

A new substance to relax polychaete worms (Annelida) prior to morphological study

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Abstract

A variety of chemical substances have been used to relax and/or immobilize polychaete worms, and other invertebrates, prior to specimen preparation for morphological examination. To solve difficulties encountered during the study of nereidid polychaetes (Annelida: Phyllodocida), an experiment was designed and carried out to investigate a new relaxing agent to immobilize nereidid specimens and stimulate pharynx eversion. The new substance, Dentol® (Khoraman laboratory, Iran), a dental anesthetic and antiseptic medicine containing 10% Carvacrol as the effective ingredient, was used for the first time and compared with other substances that have been used traditionally in polychaete studies. Crosstab analysis showed significant differences between different treatment groups, with Dentol® providing much better results for all considered criteria.

Keywords

Carvacrol, narcotize, Polychaeta, specimen preparation

Introduction

In order to properly examine nereidid polychaetes, and other Phyllodocida (Annelida), they first should be relaxed, to expose their eversible pharyngeal organ, and in the case of nereidids their pharyngeal armature (paragnaths and/or papillae) and jaws. Anesthetization facilitates pharyngeal organ eversion (Oliveira et al. 2010). Various anesthetics, including Magnesium Chloride (Fauchald 1977; Rouse and Pleijel 2001), Menthol crystals (Coutinho and Santos 2014; Glasby and Hsieh 2006; Russell 1963), MS222 (Tricaine Methane Sulphonate) (Clark 1961; Fauchald 1977), Phenoxethanol (Hsieh and Li 1998), Ethyl Alcohol (Russell 1963), and various techniques such as cooling the worms (Costa-Paiva et al. 2007), have been applied to immobilize and/or relax polychaetes. The benefits of anesthetization include reducing the risk of morphological damage (e.g., loss of body segments and parapodial appendages) during fixation, and slowing the specimen down to enable morphological examination of live specimens and photography. Disadvantages include the additional time required for sample preparation (time of relaxation varies with taxa from minutes to hours), and the possibility of distortion of morphological features. For example, inadequate fixation methods or using different anesthetics have resulted in erroneous taxonomical observations of *Laeonereis* (Oliveira et al. 2010) and sabellid polychaetes (Costa-Paiva et al. 2007). A suitable anesthetic substance should be safe, environmentally friendly, readily available, easy to use, economically feasible, effective, efficient, and without any morphological modifications resulting from over-relaxation, for example (Costa-Paiva et al. 2007, Williams and Van Syoc 2007).

During a survey of nereidids of the Persian Gulf, we encountered some difficulties concerning anesthetic substances, mainly poor results for pharyngeal eversion and a long elapsed-time to relaxation. Most previous studies concerning anaesthetization methods are about the maintenance of living animals for transportation rather than preparation for morphological studies (Oliveira et al. 2010). Consequently, in search for better results, we designed a study to find a more effective, efficient and readily available anesthetic agent to relax specimens and stimulate nereidids to evert their pharynx. In this study we compare three conventional substances previously used in other studies (Beesley et al. 2000; Fauchald 1977; Russell 1963) with a new substance, Dentol[®], a dental anesthetic and antiseptic medicine containing 10% pure Carvacrol; Although the availability of Dentol[®] outside of Iran has not been determined, Carvacrol at least is present in different concentrations in oil of oregano, oil of thyme, oil obtained from pepperwort, and wild bergamot and many other plant extracts, available worldwide (see Discussion).

Material and methods

Nereidid worms were collected from the intertidal zone of two islands, Qeshm (26°58'17"N, 56°15'32"E) and Hengam (27°03'01"N, 56°29'58"E), west of the Strait of Hormuz, the Persian Gulf.

A total of 60 specimens of different species were randomly separated into four equal groups, each containing 15 specimens. The first group was administered with 8% MgCl_2 in seawater, the second group with 8% MgSO_4 in seawater, the third group with 8% Menthol crystals in sea water, and the fourth group with 8% Dentol® (10% pure Carvacrol, or cymophenol, $\text{C}_6\text{H}_3\text{CH}_3(\text{OH})(\text{C}_3\text{H}_7)$, a monoterpenoid phenol) in seawater. Specimens in each group were exposed to the relevant substance and the time to immobilization recorded. After 30 minutes, specimens in each treatment group were fixed, separately, in 5% formaldehyde diluted in seawater, for 24h. After fixation, specimens were washed in tap water and transferred into 70% ethyl alcohol for storage. Prepared specimens were studied under a stereo microscope, to compare how the different treatments affected morphology.

In all groups, based on the amount of proboscis eversion, specimens were classified into three categories, not-everted (Figure 1a), partially-everted (Figure 1b), and fully-everted (Figure 1c). Specimens in each category were counted, their length and width were measured, to the nearest millimeter, and then weighed individually to the nearest milligram. Measurements were entered into SPSS (ver. 22) and statistical analyses including crosstab, chi-square, t-test and ANOVA were carried out.

Results

Mean and standard deviation for length, width, and weight of specimens for each treatment group were measured to the nearest millimeter. ANOVA analysis showed no significant difference between the four treatment groups for length ($F=2.011$, $df=3$, $P=0.052$), width ($F=0.892$, $df=3$, $P=0.451$), and weight ($F=1.686$, $df=3$, $P=0.180$) (Table 1).

Examining the number of specimens with an everted pharynx for each treatment showed that Dentol® treated worms had 15 specimens (100%) with a fully everted

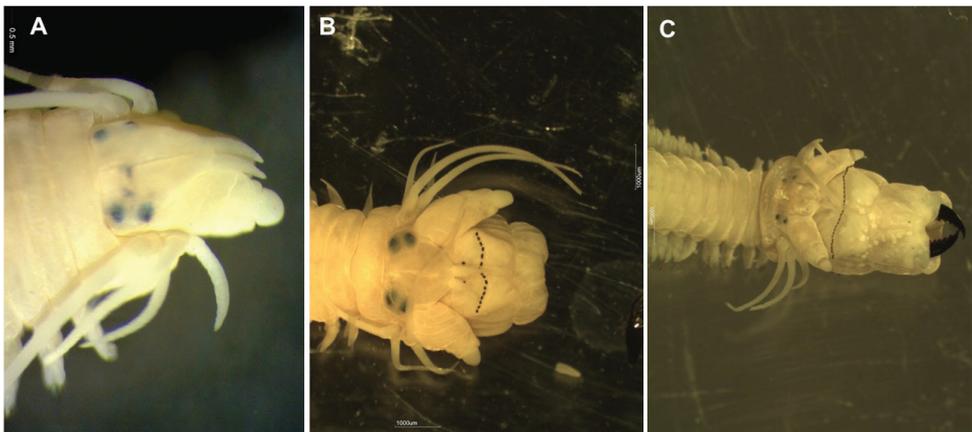


Figure 1. Categorization of nereidid worms based on the amount of proboscis eversion, after anaesthetic applications; not-everted (a), partially-everted (b), and fully-everted (c).

Table 1. Descriptive statistics, ANOVA and crosstab analysis for the effect of different substances on pharynx eversion in the four treatment groups.

Treatments	Number of specimens		Length (mm)		Width (mm)		Weight (gr)		ANOVA			Everted No. (percentage)		Crosstab	
	M	SD	M	SD	M	SD	M	SD	length	width	weight	Not-everted	Partly-everted		Fully-Everted
Dentol	15	61.21	15.87	3.69	0.58	0.125	0.058					0	0	15 (100%)	
Menthol	15	73.00	16.69	3.43	0.45	0.164	0.054	F=2.011, df=3, P=0.052	F=0.892, df=3, P=0.451	F=1.686, df=3, P=0.180	15 (100%)	0	0	$\chi^2=78.462$, df=6, P<0.001	
MgCl ₂	15	77.19	16.75	3.55	0.44	0.153	0.045				15 (100%)	0	0		
MgSO ₄	15	60.71	15.15	3.71	0.63	0.165	0.062				9 (60%)	6 (40%)	0		

pharynx. By comparison, Menthol and $MgCl_2$ treatments had no specimens with an everted pharynx after a 30 minute exposure period. In the $MgSO_4$ treatment group nine specimens (60%) had an uneverted pharynx, while six specimens (40%) had a partially-everted pharynx. Crosstab analysis showed significant differences between different treatment groups in the number of everted pharynges ($\chi^2=78.462$, $df=6$, $P<0.001$) (Table 1). Further, specimens of the Dentol® treated group became motionless faster than those with other treatments (less than one minute compared to more than one minute).

Discussion

Most polychaetes have an eversible pharynx, the anterior part of digestive tract (Fauchald 1977), which has taxonomically useful features on its surface. Members of Nereididae have small horny teeth (paragnaths) and/or papillae whose various shapes, patterns and numbers can be used in generic and species identification (Bakken et al. 2009; Fauchald 1977; Uschakov 1955). The problem, however, is that the pharynx is not readily everted and its taxonomically-useful characteristics are often not visible in preserved specimens. In these cases dissection with iridology scissors is the only means to reveal and observe the pharyngeal characters; however, specimens less than about 1 mm are impossible to dissect even with the finest scissors. As a result, different substances have been used to facilitate pharynx eversion. The most frequently used substance, mentioned in scientific papers, is 7–8% Magnesium Chloride ($MgCl_2$) in seawater ($MgCl_2 \cdot 6H_2O$) (Bonyadi and Rahimian 2009; Darbyshire 2014; Fauchald 1977; Williams and Van Syoc 2007), although Rouse and Fauchald (1997) recommend dilution in fresh water. Time to relaxation is at least 30 minutes on small-sized worms. Magnesium chloride works because Magnesium ions can relax muscles through direct action on cell membranes; Mg^{2+} ions block certain types of Calcium channels, which conduct a positively charged Calcium ion into neurons. An excess of Magnesium, causes more channels to be blocked resulting in nerve cells having less activity (Slutsky et al. 2010), hence muscle relaxation and pharynx eversion. Menthol crystals are a slower acting anesthetic, needing about 12 hours (Wilson et al. 2003) to take the desired effect. Menthol works by blocking voltage-sensitive sodium channels, hence reducing neural activity that may stimulate muscles (Haeseler et al. 2002). MS-222, Tricaine methanesulfonate (a chemical commonly used for anesthesia, sedation, or euthanasia of fish) (Wayson 1976), has also been used to relax polychaetes prior to examination (Fauchald 1977; Fidalgo e Costa 1999). MS-222 can simultaneously block pain sensation, paralyze the animal by muscle relaxation and exert a general anesthesia by blocking Na^+ -conductance of cellular elements comprising the neuromuscular system (brain and muscles) (Ramlochansingh et al. 2014). A 0.05 percent solution of MS-222 in sea-water completely anesthetizes medium-sized worms within 5–7 minutes (Clark 1961). Other substances including Phenoxyethanol (McKay and Hartzband 1970), Ethyl Alcohol 70% (Russell 1963), and Clove oil (Hutchings and Glasby 1999; Rouse and Fauchald 1997) have also been used to evert a polychaete's

pharynx, but much less frequently. Finally, fast acting anesthetics are preferable in general, in order that the integrity and quality of the specimens is maintained, particularly in tropical areas where delays can result in specimen degradation.

Dentol[®], contains 10% pure Carvacrol, an essential oil, or terpene, present in various aromatic plants. Carvacrol is the main oil constituent (86%) in *Satureja khuzistanica* Jamzad (Marzeh khuzestani in Persian, family Lamiaceae). *S. khuzistanica* is an endemic plant widely distributed in the southern parts of Iran (Jamzad 1994) and has been traditionally used as an analgesic and antiseptic agent (Farsam et al. 2004). The effective agent of this plant, Carvacrol, has anti-inflammatory, antinociceptive, antidiabetic, and antioxidant properties (Baser 2008; Clark 1961; Rahman 2013). Moreover, the pharmacological inhibition of smooth muscular activity has been attributed to the prostaglandin inhibition effects of Carvacrol (Kintzios 2003).

Carvacrol is also present in two other well-known, herbaceous plant derived oils, although in lesser amounts: Oregano Oil (*Oreganum compactum*, 50%), and Clove Oil (*Eugenia caryophyllata*, <1%) (Charai et al. 1996; Chaieb et al. 2007). Since Carvacrol is present in all three plant extracts, a comparison of their anesthetic effects on polychaetes would be useful, and may assist in determining whether Carvacrol itself, or one of the other aromatic oils, or perhaps a synergistic combination of oils, is responsible for the anesthetic effect. One possible disadvantage of the use of essential oils for anesthetization is that specimens may be coated with an oily film that does not dissolve readily in ethanol preservative and so could be problematic especially in molecular studies; this possibility, however, needs to be investigated.

Results of the present experiment showed significant differences between different substances used to narcotize/relax polychaete worms. In examining polychaete worms it is important not only to immobilize the specimen but also to have its pharynx everted to be able to observe its taxonomically important characteristics.

Like Nereididae, other members of Phyllodocida have a symmetrical axial pharynx with a strong muscular region that is often protractible (Dales 1962; Rouse and Fauchald 1997). Therefore Dentol[®] may be an effective anesthetizing agent for other Phyllodocida families, such as Hesionidae, Pilargidae, Syllidae, etc., although the precise details of their response would need to be examined and compared to that in Nereididae.

The mechanism of pharynx eversion appears to be the same in all groups of nereidids though there are differences in detail (Dales 1962), and knowing that samples tested included different species, it is likely that some of the variation between treatments could be the result of variability between species.

One ‘manual’ way to achieve pharynx eversion is to anesthetize the worm and then encourage them to evert the pharynx by stimulating, pressing or otherwise forcing it to eject the pharynx. That, however, is not an easy task requiring considerable microscopic manipulation skills, and is time consuming particularly if many specimens are to be treated. Using Dentol[®] has the advantages of both immobilizing the specimen (for example for photography) and everting the pharynx, both in a short time. This substance, and/or its active ingredient Carvacrol, is effective, safe, reasonably cheap, and usually easy to obtain in pharmacies in many countries around the world.

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A new family Lepidocharontidae with description of *Lepidocharon* gen. n., from the Great Barrier Reef, Australia, and redefinition of the Microparasellidae (Isopoda, Asellota)

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Abstract

Lepidocharontidae Galassi & Bruce, **fam. n.** is erected, containing *Lepidocharon* Galassi & Bruce, **gen. n.** and two genera transferred from the family Microparasellidae Karaman, 1934: *Microcharon* Karaman, 1934 and *Janinella* Albuquerque, Boulanouar & Coineau, 2014. The genus *Angeliiera* Chappuis & Delamare Deboutteville, 1952 is placed as genus *incertae sedis* in this family. The Lepidocharontidae is characterised by having rectangular or trapezoidal somites in dorsal view, a single free pleonite, a tendency to reduction of the coxal plates, and the unique uropodal morphology of a large and long uropodal protopod on which the slender uropodal exopod articulates separately and anteriorly to the endopod. *Lepidocharon* Galassi & Bruce, **gen. n.** has a 6-segmented antennula, a well-developed antennal scale (rudimentary exopod), long and slender pereopods 1–7 directed outwards, coxal plates rudimentary, incorporated to the lateral side of the sternites, not discernible in dorsal view, the single pleonite narrower than pereionite 7, scale-like elements bordering the proximal part of male pleopod 1 on posterior side, and stylet-guiding grooves of male pleopod 1 which run parallel to the outer lateral margins of the same pleopod. *Lepidocharon priapus* Galassi & Bruce, **sp. n.**, type species for the genus, and *Lepidocharon lizardensis* Galassi & Bruce, **sp. n.**

are described from Lizard Island, northern Great Barrier Reef. The most similar genus is *Microcharon*, both genera sharing the same general organization of the male pleopods 1 and 2, topology and architecture of the stylet-guiding groove of male pleopod 1, morphology of female operculum, presence of 2 robust claws of different lengths on pereopodal dactylus 1–7, not sexually dimorphic. *Lepidocharon* **gen. n.** differs from *Microcharon* in the shape of the pereionites, very reduced coxal plates, the presence of imbricate scale-like elements bordering the proximal postero-lateral margins of the male pleopod 1, and the topology of the pereopods, which are ventro-laterally inserted and directed outwards in *Lepidocharon* **gen. n.** and dorso-laterally inserted and directed ventrally in *Microcharon*. *Lepidocharon* shares with the genus *Janinella* the morphology of the tergites and the reduced *lacinia mobilis* of the left mandible, but differs significantly from *Janinella* in having a well-developed antennal scale, very reduced coxal plates also in females bearing oostegites, the general morphology and spatial arrangement of the stylet-guiding groove of male pleopod 1 and the possession of a 6-segmented antennula. The family Microparasellidae is redefined as monotypic, the only genus being *Microparasellus* Karaman, 1933.

Keywords

Isopoda, Microparasellidae, Lepidocharontidae new family, *Lepidocharon* new genus, coral reef, Australia

Introduction

Kensley (2001) stated that 84% of the species are endemic to the Indian Ocean overall, but only 18% of the genera. This level of endemism is generally true for free-living marine Isopoda, and consistent with that observed in the Great Barrier Reef isopod fauna. Data for coral-reef Asellota are few and at present all Great Barrier Reef Asellota are endemic at species level and genus level with the only apparent endemic genus, *Prethura* Kensley, 1982 having been recently reported from Japan (Shimomura and Naruse 2015). Species-level endemism is high among marine isopods, but generic endemism is generally low, so the discovery that the two new species from Great Barrier Reef belong to a new genus is noteworthy.

The marine isopod fauna of Queensland is diverse with 343 recorded species. Of that total only 22 species (6.4%) are Asellota, the suborder clearly being under-documented for the region as worldwide (Poore and Bruce 2012, Schotte et al. 2013). They constitute approximately 33% of all isopod species. Of those 22 species, 16 have been described since 2009 (Bruce 2009, Shimomura and Bruce 2012, Bruce and Buxton 2013, Bruce and Cumming 2015) highlighting the potentially high diversity of the suborder (Roberts et al. 2002). Tropical regions from northern and western Australia have an even lower level of recorded species. Given the low level of reporting from the region it is unsurprising therefore to discover both new genera and also families not previously recorded from tropical Australia. The discovery of what appeared to be the first marine Microparasellidae Karaman, 1934 from the Great Barrier Reef (GBR) and Australia was in itself not surprising and furthermore the family was known from coral reefs in New Caledonia (Coineau 1970) and the Caribbean (Kensley 1984).

Describing what proved to be a new genus of Asellota led to a reappraisal of the Microparasellidae, and to the conclusion that the family should be split, with the new genus being placed in the Lepidocharontidae Galassi & Bruce, fam. n. A further three

species of Lepidocharontidae have been collected from the Great Barrier Reef, including Heron Island in the south, these being undescribed species of *Microcharon* and *Lepidocharon*, respectively, and a distinctive new species of uncertain generic identity, yielding a total of at least five species in three genera for the GBR. The presence of this number of species suggests that with appropriate collecting in suitable habitats this family may be more diverse in marine habitats than previously believed.

Methods

Collection methods have recently been described by Bruce (2015) and Bruce and Buxton (2013). CReefs samples were all preserved in high-grade ethanol (a requirement of the CReefs program), without first fixing in formalin. Drawings and measurements were made using a camera lucida on a Leica DM 2500 phase contrast and interferential microscope. Some details gained from scanning electron microscopy (SEM) are added to line drawings. Scanning Electron Microscope specimens of *Lepidocharon priapus* sp. n. were dehydrated in a graded ethanol series, critical point dried in a Balzers Union CPD 020 apparatus and sputter coated with gold in a Balzers Union SCD 040. Observations were made with a Philips SEM XL30 CP scanning electron microscope.

Sampling was carried out under GBRMPA Permit G08-27858.1 and General Fisheries Permit (QLD DPI) 95152.

The type material is deposited in the Museum of Tropical Queensland, Australia (MTQ).

Results

Taxonomy

Asellota Latreille, 1803

Janiroidea Sars, 1897

Lepidocharontidae Galassi & Bruce, fam. n.

<http://zoobank.org/B21B701E-73AE-4433-84C3-1EA34618814B>

Diagnosis. Male. Body dorsally flat, slender, $\sim 4\text{--}10\times$ long as wide, without chromatophores; somites all subsimilar in width, somites sub-rectangular or trapezoidal, lateral margins of head and pereionites sub-parallel. Pleon of one segment, with free lateral margins. Head with weak or absent rostrum, without pseudorostrum. Eyes absent. Antennula with maximally 4 flagellar articles. Antenna flagellum longer than podomeres. Antennal scale (rudimentary exopod) present, even if more or less developed among genera. Mandible incisor with 2 to 8 cusps; molar process subconical, without grinding surface, with apical unequal smooth and pinnate setae; spine row and *lacinia mobilis*

present, the latter only on left mandible. Maxilliped slender, covering entire mouthpart field, endite distal margin narrowly rounded; epipod slender, quadrate or distally acute; palp composed of 5 articles; 2 stiff pectinate setae always present on maxilliped distal article. Pereiopods 1–7 subsimilar, always without subchela; all pereiopods with 2 dactylar claws; pereiopods articulating dorso-laterally or laterally, and projecting ventrally (in *Janinella* and *Microcharon*) or outwards (in *Lepidocharon*). Penial processes with openings coalescent and medial. Male pleopods 1 and 2 not operculate; male pleopod 1 distally rounded or subtruncate, with or without acute distolateral lobes; proximal part of the pleopod with or without scale-like elements on postero-lateral margins; stylet-guiding grooves running parallel to the lateral free distal margin of pleopod 1 and folded by a hyaline lamella (transversal and unfolded in *Janinella*); pleopod 3 endopod with 3 plumose setae (marine taxa) or without plumose setae (freshwater taxa), exopod slender. Pleopod 4 globular, pleopod 5 absent. Uropods biramous, ventrally inserted on pleotelson, protopod large, *c.* 0.5–1.3 as long as pleotelson; protopod length/width ratio *c.* 2.5–4.5; rami slender with exopod articulating anteriorly to endopod. Anus terminal, not covered by pleopods. Anus outside pleopodal chamber, between bases of uropodal protopods.

Female. Operculum (pleopod 2) from sub-quadrate (as long as wide) to more than 2 times longer than wide, with free distal margin deeply incised medially, faintly incised, or without medial incision, armed with 4 or 2 setae, or unarmed.

Genera included. *Microcharon* Karaman, 1934; *Janinella* Albuquerque, Boulanouar & Coineau, 2014; *Lepidocharon* Galassi & Bruce, gen. n.

Genus *incertae sedis*. *Angeliara* Chappuis & Delamare Deboutteville, 1952.

Remarks. Wilson and Wägele (1994) and Wilson (1994) critically discussed the status of the Microparasellidae in relation to the Janiridae on a cladistic phylogenetic basis. Wilson and Wägele (1994) in their review of the Janiridae analysed the status of the family Microparasellidae in detail, reviewing the history of the debate over the status of the family. Wilson (1994) also included the Microparasellidae in his cladistic analysis of the phylogeny of the Janiridae. Wilson and Wägele (1994: page 721) stated “*The family concept of the Microparasellidae may be open to challenge because Microparasellus is distinct from the other three genera in these autapomorphies*”, these being the differences in the somatic and uropodal morphology. These authors went on to say “*the composition of this family will require further study.*” In Wilson’s (1994) analysis, there were no supporting apomorphies for the Microparasellidae as then constituted, but there were separate supporting apomorphies for the genus *Microparasellus* and the clade holding the remaining genera, strongly suggesting that potentially these were two monophyletic clades, albeit the Microparasellidae being monogeneric. The principle basis for this is that each group had unique and derived uropod morphology and substantial differences in body morphology.

The description of the new genus *Lepidocharon* Galassi & Bruce, gen. n. led to a reappraisal of the taxonomic status of the Microparasellidae and its constituent genera. We conclude that the Microparasellidae is a mono-generic family supported by a prominent acute or narrowly rounded rostrum, the antennal flagellum shorter than podomeres,

all somites with straight lateral margins that also have scales, an indisputable ventral position of the pereopods, the unique uniramous and short uropods (see Appendix 1). The remaining genera are housed in the new family Lepidocharontidae fam. n., the diagnostic characters being the elongate body (up to 10× as long as maximum width), a weak or absent rostrum, the antennal flagellum longer than podomeres, the pereionites rectangular or trapezoidal in dorsal view, with sub-parallel lateral margins, a lateral or dorso-lateral position of the pereopods, a tendency to reduction of pereopodal coxal plates, and the uropod with a large protopod with the exopod articulating anteriorly and separately to the endopod.

Within the family Lepidocharontidae there is great uniformity of the diagnostic characters among all the genera. The genus *Angeliara* has been placed *incertae sedis* in the Asellota on the basis of marked differences in several morphological features that set this genus far away the basic body plan observed in Lepidocharontidae.

The largest genus in the Lepidocharontidae is *Microcharon* with 77 species, both marine and freshwater. Many species lack full descriptions, and there are inconsistencies in the distribution of certain characters within the genus. A dorsal view of the head is not routinely figured; when figured, it can be seen that some species do have a rostral point or rostrum, while others have the anterior margin of the head weakly concave.

The generic name *Microcharon* is unavailable under the ICZN (1999)'s rules because the genus was established by Karaman (1934) without type species designation. This prevents the use of "Microcharontidae" (from the most speciose and well-known genus *Microcharon*) as the family name because ICZN (1999)' article 13.2 unambiguously states: "To be available, every new family-group name published after 1930 must satisfy the provisions of Article 13.1 and must be formed from an available genus-group name then used as valid by the author in the family-group taxon [Arts. 11.7.1.1, 29]". Therefore we here propose the name Lepidocharontidae fam. n.

The family name Microparasellidae was first introduced by Karaman (1934: page 44) when describing the genus *Microcharon*, although the family had been earlier diagnosed by Karaman (1933: page 17) with the accompanying statement "Microparasellus n. fam., n. gen.", but without type-species designation for the genus *Microparasellus* (see Karaman 1933). According to the ICZN (1999: Article 13.2) the family name Microparasellidae proposed by Karaman (1934) is thus a *nomen nudum*, because the family was erected on the unavailable generic name *Microparasellus* Karaman, 1933 that lacked type-species designation. Nevertheless, Article 13.2.1 states that "A family group name first published after 1930 and before 1961 which does not satisfy the provisions of Article 13.1 is available from its original publication only if it was used as valid before 2000, and also was not rejected by an author who, after 1960 and before 2000, expressly applied Article 13 of the then current editions of the Code". The family name was considered valid until 2000, and for this reason it is an available name as Microparasellidae Karaman, 1934. The family name Microparasellidae is then valid.

Conversely, we provisionally maintain current and common usage of the names *Microparasellus* and *Microcharon*, and this is discussed in more detail together with a new diagnosis for the Microparasellidae (see Appendix 1). As the nomenclature within

the family Microparasellidae is well established and widely used, a proposition (Galassi and Bruce in preparation) will be submitted to the ICZN Commission for maintaining the stability of the current nomenclature and related authorities.

Key to Microparasellidae and genera of Lepidocharontidae fam. n.

- 1 Uropods uniramous, short; antennal podomeres longer than flagellum, scale missing; head with prominent rostrum...**Microparasellidae** (*Microparasellus*)
- Uropods biramous, large; antennal podomeres shorter than flagellum; rostrum small or absent **2**
- 2 Pereionites 1–7 cylindrical, free pleonite as wide as pereionite 7**Microcharon**
- Pereionites 1–7 dorsally flat and trapezoidal, except pereionite 4; free pleonite narrower than pereionite 7 **4**
- 3 Male pleopod 1 with transverse stylet-guiding grooves, unfolded; proximal postero-lateral margins of male pleopod 1 without scale-like elements
..... **Janinella**
- Male pleopod 1 with distal stylet-guiding grooves parallel to the lateral margins, folded by hyaline lamella posteriorly; proximal lateral margins of male pleopod 1 armed with scale-like elements **Lepidocharon gen. n.**

Lepidocharon Galassi & Bruce, gen. n.

<http://zoobank.org/6263F663-8F45-43A2-8AFC-678DE31BD450>

Type species. *Lepidocharon priapus* Galassi & Bruce, sp. n.; here designated.

Other species. *Lepidocharon lizardensis* Galassi & Bruce, sp. n.

Diagnosis. Male. Body slender, 8.5–9.7 as long as wide. Free pleonite narrower than pereionites and pleotelson, visible in dorsal view. Cephalon medio-frontal margin not produced, anterior margin straight, rostrum absent. Pereionites 1–3 anteriorly widest, with distinct anterolateral angle, pereionite 4 sub-rectangular, pereionites 5–7 posteriorly widest, lateral margin with distinct posterolateral angle. Pereionites dorsally ornamented by paired setae. Cuticle with small semicircular thickening present or absent both dorsally and ventrally. Antennula 6-segmented; long aesthetascs on articles 5 and 6, long brush seta on article 2 extending to tip of article 6. Antenna with 6 podomeres; article 3 with long blade-like or candle flame-like scale, reaching article 5; lateral margin with 2 setae; flagellum with 8–12 articles.

Mandible palp article 1 unarmed, article 2 with 2 stiff spinulose setae, article 3 with 5 stiff spinulose setae. Right mandible: incisor with 6 to 9 cusps; *lacinia mobilis* absent; molar process conical, with 3 setae. Left mandible: incisor with 2 to 3 cusps; *lacinia mobilis* present and produced in 2 cusps; molar process conical, with 3 setae. Maxillula: mesial lobe slender and tapering at distal part, bearing 1 short apical seta accompanied by subapical shorter setae and lateral thin and short setae. Lateral lobe sub-rectangular in shape. Apical setation composed by a variable number of setae.

Maxilla: mesial ramus with 8–9 setae; 1 apical comb-like seta, strong, unipinnate and ornamented with fine regularly-spaced setules parallel to one another. Lateral rami close-set, each bearing 4 slender and simple setae, respectively. Maxilliped palp wider than endite, mesial margin of articles 2 and 3 expanded. Pereiopods all subequal in length, all subsimilar in size and general morphology; all with 2 dactylar claws; pereiopod 1 dactylar claws subequal; pereiopods 2–7 superior claw slender (3.8–4.3× basal width), inferior claw robust (2.3–2.6× basal width). Coxae rudimentary reduced to small sclerites, not discernible in dorsal view, coalescent to body wall of the sternites, located on the anterior margin of the concavity which houses the propodus, and apparently not articulated to the sternites.

Pleotelson 1.3–1.7 as long as wide, 1.7–1.9 as long as pereionite 7. Penial papillae opening on postero-medial margin of sternite 7. Pleopod 1 rami proximally fused; proximolateral margins with cuticular imbricate scales on posterior side; distolateral margins convex; stylet-guiding groove represented by a folded hyaline lamella running sub-parallel to free lateral margin of rami; pleopod 1 transverse stylet-guiding grooves absent, unlike *Janinella*. Pleopod 2 stylet long and slender, of variable length. Pleopod 3 endopod bearing 3 distal plumose setae; exopod elongate, lateral margin with thin setae, article 2 with 1 subapical seta; pleopod 4 rudimentary, ovoid, uniramous. Uropodal protopod as long as the pleotelson, slender (length/width ratio: 4.3), not sexually dimorphic, with long and slender exopod and endopod.

Female. As for the male, except for sexual characters. Operculum (pleopod 2) longer than broad, with surface smooth or with semicircular thickening, with distal margin faintly incised, and 4 apical setae.

Etymology. The generic name is derived from the ancient Greek name *λεπις*, *λεπιδος* meaning “scale”, which refers to the unique rim of scale-like elements bordering the proximal part of the first male pleopod on the posterior side, combined with the mythological name *Charon*, *Charontis* referring to the Ferryman of Hades. Gender: masculine.

Remarks. *Lepidocharon* gen. n. is most similar to the genus *Microcharon*, the two genera sharing the following characters: well-developed uropods with slender endopod and exopod; pereiopodal coxal plates not discernible in dorsal view, small, incorporated to the sternite body wall; male pleopod 1 with similar general organization, the distal lateral lobe with a folded hyaline lamella (stylet-guiding groove) running almost parallel to the lateral margins of the pleopod (this orientation and structure of the stylet-guiding groove appears different from that of *Janinella*, where a transversal and oblique groove hosts the stylet of the male pleopod 2 which seems not to be enveloped by a hyaline lamella); pleopod 2 identical in the development of both exopod and endopod, the latter ending with a stylet of different length depending on the species; penial papillae small and located at the posteromesial margin of sternite 7; female operculum as long as the pleotelson, faintly incised, bearing 4 apical setae (a condition shared by some predominantly marine, and rarely freshwater, *Microcharon* species).

Lepidocharon gen. n. shares with *Janinella* the morphology of the tergites, the first three pereionites with antero-lateral protrusions, the fourth sub-rectangular in shape,

and the last three postero-laterally protruded, together with the lateral insertion of pereopods 1–7 oriented outwards (*vs.* ventrally in *Janinella*, see Albuquerque et al. 2014); pereopodal coxal plates small in *Janinella*, very reduced and incorporated to body wall in *Lepidocharon* gen. n. The female operculum of *Lepidocharon* is more than twice as long as wide, as long as the pleotelson, only faintly incised and bearing 4 apical setae, the mesial pair being close-set. These apical setae resemble that of *Janinella* species, where only the two close-set setae are present.

Lepidocharon gen. n. differs from all the other lepidocharontid genera by the combination of the following morphological characters: (1) the unique presence of scale-like elements bordering the postero-lateral margins of the proximal part of the male pleopod 1; (2) pereionites with coxal plates hardly discernible, small, and incorporated into the sternite body wall, not visible in dorsal and lateral views; (3) the long and slender pereopods that are inserted laterally and directed outwards; (4) pereionites that are not cylindrical, except pereionite 4 (*vs.* cylindrical in *Microcharon*); (5) the presence of elongate antennal scale (*vs.* rudimentary in *Janinella*, and generally reduced in *Microcharon*); (6) the mandible incisor with up to 9 cusps (lower number of cusps in *Microcharon* and *Janinella*).

Microcharon galapagoensis Coineau & Schmidt, 1979 is closer to *Lepidocharon* gen. n., especially in the general body morphology, pereionite shape, slender pereopods directed outwards, a long antennal scale, and slender and elongate uropods; however the unique scale-like elements of the male pleopod 1 of *Lepidocharon* are not present in this species. These scales are not easily detected under optical microscopy (if not at 100× magnification), nor were they seen using SEM because located on the posterior side of the pleopod.

The mid-section of the male pleopod 1 shows lateral margins expanded in both species of *Lepidocharon* gen. n. In contrast to the new genus, *M. galapagoensis* shows a 5-segmented antennula. Coineau and Schmidt (1979) had informally proposed the creation of an intermediate group between the genus *Microcharon* and *Janinella* (formerly *Paracharon* Coineau, 1970) for *M. galapagoensis* Coineau & Schmidt, 1979 (from marine interstitial habitats in the Galapagos), *M. salvati* Coineau, 1970 (from coral sands in New Caledonia) and *M. herrerae* Stock, 1977 (from brackish groundwater in the Netherlands Antilles) (Stock 1977).

***Lepidocharon priapus* Galassi & Bruce, sp. n.**

<http://zoobank.org/C6CDCAD6-3118-4E4A-A918-9B922E58E17D>

Figures 1–8, 15A

Material examined. *Holotype* here designated. Adult ♂ (1.3 mm) completely dissected and mounted in polyvinyl lactophenol on one slide (MTQ W28329), 17 February 2009, coll. N.L. Bruce and M. Błażewicz-Paszkowycz.

Type-locality: Australia, Great Barrier Reef, off Coconut Beach, Lizard Island, reef front, sand adjacent to bommies, 4 m, stn LIZ 09–09A, 14.68441°S, 145.47197°E.

Paratypes. 2 ♂♂ (0.9, 1.1 mm), 2 ♀♀ (1.1, 1.4 mm), Great Barrier Reef, Australia, completely dissected and mounted in polyvinyl lactophenol; 2 ♂♂, 1 ♀, same data as holotype preserved in alcohol; 1 ♂, 1 ♀ mounted on SEM stubs (all MTQ W30933).

Etymology. The epithet is derived from the god *Priapus* (Πριάπος) of the Greek mythology. He was considered the protector of livestock, fruit plants, gardens and male genitalia. He was famous for his largely phallic character and the specific names is here referring to the extraordinary length of the stylet of male pleopod 2.

Description of male. Body length, measured from tip of cephalon to end of pleotelson, from 0.9 to 1.3 mm (n=6). Body 7.0–8.0 times longer than wide, dorsally flat. Cephalon (Fig. 1A) longer than wide (length/width ratio: ~1.3), as large as pereionites, lateral margins sub-parallel. Pereionites 1–3 with anterolateral margins of tergites protruding; pereionite 4 rectangular, without tergite protrusions, pereionites 5–7 with posterolateral margins of tergites protruded. Pereiopods inserted close to lateral closure of tergites (Figs 1A, 2G–H) and extended outwards, coxal plates not discernible on dorsal and lateral views, small, totally incorporated to the border of the concavity housing the pereiopods (Fig. 2D, G–H).

Paragnaths (Fig. 3C) large, free distal margin with long slender simple setae and marginal and submarginal rows of scale setae. Labrum ovoid (Fig. 3D), ornamented with fine spines on free anterior margin.

Antennula (Fig. 1B) composed of 6 articles; article 1 1.5 as long as wide, directed anteriorly, with 2 simple setae; article 2 1.5 as long as wide, 0.8 as wide and 0.8 as long as article 1, with 4 setae inserted at distal third of article, 2 of which penicillate setae plus 1 long penicillate seta inserted on a lateral protrusion of article, accompanied by a short and thin naked seta on its basis; article 3 unarmed; article 4 with 1 lateral seta and 2 surface penicillate setae; article 5 shorter than article 4, bearing 1 long aesthetasc and 1 simple seta at basis of aesthetasc-bearing protrusion; article 6 short, 0.5 as long as article 5 and clearly articulated with article 5 (Fig. 2A), bearing 5 setae in total, two surface seta (one of which penicillate), one apical and one subapical simple setae; one long aesthetasc and a robust and long seta apically, both incorporated to article 6.

Antenna (Fig. 3A–B) with 6 podomeres, 2 proximal articles short and stout, article 1 with 1 apical outer seta; article 2 naked; article 3 robust, length/width ratio: 1.25, with mesial apical seta and long exopod quite overreaching segment 4, knife-blade shaped and bearing 2 long and thin setae inserted on lateral margin at middle of exopod; article 4 stout and curved outward, 0.5 as long as article 3, with 2 apical mesial setae; articles 5 and 6 slender and long, article 6 longest, bearing 6 and 10 simple setae, respectively; flagellum composed of 9 articles in holotype; 11 articles in one male paratype; all flagellar articles with distal hyaline lamella partially covering insertion of following article, all armed with setae on distal margin, except article 9 ending with 3 simple and 1 penicillate setae.

Mandible palp (Fig. 3E) on short cuticular projection; palp article 1 without setae, article 2 longest, about 2.5 times longer than wide, with 2 pinnate robust setae laterally, their insertion more or less coalescent with article; article 3 curved laterally, with 5 pinnate robust setae, distalmost seta longest; 2 cuticular comb rows on lateral margin

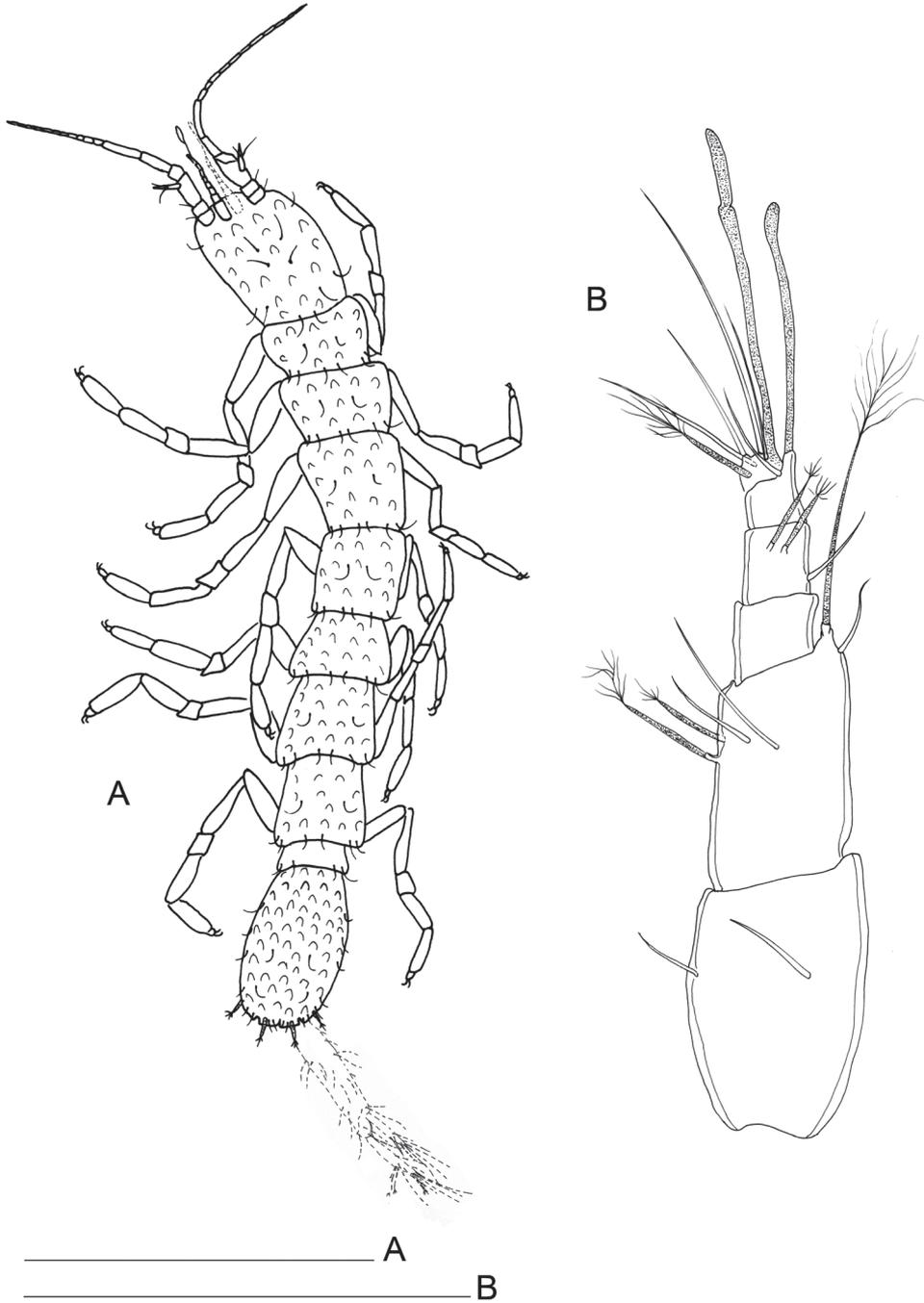


Figure 1. *Lepidocharon priapus* gen. n., sp. n. ♂ holotype. **A** habitus **B** antennula (scale bars: **A** 0.5 mm; **B** 0.1 mm).

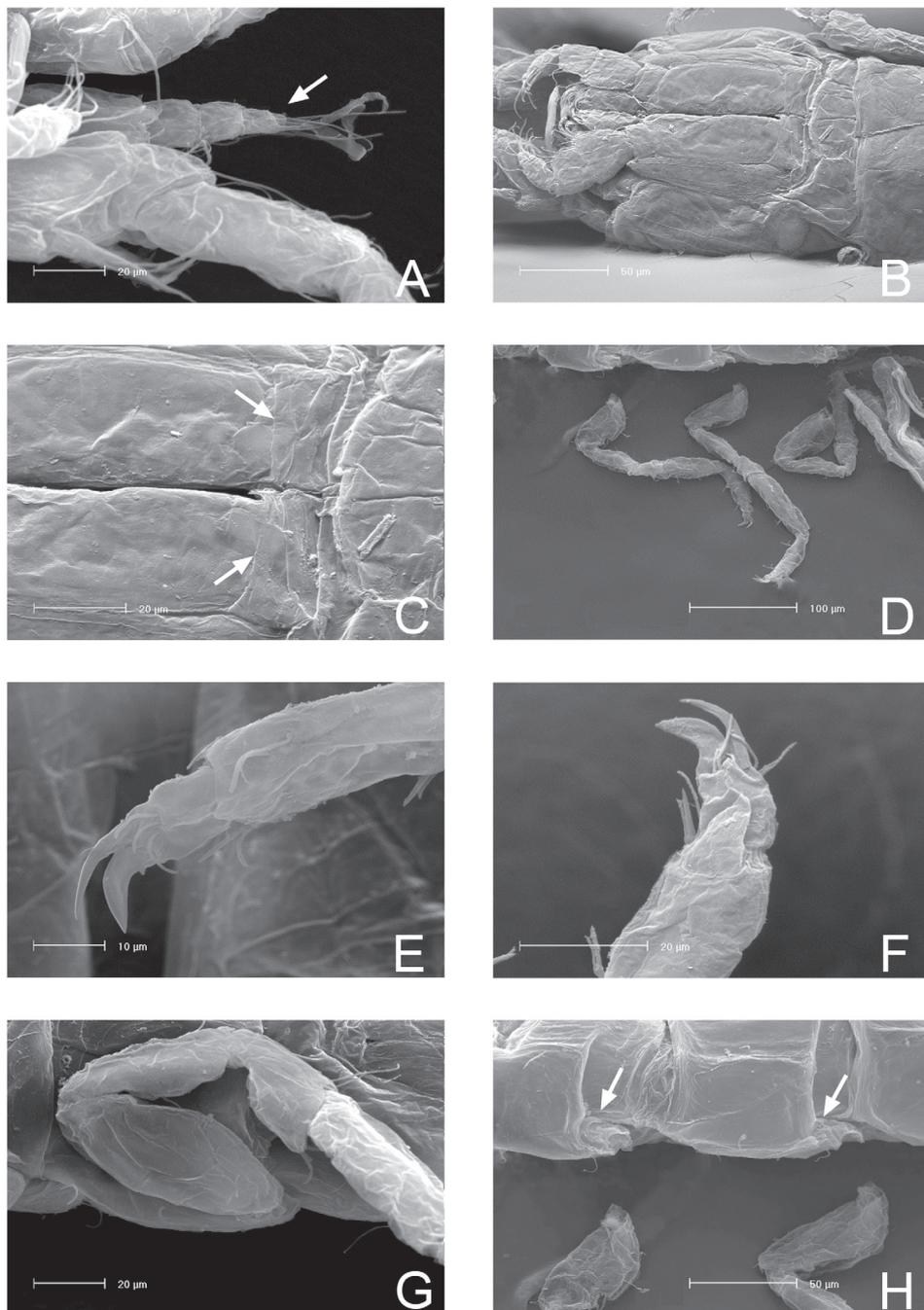


Figure 2. SEM micrographs of *Lepidocharon priapus* gen. n., sp. n. ♂ paratype. **A** antennula, sixth segment arrowed **B** maxillipeds, general view **C** maxilliped endite and rudimentary sympod (?) arrowed **D** coxal plates of pereionites 5–7, ventral view **E** pereiopod 1, detail of dactylus and reduced sclerite **F** pereiopod 7, detail of dactylus and articulated sclerite **G** pereiopods, lateral view **H** rudimentary coxal plates, ventral view, arrowed.

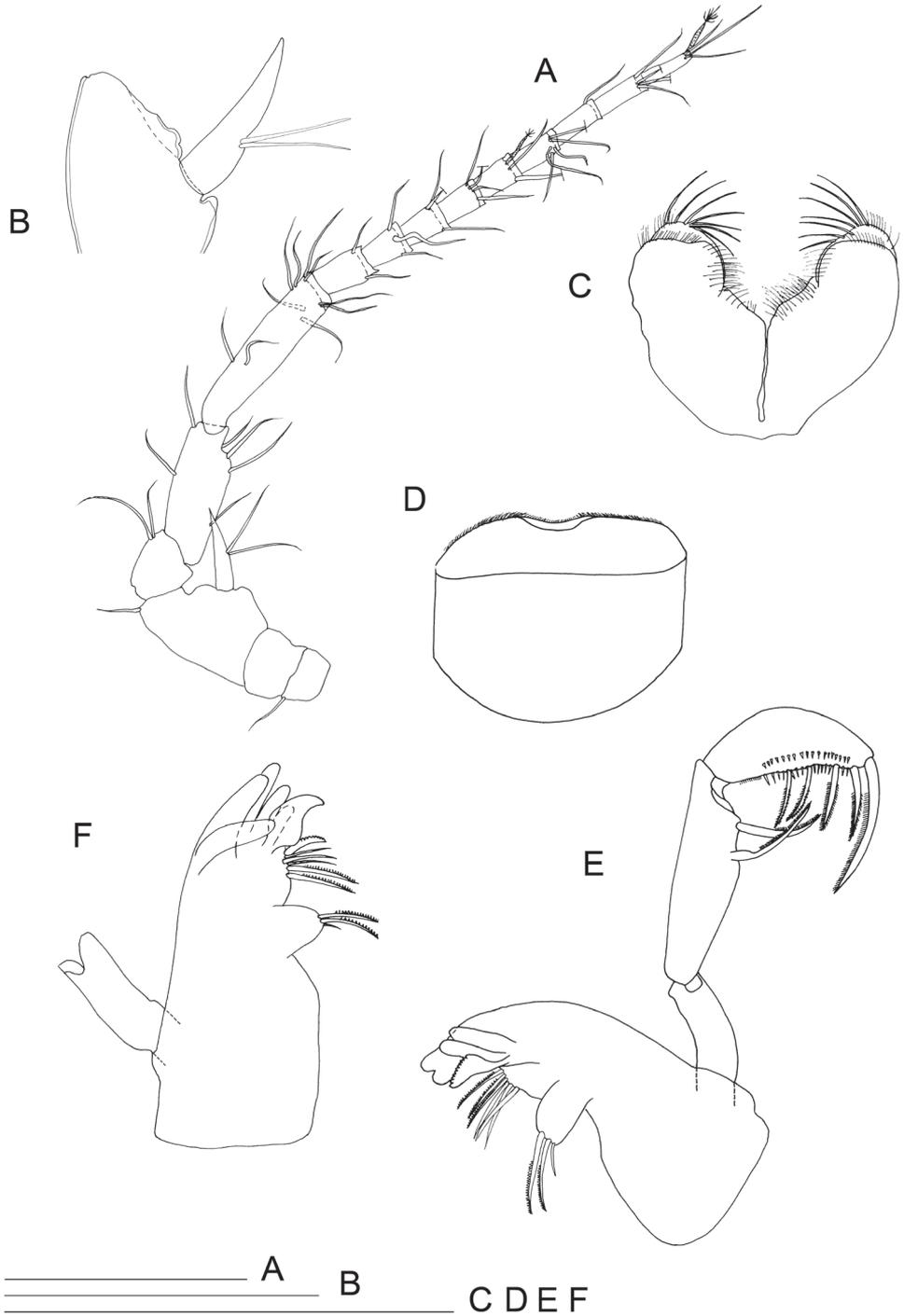


Figure 3. *Lepidocharon priapus* gen. n., sp. n. ♂ holotype. **A** antenna **B** detail of the antennal scale **C** paragnaths **D** labrum **E** left mandible and maxilliped **F** right mandible (scale bars: 0.1 mm).

of article. Left mandible (Fig. 3E): incisor with two strong and large cusps; *lacinia mobilis* as in the genus; molar process with two long unipinnate setae accompanied by 1 short simple spine. Between *lacinia mobilis* and molar process 3 thin, long and simple spines and 3 unipinnate spines are present, 1 modified seta, cockscomb-shaped, close to *lacinia mobilis*, with total of 7 elements. Right mandible (Fig. 3F) incisor with 6 robust cusps; 2 long unipinnate and 1 short simple seta. Between incisor and molar process 6 spines are inserted, apicalmost robust, curved and unipinnate, 3 naked and 2 unipinnate spines.

