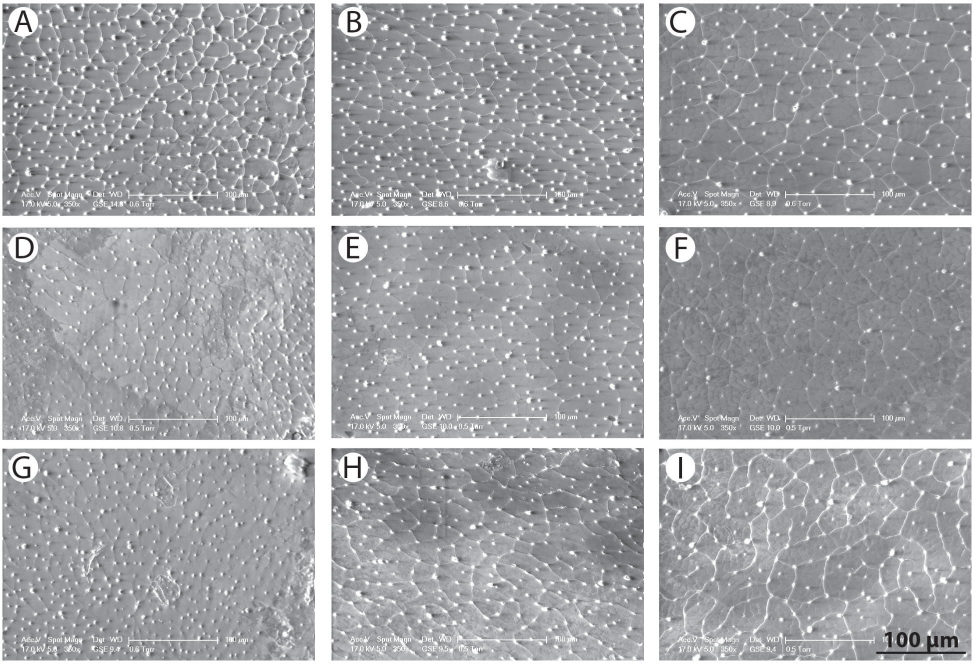

Figure 3. SEM images of pronotal and elytral microstructure: **A–C** *Rhantus manjakatompo* pronotum (**A**) anterior part of elytron (**B**) and posterior part of elytron (**C**) **D–F** *Rhantus bouvieri* pronotum (**D**) anterior part of elytron (**E**) and posterior part of elytron (**F**) **G–I** *Rhantus latus* pronotum (**G**) anterior part of elytron (**H**) and posterior part of elytron (**I**).

Diagnosis. Pronotum entirely testaceous or with two small dark spots medially (Figure 2a–b). Black irrorations of elytra somewhat confluent subapically (Figure 2a–b). Male protarsal claws equally long, evenly curved, apex acute (Figure 5a–b). Male mesotarsal claws equally long but posterior claw distinctly thicker than anterior claw (Figure 6a–b). Penis short and robust (Figure 7a–b), Parameres evenly curved and tapering to apex (Figure 7g). 11.5–12.5 mm long.

Description. *Size*: ML 11.5–12.5 mm; MW 5.7–5.9 mm; Lp 1.4 mm; Epb 4.7–4.9 mm; Le 8.4–9.0 mm (n = 5).

Head. Testaceous with black areas posteriorly and inside eyes. Interocular black pattern narrowly separated medially (Figure 2a–b). Dense reticulation of well impressed meshes, very fine punctuation within and at edges of meshes.

Pronotum. Testaceous to ferrugineous with two small dark spots medially (Figure 2b), which may be absent (Figure 2a). Rim at lateral margin clearly visible to indistinct. Dense reticulation of well impressed meshes, very fine punctuation within and at edges of meshes (Figure 3a).

Elytra. Testaceous with black irrorations, leaving paler sides and yellow sutural lines (Figure 2a–b). Black irrorations somewhat confluent subapically. Reticulation of polygonal meshes simple anteriorly and double at middle and posteriorly, meshes are

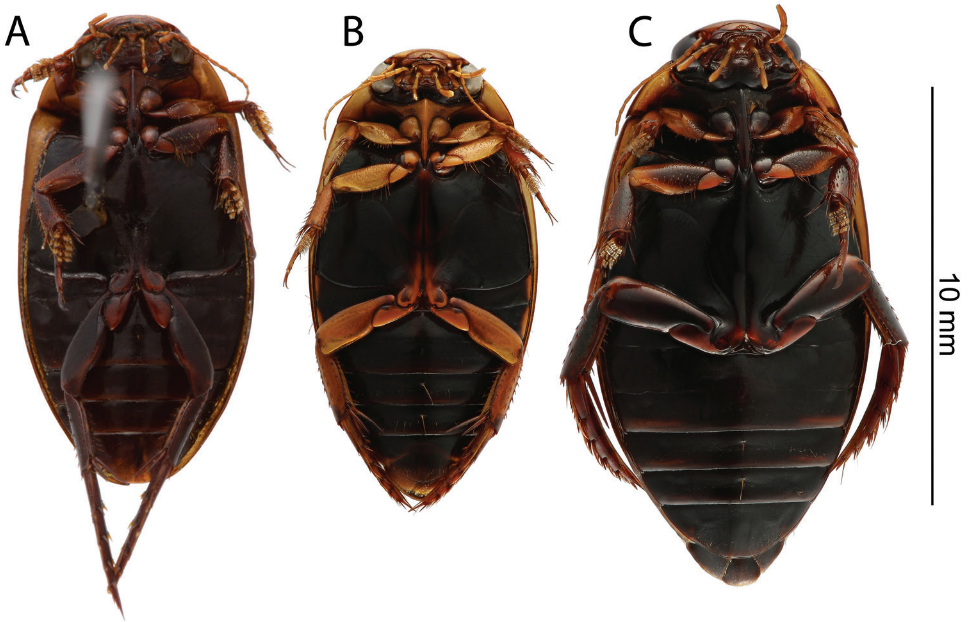

Figure 4. Habitus, ventral view: **A** *Rhantus manjakatompo* holotype **B** *Rhantus bouvieri* **C** *Rhantus latus.*

somewhat less impressed than on pronotum and larger posteriorly. Very fine punctuation within and at edges of meshes (Figure 3b–c).

Ventral side. Dark brown to black. Epiplura testaceous. Metafemora infuscated (Figure 4a).

Male. Protarsal claws equally long, evenly curved, apex acute (Figure 5a–b). Mesotarsal claws equally long but posterior claw distinctly thicker than anterior claw (Figure 6a–b). Penis short and robust, shape as Figure 7a–b. Parameres evenly curved and tapering to apex (Figure 7g).

Remarks. In 2004 David Lees and Ravomiarana Ranaivosolo collected material of what seemed to be a new *Rhantus* species from the mountain massif of Tsaratanana, North Madagascar. *R. manjakatompo* was described in 2009 based on a single male specimen from Ankaratra mountains, 70km south of Antananarivo in central Madagascar. Despite variation in color and impression of a lateral rim of pronotum, molecular data indicate that the studied material from Ankaratra and Tsaratanana are conspecific or at least not an old enough divergence to be recognised based on *COI* sequence (genetic distance 0.008–0.011). As also male tarsal claws, aedeagus and parameres were identical, we consider the specimens from Tsaratanana mountains conspecific with the holotype of *R. manjakatompo*.

Habitat. Associated with sources and streams, and surrounding water assemblages like nearby pools and marshes at altitudes between 1700 to 2070 m a.s.l. In Manjakatompo, the species was most numerous at elevations above 2000 m.

Distribution. Endemic to Madagascar. Only known from Tsaratanana mountains and Manjakatompo forestry station in the Ankaratra mountains (Figure 8).

Rhantus bouvieri Régimbart, 1900

http://species-id.net/wiki/Rhantus_bouvieri
Figures 2c, 3d–f, 4b, 5c–d, 6c–d, 7c–d, h

Rhantus bouvieri Régimbart, 1900: 374
Rhantus Bouvieri Régimbart, 1899: Zimmerman (1920: 199); Guignot (1961: 754).
Rhantus bouvieri Régimbart, 1899: Nilsson (2001: 48).

Type locality. Madagascar, Fianarantsoa.

Type material (MNHN). **Lectotype** ♂, here designated to fix the concept of *Rhantus bouvieri* and to ensure the universal and consistent interpretation of the same: "Data in NHRS-JLKB000030144", "Rhantus Bouvieri Rég. M. Régimbart det.", "Museum Paris Madagascar Fianarantsoa Grandidier 1852–91", "Rhantus bouvieri Régimbart, 1900 Det. J. Bergsten 2011" our lectotype label.

Paralectotypes 2♀: "Data in NHRS-JLKB000030405 and Data in NHRS-JLKB0000304283", "Museum Paris Madagascar Fianarantsoa Grandidier 1852–91", our paralectotype labels.

Additional material studied (NHRS, BMNH, MNHN, NMW, DEAU, see Appendix):

Matsiatra Ambony (Haute Matsiatra) region (former provinces: Fianarantsoa): 2♂ (Cat. No. BMNH-743391, 743392), Andringitra NP [Zomandao river, by bridge on road to camp Belamba], 22.1043S, 46.9207E, 1420m, 09.v.2006, Bottle-trap, Leg Bergsten et al.

Vakinankaratra region (former provinces: Antananarivo): 1♂, 1♀ (Cat. No. NHRS-JLKB000030410, 30411), Manjakatompo [pond by], 10.x.1968, Leg. Starmühlner, 2spp. (Cat. No. NHRS-JLKB000010272), Manjakatompo [Analafandriana, 500m S fish farm by the road][grassy pond][MAD11–14], 19.3619S, 47.3150E, 1730m, 03.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 8spp. (Cat. No. NHRS-JLKB000010275), Manjakatompo [500m E Lac Froid by the road][pond and inlet stream][MAD11–16] 19.3449S, 47.3338E, 1620m, 04.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 16spp. (Cat. No. NHRS-JLKB000010276), Manjakatompo [500m E Lac Froid by the road][pond and inlet stream][MAD11-16], 19.3449S, 47.3338E, 1620m, 04.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 10spp. (Cat. No. NHRS-JLKB000010273), Manjakatompo [2km NE Amparandraindrisa][pond and stream][MAD11-18], 19.3607S, 47.3009E, 1770m, 05.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 4spp. (Cat. No. NHRS-JLKB000021024), Manjakatompo [Anosiarivo][lake near source][MJK12-13], 19.3449S, 47.3041E, 2070m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 7spp.

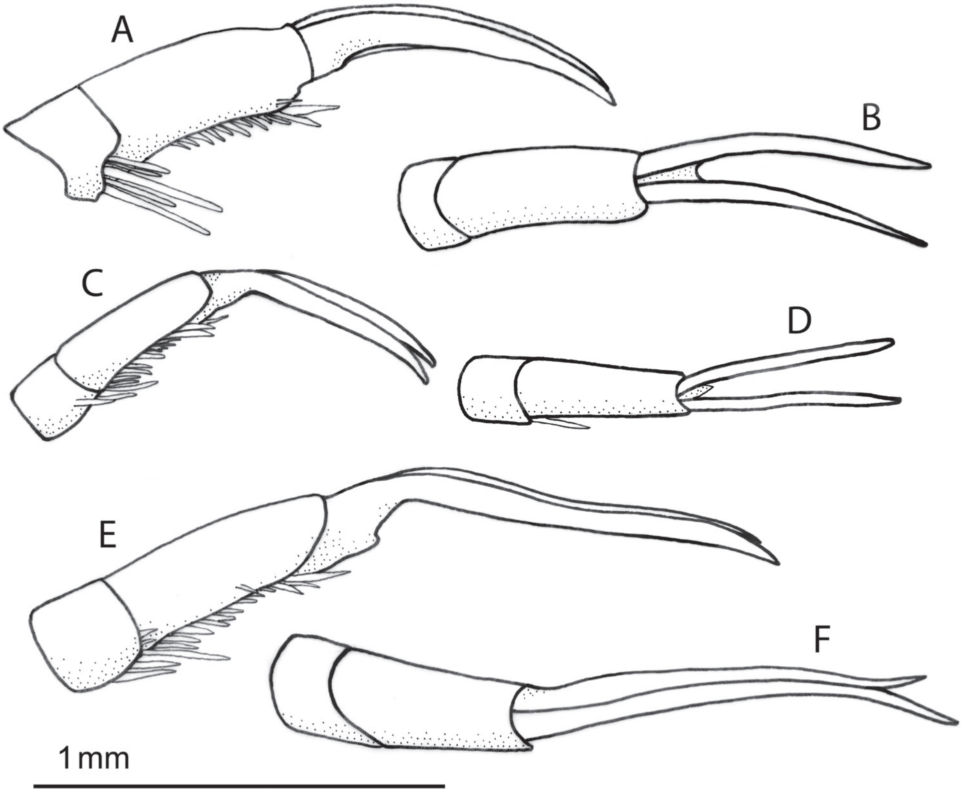

Figure 5. Left protarsal claws: **A–B** *Rhantus manjakatompo*, lateral (**A**) and dorsal (**B**) view **C–D** *Rhantus bouvieri*, lateral (**C)** and dorsal (**D**) view **E–F** *Rhantus latus*, lateral (**E**) and dorsal (**F**) view.

(Cat. No. NHRS-JLKB000021025), Manjakatompo [Poste][lake with grass][MJK12-14], 19.3542S, 47.3081E, 1800m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja.

Madagascar (region indecisive): 3♂, 6♀ (Cat. No. NHRS-JLKB000030139, 30140, 30141, 30142, 30144, 30400, 30401, 30402, 30403), Madagascar, Leg. Catat, 2♀ (Cat. No. NHRS-JLKB000030404, 30143) Madagascar [Centre-Sud], 1901, Leg. Alluaud, 1♀ (Cat. No.: NHRS-JLKB000030138) Antananarivo [city or province, indecisive], Leg. Sikora, 1♀ (Cat. No. NHRS-JLKB000030287) Forêt Tanala [province indecisive], Leg. Alluaud

Diagnosis. Pronotum with two elongated rectangular dark spots, narrowly (or partly) separated in middle (Figure 2c). Male protarsal claws equally long, straight in lateral view apart from at base and apex (Figure 5c–d). Male mesotarsal claws curved in lateral view, equally thin, the anterior claw somewhat longer than the posterior (Figure 6c–d). Penis in dorsal view evenly tapering towards apex, in lateral view with a relatively sharp angle at the base (Figure 7c–d). Parameres with inner margin undulated (Figure 7h). 9.4–10.8 mm long.

Description. *Size*: ML, 9.4-10.8 mm ; MW, 5.2–5.7 mm; Lp, 1.0–1.4 mm; Wp, 3.8–4.1 mm; Le, 7.2–8.1 mm (n = 18).

Head. Testaceous with black areas inside and posterior of eyes. Interocular black pattern tapering towards the interior and narrowly separated (Figure 2c). Dense micropunctation and incomplete microreticulation.

Pronotum. Testaceous ferrugineous with two elongated rectangular black areas that are narrowly separated medially (Figure 2c). Lateral rim present, distinct. Dense micropunctation and incomplete microreticulation (Figure 3d).

Elytra. Testaceous with black irrorations, leaving paler sides and yellow sutural lines (Figure 2c). Somewhat confluent to form two small black areas subapically. Microreticulation double but somewhat indistinct. Very fine punctuation within and at edges of meshes (Figure 3e–f).

Ventral side. Dark brown to black, abdominal segments with testaceous spots along the lateral edges. Epiplura and legs, including metafemur, testaceous (Figure 4b).

Male. Protarsal claws equally long, medially straight in lateral view (Figure 5c-d). Mesotarsal claws curved in lateral view, equally thin, the anterior claw somewhat longer than the posterior (Figure 6c–d). Penis in dorsal view evenly tapering towards apex (Figure 7d). In lateral view not evenly curved but almost angulate at base (Figure 7c). Parameres with inner margin undulating (Figure 7h).

Remarks. In the original description, Régimbart refers to specimens from Fianarantsoa collected by Grandidier, but the holotype is not designated. The three specimens labelled “Fianarantsoa Grandidier” at MNHN are therefore considered to be syntypes and a male with genitalia extracted is designated here as the lectotype. Lectotype and paralectotypes were labelled as such.

Habitat. Known from a river in Andringitra and streams and grassy ponds in Manjakatompo, in both cases at altitudes between 1420 to 2070 m a.s.l.

Distribution. Endemic to Madagascar. Precise localities only known from Manjakatompo forestry station in the Ankaratra mountains and the Andringitra mountains (Figure 8). Forêt Tanala, Fianarantsoa and Tananarive are less precise region descriptions which includes Manjakatompo (Antananarivo) and Andingitra (Fianarantsoa, Forêt Tanala).

***Rhantus latus* (Fairmaire, 1869)**

http://species-id.net/wiki/Rhantus_latus
Figures 2d, 3g–i, 4c, 5e–f, 6e–f, 7e–f, i

Colymbetes latus Fairmaire, 1869: 191
    Type locality: Madagascar
Rhantus goudoti Sharp, 1882: 623. Synonymized by Branden (1885: 91)
    Type locality: Madagascar
Rhantus stenonychus Régimbart 1895: 179 **syn. n.**
    Type locality: Madagascar, lake Ambodinandohalo

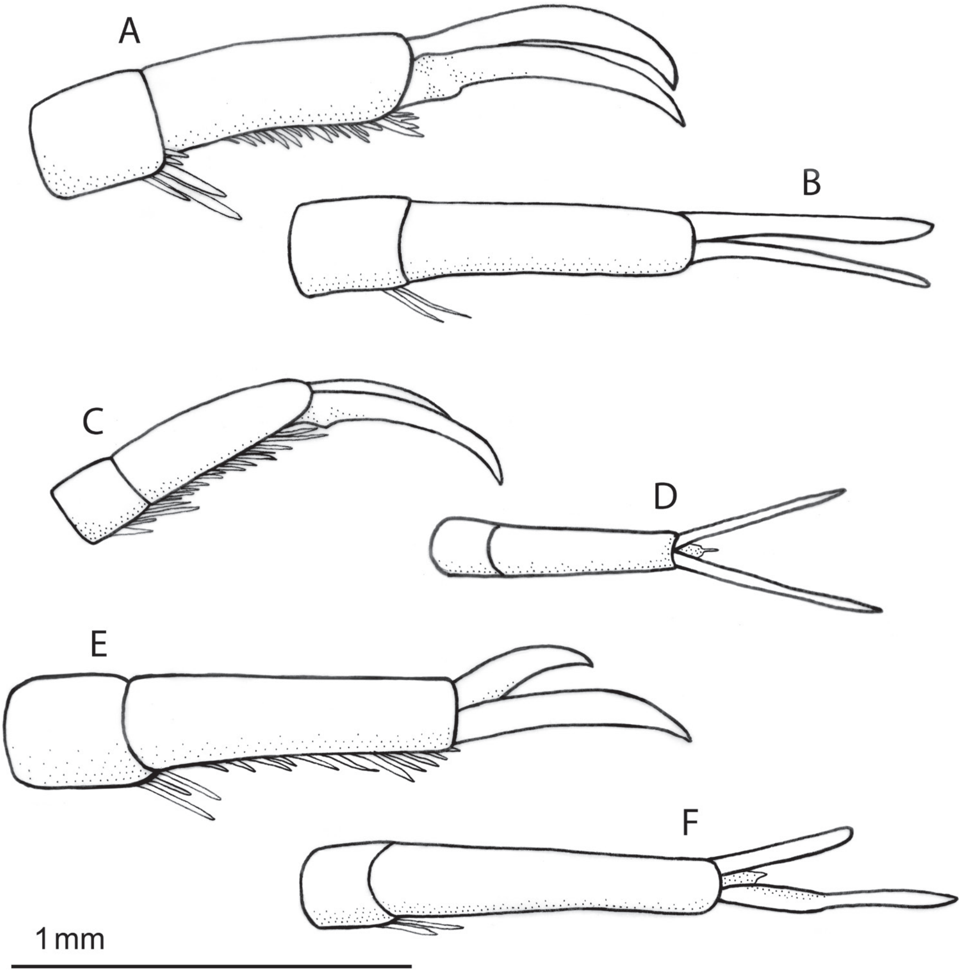

Figure 6. Left mesotarsal claws: **A–B** *Rhantus manjakatompo*, lateral (**A**) and dorsal (**B**) view **C–D** *Rhantus bouvieri*, lateral (**C**) and dorsal (**D**) view **E–F** *Rhantus latus*, lateral (**E**) and dorsal (**F**) view.

Rhantus latus (Fairmaire, 1869): Régimbart (1895: 183); Zimmermann (1920: 202); Guignot (1961: 764); Nilsson (2001: 51).

Type material studied. Typematerial of *Colymbetes latus* could not be localized in the Paris collections. We have no reasons to believe the type material is lost or destroyed, but to localize and identify the material that Fairmaire studied proved difficult as it was not found in Fairmaire's collection. The type material was collected by medicine doctor and entomologist Charles Coquerel who died in 1867.

Rhantus stenonychus: **Holotype** ♂ for *R. stenonychus* (MNHN): "Madagascar, Lac Ambodo, R.P. Camboue", "Data in NHRS-JLKB000030296".

Rhantus goudoti: **Lectotype** ♂ for *R. goudoti*, here designated to fix the concept of Rhantus goudoti Sharp and to ensure the universal and consistent interpretation of the same (BMNH): "905", "C. latus Fairm. seems very close to this, but is a little shorter in form, +paler" [handwritten note on the specimen], "Sharp Coll. 1905–313.", "Type", "Type 905 Goudoti Dej. Madagascar." "Data in NHRS-JLKB 000030415", our lectotype label. **Paralectotype** ♀ (BMNH) "Sharp Coll. 1905–313.", "Co-type", "Madagascar. 905" "Data in NHRS-JLKB 000030413", our paralectotype label. **Paralectotype** ♂ (BMNH) "37" "Co-type", "Madagascar 905", "Sharp Coll. 1905–313." "Data in NHRS-JLKB 000030414"our paralectotype label. 1♂ **Paralectotype** (MNHN): Data in NHRS-JLKB 000030297, Colymbetes goudotii mihi Madagascar, D. Sharp Monogr., Ex Musæo Dejean, our paralectotype label.

