Zoological Studies **61**:27 (2022)
doi:10.6620/ZS.2022.61-27

Zoological Studies

Open Access

A New Species of *Elpidium* (Crustacea: Ostracoda: Limnocytheridae) from Brazil and a Morphological Phylogenetic Proposal for the Genus

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Received 23 October 2021 / Accepted 13 April 2022 / Published 15 July 2022
Communicated by Ryuji Machida

Elpidium species exclusively inhabiting confined and temporary environments, such as those of tank-bromeliads, are a source of interesting and diverse studies on taxonomy, evolution and ecology, to name a few. However, despite its great diversity of species or potential for study, this genus (and other phytotelm members) has been poorly studied. In the last years, however, description of *Elpidium* species increased from six before 2013 to 11 today. This study is an effort to keep uncovering its great diversity and to go further in order to deeply understand the genus *Elpidium*. To this end, this study describes another species in the genus, *Elpidium litoreum* sp. nov., and proposes a phylogenetic reconstruction of it based on morphological characters. Our results point to the monophyly of *Elpidium* and puts *Intrepidocythere ibipora* as its sister-group. Although the phylogeny revealed some interesting relations, it also exposed some incongruities that ultimately demonstrate how superficial the current knowledge about the genus is. All these questions are discussed in detail. We see this work as at the same time an effort to better understand *Elpidium* and a stimulus to other researches to turn their attention to the historically neglected phytotelmata community.

Key words: Limnocytheridae, Taxonomy, Evolution, Bromeliad fauna, Microcrustaceans.

BACKGROUND

Ostracods are microcrustaceans with an outstanding diversity in terms of described genera and species; they also inhabit an impressive variety of environments (Martens et al. 2008; Gusakov et al. 2021). The group has experienced a shift from marine to freshwater habitats several times over its evolutionary history. One of the most successful families in freshwater environments is the Limnocytheridae, with remarkable diversification and worldwide distribution (Park et al. 2002; Martens et al. 2008).

Among limnocytherids, the genus *Elpidium* Müller, 1880 matchlessly adapted to living in bromeliad water tanks. Plants or plant structures capable of holding limited amounts of water—such as pitcher-plants, bamboos and tank-bromeliads—are called phytotelmata (Kitching 2001; Srivastava et al. 2004). *Elpidium* was first described by Müller (1880) with the publication of type-species *Elpidium bromeliarum* Müller, 1880,

Citation: Pereira JS, Rocha CEF, Pinto RL, DaSilva MB. 2022. A new species of *Elpidium* (Crustacea: Ostracoda: Limnocytheridae) from Brazil and a morphological phylogenetic proposal for the genus. Zool Stud **61**:27. doi:10.6620/ZS.2022.61-27.

from southern Brazil. Later on, Tressler (1941 1956) described *E. maricaoensis* (Tressler, 1941) and *E. laesslei* (Tressler, 1956), respectively, from Jamaica and Puerto Rico. Pinto and Sanguinetti (1962) compared the carapace morphology of several living and fossil genera of Timiriaseviinae, including *Elpidium* and proposed diagnostic features for each genus. In 1970, new material from southern Brazil allowed the proposition of a neotype and neoparatypes for *E. bromeliarum* by Pinto and Purper (1970), along with detailed redescriptions. Danielopol (1975) subsequently reported on three new species from Cuba but left them in open nomenclature. These three species were eventually formalized as *E. inaequivalve* Danielopol, 1981; *E. purperae* Danielopol, 1981; and *E. pintoi* Danielopol, 1981 (Colin and Danielopol 1981). For information about corrections in the specific epithet spellings and publication dates of the three latter species, see Meisch et al. (2019). Pinto and Jocqué (2013) also described *E. merendonense* Pinto and Jocqué, 2013 from Honduras.

Interest in the evolutionary biology of *Elpidium* was advanced mainly by Little and Hebert (1996). Based on morphological and genetic aspects of Jamaican species, these authors suggested that bromeliads act as ecological islands, favoring high speciation and endemicity in *Elpidium*. The material used by Little and Hebert (1996) received formal taxonomical treatment in two publications. Danielopol et al. (2014) described *E. martensi* Danielopol, Pinto, Gross, Pereira and Riedl, 2014 and Pereira et al. (2019) added another three descriptions, *E. littlei* Pereira, Rocha and Pinto, 2019 *E. heberti* Pereira, Rocha and Pinto, 2019 and *E. wolfi* Pereira, Rocha and Pinto, 2019. Thus, the genus currently comprises 11 valid species. However, the sampling effort for phytotelmata inhabitants is still scarce (Jocqué et al. 2013). Consequently, this figure possibly represents an underestimation of the actual *Elpidium* diversity.

In terms of ecology, in the original discovery of *Elpidium*, Müller (1880) proposed a probable phoretic behavior. Indeed, Lopez et al. (1999 2002 2005) later demonstrated that *Elpidium* species disperse between bromeliads by using amphibians as phoretic vectors.

In the present work, we describe a new species of *Elpidium* and present a morphological phylogenetic analysis of the genus. We see this study as an effort to do a more pluridisciplinary study, following the proposition of Danielopol et al. (2014) to integrae different biological aspects that can ultimately lead us to a deeper understanding of *Elpidium*.

MATERIALS AND METHODS

Specimens were collected from unidentified tank-bromeliads in Arraial do Cabo, Rio de Janeiro, Brazil. Two populations of the new species were found, one from sandy beaches in Praia Grande and another one from a rocky shore in Praia do Forno. All illustrations and type-series presented below were derived from the material collected in Praia Grande.

Water samples were collected from bromeliad tanks with the aid of a pipette and taken to the laboratory for ostracod sorting. Specimens were preserved in alcohol 90%. Adult specimens were dissected under a stereomicroscope; soft parts were mounted in permanent slides using CMC-9AF mounting medium and valves were stored dry in micropaleontological slides. Appendages were drawn under the microscope with the aid of a camera lucida. Both valves of dissected specimens and closed carapaces of males and females were photographed using scanning electron microscopy (SEM).

All material used for the description is deposited in the crustacean collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP). Higher taxonomy follows Horne et al. (2002) and Danielopol et al. (2014).

For the phylogenetic analysis, 77 morphological characters were erected (Table S1) and organized in two different matrices, one for continuous (Table S2) and another for discrete characters (Table S3). With the software Mesquite 3.6 (Maddison and Maddison 2018), the two matrices were generated and subsequently exported to a text (.txt) file. Both text files were then manually combined into one mixed matrix, composed of 16 species and 77 characters. Three of the 16 species were used as outgroup—*Cytheridella* sp., *Gomphocythere huwi* Martens, 2003 and *Thaicythere srisumonae* Savatenalinton et al., 2008—while the remaining 13 species were treated as the internal group: all species of *Elpidium* and *Intrepidocythere ibipora* Pinto et al., 2008, since this monospecific genus is morphologically very similar to *Elpidium*. Of the 77 morphological characters, 35 were extracted from the carapace and 42 from appendages, from which 11 were extracted specifically from sexual appendages. Five of these 77 morphological characters were continuous, and measurements were made with the software Zeiss Axiovision 4.8 using the holotype and allotype of each species. Whenever possible, new dissections of fresh material or available dissected specimens were used for direct verification of character states. The material used is detailed below.

In the outgroup, we directly verified the characters of *Cytheridella* from available material sampled in

Lake Paranoá, Brasíla, Brazil (taxonomy of this species will be presented elsewhere). We relied on the original descriptions for *Gomphocythere huwi* and *Thaicythere srisumonae*, since they present thorough descriptions and illustrations.

In the internal group, both the original description and direct examination of dissected specimens were used for *I. ibipora*. Within *Elpidium*, only published descriptions were used for *E. inaequivalve*, *E. purperae* and *E. pintoi* and for the type species of the genus, *E. bromeliarum*. For *E. maricaoensis*, *E. merendonense* and *E. martensi*, original descriptions and stored material were used. Concerning *E. laesslei*, *E. wolfi*, *E. littlei* and *E. heberti*, we directly examined the material sampled by Little and Hebert (1996) and re-described by Pereira et al. (2019). All the sources used for each species are fully detailed in table 1.

The combined matrix of discrete and continuous characters was analyzed with the phylogenetic software Tree analysis using New Technology (TNT) (Goloboff and Catalano 2016). The analysis was performed using the exact solution algorithm "Implicit Enumeration", applying the same weight to all characters. Characters 13, 19, 21–24, 38 and 72 were treated as additive, remaining characters were all non-additive. Characters 1 to 5 were analyzed without *a priori* discretization, following Goloboff et al. (2006). No consensus method was applied since a single most parsimonious tree was found (see RESULTS and DISCUSSION).