Maxillula (Fig. 4A): mesial lobe slender and tapering at distal part bearing 1 short apical seta accompanied by 2 subapical shorter setae and two lateral thin and short setae. Lateral lobe sub-rectangular in shape, bearing scale-like elements on both lateral and mesial margins. Apical setation composed by 11 elements; 3 simple setae in sub-apical position (surface apical setae); 2 mesial setae with apical tuft; remaining 6 distal setae unipinnate.

Maxilla (Fig. 4B): mesial ramus with 9 setae, 3 naked and slender setae on mesial margin, 1 surface seta short; 5 apical setae, mesialmost and 3 lateralmost apical setae simple and slender; second apical setae (starting from mesial margin) comb-like, strong, unipinnate and ornamented with fine regularly-spaced setules parallel to one another. Lateral rami close-set, each bearing 4 slender and simple setae, respectively.

Maxilliped (Figs 4C, D, 2B, C): palp robust and curved inwards; article 1 sub-rectangular in shape, bearing 1 mesial and 1 lateral setae; article 2 longest, robust, rounded on mesial margin, with 2 setae at distomesial angle; article 3 shorter than article 2 with 1 distolateral and 3 mesial setae; article 4 angled mesially, with 1 seta on distolateral margin, 1 seta on mesial margin and 3 setae on distal margin; article 5 about 0.5 as long as article 4, with 8 apical setae, of which 6 are simple slender setae and 2 are stiff pectinate setae. Endite almost reaching end of palp article 2; mesial margin ending in a pointed protrusion, with numerous hair-like setules and 2 coupling hooks medially; apical free distal margin with 4 bipinnate, spine-like, stout setae and 1 simple non-tapered seta; 3 subapical fan setae are present; epipod ovoidal, reaching distal part of palp article 1.

Pereiopod 1 (Figs 2E, 4E): coxal plate hardly discernible, basis slightly enlarged, relatively short in comparison to length of same segment of pereiopods 2–7, with 3 short setae, one of which is a penicillate seta; 2 opposite setae on ischium, transformed in sensorial penicillate setae; merus trapezoidal, shorter than all other articles, bearing 4 long and large setae on mesial and lateral apical margins; carpus longer than merus bearing 2 opposite long slender setae; mesiodistal margin with spinule row; propodus longer and slender than merus, ending with a small sclerite (Fig. 2E), with mesial hyaline lamella, bearing 1 bifid robust seta and 5 slender setae; dactylus with 6 slender sensorial setae, 3 of which inserted on surface of dactylus at base of insertion of longer claw, 2 surface setae inserted at basis of shorter claw, 1 seta on mesial distal margin of dactylus. Pereiopods 2–7 with strong dactylar claws with rounded tip. Morphology, relative length of pereiopodal segments and their armature apparently identical. Pereiopods 2–7 (pereiopod 7 figured; Fig. 5A) with coxal plate hardly discernible,

basis slender than in pereopod 1, bearing 4 setae, 2 of which penicillate, and 2 not transformed slender setae; ischium longer than in pereopod 1, rectangular in shape, bearing 3 setae, 2 of which penicillate; merus shorter than articles, trapezoidal, and stouter than in pereopod 1, bearing 1 sensorial seta on mesial margin, and 2 robust spiniform setae on apical mesial and lateral margins, respectively; a pointed protrusion discernible on mesial margin in subapical position accompanied by 1 thin seta; carpus longest; longer than both merus and propodus, bearing 4 elements; 1 proximal mesial simple seta and 1 bifid spine; 2 lateral setae located on apical lateral margin, 1 of which thin and long, the latter transformed in a penicillate seta; propodus long and slender than carpus, ending with elongate sclerite (Fig. 2F), bearing 2 bifid stout spines on mesial margin and 4 simple thin setae of different lengths; dactylus ending with two stout claws subequal in length, armed with 5 thin setae likely with sensorial function, 3 of which inserted on surface of dactylus at base of the insertion of longer claw, 2 surface setae inserted at basis of shorter claw.

Pleonite 0.29 as long as and 0.84 as wide as pereonite 7 (Figs 1A, 6A), small, narrower and shorter than pereonites and pleotelson; well discernible on dorsal and ventral views, partially covered by pereonite 7 on ventral view (Fig. 6A).

Penial papillae, as for the genus, coalescent and located at the middle of the free posterodistal margin of pereonite 7, with undulated free outer margins and a medial channel (Fig. 6A–B).

Pleotelson 1.37 as long as maximal width (Figs 8D, 15A), longer than wide (length/width ratio: from 1.75 to 2.00, $n = 5$). Dorsal side with semicircular thickening, as dorsal and ventral body surfaces; with 4 dorsal setae, arranged in two pairs; lateral margins each bearing 3 slender setae. Pleotelson distal margin with 12 marginal setae, inserted in apical or subapical position; 2 of them are penicillate setae.

Male pleopods 1 elongate and slender, fused proximally, with sperm tube medially with an anterior opening ornamented by small spines (Figs 5B, 6A, C), approximately 3.3 as long as maximum width (measured at widest section of proximal part of pleopod). Proximal part of pleopod large and gradually tapering at distal part, bordered by paired rows of 10 imbricate scale-like elements on posterior surface. Middle part of pleopod with free lateral margins smooth, parallel, and slender, ending with convex rounded margins, tapering apically, with paired well-developed distal protrusions, each ending with rounded apex, a hyaline membrane, crenulated on lateral margin, and smooth on mesial margin. Stylet-guiding groove parallel to lateral margins of pleopod, and folded by hyaline lamella, sclerotized in terminal part on both lateral and mesial sides of distal part of pleopod. Distal part of pleopod with 7 setae.

Male pleopod 2 (Fig. 7A): protopod elongate, sub-rectangular at its proximal part, and with rounded mediobasal corner; surface of protopod with semicircular thickening (Fig. 7B), exopod extruding partially from distal part of protopod, *appendix masculina* (endopod) extraordinarily long, more than 3 times (3.0–3.3; $n = 4$) the protopod length, ending with a long stylet with a sclerotized rib that runs along its entire length; terminal part with inflated lateral margins tapering to an acute tip. Stylet quite overreaching distal part of protopod, and reaching in length distal part of uropods.

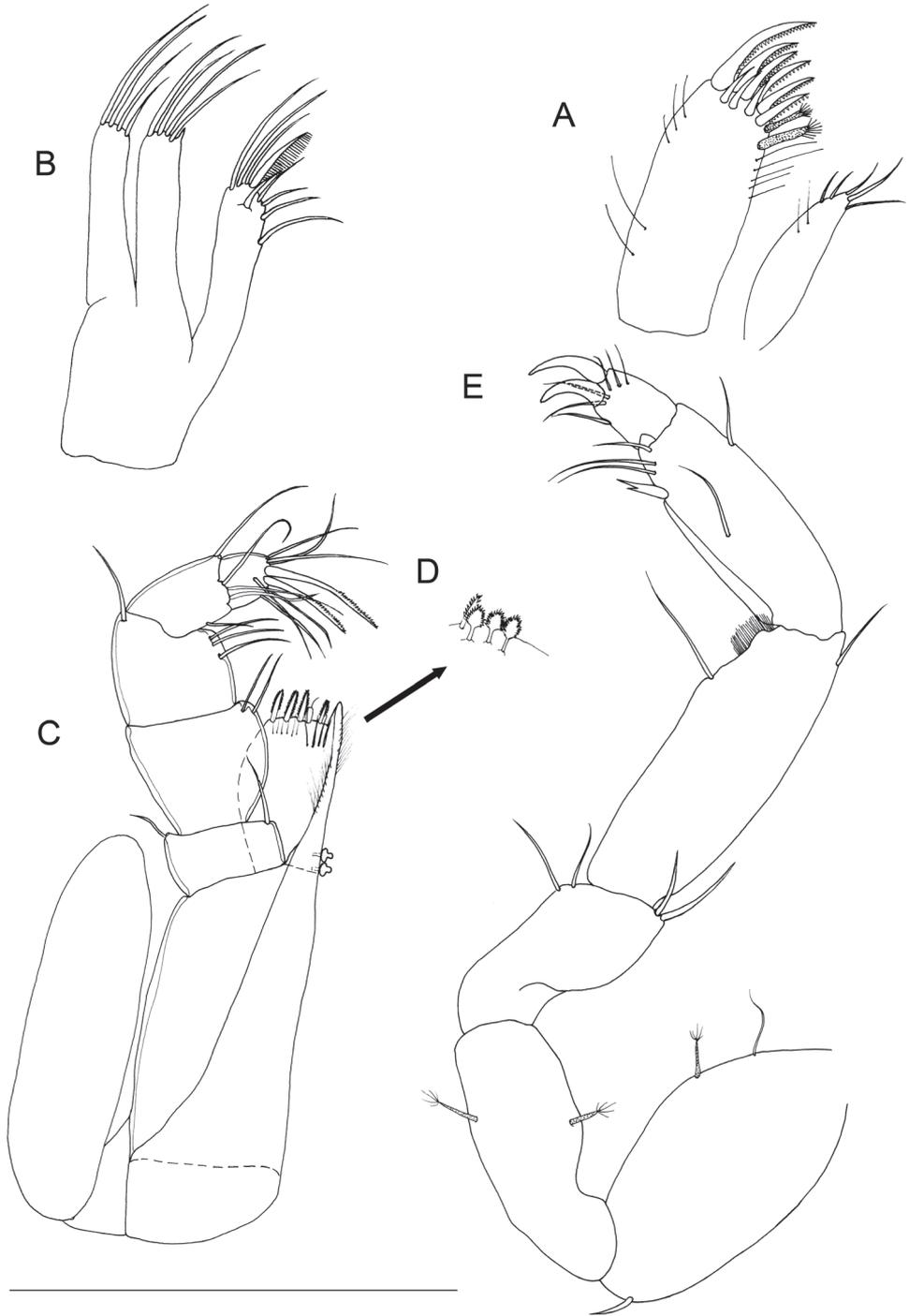


Figure 4. *Lepidocharon priapus* gen. n., sp. n. ♂ holotype. **A** maxillula **B** maxilla **C** maxilliped **D** maxilliped, detail of the subapical setae morphology of the endite **E** pereiopod 1 (scale bar: 0.1 mm).

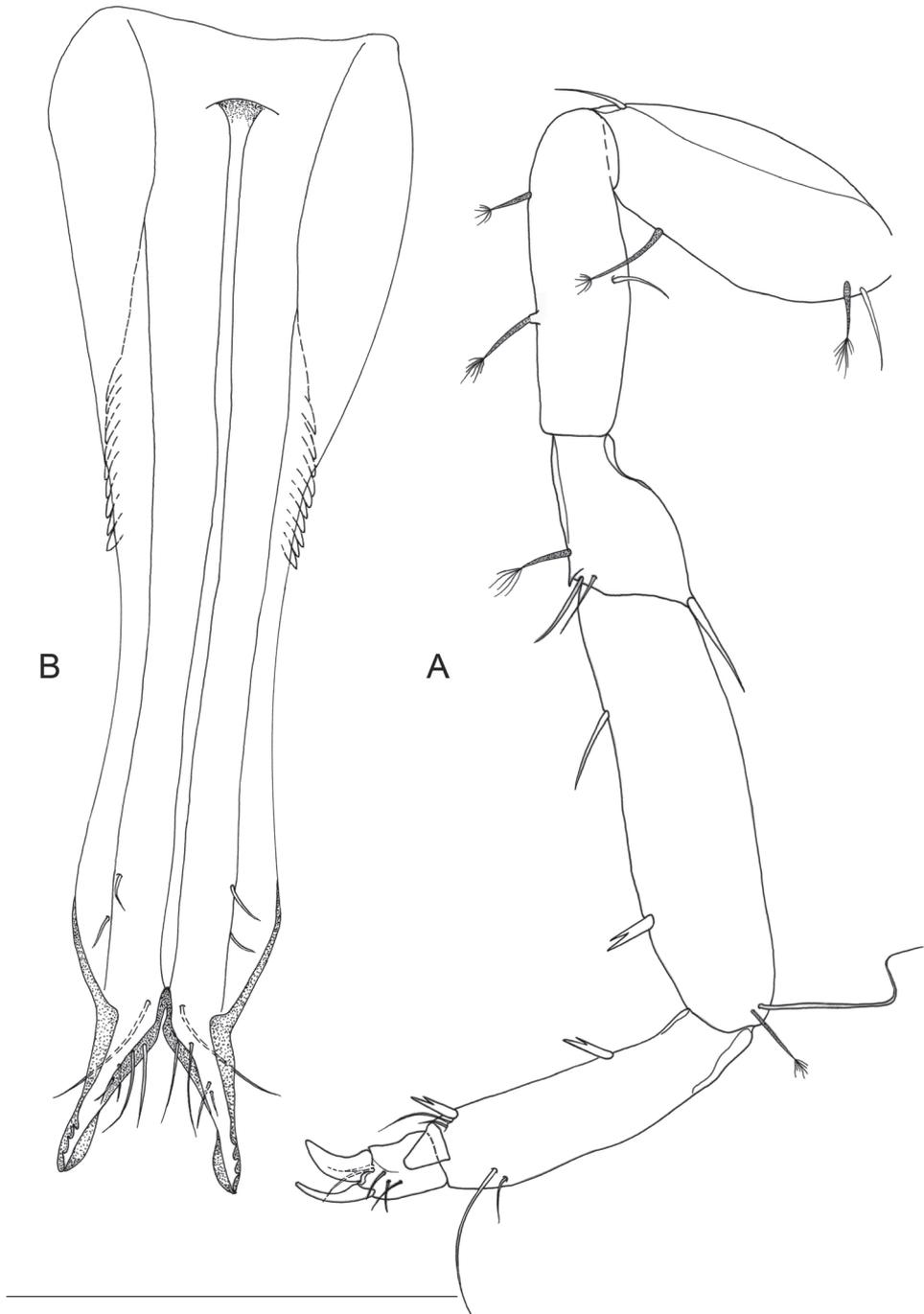


Figure 5. *Lepidocharon priapus* gen. n., sp. n. (♂ holotype). **A** pereopod 7 **B** pleopod 1 (scale bar: 0.1 mm).

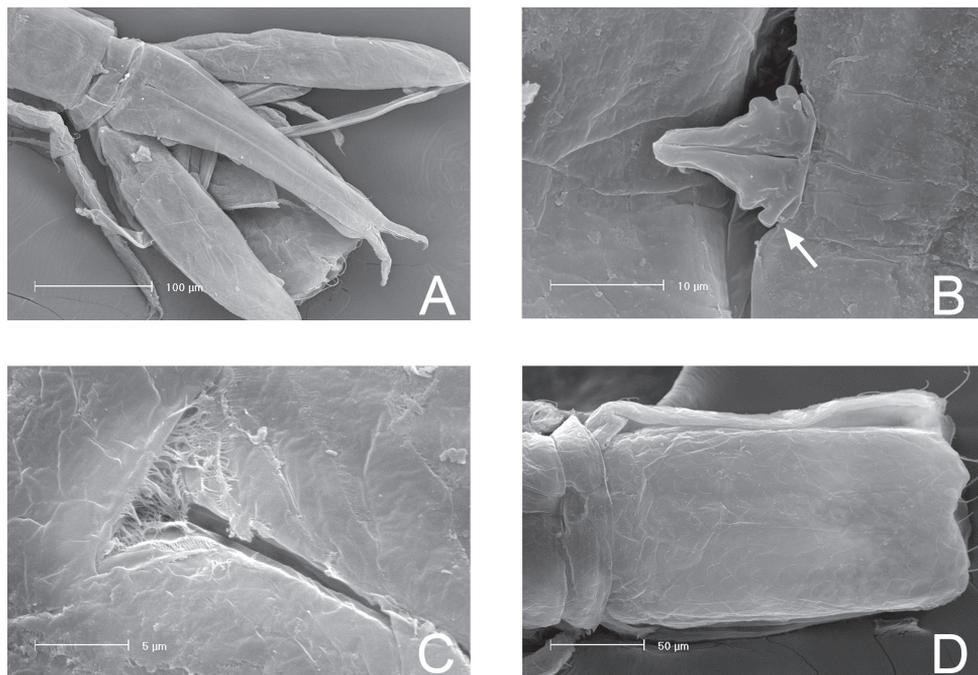


Figure 6. SEM micrographs of *Lepidocharon priapus* gen. n., sp. n. **A, B, C** ♂ paratype. **A** general view of free pleonite (ventral view), and pleopod 1 and 2 **B** penial papillae with ventral groove (arrowed) **C** pleopod 1, detail of the anterior proximal opening to the mesial channel **D** ♀ paratype, operculum.

Pleopod 3 (Fig. 7C, D) with endopod bearing 1 apical, 1 mesial subapical and 1 lateral plumose setae; exopod 2-segmented, with setulose hyaline lamella on lateral margin; exopod 1 elongate, 2.8 times longer than exopod 2, the latter ending with short simple seta.

Pleopod 4 (Fig. 8A) rudimentary, ellipsoidal, uniramous.

Uropods long and slender (Fig. 8B), approximately two times longer than pleotelson. Protopod long, two times longer than endopod. Exopod and endopod long and slender; endopod longer than exopod, the latter inserted in subapical position on protopod. Mesial margin of protopod armed with 5 setae; lateral margin with 8 setae, 8 surface setae, 4 of which are slender and located on ventral surface, remaining setae on dorsal surface large. Endopod with long setae on distal third, 6 of which are penicillate setae, remaining 7 setae slender, simple, with rounded tip. Exopod armed with 9 setae on distal third, all of which slender; apicalmost setae longest.

Female. Body length generally similar to male (Fig. 15A). Body length measured from tip of cephalon to end of pleotelson from 0.9 to 1.4 mm. ($n = 3$). Length/width ratio: ~ 8.5 . Female operculum elongate (Figs 6D, 8C), sub-rectangular in shape, with parallel lateral margins, proximal margin straight; distal part medially incised, bearing 2 close-set short medial setae and 2 setae in apical lateral position. Operculum surface with semicircular thickening (Fig. 6D).

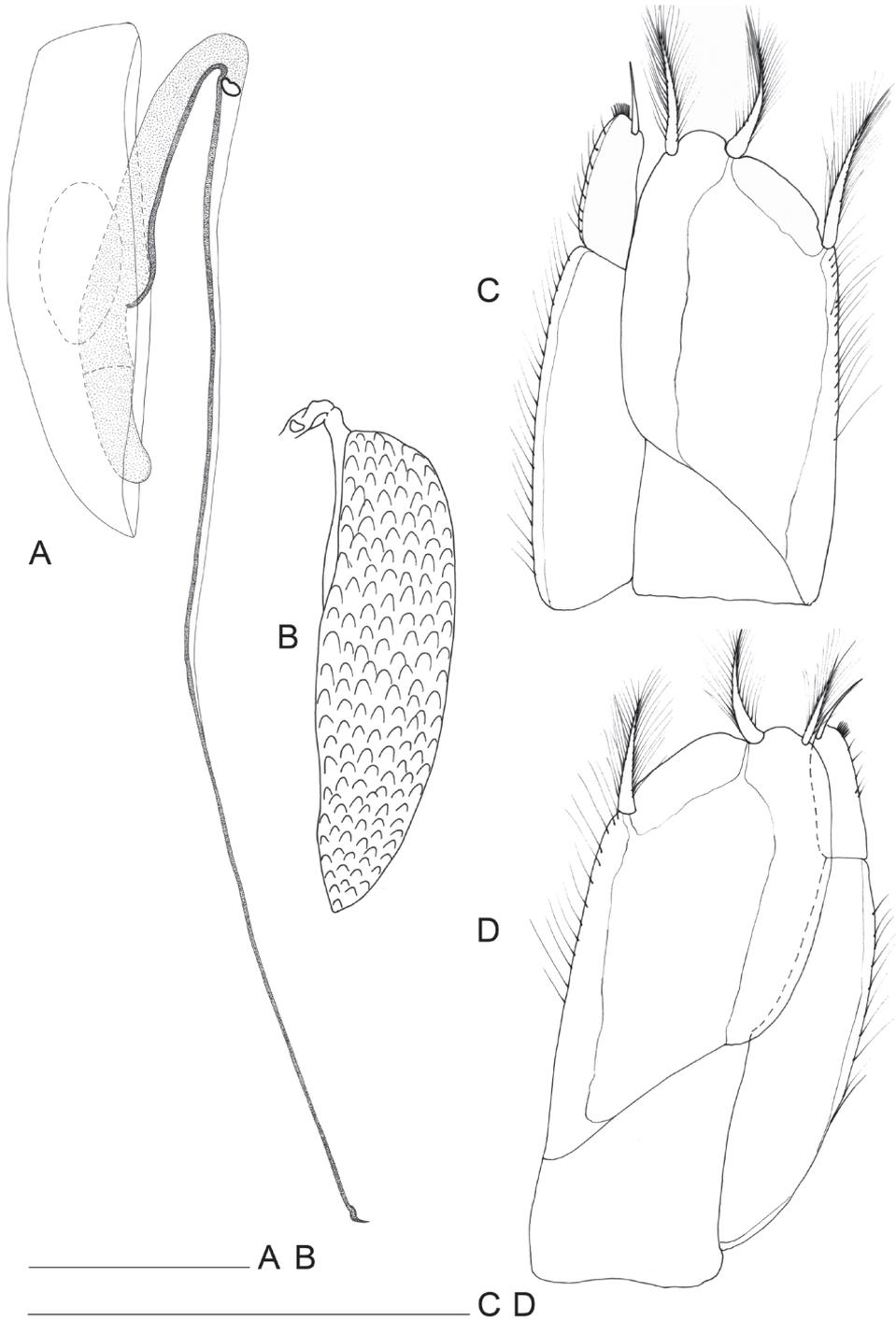


Figure 7. *Lepidocharon priapus* gen. n., sp. n. **A, C, D** ♂ holotype. **A** pleopod 2 **B** ♂ paratype, pleopod 2, protopod, **C** right pleopod 3 **D** left pleopod 3 (scale bar: 0.1 mm).

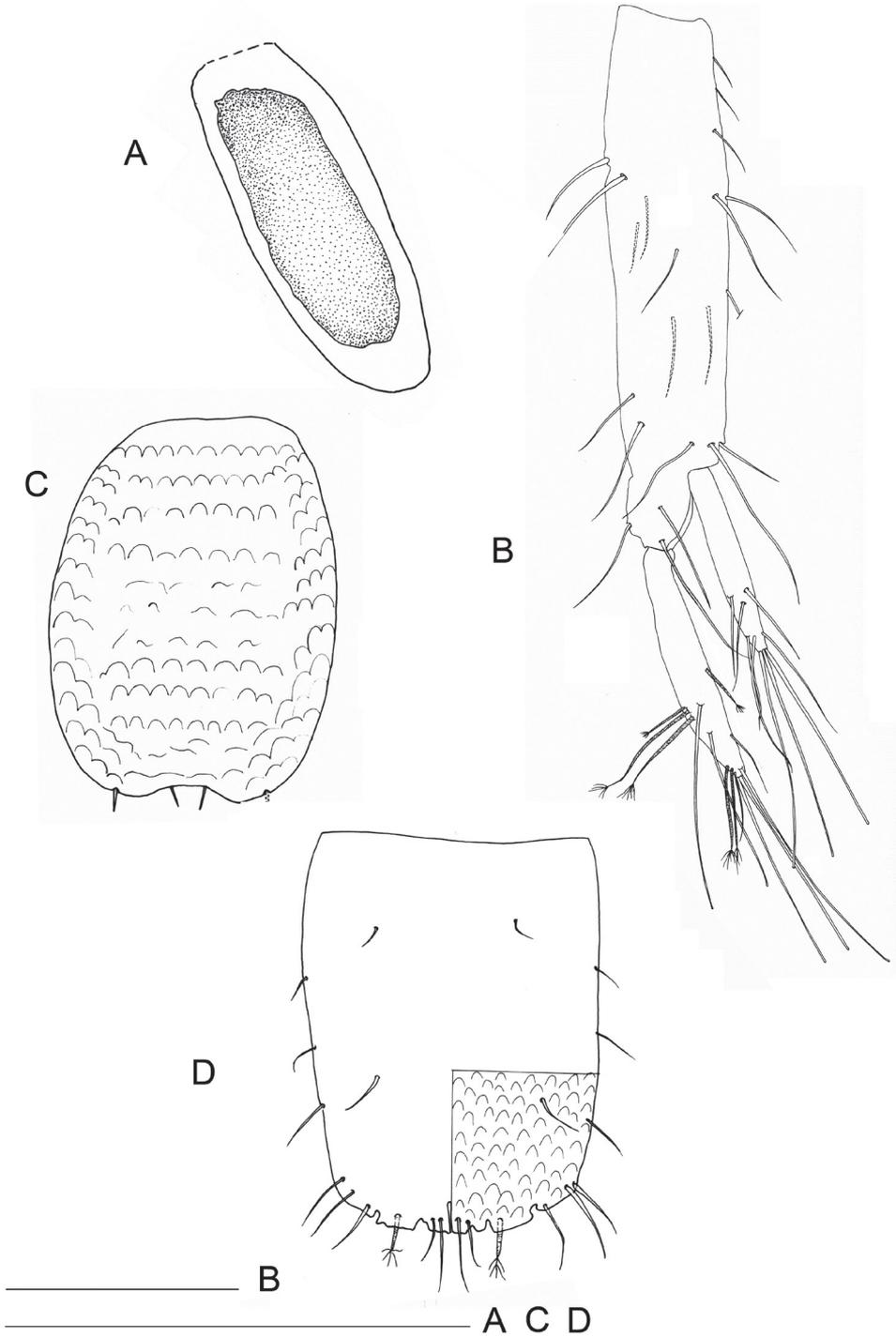


Figure 8. *Lepidocharon priapus* gen. n., sp. n. **A, B, D** ♂ holotype. **A** pleopod 4 **B** uropod **C** ♀ paratype, pleopod 2 (operculum) **D** pleotelson (scale bars: 0.1 mm).

Remarks. Detailed comparison between the two species is given in the remarks for *Lepidocharon lizardensis* sp. n.

***Lepidocharon lizardensis* Galassi & Bruce, sp. n.**

Figures 9–13, 15B

<http://zoobank.org/CE092992-8ECA-4891-B2A7-1396F8263D3F>

Material examined. *Holotype* here designated. Adult ♂ (1.1 mm), completely dissected and mounted in polyvinyl lactophenol on one slide, 17 February 2009; coll. N.L. Bruce and M. Błażewicz-Paszkowycz (MTQ W28330).

Type-locality: Australia, Lizard Island, off Coconut Beach, 14.68441°S, 145.47197°E, reef front, sand adjacent to bommies, 4 m, stn Liz 09-09A.

Paratypes. 1 ♂ (0.9 mm), 1 ♀ (1.2 mm) completely dissected and mounted in polyvinyl lactophenol, same data as holotype (all MTQ W28331).

Etymology. The epithet *lizardensis* is after the type locality, Lizard Island, northern Great Barrier Reef, Queensland.

Description of male. Body length measured from tip of cephalon to end of pleotelson 0.9–1.1 mm (n = 2). Body dorso-ventrally flattened, stocky, small-sized, about 6.5 times longer than wide (Figs 9A, 15B). Cephalon as long as wide (length/width ratio: ~1), as large as pereionites, lateral margins sub-parallel; anterior margin not projecting, rostrum absent. Dorsal surface of cephalon, pereionites and pleotelson (except free pleonite) ornamented with dorsal setules, arranged symmetrically and in pairs (Fig. 9A).

Pereionites 1–7 subequal in width (Fig. 9A); pereionites 1–3 with anterolateral margins of tergites only slightly protruding; pereionite 4 rectangular, without protrusions, pereionites 5–7 with posterolateral margins of tergites slightly protruded. Pereiopods inserted on lateral margins of tergites, visible in dorsal view (Fig. 9A); coxal plates rudimentary, incorporated to sternites.

Paragnaths (Fig. 9B) consisting of 2 large rounded lobes, deeply incised on medial side, ornamented with long setules on free mesial margins; thin simple setae are accompanied by small setules. Lateral margins with three short spinule rows. Labrum ovoid (Fig. 9C), with free anterior margin convex and medially thickened, with pair of thin scale-setae inserted symmetrically on the outermost sides of the free distal margin.

Antennula (Fig. 9D) composed of 6 articles; article 1 broadest, 1.6 as long as wide, directed anteriorly, with 2 simple and 2 penicillate setae; article 2 narrow, 1.6 as long as wide, 0.6 as wide and 0.7 as long as article 1, with 5 setae inserted at distal third of article, two of which penicillate; 1 long sensorial aesthetasc-like seta inserted on lateral protrusion, accompanied by a short and slender simple seta on its basis; article 3 unarmed; article 4 with 1 lateral simple seta and 2 penicillate setae inserted in apical position; article 5 slightly shorter (0.86) than article 4, bearing 1 long aesthetasc and 1 simple long slender seta at base of aesthetasc-bearing protrusion; 2 simple setae inserted at surface of article; article 6 very short, clearly articulated with article 5, bearing

2 subapical lateral setae, one of which aesthetasc-like penicillate seta, and 1 long apical seta close to 1 long aesthetasc and 1 subapical slender seta between them.

Antenna (Fig. 10A–B): with 6 podomeres, articles 1 and 2 short, article 1 with mesial seta; article 2 with short lateral seta; article 3 robust, with mesial apical seta and long exopod overreaching segment 4, candle-flame shaped and bearing 2 short and slender setae inserted at middle of exopod; article 4 stout and curved with 2 apical mesial setae; articles 5 and 6 slender, article 6 longest, bearing respectively 7 and 12 armature elements, 5 of which transformed in penicillate setae with apical tuft; flagellum composed of 12 articles, all flagellar articles with setae on distal margins, most setae simple; some on flagellar articles 1 and 4 are penicillate setae.

Mandible. Palp (Fig. 10C) on short cuticular projection. Palp article 1 naked, article 2 longest, about 2.5 times as long as wide, with 2 pinnate robust setae laterally, their insertion more or less coalescent with article; article 3 curved laterally, with 4 spinulose and 1 apicalmost unipinnate setae, distalmost longest and stout; 2 setule rows on lateral margin of article. Left mandible (Fig. 10D): incisor with 3 cusps; *lacinia mobilis* with 2 teeth; molar process with 2 long unipinnate spines and 1 short smooth seta. Between *lacinia mobilis* and molar process 2 transformed crested setae are present; 6 slender, long simple setae complement total pattern of 8 elements. Right mandible (Fig. 10E) incisor with 8 robust cusps; molar process with 3 apical spines, 2 of which unipinnate and robust, proximalmost naked and shorter; between incisor and molar process 6 spines are inserted, proximalmost curved, robust and uniserrate; remaining spines simple.

Maxillula (Fig. 11A) with slender mesial lobe tapering at distal part bearing 1 short apical seta accompanied by secondary subapical short seta and hair-like setules. Lateral lobe sub-rectangular in shape, bearing scale-setae on both lateral and mesial margins. Apical setation composed of 13 elements; 1 mesial slender seta, 1 mesialmost apical seta with bifid tip; 1 penicillate seta, 7 uniserrate setae, and 3 subapical surface simple setae.

Maxilla (Fig. 11B) mesial ramus with 8 setae, 2 naked setae on mesial margin, 6 apical setae, mesialmost naked, 1 uniserrate, third seta comb-teeth shaped, unipinnate, ornamented by fine setules regularly spaced and parallel to one another; remaining 3 setae simple. Lateral rami close-set, each bearing 4 setae of different lengths; lateralmost ramus with 3 spinulose and 1 short mesial setae.

Maxilliped (Fig. 11C–D) palp robust and curved mesially; article 1 sub-rectangular in shape, bearing 1 lateral and 1 mesial short setae; article 2 robust, bearing 1 apical mesial seta; article 3 as long as article 2, with 1 lateral apical and 3 mesial setae inserted on mesial margin; article 4 curved inwards, slender, with 1 lateral and 4 apical setae inserted at boundary line between article 4 and 5; article 5 short and narrow, with 2 apical and 3 subapical setae, of which 3 are simple slender setae of different lengths and 2 are robust and large, stiff pectinate setae. Endite almost reaching end of palp article 2; mesial margin ending in pointed protrusion, with numerous hair-like setules and 2 coupling hooks mesially; apical free distal margin with 3 spine-like, serrate setae, 1 simple non-tapered seta, and 2 surface fan setae; epipod ovoidal, overreaching distal part of palp article 1, bearing 1 subapical short, minute seta (this seta not found in other specimens).

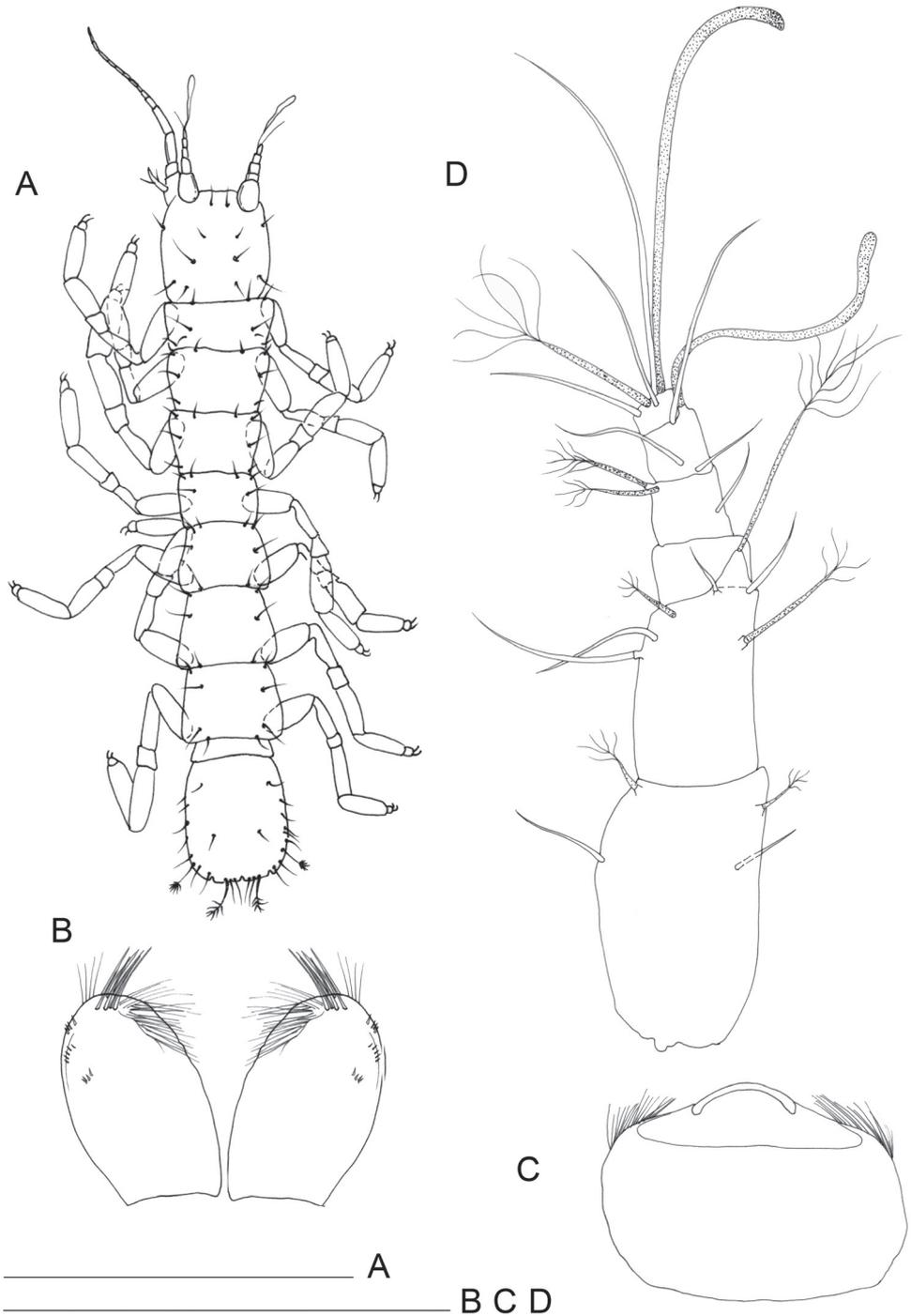


Figure 9. *Lepidocharon lizardensis* gen. n., sp. n. ♂ holotype. **A** habitus **B** paragnaths **C** labrum **D** antennula (scale bars: **A** 0.5 mm; **B, C, D** 0.1 mm).

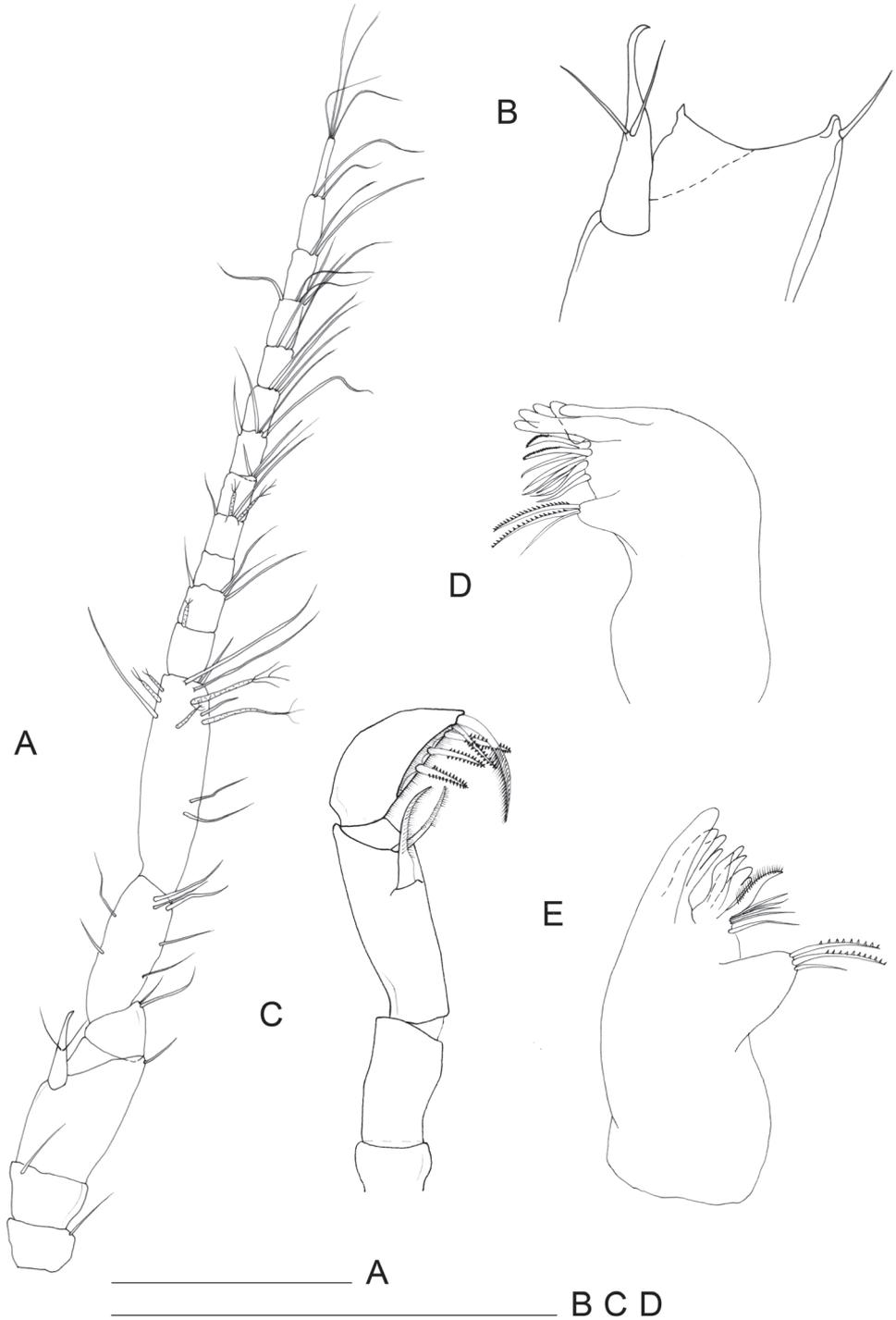


Figure 10. *Lepidocharon lizardensis* gen. n., sp. n. ♂ holotype. **A** antenna **B** detail of the antennal scale **C** mandibular palp **D** left mandible **E** right mandible (scale bars: 0.1 mm).

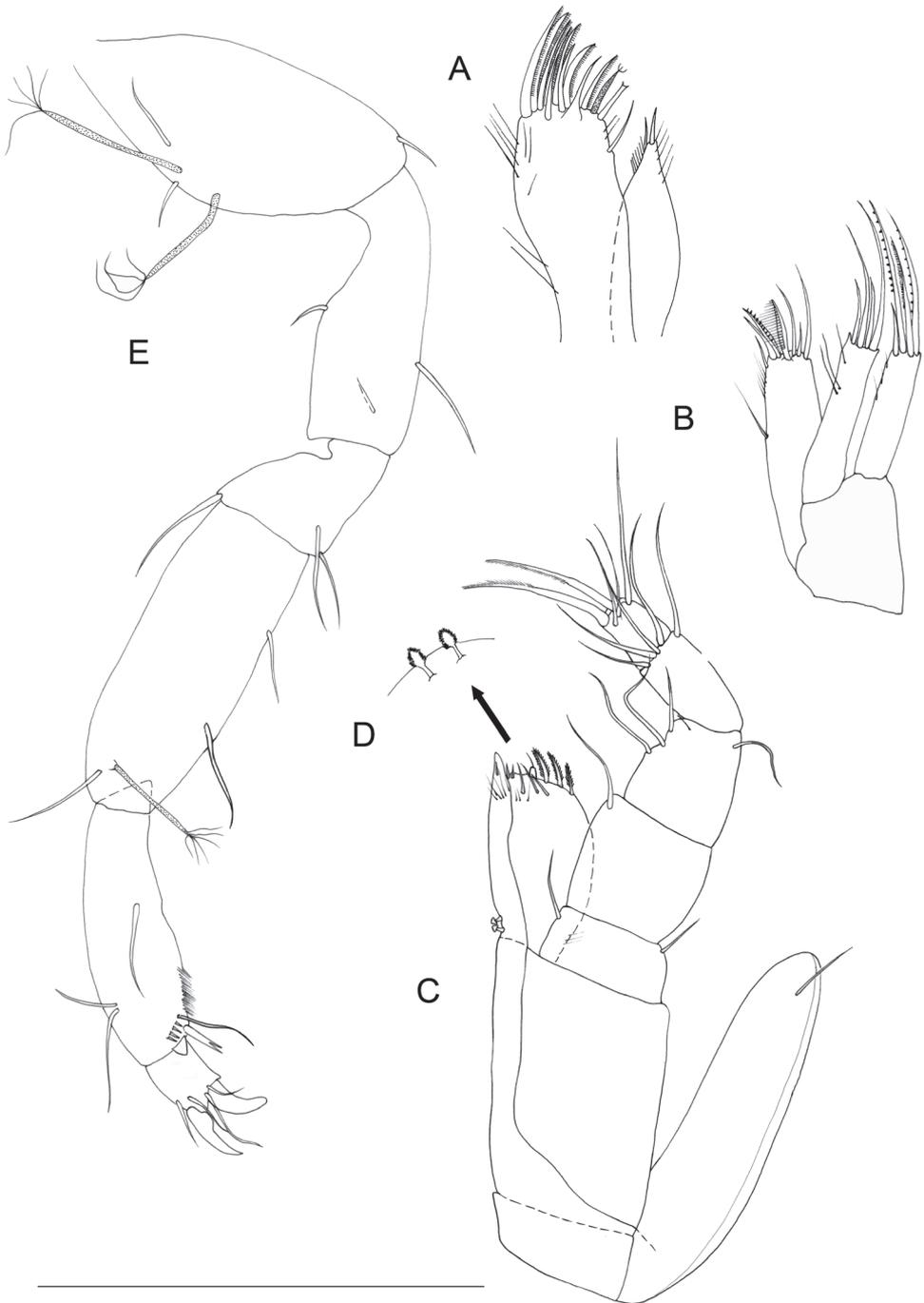


Figure 11. *Lepidocharon lizzardensis* gen. n., sp. n. ♂ holotype. **A** maxillula **B** maxilla **C** maxilliped **D** maxilliped, detail of the subapical setae morphology of the endite **E** pereopod 1 (scale bar: 0.1 mm).

Pereiopod 1 (Fig. 11E) coxal plate hardly discernible; basis slightly enlarged, shorter than that of P2–P7, with 2 short setae on mesial surface and 1 short simple seta on distolateral margin, and 2 penicillate setae; 2 opposite setae on ischium, one of which longer, a tubular sensorial seta on surface; merus shorter than other articles, trapezoidal, bearing 3 long setae; carpus slender and longest, mesial margin with 2 thin setae; distolateral margin with 1 simple slender seta and 1 penicillate seta; propodus slender than carpus, ending with a small elongate sclerite, with 2 mesial slender setae and 1 bifid spiniform seta; spinule row on free distal mesial margin; 3 simple setae on anterior surface; a spinule row at insertion of bifid spine; dactylus with 4 thin sensorial setae, inserted in pairs at base of each claw. Pereiopods 2–7 (pereiopod 7 figured; Fig. 12A) with coxal plate rudimentary and incorporated to the sternite, basis slender than in pereiopod 1, bearing 5 setae, three of which penicillate and two slender simple setae; ischium slightly longer and slender than in pereiopod 1, rectangular in shape, bearing 3 setae, 1 of which transformed in penicillate seta; merus shorter than all other leg segments, trapezoidal, and slightly longer and slender than in pereiopod 1, bearing 2 setae on mesial margin, 1 robust seta on apical lateral margin and 1 slender simple seta on surface at boundary between merus and carpus; carpus almost as long as propodus; robust, bearing 1 simple thin seta and a bifid spine along mesial margin, and 2 short thin setae on lateral margin, a penicillate seta inserted close to apicalmost lateral seta; propodus slender than carpus, with small elongate sclerite, bearing 2 bifid stout spine-like setae on mesial margin, 2 surface simple setae of different lengths and a surface penicillate seta at distal third of article; dactylus ending with 2 strong claws with rounded tip, slightly subequal in length, lateral claw slender, mesial stouter and shorter; dactylus armed with 5 thin setae likely with sensorial function, 3 of which inserted at surface of article, at base of insertion of the longer claw, 2 surface setae inserted at basis of shorter and stouter claw.

Pleonite length 0.27 pereionite 7 length, width 0.86 pereionite 7 width (Figs 9A, 14A, 15B).

Pleotelson longer than wide (Fig. 14A, 15B) (length/width ratio: from 1.33 to 2.00, $n = 2$). Dorsal side with 6 setae, a pair located on proximal part of pleotelson, other 2 pairs of setae arranged in close-set two pairs, both pairs located on distal third of pleotelson. Lateral margins bearing 3 slender setae at each side; 16 setae bordering free distal margin of pleotelson, inserted in apical or subapical position; 4 of them are penicillate setae.

Male pleopods 1 (Fig. 12B) elongate, coalescent in proximal part, with sperm tube medially, length about 4.5 times longer than maximum width (measured at widest section of proximal part of pleopod). Proximal part of pleopod large and gradually tapering at its distal part, bordered by paired rows of 4 scale-like elements. Middle part of pleopod with free distal margins smooth, parallel and slender, ending with slightly inflated sub-distal rims, tapering in apical part with paired well-developed sub-rounded bilobate tips. Stylet-guiding groove folded by hyaline lamella, running parallel to lateral margins of pleopod, only slightly sclerotized and ending with a transversal straight margin. Set of distal setae composed by 6 elements only.

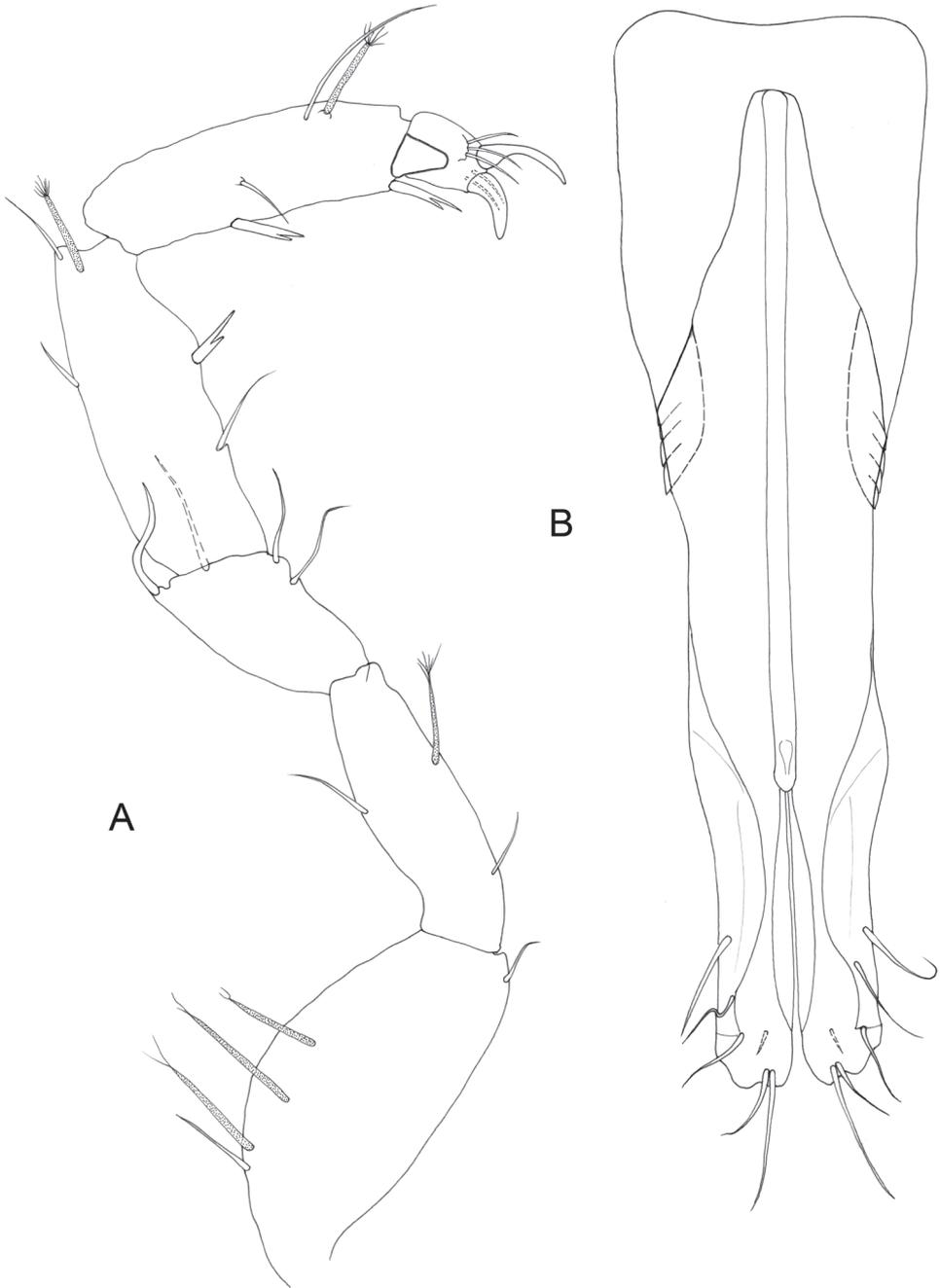


Figure 12. *Lepidocharon lizardensis* gen. n., sp. n. ♂ holotype. **A** periepod 7 **B** pleopod 1 (scale bar: 0.1 mm).