Additional material studied (for full details see Appendix).

Vakinankaratra region (former provinces: Antananarivo): 15spp. (Cat No. NHRS-JLKB000010277), Manjakatompo [2km NE Amparandraindrisa][pond and stream][MAD11-18], 19.3607S, 47.3009E, 1770m, 05.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 24 spp. (Cat. No. NHRS-JLKB000010278), Manjakatompo [500m E Lac Froid by the road][pond and inlet stream][MAD11-16], 19.3449S, 47.3338E, 1620m, 04.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 12 spp. (Cat. No. NHRS-JLKB000010274), Manjakatompo [Analafandriana, 500m S fish farm by the road][grassy pond][MAD11-14], 19.3619S, 47.3150E, 1730m, 03.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 1sp. (Cat. No. NHRS-JLKB000010269), Manjakatompo [Analamitana, by bridge close to SKOL facility][stream and stagnant pool][MAD11-19], 19.3646S, 47.2989E, 1750m, 05.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 2sp. (NHRS-JLKB000010270), Manjakatompo [Analafandriana close to fish farm][stream and wet field][MAD11-13], 19.3581S, 47.3140E, 1730m, 03.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 1sp. (Cat. No. NHRS-JLKB000021026), Manjakatompo [Tavolatara][pool near stream][MJK12-09], 19.3491S, 47.2780E, 2050m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 2spp. (Cat. No. NHRS-JLKB000021027), Manjakatompo [Analamitana] [swamp near stream][MJK12-02], 19.3640S, 47.2991E, 1760m, 22.i.2012, Leg. Ranarilalatiana, Randriamihaja, 2spp. (Cat. No. NHRS-JLKB000021028), Manjakatompo [Tavolatara][swamp near source][MJK12-10], 19.3496S, 47.2779E, 2050m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 3spp. (cat. No. NHRS-JLKB000021029), Manjakatompo [Anosiarivo][lake near source][MJK12-13], 19.3449S, 47.3041E, 2070m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 2spp. (Cat. No. NHRS-JLKB000021030), Manjakatompo [Poste][lake with grass][MJK12-14], 19.3542S, 47.3081E, 1800m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 5sp. (Cat. No. NHRS-JLKB000021031), Manjakatompo [Andohariana][small lake][MJK12-07], 19.3677S, 47.3143E, 1710m, 24.i.2012, Leg. Ranarilalatiana, Randriamihaja, 10spp. (Cat. No. NHRS-JLKB000021032), Manjakatompo [near camping][big temp. lake][MJK12-15], 19.3630S, 47.3171E, 1710m, 25.i.2012, Leg. Ranarilalatiana, Randriamihaja.

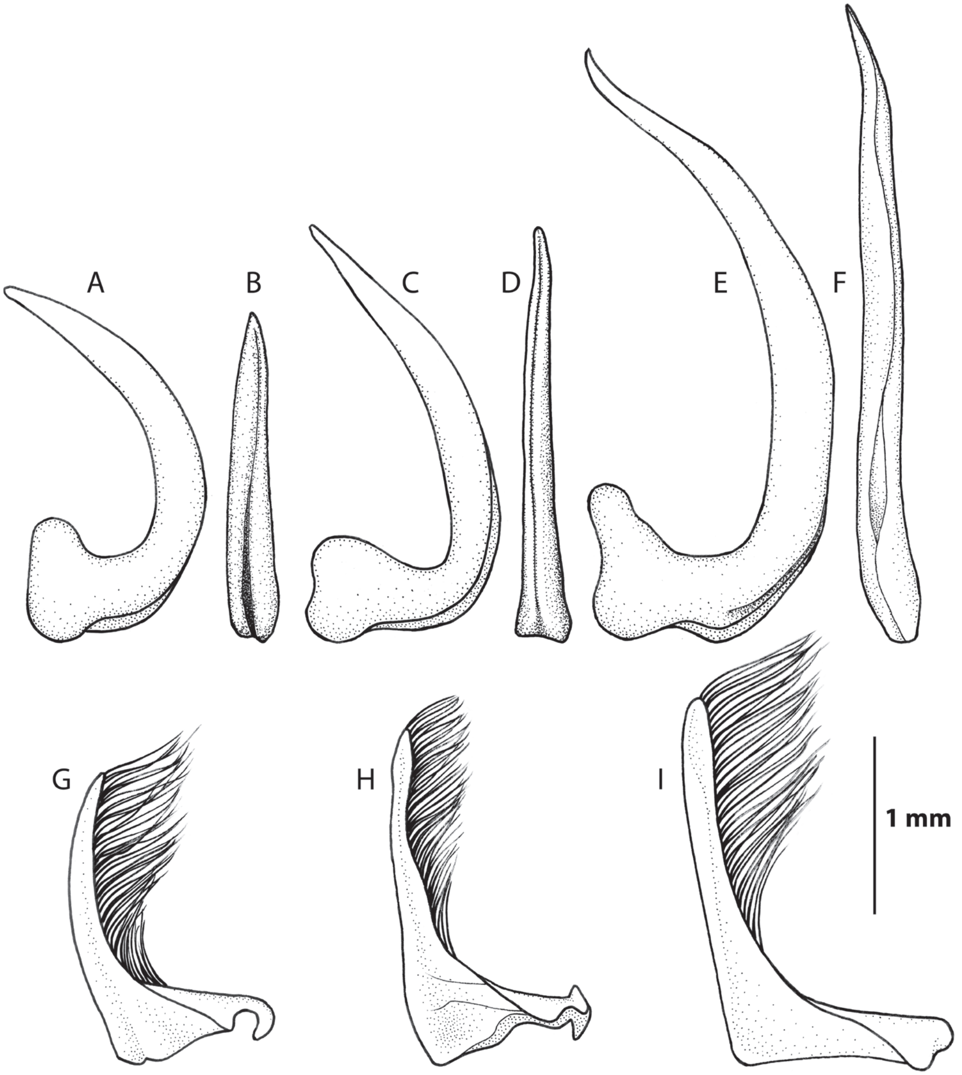

Figure 7. Male genitals, penis in lateral and dorsal view: **A–B** *Rhantus manjakatompo* **C–D** *Rhantus bouvieri* **E–F** *Rhantus latus*. Parameres in lateral view **G** *Rhantus manjakatompo* **H** *Rhantus bouvieri* **I** *Rhantus latus*.

Alaotra Mangoro region (former provinces: Toamasina): 1♂ (Cat. No. NHRS-JLKB000030407), Mantadia, NP, Rianila Basin, [affluent non nommé riv.], [PO670], 18.935S, 48.4167E, 920m, 29.xi.1996, Leg. Legrand, Randriamasimanana, 1sp. (Cat. No. NHRS-JLKB000010268), Mantadia NP [3km from park entrance][open pond with vegetation][MAD11-42], 18.8526S, 48.4272E, 920m, 13.xi.2011, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Bukontaite, 1♀ (Cat. No. BMNH-829991), Andasibe NP [entry to park, Anamalozaotra river and pond][P61Bi01], 18.9348S, 48.4175E, 950m, 04.i.2007, Leg. Isambert et al., 2♂ (Cat. No. NHRS-JLKB000030295), Antsianaka, 1892, Leg. Perrot, Perrot, 1♂ (Cat. No. NHRS-JLKB000030289), Ambatosoratra, env. Tananarive, vii.1934, Leg. Olsoufieff.

Matsiatra Ambony (Haute Matsiatra) region (former provinces: Fianarantsoa): 1sp. (Cat. No. BMNH-742359), Sendrisoa, S of Ambalavao, [P38], 22.0098S, 46.9504E, 1160m, 07.v.2006, Leg. Bergsten et al., 1sp. (Cat. No. BMNH-742090), Andringitra NP [Zomandao river, by bridge on road to camp Belamba], 22.1043S, 46.9207E, 1420m, 09.v.2006, Leg. Bergsten et al., 1♂ (Cat. No. BMNH-829990), Ambalavao, 15km SW of, RN7.

Amoron'i Mania region (former provinces: Fianarantsoa): 6spp. (Cat. No. BMNH-729860, 729861, 729862, 729863, 729864, 829992), Col des Tapia, 48 km N Ambositra, RN7, [P30MD33], 20.2388S, 47.1002E, 1440m, 08.xii.2004, Leg. Balke et al., 1♂ (Cat. No. BMNH-741961), Ambositra, 34km S of, RN7, 20.7719S, 47.1810E, 1690m, 06.v.2006, Leg. Bergsten et al.

Melaky region (Former provinces: Mahajanga): 1♂, 1♀ (Cat. No. NHRS-JLKB000000089, 000000090), Ambohijanahary NP [MAD09-76], 18.2685S, 45.4635E, 910m, 19.xii.2009, Leg. Bergsten, Ranarilalatiana, Randriamihaja, Jönsson

Atsimo Andrefana region (former provinces: Toliara): 3spp. (Cat. No. BMNH-742641, 742639, 742640), Zombitse-Vohibasia NP [Isoky, Ranomena, muddy pool in river basin], 22.6401S, 44.8644E, 580m, 15.v.2006, Leg. Bergsten et al., 1sp. (Cat. No. BMNH-741979), Zombitse-Vohibasia NP [edge of, Ambiamena, stagnant zebu-visited marshland, muddy & vegetation], 22.8601S, 44.6173E, 530m, 14.v.2006, Leg. Bergsten et al.

Ihorombe region (former provinces: Fianarantsoa): 1sp. (cat. No. BMNH-741810), Isalo NP [Menamaty river][river with vegetation][P41AM01], 22.5500S, 45.4012E, 760m, 11.v.2006, Leg. Bergsten et al.

Analamanga region (former provinces: Antananarivo): 1♂ (Cat. No. NHRS-JLKB000030282), Antananarivo [city or province, indecisive], 31.v.1947, Leg. Clement, 1♂ (Catl. No. NHRS-JLKB000030288), Lac Tsimbazaza, Antananarivo, 18.9333S, 47.5333E, 1410m, vii.1934, Leg. Vadon, 1♂ (Cat. No. NHRS-JLKB000030284), Andrang, Leg. Sikora, 1♂ (Cat. No. NHRS-JLKB000030285), Ambohibeloma, Leg. Sikora.

Madagascar (region indecisive): 2♂, 1♀ (Cat. No. NHRS-JLKB000030406, 30408, 30409), Madagascar, vii.1968 – ix.1968, Leg. Starmühlner, 1sp. (Cat. No. BMNH-729851), Madagascar, 1♂ (Cat. No. NHRS-JLKB000030286), Madagascar [Centre-Sud], Leg. Alluaud.

Diagnosis. Pronotum somewhat infuscated and with one wide black spot, not divided medially (Figure 2d). Male protarsal claws long and slender, almost twice the length of last tarsal segment, somewhat sinuated and unequal, anterior claw longer (Figure 5e–f). Male mesotarsal claws very unequal, the anterior almost twice the length, and very broadened, compared to posterior (Figure 6e–f). Penis long and slender, apically upturning in lateral view, in dorsal view with the apex curved to the left and sharply pointed (Figure 7e–f). Parameres with dorsal edge straight (Figure 7i). 11.6–13.0 mm long.

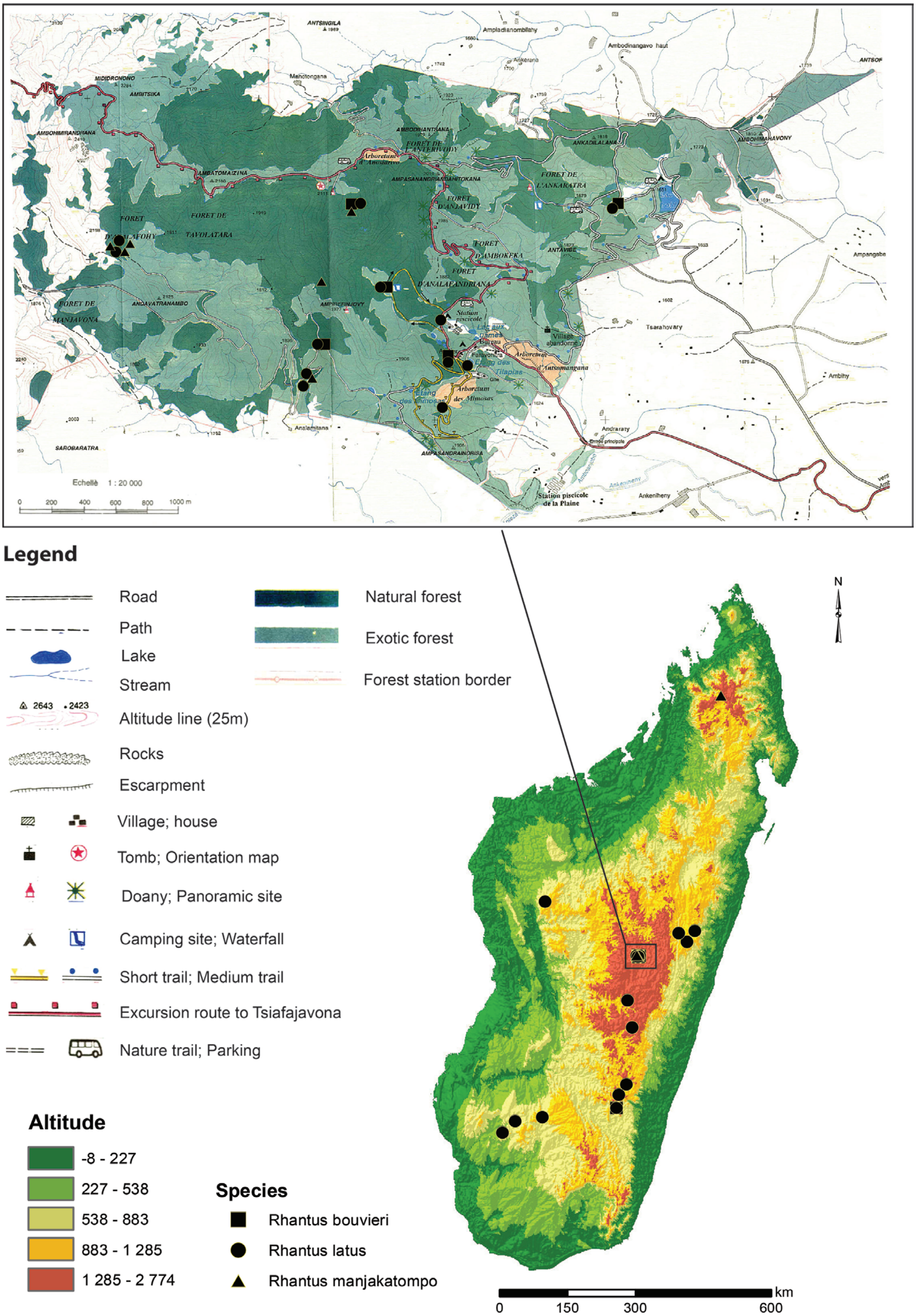

Figure 8. Distribution and all known records of the three *Rhantus* species of Madagascar with a special emphasis on Manjakatompo. Inset map of Manjakatompo adapted from FTM (1995) Extent of natural forest left may have changed since the map was made.

Description. *Size*: ML, 11.6–13.0 mm; MW, 6.2–7.0 mm; Lp,1.2–1.6 mm; Wp, 4.7–5.3 mm; Le, 9.3–10.1 mm (n = 12).

Head. Testaceous with black areas inside and posterior of eyes. Interocular black pattern often rather broadly separated medially (Figure 2d). Dense micropunctuation but no reticulation.

Pronotum. Somewhat infuscated and with one wide dark spot which is not medially divided (Figure 2d). No rim at lateral margin. Micropunctation and vague microreticulation (Figure 3g).

Elytra. Testaceous ferrugineous with black irrorations, leaving paler sides and yellow sutural lines (Figure 2d). Black irrorations regular throughout, even posteriorly with no sign of preapical black areas. Microreticulation double, meshes are well impressed posteriorly but vague anteriorly. Micropunctation within and at edges of meshes (Figure 3h–i).

Ventral side. Dark brown to black. Epiplura testaceous. Metafemur infuscated (Figure 4c).

Male. Protarsal claws long and slender, almost twice the length of last tarsal segment, somewhat sinuated and unequal, anterior claw longer (Figure 5e–f). Mesotarsal claws curved and unequal, the anterior claw is almost twice as long as the posterior and much broader (Figure 6e–f). Penis long and slender, apically upturning in lateral view, in dorsal view with the apex curved to the left and sharply pointed (Figure 7e–f). Parameres with dorsal edge straight (Figure 7i).

Remarks. The interpretation of Fairmaire's name *Colymbetes latus* is unambiguous following common usage (e.g. Régimbart 1895, Guignot 1961), even though typematerial with Coquerel as collector could not be found in Fairmaire's collection in Paris, or elsewhere. Neither Guignot (1961) nor Balke (1992b) were able to localize the holotype of *R. stenonychus* and the validity of the name, based on a single specimen, has therefore not been evaluated before but the name lingered in the literature. The details for the type in Régimbart's original description are as follows: "Madagascar: Lac Ambodinandohalo (R. P. Camboué), un seul male (coll. R. Oberthür)". In Régimbart's collection at MNHN there was a pin without a specimen but with the handwritten labels by Régimbart "stenonychus Rég." and "Madag. Coll. Oberthür". We interpret the pin with the label as a reference to the Oberthür collection for the unique type. In Oberthür's collection a single male specimen was found bearing the label "Lac Ambodo [or Ambod°], R.P. Camboue". We believe "Lac Ambodo [or Ambod°]," is an abbreviation for Lac Ambodinandohalo and that this is the holotype. We are not able to find any records of another lake named "Ambodo" in Madagascar. Lake Ambodinandohalo was a lake in the haute-ville in Antananarivo and the French Jesuit priest and missionary Paul Camboué lived just west of Antananarivo in Arivonimamo and Ambohibeloma. *Peltodytes quadratus* Régimbart, 1895 was described in the same article from the same locality (Lac Ambodinandohalo, collected by R. P. Camboué) and the type specimen in Paris bore a label with the same abbreviated locality name (see Vondel 2010; Vondel and Bergsten 2012).

The holotype is as judged by morphological characters conspecific with *R. latus*. Already in the original description similarities with *R. latus* are obvious (also see Guignot 1961) and it is odd that Régimbart did not compare the species with *R. latus* in his original description. We therefore synonymize the name *R. stenonychus* with *R. latus.*

Habitat. Occurs in a quite wide range of habitats, like streams and rivers, muddy waterpools and grassy ponds. Of the three *Rhantus* species it is the only one that can be found below 1000 m altitude and known range include 530 to 2070 m.

Distribution. Possibly endemic to Madagascar as we have not been able to verify the records from mainland Africa by Régimbart (1895) "Cafrerie, Cap" and by Guignot (1961) "Sud d'Afrique". In Madagascar rather widespread over the central high plateau (Figure 8). Known from Andasibe-Mantadia NP, Antananarivo and Ambohijanahary NP, which are the northernmost records. Further known from several localities south of Antananarivo along RN7 from Manjakatompo forestry station to Andringitra NP, and further southwest to Isalo NP and Zombitse-Vohibasia NP. Seemingly lacking from the northern third of Madagascar.

Identification key on habitus of females and males

1a Smaller (ML 9.4–10.8 mm), legs including metafemur mostly yellow (Figure 4b), pronotum yellow with two elongated rectangular black fields narrowly divided in middle (Figure 2c) .... ***R. bouvieri***

1b Larger (ML: 11.5–13.0 mm), legs mostly infuscated especially metafemur (Figure 4a, c), pronotum yellow or infuscated with or without black markings which, if present, are either not medially divided or are not elongated but small dots (Figure 2a–b, d). **2**

2a Pronotum entirely yellow without black markings (Figure 2a) or with two small black dots, medially divided (Figure 2b). Elytral black irrorations somewhat confluent subapically to form small dark fields (Figure 2a–b) .... ***R. manjakatompo***

2b Pronotum somewhat infuscated and with a single medial elongated rectangular black field not medially divided (Figure 2d). Black elytral irrorations even throughout, not forming denser black areas subapically (Figure 2d) .... ***R. latus***

Identification key for males

1a Male mesotarsal claws very unequal in length, anterior claw broad and almost twice as long as posterior claw (Figure 6e–f). Male protarsal claws very long, slender and sinuate, almost twice the length of last protarsal segment (Figure 5e–f). Penis long and slender, apically twisted (Figure 7e–f) .... ***R. latus***

1b Male mesotarsal claws equal or somewhat unequal, anterior claw thin (Figure 6a–d). Male protarsal claws shorter and not sinuated (Figure 5a–d). Penis shorter and not apically twisted (Figure 7a–d) **2**

2a Smaller (ML 9.4–10.8 mm), Male mesotarsal claws subequal, posterior claw somewhat shorter but equally thin as anterior claw (Figure 6c–d). Pronotum with two wide rectangular dark spots (Figure 2c). Penis in lateral view not evenly curved but almost angulate at base (Figure 7c). Parameres with inner margin undulating (Figure 7h) ....***R. bouvieri***

2b Larger (ML: 11.5–12.5 mm), Male mesotarsal claws equally long but posterior claw distinctly thicker than anterior claw (Figure 6a–b). Pronotum with two small dark spots (Figure 2b), or spots are absent (Figure 2a). Penis in lateral view short robust and evenly curved (Figure 7a). Parameres evenly curved and tapering to apex (Figure 7g)..........................***R. manjakatompo***

Discussion

On Madagascar, *Rhantus* is a genus of the highland plateau. Like the *Rhantus* diversity in southeast Asia, Indonesia and Melanesia (Balke 2001), the genus is lacking from the lowland tropics in Madagascar. The central highland plateau of Madagascar (about 40% of the island) however, is almost completely degraded and very little of the original forests remains. Manjakatompo forestry station is one of three small forest relics remaining of the high plateau together with Ambohitantely Special Reserve and the Anjozorobe Forest. In November of 2011 and January 2012 we carried out fieldwork in Manjakatompo forestry station, investigating the aquatic beetle fauna. Although we have sampled aquatic beetles across Madagascar at hundreds of localities, nowhere else was the *Rhantus* fauna richer both in individuals and species than in Manjakatompo. All three endemic species of Madagascar existed here in healthy populations, two of which are only known from one other locality outside of Manjakatompo. Manjakatompo is clearly a small and fragile but important forest refugium which *Rhantus* and other highland fauna may depend on.