RESULTS

TAXONOMY

Class Ostracoda Latreille, 1802
Subclass Podocopa Sars, 1866
Order Podocopida Sars, 1866
Suborder Cytherocopina Baird, 1850
Superfamily Cytheroidea Baird, 1850
Family Limnocytheridae Sars, 1925
Subfamily Timiriaseviinae Mandelstam, 1960
Tribe Timiriaseviini Mandelstam, 1960

Genus *Elpidium* Müller, 1880

Type species. *Elpidium bromeliarum* Müller, 1880.

Table 1. Source of information used for each species for the character list and matrix

Taxa	Source of information
Gomphocythere huwi	Original description (Martens 2003).
Thaicythere sirsumonae	Original description (Savatenalinton et al. 2008).
Cytheridella sp.	Sample available (collected by Ricardo L. Pinto from Lake Paranoá, Brasília, Brazil).
Intrepidocythere ibipora	Original description (Pinto et al. 2008). Material stored at Museu de Zoologia da Universidade de São Paulo (MZUSP 18479, MZUSP 18480).
Elpidium bromeliarum	Original description (Müller 1880). Description of the neotypical series (Pinto and Purper 1970). Description of the ontogenetic series (Pereira et al. 2017).
Elpidium maricaoensis	Original description (Tressler 1941). Material stored at National Museum of Natural History, Smithsonian Institute (USNM 80029).
Elpidium laesslei	Re-description (Pereira et al. 2019). Sample available (collected by Little and Hebert 1996).
Elpidium inaequivalvis	Original description (Danielopol 1975).
Elpidium pintoi	Original description (Danielopol 1975).
Elpidium purperae	Original description (Danielopol 1975).
Elpidium merendonense	Original description (Pinto and Jocqué 2013). Material stored at Museu de Zoologia da Universidade de São Paulo (MZUSP 29072, MZUSP 29073).
Elpidium martensi	Original description (Danielopol et al. 2014). Material at stored at Museu de Zoologia da Universidade de São Paulo (MZUSP 32812, MZUSP 32813). Material collected by Little and Hebert (1996).
Elpidium littlei	Original description (Pereira et al. 2019). Material collected by Little and Hebert (1996).
Elpidium heberti	Original description (Pereira et al. 2019). Material collected by Little and Hebert (1996).
Elpidium wolfi	Original description (Pereira et al. 2019). Material provided by Prof. Dr. Wilhelm Foissner (University of Salzburg, Austria).
Elpidium litoreum sp. nov.	Material collected by Julia S. Pereira, Danielly G. Oliveira and Dariane I. D. Schneider (here described).

Species included in the genus: *Elpidium maricaoensis* (Tressler, 1941); *Elpidium laesslei* (Tressler, 1956); *Elpidium inaequivalve* Danielopol, 1981; *Elpidium pintoi* Danielopol, 1981; *Elpidium purperae* Danielopol, 1981; *Elpidium merendonense* Pinto & Jocqué, 2013; *Elpidium martensi* Danielopol et al., 2014; *Elpidium littlei* Pereira et al., 2019; *Elpidium heberti* Pereira et al., 2019; *Elpidium wolfi* Pereira et al., 2019; *Elpidium litoreum* sp. nov.

Diagnosis (modified after Pinto and Jocqué 2013): Medium to large sized carapace, generally with subtle ornamentation marked by minute individual or grouped foveolae (with the exception of *Elpidium laesslei*). Brownish color, varying from light to dark. Width larger than height, ventral surface flat. Bisexual, with sexual dimorphism on both carapace and appendages varying from subtle to outstanding, but always present. Males with greatest width usually at mid-length; females broader than males posteriorly due to the existence of a brooding chamber, and greatest width displaced posteriorly. Antennula 5-segmented. First segment bearing dorso-apical expansion set with pseudochaetae. Antenna with 2 biserrate claws and 1 pectinate claw in males and 3 bisserrate claws in females; hyaline formation on terminal segment in both males and females. Maxillula with 2 spatulate claws in each second and third endites. Hemipenis greatly sclerotized; caudal ramus reduced to a pair of setae; copulatory process usually a hook-like structure with ejaculatory glans and ducts united or separated; distal lobe with dorsal seta, both varying in shape and size; lower ramus present and varying in shape; upper ramus absent. Females with abdomen rounded, bearing a stiff dorsal spine; caudal ramus reduced as in males; genital operculum sclerotized.

Elpidium litoreum sp. nov. Pereira, Rocha, Pinto and DaSilva

(Figs. 1–6)
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Diagnosis: Small-sized *Elpidium*, markedly elongated (length/width ratio = 1.4; length/height ratio = 1.9–2.0). Brownish carapace surface with sparse setae, normal pore canals and subtle ornamentation, represented by minute individual foveolae. In dorsal and ventral views, carapace symmetric. Ventral surface flat. In right lateral view, left valve overlaps right one in all margins; dorsal margin slightly arched, straight on the central portion; ventral margin arched, not straight; external antero-ventral flange well marked. Sexual dimorphism outstanding: in dorsal and ventral views posterior margin rounded in males while truncate in females. Hemipenis with left and right distal lobes elongated (distal lobe basis width/ distal lobe length ratio = 0.4), but asymmetric in shape: left one subquadrate, right one with curved apex; vestigial digital expansion present medially. Copulatory process a simple short hook-like ejaculatory duct. Lower ramus with broad basis, tapering towards lancet-shaped apex.

Type material: *Holotype*: a dissected ♂ (MZUSP 38804) with valves dried and coated for scanning electron microscopy stored in a micropaleontological slide and appendages mounted in a sealed slide with glycerin. Allotype: a dissected ♀ (MZUSP 38805) stored like the holotype. Paratypes: a ♂ (MZUSP 38820) and 2 ♀ (MZUSP 38807, MZUSP 38818) dissected and stored like the holotype; 3 ♂ (MZUSP 38806, MZUSP 38808, MZUSP 38815) dissected with appendages mounted in a sealed slide with glycerin; 5 ♂ (MZUSP 38812, MZUSP 38813, MZUSP 38814, MZUSP 38817, MZUSP 38819) and 4 ♀ (MZUSP 38809, MZUSP 38810, MZUSP 38811, MZUSP 38816) dried and coated for scanning electron microscopy stored in micropaleontological slides; about 136 ♂ and 150 ♀ (MZUSP 38821) kept whole in a vial with 70% alcohol.

Type locality: Tank-bromeliads from Praia Grande, Arraial do Cabo, Rio de Janeiro, Brazil. Approximate geographical coordinates: 22°98'S 42°03'W. Material collected in 11.x.2013 by Julia S. Pereira, Danielly G. Oliveira and Dariane I. D. Schneider.

Additional material: 3 ♂ (MZUSP 38822, MZUSP 38830, MZUSP 38831) and 2 ♀ (MZUSP 38823, MZUSP 38824) dissected and stored like the holotype; 3 ♂ (MZUSP 38825, MZUSP 38826, MZUSP 38828) and 2 ♀ (MZUSP 38827, MZUSP 38829) dried and coated for scanning electron microscopy stored in micropaleontological slides; 5 ♀ (MZUSP 38832) kept whole in a vial with 70% alcohol.

Locality: Tank-bromeliads from a rocky shore in Praia do Forno, Arraial do Cabo, Rio de Janeiro, Brazil. Approximate geographical coordinates: 22°57'S 42°00'W. Material collected in 11.x.2013 by Julia S. Pereira, Danielly G. Oliveira and Dariane I. D. Schneider.

Derivation of name: The specific epithet “litoreum” refers to the occurrence of the species (and its host bromeliads) on the beaches of Arraial do Cabo, Rio de Janeiro, Brazil. It derives from the Latin adjective “litoreus”, meaning from the beach, coastal.