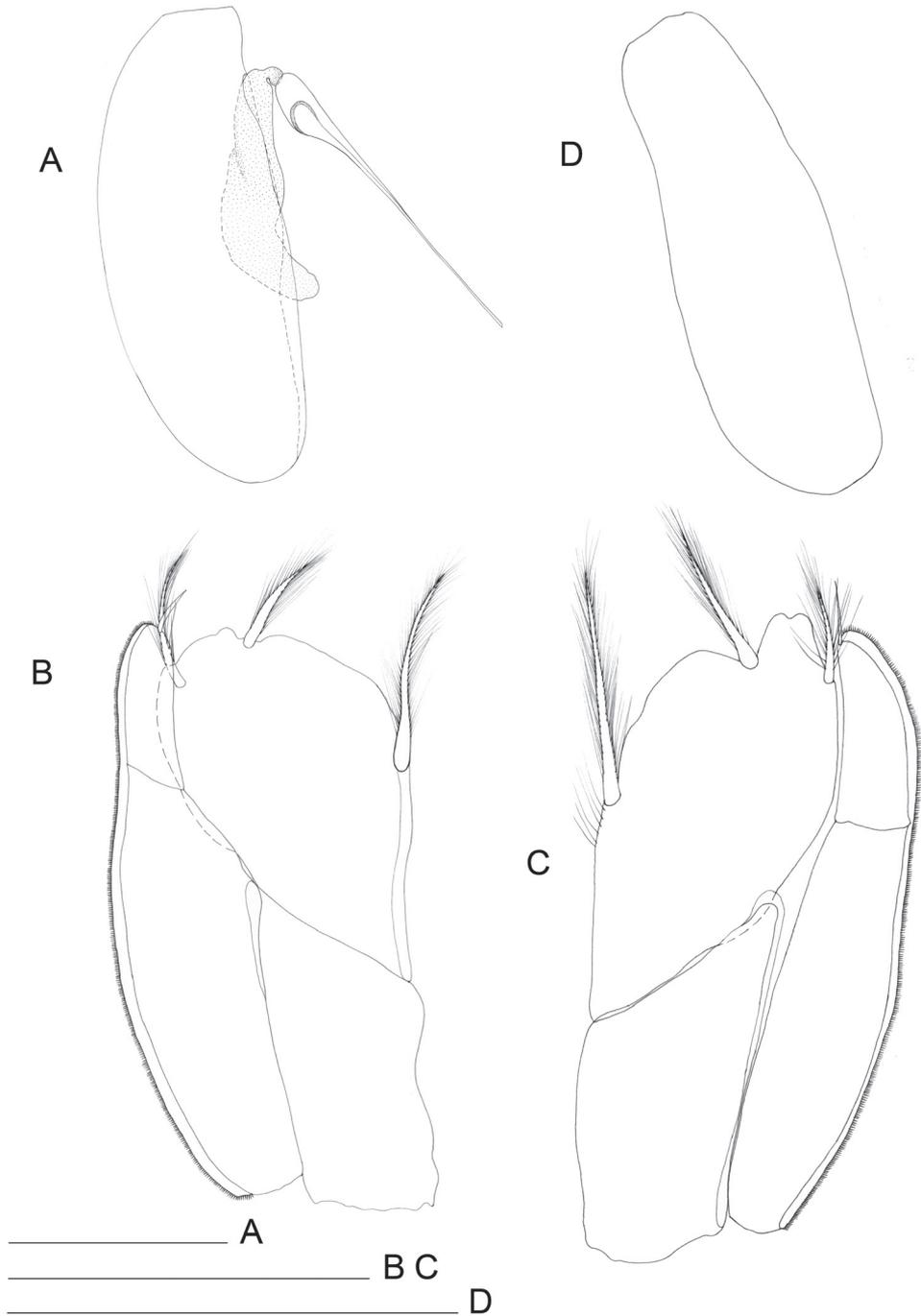


Figure 13. *Lepidocharon lizardensis* gen. n., sp. n. **A, C** ♂ holotype. **A** pleopod 2 **B** ♂ paratype, right pleopod 3 **C** left pleopod 3 **D** ♂ paratype, pleopod 4 (scale bars: 0.1 mm).

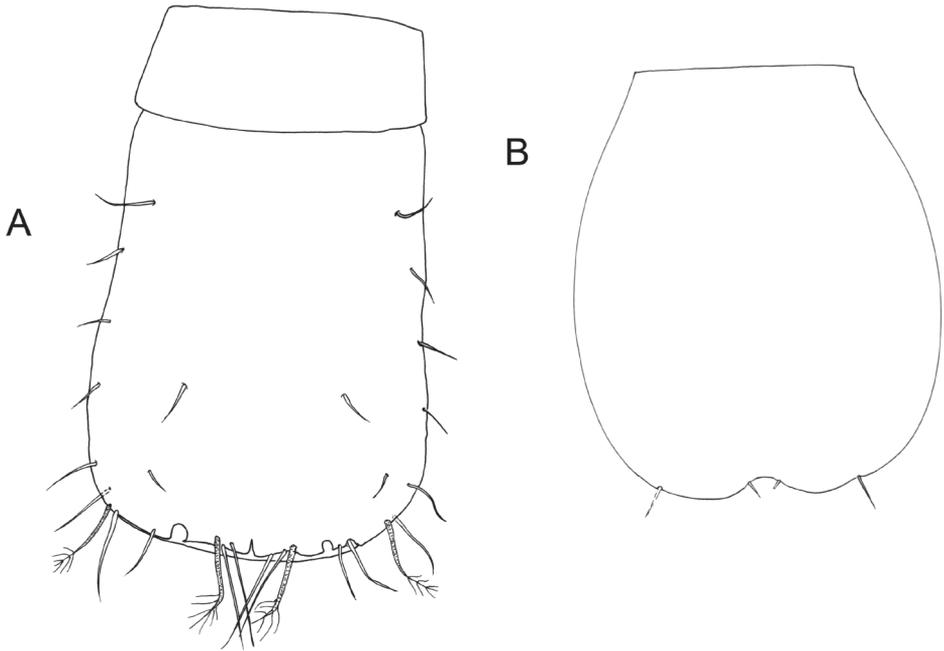


Figure 14. *Lepidocharon lizardensis* gen. n., sp. n. **A** ♂ holotype, pleonite free and pleotelson **B** ♀ paratype, pleopod 2 (operculum) (scale bar: 0.1 mm).

Male pleopod 2 (Fig. 13A): protopod elongate, sub-rectangular at proximal part, with rounded mediolateral corner; exopod protruding from protopod at middle of mesial margin, *appendix masculina* (endopod) stylet ending with skewed apex, opening oblique; stylet short, shorter than and not reaching distal part of protopod.

Pleopod 3 (Fig. 13B–C) exopod bearing 1 apical plumose seta, 1 lateral subapical medial long plumose seta and 1 subapical mesial plumose seta. Between mesial and apical setae exopod is protruded in rounded or bilobate lobes (Fig. 13B–C); endopod with setulose hyaline lamella on mesial margin bordered by fine setule row; endopod 1 elongate, about 2.2 longer than endopod 2, the latter ending with short simple subapical seta.

Pleopod 4 (Fig. 13D) rudimentary, ellipsoidal, uniramous.

Uropods unknown.

Female. Body length approximately as in male. Body length measured from tip of cephalon to end of pleotelson 1.2 mm. No sexual dimorphism observed in body morphology, cephalic appendages and pereopods. Female operculum (pleopod 2) elongate (Fig. 14B), sub-ovoid, with rounded lateral margins, proximal margin straight; distal margin with medial incision, with 2 close-set short medial setae very close to medial incision and 2 longer apical setae in lateral position. Operculum surface smooth. Uropods unknown.

Remarks. *Lepidocharon priapus* and *L. lizardensis* differ from each other in several characters: 1) the morphology of the antennal scale (blade-knife shaped in *L. priapus*

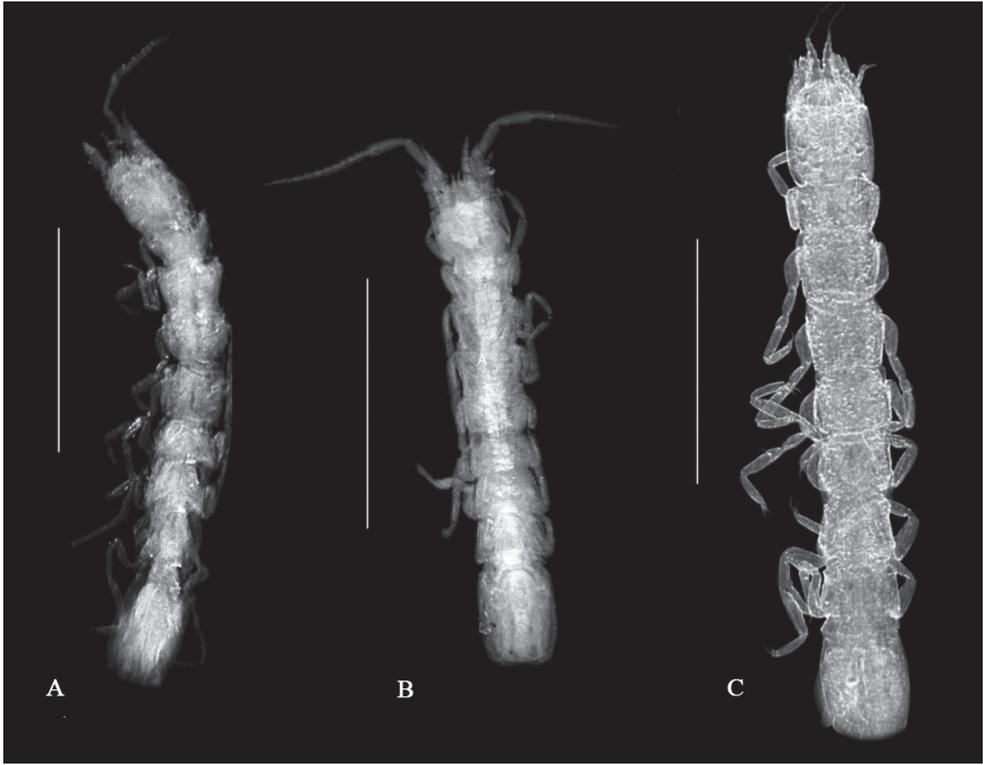


Figure 15. Stereomicroscope images of the habitus of **A** *Lepidocharon priapus* gen. n., sp. n. ♀ paratype **B** *Lepidocharon lizardensis* gen. n., sp. n. ♀ paratype **C** *Microcharon reginae* Dole and Coineau, 1987 ♀ toptype, showing the different morphology of the pereionites, the topology of pereiopods, and the different degree of development of the single pleonite free (scale bars: 0.5 mm).

vs. candle-flame shaped in *L. lizardensis*); 2) the slender body with different degree of protrusion of the pereionites 1–3 and 5–7 (markedly protruded in *L. priapus* *vs.* stouter and less protruded in *L. lizardensis*); 3) the different shape of the male pleopod 1 (with strongly protruded and pointed apical lobes and sclerotized hyaline lamella in *L. priapus* *vs.* sub-rounded and undulated apical lobes and a tiny hyaline lamella in *L. lizardensis*); 4) the setal complement of the male pleopod 1 (7 in *L. priapus* *vs.* 6 in *L. lizardensis*); 5) the morphology of the male pleopod 2 (sub-rectangular and long protopod with endopodal stylet extraordinarily long, reaching the tips of the uropods in *L. priapus* *vs.* ovoidal protopod with a short stylet, not reaching the distal part of the protopod in *L. lizardensis*); 6) female operculum sub-rectangular in shape, with straight lateral margins in *L. priapus* *vs.* oval, with convex lateral margins in *L. lizardensis*); 7) a rounded pleotelson in *L. priapus* (sub-truncate in *L. lizardensis*), and 8) body surface with visible semicircular thickening in *L. priapus* (smooth in *L. lizardensis*).

Discussion

The family Microparasellidae has been provisionally assigned to the asellote superfamily Janiroidea Sars, 1897. Its monophyletic status was debated since the interpretation of most character states in microparasellids is still doubtful, if not questionable (Bocquet and Lévi 1955, Wolf 1962, Cvetkov 1968, Wilson 1987, Wilson and Wägele 1994) in recognizing family rank for the Microparasellidae. Its monophyletic status had been hypothesised by Coineau and Schmidt (1979) and Coineau (1986, 1994). Since the original diagnosis given by Karaman (1933) (without providing the family name) and the provisional diagnosis given by Wilson and Wägele (1994) in their review of the family Janiridae, it had become clear that several diagnostic characters are weak and others have not been considered in detail (Albuquerque et al. 2014), and both the Janiridae and Microparasellidae were placed as *incertae sedis* (*sedis mutabilis* according to Wilson 1987: page 776) in the suborder Asellota.

Wilson and Wägele (1994) produced a critical review of the genera attributable to the former family Microparasellidae, arguing the definitive exclusion of the genus *Protocharon* Chappuis, Delamare Deboutteville & Paulian, 1956 from this family on the basis of the closest similarity, they claimed, between *Protocharon* and *Iais* Bovallius, 1886, with *Protocharon antarctica* Chappuis, 1958 definitively moved to the janirid genus *Iais* (Wilson and Wägele 1994: page 700), and *Protocharon arenicola* Chappuis, Delamare Deboutteville & Paulian, 1956 being the only species attributable to the now monotypic genus *Protocharon*.

Similar arguments had been anticipated by Wolf (1962) who highlighted that blind and colourless species occur in almost all the families of the suborder Asellota; species belonging to the same genus may or may not have eyes. Anophtalmy is a common convergence to lightless or extreme low-light habitats, such as the deep-sea, groundwater habitats, mud, crevices, and as such is useless in assessing phylogenetic relationships. For instance, several Janiridae are eyeless and small-sized (e.g., species of *Jaera* Leach, 1814, *Heterias* Richardson, 1904, *Austrofilus* Hodgson, 1910, *Caecijaera* Menzies, 1951, *Caecianiropsis* Menzies & Pettit, 1956), and have neither prehensile nor subchelate pereopod 1, as have all the presently known species assigned to both Microparasellidae and Lepidocharontidae.

Among the genera assigned to the former family Microparasellidae, the genus *Angeliara* uniquely has a 7-segmented antennula, antennal scale absent; the maxilliped palp 4-segmented, without apical stiff pectinate setae; most pereopods (variable among species of the genus) with three claws; pereopods 1–4 and 6–7 subsimilar, with pereopod 5 sexually dimorphic, in the male being subchelate and stout, with carpus transformed; male pleopod 1 is short and sub-quadrate and the penial processes are paired, not coalescent, arising near the base of pereopod 7 (i.e. laterally) (see for details Coineau 1968). The female operculum is shorter than pleotelson, deeply incised or concave on free distal margin, without apical setae, never reaching the free distal margin of the pleotelson. Furthermore in *Angeliara* the male pleopod 2 displays a short and truncate form, similar to that of the Vermectiadiidae Just & Poore, 1992.

The family Vermectiadiidae Just & Poore, 1992 shows superficial similarities to the genus *Angeliara* but can immediately be distinguished by having three free pleonites, laminar pleopods, none of which form an operculum, as well as small eyes (Just and Poore 1992).

Angeliara lacks the molar process, the most derived condition found in the Janiroidea. Nevertheless, similar reductions of the molar process occur in the janiroid family Katianiridae Svavarsson, 1987, where it varies from being reduced to a single spine in *Katianira acarina* (Menzies, 1962) (Svavarsson 1987) to lost in *Katianira platyura* Shimomura & Akiyama, 2006 (Shimomura and Akiyama 2006: fig. 1D, E, page 578). Similar reductions occur also in members of other janiroid families (e.g. Nannoniscidae Hansen, 1916, Desmosomatidae G.O. Sars, 1897, Macrostylidae Hansen, 1916, Munnopsidae Lilljeborg, 1864) and are likely adaptive traits related to the trophic niche of the taxa. The fourth article of the maxillipedal palp in *Angeliara* has one apical seta only instead of the two distal stiff setae present in all the other members of the family. As the maxilliped is considered homologous to the pereopods (for details see Thompson 2013), the condition showed by *Angeliara*, with only one apical seta, coalescent with the fourth article, is to be considered a derived condition in the Janiroidea.

Zemko and Kaiser (2012) discussed some affinities of the janiroidean family Thambematidae Stebbing, 1912, known from marine deep-sea habitats, and the former Microparasellidae, as previously claimed by Birstein (1961). The Thambematidae, on the basis of the similar body shape, was supposedly related to the Microparasellidae, even if the single free pleonite in Thambematidae is well-developed in comparison to the more reduced pleon of the Lepidocharontidae genera *Janinella* and *Lepidocharon*. Numerous characters separate the two families, including the morphology of the mandibular molar process, which is cylindrical in Thambematidae Stebbing, 1912 (*vs.* conical in Lepidocharontidae fam. n.), flat and prominently setose pereopods (*vs.* slender), pereopod 1 distinctly prehensile (*vs.* never prehensile neither subchelate in Microparasellidae and Lepidocharontidae fam. n.), male pleopod 1 with disto-lateral protrusions representing the extension of the transversal stylet guiding groove (only superficially resembling the general construction found in *Janinella* and in some Janiridae) and short, cylindrical uropodal sympod (*vs.* flattened) (Harrison 1987, Zemko and Kaiser 2012).

A single free pleonite is shared by the Janiridae, the Microparasellidae and the Lepidocharontidae fam. n., as well as by other phylogenetically distant families (e.g. Thambematidae Stebbing, 1912; Paramunnidae Vanhöffen, 1914; Urstylidae Riehl, Wilson & Malyutina, 2014). Members of the genus *Microcharon*, as observed in *Microcharon reginae* Dole & Coineau, 1987, possess a large free pleonite (Fig. 15C). Species of *Janinella* (Albuquerque et al. 2014) and *Lepidocharon* have a shorter and narrower pleonite.

The single free pleonite is even more reduced in the janirid *Microjaera anisopoda* Bocquet & Lévi, 1955, as highlighted by Bocquet and Lévi (1955). These authors supported the hypothesis of a strict relationship between *Microjaera* Bocquet & Lévi, 1955 and the microparasellid genus *Microparasellus* and the lepidocharontid genus

Microcharon on the basis of the elongate and slender body, the small size, and other unspecified characters (Bocquet and Lévi 1955: page 128). Conversely, the differences observed and listed by the same authors among their *Microjaera* and *Microparasellus-Microcharon* (and *Angeliara*) support only a remote affinity among these genera. Later, a second species of the genus, *Microjaera morii* Shimomura, 2005, was described, this species showing a highly reduced free pleonite, that is not discernible in dorsal view, and visible only on ventral view (Shimomura 2005: fig. 3F, page 118).

The relatively *large size of the free pleonite* in Microparasellidae (*Microparasellus*) has been considered a distinctive trait of the family by Wilson and Wägele (1994). After assessing the degree of development of this body somite, we observed that among the Lepidocharontidae, it is well-developed in *Microcharon*, being as large as pereionite 7. It is smaller and narrower in *Janinella* and *Lepidocharon*.

The *antennula* in the Lepidocharontidae is short, not sexually dimorphic, and composed of a maximum of 6 articles, this condition being derived in the Janiroidea. The segmental pattern of the antennula ranges from 6 to 5 in *Microcharon*, where the 5-segmented condition is shared by almost all the marine species of the genus, and the 6-segmented condition by fresh groundwater species, which likely retained the 6-segmented antennula of the ancient marine ancestor, in the more conservative and stable groundwater environment, according to Coineau (1986). Only the marine species *Microcharon monnioti* Bocquet, 1970 from the psammolittoral of Roscoff, France, possesses a 6-segmented antennula (even if the generic position of this species requires confirmation, N. Coineau, pers. comm.). The antennula is 5-segmented in *Janinella* and 6-segmented in *Lepidocharon*.

The *antennal exopod* (scale) is present in all the Lepidocharontidae genera. In general, the exopod is rudimentary in the Janiridae, and in members assigned to the Lepidocharontidae is ovoid and small, as in *Microcharon*, the most reduced condition being found in *Janinella* (Albuquerque et al. 2014) with a reduced scale, not reaching the tip of the third podomere. Conversely, it is long in *Lepidocharon*, reaching the fourth podomere, being candle-flame or knife-blade shaped.

The *molar process of the mandible* is always conical and reduced in the lepidocharontid genera *Microcharon*, *Janinella* and *Lepidocharon*.

In *Lepidocharon* the *mandibular palp* is inserted on short cuticular projection, as also observed in *Janiropsis* Sars, 1897 and *Janaira* Moreira & Pires, 1977 by Doti and Wilson (2010) and it is figured as a small well-defined article in *Trogloianiropsis lloberai* Jaume, 1995 (Jaume 1995: fig. 16, page 183). This condition is likely primitive in the Janiroidea, and seems to be retained by *Lepidocharon*.

The *distal article of the mandibular palp* bears from 5 to 3 robust setae, the latter status shared by the most derived groundwater species of *Microcharon*. The possession of 5 setae is shared by a few marine *Microcharon* species, when described, and the stygobiotic *Microcharon acherontis* Chappuis, 1942 is figured with 6 setae, a state not found in any other member of the genus.

In the *incertae sedis* genus *Angeliara*, the terminal segment of the mandibular palp has been described and figured without any seta or stiff setae, and some descriptions

refer to the original description of *A. phreaticola* Chappuis & Delamare Deboutteville, 1952 for which few details are available (Chappuis and Delamare 1952, Delamare Deboutteville 1960, Coineau and Rao 1972).

The *maxilliped* is 5-segmented in *Microcharon*, *Janinella* and *Lepidocharon*.

The genus *Angelierra* placed as *incertae sedis* in the Lepidocharontidae has a 4-segmented palp as other janiroid families, e.g., *Katianira* and *Natalianira* Kensley, 1984 (Katianiridae), raising questions about purported affinities between katianirids and the genus *Angelierra* (see Svavarsson 1987). The same author (Svavarsson 1987: page 717) rejected this assumption on the basis of the marked differences observed in *Katianira* and the former Microparasellidae in body shape, structure of the antenna and uropods.

The *maxilliped endite* has the same shape, recurrent in all members of the family, except for the genus *Angelierra*, where its distal part is swollen and ending with sinuous setae.

The *body morphology* is generally similar among members of the family Lepidocharontidae: pereional somites are rectangular in *Microcharon*, with a well-developed free pleonite, shorter and as wide as pereionite 7. *Janinella* and *Lepidocharon* have the anterior three pereionites markedly protruded anteriorly, the fourth almost rectangular in shape, and the last three pereionites protruded posteriorly. We assume that the morphology of the pereionites, together with the position of the pereiopods are related to a different way of locomotion of the species.

The *pereiopodal dactylus* has two unguli, showing a general tendency to be stout and subequal in length in marine species, and slender with the inner claw about $\frac{1}{2}$ the length of the lateral claw in freshwater species.

The *morphology and armature of the female operculum* differs within the family. A character considered diagnostic for species distinction within the family is the number of apical setae bordering the female operculum. In *Microcharon* the highest variation occurs: from long, more than two times longer than wide, faintly incised female operculum in marine and freshwater species, bearing 4 or 2 apical setae (Galassi 1991), to rounded and unarmed in the most derived groundwater species (Coineau 1994).

The pleopod 3 has the *distal article of the endopod* either with 3 plumose setae or completely unarmed. The marine species always have these setae and they are consistently absent in all groundwater species.

Angelierra shows the most striking derived character states, suggesting a divergent position in relation to the remaining members of the family. *Janinella* shares with *Angelierra* the topology of the outwardly directed stylet-guiding groove of the male pleopod 1 on both pleopodal rami, a character that may be phylogenetically informative, and likely primitive in the family because it is shared by members of the closely related family Janiridae (e.g. *Jaera* Forsman, 1949, *Iais*, *Ectias* Richardson, 1906). *Microcharon* and *Lepidocharon* share stylet-guiding grooves folded by a hyaline lamella that run almost parallel to the free lateral margins of the distal parts of male pleopod 1, a likely derived organization and topology of the stylet-guiding groove if compared to that of *Janinella*. The stylet guiding-groove is in this case represented by a hyaline lamella, which holds the stylet of the pleopod 2 in a precise position. In the

first case, as in *Jaera* (Wilson 1991: fig. 13.3, page 237), *Angelierea* and *Janinella*, the tip of the endopodal stylets of the second pleopod are directed outwards; conversely they run parallel to the lateral margins of pleopod 1 (Cvetkov 1968: fig. IV, page 118) in *Microcharon* and *Lepidocharon*, and the stylets are locked in a fixed position by lateral folding of the pleopods 1 (Cvetkov 1968: fig. IIA, page 115). We retain that these differences may have a phylogenetic significance, agreeing with Wilson (1991, 1994).

The penial papillae are differently organized in *Angelierea*: they are tubules which start from the insertion of the pereopods on sternite 7 and converge at the midline of the sternite of the pereonite 7, maintaining their openings separate and not coalescent (Coineau 1971). In *Microcharon*, *Janinella* and *Lepidocharon*, there is no longer trace of paired penial papillae, as in most Janiroidea, and they are located at the postero-medial margin of the sternite, are very small, coalescent, even if still separated by a medial groove, likely the remnant of their paired origin. The few data available on *Microcharon* and *Janinella* show a medial position of the penial papillae (Coineau 1971); in *Lepidocharon* they are for the first time observed with the aid of scanning electron microscopy (Fig. 6B). According to Wilson (1987, 1991) little information is available on the precise function of the male pleopods 1 and 2 in the Janiroidea with regard to sperm transfer from the penial papillae (penes) to the female genitalia, being still unclear the role of the endopodal stylet of male pleopod 2, and the mating behaviour highly variable among the widely diversified asellote isopods. On this regard, it was argued that the different modes of copulation observed in the marine isopods may have played a key-role in their high diversification in marine and freshwater environments, allowing also the colonization of the deep-sea habitats (Wilson 1991).

The description of *Lepidocharon* Galassi & Bruce, gen. n. and the establishment of the Lepidocharontidae Galassi & Bruce, fam. n. shed new light on the diversity of the morphological body plans observable in the former family Microparasellidae, allowing for a better understanding of the phylogenetic relationships of the Lepidocharontidae fam. n. and the Microparasellidae, a family placed as *incertae sedis* within the superfamily Janiroidea Sars, 1897 (see Wilson 1994; Wilson and Wägele 1994).

Conclusion

The small body size, soft cuticle, possession of the single reduced pleonite with laterally free margins, the simplification of the general structure and armature of the mandibles, the low diversification of setae morphology, the absence of pleopod 5, could be interpreted all together as derived character states, which may conversely be the result of homoplasy for adaptive convergence due to the interstitial life, or to life in microhabitats with reduced living space (Coineau 1986, 2000, Galassi et al. 1999).

In addition, the lack of morphological data in both historic and some recent contributions does not help in reconstructing an evolutionary scenario. Nonetheless, despite convergence, phylogenetic characters can be identified, and a greater degree of character resolution in family and genera definitions developed. Even if comprehensive

data for all species are not available, it is evident that the current generic composition of the Microparasellidae could not be maintained. The separation of the Lepidocharontidae fam. n. and its constituent genera resolves the former paraphyletic family into two monophyletic families, which in future should enable a more clear understanding of the relationships of the two families to the Janiridae to be developed.

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Appendix I

Microparasellidae Karaman, 1934

The family name Microparasellidae was first formally proposed by Karaman (1934: page 44) although the family diagnosis was given the previous year (Karaman 1933: page 17) when describing the new species and new genus *Microparasellus puteanus* Karaman, 1933, with the accompanying statement “*Microparasellus n. fam., n. gen.*” At that time a type species was not designated for the genus *Microparasellus* (see Karaman 1933), nor one year later (see Karaman 1934). As previously stated, the family name was considered valid until 2000, and for this reason it is an available name as Microparasellidae Karaman, 1934.

Microparasellidae Karaman, 1934

Synonymy: Karaman 1934: 44.—Wolff 1962: 35.—Coineau 1986: 465.—Kensley and Schotte 1989: 90.—Wilson and Wägele 1994: 720.

Diagnosis of male. Body slender, 4.0–6.0× long as wide, somites all subsimilar in width, head with acute or narrowly rounded rostrum. Lateral margins of head and pereionites convex, with cuticular scales; pleonite 1 laterally free; body without chromatophores. Eyes absent. Antennula flagellum with maximally 4 articles. Antennal scale absent. Antenna flagellum shorter than podomeres. Mandibular molar process distally pointed, without grinding surface, with several terminal setae. Pereiopods 1–7 not chelate or subchelate, almost identical in shape; all pereiopods with 2 dactylar

claws. Pereiopods articulated latero-ventrally. Penial processes present, coalescent, with single medial opening on posterior margin of pereionite 7. Pleopods 1 and 2 not operculate in males; female pleopod 2 operculate; pleopod 3 endopod unarmed, exopod slender. Uropods uniramous, minute, stub-like, insertion ventro-terminal; single ramus, shorter than protopod. Anus terminal, not covered by pleopods.

Remarks. The family Microparasellidae was ambiguously established by Karaman (1933) without formally stating family name anywhere in that publication. On the same occasion, the author described two new species of *Microparasellus*, namely *Microparasellus puteanus* Karaman, 1933 and *Microparasellus stygius* Karaman, 1933, but without designation of the type species for the genus *Microparasellus*, and without providing the name of the new family. One year later, Karaman (1934) formally proposed the family name Microparasellidae for the new family, also giving a family diagnosis and assigned two genera to the family: the then monotypic *Microparasellus* Karaman, 1933 (*Microparasellus puteanus*) and transferring *Microparasellus stygius* Karaman, 1933 to the new genus *Microcharon* Karaman, 1934 together with *Microcharon latus* Karaman, 1934 described in the same paper (Karaman 1934).

***Microparasellus* Karaman, 1933**

Microparasellus Karaman, 1933: 17; 1934: 44.—Wilson and Wägele 1994: 725.

Duslenia Lévi, 1950: 42.

Type species. *Microparasellus puteanus* Karaman, 1933. Type locality: Skopje, Macedonia. Karaman (1934) formally described the family Microparasellidae, at the same time establishing the genus *Microcharon* Karaman, 1934. At that point Karaman restricted the genus *Microparasellus* to the single species *M. puteanus*. This in itself does not constitute a subsequent type species designation, and Karaman's (1934) intention was clearly not to do that as he equally did not designate a type species for *Microcharon* (this was a common practice at that time).

Other species. *Microparasellus libanicus* Chappuis & Delamare Deboutteville, 1954, Lebanon; *Microparasellus aloufi* Coineau, 1968, Lebanon; *Microparasellus hellenicus* Argano & Pesce, 1979, Greece.

Remarks. The generic name *Microparasellus* established by Karaman (1933) is not an available name because, even though a diagnosis has been given, the type-species was not designated for the genus. This course of action makes the name unavailable under the provisions of the ICZN (1999: Article 67.4.1 which states: "A nominal genus-group taxon established after 1930 (or, in the case of an ichnotaxon, after 1999 [Art. 66.1]) must have its type species fixed in the original publication [Art. 13.3]". As the nomenclature within the family Microparasellidae is well established and widely used, a proposition to the ICZN Commission is needed for maintaining the stability of the nomenclature and related authorities. The same situation exists for the genus *Microcharon* Karaman, 1934, now placed in the new family Lepidocharontidae. This

state of affairs, along with the minimal standard of species descriptions, would require a detailed re-examination of the *Microparasellus* as a whole. Pending this procedure, authorities and dates of common use are hereby maintained.

The Microparasellidae can be regarded as monophyletic as here defined. While we do not enter a discussion into monophyly or otherwise of the Janiroidea, it is apparent, as shown by Wilson's (1994) analysis, that the Microparasellidae lacks the characteristic operculate pleopods shown by the Janiroidea.

Distribution. Species of the family are known from North Africa and eastern Europe only.

Two new species of *Echinoderes* (Kinorhyncha, Cyclorhagida), *E. romanoi* sp. n. and *E. joyceae* sp. n., from the Gulf of Mexico

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Abstract

Meiofauna sampling on the continental shelf of the northern Gulf of Mexico has been ongoing since 2007, on annual cruises in collaboration with the National Marine Fisheries Service laboratory in Pascagoula, Mississippi. This sampling has resulted in numerous new species of kinorhynchs from the shelf sediment, two of which are described in detail in this paper. Other species descriptions from this research effort include *Echinoderes augustae*, *E. skipperae*, and *E. charlotteae*. We now describe *Echinoderes romanoi* sp. n. and *E. joyceae* sp. n., which are unique in their spine, tube, and glandular cell outlet patterns.

Keywords

Echinoderidae, kinorhynchs, meiofauna, morphology, taxonomy

Introduction

Diversity in the phylum Kinorhyncha has been underreported from the Gulf of Mexico, though recently new investigations are adding to our knowledge of the Gulf species. Currently there are few kinorhynchs identified to species from the Gulf, though their presence and abundance are well documented. The known species diversity in the

Gulf of Mexico includes *Echinoderes steineri* (Chitwood, 1951), *E. coulli* Higgins 1977, *E. remanei* (Blake, 1930), *Kinorhynchus langi* (Higgins, 1964), *Campyloderes* cf. *vanhoeffeni* Zelinka, 1913, *Centroderes barbanigra* Neuhaus et al., 2014, *Centroderes* cf. *drakei* Neuhaus et al., 2014, *E. skipperae* Sørensen & Landers, 2014, *E. augustae* Sørensen & Landers, 2014, *E. bookhouti* Higgins, 1964 and *E. charlotteae* Sørensen et al., 2016 (Chitwood 1951, Harper et al. 1981, Shirley 2009, Neuhaus 2013, Neuhaus et al. 2014, Sørensen and Landers 2014, Fleeger et al. 2015, Sørensen et al. 2016). The last four Gulf records resulted from meiofauna surveys conducted by our labs in the Gulf in collaboration with the National Marine Fisheries Service (NMFS) lab in Pascagoula, Mississippi. This collaboration began in 2007 and continues currently, with sediment collection occurring on annual fall cruises. The first two reports from the kinorhynch analysis of this long term meiofauna study described three new species, *E. augustae*, *E. skipperae*, and *E. charlotteae*, and also provided a redescription of *E. bookhouti* (Sørensen and Landers 2014, Sørensen et al. 2016). This current contribution is the third in our series of new kinorhynch species discovered in the northern Gulf of Mexico, from continental shelf sediments, and describes *Echinoderes romanoi* sp. n. and *E. joyceae* sp. n. These new descriptions will be helpful for future taxonomic and morphological studies.

Materials and methods

Sediment was collected along the northern Gulf of Mexico continental shelf during several NOAA cruises from 2010 to 2015 in collaboration with the NMFS lab in Pascagoula, Mississippi, on NOAA ships *Gordon Gunter*, *Pisces*, and *Oregon II*. Sediment was collected in 2010–2012 using a Shipek® sediment grab, and in 2013–2015 using an Ocean Instruments® mini-multicorer. Specimens from the present study were obtained from 12 locations located along the northern Gulf of Mexico continental shelf (Fig. 1, Table 1).

The samples were fixed immediately in 5–10% formalin on the cruise, and the meiofauna was subsequently extracted by Ludox centrifugation (Burgess 2001). After sorting the animals using a counting wheel, the kinorhynchs were stored in 70% isopropanol. They were processed for light microscopy by subjecting them to increasing concentrations of glycerin before mounting them in Fluoromount G® (Sørensen and Pardos 2008). They were examined and photographed using a Nikon E600 (Troy University) or an Olympus BX51 (University of Copenhagen) light microscope equipped with Nomarski interference contrast optics using digital cameras. Line art illustrations were based on mounted specimens that were drawn using Adobe Illustrator® CS6 or Adobe Photoshop Elements® software. Measurements were made with CellSens® software. All dimensions reported in the tables are based on LM measurements. All light microscopy type material is deposited at the Natural History Museum of Denmark (Copenhagen, Denmark).

Table 1. Summary of data on stations, species identities and catalogue numbers (KIN-) for specimens deposited at the Natural History Museum of Denmark.

Station	Date	Position	Depth, Salinity (ppt)	Species	Mounting	Type status and catalogue numbers
149-2010	Nov. 21, 2010	28° 31'9"N 86° 18'54"W (28.5192°, -86.3151°)	443 m 35.2	<i>Echinoderes romanoi</i> sp. n.	LM	1 ♂ nontype
154-2010	Nov. 22, 2010	29° 22'28"N 86° 41'46"W (29.3747°, -86.6962°)	382 m 35.4	<i>Echinoderes romanoi</i> sp. n.	LM	1 ♂ paratype, KIN-967, 1 ♂ and 1 ♀ non-types
050-2013	Nov. 2, 2013	28° 14'21.12"N 90° 52'1.91"W (28.2392°, -90.8672°)	74 m 36.5	<i>Echinoderes romanoi</i> sp. n.	LM	1 ♂ paratype, KIN-899
012-2014	Nov. 13, 2014	29° 15'10"N 88° 18'23"W (29.2528°, -88.3066°)	90 m 36.3	<i>Echinoderes romanoi</i> sp. n.	SEM LM	1 ♂ nontype 1 ♂ holotype, KIN-962, 2 ♀ paratypes, KIN-963 to KIN-964
019-2014	Nov. 14, 2014	28° 43'52"N 89° 34'16"W (28.7313°, -89.5712°)	94 m 36.3	<i>Echinoderes romanoi</i> sp. n.	LM	2 ♀ paratypes, KIN-965 to KIN-966 1 ♂ nontype
026-2014	Nov. 16, 2014	28° 27'25"N 91° 31'53"W (28.4572°, -91.5314°)	52 m 36.2	<i>Echinoderes romanoi</i> sp. n.	SEM LM	1 ♀ non type 1 ♀ non type
130-2015	Nov. 4, 2015	28° 32'45"N 91° 53'21"W (28.5459°, -91.8894°)	45 m 36.0	<i>Echinoderes romanoi</i> sp. n.	SEM	1 ♀ non type
010-2010	Oct. 16, 2010	27°09'12"N 96°09'59"W (27.1534°, -96.1666°)	427 m 35.2	<i>Echinoderes joyceae</i> sp. n.	LM	1 ♂ holotype KIN-845, 1 ♀ paratype KIN-849
016-2010	Oct. 17, 2010	27°47'58"N 95°38'11"W (27.7995°, -95.8345°)	57 m 36.4	<i>Echinoderes joyceae</i> sp. n.	LM	1 ♂ paratype KIN-850
068-2012	Oct. 22, 2012	28°05'12"N 91°38'59"W (28.0868°, -91.6365°)	99 m 36.4	<i>Echinoderes joyceae</i> sp. n.	LM	1 ♂ paratype KIN-922
031-2013	Oct. 30, 2013	28°01'55"N 93°13'29"W (28.0320°, -93.2249°)	99 m 31.2	<i>Echinoderes joyceae</i> sp. n.	LM	1 ♂ paratype KIN-867
033-2014	Oct. 20, 2014	29° 32'32"N 86° 11'33"W (29.5423°, -86.1926°)	98 m 35.3	<i>Echinoderes joyceae</i> sp. n.	SEM	1 ♀ nontype

Specimens for scanning electron microscopy were observed at the Auburn University Research Instrumentation Facility (Auburn, Alabama). Specimens stored in 70% isopropanol were hydrated, post-fixed in OsO₄ vapor, then dehydrated to 100% ethanol through a graded series, critical point dried, mounted on aluminum stubs, and sputter-coated with gold. Specimens were photographed with a Zeiss EVO 50 SEM, using the backscatter and secondary electron detectors.

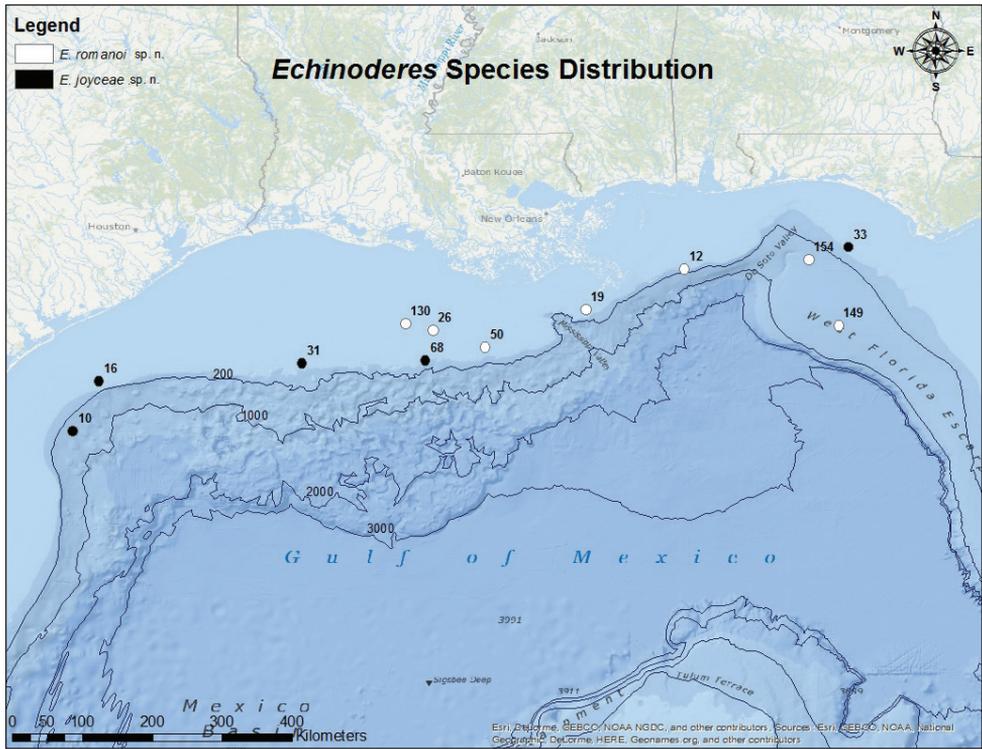


Figure 1. Map of the northern Gulf of Mexico indicating collection stations for *E. romanoi* sp. n. and *E. joyceae* sp. n.

Results

Class Cyclorhagida (Zelinka, 1896) Sørensen et al., 2015

Order Echinorhagata Sørensen et al., 2015

Family Echinoderidae Zelinka, 1894

Genus *Echinoderes* Claparède, 1863

***Echinoderes romanoi* sp. n.**

<http://zoobank.org/E6387ED6-A68B-4DB2-A835-777A585D5DEC>

Figs 2–4

Material. Holotype: Adult male (ZMUC KIN-962), collected from sediment on November 13, 2014, at station 012-2014 (Fig. 1), at 90 m depth, <100 km east of the outlet of the Mississippi River, Louisiana (29°15'10"N, 88°18'23"W), mounted in Fluoromount G®, deposited at the Natural History Museum of Denmark. Paratypes include two females (ZMUC KIN-963 and KIN-964) from station 012-2014, two females (ZMUC KIN-965 and KIN-966) from station 19-2014, one male from station 154-2010 (ZMUC KIN-967) and one male from 050-2013 (ZMUC KIN-899).

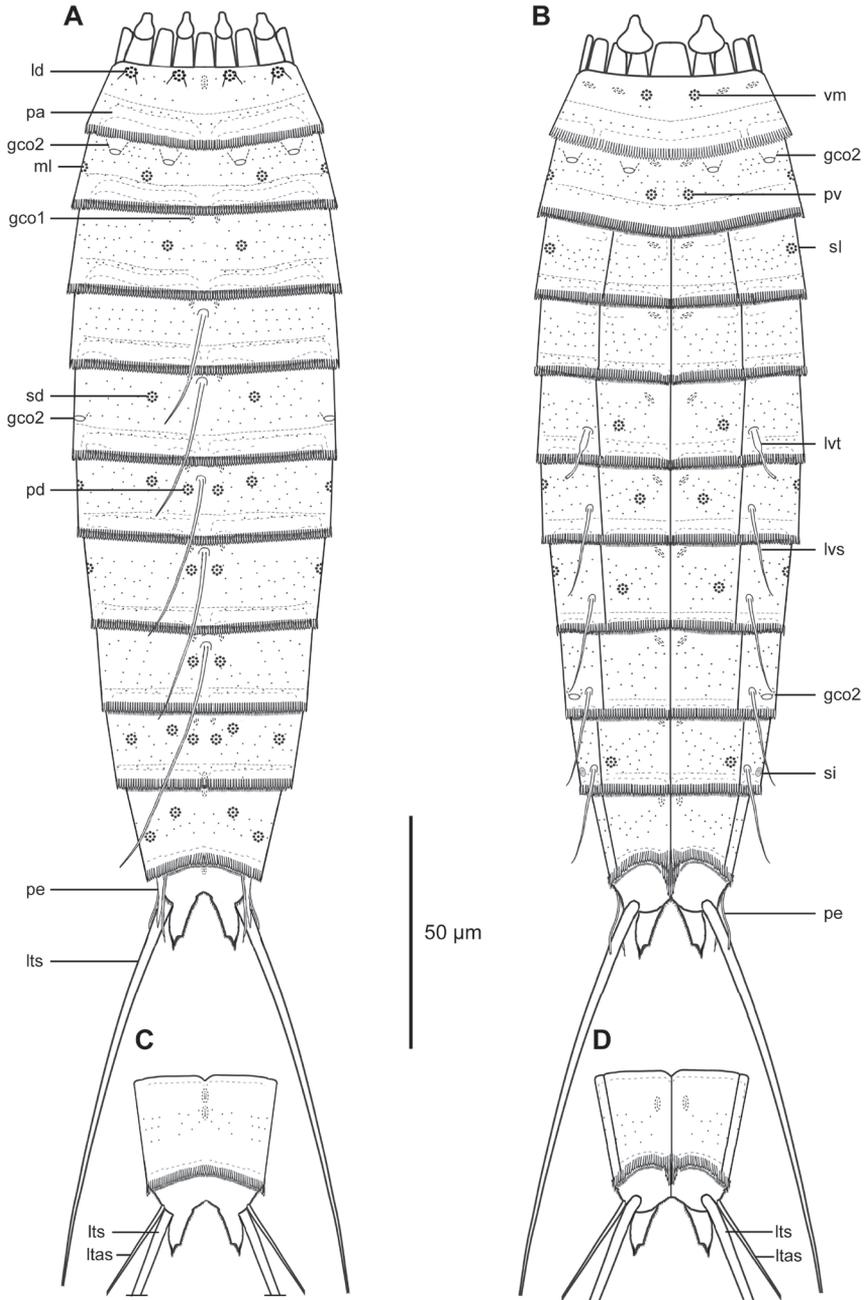


Figure 2. Line art illustrations of *Echinoderes romanoi* sp. n. **A** Male, dorsal view **B** Male, ventral view **C** Female, segments 10 to 11, dorsal view **D** Female, segments 10 to 11, ventral view. Abbreviations: gco1/2, glandular cell outlet type 1/2; ld, laterodorsal sensory spot; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tube; ml, midlateral sensory spot; pa, pachycyclycus; pd, paradorsal sensory spot; pe, penile spine; pv, paraventral sensory spot; sd, subdorsal sensory spot; si, sieve plate; sl, sublateral sensory spot; vm, ventromedial sensory spot.

All paratypes are mounted in Fluoromount G® and deposited at the Natural History Museum of Denmark. Additional nontype material is listed in Table 1. See Figure 1 for localities and Table 1 for detailed station information.

Diagnosis. *Echinoderes* with middorsal spines on segments 4–8, and spines in lateroventral positions on segments 6–9. Tubes present in lateroventral position on segment 5. Glandular cell outlets type 2 present in subdorsal, laterodorsal, sublateral, and ventrolateral positions on segment 2, in midlateral position on segment 5, and in sublateral position on segment 8.

Description. Adults with head, neck and eleven trunk segments, ranging from 196–247 µm in trunk length (Figs 2–4). For complete overview of measures and dimensions, see Table 2. Distribution of cuticular structures, i.e., sensory spots, glandular cell outlets, spines and tubes, is summarized in Table 3.

The head (Fig. 3A, B, 4B) consists of a retractable mouth cone and an introvert. The mouth cone has nine outer oral styles. The introvert sectors are defined by 10 primary spinoscalids in ring 1. Each primary spinoscalid consists of a basal sheath with approximately 7 long extensions forming a fringed margin, and a distal end piece with a blunt tip. It was only possible to obtain information about the appearance and arrangement of scalids for introvert sectors 2, 3, and 4, which have the following characteristics: single central scalids of Rings 02 and 04, and paired scalids of Rings 03 and 05.

The neck (Figs 2A, B, 3A, B) has 16 placids, measuring 10 µm in length. The midventral placid is broadest, measuring 9 µm in width at its base, whereas all other are narrower, measuring 5–6 µm in width at their bases. The trichoscalid plates, each with a trichoscalid, are present in subdorsal, laterodorsal and ventromedial positions.

Segment 1 (Figs 2A, B, 3A–C, 4A) consists of a complete cuticular ring. Sensory spots are located anteriorly in subdorsal, laterodorsal, and ventromedial positions; sensory spots minute and rounded with two anterior cuticular hairs. Glandular cell outlets type 1 present middorsally, sublaterally and lateroventrally. Cuticular hairs sparse on dorsal and ventral surface. A line of cuticular hairs is located below the intersegmental joint line. Pectinate fringe of posterior segment margin with typical fringe tips.

Segment 2 (Figs 2A, B, 3A–D, 4A) consists of a complete cuticular ring. Pachycyclus of the anterior segment margin interrupted in middorsal and lateroventral positions. Sensory spots located in laterodorsal, midlateral, and paraventral positions; sensory spots on this and following segments minute and rounded. Glandular cell outlets type 2 located in subdorsal, laterodorsal, sublateral and ventrolateral positions. Glandular cell outlets type 1 located in paraventral positions. Secondary pectinate fringe present on this segment and on the following segments 3–10.

Segment 3 (Figs 2A, B, 3A–C), and remaining segments, consisting of one tergal and two sternal plates. Pachycyclus of the anterior segment margin interrupted middorsally, midventrally, and at the tergo-sternal junctions. Sensory spots present in subdorsal and sublateral positions. Cuticular hairs evenly distributed over tergal and sternal plates, between the pectinate fringe and intersegmental joint line, with a line of cuticular hairs below the joint line. Glandular cell outlets type 1 in paradorsal and paraventral positions.