Manjakatompo is located in the province of Antananarivo, region Vakinakaratra and district of Ambatolampy, at 17 km to the west of the city Ambatolampy (19°22'S, 47°16'E). It lies on the eastern slope of the Ankaratra mountain massif of Quaternary volcanic origin. The altitude ranges between 1550 and 2643 m with the highest peak, Tsiafajavona, being the third highest on Madagascar. Forests are humid and the climate follows a pattern of cold and dry austral winter and a warm and wet austral summer (annual rainfall around 2000mm) (Vences et al. 2002). The average temperature of the coldest month is 5–10°C, but can drop below zero at higher altitudes. The station covers an area of 8320 ha, with only 650 ha of natural forest and 2300 ha replanted with exotic trees (Goodman et al. 1996). Even the natural forest portion is composed of largely secondary forest mixed with exotic trees. Manjakatompo forestry station was established in 1923 to preserve the relic of primary forests that remained at the time (Andriampenitra 2007).

The forest relics have been kept partly thanks to legal protection more or less effectively exerted by the agents of the Forestry Station, with its status as Integral Reserve

(Andriampenitra 2007). However, no part of the Ankaratra massif is part of the protected area network of reserves and national parks with a higher level of protection. According to our personal observation, and local information, the Manjakatompo forest is constantly exposed to serious incidents such as commercial operation, fire and slash and burn agriculture, so called "tavy". In 2008, about 70 ha of the forest surface was burnt (Martin 2008). Inventories of the herpetofauna in Manjakatompo also found a specialised montane fauna with some 10–15% being endemics of the Ankaratra massif (Vences et al. 2002). Vences et al. (2002) were concerned that changes like increased use of pesticide, increased cattle (zebu) grazing, or aquaculture and release of fish, could cause a serious threat to the montane fauna. All these three factors are also known causes by which the aquatic insect fauna can drastically change, leading to the replacement of endemics by widespread opportunists. Kremen et al. (2008) used distribution data of various organism groups to model and optimize where additional protected areas on the island would come best to use for conserving additional components of the endemic fauna and flora not already under protection. The Ankaratra massif was part of the proposed new areas. While the flora and fauna show similarities to the Andringitra massif, which we can confirm based on the *Rhantus* fauna, it also has unique components and characteristics (Vences et al. 2002, Goodman et al. 1996), and remains a highly prioritized area for increased protection.

Acknowledgements

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Appendix

Supplementary table include detailed information of all examined specimens. (doi: 10.3897/zookeys.350.6127.app) File format: Microsoft Excel file (xls).

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Four new species of *Oidardis* Hermann, 1912 (Diptera, Asilidae, Laphriinae, Atomosiini) from two major faunistic surveys in the Atlantic Rainforest

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Abstract

Two recent faunistic surveys in the Brazilian Atlantic Forests region, the PROFAUPAR and the Biota/FAPESP Program, have provided important material for the discovery of new taxa from Brazil. We describe herein four new species of robber-flies of the genus *Oidardis* (*O. falcimystax* **sp. n.**, *O. fontenellei* **sp. n.**, *O. maculiseta* **sp. n.** and *O. marinonii* **sp. n.**), including illustrations and details on male hypopygia and female genitalia. A distribution map and a key to the species of *Oidardis* from the Brazilian Atlantic Forests region, including *O. triangularis* (Hermann), 1912, are also provided.

Keywords

Robber-flies, taxonomy, Brazil, Neotropical

Introduction

The Atomosiini are small compact assassin flies, whose length can range from 4–12 mm. Although they seem to be the dominant group of Asilidae in Neotropical forested areas, they are easily overlooked in the field due to their small size and usual preference for shadowy environments (Fisher 1985, 2009).

Oidardis Hermann is characterized by a smoothly curved lateral eye margin, scutum densely covered by short erect setulae and the absence of spines on the scutellum (Artigas et al. 1991, Fisher 2009). Two groups of species are clearly noted, based on the degree of sexual dimorphism. Males of the highly dimorphic species bear a rather long modified seta on the hind tibiae, related to the elaborate courtship behaviour they display. Species of the less dimorphic group display a much plainer sexual behaviour (Fisher 1985, 2009, Fisher and Hespenheide 1992), and the males lack the long setae on the posterior tibiae.

Oidardis comprised seven species prior to this study, distributed from Costa Rica to Brazil. They are almost exclusively found perching on twig tips in shaded understory. Five of them occur in the dense forests in the Amazon (*O. aenescens*, *O. aveledoi*, *O.curupaoensis* and *O. gibbosa*) and Central America (*O. gibba*). For the Atlantic Forest, and Brazil as a whole, there were only two species recorded in previous works, *O. triangularis* (Hermann), 1912 and *O. nigra* (Hull), 1962 (Hull 1962, Artigas et al. 1991, Fisher 2009).

The Atlantic Rainforest is one of the world's highest diversity biomes, with a large number of endemic species; yet, it is also one of the most devastated biomes due to human occupation and development. Undisturbed habitat occupies less than 7% of the original area, and it is recognized as one of the world's hotspots for conservation (Da Fonseca 1985, Morellato and Haddad 2000, Myers et al. 2000).

In this scenario of high diversity and intense destruction, providing more knowledge on this biome is a matter of great urgency. Two major efforts seeking a wider consciousness about the insect diversity of the Brazilian Atlantic Rainforest should be noted: the projects BIOTA/FAPESP and PROFAUPAR.

The BIOTA program, funded by the São Paulo State Research Foundation (FAPESP), was primarily aimed at surveying and characterizing São Paulo state biodiversity, and guiding actions for its conservation. Since the Atlantic Forest is the most representative biome in São Paulo, it has attracted most of the effort of this survey. Some projects within the program also included expeditions to Atlantic Forest areas outside São Paulo state. Over 500 researchers were included in more than 70 projects in BIOTA/FAPESP program (Staley 2001, Metzger and Casatti 2006).

Another remarkable effort at increasing knowledge of Brazilian biodiversity was the Survey of the Entomological Fauna of Paraná State, PROFAUPAR, initiated by Dr. Renato Contin Marinoni. It was conducted from 1986 to 1988 and focused on Paraná state biodiversity in the different ecosystems that occur in its area (Marinoni and Dutra 1991).

Presented here are the illustrated descriptions, with details on male terminalia and female genitalia, of four new species of *Oidardis* from the Atlantic Forest, collected under

BIOTA/FAPESP and PROFAUPAR faunistic surveys. These descriptions represent three species of the highly dimorphic group in the genus (*O. falcimystax* sp. n., *O. fontenellei* sp. n., and *O. maculiseta* sp. n.), and one of the less dimorphic group (*O. marinonii* sp. n.). A distribution map and a key to identification of all species of *Oidardis* occurring in this biome are also provided. The following results are part of a wider ongoing research on this genus. Fauna occurring in other biomes will be included in future publications.

Material and methods

Specimens were examined with a ZEISS Stemi SV6 Stereomicroscope. Terminalia of selected paratypes were dissected and cleared in KOH at 25°C for 24 hours; dehydrated under an alcoholic series, in increasing concentration (30–95%); examined in temporary slides with glycerine; drawn under microscope with aid of a ZEISS Axioskope 40 *camera lucida*; and stored in a plastic microtube pinned with the specimen. Descriptions of the holotypes include a discussion of intraspecific variation; descriptions were generated with the software package “CSIRO DELTA (Description Language for Taxonomy) for Windows” v. 1.04 (Dallwitz 1980, Dallwitz et al. 1999). Mantis v. 2.0.1 (Naskrecki 2008) was used as a database, primary generator for material examined lists, and exporting locality data to Google Earth v. 6.1.0.5001. Distribution maps were prepared in Quantum GIS v. 1.7.0 Wroclaw, from locality data imported from Google Earth; ecorregion shapefiles for the maps follow Olson et al. (2001) and were obtained from World Wildlife Fund (WWF) website (World Wildlife Fund 2012).

The identification key includes the four new species of *Oidardis*, plus *O. triangularis. Oidardis nigra,* thought probably to be a junior synonym of *O. triangularis*, remains *inquirenda* since the type-material is lost; *O. nigra* is not included in the dichotomous key to species of *Oidardis.*

Photographs were taken under ZEISS Discovery V20 Stereomicroscope with a ZEISS AxioCam Mrc5 camera attached, connected to a desktop computer through ZEISS AxioVs40 v. 4.8.2.0 software. Image stacks were assembled in Combine ZP software (Hadley 2010). Adobe Photoshop was used for editing images. Line drawings and plates were prepared in the Adobe Illustrator software.

Terminology for general morphology follows Cumming and Wood (2009); Stuckenberg (1999) for antennal structures; and Theodor (1976) for characters of male terminalia not mentioned in Cumming and Wood (2009). Tergites are often referred to as “T” followed by the segment number (e.g. T3 for the tergite of the third abdominal segment).

Depository for the specimens is noted within parentheses under the material examined section, according to acronyms listed below:

BMNH	British Museum (Natural History), London
CNC	Canada National Collection of Insects, Arachnids and Nematodes, Ottawa
DZUP	Coleção Entomológica Padre Jesus Santiago Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba

EFISHER	Eric Fisher's private collection, El Dorado Hills
LEEID	Laboratório de Ecologia Evolutiva de Insetos de Dossel, Universidade Federal de Ouro Preto, Ouro Preto
MNRJ	Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro
MZUEFS	Museu de Zoologia, Universidade Estadual de Feira de Santana, Feira de Santana
MZUSP	Museu de Zoologia, Universidade de São Paulo, Sao Paulo
ZSM	Zoologische Staatssammlung München, Munich

Results

Identification key to the Atlantic Forest species of *Oidardis* Hermann (except for *O. nigra*, which is unrecognized)

1 Gibbosity extending through lower half of face or beyond (Fig. 5A); abdomen cup-shaped; legs yellow or light-brown. Males with modified mystax (pair of regular setae dorsally, pair of dark-brown laterally-flattened setae medially, and pair of white, sinuous, filiform setae ventrally) (Fig. 5A); modified tibial setae dark-brown, shorter than femur, with dark-brown leaf-shaped, longitudinally-striated lamella on apical 1/5, inserted on middle of hind tibiae (Fig. 2A) [Peru, Bolivia, Brazil (Goiás, Mato Grosso, São Paulo and Paraná) and Argentina] .... ***O. falcimystax* sp. n.**

– Gibbosity extending through lower third of face at most (Figs 5B–D). Males with only regular mystacal setae (Figs 5B–D). Other combination of characters **2**

2(1) Body yellow and black or light-brown and black (Figs 1C–D, G–H); anterior region of scutum, pleura and lateral margins of tergites yellow.................... **3**

– Body entirely black or dark-brown (Figs 1A–B, E–F); anterior region of scutum, pleura and lateral margins of tergites dark-brown **4**

3(2) Scutum vestiture homogeneously directed (all setulae reclinate, including posterior region of scutum); antenna usually entirely dark-brown; anterior and mid femora brown. Male with hind femur yellow; hind tibia entirely dark-brown; with modified tibial seta on posterior leg (short light-brown seta, with slightly dilated apex white) (Fig. 2B) [Brazil (Sergipe, Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, and Paraná)]... ***O. fontenellei* sp. n.**

– Scutum vestiture heterogeneously directed (setulae on posterior region of scutum proclinate); antenna with yellow or light-brown scape and pedicel; anterior and mid femora yellow. Male with hind femur dark-brown; hind tibia yellow dorsally and dark-brown ventrally; without modified tibial seta on hind leg (Fig. 2D) [Brazil (São Paulo and Paraná)] ... ***O. marinonii* sp. n.**

4(2) Legs predominantly yellow, femora dorsally and tibiae ventrally dark-brown (Fig. 2C); face with golden pollinosity (Fig. 4C). Male with modified tibial seta dark-brown, as long as femur, golf-club-shaped, with apical 1/4 as a large white lamella with black spot at apex (Fig. 2C); mystax short (Fig. 5C); mid prong of the phallus much longer than lateral prongs (Fig. 7C). [Brazil (Goiás, São Paulo and Paraná)]......................................***O. maculiseta* sp. n.**

– Legs predominantly dark-brown, anterior and mid tibiae at most slightly lighter dorsally; face usually copper-pollinose, with gibbosity silver-pollinose (rarely entirely golden-pollinose). Male without modified tibial seta; mystax as long as, or longer than, proboscis; phallus with equal-sized prongs; gonocoxite with three spines in characteristic fork-like pattern on apex [Brazil (Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina)] ....***O. triangularis* (Hermann)**

Descriptions

Oidardis falcimystax sp. n.

http://zoobank.org/7659EB6E-08DA-4831-91BF-73C934C6E774
http://species-id.net/wiki/Oidardis_falcimystax
Figures 1A–B, 2A, 3A, 4A, 5A, 6A–C, 7A, 8C, 10

Diagnosis. Gibbosity extending through lower half of face or beyond; abdomen cup-shaped; legs yellow or light-brown. Males with modified mystax (pair of regular setae dorsally, pair of dark-brown laterally-flattened setae and pair of white sinuous filiform setae ventrally); modified tibial setae dark-brown, shorter than femur, with dark-brown leaf-shaped, longitudinally-striated lamella on apical 1/5, inserted on middle of hind tibiae.

Description. Holotype. Male. Body shiny black. Total length, excluding antennae, 5 mm; length of thorax, 1.2 mm; length of wing, 4.4 mm; greatest width of abdomen, 1 mm.

Head, laterally. Face, between antennal insertion and gibbosity, plane with eye margin; gibbosity prominent, equals ventral 0.7 of face height; dorsal occipital setae dark-brown, lateral occipital setae white, ventral occipital setae white; proboscis 0.53 × the height of head, with a pair of yellow macrosetae ventrally; palpus dark-brown, with yellow setae apically. **Antenna**. Antenna 0.74 × as long as the height of eye, entirely dark-brown, with dark-brown setae and macrosetae; antennal insertion at dorsal 0.2 of head height; scape slightly longer than pedicel, with medium-sized ventral seta, numerous short setae ventrally and around the whole segment apically; pedicel oval; postpedicel oblong, 1.7 × length of basal two segments, brown-pollinose, except for silvery-yellow pollinosity on elliptical sensorial area on inner face, with dorsal spine subapical (3/4 length of postpedicel or beyond). **Head, anteriorly**. Head 1.39 × as wide as high; face 0.14 × as wide as head, silvery-pollinose, except on shiny upper half of gibbosity; mystax long (extending beyond the apex of proboscis), comprised of 6 macrosetae – dorsal pair regular and dark-brown, middle pair spatulate and dark-brown, and ventral pair sinuous-filiform and white; facial setae, other than mystax, pale-yellow; frons golden-pollinose; orbital setae dark-brown; vertex golden-pollinose;

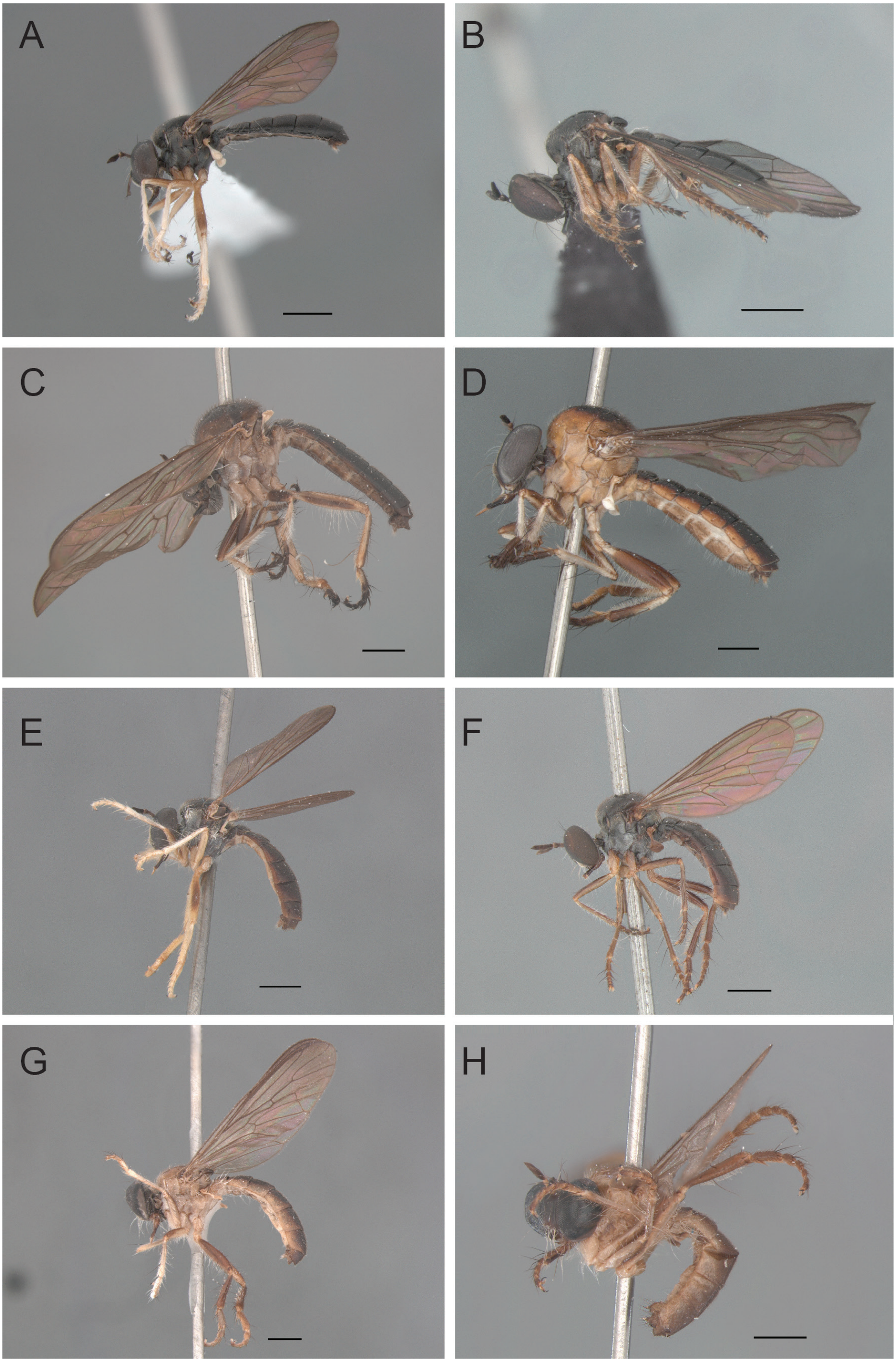

Figure 1. Habitus, lateral view: **A** *O. falcimystax* male (HT) **B** female (PT) **C** *O. fontenellei* male (HT) **D** female (PT) **E** *O. maculiseta* male (HT) **F** female (PT) **G** *O. marinonii* male (HT) **H** female (PT). Scale bar = 1 mm.

ocellar tubercle golden-pollinose, as high as vertex, 0.29 × as wide as frons, anterior ocellus 0.11 × as wide as frons by the ocellus position.

Thorax. Postpronotal lobe dark-brown; scutum shiny black, not punctate, vestiture golden, equal-sized, longest setae as long as half the scape, reclinate anteriorly and proclinate posteriorly; one dark-brown notopleural; scutellum black, scutellar margin strongly impressed, marginal scutellar macrosetae dark-brown, equal-sized, longest ones much shorter than scutellum (as long as the width of the rim); postalar callosity dark-brown, partly with bright-blue reflections; pleuron shiny dark-brown, with silvery-white pollinosity; setulae on proepisternum, katepisternum and anepisternum yellow; one anepisternal macroseta, plus fine setulae, yellow; tuft of katatergal macrosetae light-brown; anatergite with golden, hair-like setae.

Legs. Coxae orange-yellow; trochanter orange-yellow, with fine yellow setulae; femora yellow, slightly darkened dorsally—except hind femur, only darkened distally, covered with short stout yellow setulae dorsally, with dark setae on apical 1/3 dorsally, hind femur with 4 long yellow ventral macrosetae in a row along proximal half; tibiae entirely yellow, with yellow setulae, long yellow macrosetae and thick spines; hind tibia entirely covered by golden setulae, with white setulae ventrally, long dark-brown macrosetae ventrally and long dark-brown macrosetae anterodorsally; modified tibial setae attached to hind tibia at middle, dark-brown, shorter than femur, with dark-brown leaf-shaped longitudinally-striated lamella on apical 1/5; tarsi yellow, 5th tarsomere dark-brown, with stout yellow setae dorsally, and densely covered with thick spine-like golden setae, 5th tarsomere with 3 setae apically, opposite the claws and longer than them; claws yellow on base and black apically; pulvilli yellow and fringed; empodium shorter than claws.

Wing. Brownish, darker along upper margin; cell r_1 with short slightly-concave stalk (2 × the length of r-m); crossvein r-m at proximal half of cell d, aligned to the end of Sc; cell m_3 narrowing distally (M_2 and M_3 converging by the end of cell m_3), with stalk slightly longer than r-m, apex of m_3 and apex of cell d parallel and unaligned, apex of m_3 beyond apex of d; crossvein bm-cu long, base of M_3 and CuA_1 distant from each other and not appearing as an "X"; cell cu*p* with stalk shorter than r-m; posterior margin of wing slightly convex at distal half; calypters orange, with light-brown margin and fringe of short brown setae; halter with orange stem, white knob.

Abdomen. Black, punctate, with sides diverging posteriorly, T2 1.9 × wider than long; vestiture longer and lighter laterally and ventrally, several white macrosetae present on lateral margin of T1 and T2. **Male terminalia**. Hypopygium barely conspicuous; hypandrium regular-sized (2/3 the width of hypopygium or more), much wider than long, anterior margin straight to slightly convex, posterior margin sharply pointed; gonocoxites free, gonocoxal prolongation thin, smoothly curved inwards, with 2 spines at apex; gonostylus reduced, round, laterally flattened, free, attached to the base of gonocoxite; apex of phallus with three equal-sized prongs; epandrium straight in lateral view; lobes of hypoproct short.