Description of the male: Carapace (Fig. 1A–I). Small-sized *Elpidium* (length of holotype = 629.8 μm), carapace elongated (length/width ratio = 1.4; length/height ratio = 1.9). Color varying from light to dark brown. Subtle ornamentation numerous and minute individual foveolae. Normal pore canals and sparse

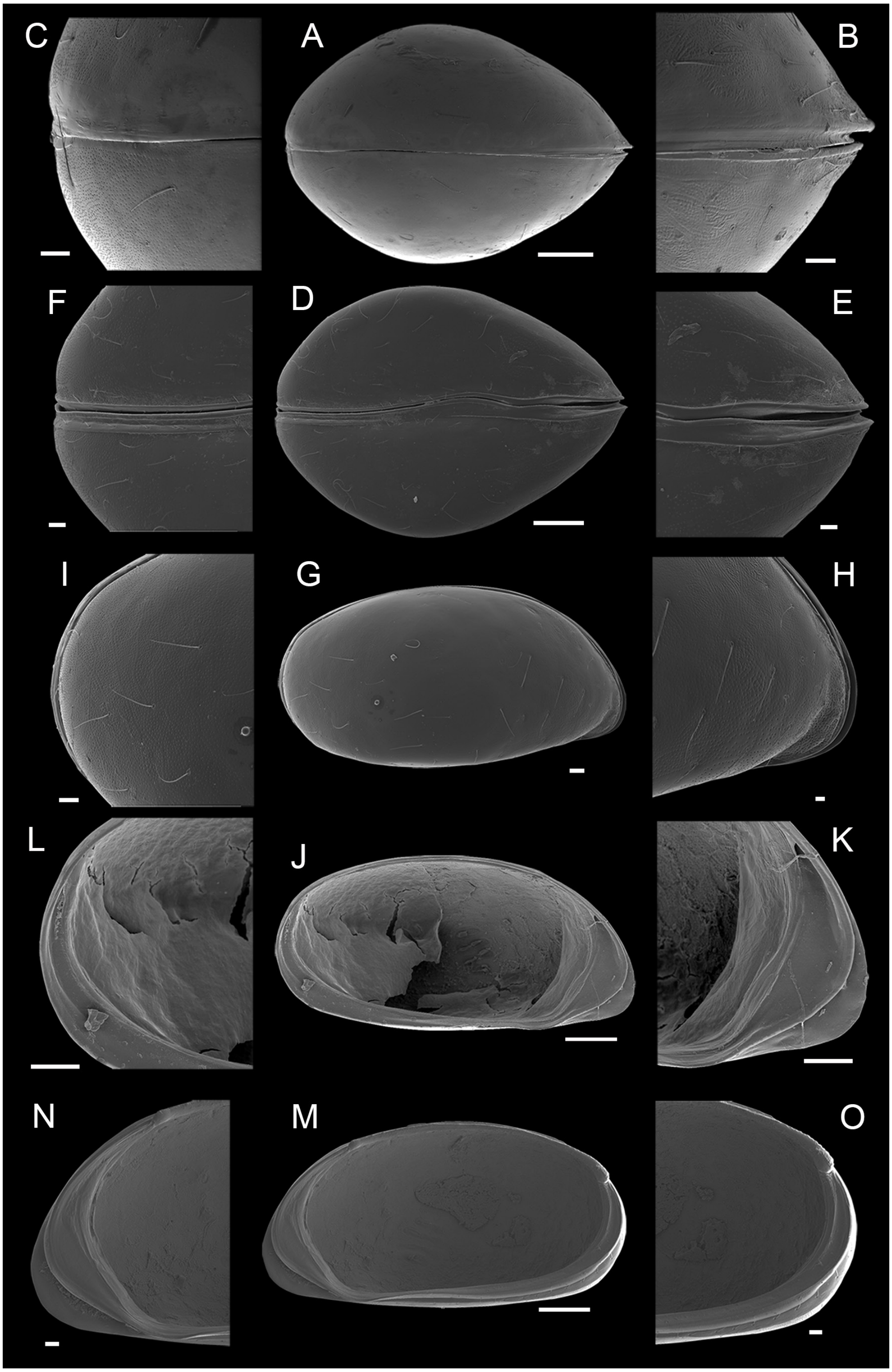

Fig. 1. *Elpidium litoreum* sp. nov., ♂, carapace. A, dorsal view (MZUSP 38812); B, dorsal view, detail of anterior region (MZUSP 38812); C, dorsal view, detail of posterior region (MZUSP 38812); D, ventral view (MZUSP 38813); E, ventral view, detail of anterior region (MZUSP 38813); F, ventral view, detail of posterior region (MZUSP 38813); G, right lateral view (MZUSP 38812); H, right lateral view, detail of anterior region (MZUSP 38812); I, right lateral view, detail of posterior region (MZUSP 38812); J, left valve, internal view (MZUSP 38819); K, left valve, internal view, detail of anterior region (MZUSP 38819); L, left valve, internal view, detail of posterior region (MZUSP 38819); M, right valve, internal view (MZUSP 38831); N, right valve, internal view, detail of anterior region (MZUSP 38831); O, right valve, internal view, detail of posterior region (MZUSP 38831). Scale bars: A, D, J, M = 100 µm; B, C, E, F, H, N, O = 20 µm; G = 30 µm; I = 10 µm; K, L = 50 µm.

setae present. In dorsal and ventral views, posterior region slightly broad; posterior margin rounded, not pointed; ventral surface flat. In right lateral view, left valve overlaps right valve on all margins; dorsal margin slightly arched, straight on the central portion; ventral margin arched, not straight; external antero-ventral flange outstanding.

Left valve (Fig. 1J–L): Flange present in anterior margin, absent in ventral and posterior margins. Selvage well marked in anterior margin; bow funnel-shaped structure in oral region. Calcified inner lamella broad in anterior and posterior regions; inner list well marked on anterior calcified inner lamella and subtle with a row of minute pseudochaetae on posterior calcified inner lamella. Vestibule broad in anterior and posterior regions. Adductor muscle scars 4-stacked spots on valve anterior third.

Right valve (Fig. 1M–O): Flange present in anterior, ventral and posterior margins, the latter one with sparse setae. Selvage well marked in anterior, ventral and posterior margins; bow funnel-shaped structure in oral region, interrupting flange. Calcified inner lamella broad in anterior and posterior regions; inner list subtle on anterior calcified inner lamella and well-marked on posterior calcified inner lamella. Vestibule broad in anterior and posterior regions. Adductor muscle scars 4-stacked spots on valve anterior third. Hinge about 2/3 of the dorsal margin extension; cardinal bar with 2 proto-teeth: posterior one more developed; bar and proto-teeth with very small crenulated ornamentation, visible only under high magnification.

Antennula (Figs. 2A, 3A - represented by female specimen): 5-segmented. First segment relatively long bearing sub-apical expansion with a tuft of tiny pseudochaetae. Second segment the longest, with a single plumose seta in ventro-proximal position reaching fourth segment. Third segment square-shaped with a unique serrate seta in dorso-apical position that reaches fourth segment at about mid-length. Fourth segment bigger in length than in width and partially subdivided slightly before mid-length; medially with 2 dorsal sub-equal serrate setae and one ventral serrate seta; apically with a very long ventral serrate seta and 3 dorsal serrate setae: 1 short and 2 long, sub-equal length. Fifth segment (terminal) with 3 serrate and thin setae, 1 long and 2 equally short, plus an aesthetasc (Ya). Third, fourth and fifth segments with a row of pseudochaetae, covering whole or part of their apical portions.

Antenna (Figs. 2B, 3B, E, F, 3C, D - represented by female specimen): Protopodite 2-segmented; coxa ring-shaped and basis long and arched, dorsally with 2 rows of tiny pseudochaetae and ventrally a triangular-shaped group of pseudochaetae. Endopodite 3-segmented. First endopodal segment relatively short, with very long serrate ventro-apical seta reaching apical portion of second endopodal segment; a group of pseudochaetae ventrally and 3 separated groups of long pseudochaetae dorsally, with each group reaching the next one in length. Second endopodal segment the longest, with a hardly visible vestigial seta apically, 2 sub-apical setae, 1 half as long as the other, dorsally and ventrally, about mid-length, a serrate seta and an aesthetasc (Y). Third segment (terminal) with 3 claws of sub-equal length, 2 serrate and 1 pectinate with a very strong row of denticles, besides tiny seta and hyaline formation. Exopodite very long and arched spinneret seta and vestigial basal seta.

Mandible (Fig. 2F–H, represented by female specimen, 3G, H): Coxa internally with 8 strong teeth, modified X1 seta (spoon-shaped) and long plumose seta plus 2 interdental setae (X2 and X3), 3 interdental spines and sub-apical plumose seta. Palp 4-segmented: basis and 3 endopodal segments; basis with 2 setae in subapical position and respiratory plate (the exopodite) with 3 long setae and 1 short, reflexed seta, all with tiny setulae; first endopodal segment with 2 apical setae, 1 half as long as the other; second endopodal segment with 4 apical setae, 2 long and 2 short; third endopodal segment (terminal) with 3 setae, 2 thin and similar in length and 1 larger and longer than the other 2.