Table 2. Measurements from light microscopy of *Echinoderes romanoi* sp. n. (in μm) from the Gulf of Mexico, including number of measured specimens (n) and standard deviation (SD). Abbreviations: (ac): acicular spine; LTAS: lateral terminal accessory spine; LTS: lateral terminal spine; LV: lateroventral; MD, middorsal; MSW-7: maximum sternal width, measured on segment 7 in this species; S: segment lengths; SW-10, standard width, always measured on segment 10; TL: trunk length.

Character	n	Range	Mean	SD
TL	10	196–247	227	15.08
MSW-7	10	37–44	39	2.28
MSW-7/TL	10	15–20.8%	17.3%	1.87%
SW-10	10	30–35	33	1.58
SW-10/TL	10	13–16.5%	14.4%	1.15%
S1	10	20–24	22	1.33
S2	10	20–24	22	1.45
S3	10	21–26	24	1.51
S4	10	21–27	24	1.75
S5	10	24–29	26	1.64
S6	10	25–30	28	1.51
S7	10	27–32	30	1.57
S8	10	27–33	31	1.77
S9	10	29–34	32	1.58
S10	10	28–33	30	1.83
S11	10	22–30	25	2.42
MD4 (ac)	10	23–49	30	7.23
MD5 (ac)	8	24–55	39	10.04
MD6 (ac)	9	34–65	46	9.30
MD7 (ac)	10	36–68	50	9.54
MD8 (ac)	10	48–73	62	8.15
LV6 (ac)	10	19–35	25	5.57
LV7 (ac)	10	19–36	27	6.00
LV8 (ac)	10	18–42	27	6.81
LV9 (ac)	8	19–46	28	7.87
LTS	10	127–232	154	32.18
LTS/TL	10	55.8–109.4%	68.4%	16.91%
LTAS	5	45–77	62	13.38

Segment 4 (Figs 2A,B, 3A, B, 4A) with acicular spine in middorsal position. Sensory spots not present. Glandular cell outlets type 1 slightly anterior to the spine insertion paradorsally and also present in paraventral positions. Pachycycli and cuticular hairs as on preceding segment.

Segment 5 (Figs 2A, B, 3A,B, 4A,C,E) with acicular spine in middorsal position and tubes in lateroventral positions. Sensory spots present in subdorsal and ventromedial positions. Glandular cell outlets type 2 in midlateral position. Pachycycli, glandular cell outlets type 1 and cuticular hairs as on preceding segment.

Segment 6 (Figs 2A, B, 3A, B, E, 4E) with middorsal and lateroventral acicular spines. Sensory spots present in paradorsal, subdorsal, midlateral, and ventromedial positions; ventromedial sensory spots slightly closer to midsternal junction than those on preceding segment. Pachycycli, glandular cell outlets type 1, and cuticular hairs as on preceding segment.

Segment 7 (Figs 2A, B, 3A, B, E, 4C,E) with middorsal and lateroventral acicular spines. Sensory spots present in paradorsal, midlateral, and ventromedial positions; ventromedial sensory spots aligned with those on segment 5. Pachycycli, glandular cell outlets type 1, and cuticular hairs as on preceding segment.

Segment 8 (Figs 2A, B, 3A, B, E, 4D, E) with middorsal and lateroventral acicular spines. Sensory spots present in paradorsal position. Glandular cell outlets type 2 in sublateral position. Pachycycli, glandular cell outlets type 1, and cuticular hairs as on preceding segment.

Segment 9 (Figs 2A, B, 3A, B, 4D,F) with acicular spines in lateroventral position. Sensory spots present in paradorsal, subdorsal, laterodorsal and ventrolateral positions. Glandular cell outlets type 1 are present in paradorsal and paraventral positions. Minute sieve plates present in sublateral position. Cuticular hairs and pachycycli as on preceding segment.

Segment 10 (Figs 2A–D, 3A, B, F, G, 4D, F) with sensory spots in subdorsal and laterodorsal positions. Glandular cell outlets type 1 in tandem at the middorsal position, and in paraventral position. Posterior margin of pectinate fringe curved slightly anteriorly at the middorsal location. Posterior margins of sternal plates slightly rounded. Cuticular hairs sparse.

Segment 11 (Figs 2A–D, 3A, B, F, G, 4D, F) with lateral terminal spines. Sensory spots not observed. Females with thin lateral terminal accessory spines. Female gonopores near anterolateral margins of sternal plates of segment 11; gonopores with rounded, intracuticular thickenings, and externally covered by fringed flap. Males with three pairs of penile spines. The dorsal- and ventral-most penile spines are thin and flexible; medial ones are more stout and rigid, tapering towards the tip. Glandular cell outlets type 1 present in tandem at the middorsal position, with the anterior outlet positioned horizontally and the posterior outlet positioned vertically. Tergal extensions elongated and curved on the lateral surface, with margin of medial sides decorated with hair-like extensions. Sternal extensions are rounded.

Etymology. This species is named after the late Dr. Frank A. Romano III, Jacksonville State University, Alabama, for his contributions to the study of meiofauna and for his initiation of our ongoing meiofauna survey.

Remarks. *Echinoderes romanoi* sp. n. is characterized by the presence of middorsal spines on segments 4 to 8, lateroventral tubes on segment 5, lateroventral spines on segments 6 to 9, and glandular cell outlets type 2 on segments 2 (4 pairs), 5, and 8. This combination of spines, tubes, and glandular cell outlets is unique among all species in the genus. The spine/tube arrangement is not unusual among congeners: 36 additional species share the presence of middorsal spines on segments 4 to 8 and lateroventral tubes/spines on segments 5 to 9, and out of these, ten also lack tubes on segment 2 as

Table 3. Summary of nature and location of sensory spots, glandular cell outlets, tubes and spines arranged by series in *Echinoderes romanoi* sp. n. Abbreviations: LA: lateral accessory; LD: Laterodorsal; LV: lateroventral; MD: middorsal; ML: midlateral; PD: paradorsal; PV: paraventral; SD: subdorsal; SL: sublateral; VL: ventrolateral; VM: ventromedial; ac, acicular spine; gco 1/2, glandular cell outlet type 1/2; ltas, lateral terminal accessory spine; lts, lateral terminal spine; pe, penile spines; si, sieve plate; ss, sensory spot; tu, tube; (♀), female and (♂), male conditions of sexually dimorphic characters.

Position Segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM	PV
1	gco1		ss	ss		gco1		gco1		ss	
2			gco2	gco2, ss	ss	gco2			gco2		gco1, ss
3		gco1	ss			ss					gco1
4	ac	gco1									gco1
5	ac	gco1	ss		gco2			tu		ss	gco1
6	ac	gco1, ss	ss		ss			ac		ss	gco1
7	ac	gco1, ss			ss			ac		ss	gco1
8	ac	gco1, ss				gco2		ac			gco1
9		gco1, ss	ss	ss		si		ac	ss		gco1
10	gco1, gco1		ss	ss							gco1
11	gco1, gco1				pe(♂)		ltas(♀)	lts			

does *E. romanoi* sp. n.: *Echinoderes angustus* Higgins and Kristensen 1988, *E. aquilonius* Higgins and Kristensen 1988, *E. tubilak* Higgins and Kristensen 1988, *E. remanei* (Blake 1930), *E. brevicaudatus* Higgins 1977, *E. cernunnos* Sørensen et al. 2012, *E. koreanus* Adrianov, 1999, *E. stockmani* Adrianov, 1999, *E. obtuspinosus* Sørensen et al. 2012, and *E. bookhouti* Higgins, 1964 (Blake 1930, Higgins 1964a, 1964b, Higgins 1977, Higgins and Kristensen 1988, Adrianov and Malakhov 1999, Sørensen et al. 2012, 2016). Many of the descriptions of these ten species do not include glandular cell outlet type 2 information, though there are a variety of characteristics that distinguish *E. romanoi* n. sp. from each of the 10 other taxa. The first three species, *E. angustus*, *E. aquilonius*, and *E. tubilak*, all described from Disko Island, Greenland, can be distinguished from *E. romanoi* n. sp. by size alone. The three Greenland species all have a trunk length and placid length much larger than the trunk length (196–247 µm) and placid length (10 µm) of *E. romanoi* n. sp. (*E. angustus* 320–475 µm, 16–20 µm; *E. aquilonius* 363–465 µm, 15–20 µm; *E. tubilak* 333–415 µm, 14–18 µm). The same distinction is true for *E. remanei*, redescribed by Higgins (1964a), which has a trunk length of 282–358 µm. *Echinoderes brevicaudatus* has lateral dorsal tubes on segment 10, and short stubby lateral terminal spines, distinct from the new species. *E. cernunnos* has glandular cell outlets type 2 located similarly to *E. romanoi* on segments 2, 5 and 8, though *E. cernunnos* also has glandular cell outlets type 2 in the midlateral position on segment 7 and additionally has elongated spinous tergal extensions. *Echinoderes koreanus* has spines in the lateral dorsal positions on segments 7 and 8, and tubes in the laterodorsal position on segment 10, unlike the new species. *Echinoderes stockmani* is distinguished by having the lateral spines on segment 8 distinctly longer than those

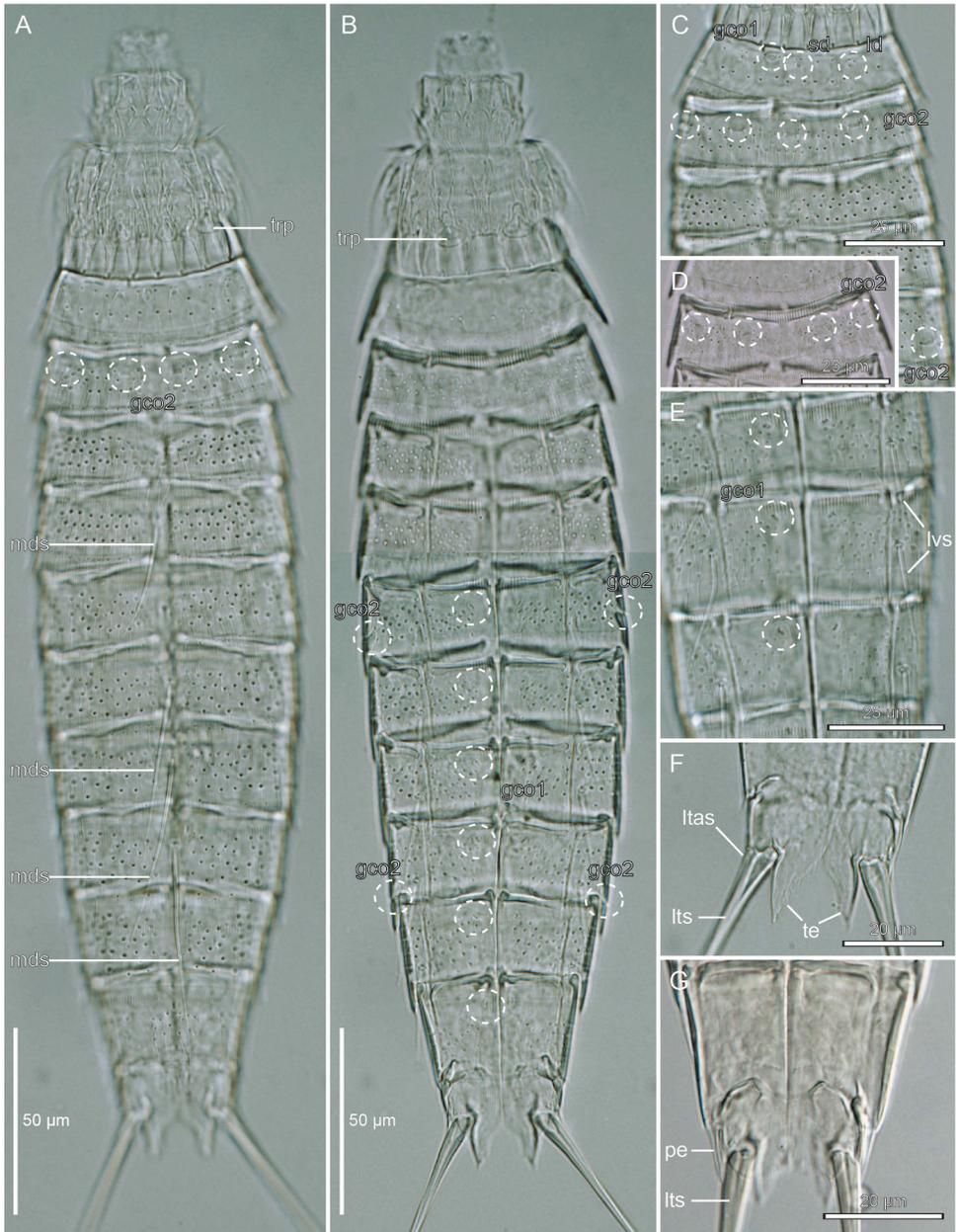


Figure 3. Light micrographs showing overviews and details in male holotype ZMUC KIN-962 (**A–B, D**), female paratypes ZMUC KIN-965 (**C, F**) and ZMUC KIN-966 (**E**) and male paratype ZMUC KIN-967 (**G**) of *Echinoderes romanoi* sp. n. **A.** Dorsal overview **B** Ventral overview **C** Segments 1 to 5, dorsal view **D** Segments 1 and 2, ventral view **E** Segments 6 to 8, ventral view **F** Segments 10 and 11 of a female, ventral view **G** Segments 10 and 11 of a male, ventral view. Abbreviations: gco1/2, glandular cell outlet type 1/2; ld, laterodorsal sensory spot; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; mds, middorsal spine; pe, penile spine; sd, subdorsal sensory spot; te, tergal extensions; trp, trichoscalid plate.



Figure 4. Scanning electron micrographs showing overviews and details in head and trunk morphology of *Echinoderes romanoi* sp. n. **A** Right lateroventral view of anterior end of a female **B** Oral stylets and introvert **C** Right lateral overview of a male **D** Right lateral view of segments 8 to 11 of a male **E** Trunk segments 5 to 8, lateral view **F** Segments 9 to 11 of a female, ventral view. Abbreviations: gco2, glandular cell outlet type 2; go, gonopore; ld, laterodorsal sensory spot; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tube; mds, middorsal spine; ml, midlateral sensory spot; pd, paradorsal sensory spot; pe, penile spine; psp, primary spinoscalid; sd, subdorsal sensory spot; sp2–3, spinoscalids of Rings 2 to 3; spf, secondary pectinate fringe; vl, ventrolateral sensory spot; vm, ventromedial sensory spot.

on segment 9, unlike *E. romanoi* n. sp. *Echinoderes obtuspinosus* has glandular cell outlets type 2 similarly to *E. romanoi* on segments 2 and 8. However, *E. obtuspinosus* has glandular cell outlets type 2 in the subdorsal position on segment 4 and none on segment 5. Further, *E. obtuspinosus* has short stubby lateral terminal spines. Finally, *E. bookhouti* has lateral accessory spines on segment 8, and lacks glandular cell outlets type two on segment 5 and in the laterodorsal and sublateral position on segment 2.

***Echinoderes joyceae* sp. n.**

<http://zoobank.org/B7ED6634-BA3F-4250-84DC-DE7CBC53A19D>

Figs 5–7

Material. Holotype: Adult male (ZMUC KIN-845), collected from muddy sediment on October 16, 2010, at station 010-2010 (Fig. 1), at 427 m depth, about 100 km east southeast of Corpus Christi, Texas (27°09'12"N 96°09'59"W), mounted in Fluoromount G®, deposited at the Natural History Museum of Denmark. Paratypes include one female (ZMUC KIN-849), collected at same time and locality as the holotype, and three males, collected at stations 016-2010 (ZMUC KIN-850), 068-2012 (ZMUC KIN-922), and 031-2013 (ZMUC KIN-867). All paratypes were mounted in Fluoromount G® and deposited at the Natural History Museum of Denmark. Additional non-type material includes one female from station 033-2014. See Fig. 1 for localities and Table 1 for detailed station data.

Diagnosis. Conspicuously small *Echinoderes* (183–209 µm) with middorsal spines on segments 4, 6 and 8, and spines in lateroventral positions on segments 6 to 9. Tubes present in ventrolateral positions on segment 2, in lateroventral positions on segment 5, and in laterodorsal positions near the posterior margin of segment 10. Glandular cell outlets type 2 present in subdorsal position on segment 2, in midlateral position on segment 6, and in sublateral position on segment 8.

Description. Adults conspicuously small (183–209 µm in trunk length), with head, neck and eleven trunk segments (Figs 5–7). For complete overview of measures and dimensions, see Table 4. Distribution of cuticular structures, i.e., sensory spots, glandular cell outlets, spines and tubes, is summarized in Table 5.

The head (Fig. 7A–C) consists of a retractable mouth cone and an introvert. Mouth cone with nine outer oral styles that alternate in length between slightly shorter and slightly longer ones. No outer oral styles present anterior to introvert sector 6. A fringe with three long spikes is located at the base of each outer oral style. It was only possible to obtain complete information about appearance and arrangement of scalids for introvert sectors 8 and 9. Sector 8: single central scalids of Rings 02 and 04, and paired scalids of Rings 03 and 05. No scalids present posterior to Ring 05, except for a single trichoscalid that attaches through a trichoscalid plate. Sector 9: single central scalids of Rings 02, 04, and 06, and paired scalids of Rings 03 and 05. No trichoscalids present.

The neck (Figs 5A, B, 6A–D) has 16 placids, measuring 11 µm in length. The midventral placid is broadest, measuring 9 µm in width at its base, whereas all other are

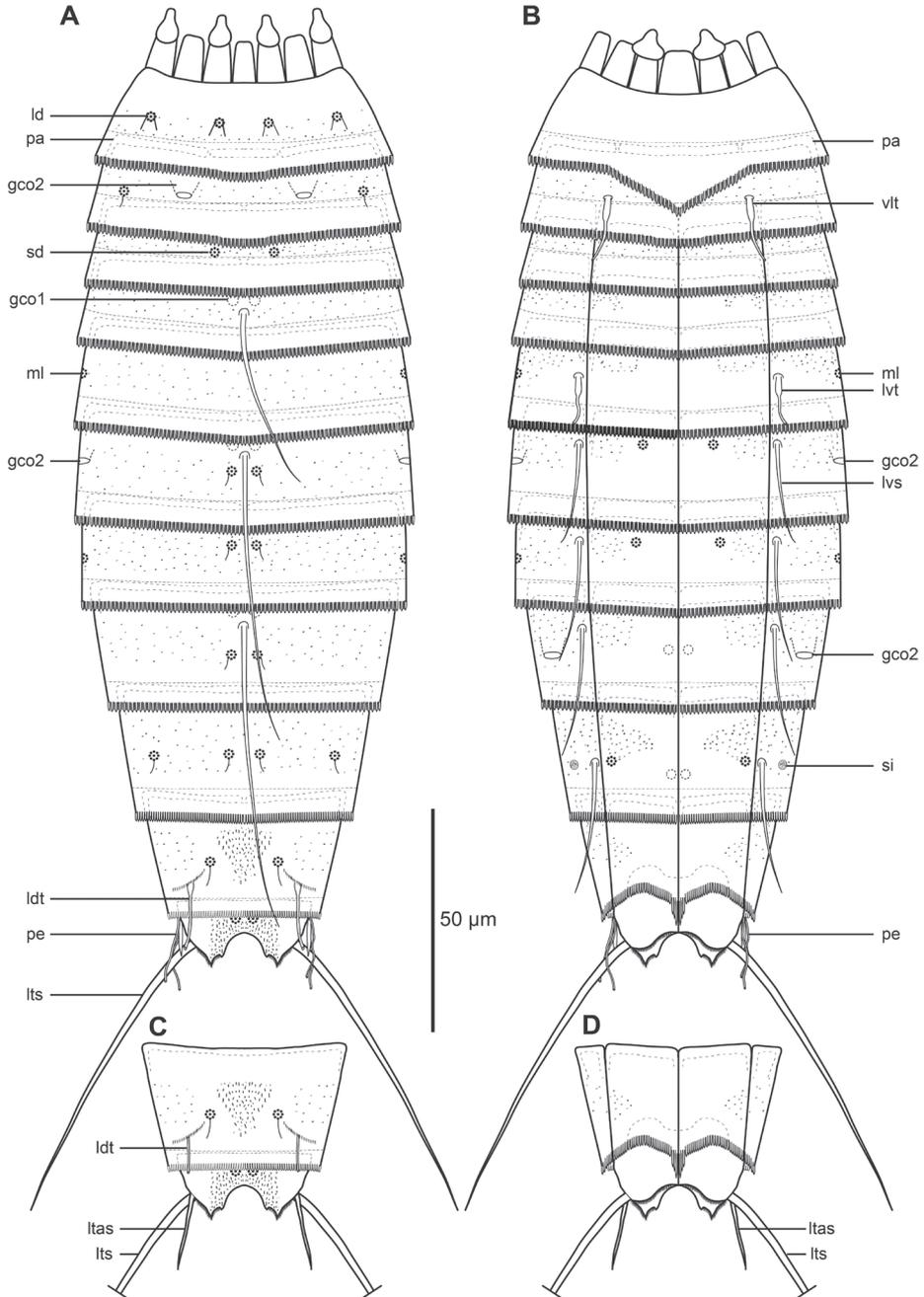


Figure 5. Line art illustrations of *Echinoderes joyceae* sp. n. **A** Male, dorsal view **B** Male, ventral view **C** Female, segments 10 to 11, dorsal view **D** Female, segments 10 to 11, ventral view. Abbreviations: gco1/2, glandular cell outlet type 1/2; ld, laterodorsal sensory spot; ldt, laterodorsal tube; lts, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; lvt, lateroventral tube; ml, midlateral sensory spot; pa, pachycyclus; pe, penile spine; sd, subdorsal sensory spot; si, sieve plate; vit, ventrolateral tube.

Table 4. Measurements from light microscopy of *Echinoderes joyceae* sp. n. (in μm) from the Gulf of Mexico, including number of measured specimens (n) and standard deviation (SD). Abbreviations: (ac): acicular spine; LTAS: lateral terminal accessory spine; LTS: lateral terminal spine; LV: lateroventral; MD, middorsal; MSW-6: maximum sternal width, measured on segment 6 in this species; S: segment lengths; SW-10, standard width, always measured on segment 10; TL: trunk length.

Character	n	Range	Mean	SD
TL	4	183–209	195	10.90
MSW-6	4	40–46	43	3.00
MSW-6/TL	3	20.4–23.0%	21.8%	1.32%
SW-10	4	34–37	35	1.26
SW-10/TL	3	16.7–19.4%	17.8%	1.38%
S1	4	23–26	25	1.50
S2	4	22–26	25	1.73
S3	4	23–25	23	1.26
S4	4	21–26	24	2.22
S5	4	23–27	26	1.73
S6	4	25–30	28	2.22
S7	4	27–31	30	1.91
S8	4	30–33	32	1.29
S9	4	30–31	31	0.58
S10	4	31–33	32	0.82
S11	4	24–27	25	1.41
MD4 (ac)	5	40–58	47	7.30
MD6 (ac)	5	59–74	66	6.07
MD8 (ac)	5	61–75	68	7.06
LV6 (ac)	4	20–25	23	2.06
LV7 (ac)	4	22–31	27	3.70
LV8 (ac)	4	29–39	32	5.12
LV9 (ac)	4	31–34	33	1.29
LTS	5	72–83	79	4.66
LTS/TL	4	39.3–42.9%	40.3%	1.75%
LTAS	1	24	N/A	N/A

narrower, measuring 6 μm in width at their bases. The trichoscalid plates, each with a trichoscalid, present in subdorsal, laterodorsal and ventromedial positions.

Segment 1 (Figs 5A, B, 6A–D, 7A, C, D) consists of a complete cuticular ring. Sensory spots are located in subdorsal and laterodorsal positions; sensory spots are rounded and flanked by a pair of cuticular hairs. Glandular cell outlets type 1 not present. Cuticular hairs are very scarce on the dorsal side, and not present at all on the ventral. The posterior segment margin is straight along the dorsal and lateral side, but extends more posteriorly ventrally, into a midventral point. Pectinate fringe of posterior segment margin with fringe tips alternating with small trichoid extensions.

Segment 2 (Fig. 5A, B, 6A–D, 7A, C, D) consists of a complete cuticular ring, with tubes located in ventrolateral position. Sensory spots (with one marginal hair) located in the laterodorsal position. Pachycyclus of the anterior segment margin of regular

Table 5. Summary of nature and location of sensory spots, glandular cell outlets, tubes and spines arranged by series in *Echinoderes joyceae* sp. n. Abbreviations: LA: lateral accessory; LD: Laterodorsal; LV: lateroventral; MD: middorsal; ML: midlateral; PD: paradorsal; PV: paraventral; SD: subdorsal; SL: sublateral; VL: ventrolateral; VM: ventromedial; ac, acicular spine; gco 1/2, glandular cell outlet type 1/2; ltas, lateral terminal accessory spine; lts, lateral terminal spine; pe, penile spines; si, sieve plate; ss, sensory spot; tu, tube; (♀), female and (♂), male conditions of sexually dimorphic characters.

Position Segment	MD	PD	SD	LD	ML	SL	LA	LV	VL	VM	PV
1			ss	ss							
2			gco2	ss					tu		
3			ss								
4	ac	gco1									
5					ss			tu			
6	ac	gco1,ss			gco2			ac		ss	
7		ss			ss			ac		ss	
8	ac	gco1,ss				gco2		ac			gco1
9		ss		ss		si		ac	ss		gco1
10			ss	tu							
11		ss			pe(♂)		ltas(♀)	lts			

thickness and interrupted in subdorsal and ventrolateral positions. Glandular cell outlets type 2 are located in subdorsal position. Secondary pectinate fringe not detected on this or any of the following segments. Bracteate cuticular hairs evenly distributed in a medial band around the segment. The posterior segment margin is straight and consists of a pectinate fringe, with fringe tips alternating with small trichoid extensions.

Segment 3 (Figs 5A, B, 6A–D, 7A, D, E), and remaining segments, consisting of one tergal and two sternal plates. Pachycyclus of the anterior segment margin interrupted middorsally; thickness on the dorsal side rather average; ventral pachycycli thicker, and interrupted at the tergo-sternal and midsternal junctions. Sensory spots (without marginal hairs) are located in subdorsal position only. Cuticular hairs evenly distributed over tergal plate, whereas the sternal plates only have a few hairs near their anterolateral corners. Posterior segment margin and pectinate fringe as on preceding segment.

Segment 4 (Figs 5A, B, 6A–D, 7A, D, E) with acicular spine in middorsal position, flanked by pair of paradorsal glandular cell outlets type 1. Sensory spots not present. Pachycycli, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 5 (Figs 5A, B, 6A–D, 7A, D, E) with tubes in lateroventral position. Sensory spots (without marginal hairs) present in midlateral positions. Pachycycli, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 6 (Figs 5A, B, 6A–D, 7A, D–F) with middorsal and lateroventral acicular spines. Paradorsal glandular cell outlets type 1 present anterior to middorsal spine, and paradorsal sensory spots (with or without marginal hairs) posterior to spine. One additional pair of sensory spots without marginal hairs present in ventromedial posi-

tion. Glandular cell outlets type 2 present in midlateral position. Pachycycli, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 7 (Figs 5A, B, 6A, B, 7A, E–G, I) with acicular spines in lateroventral position, and sensory spots in paradorsal (with one marginal hair), midlateral and ventromedial positions. Pachycycli, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 8 (Figs 5A, B, 6A, B, E, F, 7A, E–G, I) with middorsal and lateroventral acicular spines. Paradorsal glandular cell outlets type 1 present anterior to middorsal spine, and paradorsal sensory spots (with marginal hairs) posterior to spine. Additional glandular cell outlets type 1 are present in paraventral position, and glandular cell outlets type 2 in sublateral position. Pachycycli, pectinate fringe of posterior margin and cuticular hairs as on preceding segment.

Segment 9 (Figs 5A, B, 6A, B, E–G, 7H, I) with acicular spines in lateroventral position. Sensory spots with marginal hairs present in paradorsal and laterodorsal positions, and without marginal hairs in ventrolateral positions. Glandular cell outlets type 1 are present in paraventral position, and a pair of very minute sieve plates is located in sublateral position. Ventral pachycycli of anterior segment margin slightly thinner than those on preceding segment. Pectinate fringe of posterior margin only with regular fringe tips. Cuticular hairs as on preceding segment on tergal plate; sternal plates with cuticular hairs forming triangular patterns extending from the tergo-sternal junctions.

Segment 10 (Figs 5A–D, 6A–B, E–G, 7H, I) with laterodorsal tubes near posterior segment margin: tubes in males are well-developed, resembling regular tubes with thickened bases; tubes in females are about half as long and formed like simple tubes without thickened bases; tubes in both sexes emerge through slit-like openings in the cuticle at the posterior part of the segment. Sensory spots with marginal hair present in subdorsal positions. Glandular cell outlets type 1 not observed. Tergal plate with triangular middorsal patch of cuticular hair-like extensions, without perforation sites. Cuticular hairs with perforation sites in two patches going from the laterodorsal positions to the tergo-sternal junctions; hairs on the sternal plates only ventrolaterally, near the tergo-sternal junctions. Posterior margin of tergal plate straight; posterior margins of sternal plates concave.

Segment 11 (Fig. 5A–D, 6A–B, E–G, 7H, I) with lateral terminal spines. Females with thin lateral terminal accessory spines, and males with three pairs of penile spines: dorsal- and ventral-most penile spines are thin and flexible; medial ones are more stout and rigid. Sensory spots, without marginal hairs, present in paradorsal positions. Glandular cell outlets type 1 not observed. Cuticular hairs with perforation sites not present. Cuticular hair-like extensions present in patch going from the subdorsal to the middorsal areas. Tergal extensions short and pointed, with margin of inferior sides interrupted by elongated tips formed by the marginal fringes. Sternal extensions are short and broadly rounded.

Etymology. This species is named after Joyce Wright Landers—the wife of the first author.

Remarks. *Echinoderes joyceae* sp. n. is characterized by the presence of middorsal spines on segments 4, 6 and 8, ventrolateral tubes on segment 2, lateroventral tubes/



Figure 6. Light micrographs showing overviews and details in male holotype ZMUC KIN-845 (**A–F**), and female paratype ZMUC KIN-849 (**G**) of *Echinoderes joyceae* sp. n. **A** Dorsal overview **B** Ventral overview **C** Segments 1 to 6, dorsal view **D** Segments 1 to 6, ventral view **E** Segments 8 to 11, dorsal view **F** Segments 8 to 11, ventral view **G** Segments 9 to 11, dorsal view. Abbreviations: gco1/2, glandular cell outlet type 1/2; ld, laterodorsal sensory spot; ltas, lateral terminal accessory spine; lts, lateral terminal spine; lvs, lateroventral spine; mds, middorsal spine; pe, penile spine; pd, paradorsal sensory spot; sd, subdorsal sensory spot; si, sieve plate; vm, ventromedial sensory spot.

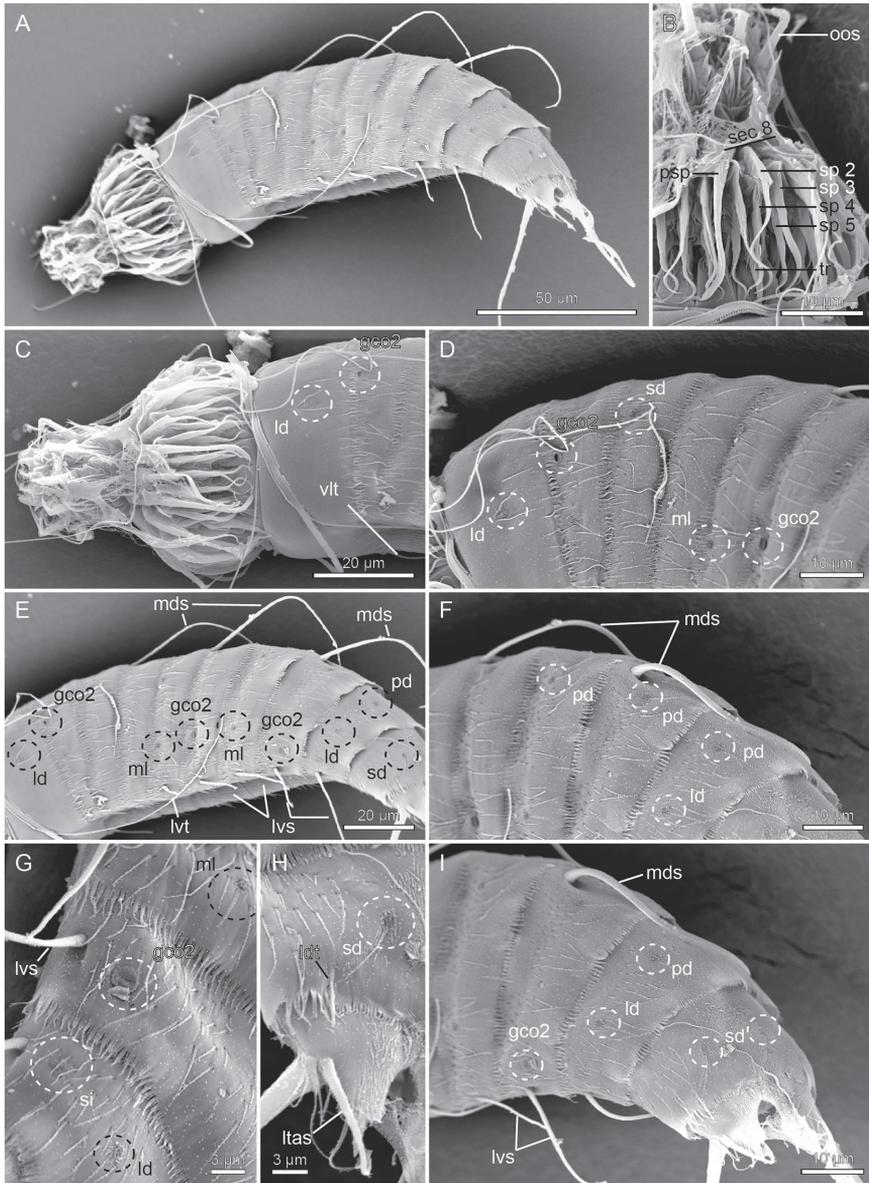


Figure 7. Scanning electron micrographs showing overviews and details in head and trunk morphology of female *Echinoderes joyceae* sp. n. **A** Lateral overview **B** Mouth cone and introvert sector 8 **C** Head and segments 1 to 2, lateral view **D** Laterodorsal parts of segments 1 to 6 **E** Trunk segments 1 to 10, lateral view **F** Laterodorsal parts of segments 4 to 7 **G** Detail showing sublateral parts of segments 7 to 9 **H** Detail showing sub- and laterodorsal parts of segment 10 and left tergal extension of segment 11 **I** Segments 7 to 11, laterodorsal and caudal view. Abbreviations: gco2, glandular cell outlet type 2; ld, laterodorsal sensory spot; ldt, laterodorsal tube; ltas, lateral terminal accessory spine; lvs, lateroventral spine; lvt, lateroventral tube; mds, middorsal spine; ml, midlateral sensory spot; oos, outer oral style; pd, paradorsal sensory spot; psp, primary spinoscalid; sd, subdorsal sensory spot; sec 8, introvert sector 8; si, sieve plate; sp2–5, spinoscalids of rings 2 to 5; tr, trichoscalid; vlt, ventrolateral tube.

spines on segments 5 to 9, and laterodorsal tubes on segment 10. This combination of spines and tubes is not unusual among congeners, and is shared with five other species: *E. bermudensis* Higgins 1982, *E. kristenseni* Higgins 1985, *E. abbreviatus* Higgins 1983, *E. hispanicus* Pardos et al., 1998 and *E. intermedius* Sørensen, 2006. (Higgins 1982, 1983, 1985; Pardos et al. 1998, Sørensen 2006). These species are distinguished from *E. joyceae* using a number of characteristics. All five of these species have tergal extensions on segment 11 distinct from *E. joyceae*. Additionally, the new species has a distinctive distribution of glandular cell outlets type 2, with locations in the subdorsal position on segment 2. Amongst the abovementioned species this is only found in *E. kristenseni*, which also has glandular cell outlets type 2 in lateroventral positions of segment 2, not present in *Echinoderes joyceae* sp. n. The most unique character combination in *Echinoderes joyceae* sp. n. though, is the presence of glandular cell outlets type 2 in midlateral positions of segment 6 and in sublateral positions of segment 8. This combination is not found in any other species of *Echinoderes*. Furthermore, *Echinoderes joyceae* sp. n. is characteristic by its minute size. With a trunk length ranging from 183 to 209 μm , *Echinoderes joyceae* sp. n. is among the smallest known *Echinoderes*.

Discussion

This study describes *E. romanoi* sp. n. and *E. joyceae* sp. n., and reports the known distribution of the two new species along the northern Gulf of Mexico continental shelf. In common with the previous shelf species reported so far during this long term meiofauna sampling is the broad distribution of the taxa. *Echinoderes romanoi* sp. n. and *E. joyceae* sp. n. are distributed across wide regions of the United States' Gulf shelf, with the location of *E. romanoi* extending from central Louisiana to western Florida, and with *E. joyceae* sp. n. extending from Texas to Florida. Similarly, *E. augustae*, *E. skipperae*, *E. charlotteae*, and *E. bookhouti* (Sørensen and Landers 2014, Sørensen et al. 2016), all reported during this survey from the northern Gulf shelf, have broad distributions either extending across half of the U.S. Gulf shelf or across the entire Gulf. As more samples are processed during our survey, more locations for all of these species will be determined. Their distribution will likely cover the entire shelf from Florida to Mexico, given the trend observed so far. Despite the broad distribution of these *Echinoderes* species across the Gulf, it is interesting that they have not been observed in coastal marshes. In a recent study on the effects of the Deepwater Horizon oil spill from 2010 in Barataria Bay, Louisiana (Fleeger et al. 2015), all identified kinorhynchs from a subsample of 100 animals were identified as *E. coulli*, with no offshore species present (identifications in the Fleeger et al. 2015 paper were made by M.V. Sørensen). *Echinoderes coulli* is an estuarine species, which has not been observed in our offshore samples. Our sampling on the continental shelf has consistently sampled sediment from high salinity waters, yielding species that may not tolerate estuarine conditions. Sampling along the U.S. shoreline in high and low salinity waters are needed to determine if the shelf species are found in the intertidal zone or marshes.

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The phylogenetic position of a new species of *Plakobranchus* from West Papua, Indonesia (Mollusca, Opisthobranchia, Sacoglossa)

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Abstract

Plakobranchus papua Meyers-Muñoz & van der Velde, **sp. n.** from West Papua (Papua Barat province, Indonesia), is described based on its external morphology, colour pattern, internal anatomy, radula and reproductive system. In a molecular phylogenetic study specimens of this new species were compared with those of ten candidate taxa under the name *Plakobranchus ocellatus* van Hasselt, 1824. DNA analyses of COI mtDNA showed a clear distinction between *P. papua* **sp. n.** and “*P. ocellatus*”. *Plakobranchus papua*, **sp. n.** also differed from all taxa that have been synonymised with *P. ocellatus*. The genus is in dire need of taxonomic revision, preferably based on an integrative analysis involving morphology and DNA of all known *Plakobranchus* varieties.

Keywords

COI, phylogeny, *Plakobranchus ocellatus*, reproductive system, Sacoglossa, taxonomy

Introduction

Sea slugs of the genus *Plakobranchus* van Hasselt, 1824 (Order Sacoglossa, Suborder Plakobranchea) have an elongated body and dorsoventrally flattened, lateral parapodia, which are folded up on the dorsal surface (van Hasselt 1824; Jensen 1992). According to Jensen (1997a) this genus possesses a number of plesiomorphic characters such as an anterodorsal anus, a pharyngeal pouch, triangular, denticulate teeth, a long, curved penial stylet, and the absence of dorsal vessels. The genus also possesses a number of autapomorphies: a broad and flat head, rhinophores located at the anterior corners, mediodorsal eyes on a small papilla, numerous longitudinal dorsal lamellae containing branches of the digestive gland, and a truncate tail; the hermaphrodite ampulla has apparently been lost.

These animals can be found in shallow sandy habitats, crawling over it or half-buried (Gosliner et al. 2008; Mehrotra et al. 2015), or on coral rubble and in rock pools (Yonow 2008) where they consume green macroalgae (Jensen 1993).

Plakobranchus ocellatus van Hasselt, 1824, feeds on a wide variety of marine green algae (Chlorophyta), including at least five species of Ulvophyceae (Wägele et al. 2011). Many studies on *Plakobranchus* deal with their kleptoplasty, the ability to retain functional chloroplasts from their green algae in their digestive gland cells (Clark et al. 1990; Jensen 1996, 1997a). Species of this genus as well as other sacoglossans belong to the few known animal species with the ability of photosynthesis (Trench 1969; Hirose 2005; Bass 2006; Händeler et al. 2009; Maeda et al. 2010; Wägele et al. 2011; Christa et al. 2013; Yamamoto et al. 2013).

Plakobranchus species are simultaneous hermaphrodites, which possess a penial stylet used in hypodermic insemination. Penial stylets and hypodermic insemination are commonly found within the Sacoglossa (Schmitt et al. 2007; Smolensky et al. 2009). Jensen (1992) also observed extensively branched prostate and albumen glands and a pair of secondary copulatory bursae in *Plakobranchus*, which are unique for the genus.

During the last decades only *Plakobranchus ocellatus* has been considered a valid species within the genus (Jensen 1992). This species was described from shallow waters in the Sunda Strait near Anyer, northwest Java, Indonesia. van Hasselt (1824) gave this name because of the blue- and yellow-centred ocellated spots covering the dorsal side and flanks of the body (Figure 1). The original description is based on the species' phenotype and some characteristics of the parapodial lamellae, heart and reproductive system as shown in the original illustrations supplied by van Hasselt (1824). The name *Plakobranchus* has occasionally been misspelled as *Placobranchus*, which started when the original description was translated from Dutch to French in 1824 (Bergh 1887; Jensen 1997a, 1997c).

Jensen (1992) studied the anatomy of several *Plakobranchus* specimens from various Indo-West Pacific locations (Red Sea, Thailand, Guam) and synonymised the previously described species. The descriptions were mainly based on external anatomy, colour pattern and geographic distribution (Yonow 1990, 2008; Debelius 1996; Jensen 1997a; Marshall and Willan 1999; Gosliner et al. 2008) and hardly included descriptions of the internal anatomy as presented by van Hasselt (1824) and Jensen (1992). *Plakobranchus ocellatus* is now considered the only known valid species of the

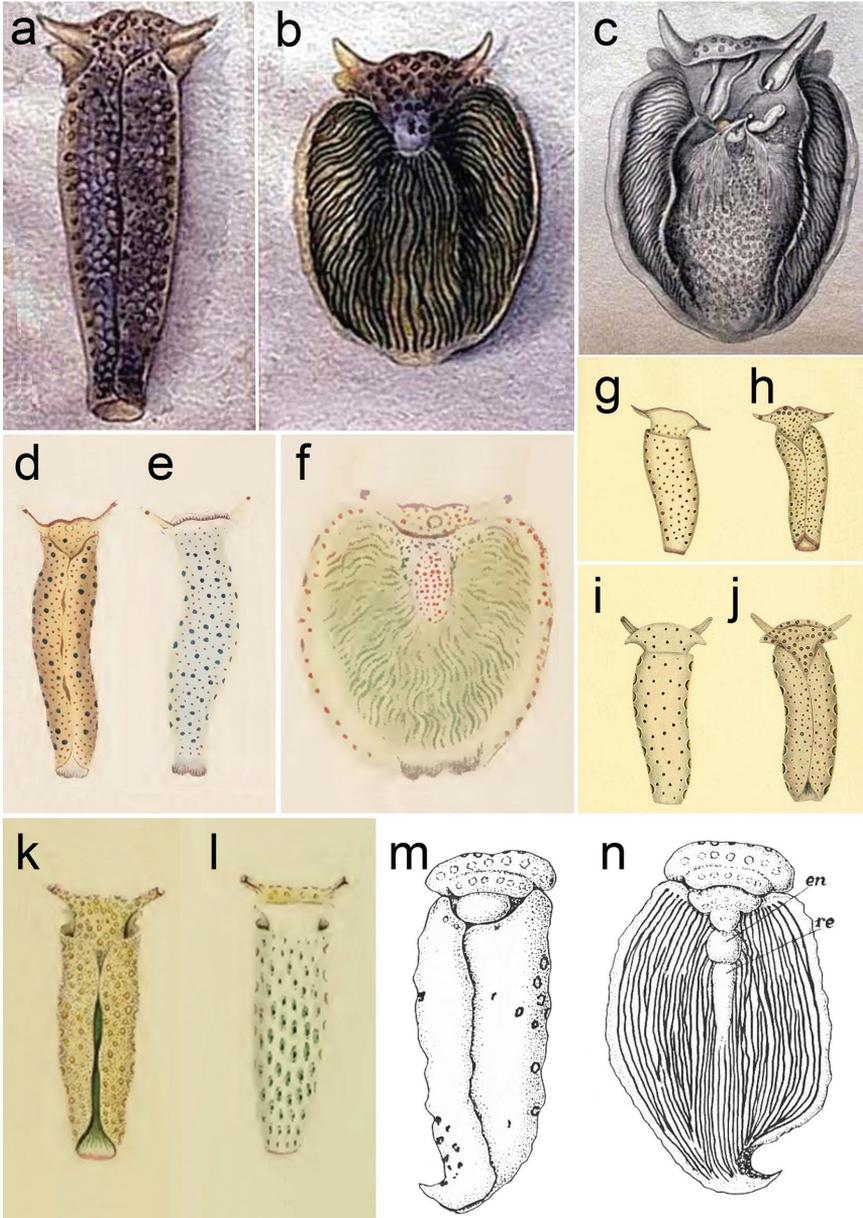


Figure 1. a–c *Plakobranchus ocellatus*, drawings by van Hasselt (1824): a dorsal view with parapodia folded up on the dorsal body surface b dorsal view with open parapodia, showing longitudinal lamellae c internal anatomy d–f *P. ianthobaptus*, drawings by Gould (1852): d dorsal view with parapodia folded up on the dorsal body surface e ventral view f dorsal view with open parapodia, showing longitudinal lamellae g–j two *Plakobranchus* species illustrated by Pease (1871) g–h *P. gracilis*: g ventral view h dorsal view i–j *P. variegatus*: i ventral view j dorsal view k–l drawings of *P. chlorophacus* by Bergh (1873): k dorsal view with parapodia folded up on the dorsal body surface l ventral view with ocellated spots m–n drawings of *P. ocellatus* by Marcus (1982): m dorsal view with parapodia folded up on the dorsal body surface n dorsal view with open parapodia, showing the longitudinal lamellae (en = pericardium; re = renal prominence).

genus, with ten synonyms and a wide Indo-Pacific distribution (Jensen 2007). This may have been premature (Jensen 1992, 1997a, 1997c), because Gosliner et al. (2008) distinguished two undescribed *Plakobranchnus* species in addition to *P. ocellatus*. Subsequently, Krug et al. (2013), who used the mitochondrial barcoding gene COI and the nuclear histone 3 gene, found ten distinct phylogenetic lineages in *Plakobranchnus*. This suggests that the taxonomy of *Plakobranchnus* still deserves further study. In the present study a new *Plakobranchnus* species from West Papua, Indonesia, is described and a phylogenetic reconstruction based on the mitochondrial barcoding COI gene is included to show its position within the genus *Plakobranchnus*.

Material and methods

Twenty specimens were collected by Gerard van der Velde in Indonesia during the 2007 Raja Ampat Expedition (Figure 2; Hoeksema and van der Meij 2008). The specimens were observed alive, photographed, and subsequently preserved in 96% ethanol. Material analysed in this study was deposited in the mollusc collection of Naturalis Biodiversity Center, Leiden, The Netherlands, and catalogued as RMNH.MOL. One specimen of *P. ocellatus* (RMNH.MOL.336426), collected in the Philippines, 4 November 1999 (Sta. CEB.01, Cebu Strait, E side of Olango Island 10°15'54"N 124°04'17"E, coll. BWH) was used for comparison of external characters with the new species in absence of the holotype of *P. ocellatus* (Figures 7b–d). The original drawings of van Hasselt (1824) are available in scientific archives of Naturalis Biodiversity Center and reprinted here (Figures 1a–c).

To study the radula, the buccal masses of two specimens were dissected and immersed in 10% NaOH until the tissue surrounding the radulae was dissolved. The radulae were rinsed in distilled water and transferred to 70% ethanol. They were subsequently examined by means of a light microscope, photographed, mounted on stubs, and gold-sputter-coated for scanning electron microscopy (SEM). Eight specimens were dissected for anatomical studies. One specimen (Table 1) was dehydrated in a graded ethanol series, embedded in paraffin, and cut into 7 µm serial sections with a manual microtome for histological observations. Sections were stained with toluidine blue to identify structures on the basis of metachromasia.

The holotype (RMNH.MOL.336417) and two paratypes (RMNH.MOL.336418–336419) of *Plakobranchnus papua* sp. n. were used for DNA analysis. A piece of foot tissue was extracted and treated according the DNeasy Blood and Tissue, spin-column protocol for the purification of total DNA. Primers (LCOI490–HCO2198) were used for the amplification of the mitochondrial gene COI (cytochrome c oxidase subunit, partial) region (Folmer et al. 1994).

PCR reactions were performed with Thermocycle Biometra T professional. All PCR reactions were carried out in 50 µl reaction volume, including 25 µl Q PerfeCTa®SYBR® Green FastMix® from Quanta BioScience Inc, (Gaithersburg, USA), 2 µl of each primer, 19µl of DPEC treated water and 2 µl of genomic DNA. Thermal

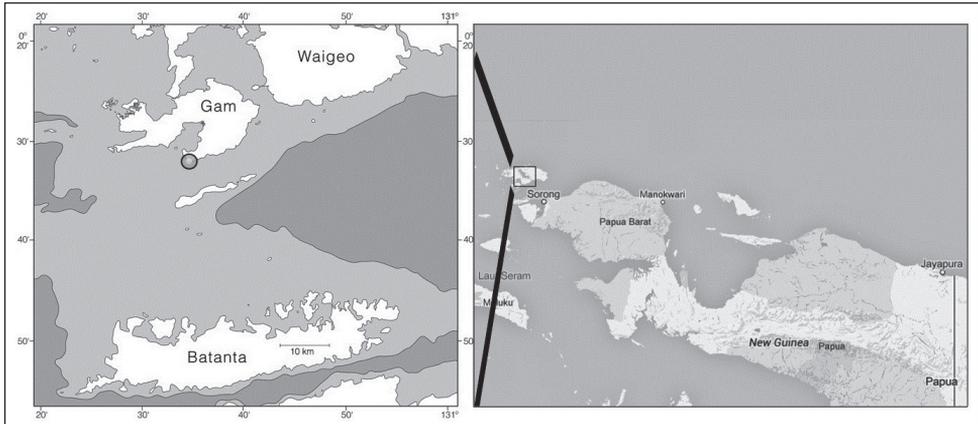


Figure 2. Map of West Papua and Papua, Indonesia, showing the area and locality where *Plakobranchus papua* specimens were collected: south Gam Island, Mangrove Creek.

cycling started with an initial melting step for 3 min at 94 °C, followed by 39 cycles at 94 °C for 15 sec, 50 °C for 30 sec, annealing using a temperature gradient from 50–60 °C for 1 min, 72 °C for 1 min, and a final elongation step at 72 °C for 5 min.