Female. Total length, excluding antennae, 5.3–6.2 mm, (n= 5); length of thorax, 1.4–1.7 mm, (n= 5); length of wing, 4.6–5.4 mm, (n= 5); greatest width of

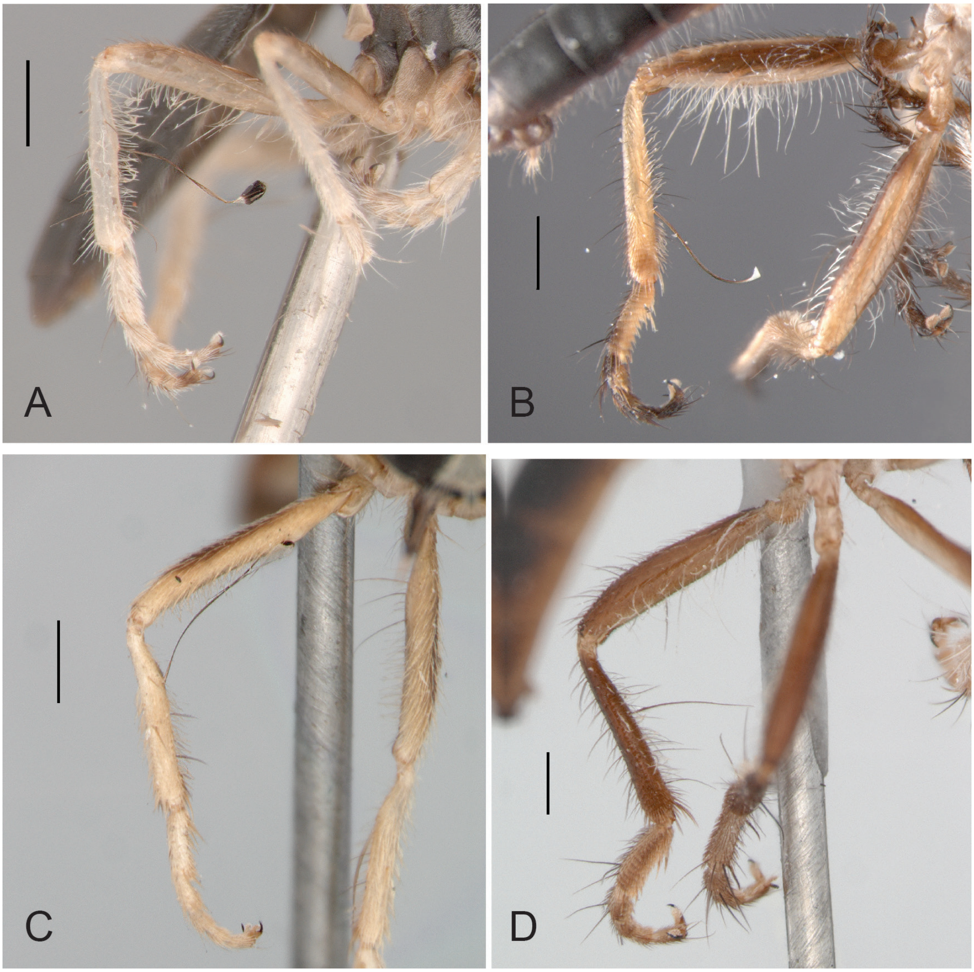

Figure 2. Hind leg, male: **A** *O. falcimystax* (PT) **B** *O. fontenellei* (HT) **C** *O. maculiseta* male (HT) **D** *O. marinonii* (HT). Scale bar = 0.5 mm.

abdomen, 1.0–1.3 mm, (n= 3). Differs from male as follows: gibbosity that equals ventral 0.5–0.64 of face height; proboscis 0.48–0.55 × the height of head; antenna 0.68–0.8 × as long as the height of eye; antennal insertion at dorsal 0.22–0.4 of head height; postpedicel 1.4–1.5 × length of basal two segments; head 1.3–1.43 × as wide as high; face 0.11–0.14 × as wide as head; mystax comprised of regular golden-brown macrosetae; ocellar tubercle 0.28–0.37 × as wide as frons; anterior ocellus 0.13–0.17 × as wide as frons by the ocellus position; hind tibiae with fine, medium-sized, golden setae ventrally, long, yellow macroseta inserted ventrally on the middle, and long, dark-brown macrosetae anterodorsally; modified tibial setae absent; T2 2.13–2.29 × wider than long; **Female genitalia.** Three spermathecae; reservoirs cylindrical, disposed in a spiral; spermathecal ducts opening independently at the bursa; genital fork rectangular, U-shaped, arms anteriorly thick, posteriorly truncate, divergent; accessory glands undistinguishable.

Morphological variation. Total length, excluding antennae, 5.0–6.3 mm, (n= 10); length of thorax, 1.2–1.5 mm, (n= 10); length of wing, 4.2–4.9 mm, (n= 9); greatest width of abdomen, 0.9–1.2 mm, (n= 9). Some specimens differed from the holotype, as follows: gibbosity that equals ventral 0.5–0.7 of face height; lateral occipital setae dark-brown; proboscis 0.35–0.53 × the height of head; proboscis with dark-brown macrosetae, ventrally; antenna 0.7–0.8 × as long as the height of eye; antennal insertion at dorsal 0.2–0.27 of head height; numerous short setae on a row around the scape; postpedicel 1.4–1.8 × length of basal two segments; postpedicel golden-pollinose; head 1.22–1.43 × as wide as high; face 0.07–0.21 × as wide as head; frons silvery-pollinose; orbital setae golden-brown; ocellar tubercle 0.29–0.33 × as wide as frons; anterior ocellus 0.11–0.14 × as wide as frons by the ocellus position; postpronotal lobe black with yellow spot dorsal to mesothoracic spiracle; scutal vestiture dark-brown; longest marginal scutellar macrosetae shorter than scutellum; postalar callosity light-brown; fore and mid tibiae with yellow setulae, long dark-brown macrosetae, and thick spines; tarsi with stout dark-brown setae dorsally and densely covered with thick spine-like golden setae; claws reddish on base and black apically; calypters white, with light-brown margin and fringe of short yellow setae; halter knob pale-yellow; T2 1.63–2.13 × wider than long.

Distribution. Peru, Bolivia, Brazil (Goiás, Mato Grosso, São Paulo and Paraná) and Argentina.

Remarks. This species share with an undescribed species from Panama–"*Oidardis signaseta*" Fisher (*nomen nudum*) (Fisher 2009: pp. 600, 604, 624, figs 46, 77)–several peculiar characters regarding the shape of gibbosity and mystax. The main difference between those two species is that "*Oidardis signaseta*" males bear two pairs of modified blade-shaped setae, whilst *O. falcimystax* males bear only one pair, along with a pair of white sinuous filiform setae.

Oidardis falcimystax, in its farther western occurrences, inhabit Peruvian and Bolivian Amazon forests, as do its congeners *O. aenescens* Hermann, 1912, and *O. gibbosa* Hermann, 1912; yet, due to the singular morphology of its facial gibbosity and mystax, and the modified tibial seta, *O. falcimystax* can be readily distinguished from these other two species. *Oidardis aenescens* have similar size and overall coloration, and even presents a similarly-prominent gibbosity, but its extent reaches no more than the ventral third of the face; besides, males of this species do not present any striking modification on mystax or tibial setae. *Oidardis gibbosa* presents a completely different color pattern, along with distinctly modified tibial seta, among other characters. *Oidardis aenescens* and *O. gibbosa* will be thoroughly presented in future publications.

This species, along with *O. maculiseta*, is the first occurrence of *Oidardis* in the Cerrado area, since the genus is almost exclusively found in dense-forest biomes. Those occurrences are, though, probably related to the higher forest environments in Cerrado, "Cerradão", and riparian forests, which have been noticed to share fauna of the Lower Diptera, at least, with Atlantic Semi-deciduous Forest areas (D. S. Amorim, unpublished data).

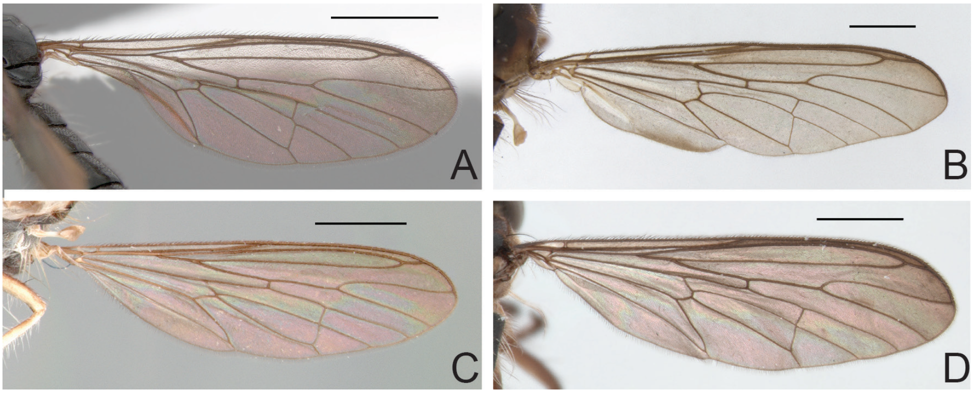

Figure 3. Wing, male: **A** *O. falcimystax* (HT) **B** *O. fontenellei* (PT) **C** *O. maculiseta* (HT) **D** *O. marinonii* (PT). Scale bar = 1mm.

Etymology. From the Latin, *falx* = scythe, and the Greek, *mystax* = moustache. Refers to the flattened blade-shaped mystacal macrosetae.

Type-material examined. Holotype: Brazil: São Paulo, Sertãozinho, elev. 550 m (21°9.14'S, 48°5.72'W), 10–24.xi.2010, coll. V. C. Silva & P. F. Donda (Frag. 2) - male (MZUSP) **Paratypes: Argentina**: Misiones, Puerto Iguazu, behind Hotel Orquídeas, (25°37'29.59"S, 54°33'2.65"W), 1–6.ii.1992, coll. S.A. Marshall - 1 female (EFISHER); **Bolivia**: Beni, Palos Blancos, elev. 600 m (15°35'S, 67°15'0"W), 11–15.i.1976, coll. L.E. Pena - 2 males (CNC); **Brazil**: Goiás, Jataí, (17°52'33.25"S, 51°43'17.19"W), xi.1972, coll. F. M. Oliveira - 1 female (MZUSP); Mato Grosso, Chapada dos Guimarães, (15°27'10.26"S, 55°44'21.02"W), 21.xi.1983, coll. Exc. Dep. ZOO - 4 males (DZUP, MZUSP); same locality, 24.xi.1983, coll. Exc. Dep. ZOO - 1 male (DZUP); Paraná, Fênix, Reserva Estadual ITCF, (23°55'0.05"S, 51°57'38.26"W), 24.xi.1986, coll. Lev. Ent. PROFAUPAR - 1 male (MZUSP); same locality, 8.xii.1986, coll. Lev. Ent. PROFAUPAR - 5 males (DZUP); same locality, 15.xii.1986, coll. Lev. Ent. PROFAUPAR - 2 males (DZUP); same locality, 22.xii.1986, coll. Lev. Ent. PROFAUPAR - 5 males (DZUP); same locality, 29.xii.1986, coll. Lev. Ent. PROFAUPAR - 5 males (DZUP); Foz do Iguaçu, (25°32'48.83"S, 54°35'17.42"W), 3.xii.1966, coll. Exc. Dep. ZOO - 1 female (DZUP); same locality, 5.xii.1966, coll. Exc. Dep. ZOO - 1 female (DZUP); same locality, 7.xii.1966, coll. Exc. Dep. ZOO - 1 female, 3 males (incl. 4 paratypes) (DZUP, MZUSP); same locality, 12.xii.1966, coll. Exc. Dep. ZOO - 3 males (DZUP); São Paulo, Sertãozinho, elev. 550 m (21°9.14'S, 48°5.72'W), 19.i.2011, coll. V. C. Silva & P. F. Donda (Frag. 1) - 1 male (MZUSP); same locality, 2.ii.2011, coll. V. C. Silva & P. F. Donda (Frag. 1) - 1 female (MZUSP); same locality, 16.ii.2011, coll. V. C. Silva, P. F. Donda, G. P. Ignácio & D. S. Amorim (Frag. 1) - 1 female (MZUSP); **Peru**: Madre de Diós, Tambopata Reserve - 30 km SW Puerto Maldonado, (12°44'50.9"S, 69°25'46.46"W), 6.xii.1982, coll. J.J. Anderson - 1 male (EFISHER).

Oidardis fontenellei **sp. n.**

http://zoobank.org/19052A06-27EA-4A04-B809-8F3973AB2625
http://species-id.net/wiki/Oidardis_fontenellei
Figures 1C–D, 2B, 3B, 4B, 5B, 6D–F, 7B, 8B, 9, 10

Diagnosis. Body shiny black and yellow; scutum with distinct arrow-like color pattern, in dorsal view; tergites with slightly paler to yellow lateral margins. Male with characteristic modified tibial seta, short light-brown with very slightly-dilated white apex.

Description. Holotype. Male. Body yellow and black. Total length, excluding antennae, 7.9 mm; length of thorax, 1.8 mm; length of wing, 6.9 mm; greatest width of abdomen, 1.3 mm.

Head, laterally. Face, between antennal insertion and gibbosity, slightly concave; gibbosity prominent; dorsal occipital setae dark-brown, lateral occipital setae dark-brown, ventral occipital setae white; proboscis 0.42 × the height of head, with a pair of yellow macrosetae ventrally; palpus dark-brown, with yellow setae apically. **Antenna**. Antenna 0.76 × as long as the height of eye, entirely dark-brown, with dark-brown setae and macrosetae; antennal insertion at dorsal 0.18 of head height; scape slightly longer than pedicel, with long ventral seta, numerous short setae on a row around the segment; pedicel round; postpedicel lanceolate, 1.9 × length of basal two segments, golden-pollinose, except for coppery-yellow pollinosity on elliptical sensorial area on inner face, with dorsal spine subapical (3/4 length of postpedicel or beyond). **Head, anteriorly**. Head 1.6 × as wide as high; face 0.19 × as wide as head, silvery-pollinose; mystax long (extending beyond the apex of proboscis), comprised of 8 golden-brown macrosetae, and few shorter setae between the rows; facial setae, other than mystax, pale-yellow; frons silvery-pollinose; orbital setae dark-brown; vertex coppery-pollinose; ocellar tubercle silver-pollinose, as high as vertex, 0.21 × as wide as frons, anterior ocellus 0.09 × as wide as frons by the ocellus position.

Thorax. Postpronotal lobe yellow; scutum shiny dark-brown medially and yellow anteriorly and laterally in an arrow-like pattern, not punctate, vestiture dark-brown, equal-sized, longest setae as long as scape, uniformly reclinate; one dark-brown notopleural; scutellum black, scutellar margin smoothly marked, marginal scutellar macrosetae dark-brown, unequal-sized, longest ones slightly longer than scutellum; postalar callosity yellow, partly with bright-blue reflections; pleuron yellow, with silvery-white pollinosity; setulae on proepisternum, katepisternum, and anepisternum golden; two anepisternal macrosetae, plus fine setulae, dark-brown; tuft of katatergal macrosetae dark-brown; anatergite with dark-brown, hair-like setae.

Legs. Coxae orange-yellow; trochanter yellow, with fine yellow setulae; femora reddish-brown – except hind femur yellow, darkened anteriorly and posteriorly—covered with short stout brown setulae dorsally, with dark setae on apical 1/3 dorsally, ventrally with weak yellow setae in two rows, hind femur with 4 long yellow ventral macrosetae in a row along distal half; anterior four tibiae entirely yellow, with white setulae, long dark-brown macrosetae and thick spines; hind tibiae orange, en-

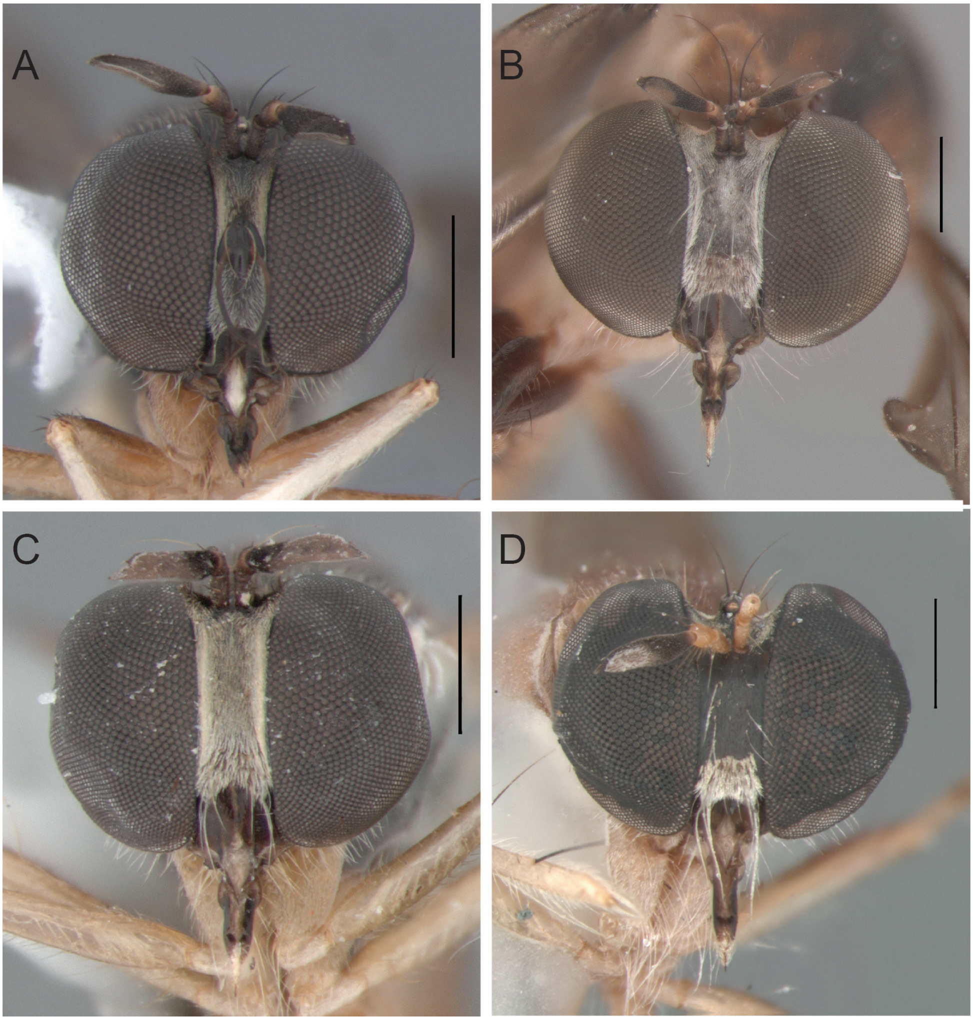

Figure 4. Head, male, anterior view: **A** *O. falcimystax* (HT) **B** *O. fontenellei* (HT) **C** *O. maculiseta* male (HT) **D** *O. marinonii* (HT). Scale bar = 0.5mm

tirely covered by dark-brown setulae, with stout dark-brown setulae, long dark-brown macrosetae anterodorsally; modified tibial setae attached to hind tibia at apical 1/3, light-brown, elongated and curved, tape-like flattened, with apical 1/7 translucent and slightly dilated; tarsi dark-brown, with stout dark-brown setae dorsally and densely covered with thick spine-like dark-brown setae, hind tarsi with flattened claw-like dark-brown setae dorsally, 5th tarsomere with 3 setae apically, opposite the claws and longer than them; claws dark-brown on base and black apically; pulvilli yellow and fringed; empodium shorter than claws.

Wing. Brownish, darker along upper margin and posteriorly on anal lobe; cell r_1 with long slightly-concave stalk (4 × the length of r-m); crossvein r-m medially in cell d, aligned to the end of Sc; cell m_3 parallel-sided distally (M_2 and M_3 parallel by the end of cell m_3), with stalk slightly longer than r-m, apex of m_3 and apex of cell d parallel and unaligned, apex of m_3 beyond apex of d; crossvein bm-cu long, base of M_3 and CuA_1 distant from each other and not appearing as an "X"; cell cu*p* with stalk slightly longer than r-m; posterior margin of wing slightly convex at distal half; calypters yellow, with light-brown margin and fringe of short brown setae; halter with yellow stem, orange knob.

Abdomen. Black, punctate, with sides nearly parallel, T2 1.5 × wider than long; vestiture longer and lighter laterally and ventrally, several light-yellow macrosetae present on lateral margin of T1, T2, and T3, one lateral marginal macroseta present on T4 and T5. **Male terminalia**. Hypopygium very conspicuous; hypandrium regular-sized (2/3 the width of hypopygium or more), much wider than long, anterior margin straight to slightly convex, posterior margin sharply pointed; gonocoxites free, gonocoxal prolongation blunt, smoothly curved inwards, with no spines at apex; gonostylus reduced, round, laterally flattened, free, attached to the base of gonocoxite; apex of phallus with three equal-sized prongs; epandrium straight in lateral view; lobes of hypoproct short.

Female. Body yellow and black. Total length, excluding antennae, 6.1–8.8 mm, (n= 4); length of thorax, 1.6–2.3 mm, (n= 4); length of wing, 6.0–7.5 mm, (n= 4); greatest width of abdomen, 1.7–1.9 mm, (n= 3). Differs from male as follows: gibbosity that equals ventral 0.27–0.35 of face height; proboscis 0.5–0.52 × the height of head; antenna 0.63–0.83 × as long as the height of eye; antennal insertion at dorsal 0.2–0.38 of head height; postpedicel oblong; postpedicel 2–2.1 × length of basal two segments; head 1.55–1.63 × as wide as high; face 0.18–0.21 × as wide as head; anterior ocellus 0.06–0.08 × as wide as frons by the ocellus position; hind femur entirely reddish-brown; hind tibiae brown on distal third dorsally, with white setulae ventrally, fine, medium-sized, dark-brown setae ventrally, long, dark-brown macroseta inserted ventrally on the middle, and long, dark-brown macrosetae anterodorsally; modified tibial setae absent; calypters with yellow margin and fringe of short yellow setae; abdomen with sides diverging posteriorly; T2 1.73–2.18 × wider than long. **Female genitalia.** Three spermathecae; reservoirs absent; spermathecal ducts opening independently at the bursa; genital fork rectangular, U-shaped, arms anteriorly thick, posteriorly slender, divergent; accessory glands undistinguishable.