Maxillula (Fig. 2E): Bearing 3 endites. First one with 3 slender setae, approximately equally long; second and third endites with 2 spatulate claws and 3 smooth and slender setae each. Palp with about 4 rows of tiny pseudochaetae medially-positioned and 2 long plumose setae plus vestigial seta on apical portion. Respiratory plate (exopodite) well developed, with minute spines centrally and 16 rays plus 1 reflexed seta, all plumose.

First thoracic limb (Figs. 4A, 5A–D): Basis long and slightly arched with several rows of tiny pseudochaetae. Dorsal margin with medium-size plumose seta plus 2 short apical pappose setae wrapped in their basal portion by an expansion of segment. Exopodite a long and plumose seta. Endopodite 3 elongated segments. First segment the longest one, with several long pseudochaetae in both sides and one, strong biserrate seta, slightly shorter than second segment length; second segment devoid of setae; third segment (terminal) with strong and arched claw, slightly biserrate on its end and with tiny vestigial seta and row of pseudochaetae on its basis. All endopodal segments with rows of pseudochaetae in their apical and lateral portions.

Second thoracic limb (Figs. 4B, 5E–G): Similar to first thoracic limb in general shape, but longer. Basis with only 1 plumose seta on its apical portion, also

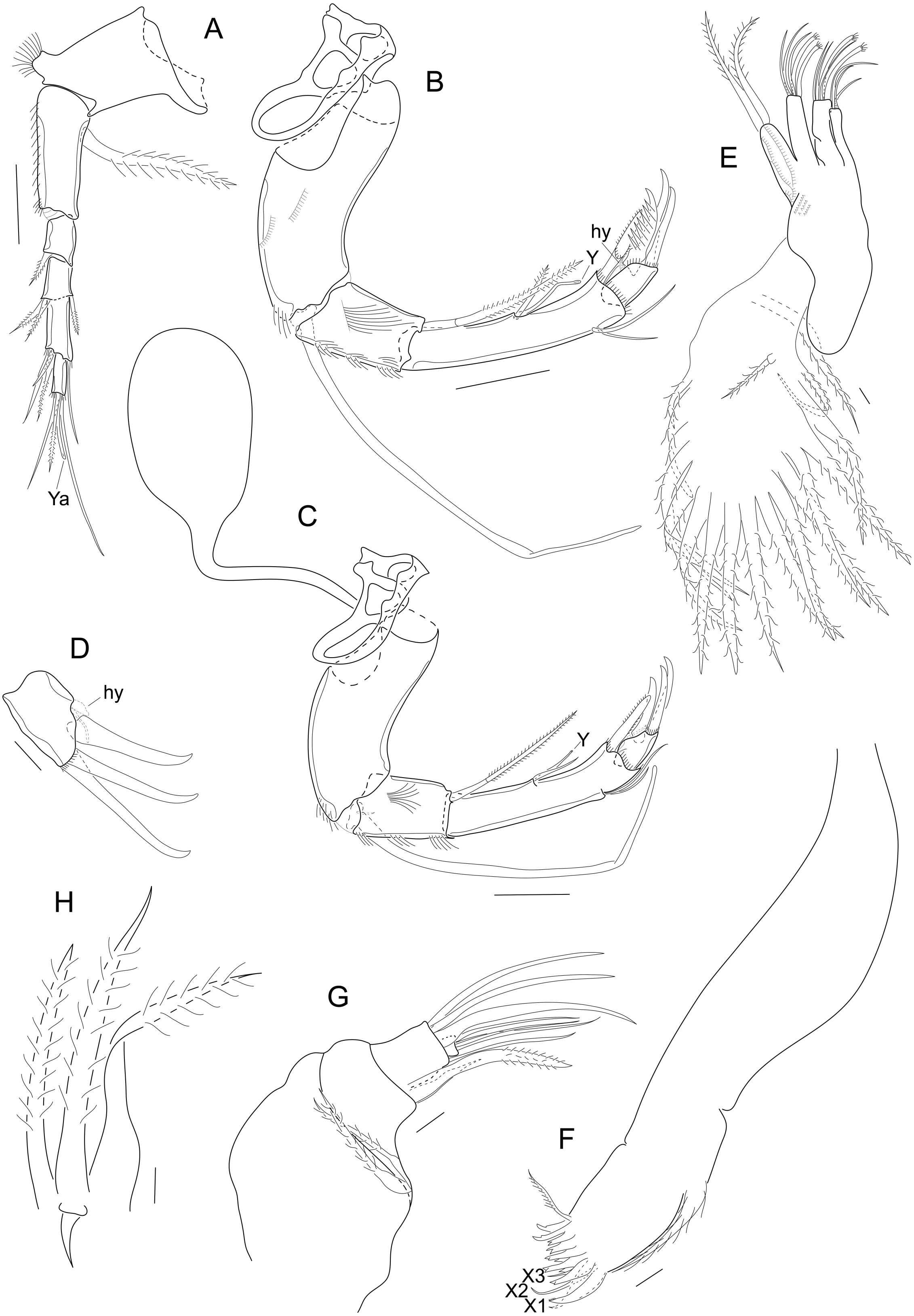

Fig. 2. *Elpidium litoreum* sp. nov., A, B, E, ♂, C, D, F–H, ♀, appendages. A, antennula (MZUSP 38815); B, antenna (MZUSP 38815); C, antenna (MZUSP 38818); D, antenna, terminal segment (MZUSP 38805); E, maxillula (MZUSP 38815); F, mandible, coxa (MZUSP 38805); G, mandible, basis (MZUSP 38805); H, mandible, respiratory plate (MZUSP 38805). Scale bars: A–C = 0.05 mm; D–H = 0.01 mm.