The PCR products were purified with a Gene Jet™ PCR Purification kit (Fermentas Life Sciences Lithuania) and sequenced directly, using the same primers as for PCR. DNA sequences were obtained using the Big Dye terminator ver. 3.1 Cycle Sequencing kit (Applied Biosystems) and analysed with the automated sequencer ABI 3130 Genetic analyzer at the sequencing facility of the Department of Anthropogenetics at the University Medical Centre, Radboud University, Nijmegen. The sequences were analysed with the program Chromas Lite (Technelysium Pty Ltd.) and deposited in Genbank (KU934191–KU934193).

Phylogenetic analyses were carried out on a dataset of 81 sequences including two outgroup species (Table 1). All COI sequences of *Plakobranchus* available on GenBank were used, and three newly obtained sequences of *P. papua* sp. n. were added. *Elysia ornata* (Swainson, 1840) (AB758962) and *Thuridilla carlsoni* Gosliner, 1995 (GQ996681) were selected as outgroups (Bass and Karl 2006). Sequences were aligned using the Guidance server (ClustalW), resulting in an alignment score of 1.00 for the dataset (Penn et al. 2010). A model selection analysis was carried out in jModeltest (Posada 2008) to select the best-fit model based on AICc (corrected Akaike Information Criterion), rendering TrN + G as the best-fit model. A maximum likelihood analysis using the GTR + G model (1000 bootstraps) was carried out in Phyml 3.1 (Guindon et al. 2010) in the Seaview platform (Gouy et al. 2010) and a majority rule consensus tree was constructed. Bayesian inferences (3,000,000 million generations) were estimated in MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) using the GTR + G model (because of unavailability of the TrN + G model). Average standard deviation of split frequencies was 0.09851. A majority-rule consensus tree was constructed in MrBayes with a burnin of 25%, and visualised in FigTree 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Table 1. COI data from *Plakobranchnus* cf. *ocellatus* from GenBank, for comparison with the sequence data of *Plakobranchnus papua* sp. n.

Species	Accession number	Publication	Collection locality
<i>Plakobranchnus</i> cf. <i>ocellatus</i>	AB501307	Maeda et al. 2010	Okinawa, Japan
	AB758968-971	Takano et al. 2013	Okinawa, Japan
	DQ237996	Klussmann-Kolb and Dinapoli 2006	Eastern Australia
	DQ471269	Bass unpubl.	Guam
	DQ471270	Bass unpubl.	Hawaii
	GQ996679-680	Händeler et al. 2009	Eastern Australia
	JX272685-720	Christa et al. 2013	Philippines
	KC573714-715	Krug et al. 2013	Okinawa, Japan
	KC573716	Krug et al. 2013	Vanuatu
	KC573717	Krug et al. 2013	Guam
	KC573718-719	Krug et al. 2013	Okinawa, Japan
	KC573720	Krug et al. 2013	Philippines
	KC573721-722	Krug et al. 2013	Guam
	KC573723-724	Krug et al. 2013	Andaman Sea, Thailand
	KC573725	Krug et al. 2013	Eastern Australia
	KC573726-727	Krug et al. 2013	Okinawa, Japan
	KC573728-729	Krug et al. 2013	Moorea, French Polynesia
	KC573730	Krug et al. 2013	Guam
	KC573731	Krug et al. 2013	Okinawa, Japan
	KC573732	Krug et al. 2013	Sulawesi, Indonesia
	KC573733	Krug et al. 2013	Philippines
	KC573734	Krug et al. 2013	Papua New Guinea
	KC573735-737	Krug et al. 2013	Philippines
	KC573738	Krug et al. 2013	Hawaii
	KC706898	Leray et al. 2013	French Polynesia
	HM187633-634	Wägele et al. 2011	Guam
HM187635	Wägele et al. unpubl.	Guam	
HM187638	Wägele et al. 2011	Guam	
<i>Elysia ornata</i>	AB758962	Takano et al. 2013	Japan
<i>Thuridilla carlsoni</i>	GQ996681	Händeler et al. 2009	Eastern Australia

The web version of ABGD (Automatic Barcode Gap Discovery, Puillandre et al. 2012) was used to estimate the genetic distance corresponding to the difference between a speciation process versus intraspecific variation. Runs were performed using the default range of priors ($p_{min} = 0.001$, $p_{max} = 0.10$) using the JC69 Jukes-Cantor measure of distance. The analysis involved 25 nucleotide sequences. All ambiguous positions were removed for each sequence pair; there was a total of 657 positions in the final dataset.

Systematics

Suborder Plakobranchea Gray, 1840

Superfamily Plakobranchoidea Gray, 1840

Family Plakobranthidae Rang, 1829

Genus *Plakobranthus* van Hasselt, 1824

Genus diagnosis (emended after Jensen 1992). Genus characterized by a truncate and flattened body shape, flat head, and enrolled rhinophores. Small admedian eyes. Parapodia folded up on the dorsal body surface, parapodial lamellae containing branches of the digestive gland, dorsal vessels absent. Anterodorsal anus. Long curved penial stylet.

Type species: *Plakobranthus ocellatus* van Hasselt, 1824

Figures 7b–e

- Plakobranthus ocellatus* van Hasselt 1824: 34–36 (near Anyer, Serang, Bantam, Indonesia; holotype lost); Cuvier 1830: 56; ed. Mason Moll.: 84, pl. 30, figs 7, 7a; Quoy and Gaimard 1833: 319; Bergh 1872: 147–151, pl. XIX figs 1–13; Bergh 1873: 75–76; Vayssi re 1912: 111–112, pl. 1 fig. 9; Baba 1936: 19–20; Burn 1972: 177, fig 11; Kay 1979: 454, fig. 144F; Bertsch and Johnson 1981: 20–21; Marcus 1982: 17–18, figs 35–38; Yonow 1990: 288, pl. 2; Colin and Arneson 1995: 176–177, figs 811–812; Debelius 1996: 159, 170; Erhardt and Baensch 1998: 634, 668; Marshall and Willan 1999: 37–38, fig. 48; Coleman 2001: 131; Hirose 2005: 905–916, figs 1–29; Jensen 2007: 278; Coleman 2008: 88–89; Yonow 2008: 56, 124–125, fig: 47; Gosliner et al. 2008: 93–94; Apte 2009: 169, fig. 2a; Herv  2010: 115; Ramakrishna et al. 2010: 53–54, figs 31, 31 (two images with same number); Yonow 2012: 20, pl. 17; Trowbridge et al. 2011: 2, 6, 8; Sreeraj et al. 2012: 2501.
- Placobranthus ianthobaptus* Gould 1852: 307, pl. 26, figs 407a–c (Honolulu, Hawaii); Bergh 1872: 166; Vayssi re 1912: 47; Ostergaard 1955: 120–122, fig. 8a–f.
- Placobranthus guttatus* Stimpson 1855: 378–379 (Loo Choo Is.); Trowbridge et al. 2011: 2.
- Elysia ocellata* Pease 1860: 35 (Sandwich Island, Honolulu).
- Placobranthus gracilis* Pease 1871: 303, pl. 21, figs 1a–b (Tahiti, French Polynesia); Bergh 1872: 166.
- Placobranthus variegatus* Pease 1871: 303–304, pl. 21, figs 2a–b (Huaheine, French Polynesia); Bergh 1872: 166–167.
- Plakobranthus argus* Bergh 1872: 151–165, pls. IX figs 6–9, XVII, XVIII (Honolulu, Hawaii); Vayssi re 1912: 111.
- Plakobranthus camiguinus* Bergh 1872: 167–169, pl. XIX, figs 14–19 (Luzon, Philippines).
- Plakobranthus laetus* Bergh 1872: 171–173, pl. XIX, figs 28–31, pl. XX, figs 1–7 (Masoloc, Philippines).

Plakobranthus priapinus Bergh 1872: 173–174, pl. XVIII, figs 17–18, pl. XX, figs 8–13 (Bohol, Philippines); Bergh 1905: 81–82, pl. II, fig. 21, pl. XIII, figs 18–19.

Plakobranthus punctulatus ? Bergh 1872: 169–171, pl. IX, figs 11–12, pl. XIX, figs 20–27 (Masoloc, Philippines); Bergh 1905: 82.

Plakobranthus chloropacus Bergh 1873: 76–77 (148–149), pl. IX, fig. 5–6, pl. X, fig. 22–25, pl. XI, figs 3–6 (Huaheine, French Polynesia); Barash and Zeniper 1994: 7.

Placobranthus ocellatus Bergh 1887: 310–311, pl. 6, fig. 5; O'Donoghue 1928: 714; Rao 1962: 253–254, figs 1, 2e, f; Heller and Thompson 1983: 332–334, figs 6A–C; Hughes 1977: 92; Jensen 1992: 283–285, figs 22B, 23, 24D–E; Richmond 1997: 266–267.

Plakobranthus sp. Bergh 1905: 82–83 (Kur I., Indonesia); Trowbridge et al. 2011: 3.

***Plakobranthus papua* Meyers-Muñoz & van der Velde, sp. n.**

<http://zoobank.org/E6FB98EC-AD98-4675-9FC4-0E205A6E3A2F>

Figures 2–7a

Type material. Holotype RMNH MOL.336417, length 35 mm (COI, anatomy. Genbank Accession number: KU934191). Paratype RMNH MOL.336418, length 30 mm (COI, anatomy, radula. Genbank Accession number: KU934192). Paratype RMNH MOL.336419, length 34 mm (COI, anatomy. Genbank Accession number: KU934193).

Additional material. RMNH MOL.336420, length 28 mm (anatomy, radula). RMNH MOL.336421, length 25 mm (anatomy, radula, penial bulb). RMNH MOL.5010422–5010434, slides of a single specimen, length 30 mm, northeast of Pulau Mansuar (S00°34.083', E130°38.525), Expedition Sta. RAJ.38, 30 November 2007 (histology). RMNH MOL.336423 (n = 7), length 15–26 mm, east side of Pulau Kri, Sorido Resort lagoon, near Jetty (S00°33.347', E130°41.225), Expedition Sta. RAJ.02, 4 December 2007 (anatomy, penial bulb). RMNH MOL.336424, length 41 mm, south side of Pulau Kri (S00°33.536', E130°41.258'), Expedition Sta. RAJ.03, 4 December 2007 (anatomy, penial bulb). RMNH MOL.336425 (n = 6), length 15–26 mm, west side of Pulau Yeben Kecil (S00°29.344', E130°30.081'), Expedition Sta. RAJ.48, 6 December 2007 (anatomy, radula).

Type locality. Mangrove Creek, south Gam Island (0°30'403"S, 130°38'986"E), West Papua, Indonesia, 25 November 2007.

Habitat. The specimens were collected in sea grass beds and on coral reef sand between 1 and 10 m depth.

Etymology. The specific name *papua* of this species is based on the name of the Indonesian part of New Guinea (provinces Papua and West Papua) where the type material was collected.

Description. *External morphology* (Figure 3). Body elongated, dorsoventrally flattened with wide parapodial flaps folding along the dorsal midline (Figure 3a). Rhinophores extended from lateral edges of the head long, smooth, rolled (Figure 3b).

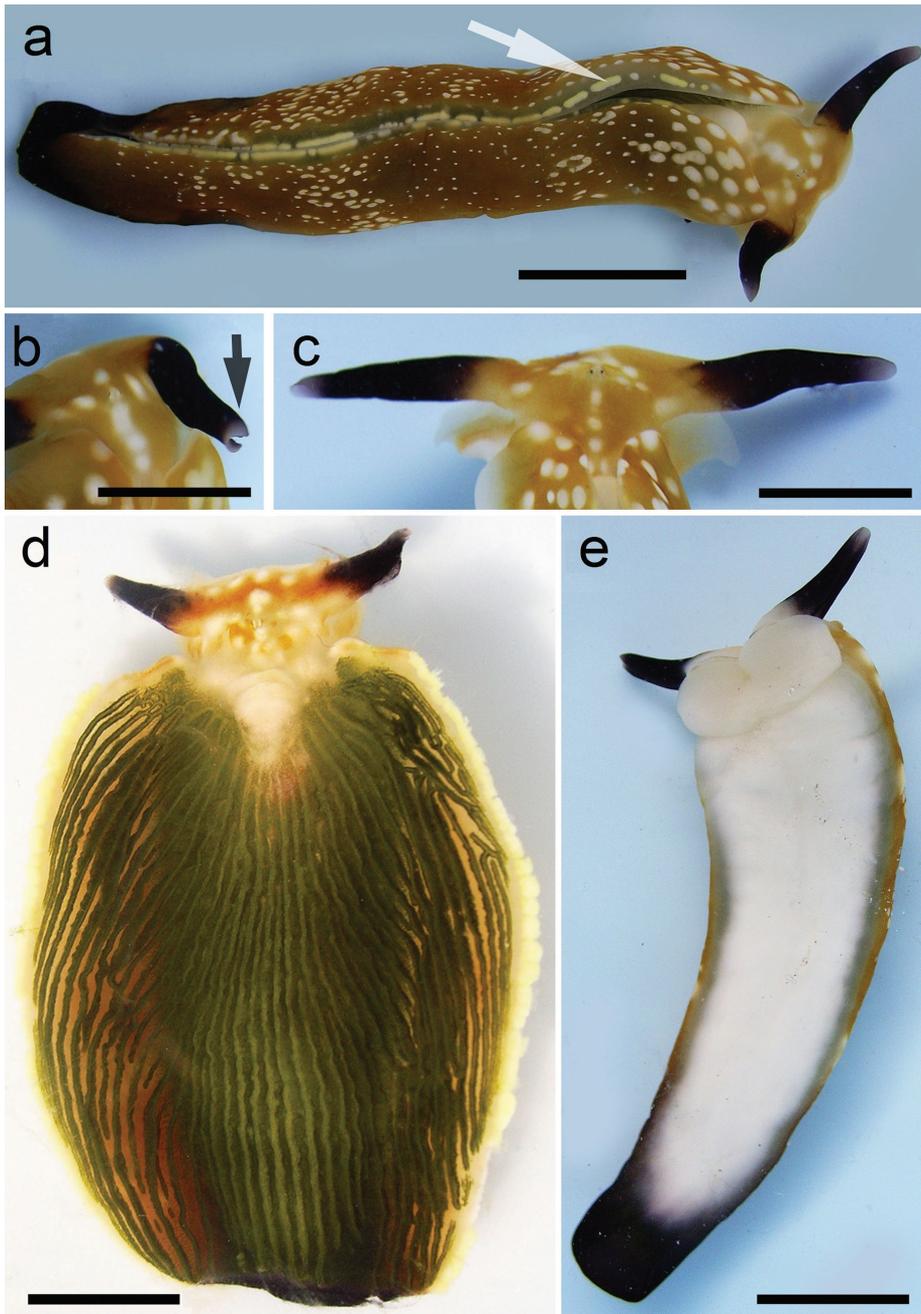


Figure 3. *Plakobranchus papua*. Images taken from live animals (external morphology). **a** Dorsal view, parapodia folded up on the dorsal body surface; arrow shows short yellow rod-like spots along the parapodial border **b** Dorsal view with detail of the rolled rhinophore indicated by an arrow **c** Detail of head, rhinophores, and pedal tentacles **d** Open parapodia with lamellae containing branches of the digestive gland and showing renopericardial area **e** Ventral view of bilobed oral prominence, narrow foot, and truncated black tail. Scale bars: **a, d, e** = 10 mm; **b, c** = 5 mm

An eye pair belonging to the central nervous system (CNS) is visible on the head. Eyes situated very close to each other, in front of a prominent, elongated white spot (Figure 3c). The voluminous renopericardial prominence is short, whitish (Figure 3d), and covered by the parapodial flaps. The folded parapodia show a smooth surface from a dorsal view. The parapodia margin at the mid-line shows short yellow rod-like spots along the complete parapodial border (Figure 3a) such as those visible in '*P. ocellatus*' (Wägele et al. 2011: fig. 1a). When the parapodia are open, the edges appear as soft yellow pectinate margins (Figure 3d). Internally, the parapodia have thick parallel longitudinal lamellae. Only the mid-central lamellae connect directly to the renopericardial prominence and run parallel to each other towards the posterior side of the body. The most external anterior lamellae are shorter, connected through anastomosing lamellae with the most internal lamellae and through them connected with the renopericardial prominence (Figure 3d). These fine dorsal anastomosing lamellae can only be recognized in live and in freshly collected, non-preserved specimens. In preserved contracted specimens it is not possible to distinguish these dorsal anastomosing lamellae. The lamellae possess visible internal granulations corresponding to the digestive gland ramifications, which contain chloroplasts. The genital opening is situated on the right anterior side of the body, just in front of the anterior part of the parapodia, and immediately behind the rhinophore. The anal opening is located on the right anterior side of the pericardium. Ventrally a bilobed oral prominence (Figure 3e) with a very fine, undulating black line boarding the upper lip is present. Pedal tentacles are short (Figure 3c). The narrow foot has a smooth surface and truncated tail (Figure 3e).

Colouration (Figure 3). Intense ochre body with white spots scattered all over the dorsum, head, and flanks: closest to the head region, on the anterior side corresponding to $1/4$ of the body length, the spots are largest, on $2/4$ and $4/4$ of the body length the dots are smaller, and on the $3/4$ of the body length they are larger, but not as large as on the anterior part of the body. Spots are pale white with a thin yellow outline (Figure 7a). Rhinophores black, the outer tips purplish. Internal parapodial flaps ridges bright green to olive green in colour, owing to chloroplasts in the digestive gland. The pericardium region is hyaline white. The pedal tentacles are translucent white. The foot sole is white with a black tail.

Digestive system (Figure 4). The masticatory apparatus was studied in four preserved specimens (RMNH.MOL.336418, 336420, 336421, 336425). The pharynx is connected to the stomach through a short muscular lightly bent oesophagus. The stomach is approximately 25% smaller than the pharynx. A pair of salivary glands inserted and extended along the oesophagus, reaching the first third of the stomach. The pharynx has prominent dorsal septate muscles. It consists of a large cuticular pharyngeal cavity, the radular sac, and one large ascus, which are also embedded in cuticular material. Uniserial radula, small, approximately 0.50 to 0.75 mm in length with 15 teeth, eight in the ascending series and seven in the descending series (Figure 4b), and an ascus-sac which varies in size (Figures 4b, c). Teeth are sharp with triangular cusps and 10–14 denticles at each margin side, the teeth measuring 70–75 μm (Figure 4d–g).

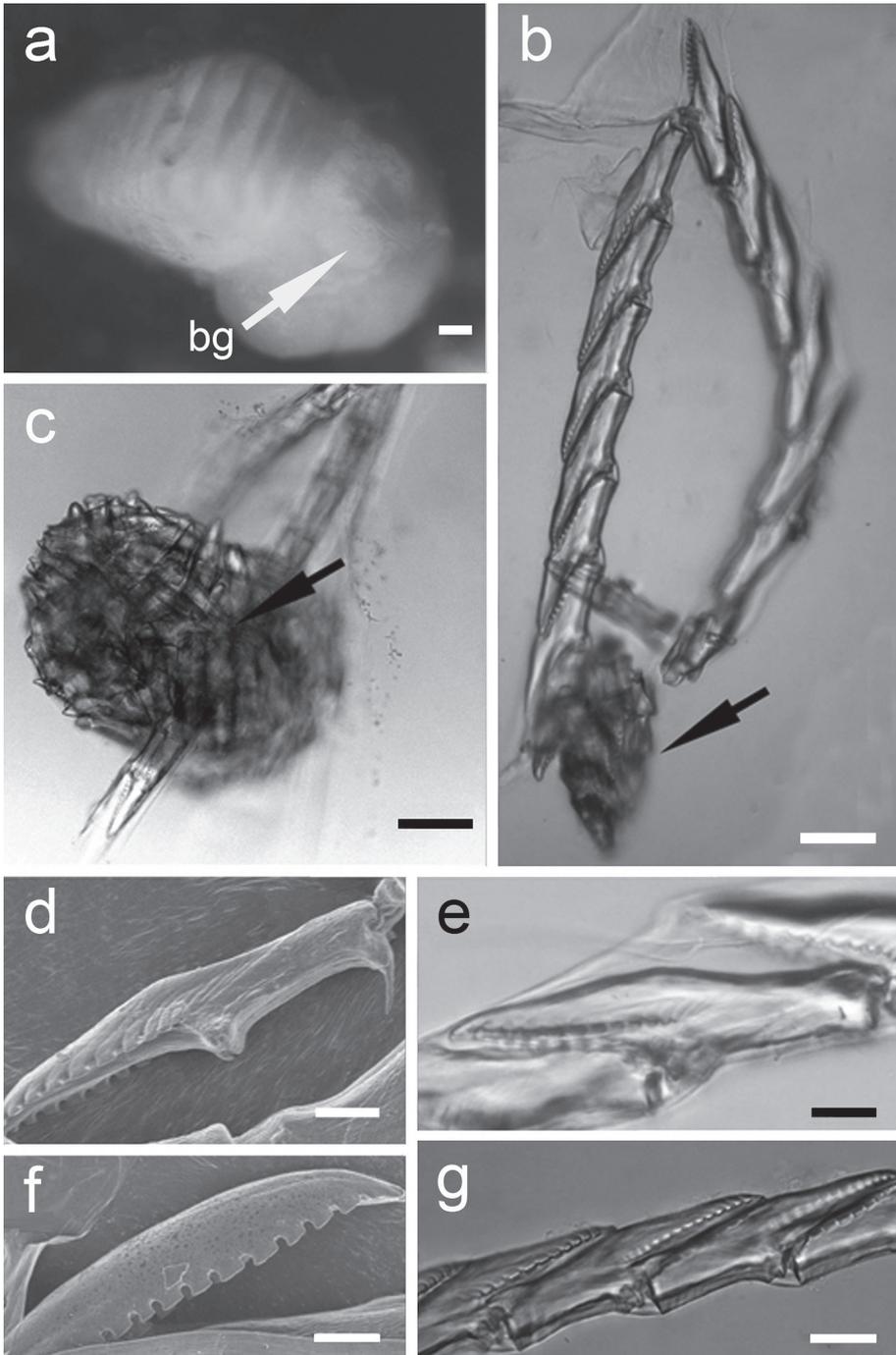


Figure 4. *Plakobranchus papua*. Masticatory apparatus. **a** Pharynx; white arrow points to the buccal ganglion (bg) **b** Radula with ascus-sac (arrowed). **c** Detail of the ascus-sac which contain used teeth (arrowed) **d** Tooth, scanning electronic photograph **e** Tooth, light microscopy photograph **f** Detail of the denticles (SEM) **g** Row of teeth (LM). Scale bars: **a, c** = 50 μm ; **b** = 25 μm ; **d-e** = 10 μm ; **f** = 5 μm ; **g** = 15 μm .

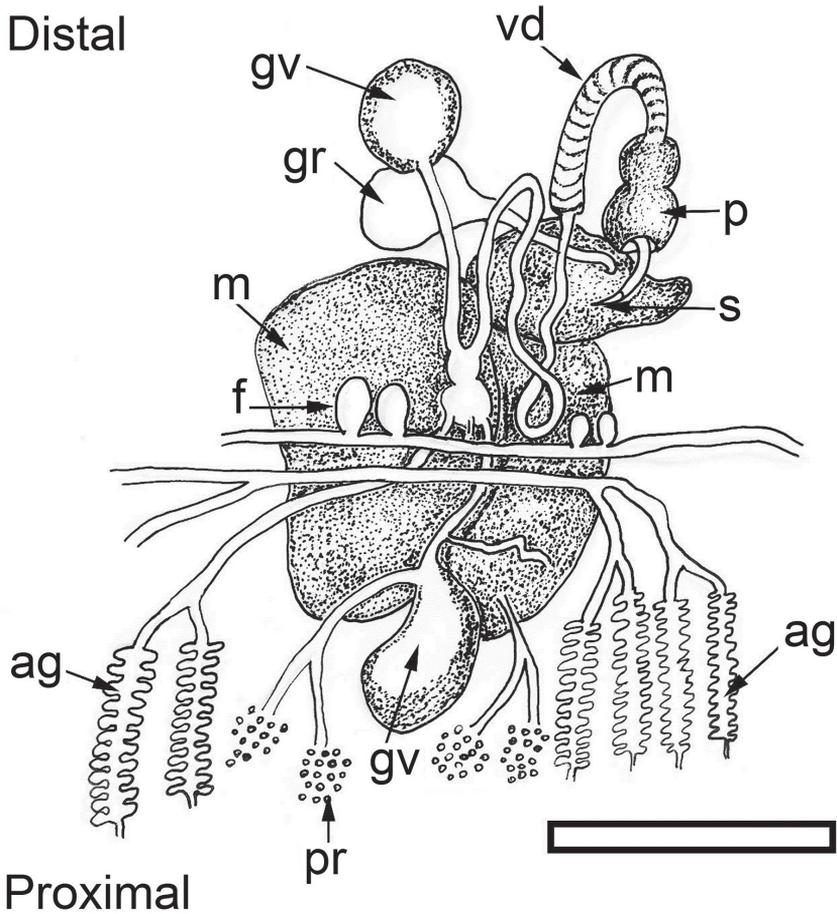


Figure 5. *Plakobranchus papua*. Schematic drawing of reproductive system (terminology after Jensen 1992). Scale bar: 250 μ m. Abbreviations: **ag** = albumen gland, **f** = follicles, **gr** = genital receptacle, **gv** = gametolytic vesicle ('bursa copulatrix'), **m** = mucus gland, **p** = penial bulb, **pr** = prostate, **s** = penial stylet, **vd** = vas deferens.

Reproductive system (Figures 5–6). Occupies almost the central anterior part of the body, at nearly 1/4 of the body length. The distal part of the reproductive system is situated below the heart and directly behind the central nervous system. The penial bulb, which is only approximately 0.5 mm long (Figure 5: p), is situated below the rhinophores at the same level as the eyes. It possesses a sharp cuticular stylet, which is hollow, with an oblique orifice at the tip (Figure 6). The vas deferens (Figure 5: vd) bends over the mucus gland (Figure 5: m), and is orientated towards the proximal area. The distal part of the vas deferens, which is in direct connection with the penial bulb, is muscular and arched. Its proximal part is thin and coiled and is connected to two spherical genital vesicles (Figure 5: gv). After this intersection the vas deferens continues in a proximal

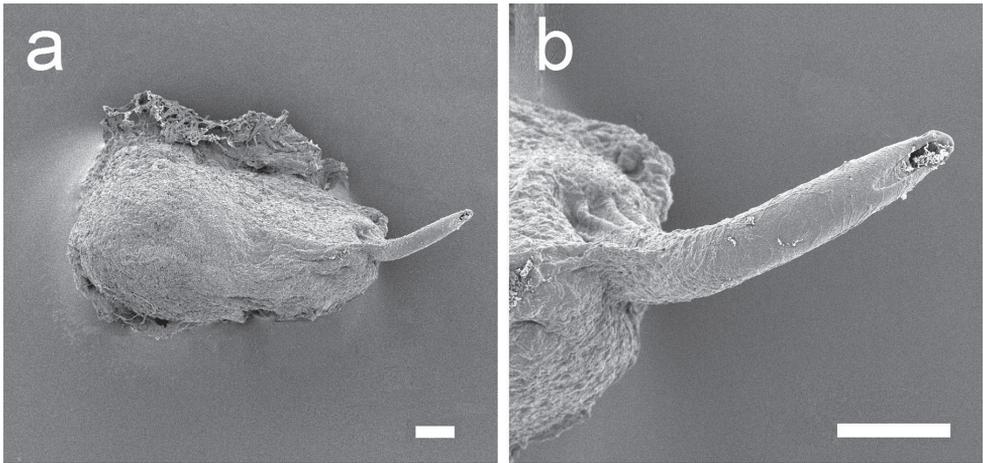


Figure 6. *Plakobranchus papua*. **a** Muscular penial bulb and stylet **b** Detail of the opening of the stylet. Scale bars: 100 μ m.

direction, where it appears as a short bulky extension, continuing in a thin duct attached to numerous ramifications, the follicles. Underneath this vesicle one large piriform white genital receptacle is present (Figure 5: gr), connected directly to the mucus gland. The mucus gland is large and divided in two lobes. The distal lobe is smaller, a little narrow and coiled. The prostate (Figure 5: pr) and albumen glands (Figure 5: ag) are extensively branched. The terminology used here is similar to the one in the schematic drawing of the reproductive system of *P. ocellatus* by Jensen (1992: fig. 22B).

Central nervous system. The central nervous system is located at the anterior part of the oesophagus and forms a circumoesophageal ring, consisting of a very small pair of buccal ganglia, a large pair of fused cerebro-pleural ganglia, and a pair of pedal ganglia. The CNS consists for the main part of the cerebral and pleural ganglia. The eyes of the CNS are situated very close to each other.

DNA analyses (Figure 8). The molecular phylogeny reconstruction of the genus *Plakobranchus* contains all the currently available sequences on GenBank. The analyses in MrBayes (Bayesian inference) and Phyml (maximum likelihood) resulted in trees in which the specimens were assigned to the same clades. In Phyml the tree formed a polytomy, whereas the analyses in MrBayes showed a topology with highly resolved clades. Ten different clades can be distinguished, of which three are represented by single specimens. The largest clade contains 49 sequences. Our new species groups with *Plakobranchus* sp. 1 of Krug et al. (2013) from Sulawesi, Indonesia and Panglao, Philippines. Over 580 base pairs there is a difference of 1.2% (7 bp) between our specimens and the specimens of *Plakobranchus* sp. 1 from Krug et al. (2013).

The ABGD analysis resulted in prior maximal intraspecific divergence of ca. 0.07. Values higher than the maximal intraspecific divergence resulted in 10 Molecular Operational Taxonomic Units (MOTUs) in both the recursive and initial partition. Each

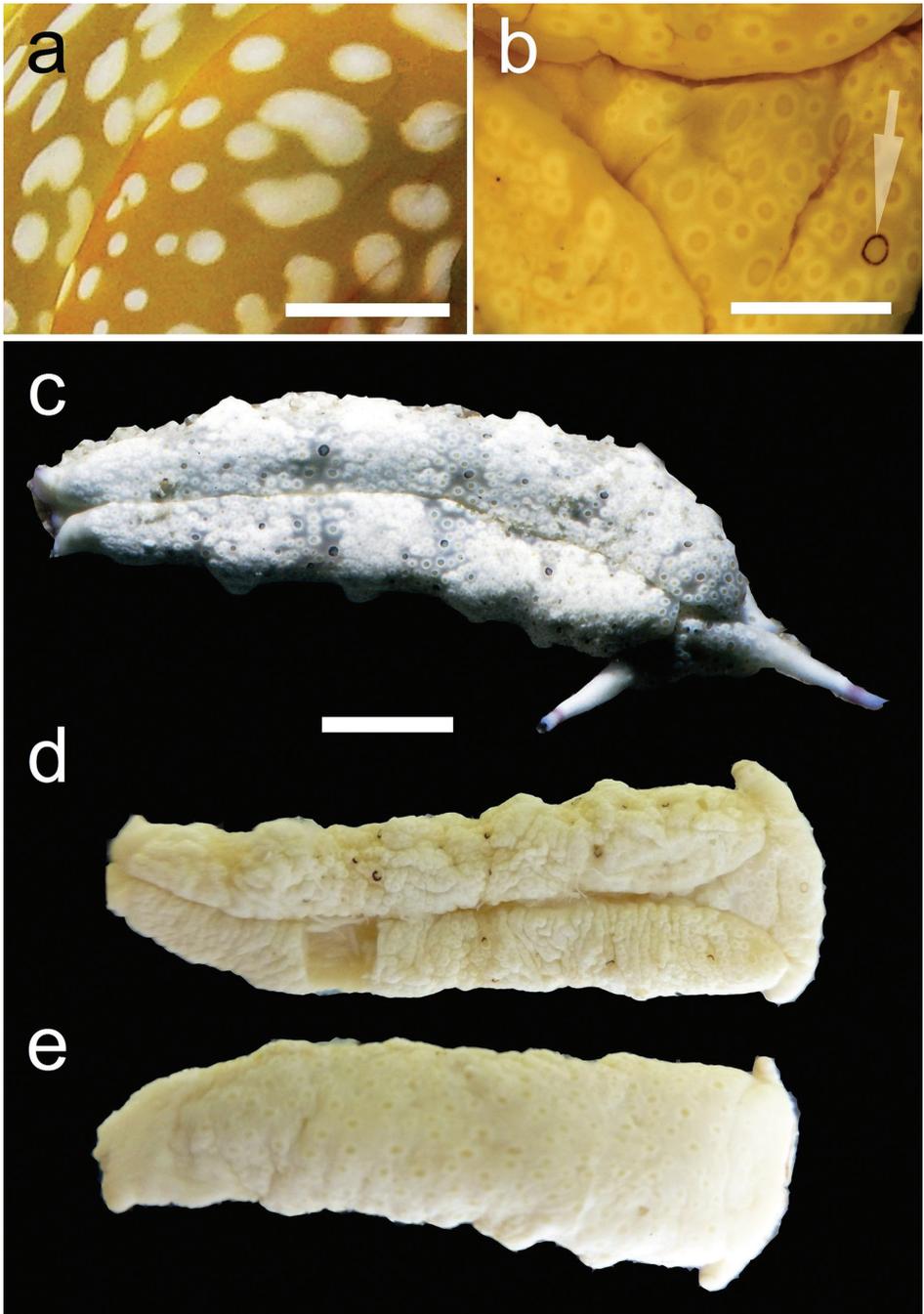


Figure 7. **a** Dorsal view of a preserved *Plakobranchus papua* showing white dots surrounded by a yellow outline. **b** Preserved *P. ocellatus*, ocellated spots with dark pigment surrounded by a white ring, some with a black ring (arrowed). **c** *P. ocellatus*, dorsal view of a live animal (Cebu, Philippines, 1999, photo BWH). *P. ocellatus*, **(d)** dorsal view and **(e)** ventral view of preserved animal. Scale bars: **a**, **b** = 5 mm, **c–e** = 10 mm.

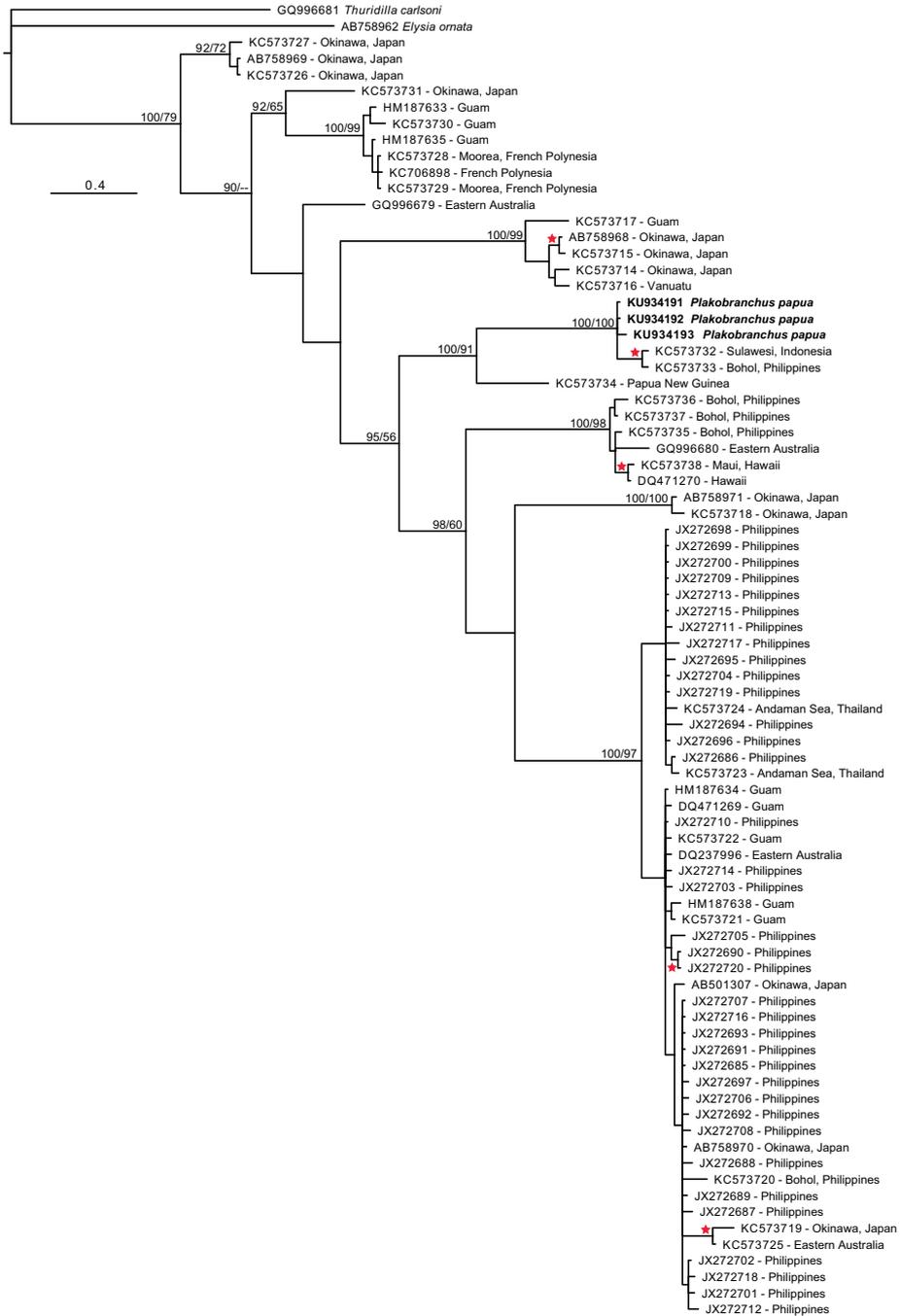


Figure 8. Phylogram of the *Plakobranchus ocellatus* species complex, topology derived from MrBayes analysis. Support values represent Bayesian posterior probabilities / ML. Numbers refer to GenBank codes. Red stars represent nodes with Bayesian probability values > 90 and high ML values.

of these MOTU's corresponds to a clade in the phylogeny reconstruction (Figure 8). The three singletons (GQ996679, KC573731, KC573734) from the ABGD analysis were also retrieved as singletons in the molecular phylogeny.

Discussion

Plakobranthus papua sp. n. differs not only from *P. ocellatus* as illustrated by van Hasselt (1824), but also from several other *Plakobranthus* colour varieties, which can be found at SeaSlugForum and NudiPixel. Colour variations of *Plakobranthus* and geographical distributions are presented in Table 2. Based on this data several species of *Plakobranthus* are to be expected. The new species differs from all other descriptions and illustrations of individuals ascribed to *P. ocellatus* by a clearly different colour pattern, with black rhinophores and tail, non-ocellated spots, and a foot sole without spots. *Plakobranthus papua* sp. n. also differs externally from all taxa that have been synonymised with *P. ocellatus* in the colouration, distribution of spots, and the absence of real ocellated spots. DNA analyses of COI mtDNA show a clear distinction between *P. papua* sp. n. and *P. ocellatus* s.l.

The original drawings of *P. ocellatus* made by van Hasselt (1824) do not show many details (Figure 1), but enough to separate it from *P. papua* sp. n. The dorsum and flanks of *P. papua* sp. n. have white spots, while *P. ocellatus* possesses ocellate spots with dark pigment surrounded by a yellow ring or surrounded by a black ring (Figures 1, 7). These large ocellate spots of *P. ocellatus* are also present all over the foot sole, while in *P. papua* sp. n. the foot sole is white without any spots. Furthermore, the ocellate spots of *P. ocellatus* are very abundant all over the dorsum in combination with broad transverse stripes of darker or lighter pigment. These ocellate spots are abundant on the dorsum of the preserved specimen (Figure 7c, d) and less abundant on the ventral part of the body (Figure 7e). The tail of *P. papua* is black, while in *P. ocellatus* only the margin of the tail has dark pigment. Bergh (1872) described the radula of *P. ocellatus* as consisting of 19 teeth, eight ascendant teeth, one not developed, one tooth at tip and nine descendant teeth, and between 90 to 100 teeth inside the ascus. The teeth possessed up to 14 strong denticles at each side. In *P. papua* the shape of the teeth appears more arched than the teeth of *P. ocellatus* described and figured by Jensen (1997a). In our specimens of *P. papua* the number of teeth is similar, and the lateral denticles ranged between 10 and 14 in number. In the SEM photos of *P. ocellatus* by Jensen (1992) at least 10 denticles can be counted. The illustrations presented by Bergh (1873: Plate XI figs 5, 6) for *P. chlorophacus* show 10–11 denticles. Within Sacoglossa, species like *Elysia viridis* (Montagu, 1804) are able to modify the teeth size and shape in response to changes in their diet (Jensen 1993) but it is not known if this ability also exists in *Plakobranthus*.

Wägele et al. (2010) discovered special glandular structures on the dorsoanterior to lateral parts of the pharynx in *P. ocellatus*. Similar glands were found surrounding the pharynx in *P. papua*, and stained dark blue when treated with toluidine blue.

Jensen (1997a) described a pair of copulatory bursae in the reproductive system of *P. ocellatus*, which were also found in *P. papua* sp. n. (Figure 5: gv).

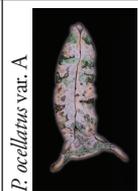
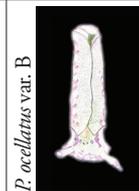
When comparing the drawings of the *P. ocellatus* holotype (Figure 1a–c) and those by Marcus (1982) of specimens collected in the Farasan Islands, Red Sea (Figure 1m, n), differences can be observed between both sets of illustrations. The schematic drawing made by Marcus (1982) is based on one preserved specimen and shows some scattered large ocellated dorsal spots and a line of ocelli along the head (Figure 1m). In the *P. ocellatus* described by van Hasselt (1824) the renopericardial area is fused together and appears as a nearly rounded prominence, while in the specimen illustrated by Marcus (1982) the pericardium is separated with respect to the renal prominence and this renal prominence is very elongated (Figure 1n), which is not confirmed in the text (Marcus 1928). Since Red Sea specimens externally resemble *P. ocellatus* s.s. (Yonow 1990, 2008), these differences need more study before they can be considered reliable. In this regard, it is noteworthy that *Plakobranchus papua* possesses a shorter renopericardial area as that illustrated by van Hasselt for *P. ocellatus*.

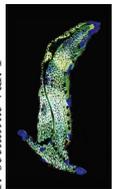
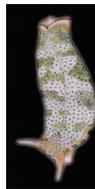
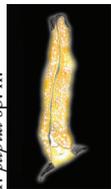
Marcus (1982) used the absence of dorsal vessels in *Plakobranchus* to separate this genus from *Pattyclaya* (which possesses clear dorsal vessels) and Jensen (1992) also used this same absence to separate *Plakobranchus* from *Elysia*. *Plakobranchus* species have tiny anastomosing lamellae connecting the external lamellae to the most internal ones. In *P. papua* fine short dorsal anastomosing lamellae connect the anteriormost external lamellae with the most internal ones, which are in direct connection with the renopericardial area. This is endorsed by Wägele et al. (2010), who stated that the Plakobranchidae are characterized as follows: “a number of pericardial vessels are found branching from the pericardium along the dorsal surface of the body and parapodia”.

The new COI sequences were used in a phylogenetic analysis together with the 76 sequences available in GenBank under the name *P. ocellatus* from various Indo-West Pacific localities (Figure 8). The phylogenetic analysis shows that *P. papua* with *Plakobranchus* sp. 1 from Krug et al. (2013) are in the same cluster, and separated from all other *P. ocellatus* sequences. Solely based on COI, *P. papua* could be considered the same species as *Plakobranchus* sp. 1 of Krug et al. (2013). This would imply that *P. papua* is not restricted to West Papua but also occurs at Panglao Island (Philippines) and Sulawesi (Indonesia).

The ten groupings retrieved in our ABGD analysis and phylogeny reconstruction (Figure 8) agree with the results of Krug et al. (2013). Thus, although morphological analyses indicate a single highly polymorphic *P. ocellatus* (Jensen 1992, 1993, 1996, 1997a, 1997b, 2007), molecular analyses indicate a species complex containing ten candidate species. Christa et al. (2013: fig. 1b) identified a specimen from Guam as *P. ocellatus*, but according to our *Plakobranchus* colour pattern and distribution review (Table 2), this specimen resembles *P. ocellatus* var. A more closely. Unfortunately the GenBank accession numbers cannot be linked to their illustrated specimens. Krug et al. (2013) stated that some differently coloured Japanese specimens could also be considered separate species. The eleven varieties of *P. ocellatus* found in the web and publications (one of which is *P. papua* sp. n.) could not be linked because Krug et al. (2013)

Table 2. Distribution, external morphology, and colour pattern of *Plakobranchus* varieties identified as *P. ocellatus* and their references.

Taxon	Colour pattern	Distribution	References
<p><i>P. ocellatus</i> var. A</p> 	<p>Small ocellate spots and white spots scattered over the head and dorsal flaps. Transverse dark stripes. Foot sole with some white and dark spots and some scattered ocellate spots.</p>	<p>Okinawa, Japan; Bai Su, Vietnam; Milne Bay and New Hanover, Papua New Guinea; Cebu, Philippines; Ambon, Indonesia</p>	<p>Adams 2000; Ono 2005; Krampf 2006; Gosliner et al. 2008;</p>
<p><i>P. ocellatus</i> var. B</p> 	<p>Body white cream, ocellate spots with red brown rings present over the head and lateral flanks. Dorsal white spots. Foot sole with black spots.</p>	<p>Okinawa, Japan</p>	<p>Ono 2005</p>
<p><i>P. ocellatus</i> var. C</p> 	<p>Translucent white rose body with few dark red ocellate spots over the parapodia and head. Foot sole with small dark dots green and brown.</p>	<p>Okinawa, Japan; Mayotte</p>	<p>Ono 2005; Deuss 2009</p>
<p><i>P. ocellatus</i> var. D</p> 	<p>Dorsal body white-green, scattered with yellow spots. Foot sole white green, without spots.</p>	<p>Okinawa, Japan</p>	<p>Ono 2005</p>
<p><i>P. ocellatus</i> var. E</p> 	<p>Translucent olive-green body, covered with large white-cream spots, rhinophores, oral tentacles and tail with bluish purple tips. Tail tip also black. Foot sole blue.</p>	<p>West Papua and Sulawesi, Indonesia; Cebu, Philippines; Palau; Milne Bay and New Hanover, Papua New Guinea; Nha Trang, Vietnam; Okinawa, Japan</p>	<p>Present study, Colin and Arneson 1995; Adams 2000; Warren 2000; Krampf 2006; Coleman 2008; Gosliner et al. 2008</p>
<p><i>P. ocellatus</i> var. F</p> 	<p>Rhinophores, oral tentacles and tail with black tips. Head and parapodia cover with many ocellate spots, small yellow and medium dark. Lateral flanks with largest black ocelli. Foot translucent green with few black ocelli.</p>	<p>Okinawa and Kagoshima, Japan</p>	<p>Imamoto 2005</p>

Taxon	Colour pattern	Distribution	References
<p><i>P. ocellatus</i> var. G.</p> 	<p>Body rose-brown, with white spots and some scattered brown and blue spots.</p>	<p>Moorea, French Polynesia</p>	<p>Greiger 2001</p>
<p><i>P. ocellatus</i> var. H</p> 	<p>Body white-light green with a little rose on head. Yellow ocellate spots all over the parapodia, only two on head. Lateral flanks with large green ocellate spots. Oral tentacles and rhinophores tips blue. Rhinophore with a fuchsia sub-terminal ring.</p>	<p>Cebu, Philippines</p>	<p>Raabe 2006</p>
<p><i>P. ocellatus</i> var. I</p> 	<p>Body dark green with tiny white spots, lateral flanks, tail and rhinophores tips blue or violet, over the midline of the head with blue/violet and some scattered black spots.</p>	<p>Thailand; Bali and Gorontalo, Indonesia; Hawaii</p>	<p>Gould 1852; Coleman 2008; Gosliner et al. 2008; Supapong 2008</p>
<p><i>P. ocellatus</i> s.s.</p> 	<p>Body pale yellow-green. Head and parapodial dorsal surface covered with ocellate spots (coloured rings). Whittish, purple rhinophores, green-blue ocellata on flanks. Front of the head and foot sole with dark ocelli.</p>	<p>Eastern Australia; Bohol and Cebu, Philippines; Guam; Indonesia; Mayotte; Danang City, Vietnam; Red Sea; Japan; Thailand; Maui, Hawaii</p>	<p>van Hasselt 1824; Jensen 1992; Colin and Arneson 1995; Erhardt and Baensch 1998; Rudman 1998; Koehler 1998; Marshall and Willan 1999; Coleman 2001; Jacobson 2003; Rudman 2003; Wyatt 2003; Groeneveld 2006; Raabe 2006; Tuyen 2008; Yonow 2008; Deuss 2009</p>
<p><i>P. papua</i> sp. n.</p> 	<p>Body yellow-orange with white spots on the dorsum. Rhinophores and tail black; Open parapodia green. Foot sole white with black end.</p>	<p>West Papua, Indonesia</p>	<p>Present study</p>

did not provide enough information on the morphology and colouration pattern of their specimens, nor did they include illustrations. Based only on phenotypes of images retrieved from the internet, eleven varieties of *Plakobranthus* were distinguished, including *P. ocellatus* (*sensu stricto*) and *P. papua* sp. n. (Table 2). All *Plakobranthus* species and varieties were recorded from the Indo-West Pacific, including the Red Sea.

Further research on the genus *Plakobranthus* based on morphology and DNA is necessary to unravel the phylogenetic and taxonomic relationships within this genus, with clear illustrations included of the external morphology. The present study shows that *in situ* photographs are indispensable to morphologically differentiate between *Plakobranthus* species because of their highly variable colour patterns, since ethanol-preserved specimens tend to lose much of their original colouration (Figure 7). Museum collections as a source for taxonomic work, studies on historical ecology, or other kinds of biodiversity studies (Rainbow 2009, Hoeksema et al. 2011, Rocha et al. 2014), may not be sufficient without photographic documentation of the specimens *in situ*. It is suggested that illustrations be included in DNA research as well as included in GenBank data.

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A review of the *sarawakensis* species group of the ground beetle genus *Orthogonius* MacLeay, 1825 (Coleoptera, Carabidae, Orthogoniini)

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Abstract

The *sarawakensis* species group of the termitophilous carabid genus *Orthogonius* MacLeay, 1825 is defined and reviewed. Members of this species group are distributed in Southeast Asia and represented by four species, including two new species: *Orthogonius sabahicus* **sp. n.** (Sabah, northern Borneo, Malaysia) and *Orthogonius morvanianus* **sp. n.** (southern Thailand). A key to all species of the species group is also provided.