Morphological variation. Total length, excluding antennae, 6.4–8.2 mm, (n= 10); length of thorax, 1.7–2.2 mm, (n= 10); length of wing, 6.3–7.7 mm, (n= 9); greatest width of abdomen, 1.2–1.4 mm, (n= 10). Some specimens differed from the holotype, as follows: face, between antennal insertion and gibbosity, plane with eye margin; gibbosity equals ventral 0.31–0.46 of face height; ventral occipital setae yellow; proboscis 0.42–0.55 × the height of head; antenna 0.61–0.99 × as long as the height of eye; light-brown scape and pedicel, dark-brown postpedicel; antennal insertion at dorsal 0.18–0.26 of head height; postpedicel 1.4–2.1 × length of basal two segments; head 1.35–1.63 × as wide as high; face 0.12–0.21 × as wide as head; face pale-yellow-pollinose or silvery-pollinose or golden-pollinose; mystax comprised of 8–10 macrosetae;

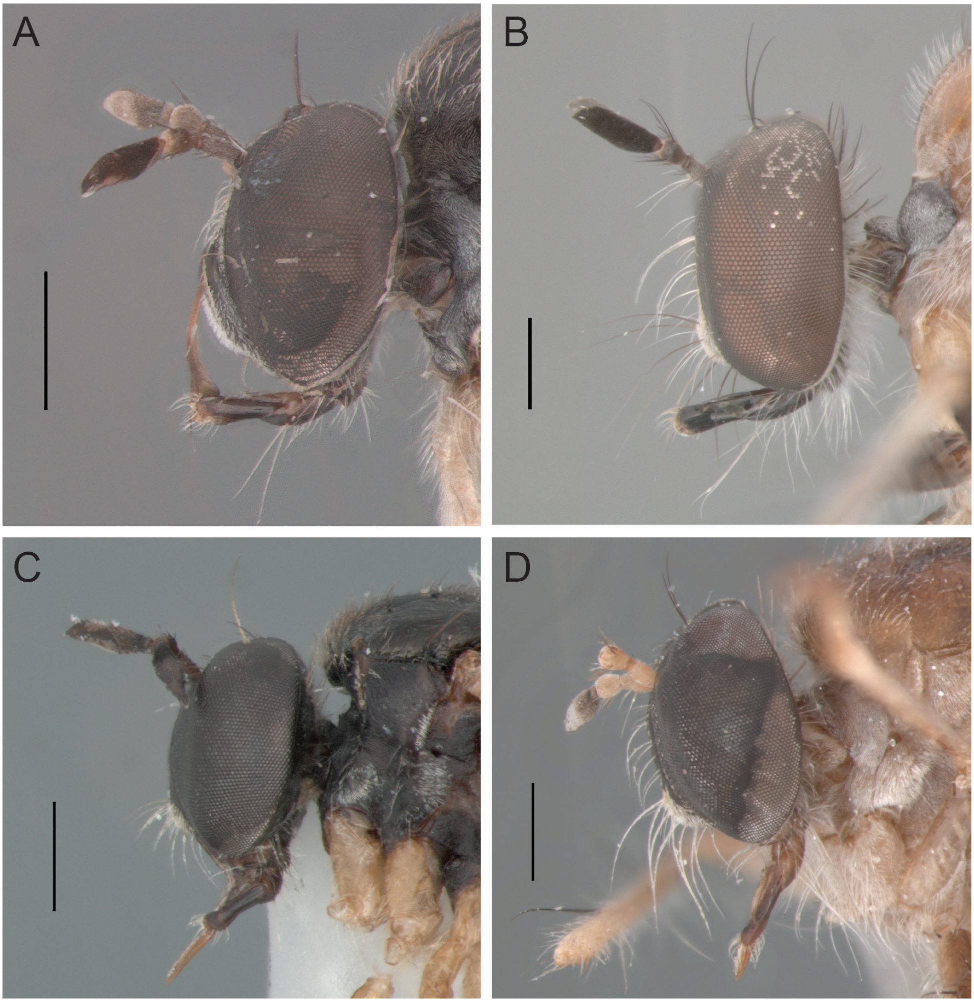

Figure 5. Head, male, lateral view: **A** *O. falcimystax* (HT) **B** *O. fontenellei* (PT) **C** *O. maculiseta* (PT) **D** *O. marinonii* (HT). Scale bar = 0.5 mm.

orbital setae golden-brown; anterior ocellus 0.06–0.09 × as wide as frons by the ocellus position; scutal vestiture white anteriorly and dark-brown posteriorly, unequal-sized; two notopleural macrosetae; scutellar margin strongly impressed; three anepisternal macrosetae; tuft of katatergal macrosetae light-brown; anatergite with golden, hair-like setae; coxae yellow; trochanter with fine, yellow setulae, and dark brown seta dorsally; tibiae yellow dorsally and brown ventrally; tarsi reddish-brown, with claw-like dark-brown setae ventrally, stout dark-brown setae dorsally, and densely covered with thick spine-like dark-brown setae; pulvilli brown; empodium well developed; cell r_1 with long, slightly concave stalk (3.5–4 times length of r-m); calypters white; white knob; abdomen black with yellow lateral margins; T2 1.41–1.92 × wider than long; several white; one lateral marginal macroseta present on T4, T5, T6, and T7.

Distribution. Brazil (Sergipe, Bahia, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo and Paraná).

Remarks. Although this species shares with *O. marinonii* the lighter color of the body, with thorax and abdomen yellow laterally, they differ in a very conspicuous character, the modified seta ventrally on the hind tibia of males—even though *O. fontenellei* bears the most discrete modified tibial seta observed in the genus. *Oidardis fontenellei* males display an elaborate courtship behavior: it approaches the female, perching on a lower position of the same twig its target is found; the male then hovers shortly behind the female, touching it quickly several times. (Guilherme Ide & Julia Almeida, pers. comm.)

Oidardis fontenellei occur preferentially in the Coastal Atlantic Forest, through its whole extension. The species was also found in Atlantic Semi-deciduous Forests in Minas Gerais and Bahia states, in localities no farther than 250 km from the coastline.

Etymology. Honors Dr. Julio Fontenelle, for his efforts on a long-term survey on Diptera at Parque Estadual do Rio Doce, the largest fragment of preserved Atlantic Rainforest in Minas Gerais state.

Type-material examined. Holotype: Brazil: Minas Gerais, Marliéria, Parque Estadual do Rio Doce, Trilha Tereza, elev. 253 m (19°42'10.69"S, 42°30'45.97"W), 25.x.2001, coll. J.C.R. Fontenelle - male (MZUSP) **Paratypes: Brazil**: Bahia, Camacan, RPPN Serra Bonita II Faz. Paris, elev. 190 m (15°25'12"S, 39°32'33"W), 3–7.ii.2009, coll. Nihei, Figueiredo, Almeida & Cezar - 1 male (MZUSP); Itapetinga, (15°15'23.24"S, 40°15'27.49"W), xi.1969, coll. F. M. Oliveira - 3 females, 9 males (MNRJ); Porto Seguro, Veracel, (16°27'3.98"S, 39°3'52.57"W), 2–5.xii.2002, coll. I. Castro - 1 female (MZUEFS); Espírito Santo, Santa Tereza, Estação Biológica Santa Lúcia, elev. 867 m (19°58'37.3"S, 40°32'22.5"W), 9–12.iv.2001, coll. C.O. Azevedo & eq. - 1 female (MZUSP); São Mateus, (18°43'16"S, 39°51'33.8"W), i.1971, coll. P. C. Elias - 1 female (MZUSP); Minas Gerais, Marliéria, Parque Estadual do Rio Doce, Trilha Tereza, elev. 253 m (19°42'10.69"S, 42°30'45.97"W), 25.x.2001, coll. J.C.R. Fontenelle - 7 males (LEEID, MZUSP); same locality, 1.xi.2001, coll. J.C.R. Fontenelle - 3 females, 1 male (LEEID, MZUSP); same locality, 8.xi.2001, coll. J.C.R. Fontenelle - 3 females, 3 males (LEEID, MZUSP); same locality, 9.xi.2003, coll. J.C.R. Fontenelle - 1 female (MZUSP); same locality, 31.x.-5.xi.2010, coll. J. C. Almeida & G. Ide - 6 females, 7 males (BMNH, MZUSP, ZSM); Paraná, Antonina, Reserva Sapitanduva, (25°25'46.77"S, 48°42'42.49"W), 1.xii.1986, coll. Lev. Ent. PROFAUPAR - 1 male (DZUP); same locality, 29.xii.1986, coll. Lev. Ent. PROFAUPAR - 1 male (DZUP); Rio de Janeiro, Nova Iguaçu, Reserva Biológica do Tinguá, (22°34'37"S, 43°26'6.6"W), 5–8.iii.2002, coll. S.T.P. Amarante & eq. - 2 males (MZUSP); Rio de Janeiro, (22°54'12.74"S, 43°12'34.51"W), i.1939, coll. Serviço Febre Amarela - 2 males (MZUSP); Rio de Janeiro, Jacarepaguá, Represa Rio Grande, (22°57'S, 43°21'0"W), xii.1969, coll. M. Alvarenga - 6 males (MNRJ); São Paulo, Bertioga, Praia de Guaratuba, (23°45'49.44"S, 45°53'45.28"W), xi.1972, coll. N. Papavero & F. Val - 1 male (MZUSP); Guarujá, (23°59'40.56"S, 46°15'24.72"W), i.1942, coll. M. Carrera - 2 females, 1 male (MZUSP); Juquiá, (24°19'17.16"S, 47°38'14.43"W),

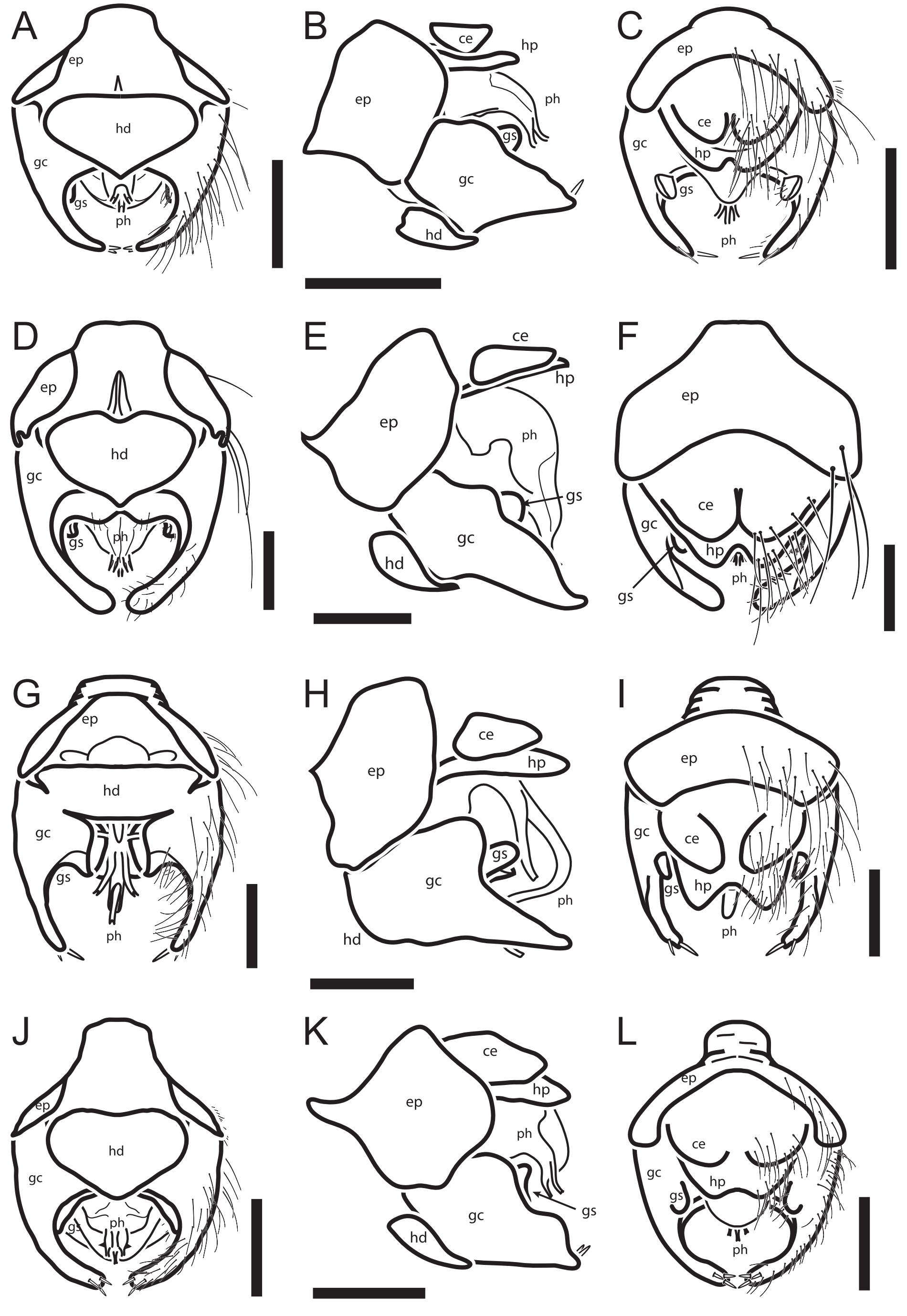

Figure 6. Male terminalia: **A–C** *O. falcimystax* **A** ventral **B** lateral and **C** dorsal views **D–F** *O. fontenellei* **D** ventral **E** lateral and **F** dorsal views **G–I** *O. maculiseta* **G** ventral **H** lateral and **I** dorsal views **J–L** *O. marinonii* **J** ventral **K** lateral and **L** dorsal views. *ep*, epandrium; *gc*, gonocoxite; *gs*, gonostylus; *hp*, hypandrium; *ph*, *phallus*. Scale bar: 0.2 mm.

i.1932, coll. J. Lane - 1 male (MZUSP); Ubatuba, Parque Estadual da Serra do Mar, (23°21'43"S, 44°49'22"W), 21.i.2002, coll. N.W. Perioto & eq. - 1 female (MZUSP); same locality, 24.i.2002, coll. N.W. Perioto & eq. - 1 female, 1 male (MZUSP); Sergipe, Santa Luzia do Itanhy Crasto , (11°22'32.8"S, 37°25'0"W), 1–4.viii.2001, coll. M. T. Tavares & eq. - 1 male (MZUSP).

Oidardis maculiseta **sp. n.**

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http://species-id.net/wiki/Oidardis_maculiseta
Figures 1E–F, 2C, 3C, 4C, 5C, 6G–I, 7C, 8A,10

Diagnosis. Leg color pattern: coxae yellow, femora dark-brown dorsally and tibiae yellow dorsally; facial pollinosity golden. Male with dark-brown modified tibial seta, as long as femur, golf-club-shaped with apical 1/4 as a large white lamella with black spot at apex; mid prong of the phallus much longer than lateral prongs; mystax short.

Description. Holotype. Male. Body shiny black. Total length, excluding antennae, 5.4 mm; length of thorax, 1.3 mm; length of wing, 4.7 mm; greatest width of abdomen, 1 mm.

Head, laterally. Face, between antennal insertion and gibbosity, plane with eye margin; gibbosity slightly prominent, equals ventral 0.26 of face height; dorsal occipital setae dark-brown, lateral occipital setae white, ventral occipital setae white; proboscis 0.48 × the height of head, with numerous white macrosetae ventrally; palpus dark-brown, with yellow setae apically. **Antenna**. Antenna 0.85 × as long as the height of eye, entirely dark-brown, with dark-brown setae and macrosetae; antennal insertion at dorsal 0.2 of head height; scape slightly longer than pedicel, with medium-sized ventral seta, numerous short setae on a row around the segment; pedicel round; postpedicel elongate, 2.1 × length of basal two segments, brown-pollinose, except for silvery-yellow pollinosity on elliptical sensorial area on inner face, with dorsal spine subapical (3/4 length of postpedicel or beyond). **Head, anteriorly**. Head 1.35 × as wide as high; face 0.17 × as wide as head, golden-pollinose; mystax short, comprised of 10 golden macrosetae, and few shorter setae between the rows; facial setae, other than mystax, pale-yellow; frons coppery-pollinose; orbital setae dark-brown; vertex coppery-pollinose; ocellar tubercle coppery-pollinose, as high as vertex, 0.39 × as wide as frons, anterior ocellus 0.14 × as wide as frons by the ocellus position.

Thorax. Postpronotal lobe black with yellow spot dorsal to mesothoracic spiracle; scutum shiny black, not punctate, vestiture dark-brown, unequal-sized, reclinate anteriorly and proclinate posteriorly; one; scutellum black, scutellar margin strongly impressed, longest ones slightly longer than scutellum; postalar callosity dark-brown, partly with bright-blue reflections; pleuron shiny dark-brown, with silvery-white pollinosity; setulae on proepisternum, katepisternum, and anepisternum; two anepisternal macrosetae, light-brown; tuft of katatergal macrosetae light-brown; anatergite with golden, hair-like setae.

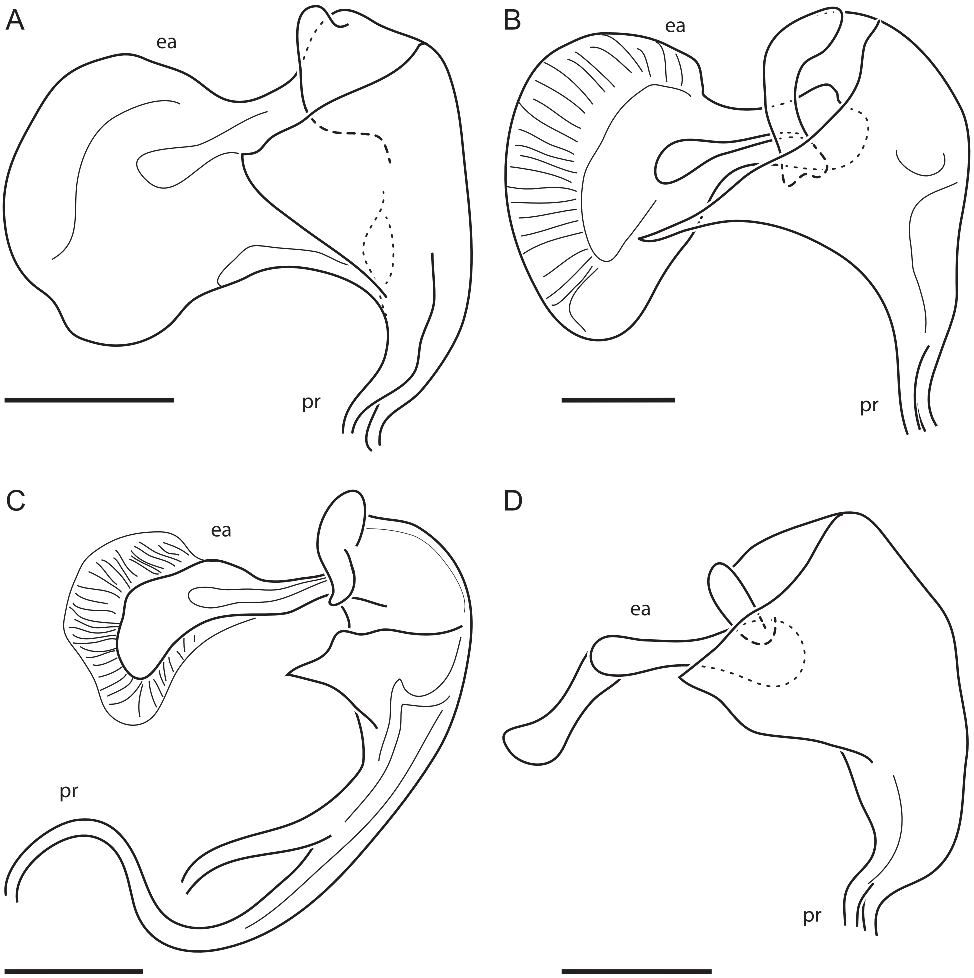

Figure 7. *Phalli*, lateral view: **A** *O. falcimystax* **B** *O. fontenellei* **C** *O. maculiseta* male **D** *O. marinonii*; *ea*, ejaculatory apodeme; *pr*, prongs. Scale bar: 0.1 mm.

Legs. Coxae orange-yellow; trochanter yellow, with fine yellow setulae; femora yellow, slightly darkened dorsally, covered with short stout yellow setulae dorsally, with dark setae on apical 1/3 dorsally, ventrally with weak yellow setae in two rows, hind femur with 3 long dark-brown ventral macrosetae; anterior four tibiae yellow dorsally and brown ventrally, with white setulae, long yellow macrosetae and long dark-brown macrosetae; hind tibiae yellow, entirely covered by golden setulae, and medium-sized fine dark-brown setae ventrally; modified tibial setae attached to hind tibia at basal 1/3, dark-brown, as long as femur, golf-club-shaped with apical 1/4 as a large white lamella with black spot at apex; tarsi yellow, with stout yellow setae dorsally and densely covered with thick yellow setae, 5th tarsomere with 3 setae apically, opposite the claws and subequal to them; claws yellow on base and black apically; pulvilli yellow and fringed; empodium shorter than claws.

Wing. Brownish, darker along upper margin; cell r_1 with long slightly-concave stalk (2.5 × the length of r-m); crossvein r-m medially in cell d, distal to the end of Sc; cell m_3 narrowing distally (M_2 and M_3 converging by the end of cell m_3), with stalk slightly longer than r-m, apex of m_3 and apex of cell d parallel and aligned; crossvein bm-cu short, base of M_3 and CuA_1 arranged almost as an “X”; cell cu*p* with stalk shorter than r-m; posterior margin of wing slightly concave at distal half; calypters white, with light-brown margin and fringe of short yellow setae; halter with yellow stem, brown knob.