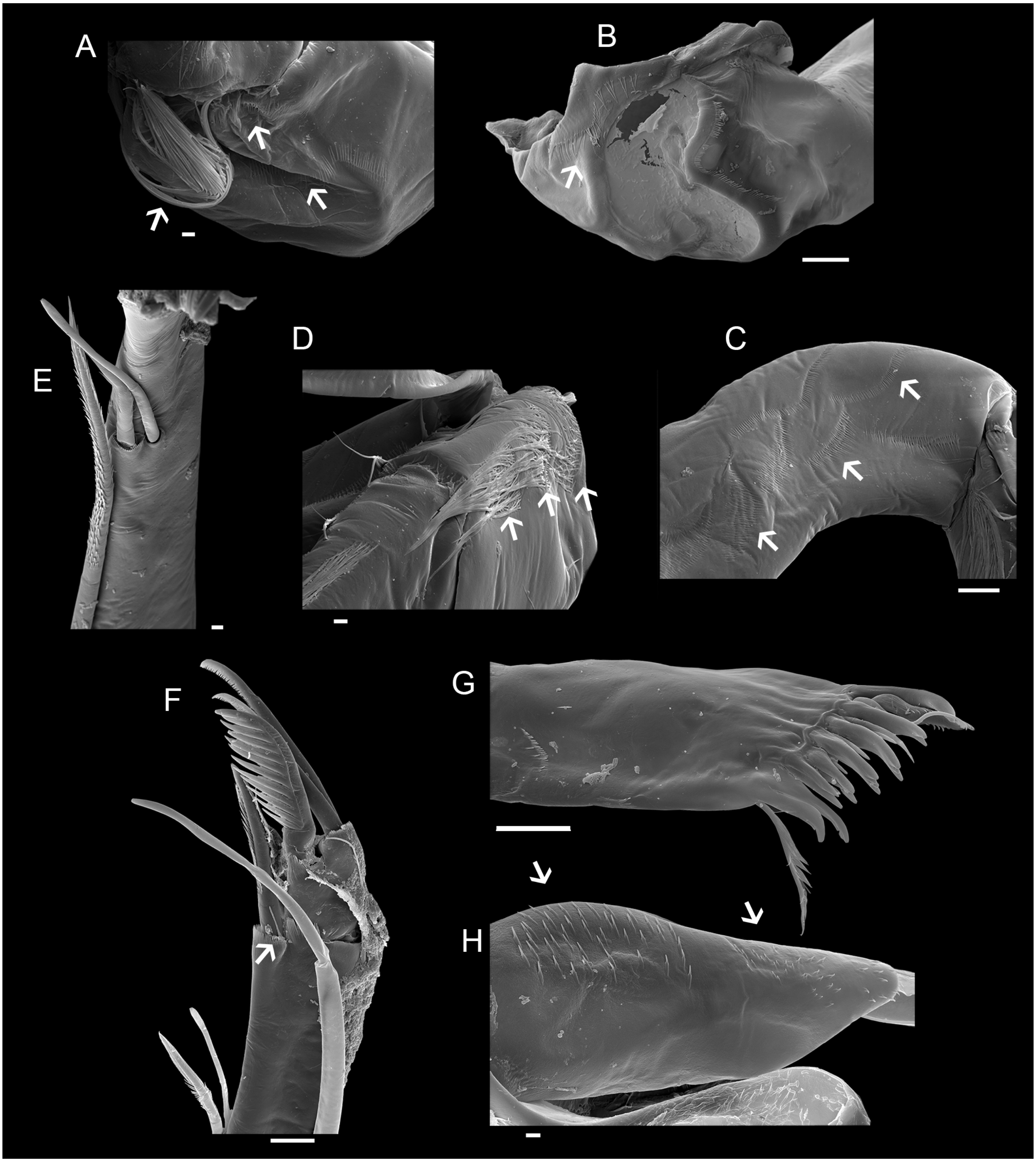

Fig. 3. *Elpidium litoreum* sp. nov., A, C, D, ♀, B, E–H, ♂, appendages. A, antennula, detail of first segment, arrows point to the sub-apical expansion structure and to the two pseudochaetae rows (MZUSP 38816); B, antenna, detail of first protopodite segment (coxa), arrow points to the pseudochaetae row (MZUSP 38817); C, antenna, detail of second protopodite segment, arrows point to the numerous pseudochaetae rows (MZUSP 38816); D, antenna, first endopodal segment, arrows point to the three pseudochaetae groups (MZUSP 38816); E, antenna, second endopodal segment showing the aesthetasc (MZUSP 38817); F, antenna, portion of second endopodal segment and third endopodal segment, arrow points to the tiny vestigial seta (MZUSP 38817); G, mandible, portion of coxa (MZUSP 38817; H, mandible, portion of coxa, arrows point to the tiny spines (MZUSP 38817). Scale bars: A, D, E, H = 2 µm; B, C, F, G = 10 µm.

wrapped by a segment expansion, but incompletely. Biserrate seta of the first endopodal segment and second segment equally long. Third segment (terminal) with strong apical claw, slightly longer and more arched than claw present on first thoracic limb terminal segment.

Third thoracic limb (Figs. 4C, 5H–J): Basis with 3 setae: 2 slender setae dorsally (1 in medial and 1, pappose, in apical position) plus 1 plumose exopodial seta ventrally in medio-proximal position. All 3 endopodal segments with length greater than width and their total lengths greater than in the first and second thoracic limbs. First segment with a unique biserrate seta equal in length to second segment and slightly more slender than its homologous structure in first and second thoracic limbs; second segment with transversal row of pseudochaetae on its lateral portion (structure absent from first and second thoracic limbs) and without seta; third segment (terminal) with very long and thin claw with vestigial seta on its basis. This latter structure biserrate as in first and second thoracic limbs, but not only in its end but in approximately 2/3 of its length. All 3 endopodal segments with rows of pseudochaetae in their apical and lateral portions, longer than those on first and second thoracic limbs.

Hemipenis (Figs. 4D, 5K–M): Large and sclerotized muscular body with copulatory complex (copulatory process and distal lobe) and furcal lobe as main structures. Furcal lobe with 2 pairs of medium-sized setae with numerous pseudochaetae. Distal lobe asymmetric; left one subquadrate, slightly longer than

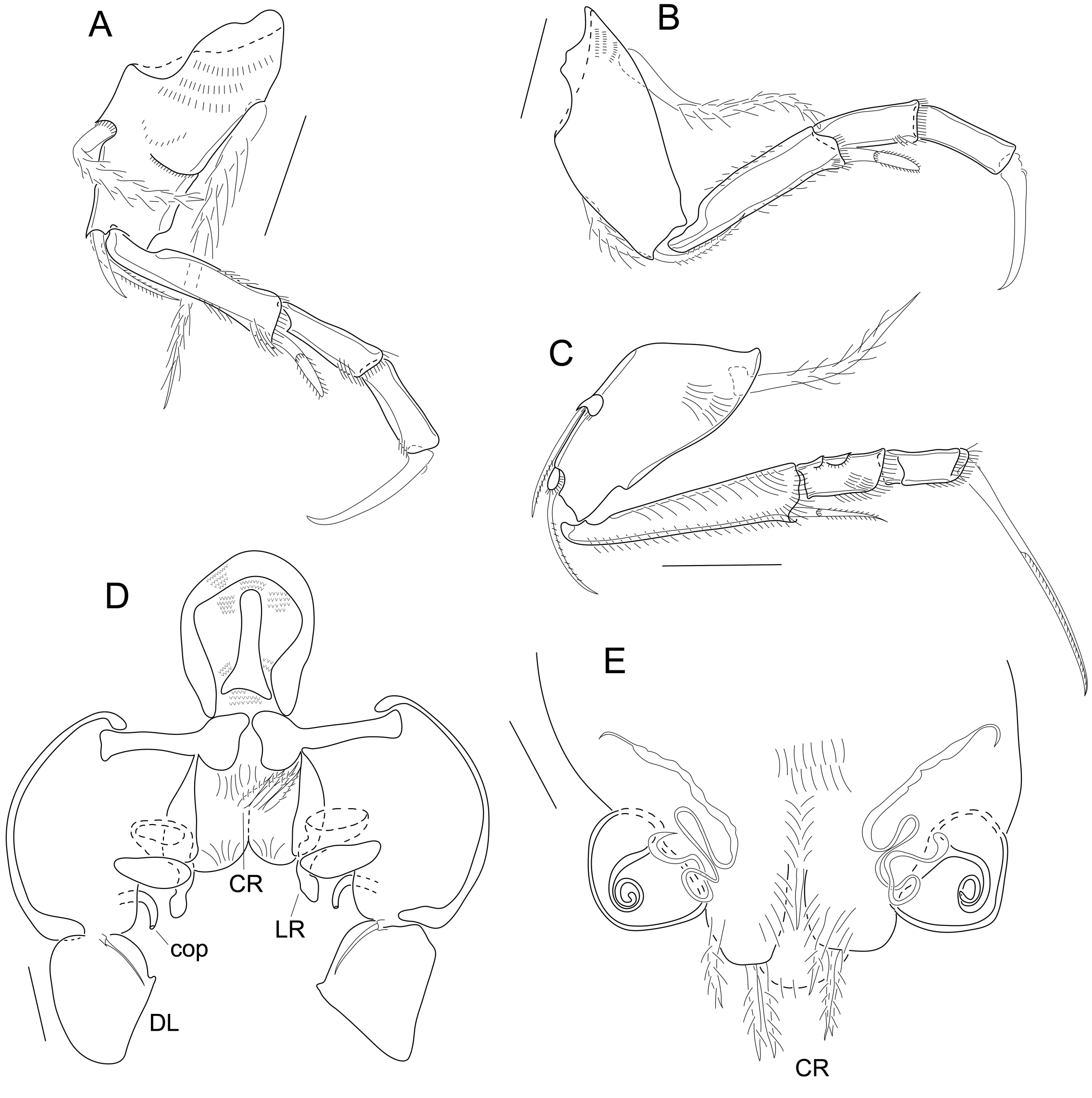

Fig. 4. *Elpidium litoreum* sp. nov., A–D, ♂, E, ♀, appendages. A, first thoracic limb (MZUSP 38815); B, second thoracic limb (MZUSP 38815); C, third thoracic limb (MZUSP 38815); D, hemipenis (MZUSP 38815); E, abdomen (MZUSP38805). Scale bars: A–E = 0.05 mm.