Keywords

Ground beetle, termitophilous, taxonomy, new species, Southeast Asia

Introduction

As part of the series works on the dominant genus *Orthogonius* MacLeay, 1825 in the tribe Orthogoniini, the *lancangjiang* and *baconii* species groups have been reviewed respectively (Tian and Deuve 2013, 2016). In this paper, the *sarawakensis* species group is dealt with.

The members of the *sarawakensis* species group are medium to large sized, broad and brown ground beetles having large and prominent eyes, rounded off pronotal

hind angles, flat intervals, and median and posterior setiferous pores in the 3rd elytral interval located on median portion of the interval, instead of close to the 2nd stria as in other *Orthogonius* species.

All of the four species of this group are distributed in Southeast Asia. The first species of this group is *O. sarawakensis* Tian & Deuve, 2006 recorded from Sarawak, northern Borneo, eastern Malaysia. Then, the second species *O. perakicus* Tian & Deuve, 2007 was described from Perak, western Malaysia. In the present paper, further two new species are discovered from Sabah, northern Borneo, Malaysia and from Khao Sok NP, southern Thailand respectively, and hereinafter described.

Material and methods

All specimens for this study are dry and mounted material. Dissection and observation of the specimens were made using a WILD M32 binocular microscope. Detailed descriptions are provided for the new species, while only diagnostic character states are given for the known species. Habitus and male genital illustrations for all species are also presented. Digital photographs were taken and processed as in Tian and Deuve (2013).

Body length was measured from apex of right mandibles to apex of elytra; body width = width of elytra.

Abbreviations of measurements used in the text are as followings:

HL	head length (from apex of right mandible to base of vertex);
HW	head width (maximum distance across head, including eyes);
PL	length of pronotum (measured from front to basal margins, through midline);
PW	width of pronotum (greatest width of pronotum);
EL	length of elytra (measured from base to apex of elytra, through suture);
EW	width of elytra (greatest transverse distance across both elytra).

The depository abbreviations used in the text are as following:

CIB	Collection of Dr. Ingo Brunk, Dresden, Germany;
CPM	Collection of Mr. Pierre Morvan (Carentoir, France);
MNHN	Muséum national d'Histoire naturelle, Paris, France;
SCAU	South China Agricultural University, Guangzhou, China.

Taxonomy

Characteristics of the *sarawakensis* species group

Large-sized and robust; body brown or yellowish brown, moderately convex; glabrous, impunctate on head and pronotum; head wide, eyes moderate large and prominent;

mentum bisetose in most species (except for *O. sabahicus* sp. n. in which the mentum is asetose); apical margin of elytra broadly rounded, not truncate, and so without outer angles, inner angles broad in most species (but acute in *O. sarawakensis*); hind angles of pronotum widely rounded off, lateral expanded margins well-marked, subequal in width in middle, from flat (*O. sarawakensis*) to evidently reflexed throughout (*O. morvanianus* sp. n.); elytra convex, base well-bordered, striae moderately deep, intervals almost flat; 7th interval simple, not carinated; at least median and posterior setiferous pores of the 3rd interval located on median portion, instead of closing to the 2nd stria as in most *Orthogonius* species, anterior pores present (but absent in *O. sabahicus* sp. n.); the 2nd–7th intervals subequal wide in middle; protarsi more developed than meso- and metatarsi; middle tibiae not expanded in male; middle coxae asetose medially; apical spurs of hind tibiae long and sharp; hind femora moderately dilated, with two setae posteriorly; the 1st and 3rd hind tarsomeres longer than the 2nd and 4th respectively, the 4th tarsomere deeply and asymmetrically emarginated, outer lobe longer than the inner; all tarsal claws pectinate; prosternal process well-bordered at apex (but unbordered in *O. sarawakensis*); male genitalia robust, notably expanded in median portion, deeply arcuate ventrally, and then gradually narrowed towards apex which is more or less pointed, or suddenly contracted at tip, dorsal opening long and large; the apical lamella long or short, broadly blunt at apex.

Sexual dimorphism. In male, the 1st–3rd protarsomeres with two rows of spongy setae ventrally (Fig. 1A); ventrite VII slightly and shallowly emarginated at apical margin (Fig. 1B).

Distribution. Southeast Asia.

Key to species of the *sarawakensis* species group

- 1 Median and posterior setiferous pores of the 3rd elytral interval located on median portion, never close to the 2nd elytra stria..... **2**
- Median and posterior setiferous pores of the 3rd elytral interval close to the 2nd elytra stria **other *Orthogonius* groups**
- 2 Mentum asetose, the 3rd elytral interval with only median and posterior setiferous pores, anterior one wanted..... ***O. sabahicus* sp. n.**
- Mentum bisetose, the 3rd elytral interval with three setiferous pores..... **3**
- 3 Prosternal process unbordered at apex, inner angle of elytra sharp ***O. sarawakensis* Tian & Deuve, 2006**
- Prosternal process well-bordered at apex, inner angle of elytra blunt..... **4**
- 4 Labrum slightly emarginated at frontal margin, the 4th hind tarsomere with a deeper emargination, outer lobe half as long as the joint ***O. perakicus* Tian & Deuve, 2007**
- Labrum strongly emarginated at frontal margin, 4th hind tarsomere with a shallower emargination, outer lobe shallower, about 1/3 as long as the joint... ***O. morvanianus* sp. n.**

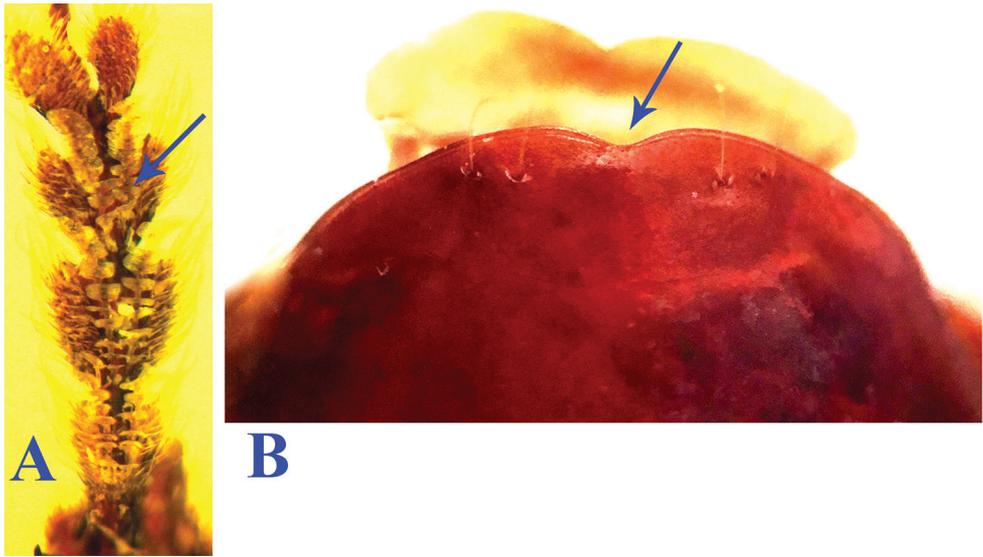


Figure 1. Protarsi and abdominal ventrite VII in male of *Orthogonius* **A** protarsi of *Orthogonius morvanianus* sp. n. **B** abdominal ventrite VII of *O. sarawakensis*.

***Orthogonius sarawakensis* Tian & Deuve, 2006**

Figs 1B, 2A–C

Orthogonius sarawakensis Tian & Deuve, 2006: Tian and Deuve 2006: 133.

Type material. Length: 15.0 mm; width: 7.0 mm. Habitus as in Fig. 2A.

Diagnosis. Head dark brown, other parts of the body yellowish brown; head stout, as long as wide; labrum slightly emarginated at frontal margin; clypeus even near base, with two longitudinal furrows along both side, joining to frontal impressions respectively; mentum bisetose; pronotum transverse, PW/PL = 1.55, widest at about middle, strongly convex; lateral expanded margins almost flat, equal wide throughout; elytra elongate ovate, EL/LW = 1.54; base well-bordered; the 3rd interval with three setiferous pores, all are located on the interval; tarsal claws pectinate; prosternal process unbordered at apex, abdominal ventrite VII shallowly emarginated at apical margin in male.

Male genitalia (Fig. 2B, C): Short and robust, expanded in median portion; in dorsal view, apical lobe elongated, gradually narrowed towards apex, with apical lamella evidently longer than wide.

Female: Unknown.

Remarks. Easily separated from its congeners, *O. perakicus* Tian & Deuve, 2007 and *O. morvanianus* sp. n., by its unbordered prosternal process at apex, pointed elytral apex, and more elongated apical lobe of aedeagus.

Material examined. 1 male, the holotype, “Nord Borneo, Mont Kina Balu, 5-8, 1903, John Waterstradt”, in MNHN; 1 male, same data as in holotype, in SCAU.

Distribution. Malaysia (Sarawak).

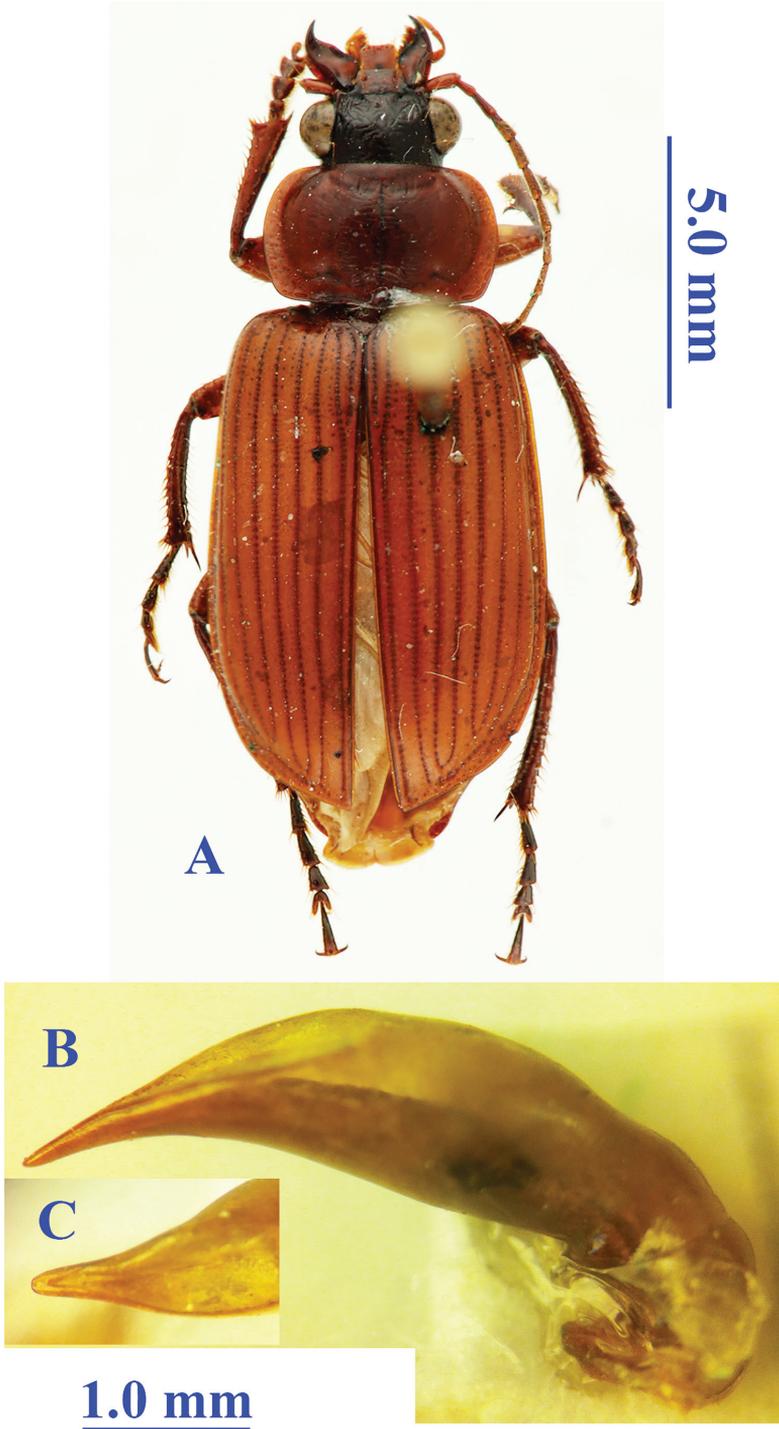


Figure 2. *Orthogonius sarawakensis* Tian & Deuve, 2006 **A** habitus, male, holotype **B** median lobe, right lateral view **C** apical lamella, dorsal view.

***Orthogonius perakicus* Tian & Deuve, 2007**

Fig. 3

Orthogonius perakicus Tian & Deuve, 2007: Tian and Deuve 2007: 239.**Type material.** Length: 14.0 mm; width: 5.5 mm. Habitus as in Fig. 3A.**Diagnosis.** Elytra and sides of pronotum yellowish brown, head dark brown, pronotum light dark brown on disc; head as long as wide, clypeus smooth, front margin of labrum slightly emarginated; mentum bisetose; pronotum strongly transverse, PW/PL = 1.62; lateral expanded margins narrow and reflexed throughout, evenly widened; elytra well-bordered at base, elongate ovate, EL/EW = 1.58; apex broadly truncate, without outer angles, inner angles broadly obtuse; intervals flat; the 3rd interval with three setiferous pores, all on median portion of the interval; legs moderately developed; the 4th hind tarsomere emarginated as deep as 1/3 of the joint; all tarsal claws pectinate; prosternal process well-bordered at apex; abdominal ventrite VII in male slightly emarginated at apical margin.

Male genitalia (Fig. 3B, C): Median lobe of aedeagus stout, more or less bent near apex, apex pointed in profile, but obtuse in dorsal aspect, apical lamella longer than wide.

Remarks. Allied to *O. morvanianus* sp. n. Differs from the latter by its labrum shallowly emarginated at front, the 4th hind tarsomere having a deeper emargination, and apical lamella of aedeagus blunt at apex (see below for detail).**Material examined.** 1 male, the holotype, “Perak”, in the Collection of Bates, MNHN.**Distribution.** Malaysia (Perak).***Orthogonius morvanianus* sp. n.**<http://zoobank.org/2695C6FD-230F-41D4-A765-25DFD75E49C2>

Figs 1A, 4A–C

Holotype. Male, “Thailand South, near Khao Sok NP, 3-6. II. 1997, A. Kudrna lgt”, in CPM.

Length: 14.0 mm; width: 5.5 mm. Habitus as in Fig. 4A.

Description. Yellowish brown, with head dark brown, the 2nd–11th antennomeres, disc of pronotum, tibiae and tarsi of legs brown; surface glabrous, impunctate, head and pronotum finely striate, the 3rd, 5th and 7th elytral intervals each with a few tiny sparse punctures; surface moderately shiny; microsculptural engraved meshes densely isodiametric on the surface of head, pronotum and elytra; body rather flat.

Head stout, as long as wide; eyes very large and prominent; frons flat, vertex slightly convex, smooth; frontal impressions very small, foveate; clypeus bisetose, rather smooth near base; labrum 6-setose, evidently emarginate at apical margin; ligula very small and narrow, bisetose at apex; palps slender or elongate, subcylindrical, normally setose; palpiger asetose, mentum edentate, mentum and submentum each with one

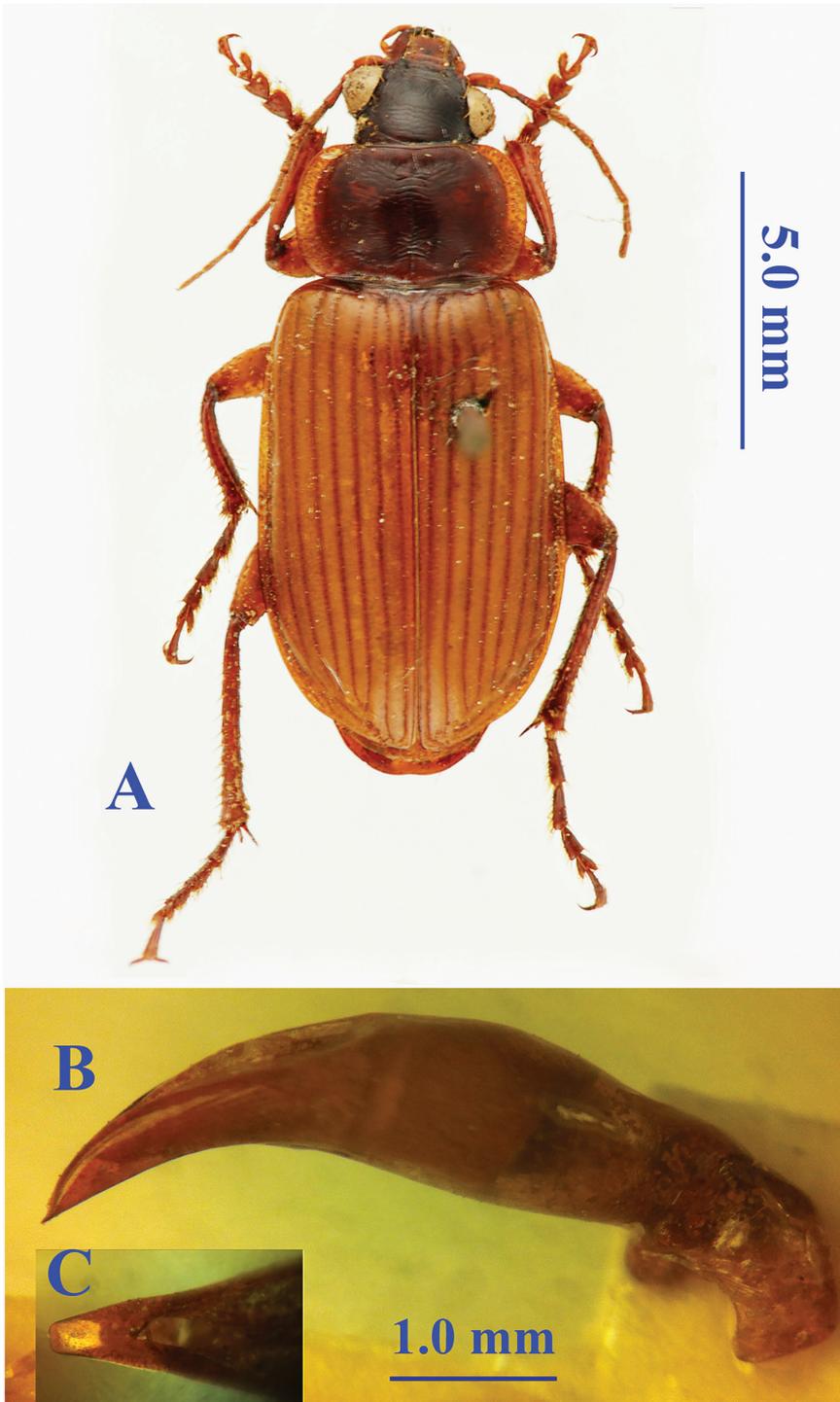


Figure 3. *Orthogonius perakicus* Tian & Deuve, 2007 **A** habitus, male, holotype **B** median lobe, right lateral view **C** apical lamella, dorsal view.

pair of setae, mental ones much shorter; antennae slender and long, reaching 1/4 of elytra from base, pubescent from apical 2/3 of the 4th antennomere; the 3rd antennomere as long as the 4th, both shorter than the 1st; the 1st–3rd antennomeres glabrous; evidently expanded laterally in the 4th and 5th antennomere; the 1st antennomere 1.6 times as long as the 2nd.

Pronotum strongly transverse, PW/PL = 1.78, sides evenly rounded, widest at about middle; both basal and fore margins beaded; lateral expanded margins well defined, subequal wide throughout, strongly reflexed, smooth though with punctate-like structures; both fore and hind angles rounded off; disc moderately convex, fore transversal impression evident, while hind one well-marked, joining basal foveae and the lateral expanded margins.

Elytra elongate ovate, much longer than wide, EL/EW = 1.63, moderately convex, apex broadly truncate, without outer apical angle, inner apical angle broadly obtuse; basal borders well-bordered; sides more or less paralleled in middle portion; striae quite deep; intervals nearly flat, subequal in width; the 3rd interval with three setiferous pores, anterior one close to the 3rd stria, while other two, median and posterior ones, on median portion of the interval; the 7th interval not carinated and without setae throughout.

Legs moderately developed; hind femur long and elongate, with 2 setae posteriorly; middle and hind coxae smooth and glabrous; fore tibia deeply sinuate at apical margin, outer angle sharp, outer margin distinctly serrate; middle tibia slender, not dilated medially in male; hind tibia slender, apex slightly dilated, apical spurs very long and sharp, the 1st tarsomere slightly longer than the 2nd, the 3rd tarsomere about 1.2 times longer than the 4th which is bilobed at apex, with outer lobe slightly shorter than half as long as the joint, outer lobe evidently longer than the inner; all tarsal claws moderately pectinate.

Male genitalia (Fig. 4B, C): Robust, expanded in median portion, then deeply concave on ventral margin, apex suddenly contracted, sharp, dorsal opening long and large, apex more or less pointed, with a fine tooth at subapex; in dorsal view the apical lamella quite long, slightly longer than wide, roundly obtuse at apex.

Prosternal process bordered at apex; apical margin of abdominal ventrite VII feebly emarginated in male.

Female: Unknown.

Remarks. Close to *O. perakicus* Tian & Deuve, 2007, but labrum strongly emarginated at frontal margin (slightly emarginated in *O. perakicus*), the 4th hind tarsomere deeply emarginated, but with outer lobe about 1/3 as long as the joint (half as long as the joint in *O. perakicus*).

Etymology. To be dedicated to Mr. Pierre Morvan (Carentoir, France), a good expert of Carabidae.

Distribution. Southern Thailand.

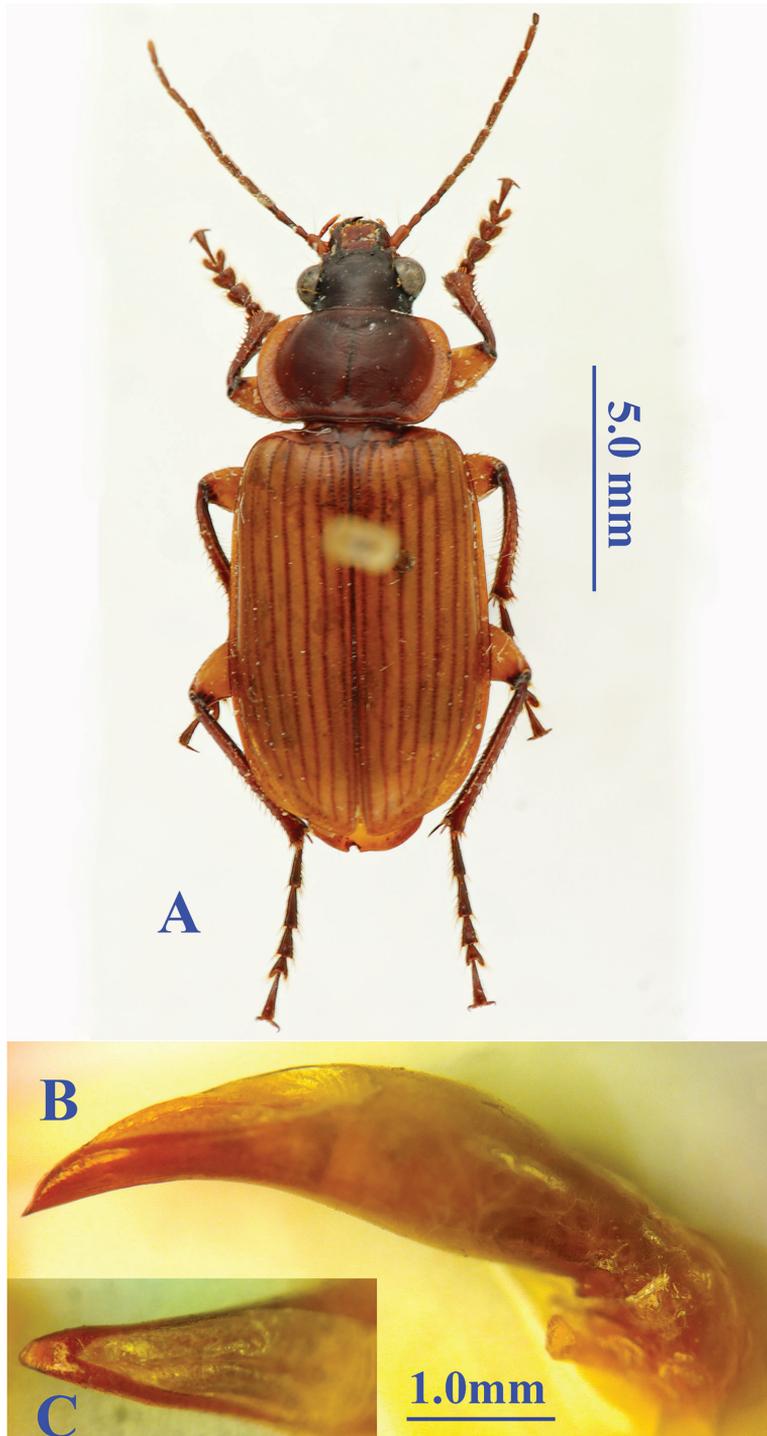


Figure 4. *Orthogonius morvanianus* sp. n. **A** habitus, male, holotype **B** median lobe, right lateral view **C** apical lamella, dorsal view.

***Orthogonius sabahicus* sp. n.**

<http://zoobank.org/C8EA2EDC-08D7-42E2-962E-347D379DCF29>

Fig. 5

Holotype. male, “Malaisie, Sabah, Crocker Range, Avril 94, Chew”, in MNHN; paratypes: 1 male, “Malaysia, N. Borneo, Sabah, Keningau distr. Trus Madi Mt. 1150 m, 7.VII.2011, A. Klimenko legit”, in CIB; 1 female, IBID, in SCAU.

Length: 16.0–17.0 mm; width: 6.5–6.6 mm. Habitus as in Fig. 5A.

Description. Head dark brown, other parts reddish brown; smooth and glabrous, impunctate, elytral intervals covered with fine and sparse punctures; head and pronotum faintly wrinkled; microsculptural engraved meshes isodiametric on head, pronotum and elytra.

Head stout, slightly longer than wide, HL/HW = 1.05–1.10; vertex convex; labrum quite long, nearly as long as wide, widely and feebly emarginated at frontal margin, 6-setose; clypeus bisetose, base raised and sides longitudinal furrowed, joining to the frontal impressions; frontal impressions pit-like; eyes moderately developed and prominent; palps slender, subcylindrical, the 3rd maxillary palpomere as long as the 4th, both glabrous, with two short setae at apex of the 3rd; the 2nd labial palpomere slightly longer than the 3rd, bisetose on inner margin, the 3rd faintly and sparsely setose; ligula narrow, bisetose at apex; mentum and palpigens unsetose, submentum bisetose; antennae extending beyond basal 1/5 of elytra, pubescent from the 4th antennomere, starting from basal 1/4; the 1st–3rd antennomeres and basal 1/4 of the 4th antennomere glabrous; the 1st antennomere stouter than other, with a long seta at subapex, several shorter setae present at apexes of each joint from the 2nd antennomere; the 1st antennomere less than twice as long as the 2nd, and slightly longer than the 3rd.

Pronotum transverse, notably wider than long, PW/PL = 1.66–1.68, widest at about middle; lateral expanded margins flat, even, feebly reflexed throughout; front and base well bordered; fore transversal impression faint, basal one evident; basal foveae small; disc markedly convex; front margin nearly straight at middle, basal margin feebly bisinuate; hind and front angles completely rounded off; base slightly shorter than front.

Elytra elongate-ovate, distinctly longer than wide, EL/EW = 1.61–1.63; nearly parallel-sided, widest at middle; basal border interrupted against the 1st–3rd intervals; humeri rather square; apex roundly truncate, without outer angles, inner angle broadly blunt; disc convex; striae finely and deeply striate; intervals convex, subequal in width; the 3rd interval with median and posterior setiferous pores (anterior one wanted), both are located on middle of the interval.

Femora moderately expanded, hind femur bisetose posteriorly; for tibia dilated at apex, apical margin obtusely truncate or sinuate towards outer angle, outer margin notably serrate; middle tibia slightly curved; hind legs slender, apical spurs long and sharp, the 1st and 3rd tarsomeres much longer than the 2nd and 4th respectively, the 4th tarsomere deeply emarginated at apex, with lobes about half as long as the joint; fore and middle tarsal claws weakly pectinate, hind claws simple.

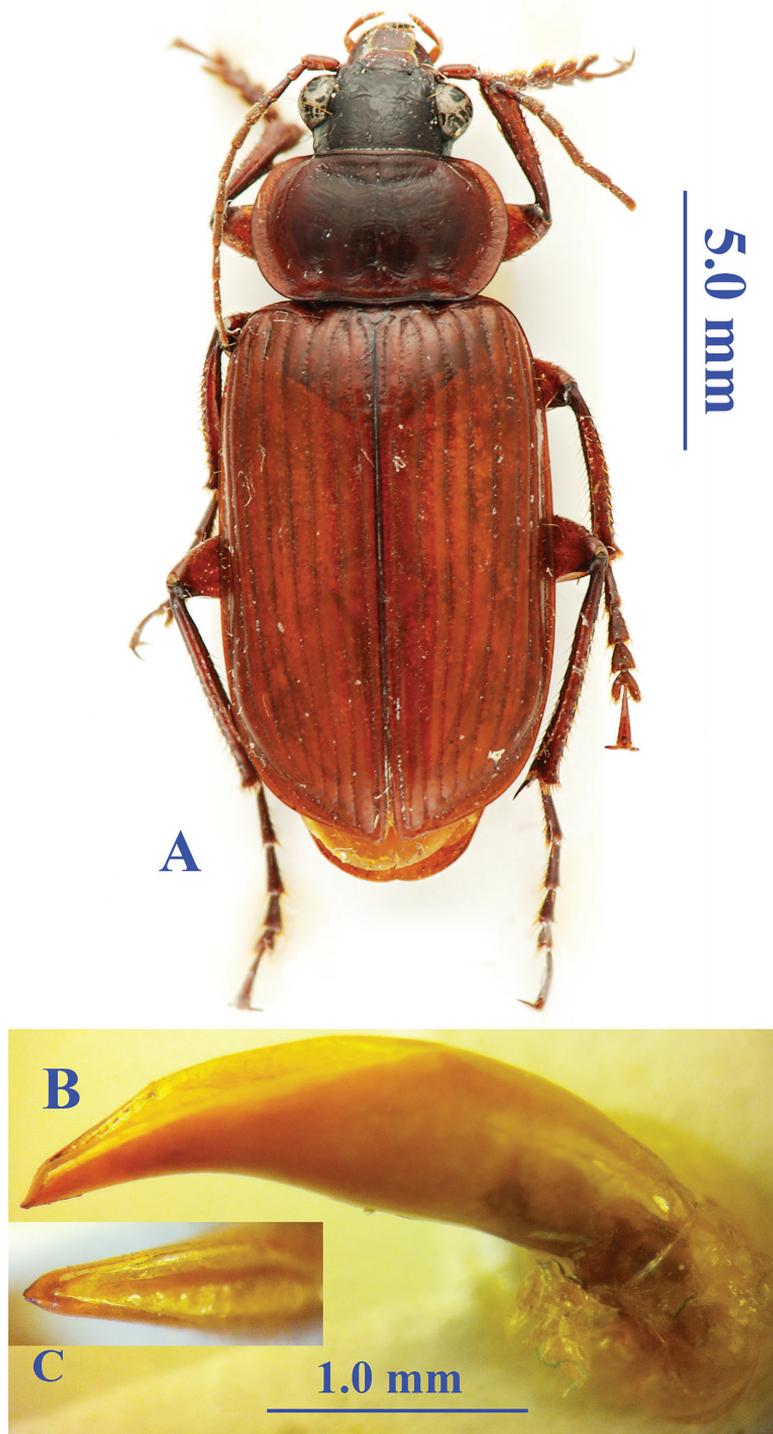


Figure 5. *Orthogonius sababicus* sp. n. **A** habitus, male, holotype **B** median lobe, right lateral view **C** apical lamella, dorsal view.

Prosternal process bordered at apex; abdominal ventrite VII finely and shallowly emarginate at apical margin in male.

Male genitalia (Fig. 5B, C): Stout and robust, apex suddenly narrowed and pointed in lateral view; the apical lamella short, wider than long.

Remarks. It is a very peculiar species, differing from other congeners by its mentum asetose, elytral base incompletely bordered, anterior dorsal pores missed on the 3rd interval, and hind tarsal claws simple.

Etymology. To refer to the type locality.

Distribution. Malaysia (Sabah).

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Caloparyphus palaearticus sp. n. (Diptera, Stratiomyidae), the first record for the soldier fly genus in the Palearctic

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Abstract

Caloparyphus palaearticus sp. n. is described from Russia and two localities in Mongolia and is the first representative of this genus in the Palearctic and the only species found outside the New World. The morphological characters of the species are described and illustrated, and relationships to related species of *Caloparyphus* are discussed.

Keywords

Palearctic Region, *Caloparyphus*, Oxycerini, taxonomy, new species, Russia, Mongolia, soldier fly

Introduction

The genus *Caloparyphus* belongs to the subfamily Stratiomyinae and the tribe Oxycerini. James (1939) described it originally as a subgenus of *Euparyphus* Gerstaecker and later it was treated as a distinct genus by Quist and James (1973). Sinclair (1989) described madicolous larvae of *C. greylockensis* (Johnson, 1912) and *C. tetraspilus* (Loew, 1866), and added adult characters of some further species. Some species of *Caloparyphus* are still insufficiently known or based on the female holotypes with the relevant males described

only superficially or not at all. Moreover, a precise identification is complicated by the known sexual dimorphism especially in the shape and the colour pattern of the head. According to the World Catalogue of the Stratiomyidae (Woodley 2001, 2011) twelve almost exclusively Nearctic species of *Caloparyphus* are known, with only *C. decemmaculatus* (Osten Sacken, 1886) penetrating into Mexico.

Recently we obtained males and females from Palaearctic Asia (Russian Kamchatka and Mongolia) that undoubtedly belong to *Caloparyphus*. They display all the main diagnostic characters of the genus (apical flagellomere style-like, antennal pedicel not elongated, scutellar spines separated by a distance as great as the length of spines, abdomen black with yellow oblique lateral spots on tergites 3 and 4, aedeagus tripartite distally). We can thus prove the occurrence of *Caloparyphus* in the Palaearctic Asia and describe a new species.

Methods

Part of the material was collected during the PIRE Mongolia project (<http://mongolia.bio.upenn.edu>), University of Pennsylvania (<http://mongolia.bio.upenn.edu>), and deposited in the collections of the Mongolian Aquatic Insect Survey (<http://clade.acnatsci.org/mongolia>) (Principal Investigator and Director of MAIS: Jon K. Gelhaus), the latter a project to document the Mongolian aquatic invertebrate diversity with respect to evolution, ecology and water quality (http://clade.ansp.org/entomology/mongolia/mais_home.html). More than 600 specimens of Mongolian Stratiomyidae have been examined and identified through the MAIS project and results are being prepared for publishing.

The notation in brackets for Arkhangai Mongolia paratypes refers to their map coordinates on the Mongolian national government topographic map 47T

The examined specimens were studied with Olympus and Nikon SMZ 1500 Stereomicroscopes. Photographs were taken through a Canon 450D and a Nikon DS-5M camera and were edited by CombineZ, Helicon Focus and Adobe Photoshop CS 4 software. The terminalia of the examined specimens were macerated in 10% KOH, rinsed with water and then preserved in glycerin and placed in a microvial on the specimen pin.

Morphological terminology follows that of McAlpine (1981) as modified by Cumming and Wood (2009). Body lengths are given exclusive of antennae.

Collection acronyms

- ANSP** The Academy of Natural Sciences of Drexel University, Philadelphia, PA, USA
CSCA California State Collection of Arthropods, Department of Food and Agriculture, Sacramento, CA, USA
USNM Department of Entomology, Smithsonian Institution, Washington DC, USA

Taxonomy

Caloparyphus palaearticus Rozkošný, Hauser & Gelhaus, sp. n.

<http://zoobank.org/92DFC733-E84E-44E6-BEE7-18323D9F8247>

Figs 1–8, 10–11

Type material. Holotype male (Figs 1–6), **Russia:** Kamchatka, okrestnosti [=environs] ESSO, lesnaja doroga [=forest road], 4.vii.2008, V. Mutin (label in Cyrillic) (deposited in CSCA).

Paratypes: 1 male, **Mongolia:** Hövsgöl Aimag, Hövsgöl Nuur (lake), east shore area, Dalbay Gol (river) valley, 51°01'40.5"N, 100°45'60.0"E, 1670 m, 22.vii.2007, D. Song (ID 263) (deposited in ANSP). 3 females, **Mongolia:** 3 females, Mongolia: Arkhangai Aimag, 17 km SW Tsenher, 1820m, swamp along stream, ca. 47°21'N, 101°33'E [47T 698.051 5243.562], 21.vii.2014 A.v.Eck (deposited in CSCA and USNM).



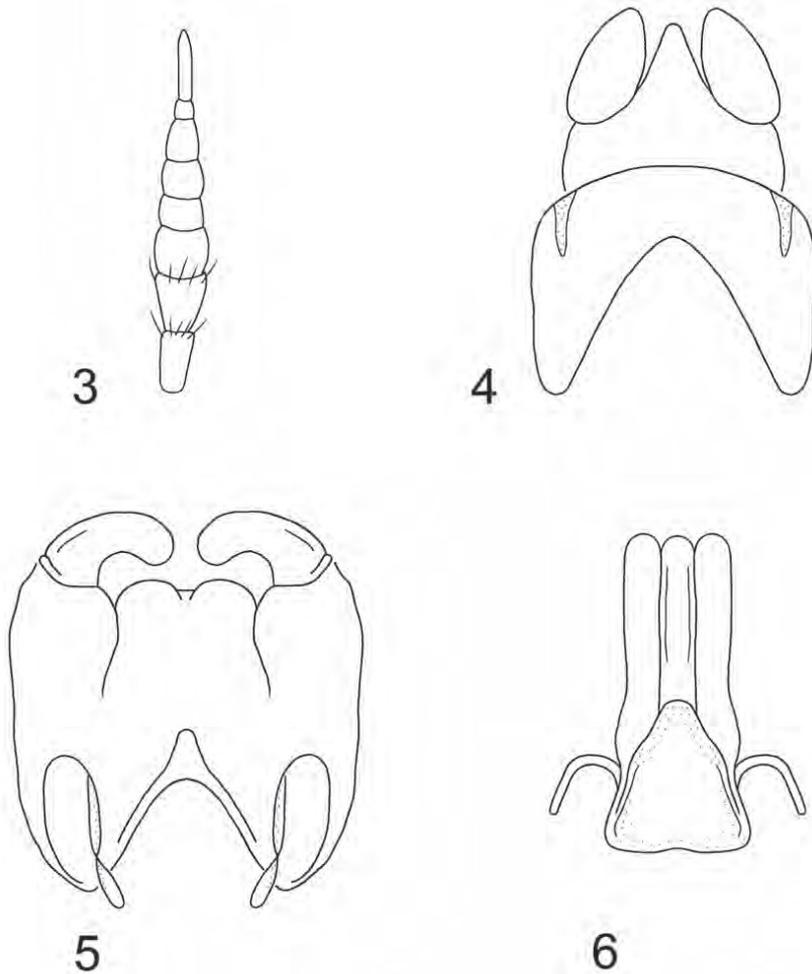
Figure 1. *Caloparyphus palaearticus* sp. n. holotype, male, habitus lateral view.



Figure 2. *Caloparyphus palaearticus* sp. n. holotype, male, habitus dorsal view.

Diagnosis. *Caloparyphus palaearticus* is the only species in the genus found in the Palaearctic Region. The males can be easily distinguished from all the other species of this genus by the two distinct brownish spots of denser microtrichia on the wing membrane (Fig. 1) in the lower distal corner of the basal medial cell and in the middle at the lower margin of the discal cell. In both sexes the antennae are as long as the head (Figs. 2, 7), and in the wing, vein R4 is absent and the discal cell is completely covered with microtrichia and has denser microtrichia patches in the males (Fig. 10).

Description. Male. (Figs 1–6) *Head:* rounded, 1.3 times higher than long in profile and 1.6 times broader than high in frontal view. Compound eyes touching in middle third of frons and consisting of larger facets on greater than lower half of its surface. Distinct border between differentially large facets and smaller facets at level of antennal insertion. Ocellar triangle slightly longer than wide, prominent in lateral view. Upper frons barely twice as wide as anterior ocellus. Lower frons somewhat broader than long, predominantly shining yellow but small upper corner black. Antenna (Fig. 3) about as long as head in profile, relative length of antennal segments scape: pedicel: flagellomere 1: flagellomere 2: flagellomere 3: flagellomere 4: flagellomere 5: flagellomere 6 as 2.7: 2.5: 2.4: 1.8: 1.8: 2.4: 1:0: 2.6. Face shining black with narrow whitish lateral stripes along inner eye margin reaching yellow frontal spots and dilated in lower half of face.



Figures 3–6. *Caloparyphus palaearticus* sp. n. **3** Antenna **4** Epandrium **5** Genital capsule **6** Aedeagal complex.

Proboscis yellow with shining black theca, black palpus very short. Ventral part of head and postgenal area black but latter grayish dusted. Head pile mostly inconspicuous, pale yellowish to white, hairs on occipital margin black.

Thorax: Shining black with two pairs of bright yellow scutal vittae. Dorsal vittae dilated in anterior third and reaching beyond transverse suture. Each lateral vitta touching yellow postpronotal callus anteriorly and transverse suture posteriorly. Also postalar callus intensively yellow, with a pointed anterior projection. Scutellum yellow but its narrow base and lateral parts black, scutellar spines yellow but blackish distally. Pleural part of thorax predominantly shining black, yellow line along upper margin of anepisternum abruptly dilated in front of wing base. Katepimeron and upper posterior part of katepisternum contrastingly yellow. Thoracic pile moderately long, mainly whitish but



Figure 7. *Caloparyphus palaeartcticus* sp. n. paratype, female, habitus lateral view.



Figure 8. *Caloparyphus palaeartcticus* sp. n. paratype, female, habitus dorsal view.

black and upright on scutum though similar whitish hairs on anterior part and along notopleura also visible. Wing membrane hyaline, veins brownish to pale yellow, stigma yellowish. No vein arising from discal cell reaching wing margin. Wing microtrichia considerably reduced in basal half of wing membrane, limited to small distal areas in



C. currani

9



C. palaearticus

10

Figures 9–10. *Caloparyphus* spp. wing. 9 *C. currani*, holotype 10 *Caloparyphus palaearticus* sp. n., paratype, female.



11



12

Figures 11–12. *Caloparyphus* spp. female head. 11 *Caloparyphus palaearticus* sp. n., paratype, female 12 *C. currani*, holotype.

basal radial and basal medial cells, sparse microtrichia in central area of posterior cubital cell and distal half of anal cell. Apical half of wing membrane almost completely covered with dense microtrichia but anterior cubital cell bare along upper and inner margins.



Figure 13. Habitat of *Caloparyphus palaeartcticus* sp. n. paratype, Mongolia, Hövsgöl Aimag, Hövsgöl Nuur (lake), Dalbay Gol (river) valley.



Figure 14. Distribution map of *Caloparyphus palaeartcticus* sp. n.

Especially dense microtrichia visible at distal part of basal medial cell and along anterior lower corner of discal cell as two distinct darkened microtrichial patches (cf. Figs 1 and 2). Calypters inconspicuous, dark, with long and upright whitish hairs. Halteres yellow, only the stem darkened basally. Legs black and yellow: coxae black, femora black with yellow tips, tibiae yellow with darkened ring on mid and hind pairs, tarsi predominantly darkened, only all basitarsi yellow. Pile on legs mainly short, whitish to yellow, often predominantly black on darkened parts, pile on basal halves of femora longer.

Abdomen: About as long as broad, sub-circular, black with yellow pattern (Fig. 1), venter black. Abdominal pile short, black and mostly semi-appressed, longer hairs visible in anterior corners, yellow spots bare. Ventral hairs whitish and mostly appressed.

Terminalia: Simple, without distinct modifications. Epandrium with a membranous incision before posterior corner on each side distally, proctiger subtriangular and cerci relatively short, oval (Fig. 4). Genital capsule (Fig. 5) with two rounded medial lobes distally and aedeagal complex (Fig. 6) simple, tripartite.

Length: body 6.5–6.7 mm, wing 5.7–5.8 mm.

Female. Similar to male, except for typical sexual dimorphism. Face yellow (Fig. 11) with a dark pentagonal mark on the frons widening from the ocellar triangular towards the antennal base, and narrowing on the lower frons before reaching the antennae. Area around the mouth black, and extending laterally upwards to the eye margin beyond antennae. Lower half of eye margin with a narrow vitta of dense silver pubescence. Hairs on lower face white, and black on frons. Gena and occiput mainly yellow, black around ocellar triangle, this black area extends to the upper eye margin and is also connected to the black pentagonal mark on the frons. The yellow spot on tergite one (Fig. 8) is more extant than in the male, also the lateral spots on tergite two are larger and more rounded (Fig. 7).

Length: body 7.9–8.0 mm, wing 6.9–7.0 mm.

Variability. There are no doubts that the male holotype and paratypes are conspecific but some small differences in color pattern were found in the male paratype from Mongolia (e.g. dorsolateral vittae are separated from the yellow postpronotal calli and a spot at the katepisternum and a small yellow basal spot on the abdomen are missing). Similar variability is commonly known in many other Oxycerini. Differences in color pattern between the female and male adults are noted in the description.

Etymology. The species epithet indicates the distribution of this species in the Palaearctic Region, i.e. in a different biogeographic realm in comparison with all other known species of this genus.

Distribution. Eastern part of the Palaearctic Region from Mongolia to Russian Kamchatka (Fig. 14).

Ecology. The male Mongolian specimen was collected in an area of mixed steppe grassland, riparian shrubs and *Larix siberica* forest (Fig. 13). Insects were collected visiting flowers on the south facing slope of the steppe dominated by a mixture of sedges (e.g. *Carex* sp.), grasses (e.g. *Festuca lenensis*, *Poa attenuata*) and forbs (e.g. *Aster alpinus*, *Potentilla* spp., *Artemisia commutata*, *Thymus gobicus*). Further information about the study site can be found in Song (2015).

Discussion

The species of the genus *Caloparyphus* were only revised by James (1939), when he erected “the species of *Euparyphus* related to *crotchii*” as a subgenus of *Euparyphus*. In that paper three species considered today part of the genus were not included: *C. tetraspilus*, *C. atriventris* (Coquillett, 1902), and *C. greylockensis*.

Of the twelve valid species of *Caloparyphus* (Woodley 2001) the following types have been examined by M. Hauser: *C. currani* (James, 1939), *C. flaviventris* (James, 1932), *C. mariposa* (James, 1939), and Norm Woodley: *C. amplus* (Coquillett, 1902), *C. atriventris*, *C. crucigerus* (Coquillett, 1902), and *C. tahoensis* Coquillett, 1902 (synonym to *C. crucigerus*). Most other species have been studied using specimens from several collections and comparing with the type series of the new species. The following differences might help in distinguishing this new Palearctic species from the Nearctic species:

- C. decemmaculatus*: The scape and pedicel distinctly elongated and tergites 2–4 have central spots (this species might not belong into this genus);
- C. tetraspilus* has a black scutellum as well as four, two or no central spots on tergites 3–4 and no extended lateral markings on the tergites;
- C. crotchii* (Osten Sacken, 1877), *C. flaviventris*, *C. major* (Hine, 1901), *C. mariposa* and *C. pretiosus* (Banks, 1920) have the antennae distinctly longer than the head and vein R_4 present;
- C. greylockensis* has no or very short vittae on the mesonotum;
- C. crotchii* has the scape twice as long as the pedicel, the mesonotal vittae ending at the suture and the male has the hind metatarsus at the apex enlarged;
- C. amplus* has the wing mainly bare (especially cell d) and vein R_4 present;
- C. atriventris*, *C. currani* and *C. crucigerus* have the wing mainly bare, especially the discal cell which is devoid of microtrichia.

The species which seem to be most similar to *C. palaeartcticus* sp. n. are the members of the *crucigerus*-group (*C. crucigerus*, *C. atriventris*, *C. currani*) and within it especially *C. currani*. But the yellow coloration of the face of these two species (Figs 11–12) is distinctly different. Although the extent of the yellow coloration shows variation in other species of this genus, it is constant in all the female paratypes of *C. palaeartcticus* sp. n. and there is no other specimen known besides the female holotype for *C. currani*. The major differences next to the coloration of the head is that the apical half and most of the posterior portion of the wing in *C. palaeartcticus* sp. n. (Fig. 10) is covered with microtrichia, especially the discal cell and cell r_{4+5} , the apical part of cell br and the apical half of cell cup, with most of these areas bare in *C. currani* (Fig. 9). The dark coloration on the femora and the apical segments of the tarsi are much darker in *C. palaeartcticus* sp. n. (Figs 1, 7) than in *C. currani*.

It is remarkable that there is no other specimen of *C. currani* found so far except the holotype. The holotype might be just a large specimen of another described species in the *crucigerus*-group. The Nearctic species of the *crucigerus*-group need to be revised,

as there are several potential new species, one in southern California, and one in Canada, and the status of *C. taboensis*, which is currently a synonym of *C. crucigerus*, should be reexamined. But this is beyond the scope of this publication, in which we wanted show that the only Palearctic species is distinct from all described Nearctic taxa.

This disjunct distribution of *Caloparyphus palaearticus* sp. n. is similar to other insects found in northern Mongolia and the Russian Far East. For example, Gelhaus and Podenas (2006) in a study of crane flies (Tipuloidea) from the same Lake Hövsgöl watershed noted that those species with a disjunct distribution in northern Mongolia and the Russian Far East comprised 9.4% of the total crane fly fauna, or 8 out of 85 species found. A similar disjunction was again recently noted where a new species was found in the crane fly genus *Heterangeus* in northern Mongolia (Hentiy mountains); the genus was known previously only from the Russian Far East, Korean peninsula and Japan (Podenas, Podeniene and Gelhaus, 2014).

Acknowledgement

Our thanks are due to Daniel Song, who collected the first specimen in Mongolia while researching pollination ecology as part of the PIRE Mongolia project at the University of Pennsylvania, Philadelphia, PA, USA and provided the habitat photo. We also thank our friends André van Eck (Tilburg, The Netherlands) for donating his interesting Mongolian Stratiomyidae collection to CSCA, which included the only known females of the new species and Valeri Mutin (Komsomolsk-on-Amur, Russia) for the gift of the only known male from Kamchatka. Ms. Bolortsetseg Erdene (Drexel University, Philadelphia) helped with the Mongolian locality information. The following curators are thanked for making their *Caloparyphus* material available: Jeff Skevington (Ontario, Canada), David Grimaldi (New York, NY), Steve Heydon (Davis, CA), Brian Brown, Giar-Ann Kung and Weiping Xie (Los Angeles, CA), Peter Oboyski (Berkeley, CA), and Derek Sikes (Fairbanks, AK). We are very grateful also to Norman Woodley (Washington DC), who kindly compared the holotype of the new species with other species of *Caloparyphus* deposited in the USNM and was the first to remark on the unique distribution of microtrichia on the wing membrane as a conspicuous distinguishing character. The line drawings were prepared by Jaromír Vaňhara (Department of Botany and Zoology, Masaryk University, Brno).