Abdomen. Black, not punctate, with sides diverging posteriorly, T2 1.6 × wider than long; vestiture longer and lighter laterally and ventrally, several light-yellow macrosetae present on lateral margin of T1 and T2. **Male terminalia**. Hypopygium very conspicuous; hypandrium regular-sized (2/3 the width of hypopygium or more), much wider than long, anterior margin straight to slightly convex, posterior margin smoothly convex; gonocoxites partially fused to hypandrium, gonocoxal prolongation thin, smoothly curved inwards, with 2 spines at apex; gonostylus reduced, round, laterally flattened, free, attached to the base of gonocoxite; apex of phallus with three unequal-sized prongs, mid prong much longer than the others; epandrium straight in lateral view; lobes of hypoproct protruding.

Female. Total length, excluding antennae, 5.7–7.2 mm, (n= 3); length of thorax, 1.5–1.7 mm, (n= 3); length of wing, 5.2–6.2 mm, (n= 3); greatest width of abdomen, 1.3–1.6 mm, (n= 3). Differs from male as follows: gibbosity that equals ventral 0.28–0.3 of face height; proboscis 0.39–0.56 × the height of head; antenna 0.78–0.95 × as long as the height of eye; antennal insertion at dorsal 0.19–0.2 of head height; postpedicel 1.8–2 × length of basal two segments; head 1.4–1.5 × as wide as high; face 0.16 × as wide as head; mystax long (extending beyond the apex of proboscis); ocellar tubercle 0.33 × as wide as frons; anterior ocellus 0.11–0.17 × as wide as frons by the ocellus position; postpronotal lobe dark-brown; proepisternum, anepisternum and katepisternum with golden setulae; trochanter orange-yellow; femora yellow and dark-brown dorsally; femora covered with short, stout brown setulae dorsally; hind femur with 3 long yellow ventral macrosetae; tibiae yellow dorsally and brown ventrally, hind tibia reddish-brown, yellow dorsally on basal 1/3; tibiae with white setulae, long yellow macrosetae, long dark-brown macrosetae, and thick spines; hind tibiae entirely covered by dark-brown setulae; hind tibia with light-brown setulae ventrally, yellow macroseta inserted ventrally on the middle, fine, medium-sized dark-brown setae dorsally, and long, dark-brown macrosetae anterodorsally; modified tibial setae absent; tarsi dark-brown, with claw-like dark-brown setae ventrally, stout dark-brown setae dorsally, and densely covered with thick spine-like dark-brown setae; claws reddish on base and black apically, mid tarsi with yellow-and-black claws; apex of cell m_3 and apex of cell d angled and unaligned, apex of m_3 beyond apex of d; posterior margin of wing slightly convex at distal half; halter with orange stem; T2 1.47–1.84 × wider than long; white macrosetae on T1–2; one lateral marginal macrosetae present on T4, T5, T6, and T7. **Female genitalia.** Three spermathecae; reservoirs cylindrical, coiled; spermathecal

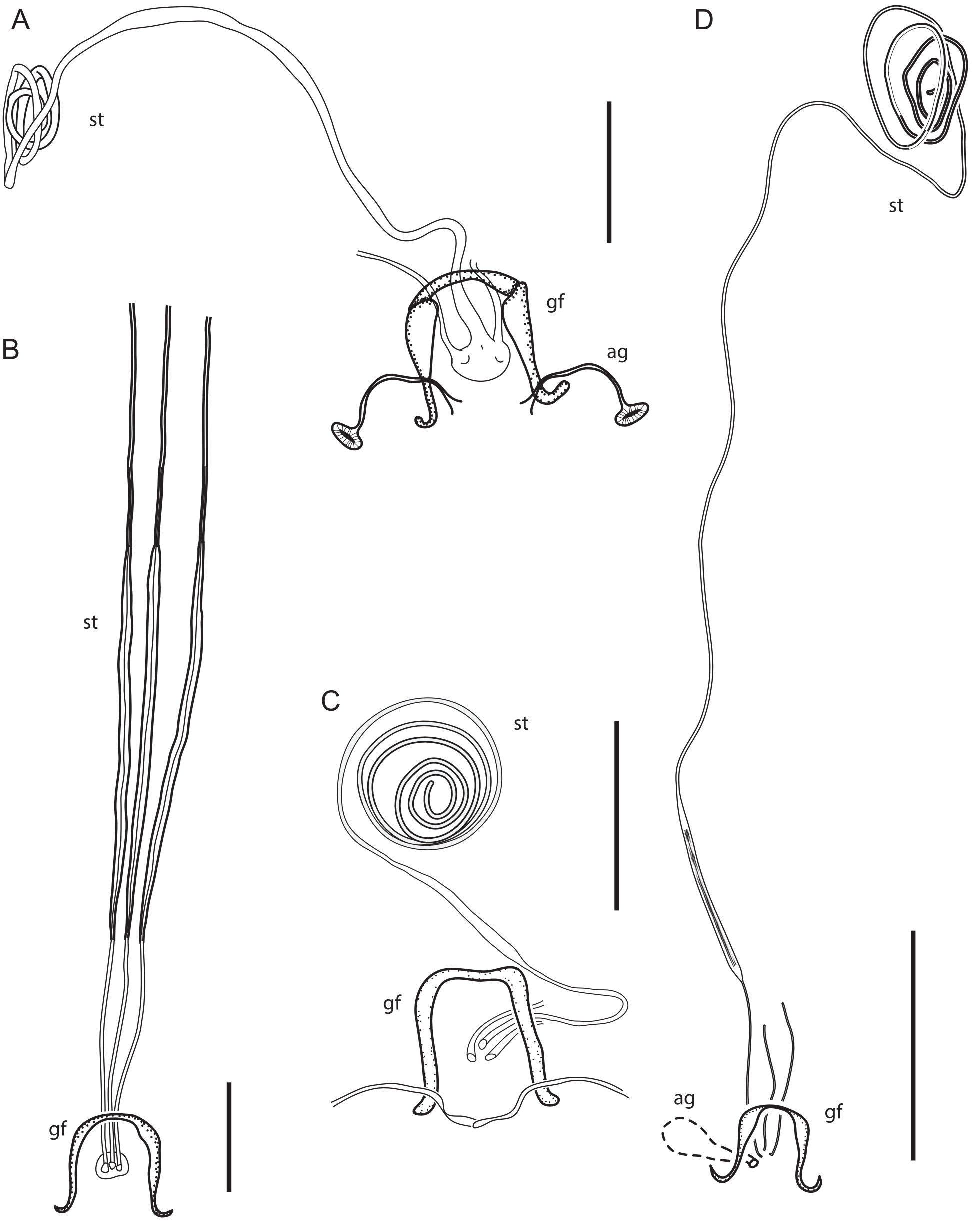

Figure 8. Spermathecae: **A** *O. maculiseta* **B** *O. fontenellei* **C** *O. falcimystax* **D** *O. marinonii*. *ag*, acessory gland; *gf*, genital fork; *st*, spermatheca. Scale bar: 0.2 mm.

ducts opening independently at the bursa; genital fork rectangular, U-shaped, arms anteriorly thick, posteriorly slender, divergent; accessory glands oval.

Morphological variation. Total length, excluding antennae, 6.0–6.6 mm, (n= 4); length of thorax, 1.4–1.5 mm, (n= 4); length of wing, 4.9–5.4 mm, (n= 4); greatest width of abdomen, 1.0–1.1 mm, (n= 4). Some specimens differed from the holotype, as follows: gibbosity that equals ventral 0.23–0.26 of face height; proboscis 0.4–0.57 × the height of head; antenna 0.82–0.86 × as long as the height of eye; antennal insertion at dorsal 0.18–0.24 of head height; postpedicel 1.8–1.9 × length of basal two segments; head 1.37–1.4 × as wide as high; face 0.15–0.17 × as wide as head; mystax comprised of 8–10 macrosetae; ocellar tubercle 0.34–0.37 × as wide as frons; anterior ocellus 0.13–0.16 × as wide as frons by the ocellus position; T2 1.42–1.56 × wider than long.

Distribution. Brazil (Goiás, São Paulo and Paraná).

Remarks. There is a group of females from Fênix (PR) that differs from the paratypes assigned by presenting an oblong postpedicel, dark-brown coxae and homogeneously directed vestiture on the scutum. Therefore, these specimens are not included as paratypes for the species, since they vary in such consistent characters; instead, these specimens are listed under “additional material examined”.

O. maculiseta occur mainly in Atlantic Semi-deciduous Forest. As noted above for *O. falcimystax*, they were also found in the Cerrado area, at Corumbá de Goiás, a locality which has a Lower Diptera fauna similar to the Semi-deciduous Forest ecorregion (D. S. Amorim, unpublished data).

Etymology. from the Latin, *macula* = spot, and *seta* = bristle. Refers to the singular morphology of the modified tibial seta.

Type-material examined. Holotype: Brazil: Paraná, Fênix, Reserva Estadual ITCF, (23°55'0.05"S, 51°57'38.26"W), 3.xi.1986, coll. Lev. Ent. PROFAUPAR - male (DZUP). **Paratypes: Brazil**: Goiás, Corumbá [de Goiás], Fazenda Monjolinho, (15°55'0.12"S, 48°46'0.12"W), xi.1945, coll. Barretto - 1 male (MZUSP); Paraná, Fênix, Reserva Estadual ITCF, (23°55'0.05"S, 51°57'38.26"W), 6.x.1986, coll. Lev. Ent. PROFAUPAR - 1 male (DZUP); same locality, 20.x.1986, coll. Lev. Ent. PROFAUPAR - 1 male (DZUP); same locality, 3.xi.1986, coll. Lev. Ent. PROFAUPAR - 2 males (DZUP, MZUSP); same locality, 10.xi.1986, coll. Lev. Ent. PROFAUPAR - 3 males (DZUP); same locality, 17.xi.1986, coll. Lev. Ent. PROFAUPAR - 2 males (DZUP, MZUSP); same locality, 24.xi.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP, MZUSP); same locality, 8.xii.1986, coll. Lev. Ent. PROFAUPAR - 2 males (DZUP); same locality, 22.xii.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP); same locality, 29.xii.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP); São Paulo, Barão de Antonina, (23°37'38.07"S, 49°33'40.68"W), i.1946, coll. Barretto - 2 females, 1 male (MZUSP); São Paulo, (23°32'56.19"S, 46°38'19.74"W), xii.1940, coll. M. Carrera (Horto Florestal) - 1 male (MZUSP). **Additional material examined. Brazil:** Paraná, Fênix, Reserva Estadual ITCF, (23°55'0.05"S, 51°57'38.26"W), 20.x.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP); same locality, 27.x.1986, coll. Lev. Ent. PROFAUPAR - 1 females (MZUSP); same locality, 10.xi.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP); same locality, 24.xi.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP); same locality, 8.xii.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP); same locality, 15.xii.1986, coll. Lev. Ent. PROFAUPAR - 1 female (DZUP); Foz do Iguaçu, (25°32'48.83"S, 54°35'17.42"W), 7.xii.1966, coll. Exc. Dep. ZOO - 1 female (DZUP).

Figure 9. *Oidardis fontenellei* sp. n., Parque Estadual do Rio Doce, Minas Gerais. Female (above) and male (below). (Photo: Guilherme Ide).

***Oidardis marinonii* sp. n.**

http://zoobank.org/3AAB47D4-535F-40DB-8496-299F27C96E2A
http://species-id.net/wiki/Oidardis_marinonii
Figures 1G–H, 2D, 3D, 4D, 5D, 6J–L, 7D, 8D, 10

Diagnosis. Pleura yellow; scutum yellow laterally and anteriorly, tergites yellow on lateral margins; scape and pedicel yellow or light brown. Males without modified tibial seta.

Description. Holotype. Male. Body yellow and black. Total length, excluding antennae, 7.3 mm; length of thorax, 1.7 mm; length of wing, 6.2 mm; greatest width of abdomen, 1.2 mm.

Head, laterally. Face, between antennal insertion and gibbosity, plane with eye margin; gibbosity slightly prominent, equals ventral 0.33 of face height; dorsal occipital setae light-brown, lateral occipital setae light-brown, ventral occipital setae yellow; proboscis 0.52 × the height of head, with a pair of yellow macrosetae ventrally; palpus dark-brown, with yellow setae apically. **Antenna**. Antenna 0.74 × as long as the height of eye, yellow scape and pedicel, dark-brown postpedicel, with dark-brown and yellow setae and macrosetae; antennal insertion at dorsal 0.28 of head height; scape slightly longer than pedicel, with long ventral seta, numerous short setae on a row around the segment; pedicel oval; postpedicel lanceolate, 1.8 × length of basal two segments, golden-pollinose, except for silvery-yellow pollinosity on elliptical sensorial area on inner face, with dorsal spine subapical (3/4 length of postpedicel or beyond). **Head, anteriorly**. Head 1.4 × as wide as high; face 0.17 × as wide as head, silvery-pollinose, on gibbosity only; mystax long (extending beyond the apex of proboscis), comprised of 10 pale-yellow macrosetae, and few shorter setae between the rows; facial setae, other than mystax, pale-yellow; frons silvery-pollinose; orbital setae dark-brown; vertex coppery-pollinose; ocellar tubercle coppery-pollinose, lower than vertex, 0.3 × as wide as frons, anterior ocellus 0.09 × as wide as frons by the ocellus position.

Thorax. Postpronotal lobe yellow; scutum shiny dark-brown posteriorly and yellow to light-brown anteriorly, not punctate, vestiture golden, unequal-sized, reclinate anteriorly and proclinate posteriorly; one golden notopleural; scutellum dark-brown, scutellar margin strongly impressed, marginal scutellar macrosetae dark-brown, unequal-sized, longest ones slightly longer than scutellum; postalar callosity light-brown, partly with bright-blue reflections; pleuron yellow, with silvery-white pollinosity; setulae on proepisternum, katepisternum, and anepisternum yellow; two anepisternal macrosetae, plus fine setulae, yellow; tuft of katatergal macrosetae light-brown; anatergite with yellow, hair-like setae.

Legs. Coxae yellow; trochanter yellow, with fine yellow setulae; femora reddish-yellow, slightly darkened dorsally, covered with short stout brown setulae dorsally, with dark setae on apical 1/3 dorsally, ventrally with weak yellow setae in two rows and fine yellow setulae apically, hind femur with 3 long yellow ventral macrosetae; anterior four tibiae entirely yellow, with yellow setulae, long yellow macrosetae, long dark-brown macrosetae and thick spines; hind tibia orange, with stout golden setulae apically, white setulae ventrally, long dark-brown macroseta inserted ventrally on

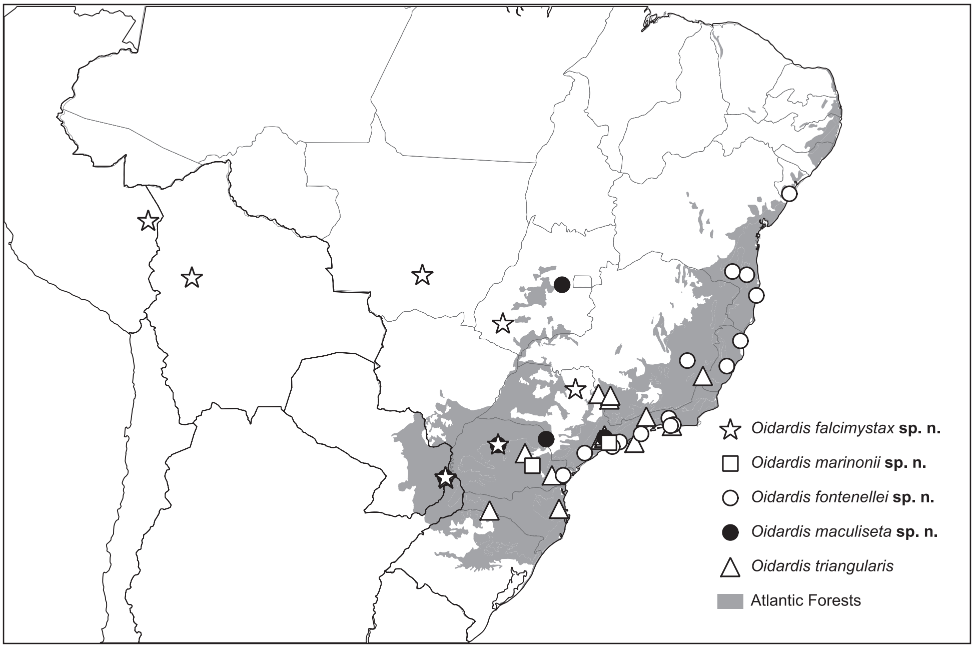

Figure 10. Distribution of *Oidardis* species throughout the Atlantic Rainforest.

the middle, long dark-brown macrosetae posteriorly, and long dark-brown macrosetae anterodorsally; modified tibial setae absent; tarsi reddish-brown, with claw-like dark-brown setae ventrally, stout dark-brown setae dorsally, and densely covered with thick spine-like dark-brown setae, 5th tarsomere with 3 setae apically, opposite the claws and longer than them; claws yellow on base and black apically; pulvilli yellow and fringed; empodium shorter than claws.

Wing. Brownish, darker along upper margin; cell r_1 with short slightly-concave stalk (2 × the length of r-m); crossvein r-m at distal half of cell d, distal to the end of Sc; cell m_3 narrowing distally (M_2 and M_3 converging by the end of cell m_3), with stalk slightly longer than r-m, apex of m_3 and apex of cell d parallel, unaligned, apex of m_3 before apex of d, and unaligned, apex of m_3 beyond apex of d (right wing) and apex of m_3 before apex of d (left wing); crossvein bm-cu long, base of M_3 and CuA_1 distant from each other and not appearing as an "X"; cell cu*p* with stalk shorter than r-m; posterior margin of wing slightly convex at distal half; calypters pale-yellow, with light-brown margin and fringe of short brown setae; halter with yellow stem, brown knob.

Abdomen. Black with yellow lateral margins, punctate, with sides nearly parallel, T2 1.46 × wider than long; vestiture longer and lighter laterally and ventrally, several light-yellow macrosetae present on lateral margin of T1, T2, T3, and T4. **Male terminalia**. Hypopygium very conspicuous; hypandrium regular-sized (2/3 the width of hypopygium or more), much wider than long, anterior margin concave, posterior margin slightly pointed (hypandrium triangular-like); gonocoxites free, gonocoxal prolongation blunt, smoothly curved inwards, with 3 spines at apex; gonostylus reduced, much wider than long, laterally flattened, fused basally to gonocoxite, attached to the base of gonocoxite; apex of phallus with three equal-sized prongs; epandrium straight in lateral view; lobes of hypoproct short.

Female. Total length, excluding antennae, 6.2 mm, (n= 1); length of thorax, 1.6 mm, (n= 1); length of wing, 7.0 mm, (n= 1); greatest width of abdomen, 1.1 mm, (n= 1). Differs from male as follows: gibbosity that equals ventral 0.3 of face height; proboscis 0.6 × the height of head; antenna 0.71 × as long as the height of eye; antennal insertion at dorsal 0.24 of head height; postpedicel 1.7 × length of basal two segments; head 1.52 × as wide as high; face 0.18 × as wide as head; mystax comprised of 8 macrosetae; tuft of katatergal macrosetae yellow; femora yellow and slightly darkened dorsally, except entirely reddish-brown hind femur; femora ventrally with weak yellow setae in two rows; tibiae with yellow setulae, long yellow macrosetae, and long dark-brown macrosetae; hind tibiae entirely covered by dark-brown setulae, with long, dark-brown macroseta inserted ventrally on the middle and long, dark-brown macrosetae anterodorsally; cell m_3 parallel-sided distally (M_2 and M_3 parallel by the end of cell m_3), and with stalk as long as r-m; apex of cell m_3 and apex of cell d angled and unaligned, apex of m_3 before apex of d; calypters white; halter with milk-coffee knob; abdominal segments narrow, T2 1.88 × wider than long; several macrosetae present on lateral margin of T1, T2, and T3; one lateral marginal macrosetae present on T4. **Female genitalia.** Three spermathecae; reservoirs cylindrical, coiled; spermathecal ducts opening independently at the bursa; genital fork rectangular, U-shaped, arms anteriorly thick, posteriorly slender, divergent; accessory glands distinguishable only for the duct and opening to bursa.

Morphological variation. Total length, excluding antennae, 6.2–7.3 mm, (n= 5); length of thorax, 1.4–1.8 mm, (n= 5); length of wing, 5.7–6.6 mm, (n= 5); greatest width of abdomen, 1.0–1.1 mm, (n= 4). Some specimens differed from the holotype, as follows: gibbosity that equals ventral 0.26–0.35 of face height; proboscis 0.56–0.64 × the height of head; antenna 0.71–0.82 × as long as the height of eye; light-brown scape and pedicel, dark-brown postpedicel; antennal insertion at dorsal 0.2–0.31 of head height; postpedicel 1.6–2.7 × length of basal two segments; head 1.42–1.53 × as wide as high; face 0.14–0.18 × as wide as head; face golden-pollinose, as a whole; mystax comprised of 8–10 golden macrosetae; ocellar tubercle 0.3–0.34 × as wide as frons; anterior ocellus 0.11 × as wide as frons by the ocellus position; one or two anepisternal macrosetae; apex of cell m_3 and apex of cell d unaligned, apex of m_3 before apex of d, or unaligned, apex of m_3 beyond apex of d; crossvein bm-cu short, base of M_3 and CuA_1 arranged almost as an "X"; T2 1.11–1.41 × wider than long.

Distribution. Brazil (São Paulo and Paraná).

Remarks. This species is similar to *Oidardis fontenellei* in general morphology and color pattern. However, since the examined specimens of *O. marinonii* come from a small collection series and are poorly preserved, the observed differences in color of antenna and legs should be used cautiously. The direction of vestiture on the posterior portion of scutum and the absence of the modified tibial seta on males in *O. marinonii* thus become all the more important for separating these species. Nevertheless, the general color pattern—paler pleura and dark scutum—is quite noticeable, even considering preservation issues of the material, and is still reliable when distinguishing both species from others in *Oidardis*.

It is also important to remark that the holotype presents an asymmetry concerning the relative position of cells *d* and m_3, when left and right wings are compared.