Fig. 5. *Elpidium litoreum* sp. nov., ♂, appendages. A, first thoracic limb, detail of protopodite and first endopodal segment, arrow points to the segment expansion that wraps the two protopodite setae basis (MZUSP 38817); B, first thoracic limb, arrow points to the short pseudochaetae group laterally placed on first endopodal segment (MZUSP 38819); C, first thoracic limb, arrow points to the short pseudochaetae group laterally placed on second endopodal segment (MZUSP 38819); D, first thoracic limb, detail of third endopodal segment and distal claw, arrow points to the transverse pseudochaetae rows on the segment (MZUSP 38817); E, second thoracic limb (MZUSP 38817); F, second thoracic limb, detail of third endopodal segment and distal claw, arrow points to the transverse pseudochaetae rows on the segment (MZUSP 38817); G, second thoracic limb, distal claw, arrows point to the transverse pseudochaetae rows on the apex and distal claw ending (MZUSP 38817); H, third thoracic limb, detail of second endopodal segment, arrows point to the pseudochaetae rows that transect the segment (MZUSP 38819); I, third thoracic limb, part of third endopodal segment, arrow points to the pseudochaetae rows that transect the segment (MZUSP 38819); J, third thoracic limb, detail of distal claw, arrows point to vestigial seta (MZUSP 38819); K, hemipenis (MZUSP 38817); L, hemipenis, detail of lower ramus and copulatory process (MZUSP 38817); M, hemipenis, detail of copulatory process (MZUSP 38817). Scale bars: A, C, D, G, J = 2 μm; B, I = 5 μm; E, H, K = 10 μm; F, M = 1 μm; L = 3 μm.

wide, apex acuminate; right one longer than wide, apex curved; both distal lobes with similar medium dorsal setae. Copulatory process simple (*i.e.*, glans and ejaculatory duct united), short, hook-like. Lower ramus with broad basis, tapering towards lancet-shaped apex. Upper ramus absent.

Description of female: Carapace (Fig. 6): Small-sized *Elpidium* (length = 622.6 µm). Carapace elongated (length/width ratio = 1.4; length/height ratio = 2). In dorsal view, posterior body region broader than in males due to the brooding chamber, posterior margin truncate. Ventral surface flattened. In right lateral view, central part of dorsal margin straighter than in males, ventral margin less rounded; external anteroventral flange greatly pronounced; left valve overlapping right one on all margins.

Antenna (Figs. 2C, D, 3C, D): Terminal segment with tiny seta, hyaline formation and 3 biserrate claws similar in length as opposed to males with 2 biserrate claws and 1 pectinate claw.

Abdomen (Fig. 4): End of body rounded with 3 main structures: spine-like seta, female genital lobes and furcal lobes. Abdominal spine-like seta very stiff, dorso-medially placed; genital lobe rounded, rough, with a net of trabeculae internally; furcal lobe rounded, not rigid, with numerous pseudochaetae and 3 setae: first 2 inserted closely together and third one placed more anteriorly.

Remaining appendages: (*i.e.*, antennula, mandible, maxillula, first, second and third toracic limbs) as described for male.

Comparison to other Elpidium species: *Elpidium litoreum* sp. nov. presents a high degree of sexual dimorphism on the carapace, like most *Elpidium* species. In all views, carapace is markedly elongated, similar to *E. merendonense*, a characteristic not usual for *Elpidium*. In dorsal view, males with posterior margin rounded as occurs in *E. littlei* and different from the usual acuminated posterior margin; females with posterior margin truncated as usual for the genus. In right lateral view, dorsal margin straight and ventral margin slightly arched as opposed to arched and straight as occurs in *E. bromeliarum* and most species of the genus; on the anterior margin an external flange is evident. *E. bromeliarum* and *E. martensi* also present this structure, although not quite evident, but slight. Distal lobes on hemipenis asymmetric, a feature hitherto unknown for the genus, at least for the species where this information can be assessed; left distal lobe somewhat similar to *E. bromeliarum* but with a longer external edge and less quadrate-shaped; right lobe resembles *E. martensi* but shorter and with a small apical projection on the internal margin. *Elpidium litoreum* sp. nov. hemipenis can also be differentiated from *E. martensi* by the morphology of the copulatory process and lower ramus. Copulatory process is simple (*i.e.*, distal glans and ejaculatory duct united) as usual for the genus.

Phylogeny

Analysis performed in TNT resulted in a single most parsimonious tree (Fig. 7), with a total length of 145.700, consistency index (CI) of 0.644 and retention index (RI) of 0.667. The reconstruction indicates three major results, supported by a number of synapomorphies: (1) *Elpidium* as a monophyletic group; (2) *I. ibipora* as its sister group; and (3) an internal division of *Elpidium* into two major groups: the Jamaican species *E. laesslei*, *E. littlei*, *E. wolfi* and *E. heberti* and, on the other hand, *E. inaequivalve*, *E. purperae* and *E. pintoi* from Cuba along with *E. merendonense* from Honduras, *E. maricaoensis* from Puerto Rico, *E. martensi* from Jamaica, *E. bromeliarum* and *E. litoreum* sp. nov. from Brazil.

DISCUSSION

Diversity

The remarkable diversity of Ostracoda in terms of the number of taxa, fossil record and inhabited environments has been repeatedly emphasized in the literature (Horne et al. 2002; Martens et al. 2008; Martens and Savatenalinton 2011). In freshwater environments, for example, ostracods can be found in extensive perennial water bodies, such as lakes or rivers, or in small and temporary water bodies, such as puddles, water film or reservoirs confined inside plant structures. Additionally, freshwater environments comprise much higher diversities when compared with marine or terrestrial ones of equivalent size (Jocqué et al. 2013). With 2330 species spread out in 270 genera, freshwater environments bear a significant portion of the extant ostracod diversity (Meisch et al. 2019). On top of that, this diversity possibly represents an underestimation of the true freshwater ostracod diversity (Martens et al. 2008; Martens and Savatenalinton 2011). The group is overall poorly investigated (Sidorov and Semenchenko 2012) in some locations due to limited funding (Meisch et al. 2007) or a lack of extensive geographical sampling (Külköylüoglu et al. 2015).

The study by Scharf and Meisch (2014) points to the same problem. In a checklist of non-marine ostracods on the Canary Islands, 15 species were recorded in La Gomera, the second smallest island, while Tenerife and Fuerteventura, the largest ones, have