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Amphibians and Reptiles of the state of Nuevo León, Mexico

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Abstract

We compiled a check list of the herpetofauna of Nuevo León. We documented 132 species (23 amphibians, 109 reptiles), representing 30 families (11 amphibians, 19 reptiles) and 73 genera (17 amphibians, 56 reptiles). Only two species are endemic to Nuevo León. Nuevo León contains a relatively high richness of lizards in the genus *Sceloporus*. Overlap in the herpetofauna of Nuevo León and states it borders is fairly extensive. Of 130 native species, 102 are considered species of Least Concern in the IUCN red list, four are listed as Vulnerable, five are listed as Near Threatened, and four are listed as Endangered. According to SEMARNAT, 78 species are not of conservation concern, 25 are subject to Special Protection, 27 are Threatened, and none are listed as in Danger of Extinction. Given current threats to the herpetofauna, additional efforts to understand the ecology and status of populations in Nuevo León are needed.

Keywords

Checklist, Conservation status, Herpetofauna, IUCN Red List

Introduction

The flora and fauna of Nuevo León is very species rich. Broadly speaking, it consists mainly of a group of species characteristic of the great deserts of North America, as well as species from the temperate forests of the Sierra Madre Oriental and subtropical species that extend their distribution northward, in some cases even from Central or South America, through the lowlands of the Atlantic slope. Despite these characteristics, there have been few studies on the diversity and distribution of the species of amphibians and reptiles in the state and those that have been conducted have focused almost entirely on the forests of the Sierra Madre Oriental and satellite mountains to the north and northeast of the city of Monterrey (Valdez-Tamez et al. 2003, Lazcano et al. 2004, 2007, 2009a, Contreras-Lozano et al. 2007, 2010, 2011a, 2012, Lemos-Espinal and Cruz 2015). The vast plains to the east of the Sierra Madre Oriental as well as the portion of the Mexican Plateau in the southwestern corner of the state remain relatively unstudied. Currently there is no systematic program to study the state's herpetofauna across different regions. Consequently, one of the main threats to the conservation of the diversity of amphibians and reptiles of the state is a lack of knowledge. Coupling this lack of knowledge of the herpetofauna of the state with problems associated with the high demand for water, energy, and food to meet the increasing needs of one of the largest and fastest growing cities in Mexico (Monterrey) does not suggest an encouraging outlook for the future of the herpetofauna of Nuevo León. To help increase the awareness of the herpetofaunal richness of Nuevo León, we gathered information on the presence of amphibian and reptile species. In addition, given the potential for increased impacts of humans on the environment, we also gathered information on the conservation status of these species. Our goal is to provide a readily accessible compilation of the herpetofaunal species and their conservation status, and to expand upon previous statewide checklists (e.g., Lemos-Espinal and Cruz 2015).

Thomas H. Webb made the earliest herpetological collections in Nuevo León in 1852, as part of the Boundary Commission Survey (see Kellogg 1932). Since then herpetological surveys of Nuevo León have been conducted with varying coverage and intensity. The work of Edward Taylor and Hobart Smith contributed greatly to increasing the knowledge of the herpetofauna of the state (e.g., Smith 1934, 1942, 1944, 1951, Taylor 1939, 1940, 1941, Taylor and Smith 1945, Smith and Hall 1974). More recently, several papers, distributional records, and natural history notes have been published documenting range extensions (e.g., Salmon et al. 2001, 2004, Price et al. 2010, Price and Lazcano-Villareal 2010, Banda-Leal et al. 2002, 2014a), behavior (Contreras-Lozano et al. 2011b), body size (Banda et al. 2005, Lazcano and Bryson 2010), parasites (García-de la Peña et al. 2004, 2005, León-Regagnon et al. 2005), morphological anomalies (Chávez-Cisneros and Lazcano 2012), diet (Castañeda et al. 2005, Lazcano et al. 2006a, 2011a, Banda-Leal et al. 2014b), sexual dimorphism (García-Bastida et al. 2013), captive husbandry (Lazcano et al. 2011b), and mortality (Lazcano et al. 2006b, 2008, 2009b, Castañeda et al. 2006, Chávez Cisneros et al. 2010).

Materials and methods

Study site

The State of Nuevo León is found in northeastern Mexico (98°26' to 101°14'W, 23°11' to 27°49'N). It shares its borders with the U.S. state of Texas and the Mexican states of Coahuila, Tamaulipas, San Luis Potosí, and Zacatecas (Fig. 1). The area of the state is 64,220 km², with an elevational range of 50 to > 3,710 m above sea level (INEGI 2010). The capital, Monterrey, forms a large metropolitan area that contains approximately 88% of the population of Nuevo León, with more than four million inhabitants (INEGI 2010). Nuevo León has an extensive road network that runs throughout most of the state (INEGI 2010).

Three topographical regions can be identified in Nuevo León (Fig. 1; see also Lemos-Espinal and Cruz 2015 for more details). The first region (area = 23,138 km²; 36% of the state's surface) is a relatively flat area with a series of small, low, scattered hills (50 to 250 m above sea level) that occurs in the central, eastern, northern, and northwestern parts of the state (Alanís-Flores et al. 1996). This region includes areas that are part of the Great Plains of North America.

The second region is the Sierra Madre Oriental, located mainly in the western portion of Nuevo León (Alanís-Flores et al. 1996). The Sierra Madre Oriental divides the low-elevation plains of the central, eastern, and northwestern parts of the state from the Mexican Plateau in the southwestern corner of the state. The Sierra Madre Oriental consists of mountain ranges that average 2000 m above sea level; with their elevation generally decreasing from south to north. The Sierra Madre Oriental is the wettest region. Near Monterrey the Sierra Madre Oriental is interrupted by valleys that create an archipelago of mountain islands (elevation not exceeding 1550 m above sea level) in the midst of arid and semiarid valleys.

The Mexican Plateau is the third region in Nuevo León, and is found in the southwestern corner of the state (Alanís-Flores et al. 1996). The altitude varies between 1500 and 2000 m, and in general is a flat and arid-semiarid area (Alanís-Flores et al. 1996, Velazco-Macías et al. 2008, Velazco-Macías 2009, INEGI 2010, Lemos-Espinal and Cruz 2015).

For most of Nuevo León, the climate is very hot, with precipitation generally not exceeding 500 mm annually (Alanís-Flores et al. 1996). Rains occur in the summer or infrequently throughout the year. The Mexican Plateau in the southwestern part of the state is also hot and very dry with annual rainfall < 200 mm. A temperate sub-humid climate is found in the Sierra Madre Oriental (Alanís-Flores et al. 1996). Here the temperature is milder (18°C–20°C) and average annual precipitation ranges from 600 to 900 mm. At high elevations (> 3000 m), alpine and subalpine climates are found. The Coastal Plain of the Gulf in the central part of the state includes tropical, sub-hot, and subhumid climates, receiving an intermediate amount of precipitation (Alanís-Flores et al. 1996).

Six vegetation types are found in Nuevo León: Chihuahuan Desert Scrub; Tamaulipan Thorn Scrub; Submontane Scrub; Montane Forest; Grassland; and Ri-

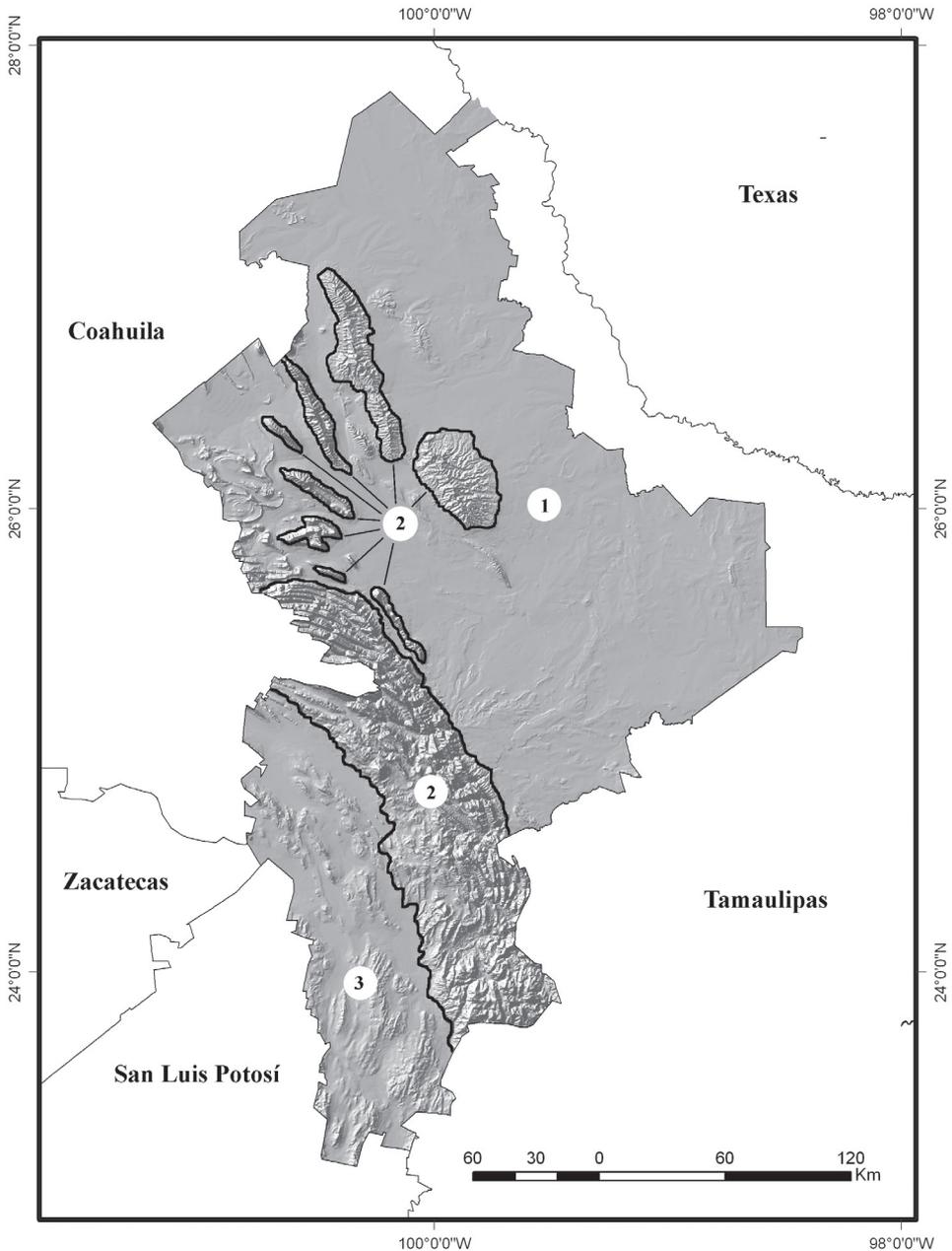


Figure 1. Topographical map of the state of Nuevo León, Mexico: **1** Flat Region **2** Sierra Madre Oriental, and **3** Mexican Plateau (INEGI 2001).

parian, Subaquatic, and Aquatic Vegetation, as well as 11 plant communities corresponding to three floristic provinces: the Mexican Plateau, the Coastal Plain of the Northeast, and the Sierra Madre Oriental (Rzedowski 1978).

Data collection

We obtained the list of amphibians and reptiles of the state of Nuevo León from the following sources: (1) specimens in the collections of the Laboratorio de Ecología-UBIPRO (LEUBIPRO); (2) databases from the Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (National Commission for the Understanding and Use of Biodiversity; CONABIO), that were the results of various scientific projects undertaken by this institution in Nuevo León and also includes records from the following 28 collections: Colección de Vertebrados, Instituto de Investigaciones de Zonas Desérticas, Universidad Autónoma de San Luis Potosí (IIZD); Colección de Vertebrados, Universidad Autónoma de Baja California (CMMEX); Colección Herpetológica Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León (UANL); Colección Herpetológica, Departamento de Zoología, Escuela Nacional de Ciencias Biológicas (ENCB); Colección Herpetológica, Museo de Zoología “Alfonso L. Herrera”, Facultad de Ciencias UNAM (MZFC-UNAM); Colección Nacional de Anfibios y Reptiles, Instituto de Biología UNAM (CNAR); Amphibians and Reptiles Collection, University of Arizona (UAZ); Collection of Herpetology, Amphibians and Reptiles Section, Carnegie Museum of Natural History, Pittsburgh; Collection of Herpetology, Biology Department, Tulane University, New Orleans (TU); Collection of Herpetology, Department of Vertebrate Zoology, National Museum of Natural History, Smithsonian Institution (USNM); Collection of Herpetology, Herpetology Department (California Academy of Sciences); Collection of Herpetology, Herpetology Department, American Museum of Natural History (AMNH); Collection of Herpetology, Herpetology Department, California Academy of Sciences (CAS); Collection of Herpetology, Museum of Comparative Zoology, Harvard University Cambridge (MCZ); Collection of Herpetology, Museum of Vertebrate Zoology, Division of Biological Sciences, University of California Berkeley (MVZ); Collection of Herpetology, Museum of Zoology, University of Michigan Ann Arbor (UMMZ); Collection of Herpetology, Texas Cooperative Wildlife Collection, Texas A&M University (TCWC); Collection of Herpetology, Texas Natural History Collection, University of Texas Austin (TNHC); Collection of Herpetology, University of Colorado Museum (UCM); Collection of Herpetology, University of Illinois Museum of Natural History (UIMNH); Division of Amphibians and Reptiles, Field Museum of Natural History (FMNH); Ernest A. Liner Collection of Herpetology (EALCH); Fort Worth Museum of Sciences and History (FWMSH); Herpetology Section, Natural History Museum of Los Angeles County (LACM); Louisiana State University, Museum of Life Sciences; Merriam Museum, University of Texas Arlington (UTAMM); Museum of Natural History, Division of Herpetology, Kansas University (MNHUK); University of Nebraska (UNO); (3) a thorough examination of the available literature on amphibians and reptiles in the state such as: Liner (1964, 1966a,b, 1991a,b, 1992a,b, 1993, 1994a,b, 1996a,b), Liner and Chaney (1986, 1987, 1990a,b, 1995a,b), Liner and Dixon (1992, 1994), Price et al. (2010), Price and Lazcano-Villarreal (2010), Rabb (1956), among others; and (4) our personal field work, primarily focused around the extreme western part of

the state on the state line between Coahuila and Nuevo León. We visited this region periodically from 2002 to 2014, taking notes on the amphibians and reptiles observed during visual encounter surveys. Taxonomy and Standard English names used here are those found in Lemos-Espinal (2015). Each species was assigned to one of three floristic provinces present in Nuevo León: the Mexican Plateau, the Coastal Plain of the Northeast, and the Sierra Madre Oriental (Rzedowski 1978).

Species were included in the check list only if we were able to confirm the record, either by direct observation or through documented museum records or vouchers in the state. Species with a questionable distribution in Nuevo León, or those that are mentioned in the literature without documented support of their presence in the state were not included in our list. In addition, we recorded the conservation status of each species based on three sources: 1) the IUCN Red List 2014; 2) Environmental Viability Scores from Wilson et al. (2013a,b); 3) listing in SEMARNAT (2010). For those neighboring states for which a recent checklist exists (Coahuila: Lemos-Espinal and Smith 2007; San Luis Potosí: Lemos-Espinal and Dixon 2013; Tamaulipas: Farr 2015; and Texas: Dixon 2015), we determined the number of overlapping species.

Results

The herpetofauna of Nuevo León includes a total of 132 species: 23 amphibians (three salamanders, 20 anurans) and 109 reptiles (six turtles, 42 lizards, 61 snakes) (Table 1; see also Lemos-Espinal and Cruz 2015). These represent 30 families: 11 of amphibians (two of salamanders and nine of frogs), and 19 of reptiles (four of turtles, eight of lizards and seven of snakes), and 73 genera: 17 of amphibians (three of salamanders and 14 of frogs), and 56 of reptiles (five of turtles, 14 of lizards and 37 of snakes) (Table 1).

Of the 132 species we documented, two are not native to Nuevo León: the Mediterranean House Gecko (*Hemidactylus turcicus*; see Rödder and Lötters 2009), and the Brahminy Blindsnake (*Indotyphlops braminus*; see Servoss et al. 2013). Thirty-four of these 132 species are endemic to Mexico, only two, the Pygmy Alligator Lizard (*Gerrhonotus parvus*) and the Nuevo León Graceful Brown Snake (*Rhadinaea montana*), are endemic to Nuevo León, where they are found in the montane forest.

Seventeen of the 34 endemics to Mexico are limited to the highlands of the Sierra Madre Oriental (*Chiropterotriton priscus*, *Pseudoeurycea galeanae*, *Eleutherodactylus longipes*, *Ecnomiophyla miotympnum*, *Sceloporus chaneyi*, *S. minor*, *S. oberon*, *S. parvus*, *Plestiodon dice*, *Scincella silvicola*, *Lepidophyma sylvaticum*, *Pituophis deppei*, *Storeria hidalgoensis*, *Rena myopica*, *Thamnophis exsul*, *Agkistrodon taylori*, and *Crotalus totonacus*). Five of these species have a narrow distribution in southeastern – eastern Coahuila and adjacent Nuevo León (*C. priscus*, *P. galeanae*, *S. oberon*, *T. exsul*), and even Tamaulipas (*P. dicei*). One more is limited to Nuevo León and adjacent Tamaulipas (*S. chaneyi*), and another 10 range from Nuevo León and Tamaulipas southward to southern Veracruz and northern Oaxaca, mainly on the Atlantic slopes of the Sierra Madre Oriental (*E. longipes*, *E. miotympnum*, *S. minor*, *S. parvus*, *S. silvicola*, *L. sylvaticum*, *S. hidalgoensis*,

Table 1. Checklist of amphibians and reptiles of Nuevo León. We also provide the Habitat type (CD = Chihuahuan Desert, SM = Sierra Madre Oriental, TS = Tamaulipan Thornscrub), IUCN Status (DD = Data Deficient; LC = Least Concern, V = Vulnerable, NT = Near Threatened; E = Endangered; CE = Critically Endangered), and Environmental Vulnerability Score (EVS; the higher the score the greater the vulnerability) from Wilson et al. (2013a,b), and conservation status in Mexico according to SEMARNAT (2010) (P = in danger of extinction, A = threatened; Pr = subject to special protection, NL – not listed). Source denotes whether the species was observed in the field by the authors (A), documented in the CONABIO data base and/or museum collections (C/M), or found in the literature (citation of source).

	Habitat Type	IUCN Status	EVS Score	SEMARNAT listing	Source
Class Amphibia					
Order Caudata					
Family Ambystomatidae					
<i>Ambystoma mavortium</i> Baird	CD	?	10	NL	Reese (1971)
Family Plethodontidae					
<i>Chiropterotriton priscus</i> Rabb	SM	?	16	Pr	Rabb (1956)
<i>Pseudoeurycea galeanae</i> Taylor	SM	NT	18	A	Taylor (1941)
Order Anura					
Family Bufonidae					
<i>Anaxyrus cognatus</i> (Say)	CD	LC	9	NL	A
<i>Anaxyrus debilis</i> (Girard)	CD	LC	7	Pr	A
<i>Anaxyrus punctatus</i> (Baird & Girard)	CD	LC	5	NL	A
<i>Anaxyrus speciosus</i> (Girard)	CD	LC	12	NL	A
<i>Incilius nebulifer</i> (Girard)	TS	LC	6	NL	A
<i>Rhinella marina</i> (Linnaeus)	TS, SM	LC	3	NL	C/M
Family Craugastoridae					
<i>Craugastor augusti</i> (Dugès)	SM	LC	8	NL	C/M
Family Eleutherodactylidae					
<i>Eleutherodactylus cystignathoides</i> (Cope)	SM	LC	12	NL	C/M
<i>Eleutherodactylus guttillatus</i> (Cope)	SM	LC	11	NL	C/M
<i>Eleutherodactylus longipes</i> (Baird)	SM	V	15	NL	C/M
Family Hylidae					
<i>Enomiophyla miotypanum</i> (Cope)	SM	NT	9	NL	C/M
<i>Smilisca baudinii</i> (Duméril & Bibron)	SM	LC	3	NL	A
Family Leptodactylidae					
<i>Leptodactylus fragilis</i> (Brocchi)	GL, Rip (CD)	LC	5	NL	C/M
Family Microhylidae					
<i>Gastrophryne olivacea</i> (Hallowell)	CD	LC	9	Pr	A
<i>Hypopachus variolosus</i> (Cope)	SM	LC	4	NL	C/M
Family Ranidae					
<i>Lithobates berlandieri</i> (Baird)	CD	LC	7	Pr	C/M
Family Rhinophrynidae					
<i>Rhinophrynus dorsalis</i> Duméril & Bibron	TS (Tamaulipan)	LC	8	Pr	C/M
Family Scaphiopodidae					
<i>Scaphiopus couchii</i> Baird	CD	LC	3	NL	A
<i>Spea bombifrons</i> (Cope)	CD	LC	10	NL	C/M

	Habitat Type	IUCN Status	EVS Score	SEMARNAT listing	Source
<i>Spea multiplicata</i> (Cope)	CD	LC	6	NL	A
Class Reptilia					
Order Testudines					
Family Emidyidae					
<i>Pseudemys gorzugi</i> Ward	CD	NT	16	A	C/M
<i>Trachemys scripta</i> (Thusberg)	CD	LC	16	Pr	C/M
Family Kinosternidae					
<i>Kinosternon flavescens</i> (Agassiz)	CD	LC	12	NL	C/M
<i>Kinosternon integrum</i> Le Conte	Riparian CD	LC	11	Pr	C/M
Family Testudinae					
<i>Gopherus berlandieri</i> (Agassiz)	TS	LC	18	A	C/M
Family Trionychidae					
<i>Apalone spinifera</i> (Le Sueur)	CD	LC	15	Pr	C/M
Order Squamata					
Suborder Lacertilia					
Family Anguidae					
<i>Barisia ciliaris</i> (Smith)	SM	?	15	NL	A
<i>Gerrhonotus infernalis</i> Baird	SM	LC	13	NL	C/M
<i>Gerrhonotus parvus</i> (Knight & Scudday)	SM	E	17	Pr	C/M
Family Crotophytidae					
<i>Crotaphytus collaris</i> (Say)	CD	LC	13	A	C/M
<i>Crotaphytus reticulatus</i> Baird	TS	V	12	A	C/M
Family Eublepharidae					
<i>Coleonyx brevis</i> Stejneger	CD	LC	14	Pr	C/M
Family Gekkonidae					
<i>Hemidactylus turcicus</i> (Linnaeus)	CD	N/A	N/A	N/A	C/M
Family Phrynosomatidae					
<i>Cophosaurus texanus</i> Troschel	CD	LC	14	A	A
<i>Holbrookia approximans</i> Baird	CD	?	14	NL	A
<i>Holbrookia lacerata</i> Cope	CD, TS	NT	14	A	C/M
<i>Phrynosoma cornutum</i> (Harlan)	CD	LC	11	NL	A
<i>Phrynosoma modestum</i> Girard	CD	LC	12	NL	A
<i>Phrynosoma orbiculare</i> (Linnaeus)	SM	LC	12	A	A
<i>Sceloporus cautilus</i> Smith	CD	LC	15	A	C/M
<i>Sceloporus chaneyi</i> Liner & Dixon	SM	E	15	NL	C/M
<i>Sceloporus consobrinus</i> Baird & Girard	CD	?	?	NL	A
<i>Sceloporus couchii</i> Baird	CD	LC	15	NL	C/M
<i>Sceloporus cyanogenys</i> Cope	CD	?	16	NL	C/M
<i>Sceloporus cyanostictus</i> Axtell & Axtell	CD	E	13	NL	Price et al. (2010)
<i>Sceloporus goldmani</i> Smith	CD	E	15	NL	C/M
<i>Sceloporus grammicus</i> Wiegmann	SM, TS	LC	9	Pr	C/M
<i>Sceloporus merriami</i> Stejneger	CD	LC	13	NL	Price and Lazcano-Villarreal (2010)
<i>Sceloporus minor</i> Cope	SM	LC	14	NL	C/M
<i>Sceloporus oregon</i> Smith & Brown	SM	V	14	NL	A

	Habitat Type	IUCN Status	EVS Score	SEMARNAT listing	Source
<i>Sceloporus olivaceus</i> Smith	TS	LC	13	NL	C/M
<i>Sceloporus ornatus</i> Baird	CD	NT	16	A	C/M
<i>Sceloporus parvus</i> Smith	CD	LC	15	NL	C/M
<i>Sceloporus poinsettii</i> Baird & Girard	CD	LC	12	NL	A
<i>Sceloporus samcolemanni</i> Smith & Hall	Grassland CD	LC	15	NL	C/M
<i>Sceloporus serrifer</i> Cope	SM	LC	6	NL	C/M
<i>Sceloporus spinosus</i> Wiegmann	CD	LC	12	NL	C/M
<i>Sceloporus torquatus</i> Wiegmann	SM	LC	11	NL	C/M
<i>Sceloporus variabilis</i> Wiegmann	SM	LC	5	NL	C/M
<i>Uta stansburiana</i> Baird & Girard	CD	LC	11	A	A
Family Scincidae					
<i>Plestiodon dicei</i> (Ruthven & Gaige)	SM	LC	7	NL	C/M
<i>Plestiodon obsoletus</i> (Baird & Girard)	CD	LC	11	NL	C/M
<i>Plestiodon tetragrammus</i> Baird	CD	LC	12	NL	C/M
<i>Scincella silvicola</i> (Taylor)	SM	LC	12	A	C/M
Family Teiidae					
<i>Aspidoscelis gularis</i> (Baird & Girard)	CD	LC	9	NL	C/M
<i>Aspidoscelis inornata</i> (Baird)	CD	LC	14	NL	C/M
<i>Aspidoscelis marmorata</i> (Baird & Girard)	CD	?	14	NL	C/M
Family Xantusidae					
<i>Lepidophyma sylvaticum</i> Taylor	SM	LC	11	Pr	C/M
Order Squamata					
Suborder Serpentes					
Family Colubridae					
<i>Arizona elegans</i> Kennicott	CD	LC	5	NL	C/M
<i>Bogertophis subocularis</i> (Brown)	CD	LC	14	NL	C/M
<i>Coluber constrictor</i> Linnaeus	Grassland in CD & SM	LC	10	A	C/M
<i>Drymarchon melanurus</i> (Duméril, Bibron & Duméril)	SM	LC	6	NL	C/M
<i>Drymobius margaritiferus</i> (Schlegel)	SM	?	6	NL	C/M
<i>Ficimia streckeri</i> Taylor	TS	LC	12	NL	C/M
<i>Gyalopion canum</i> Cope	CD	LC	9	NL	C/M
<i>Lampropeltis alterna</i> (Brown)	CD	LC	14	A	C/M
<i>Lampropeltis getula</i> (Blainville)	CD	LC		A	C/M
<i>Lampropeltis mexicana</i> (Garman)	SM	LC	15	A	C/M
<i>Lampropeltis triangulum</i> (Lacépède)	CD	?	7	A	C/M
<i>Leptophis mexicanus</i> Duméril & Bibron	SM	LC	6	A	C/M
<i>Masticophis flagellum</i> (Shaw)	CD	LC	8	A	C/M
<i>Masticophis schotti</i> Baird & Girard	CD, TS	LC	13	NL	C/M
<i>Masticophis taeniatus</i> (Hallowell)	CD	LC	10	NL	C/
<i>Ophedrys aestivus</i> (Linnaeus)	SM	LC	13	NL	C/M
<i>Oxybelis aeneus</i> (Wagler)	SM	?	5	NL	C/M
<i>Pantherophis bairdi</i> (Yarrow)	CD	LC	15	NL	C/M
<i>Pantherophis emoryi</i> (Baird & Girard)	CD	LC	13	NL	C/M
<i>Pituophis catenifer</i> Blainville	CD	LC	9	NL	A

	Habitat Type	IUCN Status	EVS Score	SEMARNAT listing	Source
<i>Pituophis depeii</i> (Duméril)	SM	LC	14	A	C/M
<i>Rhinocheilus lecontei</i> Baird & Girard	CD	LC	8	NL	A
<i>Salvadora grahamiae</i> Baird & Girard	CD	LC	10	NL	C/M
<i>Senticolis triaspis</i> (Cope)	SM	LC	6	NL	C/M
<i>Sonora semiannulata</i> Baird & Girard	CD	LC	5	NL	C/M
<i>Tantilla atriceps</i> (Günther)	CD	LC	11	A	C/M
<i>Tantilla hobartsmithi</i> Taylor	CD	LC	11	NL	C/M
<i>Tantilla nigriceps</i> Kennicott	CD	LC	11	NL	C/M
<i>Tantilla rubra</i> Cope	SM	LC	5	Pr	C/M
<i>Tantilla wilcoxi</i> Stejneger	CD	LC	10	NL	C/M
<i>Trimorphodon tau</i> Cope	CD	LC	13	NL	C/M
Family Dipsadidae					
<i>Adelphicos newmanorum</i> Taylor	SM	LC	10	Pr	C/M
<i>Anastridium sapperi</i> (Werner)	SM	LC	10	NL	C/M
<i>Diadophis punctatus</i> (Linnaeus)	SM	LC	4	NL	C/M
<i>Heterodon kennerlyi</i> Kennicott	CD	?	11	Pr	C/M
<i>Hypsiglena jani</i> (Dugès)	CD	?	6	Pr?	C/M
<i>Leptodeira septentrionalis</i> (Kennicott)	SM	?	8	NL	C/M
<i>Rhadinaea montana</i> Smith	SM	? (E)	14	Pr (E)	Chaney and Liner (1986, 1990)
<i>Tropidodipsas sartorii</i> Cope	SM	LC	9	Pr	C/M
Family Elapidae					
<i>Micrurus tener</i> Baird & Girard	CD	LC	11	NL	C/M
Family Leptotyphlopidae					
<i>Rena dulcis</i> Baird & Girard	CD	LC	13	NL	C/M
<i>Rena myopica</i> (Garman)	SM	LC	13	NL	C/M
Family Natricidae					
<i>Nerodia erythrogaster</i> (Forster)	CD	LC	11	A	C/M
<i>Nerodia rhombifer</i> (Hallowell)	CD	LC	10	NL	C/M
<i>Storeria dekayi</i> (Holbrook)	SM	LC	7	NL	C/M
<i>Storeria hidalgensis</i> Taylor	SM	V	13	NL	C/M
<i>Thamnophis cyrtopsis</i> (Kennicott)	CD	LC	7	A	A
<i>Thamnophis eques</i> (Reuss)	SM	LC	8	A	C/M
<i>Thamnophis exsul</i> (Baird & Girard)	SM	LC	16	NL	C/M
<i>Thamnophis marcianus</i> (Baird & Girard)	CD	LC	10	A	A
<i>Thamnophis proximus</i> (Say)	SM	LC	7	A	C/M
<i>Thamnophis pulchrilatus</i> (Cope)	SM	LC	15	NL	C/M
Family Typhlopidae					
<i>Indotyphlops braminus</i> (Daudin)		N/A	N/A	N/A	Guzmán and Muñiz-Martínez (1999)

	Habitat Type	IUCN Status	EVS Score	SEMARNAT listing	Source
Family Viperidae					
<i>Aegistrodon taylori</i> Burger & Robertson	SM	LC	17	A	C/M
<i>Crotalus atrox</i> Baird & Girard	CD	LC	9	Pr	A
<i>Crotalus lepidus</i> (Kennicott)	CD	LC	12	Pr	A
<i>Crotalus molossus</i> Baird & Girard	CD	LC	8	Pr	A
<i>Crotalus pricei</i> Van Denburgh	SM	LC	14	Pr	C/M
<i>Crotalus scutulatus</i> (Kennicott)	CD	LC	11	Pr	A
<i>Crotalus totonacus</i> Gloyd & Kauffeld	SM	?	17	Pr?	C/M
<i>Sistrurus catenatus</i> (Rafinesque)	CD	LC	13	Pr	C/M

R. myopica, *A. taylori*, and *C. totonacus*). One other, *P. deppei*, occurs in the Sierra Madre Oriental, the Mexican Plateau, the Transvolcanic Belt, and the Sierra Madre Occidental.

Two of the remaining 15 Mexican endemic species are limited to Coahuila and extreme western Nuevo León (*Sceloporus cyanostictus* and *Sceloporus ornatus*). Two more species are limited to scattered regions of northern Mexico: *Sceloporus couchii* to the northern Sierras of Coahuila and central western Nuevo León; and *Sceloporus goldmani* to a small area in southeastern Coahuila, adjacent Nuevo León and northeastern San Luis Potosí. Three more species endemic to Mexico are limited to the Mexican Plateau (*Sceloporus cautilus*, *S. samcolemani*, and *Lampropeltis mexicana*). Four additional species (*Kinosternon integrum*, *Sceloporus spinosus*, *S. torquatus*, and *Trimorphodon tau*) are widely distributed from central Mexico through the Mexican Plateau, and in some cases (*T. tau*) on both coasts. One species has disjunct populations in the highlands of Mexico (*Thamnophis pulchrilatus*), and another is limited to the Chihuahuan Desert of Mexico (*Holbrookia approximans*). The other two species endemic to Mexico (*Barisia imbricata* and *Phrynosoma orbiculare*), occur on the Sierra Madre Occidental and the Sierra Madre Oriental; one of them, *P. orbiculare*, ranges even into the mountains of the Transvolcanic Belt of the central part of the country.

Of the 130 native species of amphibians and reptiles in Nuevo León, 102 are considered species of Least Concern in the IUCN red list (18 amphibians, 84 reptiles), four species (1 amphibian, 3 reptiles) are listed as Vulnerable, five species (2 amphibians, 3 reptiles) are listed as Near Threatened, and four species (0 amphibians, 4 reptiles) are listed as Endangered (IUCN, 2015). Also, according to SEMARNAT listing (SEMARNAT 2010), 78 species are not listed (i.e., not of conservation concern; 17 amphibians, 61 reptiles), 25 species are Subject to Special Protection (5 amphibians, 20 reptiles), 27 species are Threatened (1 amphibian, 26 reptiles), and no species are listed as in Danger of Extinction. Several taxa are also at risk according to the EVS values. In particular, plethodontid salamanders, turtles in the families Emydidae and Testudinae, and anguid lizards have high EVS values, suggesting they may be of particular concern.

Table 2. Summary of species present in Nuevo León by Family, Order or Suborder, and Class. Status summary indicates the number of species found in each IUCN conservation status in the Order DD, LC, V, NT, E, CE (see Table 1 for abbreviations; in some cases species have not been assigned a status by the IUCN and therefore these might not add up to the total number of species in a taxon). Mean EVS is the mean Environmental Vulnerability Score, scores ≥ 14 are considered high vulnerability (Wilson et al. 2013a,b) and conservation status in Mexico according to SEMARNAT (2010) in the Order NL, Pr, A, P (see Table 1 for abbreviations).

Class	Order/ Suborder	Family	Genera	Species	Status Summary	Mean EVS	SEMARNAT	
Amphibia	Caudata		3	3	0,0,0,1,0,0	14.7	1,1,1,0	
		Ambystomatidae	1	1	0,0,0,0,0,0	10	1,0,0,0	
			Plethodontidae	2	2	0,0,0,1,0,0	17	0,1,1,0
		Anura		14	20	0,18,1,1,0,0	7.6	16,4,0,0
			Bufo	3	6	0,6,0,0,0,0	7	5,1,0,0
			Craugastoridae	1	1	0,1,0,0,0,0	8	1,0,0,0
			Eleutherodactylidae	1	3	0,2,1,0,0,0	12.7	3,0,0,0
			Hylidae	2	2	0,1,0,1,0,0	6	2,0,0,0
			Leptodactylidae	1	1	0,1,0,0,0,0	5	1,0,0,0
			Microhylidae	2	2	0,2,0,0,0,0	6.5	1,1,0,0
			Ranidae	1	1	0,1,0,0,0,0	7	0,1,0,0
			Rhynophrynidae	1	1	0,1,0,0,0,0	8	0,1,0,0
			Scaphiopodidae	2	3	0,3,0,0,0,0	6.3	3,0,0,0
		Subtotal		17	23	0,18,1,2,0,0	8.5	17,5,1,0
Reptilia	Testudines		5	6	0,5,0,1,0,0	14.7	1,3,2,0	
		Emydidae	2	2	0,1,0,1,0,0	16	0,1,1,0	
		Kinosternidae	1	2	0,2,0,0,0,0	11.5	1,1,0,0	
		Testudinae	1	1	0,1,0,0,0,0	18	0,0,1,0	
		Trionychidae	1	1	0,1,0,0,0,0	15	0,1,0,0	
		Squamata		51	103	0,79,3,2,4,0	11.3	60,17,24,0
		Lacertilia		14	42	0,28,2,2,4,0	12.7	28,4,9,0
			Anguillidae	2	3	0,1,0,0,1,0	15	2,1,0,0
			Crotaphytidae	1	2	0,1,1,0,0,0	12.5	0,0,2,0
			Eublepharidae	1	1	0,1,0,0,0,0	14	0,1,0,0
			Gekkonidae	1	1	--	--	--
			Phrynosomatidae	5	27	0,18,1,2,3,0	12.8	20,1,6,0
			Scincidae	2	4	0,4,0,0,0,0	10.5	3,0,1,0
			Teiidae	1	3	0,2,0,0,0,0	12.3	3,0,0,0
			Xantusidae	1	1	0,1,0,0,0,0	11	0,1,0,0
		Serpentes		37	61	0,51,1,0,0,0	10.3	32,13,15,0
			Colubridae	20	31	0,28,0,0,0,0	9.5	21,1,9,0
			Dipsadidae	8	8	0,4,0,0,0,0	9	3,5,0,0
			Elapidae	1	1	0,1,0,0,0,0	11	1,0,0,0
			Leptotyphlopidae	1	2	0,2,0,0,0,0	13	2,0,0,0
		Natricidae	3	10	0,9,1,0,0,0	10.4	5,0,5,0	
		Typhlopidae	1	1	--	--	--	
		Viperidae	3	8	0,7,0,0,0,0	12.6	0,7,1,0	
	Subtotal		56	109	0,84,3,3,4,0	11.5	61,20,26,0	
TOTAL			73	132	0,102,4,5,4,0		78,25,27,0	

Discussion

Nuevo León does not have a particularly large number of amphibian and reptile species, at least compared to some other states in Mexico (Ranges: amphibians 5 – 140, mean \pm 1 S.E. = 36.0 ± 5.1 ; reptiles 31 – 263, mean \pm 1 S.E. = 105.4 ± 8.9 ; Total 47 – 403, mean \pm 1 S.E. = 141.5 ± 13.7 ; see Flores-Villela and García-Vázquez 2014, Parra-Olea et al. 2014). There are relatively few species endemic to Nuevo León (only two). However, Nuevo León has a relatively high richness of lizards in the genus *Sceloporus* (N = 20, 15.3% of all species of amphibians and reptiles found in Nuevo León).

The amount of overlap in the herpetofauna of Nuevo León and the states it borders is fairly extensive. The greatest overlap is with Tamaulipas, with the two states sharing 82.3% of Nuevo León's herpetofauna, and especially its amphibians (95.7%) and to a lesser extent, its reptiles (79.4%). Nuevo León shares 79.2% of its herpetofauna with Coahuila (78.2% amphibians, 79.4% reptiles). Of Nuevo León's herpetofauna, 71.5% is shared with San Luis Potosí (78.2% amphibians, 70.1% reptiles). The state with the least overlap is Texas (overall 65.4%, amphibians 82.6%, reptiles 61.7%), which might be expected since the extent of the border is relatively small and they are separated by the Río Grande. In an analysis of the herpetofauna of the Mexican and United States Border States, including Nuevo León, Smith and Lemos-Espinal (2015) found significant similarities or clustering among Texas, Tamaulipas, Nuevo León, and Coahuila for the entire herpetofauna, with subsets of the herpetofauna (e.g., amphibians, reptiles, anurans, and lizards) showing slightly different clustering patterns; although Nuevo León frequently clustered with Tamaulipas and Coahuila. The particular similarity among Nuevo León, Tamaulipas, and Coahuila likely reflects similarities in the biotic provinces found in those states (Smith and Lemos-Espinal 2015).

The conservation status of the herpetofauna of Nuevo León has relatively fewer species listed in the IUCN red list and SEMARNAT compared to other states, at least as far as listings based on IUCN red list and SEMARNAT, as well as the EVS values provided in Wilson et al. (2013a, b). This is not to say that the herpetofauna of Nuevo León should be considered safe. Given the expanding population of Monterrey, and the potential consequences of such expansion on natural resources in the state, we must be vigilant to the threats to the herpetofauna of Nuevo León. In addition, the relative paucity of ecological studies on the amphibians and reptiles in Nuevo León mean much of our understanding of these species' status in Nuevo León is based upon information obtained in other states. We hope our summary of the species of amphibians and reptiles from Nuevo León, and their putative conservation status, will prompt further research on the ecology and status of these species in Nuevo León. Such baseline information is critical to evaluating and monitoring any changes in populations in Nuevo León that can arise due to urbanization around Monterrey and potential climate change and other threats in this area (see Biggs et al. 2010, Lavín-Murcio and Lazcano 2010, Seager and Vecchi 2010).

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A new species of *Pristimantis* (Amphibia, Anura, Craugastoridae) from the foothills of the Andes in Manu National Park, southeastern Peru

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Abstract

We describe a new species of *Pristimantis* from the humid sub-montane forest of the *Región* Cusco in Peru. *Pristimantis pluvialis* **sp. n.** was collected in the Kosñipata and Entoro valleys at elevations from 740 to 1110 m a.s.l., near the borders of Manu National Park and within the Huachiperi Haramba Queros Conservation Concession. The new species can be distinguished from other members of the genus *Pristimantis* by its rostral tubercle, smooth dorsal skin, and by its advertisement call. *Pristimantis lacrimosus* and *P. waorani* superficially most resemble the new species, but *P. pluvialis* **sp. n.** differs from both species by having a rostral tubercle (absent in *P. waorani* and variable in *P. lacrimosus*) and larger size, from *P. lacrimosus* by its call emitted at a lower frequency, and from *P. waorani* for its dorsal coloration with dark markings. Two other species have partially overlapping distributions and resemble the new species, *P. mendax* and *P. olivaceus*, but they produce advertisement calls with much higher dominant frequencies than the advertisement call of the new species. Furthermore, *P. mendax* differs from the new species by lacking a rostral tubercle and by having a sigmoid inner tarsal fold, whereas *P. olivaceus* differs by being smaller and by having dorsal skin shagreen with scattered tubercles. The new species has snout-vent length of 21.8–26.9 mm in males ($n = 12$) and 28.8 mm in a single female.

Resumen

Describimos una nueva especie de *Pristimantis* de los bosques sub-montanos de la Región Cusco, en Perú. *Pristimantis pluvialis* **sp. n.** fue colectada en los valles de Kosñipata y Entoro a altitudes entre 740 y 1110 m.s.n.m., cerca del límite del Parque Nacional del Manu y dentro de la Concesión para Conservación Huachiperi Haramba Queros. La nueva especie se diferencia de otros miembros del género *Pristimantis* por su tubérculo rostral, piel dorsal lisa, y por su canto de anuncio. Superficialmente, *Pristimantis lacrimosus* y *P. waorani* se asemejan a la nueva especie, pero *P. pluvialis* **sp. n.** difiere de ambas por tener un tubérculo dorsal (ausente en *P. waorani* y variable en *P. lacrimosus*) y mayor tamaño; además, se diferencia de *P. lacrimosus* por tener un canto de anuncio producido a frecuencias más bajas, y de *P. waorani* por su coloración dorsal con manchas oscuras. Dos especies con distribución parcialmente simpátrica se asemejan morfológicamente a la nueva especie, *P. mendax* y *P. olivaceus*. Ambas especies producen cantos de anuncio a frecuencias mucho más altas que la nueva especie. Además, *P. mendax* se distingue de *P. pluvialis* **sp. n.** por carecer de tubérculo rostral y por tener un pliegue tarsal interno sigmoideo, mientras que *P. olivaceus* se distingue por su menor tamaño, y por tener piel finamente granular con algunos tubérculos diseminados en la parte dorsal. La nueva especie tiene una longitud hocico-cloaca de 21.8–26.9 mm en machos (n = 12) y de 28.8 mm en una hembra.

Keywords

Frog, Cusco, Paucartambo, *Pristimantis pluvialis*, new species

Palabras clave

Rana, Cusco, Paucartambo, especie nueva, *Pristimantis pluvialis*

Introduction

The wet tropics are a region of incredibly high biodiversity. The combination of historical, climatic and geographic characteristics foster high speciation rates. In particular, Manu National Park and its surrounding areas have one of the highest herpetofaunal diversity in the world (Catenazzi et al. 2013). Over 155 amphibian species are known from this region, comprising over 2% of known amphibians (Catenazzi et al. 2013). Despite intensive survey efforts, new amphibian species are frequently discovered (Catenazzi et al. 2012; Chaparro et al. 2015; De la Riva et al. 2012).

Manu NP is particularly rich in members of the genus *Pristimantis* (Craugastoridae), as are other regions in the upper Amazon Basin and the eastern slopes of the Andes. This is one of the largest genera of all vertebrates, and is incredibly understudied. It contains nearly 500 species distributed throughout the New World (AmphibiaWeb 2016; Hedges et al. 2008). *Pristimantis* and most members of the Craugastoridae are primarily terrestrial and are generally assumed to be direct-developing, lacking an aquatic tadpole stage (Duellman and Lehr 2009).

A relatively recent divergence and morphological similarities among species may indicate remarkable cryptic diversity within *Pristimantis* (Ortega-Andrade et al. 2015). It can be difficult to discern new species without genetic information, particularly in Peru where this genus is diverse (Aguilar et al. 2010) and counts up to 125 species

(AmphibiaWeb 2016). However, surveys sometimes reveal species with unique morphological traits, such is the case of a new, relatively large *Pristimantis* species bearing a rostral tubercle, related to *P. lacrimosus*, that we discovered during surveys in the Kosñipata Valley near Manu NP and within the Huachiperi Haramba Queros Conservation Concession. Here we describe this new species.

Methods

The format of the diagnosis, measurements and description follows Duellman and Lehr (2009). Taxonomy follows Hedges et al. (2008), except that we followed Pyron and Wiens (2011) for family placement. Specimens were fixed in 10% formalin and preserved in 70% ethanol. Sex and maturity of specimens were determined by observing sexual characters and gonads through dissections. We measured the following variables to the nearest 0.1 mm with digital calipers under a stereomicroscope: snout–vent length (SVL), tibia length (TL), foot length (FL, distance from proximal margin of inner metatarsal tubercle to tip of Toe IV), head length (HL, from angle of jaw to tip of snout), head width (HW, at level of angle of jaw), eye diameter (ED), tympanum diameter (TY), interorbital distance (IOD), upper eyelid width (EW), internarial distance (IND), and eye–nostril distance (E–N, straight line distance between anterior corner of orbit and posterior margin of external nares). Fingers and toes are numbered preaxially to postaxially from I–IV and I–V respectively. We compared the lengths of toes III and V by adpressing both toes against Toe IV; lengths of fingers I and II were determined by adpressing the fingers against each other. Photographs taken by A. Shepack and A. Catenazzi in the field were used for descriptions of coloration in life, and have been deposited at the Calphotos online database (<http://calphotos.berkeley.edu>).

We used two sets of recordings to describe the advertisement call. Holotype, CORBIDI 16510 (SVL 24.6 mm; recording #9846 deposited at the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales, Madrid, www.fonozoo.com) was recorded at the type locality at 20:30 on 16 January 2015 ($T_{\text{air}} = 21.4\text{ }^{\circ}\text{C}$), along with several unvouchered males. We used a digital recorder (Zoom H2; WAV format, 44 KHz, 24 bit) to record these advertisement calls in 2015. In a second set of recordings, we recorded paratype MUSM 35217 at 21:00 on 2 September 1999 (SVL = 22.5 mm; $T_{\text{air}} = 20.2\text{ }^{\circ}\text{C}$; recording #9847 deposited at the Fonoteca Zoológica, Museo Nacional de Ciencias Naturales, Madrid), along with several uncaptured males, using a portable cassette recorder (Aiwa HS-F150), a small microphone, and audiotapes.

We used Raven, version 1.4 (Cornell Laboratory of Ornithology) to analyze call length, peak frequency, and calling rate. The Hamming window function for the spectrogram was set at 256 bands. We report means \pm SD. We analyzed a total of 380 calls.

We determined the phylogenetic position of the new species with respect to other *Pristimantis* species through analysis of DNA sequence data. Our analysis included the 16S rRNA mitochondrial fragment and the protein-coding gene cytochrome c oxidase subunit I (COI). We used tissue samples from specimens collected in southern Peru

(Cusco and Madre de Dios regions) to obtain DNA sequences for the new species and another undescribed *Pristimantis* from the foothills of Manu National Park (Appendix I). Additionally, we downloaded sequences from Genbank (Appendix I) of morphologically similar species (*P. bromeliaceus*, *P. galdi*, *P. mendax*, *P. mindo*, *P. moro*, *P. omeviridis*, *P. schultei*, *P. subsigillatus*) in the putative *P. lacrimosus* group (sensu Artega et al. 2013); but see (Padial et al. 2014). Sequences for several other species of *Pristimantis* that possess a rostral tubercle were not available in Genbank. We included the distantly related *Pristimantis ridens* as outgroup (Padial et al. 2014).

Extraction, amplification, and sequencing of DNA followed protocols previously used for *Pristimantis* species (Hedges et al. 2008). We used the 16SA (forward) primer (5'-3' sequence: CGCCTGTTTATCAAAAACAT) and the 16SB (reverse) primer (5'-3' sequence: CCGGTCTGAACTCAGATCACGT) to amplify 16S (Palumbi et al. 2002), and we used the dgLCO1490 (forward) primer (GGTCAACAAATCATAAAGAYATYGG) and the dgHCO2198 (reverse) primer (TAAACTTCAGGGTGACCAAARAYCA) to amplify COI (Meyer et al. 2005). We employed the following thermocycling conditions to amplify DNA using the polymerase chain reaction (PCR): 1 cycle of 96 °C/3 min; 35 cycles of 95 °C/30 s, 55 °C/45 s, 72 °C/1.5 min; 1 cycle 72 °C/7 min. We completed the cycle reactions by using the corresponding PCR primers and the BigDye Terminator 3.1 (Applied Biosystems), and obtained sequence data by running the purified reaction products in an ABI 3730 Sequence Analyzer (Applied Biosystems). We deposited the newly obtained sequences in GenBank (Appendix I).