Oidardis marinonii probably occur in the understory of dense forests, since it is recorded for Cubatão. This locality is situated in the coastal forests of Serra do Mar, a typical Ombrophilous Dense Forest. Therefore, in Ponta Grossa—situated in Araucaria Moist Forest area, but largely covered by grasslands ("campos limpos")—*O. marinonii* possibly occupies patches of Araucaria woodlands and riparian forests (Marinoni and Dutra 1991, Olson et al. 2001).

Etymology. Honors late Dr. Renato Marinoni, for his efforts on promoting, besides other projects, an important zoological survey in Paraná State (PROFAUPAR), that made available specimens for this species, and many other, to be recognized and described.

Type-material examined. Holotype: Brazil: Paraná, Ponta Grossa, Parque Estadual de Vila Velha - IAP, (25°2'37.29"S, 50°14'52.83"W), 22.xii.1986, coll. Lev. Ent. PROFAUPAR - male (DZUP). **Paratypes: Brazil**: Same locality as holotype, 15.xii.1986, coll. Lev. Ent. PROFAUPAR - 1 female, 3 males (DZUP, MZUSP); same locality, 22.xii.1986, coll. Lev. Ent. PROFAUPAR - 5 males (DZUP, MZUSP); same locality, 29.xii.1986, coll. Lev. Ent. PROFAUPAR - 3 males (DZUP); São Paulo, Cubatão, (23°53'44.02"S, 46°25'32.28"W), 15.xii.1955, coll. Pereira, Martinez, Werner & d'Andretta - 1 male (MZUSP).

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Two new species of gorgonian octocorals from the Tropical Eastern Pacific Biogeographic Region (Cnidaria, Anthozoa, Gorgoniidae)

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Abstract

The gorgoniid *Eugorgia* is exclusively an eastern Pacific genus. It has a wide geographic and bathymetric range of distribution, found from California to Perú and extends down to 65 m deep. Two new species are herein described. The morphological characters were analyzed and illustrated by light and scanning electron microscopy. *Eugorgia beebei* **sp. n.** can be distinguished by its white, ascending, sparse colony growth. *Eugorgia mutabilis* **sp. n.** can be distinguished by its white colony that changes color after collection, and the conspicuous sharp-crested disc sclerites. From a morphological point of view the new species are related to the *daniana*-group, the *rubens*-group and the *siedenburgae*-group of Eugorgia; their affiliations, and the proposal of a new group are discussed. These new species increases the number of species in the genus to 15, and contribute to the knowledge of the eastern Pacific octocoral biodiversity.

Keywords

Eugorgia, eastern Pacific, gorgonian, soft corals, taxonomy, white species

Introduction

Eugorgia is a gorgonian octocoral (family Gorgoniidae) with 13 valid species. The genus is considered to be exclusively eastern Pacific and is distributed from southern California to Perú, and found in oceanic islands. It presents a wide bathymetric range of distribution, found in shallow waters (down to 40 m), and in the mesophotic region (down to 65 m) (Breedy and Guzman 2013). *Eugorgia* is characterized by having flabellate to bushy colonies with one or multiple planes. Branching is lateral, partially dichotomous, or pinnate-like, often bushy, and combinations of them; branch anastomosis is absent, but pseudo-anastomosis frequently occurs (Breedy et al. 2009). Colonies could be attached to hard substrates, debris, and coarse sand or muddy sediments. Polyps are fully retractile into the coenenchyme in slightly raised to prominent mounds arranged in series of longitudinal rows, or evenly distributed on the branches. Coenenchymal sclerites are of various types: spindle, disc-spindle, capstan, and the most dominant form that defines the genus is the characteristic double disc. Double discs could be incomplete, when the sclerite tubercles have a partial fusion, or complete, when the fused tubercles of the sclerites form wheels like flying saucers. Anthocodial sclerites are rarely found. The color of the sclerites is variable: brownish, orange, red, violet, white, yellow or combinations of these (Breedy et al. 2009). The colonies are orange, pink, purple, red, white, or yellow, some have with colored rings around the polyp mounds. They are produced by the arrangement of darker or lighter color sclerites around the polyp aperture, in some cases they are not surrounding the polyps, just sparsely distributed giving a sprinkled appearance to the branches. According to the morphological features, the species are proposed to form three groups, the *daniana*-group, the *ampla*-group and the monospecific *rubens*-group (see Breedy et al. 2009). A new group characterized by bushy, irregularly pinnate, bicolored colonies has been proposed for the recently described species *Eugorgia siedenburgae* Breedy & Guzman, 2013.

Eugorgia is recognised for their bright colored colonies. The white color has been reported only for one species, *Eugorgia alba* Bielschowsky, 1929 in the *ampla*-group (Breedy et al. 2009), although white specimens have been observed either in collections or in the field. Herein we describe two new species that were previously recorded as color varieties (Breedy et al. 2009, E. Deichmann as a museum label).

Materials and methods

Repository abbreviations

CAS	California Academy of Science, California, USA
UCR	Museo de Zoología, Universidad de Costa Rica
STRI	Smithsonian Tropical Research Institute, Panama
USNM	National Museum of Natural History, Washington, USA

The specimens used in this study belong to the octocoral collections of the above cited museums.

Morphological analysis. Preserved specimens were photographed for later detailed observation. Sclerites were obtained by dissolving tissues from branches with 3.5% sodium hypochlorite (household bleach). Sclerites were rinsed many times with distilled water then 100% ethanol, dried, and mounted on stubs for scanning electron microscopy (SEM), and coated with 60–80 nm Pt/Pd. They were observed and photographed using an Hitachi 3700 SEM operated at 15kV. For light microscopy, clean sclerites were mounted in water or glycerin and observed and photographed using an Olympus LX 51 inverted stereoscope.

We followed Verrill (1868) and Breedy et al. (2009) for characters assessment. The terminology used in descriptions mostly follows Bayer et al. (1983), Breedy and Guzman (2002), and Breedy et al. (2009).

Morphological characters of colonies and the most abundant sclerite types of the species examined here are presented in Table 1. The most abundant sclerites in these species are disc-spindles and double discs that present various degrees of tubercle fusion. The illustrations of the sclerites are presented in different planes to provide a better idea of their architecture (Figs 3, 5). Comparison is made with the closest morphological groups, in this case, the *daniana*-, *siedenburgae*-, and *rubens*-groups (Table 1).

Taxonomy

Class Anthozoa Ehrenberg, 1831
Subclass Octocorallia Haeckel, 1866
Order Alcyonacea Lamouroux, 1812
Family Gorgoniidae Lamouroux, 1812
Genus *Eugorgia* Verrill, 1868

***Eugorgia beebei* sp. n.**
http://zoobank.org/8B75AC49-5089-4BEF-BE80-B76198B9D0E8
http://species-id.net/wiki/Eugorgia_beebei
Figs 1–3

Eugorgia rubens var. *beebei* (species name suggested by E. Deichmann in a museum label, unpublished)

Material examined. Holotype. CASIZ 75783, ethanol preserved, Los Frailes, Baja California sur, México, 52 m, coll. R. Adcock, 18 June 1979.

Paratypes. MCZ 36106, dry, Paita, Piura, Perú, no more data available. USNM 56879, ethanol preserved, El Alto, Piura, Perú, 1860-1815 m but depth data dubious (F. M. Bayer's note on label: 'specimen probably from previous shallow station'), *Anton Bruun* Cruise , 18B, Sta. 766, 4°10'S., 81°27'W, 9 September 1966.

Type locality. Baja California sur, México.

Diagnosis. Ascending colony sparse growing, branching irregularly pinnate, and multiplanar, subdividing up to 11 times, some pseudo-anastomosis present. Prominent polyp-mounds up to 0.70 mm tall, dome-shaped, arranged irregularly, and closely placed on branchlets, and very distant on thick branches. Colony and sclerites white. Spindles and disc-spindles up to 0.14 mm in length, double discs up to 0.07 mm long, and 0.05 mm wide. Anthocodial rods absent.

Description. Holotype 24 cm tall, and 20 cm wide, ascending, sparse growing, (Fig. 1A). Branching irregularly pinnate, and multiplanar, several pseudo-anastomosis occurs in branchlets and branches (Fig. 1A–B). Main stem 4 mm diameter at base, slightly compressed, and short, about 80 mm long arising from a fragment of holdfast, 0.6 mm diameter. Main stem gives off several branches and stumps. The three main branches, 3.0–4.0 mm in diameter, emerging at angles of 45–90°and producing secondary branches subdividing and giving off thin branchlets, up to 2.5 mm diameter, including polyp-mounds. Branchlets irregularly arranged, separated 5–16 mm, and giving off 2 or 3 lateral, secondary branchlets, of same thickness and arrangement. Colony branching up to 11 times. Unbranched terminal twigs blunt, and reaching up to 50 mm long (Figs. 1A–B). Polyp-mounds prominent, up to 0.7 mm height and 1 mm in diameter, dome-shaped, with slit-like apertures, arranged irregularly, close together along the branchlets, and very distantly distributed or absent along the thick branches (Fig. 1B). Holdfast devoid of polyps. Colony white (Fig. 1A–B). Sclerites of coenenchyme white, mostly double discs (Fig. 1C). Spindles and disc-spindles, up to 0.14 mm long and 0.04 mm wide, with 4 or 5 whorls of warty tubercles, the ends mostly blunt (Fig. 3A). Double discs up to 0.07 mm long, and 0.05 mm wide (Fig. 3B). Crosses about 0.08x0.06 mm, scarce on samples (Fig. 3C). No anthocodial sclerites present in the samples.

Variability. Paratype MCZ 36106 reaches up to 34 cm tall, and 31 cm wide, the main stem 0.7 mm diameter, slightly compressed, and short, about 1.0 cm long arising from an oval holdfast 3.2 cm diameter, and 0.2 cm thick (Fig. 2A–B). Sclerites as in the holotype (Fig. 2C). The other examined specimens are smaller, but very consistent in all aspects with the holotype.

Discussion. The morphology of the colony, i.e., irregular-pinnate branching and prominent polyps, immediately segregates the new species from the *ampla*-group, and suggest a similarity with *daniana*- , *rubens*- and *siedenburgae*-groups. *Eugorgia beebei* and *E. siedenburgae* differ from the species in the *daniana*-group, including *Eugorgia mutabilis* sp. n. (described below), firstly, in the colony growth, which is sparse and ascending in *E. beebei* sp. n. but bushy and profuse in *E. siedenburgae*, not flabellate as it is in the *daniana*-group species. Secondly, it differs in the branching patterns because branchlets in the *daniana*-group form flat pinnate fronds with pinnae projecting in the same plane. That is not the case in *E. beebei* and *E. siedenburgae* where the secondary branchlets stick out in several, irregular planes.

Figure 1. *Eugorgia beebei* (CASIZ 75783) holotype. **A** entire colony **B** detail of branches **C** SEM sclerites.

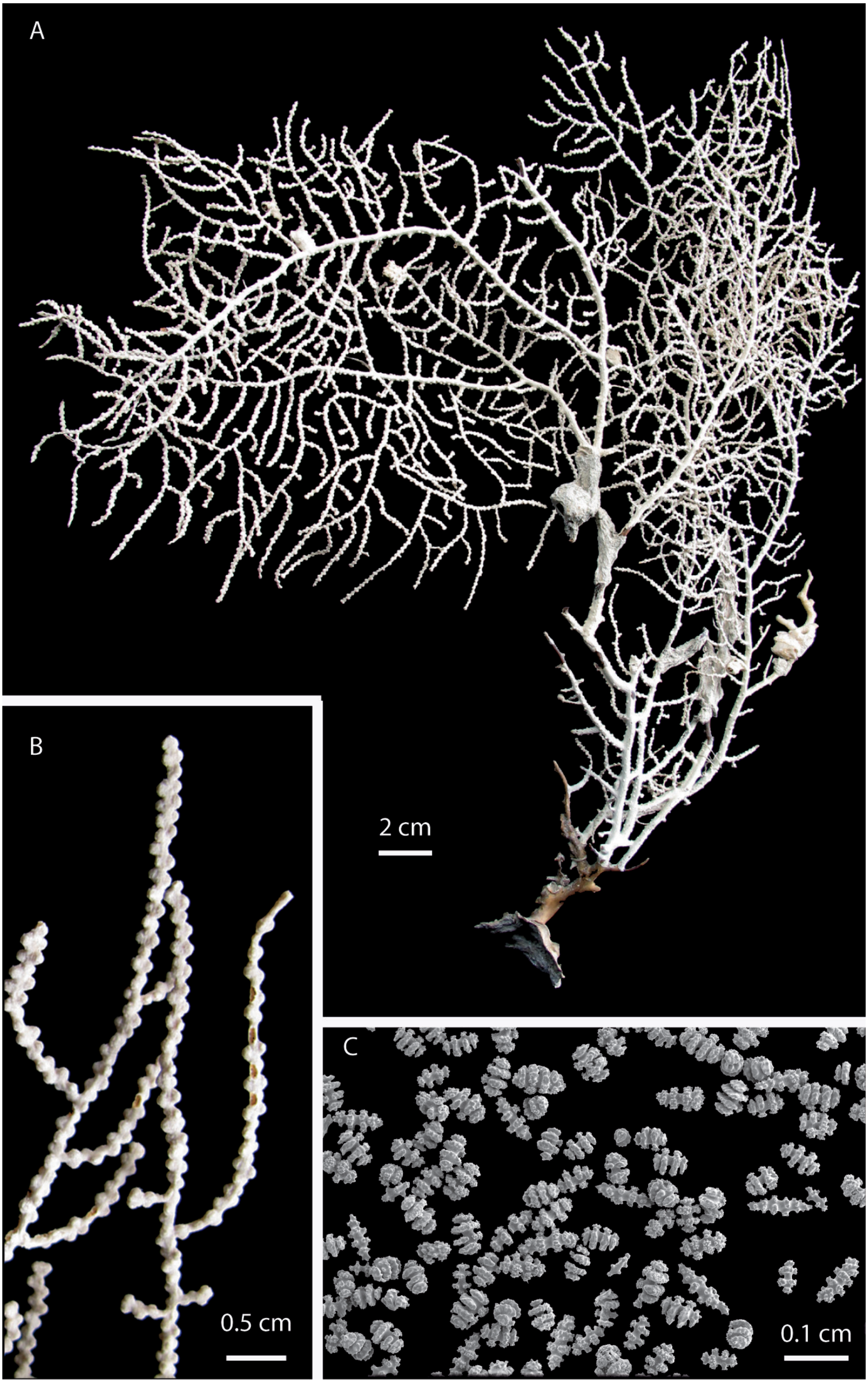

Figure 2. *Eugorgia beebei* (MCZ 36106) paratype. **A** entire colony **B** detail of branches; SEM sclerites.

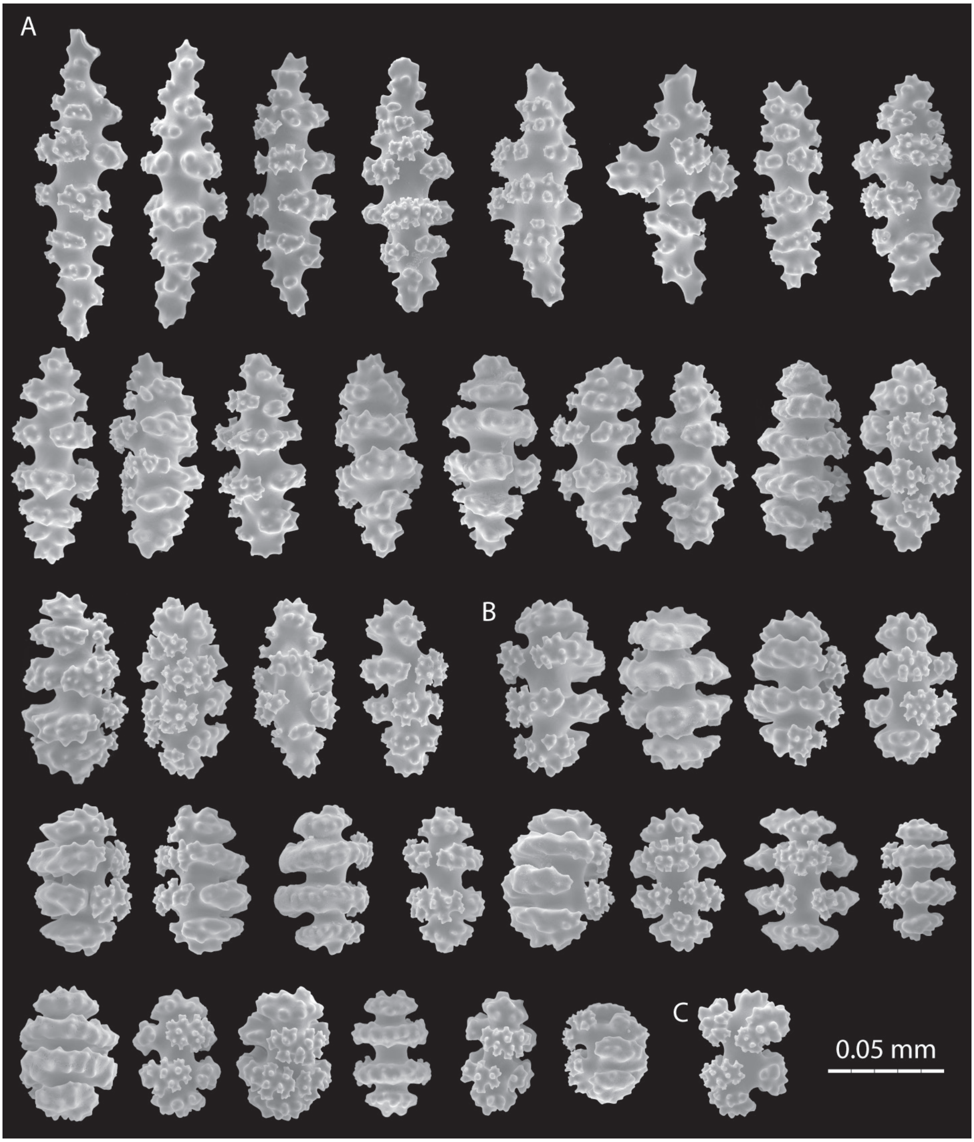

Figure 3. *Eugorgia beebei* (CASIZ 75783) holotype, SEM coenenchymal sclerites. **A** spindles and disc-spindles **B** double discs **C** cross.

Eugorgia siedenburgae and *E. rubens* form monospecific groups, they differ especially in the colony growth. The *rubens*-group have pink, sparse and laterally branched colonies, and the *siedenburgae*-group, have bushy, bicolored colonies (Breedy and Guzman 2013).

Eugorgia beebei and *E. siedenburgae* are very similar in sclerite content (Table 1), but they are different especially in the growth form and in the color. The conspicuous bushy colony immediately distinguished it from *E. beebei*; additionally, *E. beebei* has

Table 1. Comparative features of the new species, *E. beebei* sp. n. and *E. mutabilis* sp. n. within the *daniana*-group, the *rubens*-group, and the *siedenburgae*-group. Characteristics are based on the holotypes and lectotypes (Verrill 1868, Breedy et al. 2009, Breedy and Guzman 2013). Sclerite sizes represent the maximum length or the common range found in the samples. Measurements are given in mm.

Species	**Colony growth**	**Branching type**	**Max number. of branching**	**Pinna-like arr branchlets**	**Branchlet distance**	**Branchlet diameter**	**Branchlet length**	**Polyp distribution**	**Double disc**	**Capstans**	**Disc-spindle**	**Spindles**	**Bent spindles**	**Crosses**	**Anth. rods**	**Colour of colony**	**Bicolour colony**	**Coenenchymal sclerite colour**	**Colour rings**
E. aurantiaca (Horn, 1860)	fla	irr-pi	6	X	1.5–8	1–2.5	6–30	irr	0.04–0.07	0.07	no	0.11	X	0.06 × 0.06	not found	do, r		r, y	y
E. daniana Verrill, 1868	fla	irr-pi	7	X	1–4	1–1.5	1–15	irr	0.065–0.08	0.07	0.13	0.13	X	0.075 × 0.065	not found	r		r, y	
E. multifida Verrill, 1870	fla	irr-pi	7	X	1–4	1–1.5	1–10	reg	0.07	0.07	0.13	0.13	X	0.06 × 0.06	0.08 mm	do, r		r, y	
E. rubens Verrill, 1868	spa	lb	5	X	6–20	1.5–2	2–30	reg	0.06–0.07	no	0.10	0.10		no	not found	p		p	
E. siedenburgae Breedy & Guzman, 2013	bu	irr-pi	10	no	1–15	1–1.5	2–30	irr	0.08–0.05	0.07	0.11	0.11		0.08 × 0.07	not found	p,o	X	p,y	
E. mutabilis sp. n.	fla	irr-pi	7	X	1–4	1–2	1–10	irr	0.045–0.075	no	0.15	0.14		no	not found	w		w	
E. beebei sp. n.	spa	irr-pi	10	no	5–13	1–2.5	2–50	irr	0.07–0.06	no	0.14	0.14		0.08 × 0.06	not found	w		w	

Colony growth: bu, bushy; fla, planar growth, flabelliform; spa, sparse growth

Branching type: irr-pi, irregularly pinnate; lb, laterally branched

Polyp distribution: irr, arrangement mostly in irregular longitudinal rows; reg, arrangement mostly in regular longitudinal rows

Colors: dark orange (do), orange (o), pink (p), red (r), yellow (y), white (w)

X: character present

Blank space: character absent or not found thicker branches and branchlets than *E. siedenburgae*; the polyp mounds are pointed and higher in the latter, and are more rounded in *E. beebei*. Branchlets in *E. beebei* are longer than in *E. siedenburgae* (Table 1).

Remarks. We found the paratype in the MCZ (36106), labelled in Elisabeth Deichmann's handwriting (Ardis Johnston, pers. comm.) as a variety of *E. rubens*, however, she certainly had not published anything on this genus, thus the variety or the species was never established. The specimen was part of an MCZ public exhibition, the only data we have are the locality. It is probable that this specimen examined by Deichmann came from the Zaca expedition of 1937 and 1938. We do not consider *E. beebei* as a variety of *E. rubens* because they differ in the traits that have been shown to be informative to separate species in the genus: color, branching pattern, colony growth and sclerite content (see Table 1), as mentioned above.