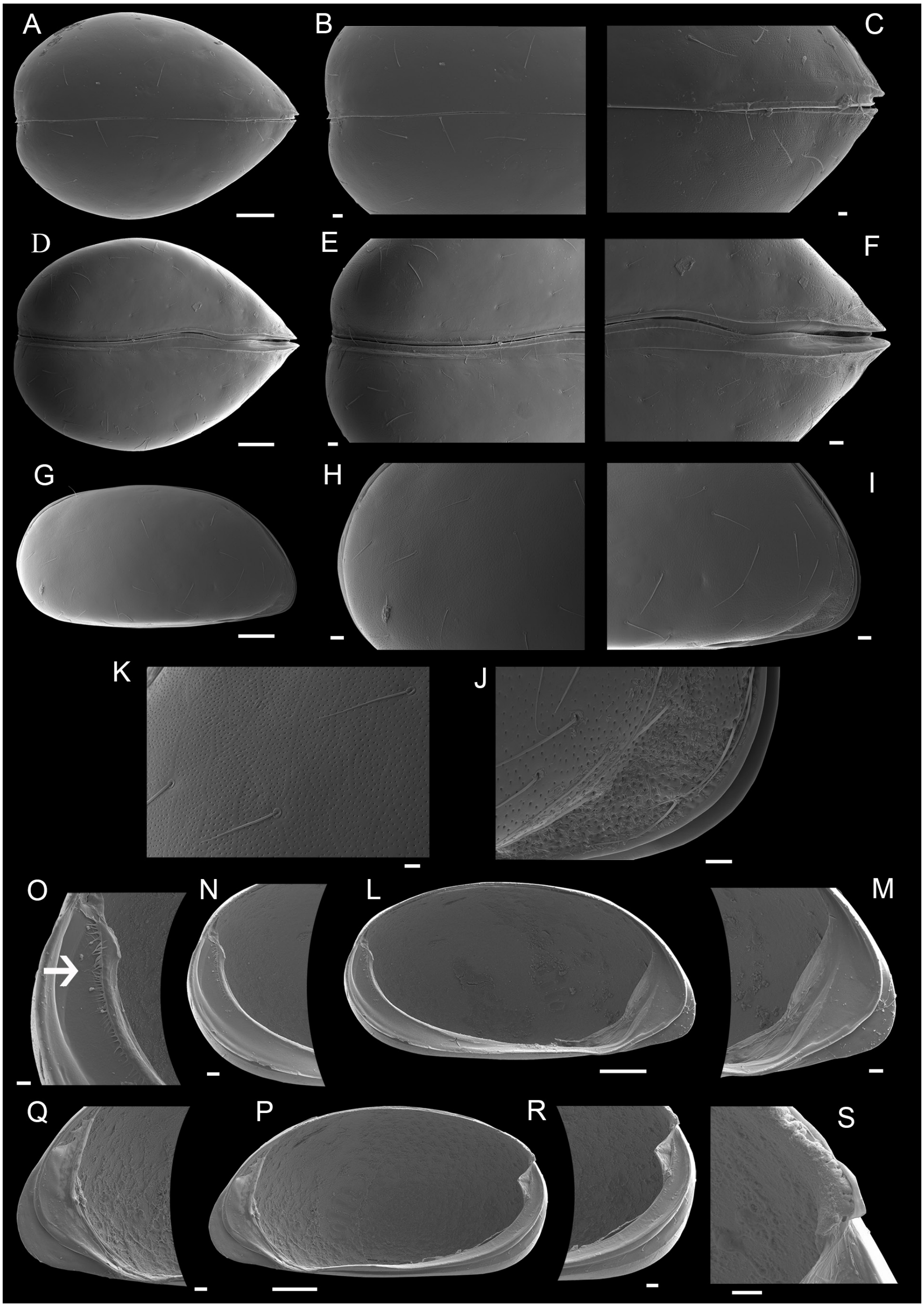

Fig. 6. *Elpidium litoreum* sp. nov., ♀, carapace. A, dorsal view (MZUSP 38811); B, dorsal view, detail of posterior region (MZUSP 38811); C, dorsal view, detail of anterior region (MZUSP 38811); D, ventral view (MZUSP 38810); E, ventral view, detail of posterior region (MZUSP 38810); F, ventral view, detail of anterior view (MZUSP 38810); G, right lateral view (MZUSP 38809); H, right lateral view, detail of posterior region (MZUSP 38809); I, right lateral view, detail of anterior region (MZUSP 38809); J, right lateral view, flange (MZUSP 38809); K, right lateral view, carapace ornamentation (MZUSP 38809); L, left valve, internal view (MZUSP 38807); M, left valve, internal view, detail of anterior region (MZUSP 38807); N, left valve, internal view, detail of posterior region (MZUSP 38807); O, left valve, internal view, detail of posterior region, arrow points to the tiny pseudochaetae row (MZUSP 38807); P, right valve, internal view (MZUSP 38816); Q, right valve, internal view, detail of anterior region (MZUSP 38816); R, right valve, internal view, detail of posterior region (MZUSP 38816); S, right valve, internal view, detail of posterior hinge proto-tooth (MZUSP 38816). Scale bars: A, D, G, L, P = 100 µm; B, E, F, H, I, M, N, Q, R = 20 µm; C, J, K, O, S = 10 µm.

11 and 13 species occurrences, respectively – the lack of a correlation between species number and island size most probably originates from the considerably larger sampling effort in La Gomera (Scharf and Meisch 2014).

Phytotelmata are plant structures that act as freshwater reservoirs, constituting an environment for aquatic biotas, either permanent or occasional (Jocqué et al. 2013). They include habitats such as tree holes, pitcher plants, inflorescences of Heliconiaceae and tank bromeliads. Biodiversity in such phytotelmata, including the ostracod fauna, remains largely undersampled. These freshwater environments have been often ignored due to their cryptic nature, which does not draw immediate attention (Jocqué et al. 2013). According to the checklist by Jocqué et al. (2013), Crustacea had 108 species recorded in phytotelmata and, from these, solely 14 corresponded to Ostracoda, of which eight belonged to the bromeliad inhabiting *Elpidium*. Currently, *Elpidium* includes 12 formally described species, which still seems deficient. There is an expectation that small confined water bodies would prevent gene flow and favor speciation (Little and Hebert 1996), especially in taxa with reduced mobility and dispersal like *Elpidium*, which depends on other animals such as amphibians to disperse (Lopez et al. 1999 2002 2005; Sabagh et al. 2011). In this way, finding new *Elpidium* species should be expected as long as the Neotropical phytotelmata remain ill-unexplored.

If Little and Hebert (1996) were correct in postulating that *Elpidium* has high levels of diversity and endemicity, the genus would stand out as an ideal model for determining areas of endemism and biogeographical evolution in the Neotropical region. The potential is even more significant if we consider the possibility of integrating these biogeographical studies with those based on bromeliads and amphibians, which provide, respectively, habitat and means of dispersal for *Elpidium*. However, the scarcity of adequate geographical sampling efforts hampers the use of *Elpidium* in biogeographical studies for now. The majority of *Elpidium* species are known from their type localities alone. Expanding taxonomic studies within phytotelmata in the Neotropics is necessary for determining the actual areas of occurrence of each species.

Phylogeny

Phylogenetic trees for the genera within Timiriaseviinae, including *Elpidium*, have been published (Savatenalinton et al. 2008; Karanovic and Humphreys 2014). However, no phylogenetic analysis of *Elpidium* species had been attempted. Our results can thus be discussed in terms of the morphological character matrix as well as of the relationships between *Elpidium* species.

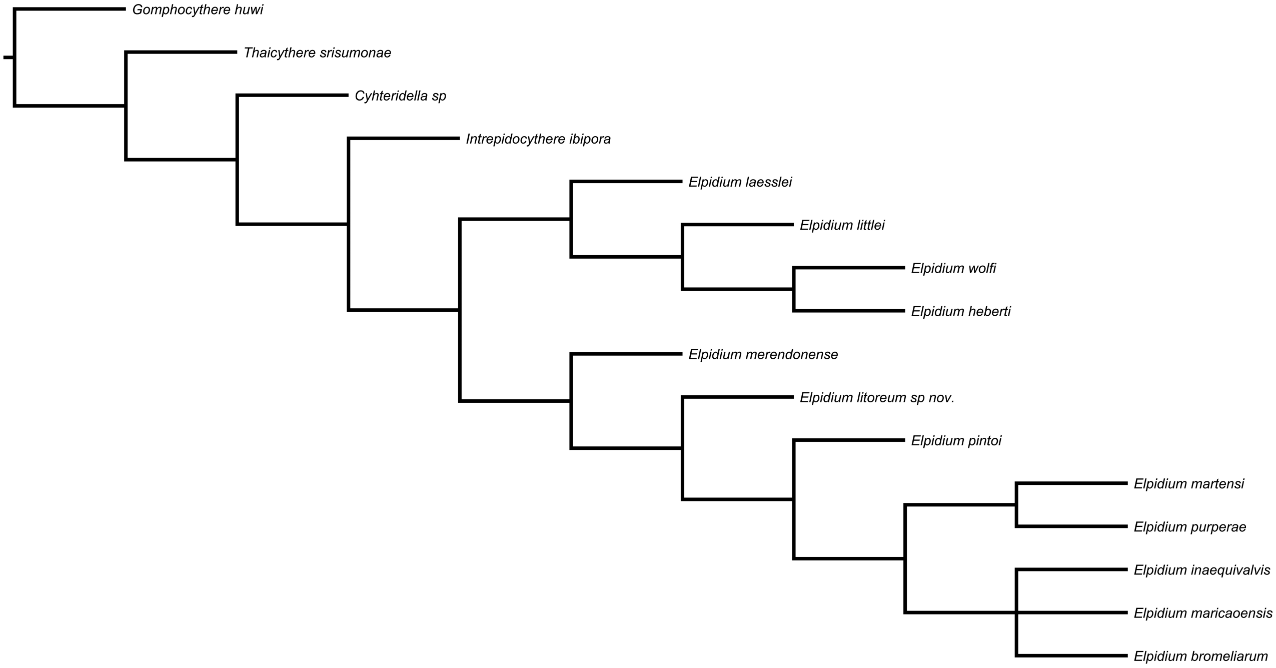

Fig. 7. Most parsimonious tree showing monophyly of *Elpidium* as sister-group to *Intrepidocythere* and internal phylogenetic relationships within *Elpidium*. Tree obtained with software TNT (details in MATERIALS AND METHODS).