We used Geneious R6, v. 6.1.8 (Biomatters, <http://www.geneious.com/>) to align the sequences with MAFFT, v. 7.017 (Katoh and Standley 2013) alignment program. Prior conducting phylogenetic analysis, we used PartitionFinder, v. 1.1.1 (Lanfear et al. 2012) to select the appropriate models of nucleotide evolution and determined the best partitioning scheme and substitution model for each gene with a Bayesian information criterion (BIC) to. We employed a Maximum Likelihood (ML) approach using RaxML, v. 8.2.4 (Stamatakis 2006) to infer a molecular phylogeny. We used the “f-a” function to conduct a bootstrap analysis and search for the optimal likelihood tree. Our analysis included 20 terminals and a 1064 bp alignment for the concatenated dataset. We used the GTR + Γ model of nucleotide substitution, performed 200 tree searches, and assessed node support using 1000 bootstrap replicates. Additionally, we used the R package APE (Paradis et al. 2004) to estimate uncorrected p-distances (i.e., the proportion of nucleotide sites at which any two sequences are different).

We quantified infection by *Batrachochytrium dendrobatidis* (Bd) by swabbing frogs with a synthetic dry swab (Medical Wire & Equipment, #113) using a standardized swabbing protocol. Swabs were stroked across the skin a total of 30 times: 5 strokes on each side of the abdominal midline, 5 strokes on the inner thighs of each hind leg, and 5 strokes on the foot webbing of each hind leg (total of 30 strokes/frog). We used a real-time Polymerase Chain Reaction (PCR) assay on material collected on swabs to quantify the level of infection (Boyle et al. 2004). After extraction using PrepMan Ultra, we analyzed DNA amplification in a Life Technologies StepOne Plus qPCR instrument following the protocol outlined in Hyatt et al. (2007) and Boyle et

al. (2004), except that extracts were analyzed once. We calculated ZE, the genomic equivalent for Bd zoospores by comparing the qPCR results to a set of standards, and considered any sample with $ZE > 1$ to be infected or Bd-positive.

Specimens examined are listed in Appendix II; codes of collections are: CORBIDI = Herpetology Collection, Centro de Ornitología y Biodiversidad, Lima, Peru; KU = Natural History Museum, The University of Kansas, Lawrence, Kansas, USA; MUSM = Museo de Historia Natural Universidad Nacional Mayor de San Marcos, Lima, Peru; MHNG = Muséum d'Histoire Naturelle, Genève, Switzerland; MTD = Museum für Naturkunde Dresden, Dresden, Germany; ROM = Royal Ontario Museum; USNM = National Museum of Natural History (USA).

Results

Pristimantis pluvialis sp. n.

<http://zoobank.org/2C675BB5-46BD-4481-BBBF-8D332BD0F562>

Holotype (Figs 1–3). CORBIDI 16510, an adult male from Quitacalzón, 13°01'31.80"S, 71°30'00.72"W (WGS84), 1050 m a.s.l., Distrito Kosñipata, Provincia Paucartambo, Región Cusco, Peru, collected by A. Shepack, A. Ttito, and A. Catenazzi on 16 January 2015.

Paratopotypes (Fig. 4). CORBIDI 16511, an adult female; CORBIDI 16512 and MHNC 15489–90, two adult males, collected by A. Shepack, A. Ttito, and A. Catenazzi on 16 January 2015.

Paratypes (Fig. 5). Eight adult males, all from Distrito Kosñipata: MUSM 35217 and MHNG 2607.12–13 from Río Entoro, 13°00'45"S; 71°21'44"W (WGS84), 740 m a.s.l., collected on 2 September 1999 by A. Catenazzi and R. von May; CORBIDI 11862 from near Chontachaca, 13°01'33"S, 71°29'03"W (WGS84), 930 m a.s.l., collected by A. Catenazzi on 11 August 2012; CORBIDI 17014–15 from near Chontachaca, 13°01'33"S, 71°29'05"W (WGS84), 940 m a.s.l., collected by A. Catenazzi and A. Ttito on 3 March 2016; CORBIDI 16695 from between Chontachaca and Quitacalzón, 13°01'33"S, 71°29'07"W (WGS84), 950 m a.s.l., collected by A. Catenazzi and A. Ttito on 25 January 2014; MHNG 2607.11 from near Radiochayoc, 13°02'07"S, 71°30'46"W (WGS84), 1110 m a.s.l., collected on 25 February 1999 by A. Catenazzi, J. L. Martínez Ruiz and W. Qertehuari Dariquebe.

Generic placement. We assign this species to *Pristimantis* on the basis of general morphological similarity to other members of the genus and molecular data. The genus *Pristimantis* lacks any diagnostic morphological synapomorphies (Hedges et al. 2008), but molecular phylogenetic analyses support the placement of the new species within the genus (Fig. 6).

Diagnosis. A new species of *Pristimantis* characterized by (1) skin on dorsum smooth, skin on belly areolate, discoidal and dorsolateral folds absent; (2) tympanic membrane differentiated, tympanic annulus distinct; (3) snout moderate in length,

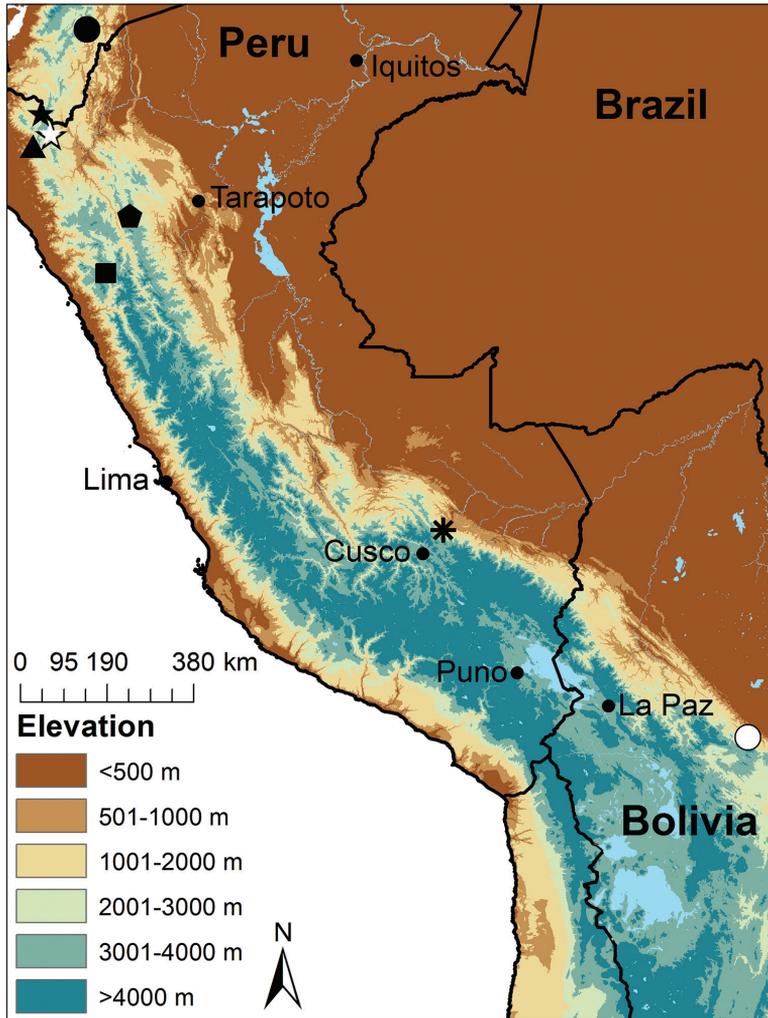


Figure 1. Map of Peru indicating the type localities of Peruvian species of *Pristimantis* known to possess a rostral tubercle or papilla: *P. proserpens* (black circle), *P. caeruleonotus* and *P. coronatus* (black star), *P. aquilonarius* (white star), *P. anemerus* (triangle), *P. corrugatus* (pentagon), *P. cordovae* (square), *P. pluvialis* sp. n. (asterisk), and *P. olivaceus* (white circle).

with small rostral tubercle, subacuminate in dorsal view and rounded in profile; (4) upper eyelid with minute conical tubercles, narrower than IOD; cranial crests absent; (5) dentigerous process of vomers barely noticeable; (6) vocal slits present; nuptial pads absent; (7) Finger I shorter than Finger II; discs broadly expanded and elliptical; (8) fingers with narrow lateral fringes; (9) single, minute ulnar tubercle present; (10) heel and tarsus lacking tubercles; (11) inner metatarsal tubercle ovoid, of higher relief and about 2.5 times the size of conical, rounded outer metatarsal tubercle; supernumerary plantar tubercles present; (12) toes with narrow lateral fringes; webbing absent; Toe V

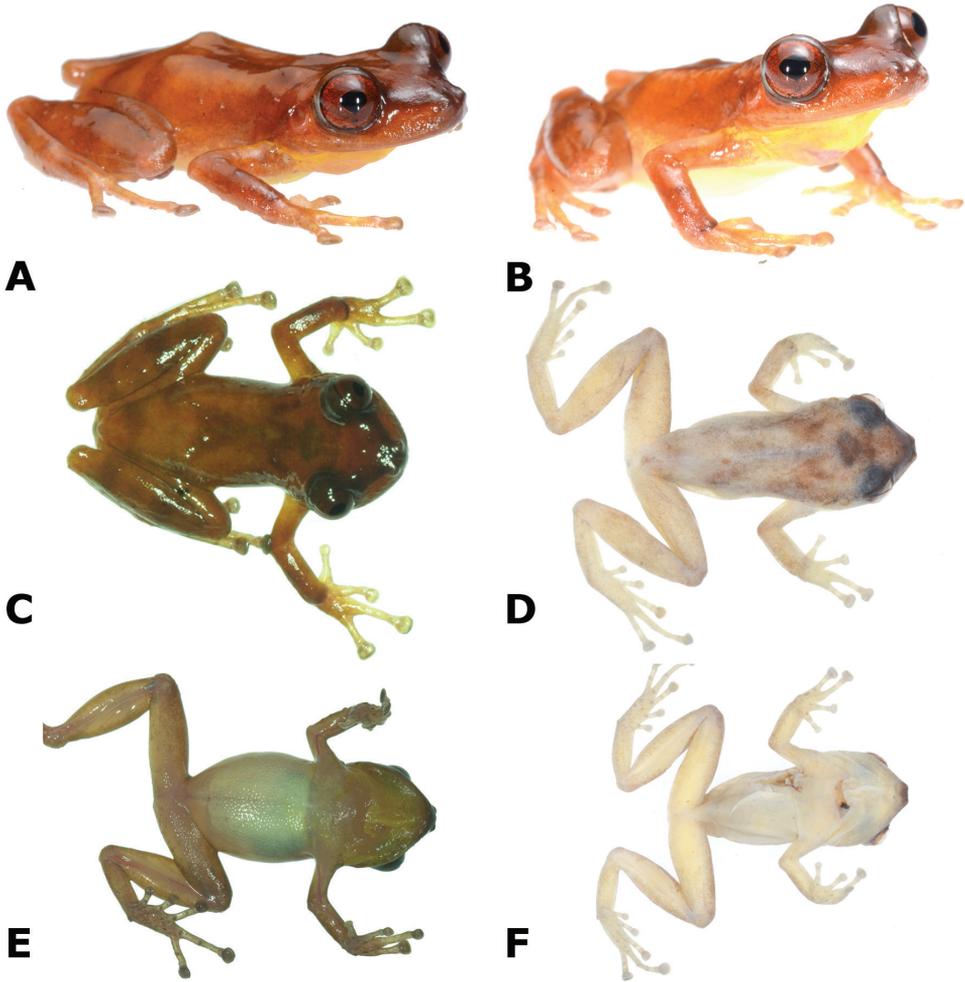


Figure 2. Holotype of *Pristimantis pluvialis* sp. n., male CORBIDI 16510 (SVL 24.6 mm) in dorsolateral view (A–B); dorsal (C–D) and ventral (E–F) views of specimen alive and fixed. Photographs by A. Shepack.

longer than Toe III; tips of digits expanded, truncate; (13) dorsum beige to reddish-brown with or without dark brown markings; interorbital bar present; venter cream; (14) SVL 21.8–26.9 mm in 12 males, 28.8 mm in one female (Table 1).

Comparisons. We tentatively assign *P. pluvialis* to the putative *P. lacrimosus* group sensu Arteaga et al. (2013) because of its smooth dorsal skin, presence of rostral tubercle, subacuminate snout profile, moderately long limbs, Finger I shorter than Finger II, expanded digital disks, and distinct tympanic membrane. Furthermore, our phylogenetic analysis (Fig. 6, Tables 2–3) supports the distinctiveness of *P. pluvialis* from other closely related taxa, including *P. bromeliaceus*, *P. galdi*, *P. cf. mendax*, *P. omeviridis*, and two undescribed species (Fig. 6). We found substantial genetic distances (uncorrected p-distances of 0.06–0.15 for 16S and 0.23–0.27 for COI; Tables 2–3)

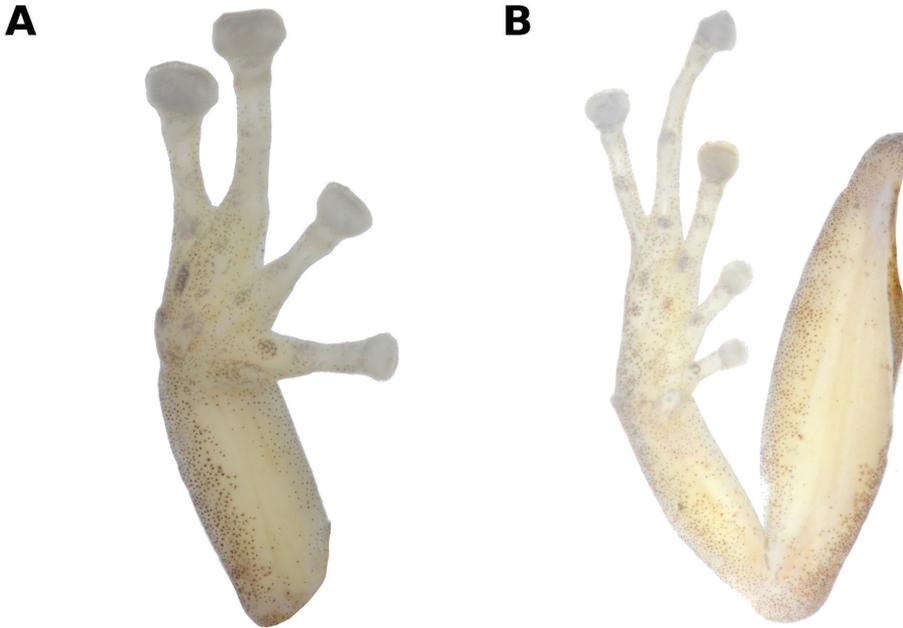


Figure 3. Ventral view of hand and foot of holotype of *Pristimantis pluvialis* sp. n., male CORBIDI 16510 (hand length 5.1 mm, foot length 9.9 mm). Photographs by A. Shepack.

Table 1. Measurements (in mm) of holotype and paratopotypes of *Pristimantis pluvialis* sp. n. from Quicalcázón, 1050 m a.s.l., Distrito Kosñipata, Provincia Paucartambo, Region Cusco, Peru.

Characters	Holotype, male	Paratopotype, male	Paratopotype, male	Paratopotype, male	Paratopotype, female
	CORBIDI 16510	MHNC 15489	MHNC 15490	CORBIDI 16512	CORBIDI 16511
SVL	24.6	22.8	23.4	23.9	28.8
Tibia length	12.9	13.1	12.9	12.8	15.0
Foot length	9.9	10.9	9.8	9.7	12.9
Head length	8.9	8.8	8.5	8.4	11.3
Head width	8.3	7.6	7.9	7.9	9.8
Interorbital distance	3.3	3.4	3.3	4.0	4.5
Upper eyelid width	2.2	2.2	1.9	1.9	2.2
Internarial distance	1.7	1.8	1.6	1.8	2.1
Eye to nostril distance	2.9	3.0	2.8	2.9	3.5
Snout to eye distance	3.8	3.9	3.7	3.5	4.8
Eye diameter	0.8	1.0	0.8	0.8	1.1
Tympanum diameter	3.0	3.2	3.3	3.3	3.7
Eye to tympanum distance	1.3	1.5	1.5	1.3	1.5
Forearm length	1.0	0.9	0.9	0.8	1.2
Hand length	5.1	5.3	4.9	5.0	6.4
Finger I length	6.6	7.0	6.7	6.4	7.6
Finger II length	2.5	3.0	2.3	2.3	3.1

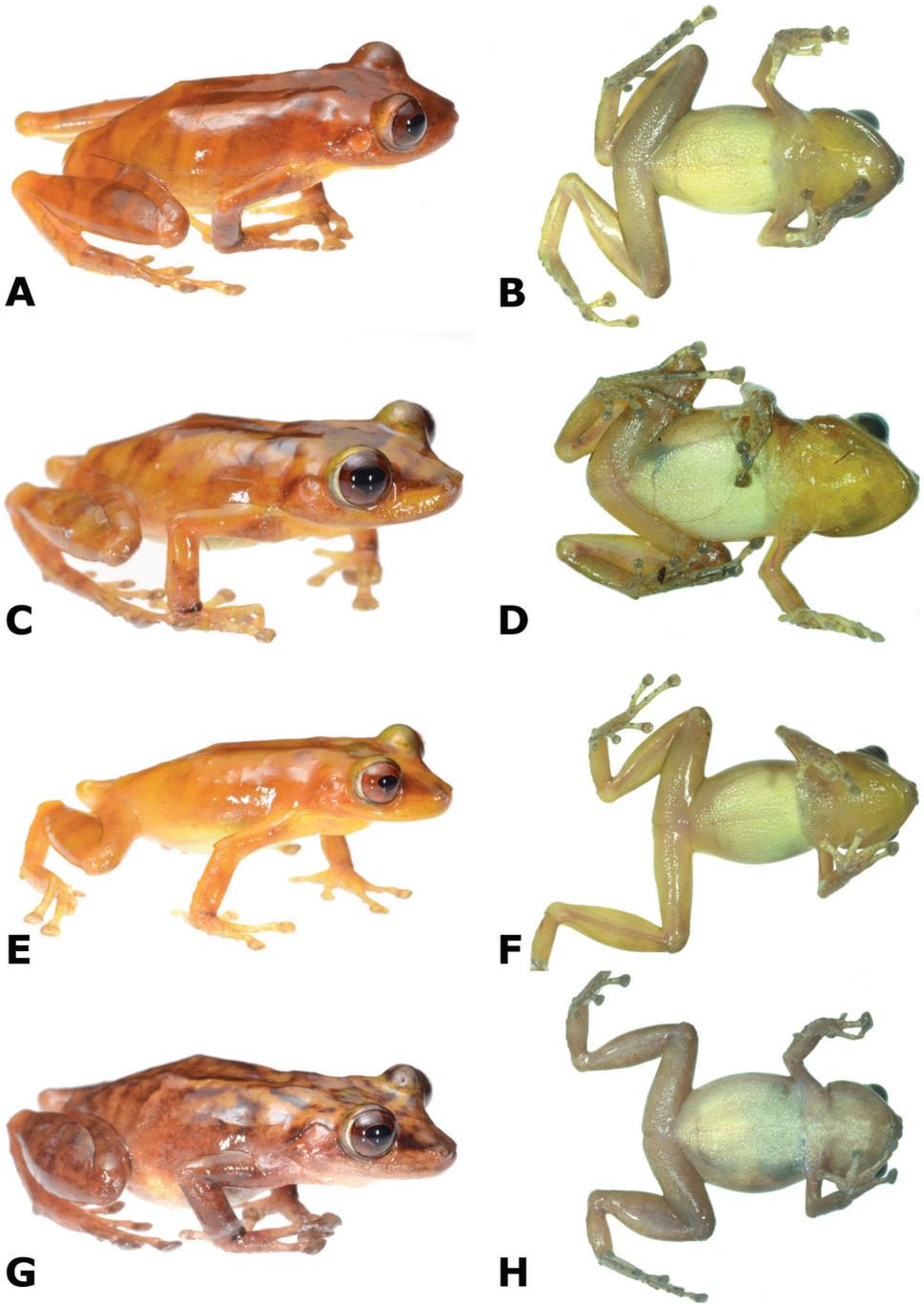


Figure 4. Dorsal and ventral views of *Pristimantis pluvialis* sp. n. paratopotypes; female CORBIDI 16511 (**A–B**); male MHNC 15490 (**C–D**); male CORBIDI 16512 (**E–F**); male MHNC 15489 (**G–H**). Photographs by A. Shepack.

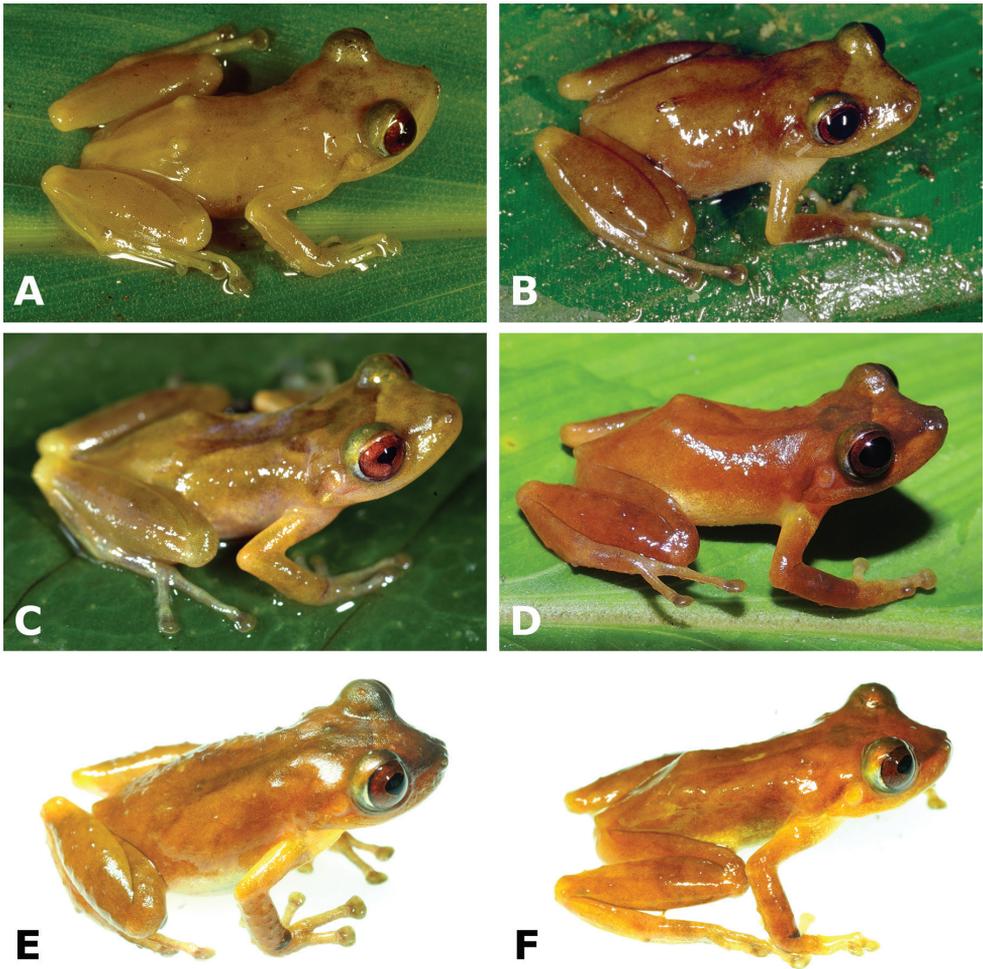


Figure 5. Dorsolateral view of six live male paratypes of *Pristimantis pluvialis* sp. n.: MHNG 2607.11 (A), MUSM 35217 (B), CORBIDI 11862 (C), CORBIDI 16695 (D), CORBIDI 17014 (E), and CORBIDI 17015 (F). Photographs by A. Catenazzi.

between *P. pluvialis* and the most closely related species for which mitochondrial sequence data were available. Given our taxon sampling, we emphasize distances for 16S. *Pristimantis pluvialis* is most closely related to two undescribed *Pristimantis*, one from near the type locality (CORBIDI 17473, 16S uncorrected p-distance: 0.06), and another from Guyana (ROM 43978, 16S uncorrected p-distance: 0.07). This species was previously identified as *P. zeuctotylus* by Hedges et al. (2008), but was treated as *Pristimantis* sp. by Padial et al. (2014). Other closely related species are *P. moro* (16S uncorrected p-distance: 0.08–0.11), *P. schultei* (0.10), *P. bromeliaceus* (0.11), and *P. mendax* (0.11).

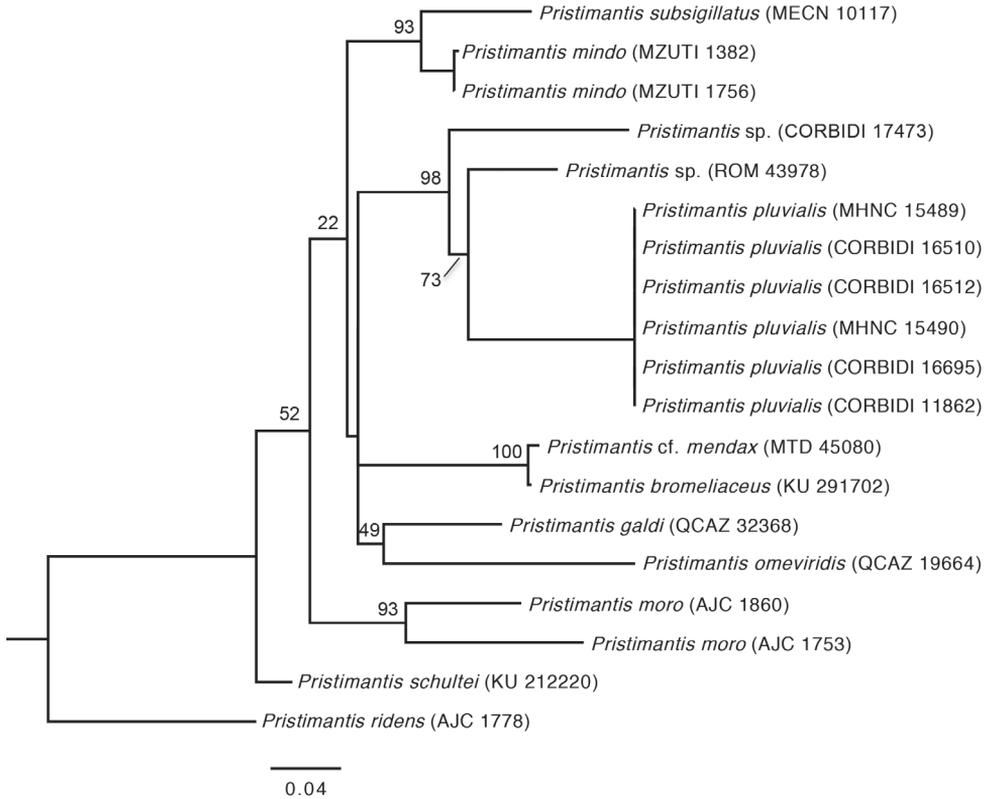


Figure 6. Maximum Likelihood (ML) phylogeny (best tree) based on the combined dataset (16S ribosomal RNA and COI genes). ML bootstrap values are indicated at each node.

The new species differs from most known Peruvian species of *Pristimantis* by having a rostral tubercle. Fewer than 20 species of Peruvian *Pristimantis* possess a rostral papilla or tubercle (Duellman and Lehr 2009): *P. acuminatus*, *P. aquilonaris*, *P. bromeliaceus*, *P. caeruleonotus*, *P. cordovae*, *P. coronatus*, *P. lacrimosus* (variable), *P. olivaceus*, *P. omeviridis*, *P. pardalinus*, and *P. proserpens*, *P. rhodostichus*, and *P. schultei*. *Pristimantis pluvialis* differs from all these species by having smooth dorsal skin and by its larger snout-vent length reaching 24.9 mm in males (except for *P. cordovae*, the largest *Pristimantis* bearing a rostral tubercle, and whose males reach 27.1 mm in SVL).

The two species that superficially most resemble *P. pluvialis* are *P. lacrimosus* and *P. waoranii*. However, *P. pluvialis* differs from both species by having a rostral tubercle (absent in *P. waoranii* and variable in *P. lacrimosus*), and by its larger size. Furthermore, it differs from *P. lacrimosus* by its call with lower dominant frequency (~2500 Hz). Calls of *P. lacrimosus* available at AmphibiaWeb Ecuador (Read 2012; Ron et al. 2016) have higher dominant frequency ranging from 3100–3273 Hz (n = 6). Furthermore, the

Table 3. Uncorrected p-distances of the protein-coding gene cytochrome c oxidase subunit I (COI). Comparisons between *P. pluvialis* and other taxa are indicated in bold.

	<i>P. moro</i> AJC1753	<i>P. moro</i> AJC1860	<i>P. pluvialis</i> CORBIDI 16695	<i>P. pluvialis</i> CORBIDI 16510	<i>P. pluvialis</i> MHNC 15489	<i>P. pluvialis</i> MHNC 15490	<i>P. pluvialis</i> CORBIDI 11862	<i>P. pluvialis</i> CORBIDI 16512	<i>Pristimantis</i> sp. CORBIDI 17473	<i>P. ridens</i> AJC1778
<i>P. moro</i> AJC1753	0.00									
<i>P. moro</i> AJC1860	0.18	0.00								
<i>P. pluvialis</i> CORBIDI 16695	0.23	0.23	0.00							
<i>P. pluvialis</i> CORBIDI 16510	0.23	0.23	0.00	0.00						
<i>P. pluvialis</i> MHNC 15489	0.23	0.23	0.00	0.00	0.00					
<i>P. pluvialis</i> MHNC 15490	0.23	0.23	0.00	0.00	0.00	0.00				
<i>P. pluvialis</i> CORBIDI 11862	0.23	0.23	0.00	0.00	0.00	0.00	0.00			
<i>P. pluvialis</i> CORBIDI 16512	0.23	0.23	0.00	0.00	0.00	0.00	0.00	0.00		
<i>Pristimantis</i> sp. CORBIDI 17473	0.26	0.24	0.23	0.23	0.23	0.23	0.23	0.23	0.00	
<i>P. ridens</i> AJC1778	0.26	0.24	0.26	0.26	0.27	0.26	0.26	0.26	0.30	0.00

new species differs from *P. waoranii* by having dark bands or markings on the dorsum (absent in *P. waoranii*). Another morphologically similar species, *P. schultei*, has an acuminate snout in dorsal view (subacuminate in *P. pluvialis*), skin on dorsum shagreen (generally smooth), and heel and outer edge of tarsus bearing many low tubercles (tubercles absent). Furthermore, *P. schultei* occurs in northern Peru at elevations above 2400 m (below 1110 m for *P. pluvialis*), and its advertisement call consists of a double note, “ping-ping” (Duellman 1990), in contrast with the single, low frequency note produced by *P. pluvialis*.

Two species related to *P. lacrimosus*, *P. mendax* and *P. olivaceus*, occur near the type locality of *P. pluvialis* in Manu NP and surrounding areas in southern Peru (Catenazzi et al. 2013; Duellman 1978b; Duellman and Lehr 2009; Köhler et al. 1998). In addition to the characters listed in the previous paragraph, *P. olivaceus* further differs from *P. pluvialis* by being smaller (17.7–22.1 mm in males, and up to 25.5 mm in females; Duellman and Lehr 2009) and by having dorsal skin shagreen with scattered tubercles and dorsal coloration brownish green or olive green. Both species produce advertisement calls with higher dominant frequencies (4000–5320 Hz; see FonoZoo recording #875 for *P. mendax*, and Köhler et al. 1998 and Márquez et al. 2002 for

P. olivaceus) than the advertisement call of the new species (~2500 Hz). *Pristimantis mendax* further differs from *P. pluvialis* by lacking a rostral tubercle, by possessing a sigmoid inner tarsal fold and by having dorsal skin shagreen with scattered spicules. Furthermore, *P. mendax* occurs in montane cloud forests above 1400 m (Duellman and Lehr 2009), an elevational distribution range that does not appear to overlap with that of *P. pluvialis*.

Description of holotype. Adult male (24.6 mm SVL); head narrower than body, its length 36.3% of SVL; head slightly longer than wide; head width 33.6% of SVL; snout short, squared in dorsal view, subtruncate in lateral view (Fig. 2); eye large, 33.9% of head length, its diameter 0.97 times its distance from the nostril; nostrils slightly protuberant, situated close to snout; canthus rostralis weakly concave in dorsal view, rounded in profile; loreal area flat; lips rounded; dorsal surface of head smooth and upper eyelids with minute tubercles; upper eyelid width 65.7% of interorbital distance; supratympanic fold absent; tympanic membrane not differentiated, tympanic annulus visible; postrictal ridges or tubercles absent. Choanae round, very small, positioned far anterior and laterally, widely separated from each other, not concealed by palatal shelf of maxilla; dentigerous processes of vomer and vomerine teeth barely noticeable.

Skin on dorsum smooth; no dorsolateral folds; skin on flanks smooth; skin on ventral surfaces and gular regions areolate; pectoral and discoidal folds absent; cloacal sheath absent, cloaca not protuberant; cloacal region lacking tubercles. Ulnar tubercle present, minute; palmar tubercle flat and bifurcate, its inner lobe much larger than outer lobe; palmar tubercle approximately twice the size of elongate, thenar tubercle; supernumerary palmar tubercles present; subarticular tubercles prominent, ovoid in ventral view, rounded in lateral view; fingers with narrow lateral fringes; fingers length when adpressed, $3 > 4 > 2 > 1$ (Fig. 3); tips of digits broadly expanded and elliptical, pads with well-defined circumferential grooves (Fig. 3); forearm without tubercles.

Tibia length 52.5% of SVL; foot length 40.3% of SVL; upper and posterior surfaces of hindlimbs smooth; heel without tubercles; outer surface of tarsus without tubercles; inner metatarsal tubercle ovoid, of higher relief and about 2.5 times the size of conical, rounded outer metatarsal tubercle; supernumerary plantar tubercles present; subarticular tubercles rounded, ovoid in dorsal view; toes with narrow lateral fringes, basal webbing absent; discs of toes expanded, rounded; toes with ventral pads well-defined by circumferential grooves; toe lengths, when adpressed, $4 > 5 > 3 > 2 > 1$ (Fig. 3).

Measurements of holotype and paratopotypes are provided in Table 1. The SVL of paratypes (all males) are (in mm): MUSM 35217 = 22.5, MHNG 2607.11 = 24.2, MHNG 2607.12 = 21.8, MHNG 2607.13 = 24.2, CORBIDI 11862 = 22.9, and CORBIDI 16695 = 24.9.

Coloration of holotype in life. Dorsum orange-brown with faint brown markings (Fig. 2). Interorbital bar dark brown, forming a triangular shape posteriorly; canthus rostralis dark brown; light green on upper eyelids. Triangular brown patterning on back, not extending to venter. Hind legs with broad brown barring. Forelimbs with faint brown barring. Throat yellowish-cream; venter cream.

Coloration of holotype in alcohol. Dorsal surfaces of head, body, and limbs grayish-tan, with dark brown regions around scapulae (see Fig. 2). Interorbital as a dark blotch that extends posteriorly; canthus rostralis dark brown. Dorsal surfaces of hind limbs with dark flecking. Iris dark gray. Throat pale white; chest and belly pale white to cream; ventral surfaces of thighs the same color as chest and belly; plantar and palmar surfaces and tips of digits pale, tubercles darker gray.

Variation. Coloration in life is based on field notes and photographs taken by A. Shepack and A. Catenazzi of 13 type specimens. The dorsum is beige to reddish-brown with or without faint dark-brown markings (Figs 3–4). A dark brown interorbital bar is present in most specimens (barely visible in some individuals). The iris is bronze with dark-brown to red reticulations. Some individuals possess faint brown barring on hind legs. The throat is cream to yellowish-white while the belly is predominantly cream to white. Dorsal skin is generally smooth, but CORBIDI 16695, 17014 (Fig. 5), and MHNG 2607.13, have minute, scattered tubercles, indicating that skin texture might be a variable trait. Some individuals have small tubercles on the outer edge of tarsus.

Vocalization and reproduction. Males call from grasses, shrubs, and trees in the understory of the submontane forest, during crepuscular hours and at night, conspicuously after heavy rains. Holotype CORBIDI 16510 was calling from a broad fern leaf at 150 cm above the ground, along a trail at ~30 m from a stream ($T_{\text{air}} = 21.4^{\circ}\text{C}$). The advertisement call consisted of a note 28.7 ± 0.7 ms (range 23.0–35.0 ms, $n = 20$) in duration (Fig. 7). Pulses emitted at the highest amplitude had dominant frequencies of 2412–2584 Hz (average 2489 ± 20 Hz, $n = 20$) and were located in the first half of the note (Fig. 7). The calling rate was 0.70 calls/second at a temperature of 21.4°C . Male MUSM 35217 was perched on a shrub at 2 m, near a stream ($T_{\text{air}} = 20.2^{\circ}\text{C}$), and produced single note calls 36.0 ± 0.5 ms (range 24.0–58.0 ms, $n = 102$) in duration, with dominant frequencies of 2067–2756 Hz (average 2407 ± 19 Hz, $n = 102$), at a calling rate of 0.64 calls/s. At least three unvouchered males were recorded at the type locality near the holotype ($T_{\text{air}} = 21.4^{\circ}\text{C}$). Their calls were 40.7 ± 0.2 ms (range 26.0–47.0 ms, $n = 220$) in duration, with dominant frequencies of 2067–2756 Hz (average 2586 ± 20 Hz, $n = 220$); call rate could not be determined. Similarly, several unvouchered males recorded near MUSM 35217 ($T_{\text{air}} = 20.2^{\circ}\text{C}$) produced calls with dominant frequencies of 2067–2584 Hz (average 2312 ± 13 Hz, $n = 104$), but their note durations and call rates could not be determined. Overall, the call of the new species can be described as a single “tock” note, 23–58 ms in duration, emitted at a rate of 0.64–0.70 calls/s, and with peak frequency ranging from 2312–2756 Hz. The call has amplitude and frequency modulation (Fig. 7): a short, high energy pulse with frequency decreasing from dominant frequency (~2500 Hz, see above) to ~2000 Hz is followed by a low energy pulse with frequency increasing from ~2000 Hz to ~2500 Hz.

Etymology. The name of the new species is a Latin word and refers to the high rainfall recorded at the type locality, which averages ~6 m annually, and represents the peak rainfall amount along the elevational transition from the Amazon lowlands to the Andean peaks. Furthermore, males of *P. pluvialis* typically call during or immediately after heavy rains.

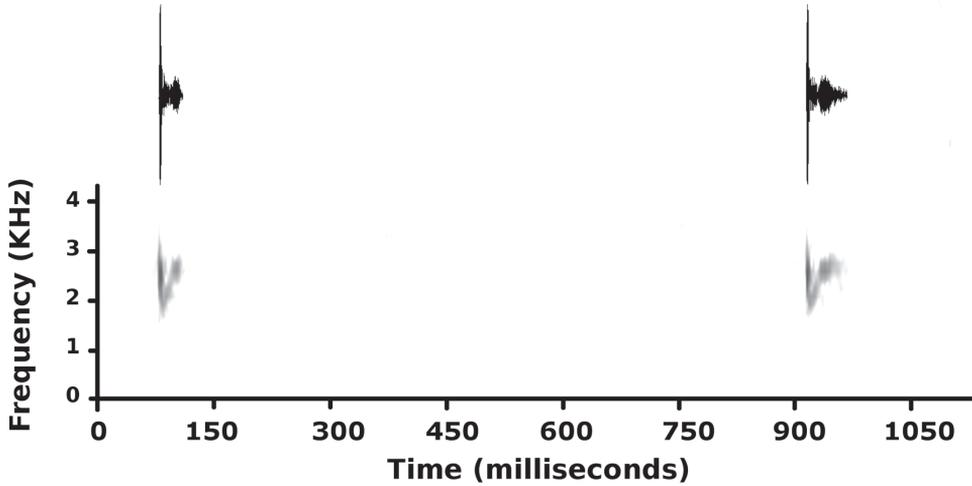


Figure 7. Advertisement call (two calls) of male CORBIDI 16510 (SVL 24.6 mm), holotype of *Pristimantis pluviialis* sp. n., recorded at the type locality on 16 January 2015 ($T_{\text{air}} = 21.4^{\circ}\text{C}$).

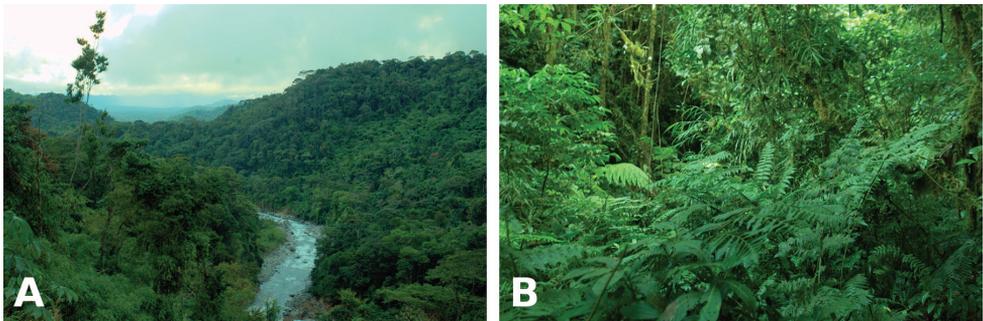


Figure 8. Habitat of *Pristimantis pluviialis* sp. n. at 1050 m a.s.l. Males and females were found on vegetation between 1–2 m above the ground.

Distribution, natural history, and threats. The new species was found during surveys in the humid sub-montane forests of the Kosñipata and Entoro valleys (Fig. 8). Observers made intensive visual searches of vegetation and leaf litter during evenings (18h30–0h00). Individuals were found after rains, calling on vegetation up to 2 m above the ground. Male CORBIDI 16511 and female CORBIDI 16512 were captured while in amplexus, during one such choruses on 16 January 2015. Average individual mass was $0.97 \text{ g} \pm 0.06$ for males ($n = 12$) and 2.1 g for one female. The oviducts of this female contained 22 unpigmented eggs, about 2.5 mm in diameter. Additionally, four out of ten individuals (MHNC 15490 and CORBIDI 11862, 16512 and 16695) tested positive for the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. This fungus has been implicated in population declines of numerous other species in this region, although it is unknown what effect it has had on this species,

and there is no evidence for declines in the populations of *P. pluvialis* (Catenazzi et al. 2011; Catenazzi et al. 2014). In addition to being found near, and likely within, Manu NP, *P. pluvialis* has been found within the Huachiperi Haramba Queros Conservation Concession, a protected area consisting of state-owned lands given in concession to private organizations with the goal of preserving biodiversity. The Huachiperi Haramba Queros concession, legally recognized in 2006 was the first concession to be granted to an indigenous community in Peru. Sympatric frog species at the type locality include *Cochranella nola*, *Hypsiboas gladiator*, *Osteocephalus mimeticus*, *Pristimantis platydactylus*, *P. reichlei*, and *Rulyrana spiculata*. Other species found around the type locality are *Allobates alessandroi*, *Ameerega simulans*, *Dendropsophus parviceps*, *Hyalinobatrachium bergeri*, *Hyloscirtus phyllognathus*, *Hypsiboas lanciformis*, *Noblella* sp., *Oreobates granulatus*, *Pristimantis danae*, *P. fenestratus*, *P. mendax*, *P. toftae*, *Ranitomeya sirensis*, *Rhinella leptoscelis*, *R. margaritifera*, *R. tacana*, *Rulyrana spiculata* and *Scinax ruber*.

Discussion

Our phylogenetic analysis indicates that *P. pluvialis* is most closely related to two undescribed *Pristimantis* species, one from the same valley near the type locality of *P. pluvialis*, and one from Guyana (Fig. 6; Padial et al. 2014). The species from Guyana (*Pristimantis* sp. ROM 43978) had previously been identified as *P. zeuctotylus* (Hedges et al. 2008). Furthermore, four other species, *P. omeviridis*, *P. galdi*, *P. mindo*, and *P. subsigillatus*, are closely related to *P. pluvialis* (Fig. 6). The most comprehensive molecular phylogenetic study of terraranas to date also found that most of these taxa form one clade (Padial et al. 2014). Nevertheless, given that there was low resolution in some nodes, both in the study by Padial et al. (2014) and this study, analyses including additional genes are needed to further examine the relationships among species in this group.

The morphologically similar *P. lacrimosus* presumably is a complex of at least two species, formed by lowland populations of small-sized individuals around the type locality in Ecuador, and larger individuals from the Andean slopes (Duellman 1978a; Lynch and Duellman 1980), which possibly form a distinct and still unrecognized species. *Pristimantis pluvialis* is larger, calls at lower frequency, and occurs at higher elevations than *P. lacrimosus*. Furthermore, the locality of the neotype of *P. lacrimosus*, collected at Santa Cecilia (Heyer and Hardy 1991), is 1600 km NW of the type locality of *P. pluvialis*, a very long distance considering the degree of endemism in montane and sub-montane *Pristimantis*. We lack sequences for *P. waorani*, but the absence of rostral tubercle and dark dorsal markings in this species differentiates it from all individuals of *P. pluvialis* in our type series.

Pristimantis pluvialis is morphologically similar to *P. olivaceus*, which has previously been reported from Manu NP (Duellman and Lehr 2009) and southeastern Peru (Köhler et al. 1998). In light of our present description, a closer examination of existing museum specimens, along with DNA sequence data and call recordings for new observations of putative *P. olivaceus* from southeastern Peru, are needed to confirm assignment of museum specimens to this species, and to properly identify newly

collected specimens. Of the characters we have mentioned in the diagnosis and comparisons, skin texture should be used with caution, because previous work shows that this trait is variable in some *Pristimantis* (Guayasamin et al. 2015), and because three of our types, despite not displaying the shagreen skin texture seen in *P. olivaceus*, differ from other smooth-skin types by having minute, scattered tubercles on dorsal surfaces.

Pristimantis pluvialis and *P. olivaceus* are most easily distinguished by their overall coloration, which is reddish-brown in *P. pluvialis* and green in *P. olivaceus*, by the larger size of *P. pluvialis* (SVL of males <22 mm in *P. olivaceus*, and >22 mm in *P. pluvialis*), and most notably by differences in their advertisement calls. Both species produce calls consisting of short, single notes, but whereas *P. pluvialis* emits “tocks” with a dominant frequency at ~2500 Hz during an initial burst followed by downward frequency modulation in the middle of the call, *P. olivaceus* emits “chirps” with a dominant frequency at ~4900 Hz with increasing frequency during the call but little amplitude modulation (Köhler et al. 1998).

With the addition of *P. pluvialis* the genus *Pristimantis* now contains 474 known species (AmphibiaWeb 2016). This cryptically diverse group surely contains even more undescribed species (Ortega-Andrade et al. 2015). We discovered *P. pluvialis* in a region where multiple researchers, including the authors, have worked previously. This suggests that continued surveying efforts are necessary to achieve a full understanding of herpetological diversity in this area (Catenazzi et al. 2013; Catenazzi and von May 2014).

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Appendix I

Gene sequences for molecular analyses

GenBank accession numbers for the taxa and genes sampled in this study. ^A*Pristimantis* sp. (ROM 43978) was previously identified as *Pristimantis zeuctotylus* by Hedges et al. (2008a), but is treated herein as *Pristimantis* sp. following Padial et al. (2014).

Taxon	Voucher Nbr.	16S	COI
<i>Pristimantis omeviridis</i>	QCAZ19664	EU130579	—
<i>Pristimantis bromeliaceus</i>	KU 291702	EF493351	—
<i>Pristimantis galdi</i>	QCAZ 32368	EU186670	—
<i>Pristimantis</i> cf. <i>mendax</i>	MTD45080	EU186659	—
<i>Pristimantis mindo</i>	MZUTI 1382	KF801584	—
<i>Pristimantis mindo</i>	MZUTI 1756	KF801581	—
<i>Pristimantis moro</i>	AJC 1860	JN991454	JN991384
<i>Pristimantis moro</i>	AJC 1753	JN991453	JN991383
<i>Pristimantis pluvialis</i> sp.n.	CORBIDI 11862	KX155577	KX155580
<i>Pristimantis pluvialis</i> sp.n.	CORBIDI 16510	—	KX155581
<i>Pristimantis pluvialis</i> sp.n.	CORBIDI 16512	—	KX155582
<i>Pristimantis pluvialis</i> sp.n.	CORBIDI 16695	KX155578	KX155583
<i>Pristimantis pluvialis</i> sp.n.	MHNC 15489	—	KX155584
<i>Pristimantis pluvialis</i> sp.n.	MHNC 15490	—	KX155585
<i>Pristimantis</i> sp.	CORBIDI 17473	KX155579	— KX159303
<i>Pristimantis ridens</i>	AJC 1778	KR863320	KR863063
<i>Pristimantis schultzei</i>	KU 212220	EF493681	—
<i>Pristimantis subsigillatus</i>	MECN 10117	KF801580	—
<i>Pristimantis</i> sp. ^A	ROM 43978	EU186678	—

Appendix II

Specimens examined

Pristimantis acuminatus (5 specimens): PERU: AMAZONAS: Quebrada Kampankis, CORBIDI 11388, 11403; CUSCO: Cashiriari-3, S of Río Camisea, USNM 537763; Pagoreni, Río Camisea, USNM 537764; San Martín-3, ~5 km N Río Camisea, USNM 537762.

Pristimantis bromeliaceus (8 specimens): PERU: AMAZONAS: Chonza Alta, Bagua, CORBIDI 778; PASCO: Comunidad Campesina Chacos, CORBIDI 3859; SAN MARTÍN: Abra Patricia, CORBIDI 510–12, 516–17; Quintecocha, MUSM 24448–49.

Pristimantis lacrimosus (10 specimens): PERU: LORETO: Sierra del Divisor, CORBIDI 3941; Río Tapiche, CORBIDI 12133–38; Campamento Piedras, Putumayo, CORBIDI 5894, 5899, 5903.

Pristimantis mendax (6 specimens): PERU: CUSCO: Paucartambo: Cusco-Pilcopata road, 1480 m., AMNH 157016; USNM 345921, 346336; MUSM 21105–07.

Pristimantis olivaceus (14 specimens): PERU: CUSCO: Cashiriari-2, ~4 km S of Río Camisea, USNM 538039–43; Cashiriari-3, S of Río Camisea, USNM 537805; Konkariari Creek Camp, Río Urubamba, USNM 538044–45; Comunidad Nativa Puyentimari, CORBIDI 8296, 9765–66, Kinteroni, CORBIDI 10260; MADRE DE DIOS: Colpa de Guacamayos, Río Tambopata, USNM 332440; Pakitza, PN Manu, USNM 342614–15.

Pristimantis rhodostichus (2 specimens): PERU: AMAZONAS: Cabeceras Katerpiza, CORBIDI 9441; LORETO: Cabeceras Wee, CORBIDI 11430.

Pristimantis schultei (21 specimens): PERU: AMAZONAS: Laguna de los Cóndores, MUSM 23040–48; ACP Huiquilla, CORBIDI 368; Yuramarca, CORBIDI 452–62.