Etymology. This new species of *Eugorgia* is named for explorer/naturalist William Beebe (1877–1962) who studied the marine fauna at numerous locations along the west coast of Central America from Mexico to Columbia during the Templeton Crocker *Zaca* expedition between 1937 and 1938. Beebe subsequently wrote the book, *Book of Bays*, which chronicles the five month expedition (Gould 2004).

Distribution. Presently known from Piura, Perú and Baja California, but it is very likely that it exist along all along the geographic range. The depth range is 50–60 m, it is possible the range could extends deeper, but not as deep as reported for paratype USNM 56879, which is probably a mistake, as was remarked by F. M. Bayer (former USNM curator).

***Eugorgia mutabilis* sp. n.**

http://zoobank.org/B552B4F0-50F4-4E16-BC00-5A3CB2C565C5
http://species-id.net/wiki/Eugorgia_mutabilis
Figs 4–6

Material examined. Holotype. UCR 2297, ethanol preserved, Burbujas, between Los Potreros and Playa Arenitas, Puerto Jiménez, Golfo Dulce, Costa Rica, 11 m, O. Breedy, 9 May 2013. **Paratypes.** UCR 2298, same data as the holotype. UCR 2272, 2276, fragments, ethanol preserved, Burbujas, 12 m, C. Sánchez, May 2012. UCR 2299, fragment, ethanol preserved, Roca Matapalo, Cabo Matapalo, Golfo Dulce, 20 m, O. Breedy, 6 February 2009. STRI 408, dry, Isla Seca Grande, Gulf of Chiriquí, Panamá, 20 m, H. Guzman, 26 August 2002. STRI 444, dry, Isla Jicarita, Gulf of Chiriquí, 20 m, H. Guzman, 29 August 2002. STRI 511, dry, Isla Ladrones, Gulf of Chiriquí, 15 m, H. Guzman, 14 April 2003. STRI 1073, Santa Cruz, Coiba Island, Panamá, 15 m, H. Guzman, 27 April 2007. STRI 1076, Twin Peaks, Coiba Island, 15 m, H. Guzman, 27 April 2007. STRI 1168, fragment, ethanol preserved, Bajo Hacha, Coiba Island, 20 m, O. Breedy, 16 April 2009. STRI 1122, ethanol preserved, La Blanca, Oxaca, Mexico, 46 m, R. Abeytia, 29 August 2004.

Type locality. Puerto Jiménez, Golfo Dulce, Costa Rica, 11 m.

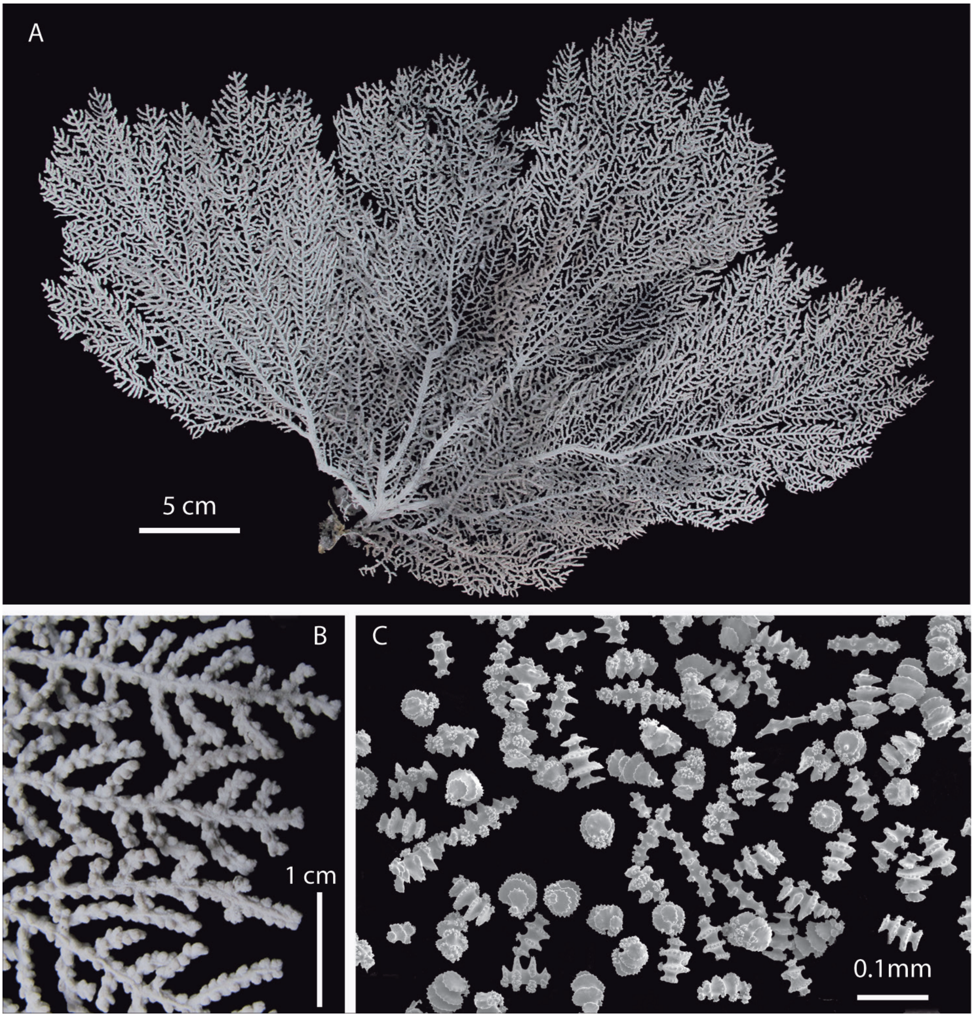

Figure 4. *Eugorgia mutabilis* (UCR 2297) holotype. **A** entire colony **B** detail of branches **C** SEM sclerites.

Diagnosis. Broad, stout, flabellate colony, main branches sinuous, branching irregularly pinnate, subdividing 5–7 times, no anastomosis present. Prominent polyp-mounds closely spaced and irregularly distributed around branches and branchlets (Figs. 4, 6A-C). Colony white, pale pink when alive (Fig. 6A–C), dark grayish when dry or ethanol preserved. Change in color after collection very conspicuous. Longitudinal grooves evident along branches and branchlets. Sclerites white. Spindles and disc-spindles up to 0.15 mm long, double discs mostly 0.05–0.08 mm long. Sclerite discs sharp, serrated and prominent. No anthocodial rods found.

Description. Holotype 30 cm tall, and 47 cm wide; colony broad, flabellate, very flexible. Branching irregularly pinnate. Main stem 6 mm diameter, laterally flattened, and short, 14 mm long. Holdfast oval, 40 mm diameter without polyps. Main stem subdividing in 5 sinuous main branches. Main branches slightly flattened on plane of colony, 3–4 mm in diameter emerging at angles of about 45°, bifurcating and diverging producing five flat pinnate fronds of long pinnate branchlets (Fig. 4A–B). Pinnae short, up to 8 mm long, and 1.5–2 mm diameter including polyp-mounds, close together 1–3 mm apart. Branching up to 7 times. Unbranched terminal twigs blunt, and reach up to 8 mm long (Fig. 4A–B). Longitudinal grooves distinct along branches and pinnate branchlets, evident in living and preserved specimens. Polyps white (Fig. 4B). Polyp-mounds prominent, up to 0.7 mm height and 0.8 mm in diameter, arranged mostly in lateral rows along the branchlets and separated by the longitudinal grooves, and more sparsely and irregularly distributed along the thicker branches (Fig. 4B). Colony white to pale pink when alive, gray to dark grayish in ethanol/dry preservation (Fig. 4A). Change in color very conspicuous possibly by liberation of black pigments after collection. Sclerites of coenenchyme white. Sclerite discs conspicuous mostly sharp, serrated and prominent (Fig. 4C, 5A–B). Disc-spindles 0.08–0.12 mm long, and up to 0.06 wide with 4–5 whorls of discs (Fig. 5B); spindles and disc-spindles, longer and thinner up to 0.15 mm long and 0.05 mm wide, with 5–7 whorls of warty tubercles, the ends acute, blunt, or both (Fig. 5A). Double discs up to 0.08 mm long, and 0.05 mm wide with prominent discs (Fig. 5B), some almost complete (Fig. 5B). No crosses, capstans or anthocodial sclerites present in samples.

Variability. The specimens present some variation in sclerite color, white sclerites being dominant, but some pale yellow hues could be observed in the samples. In all other aspects they agree with the holotype, including the change of color from bright white alive or recently collected to grayish when fixed. It is interesting that after collection, the specimens discharge a black pigment that turns the water black or the alcohol, and the colony becomes gray. The specimens from Mexico represent the deeper record; they have been observed down to 50 m, meaning that the range of depth extends from 40 to 50 m as it is for *E. rubens*, *E. siedenburgae*, and *E. beebei*, but the morphology of the colony and sclerites remain the same described for *E. mutabilis*. Current flow and depth are some of the environmental factors that could influence interspecific variability in octocorals (Fabricius and Alderslade 2001), but in this case no effect was observed. The morphology of the colony and sclerite content are persistent along the depth range.

Remarks. The species was mentioned before as a variety of *E. daniana*: 'a white variety has been observed in shoals in Costa Rica and Mexico occurring together with the red form' (Breedy et al. 2009). However, after examination of many specimens from various localities and depths, we found enough differences with respect to *E. daniana*, and to the other species in the group, especially in the color and the conspicuous sclerites, to establish *E. mutablilis* as a new species.

Habitat. The new species is found on rocky substrates, in general with other species of gorgonians, including *E. daniana*, but in some places, it is the only *Eugorgia* present (Fig. 6A–C). Other gorgonians found normally inhabiting the same localities are *Pacifigorgia irene*, *P. stenobrochis*, *Leptogorgia alba*, and *Carijoa riseii,* which were very abundant in the type locality. A variety of associated invertebrates were found

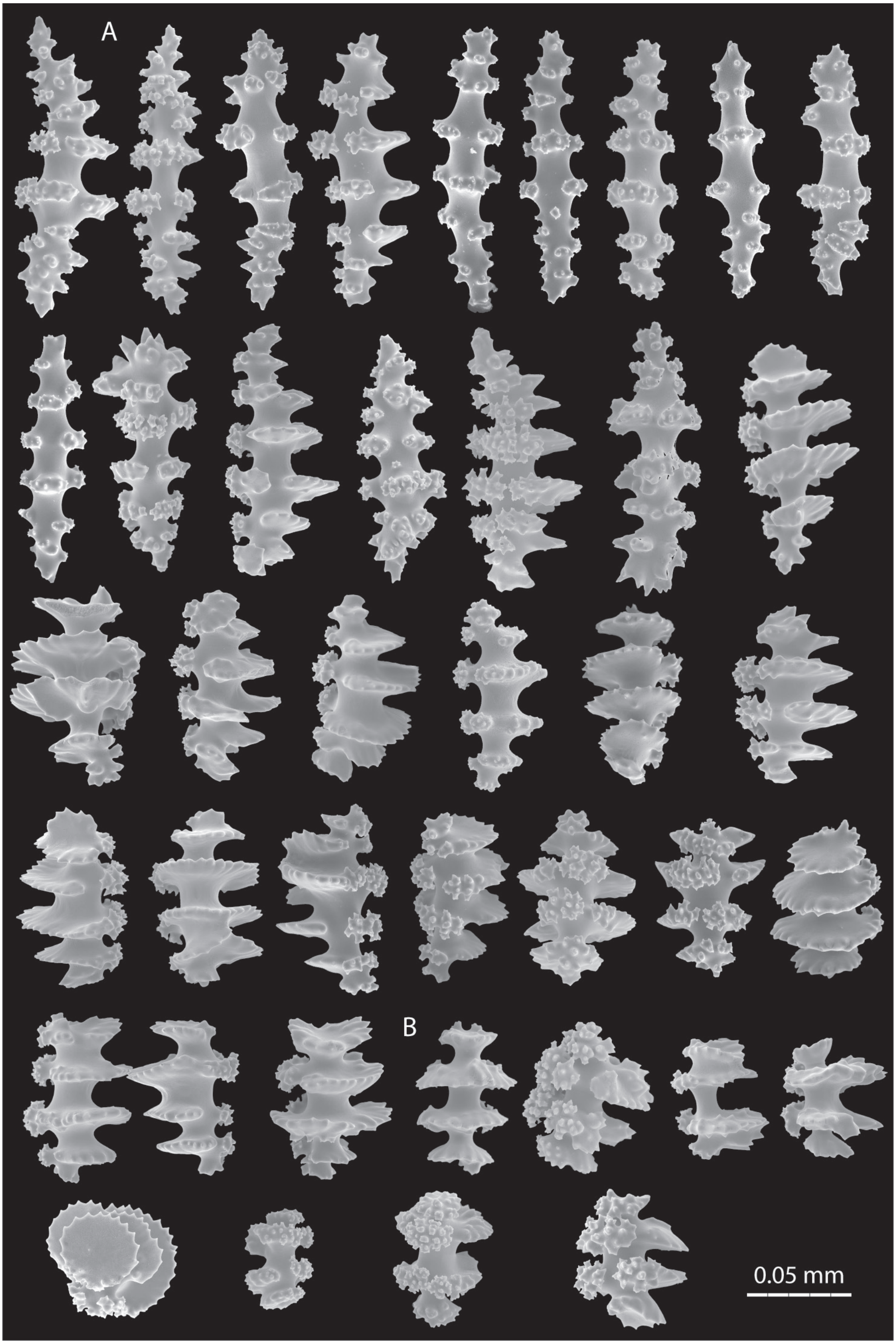

Figure 5. *Eugorgia mutabilis* (UCR 2297) holotype. SEM coenenchymal sclerites. **A, B** spindles and disc-spindles **C** double discs.

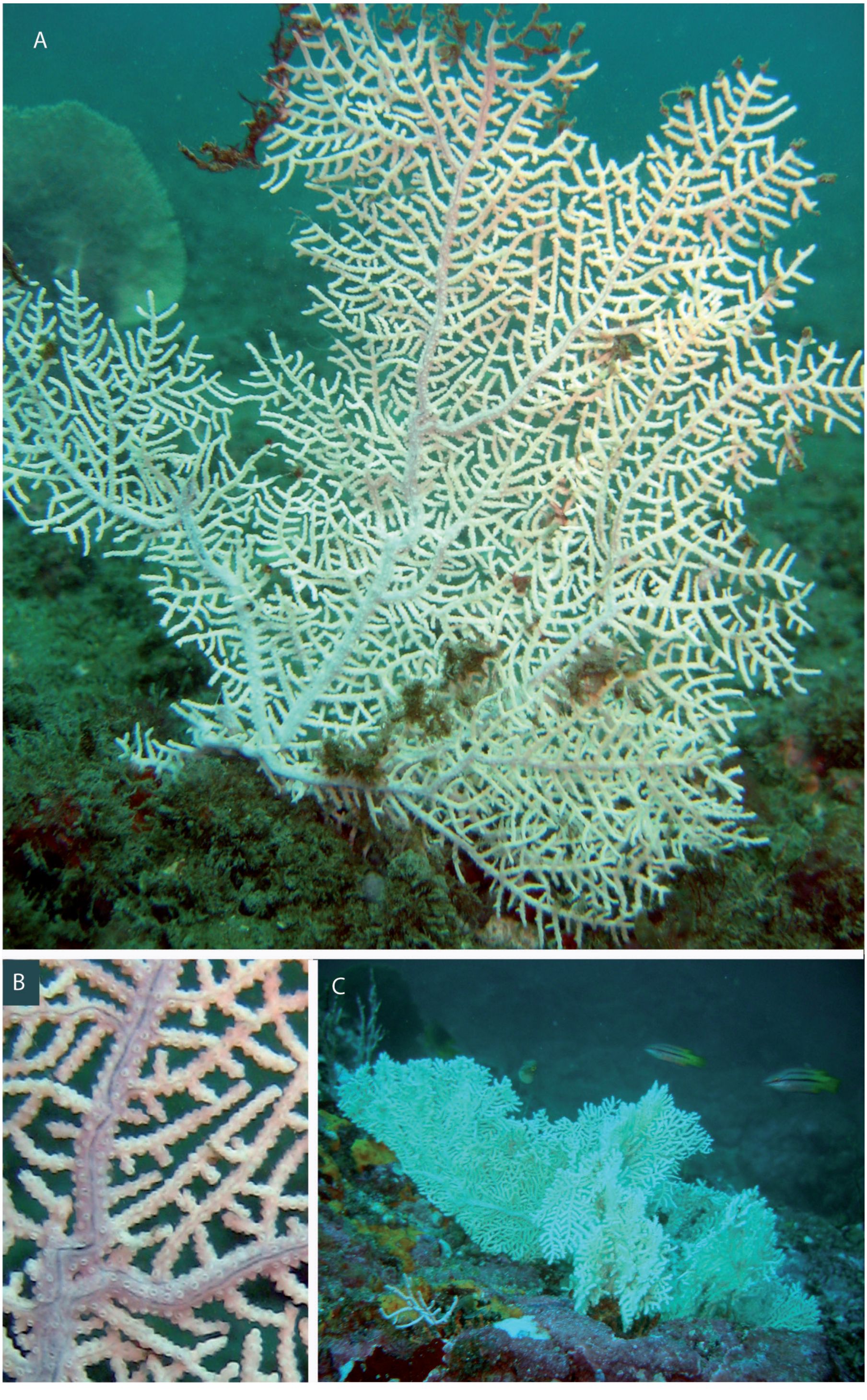

Figure 6. *Eugorgia mutabilis*, in situ colonies. **A, B** Burbujas, Puerto Jiménez, Golfo Dulce, Costa Rica, 12 m deep (photographs by C. Sánchez) **C** Montaña Rusa, Islas Contreras, Panamá, 32 m deep.

on the holotype and paratype UCR 2298, including ophiuroids, *Ophiotrhix* sp., and crustaceans, shrimps, *Periclimenaeus* sp. and abundant crabs, *Orthochela* sp.

Etymology. The specific epithet is from Latin, *mutablilis*, changeable, in allusion to the change in color after collecting.

Discussion. *Eugorgia mutabilis* belongs to the *daniana*-group with a characteristic flabellate colony composed of flat pinnate fronds, and irregular pinnate-branching pattern, and prominent polyp mounds. The white color of the colony and sclerites of *E. mutabilis* separates it from the rest of the group. However, the new species is similar to *E. daniana* in some features, e.g. maximum number of branches, branchlet distance, polyp distribution (see Table 1), but the sclerite composition is very different. The dominant sclerites in *E. mutabilis* have very sharp crested discs that are very consistent in all specimens revised from Mexico, Costa Rica and Panama, and along the depth range. These type of sclerites are distinct also from the ones in *E. beebei*, and in *E. siedenburgae*.

Distribution. Records from Costa Rica, México and Panamá suggest a wide distribution, at least from Mexico to Panamá, but this has to be further explored. The deepest record in Panama is 35 m, in Costa Rica 25 m, and in Mexico 50 m. Thus, the occurrence of this species from 11 to 50 m deep also suggests a large bathymetric range of distribution.

Final remarks

There are not many morphological characters to differentiate species in octocorals, normally the combination of growth form, and size and color of colony and sclerites are the features used for identification. However, as it was acknowledged above, colony shape can vary within species in response to environmental conditions, light availability, wave exposure and currents (Fabricius and Alderslade 2001). Thus, species delimitation are sometimes difficult to draw. For this reason, the study of a reasonable amount of samples and habitats will be of aid to decide about morphological species and varieties, especially when accessibility to collection sites is possible. However, in some cases, only old museum specimens are available but identification and recognition for biodiversity accounts is imperative.

Molecular studies have been taken to understand boundaries between species and interspecific or intraspecific phylogenetic relationships; however, a complete molecular phylogeny has not been achieved due to the lack of molecular markers with adequate resolution to distinguish species (or sometimes genera) (Cairns and Bayer 2005, McFadden et al. 2006, McFadden et al. 2010, Williams 2013, Wirshing et al. 2005).

Morphological phylogenetic studies in *Eugorgia* (Breedy et al. 2009) have shown that color of the colonies and sclerites, similar colony morphology, and ecological habits are significant characteristics to separate clades. These characters alone are not informative enough. The combination of the characters used to separate the new species that are analyzed here, showed consistency when compared with other related taxa in the genus.

Four groups of *Eugorgia* have been proposed for the eastern Pacific, four species in the *daniana*-group, one in the *rubens*-group, one in the *siedenburgae*-group, and eight in the *ampla*-group.

Although, *E. beebei* sp. n. was first mentioned as a variety of *E. rubens*, we have demonstrated that it is a different species that does not even fit in the *rubens*-group, or in the other related groups (*daniana*-, *siedenburgae*-). Thus, a new group is here proposed, the *beebei*-group characterised by white colonies and sclerites, and with ascending, sparse colony growth.

The diagnostic characters of the *daniana*-group are herein modified adding the white color for colony and sclerites, to include *E. mutabilis* sp. n. in the group. It is important to mention that occurrence of complete double discs sclerites in these species-groups is scant, and the closest example to this type are the ones in the new species. Actually, after examined many specimens especially, in the *daniana*-group, the occurrence of neat complete double discs is not frequent. It seems a more common character of the *ampla*-group. The recently described *Eugorgia ahorcadensis* Soler-Hurtado & López-González, 2012 from Ecuador should be placed in the latter group. However, there is not enough evidence to separate this species from *Eugorgia nobilis* Verrill, 1868, from which it represents a morphological variety, basically with longer branches and darker sclerites. Therefore, herein we synonymize it with *E. nobilis*.

Presently, a total of 15 valid species are recorded for the eastern Pacific but this number should increase when more geographic areas and bathymetric ranges are explored. This research is a contribution to the knowledge of the eastern Pacific octocoral biodiversity.

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Specimens from Costa Rica were collected under the MINAE 0890 permit, and from Panama under the permits: DAPVS-02-2007, DAPVS-01-2008, SE/A-71-12, SE/A-16-13 of the Panama Environmental Authority.

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