Morphological characters

From the 77 morphological characters, 35 reflect traits from carapace and 42 from appendages (Table S1). Twenty-seven of these characters were adapted from previous works about Timiriaseviinae (Colin and Danielopol 1981; Park et al. 2002; Savatenalinton et al. 2008; Danielopol et al. 2014; Karanovic and Humphreys 2014). Some of these (*e.g.*, the degree of fusion between articles '4a' and '4b' on the antennula, or the fusion between second and third segments of mandible) have been used in previous phylogenetic reconstructions of the subfamily (Savatenalinton et al. 2008; Karanovic and Humphreys 2014) and were kept here mostly without modifications. Other characters are more related to the specific literature about *Elpidium*, and we tried to convert considerations made by Colin and Danielopol (1981) and more recently by Danielopol et al. (2014) into morphological characters (*e.g.*, the varying degrees of sexual dimorphism, the characteristic bow-funnel shaped structure). A third group, still inside the 27 characters extracted from the literature, is formed by those modified in the present work. Modifications applied here aimed, for example, at encompassing the variation among *Elpidium* species, as in the case of hemipenis shape and structure. We also reinterpreted homologies or character states (as with the division of hinge type and homologization by components – anterior tooth, posterior tooth and bar). The remaining characters were newly created here. We attempted, for example, to use groups of pseudochaetae that seem to follow distribution patterns on each appendage, like the first endopodal segment on the antenna (A2). The groups of pseudochaetae used in the present work ended up being constant within the *Elpidium*. However, they may be informative when comparing to other genera of Timiriaseviinae, and future phylogenies of the subfamily could incorporate this character. Among the difficulties in producing a character matrix for *Elpidium* is the conservative morphology of most appendages, which hinders their use in the phylogeny. As a result, soft part characters concentrate predominantly on the antennula, antenna and hemipenis. In contrast, the opposite happened with characters of the hemipenis. The high degree of variation makes it challenging to homologize structures and establish character states. Such high morphological variation resulted almost only in autapomorphies.

Phylogenetic relationships

A single most parsimonious tree was obtained from the phylogenetic analysis (Fig. 7). In this tree, we first point to the monophyly of *Elpidium* and its close relationship to the monospecific *Intrepidocythere* that appeared as its sister-group, corroborating Pinto et al. (2008) and Karanovic and Humphreys (2014). *Intrepidocythere ibipora* inhabits moist leaf litter on the forest floor. Pinto et al. (2008) speculated about possible evolutionary pathways of *Intrepidocythere* and *Elpidium* concerning possible habitats of their common ancestor. Three possible scenarios were suggested. In the first scenario, *Intrepidocythere* and *Elpidium* originated from a common aquatic ancestor, and then each one colonized a different habitat (semi-terrestrial and bromeliads, respectively). Alternatively, their common ancestor colonized leaf litter and, subsequently, *Elpidium* shifted to bromeliad phytotelmata. In a third possible case, the ancestral lineage invaded bromeliad phytotelmata and, subsequently, *Intrepidocythere* colonized the moist forest floor. The phylogenetic reconstruction of the present work does not favor any of these three scenarios since the two genera are sister taxa that derived together from a typical freshwater outgroup. New findings within *Elpidium*, *Intrepidocythere* or other undiscovered lineages of Timiriaseviinae from semi-terrestrial or bromeliad habitats could help enlighten the actual sequence of habitat colonization.

Our phylogenetic tree divided *Elpidium* into two main clades. In the first one, *E. wolfi* and *E. heberti* appear are sister taxa, followed by *E. littlei* and *E. laesslei*. All four species in this clade are from Jamaica. In terms of morphology, they show a copulatory process with differentiated ejaculatory duct and distal glans (character 74). This is, in fact, the most conspicuous synapomorphy supporting the clade. Another synapomorphy of this clade is the united ventral margin and ventral ridge on posterior part of the major valve (character 17). The analysis also indicates character 32 (separated selvage and posterior outer margin of major valve, forming a flange) as a synapomorphy of this clade, but with an homoplastic appearance in *E. bromeliarum*. Finally, continuous characters 1 to 4 support this group.

The second group is formed by *E. merendonense* from Honduras, *E. inaequivalve*, *E. purperae* and *E. pintoi* from Cuba, *E. maricaoensis* from Puerto Rico, *E. martensi* from Jamaica and *E. bromeliarum* and *Elpidium litoreum* sp. nov. from Brazil. The main synapomorphy of this group is the partially calcified thickening of the ventral ridge along major valve (character 14). Results also indicate flat ventral surface (character 13) and ventral margin morphology (character 20) as synapomorphies of this clade. However, the latter two characters appear as homoplasies in *E. littlei*.

Within the latter group, we can observe a subcluster of five species: *E. bromeliarum*, *E. maricaoensis*, *E. inaequivalve*, *E. purperae* and *E.*

martensi. This clustering is supported only by female carapace length (character 2). However, it is worth noting that three of these species have very incomplete descriptions (*E. maricaoensis*, *E. inaequivalve*, *E. purperae*).

Besides morphological analyses, the geographic distribution also needs to be considered. While the geographical distribution (restricted to Jamaica) of species in the first main group are consistent with their close phylogenetic relationship, the same cannot be said about the second main group, which includes species from central America and Brazil. For example, our analysis suggests a close affinity between *E. maricaoensis*, *E. bromeliarum* and *E. inaequivalve* in a trichotomy. However, from a geographic point of view, this close phylogenetic relationship seems unlikely since *E. maricaoensis* is from Puerto Rico, *E. inaequivalve* from Cuba and *E. bromeliarum* from southern Brazil. These incongruities could be due to a significant lack of morphological information for some species. In the case of *E. maricaoensis*, *E. purperae*, *E. pintoi* and *E. inaequivalve*, incomplete original descriptions were our sole source of information (Table 1). Consequently, several characters were input as unknown in the character matrix, possibly producing spurious or unresolved relationships.

Another relevant reason is the poor knowledge about the diversity of the genus, which impacts the reconstructed evolutionary affinities between species. We highlight that uncovering this diversity will undoubtedly impact future phylogenetic studies of the genus. From this perspective, we believe that advancing the sampling efforts and alpha taxonomy of *Elpidium* will significantly improve our understanding of its evolutionary history. In particular, wider geographical samplings are necessary to unveil the distributional pattern individual species. In addition, genetic studies could help overcome some of the difficulties inherent to morphological phylogenies. *Elpidium* is a potential model for studies of biogeography, ecology, evolution, among other areas. We hope that the present contribution stimulates further research on this genus.

CONCLUSIONS

We describe a new species of *Elpidium* from the Brazilian Atlantic forest, thus taking a step forward to uncovering the actual diversity of the genus. The present work also provides the first phylogeny of *Elpidium*, based on morphological characters. Results include monophyly of the genus and its close relationship to the semi-terrestrial *Intrepidocythere*. Within *Elpidium*, the tree showed two main clades. The first one is formed by four Jamaican species that share a differentiated copulatory process. The second one includes the remaining species, but their relationships do not seem consistent with geographical distribution. Further studies with *Elpidium* are necessary to elucidate the diversity and evolution of the genus, finally allowing its use as a model group.

List of abbreviations

A1, antennule.
A2, antenna; cop, copulatory process.
DL, distal lobe.
dor, dorsal ridge.
ds, dorsal seta.
Gp, sexually dimorphic claw on antenna.
Hp, hemipenis.
hy, hyaline formation.
LR, lower ramu.
Md, mandible.
Mx, maxillula.
T1, first thoracic limb.
T2, second thoracic limb.
T3, third thoracic limb.
X1, first interdental seta on the mandibular coxa.
X2, second interdental seta on the mandibular coxa.
X3, third interdental seta on the mandibular coxa.
Y, aesthetasc from antenna.
Ya, aesthetasc from antennule.
CI, consistency index.
RI, retention index.
MZUSP, Museu de Zoologia da Universidade de São Paulo.

Acknowledgments: This work and the new species name were registered with ZooBank under urn:lsid:zoobank.org:pub:FCC640E1-2EC6-47E3-BC3A-159EC5FAEA11. Julia S. Pereira is thankful to the scholarship granted by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES – PROEX) that made this work possible, to Instituto de Biociências, Universidade de São Paulo (USP) and to Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba (UFPB) in whose facilities a part of this work was made. All authors are thankful to Danielly G. Oliveira and Dariane I. D. Schneider for helping on sampling and to Willi Hennig Society for sponsoring software TNT. Finally, authors are very grateful for the detailed analysis and significant contribution provided by an anonymous reviewer.

Authors' contributions: Julia S. Pereira, Carlos E. F. da Rocha and Ricardo L. Pinto contributed to the morphological and taxonomical analyses. Julia

S. Pereira, Ricardo L. Pinto and Marcio B. DaSilva contributed to the phylogenetic analyses.

Competing interests: Julia S. Pereira, Carlos E. F. da Rocha, Ricardo L. Pinto and Marcio B. DaSilva declare that they have no conflict of interest.

Availability of data and materials: List of morphological characters and matrix of both continuous and discrete characters are available in supplementary materials.

Consent for publication: Not applicable.

Ethics approval consent to participate: Not applicable.

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Supplementary Materials

Table S1. List of characters. (download)

Table S2. Morphological matrix of continuous characters. (download)

Table S3. Morphological matrix of discrete characters. (download)