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LAVRAS – MG

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Monografia apresentada à Universidade Federal de Lavras, como parte das exigências do Curso de Ciências Biológicas, para obtenção do título de Bacharel.

Prof. Dr. Rodrigo Lopes Ferreira
Orientador

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ABSTRACT

The Sphaeromatidae family is the most specious of the aquatic isopods, with 100 genera and approximately 700 spp**.** Most of its representatives are small and inhabit marine environments, interstitial and shallow waters including estuaries. The genus *Bilistra* consists of three species that occur in caves in karst areas in the South Island of New Zealand. Some species within this genus present troglomorphic traits, as body depigmentation and eye reduction, being, thus considered troglobites. The objective of this work is to describe a new species of *Bilistra*, which inhabits freshwater environments in one cave in Northern South Island, New Zealand.

Key-words: Troglomorphic. Taxonomy. Freshwater. New Zealand. Cave.

Summary

1 INTRODUCTION

The Sphaeromatidae Latreille, 1825 is the most specious of the aquatic isopods, with 100 genera and approximately 700 species (Wetzer et al. 2013). Most Sphaeromatids inhabit marine environments, interstitial and shallow waters including estuaries (Sket & Bruce 2004) although many species also occur in subterranean freshwaters (Prevorčnik et al. 2010). Several of its representatives are small (3–10 mm), with very few species exceeding 2 cm) (Pathan and Kadeparambil 2021).

Some morphological characters that define the family are: two segments in the pleon, the first one with two sutures that show the fusion of the segments; usually well-developed molar process, lacinia mobilis present, maxiliped with five segments and the peduncle of the uropod attached to the interior ramus (endopod) (Hurley & Jansen 1977).

Monolistra Gerstaecker, 1856, is one of the most diverse genus of Sphaeromatidae, found only in freshwaters, includes several cave-associated species, all with severe reduction or absence of eyes and lack of pigmentation. They are well known to be spread along the Dinaride and Italo-dinaride Systems, from Montenegro to the Swiss Alps.

The genus *Bilistra* was described by Sket & Bruce, 2004 and consists of three species that occur in caves in karst areas in the Nelson region, northwest of the South Island in New Zealand. The main characteristics that define the genus are the simple arcuate pleotelson, pleonal sutures running to the posterior margin of the pleon, inferior pereopods margin with a dense setulose lining, very weakly-ridged pleopods IV and V and the uropods with the exopodite about only half as long as endopodites (Sket & Bruce, 2004).

The *Bilistra millari* is the type species and inhabits surface and cave streams. *Bilistra mollicopulans* Sket & Bruce, 2004 was found only in Megamania cave, 50km away from the species *B. millari* and *Bilistra cavernicola* Sket & Bruce, 2004, separated by the non-karstic Tasman mountains. *B. cavernicola* inhabits cave waters between Motueka and East Takaka. All species of the genus were found only in freshwaters like *Monolistra*.

Many biological aspects regarding *Bilistra* species are still unknown, certainly deserving further investigations. Furthermore, a new species *Bilistra*, was discovered in a cave, in the region of Takaka (Nelson, New Zealand), which is being described in the present work. Additionally, we bring some aspects of the natural history of this new species and the conservation of their habitat.

2 MATERIAL AND METHODS

The specimens were collected with tweezers and brushes, in the Commentary cave,

located at the Takaka district (Nelson, New Zealand). The examined material is deposited in the Coleção de invertebrados subterrâneos de Lavras (ISLA), at the Federal University of Lavras (UFLA,Brazil).

For analysis and photographs, structures such as pereopods and pleopods were dissected, and assembled in temporary microscope slides. For morphometrics, five adult male specimens and one female were analyzed.

Photographs were made with Zeiss AXIO Zoom stereomicroscope V16. Scanning electron microscopy was used to observe and describe the following structures: head and antennae, pereopods, penis, pleopods, pleotelson, uropods and mouthparts such as mandible, maxilla I and II and maxilliped. The equipment used was TM4000 Tabletop Microscope HITACHI. Drawing illustrations were made using the adobe Illustrator 2021, based on photos taken with a Sony Cybershot DSC-V1 camera stereomicroscope. The CombineZP software package by Alan Hadley, was used to create composite images with depth or extended, by combining multiple images taken from different focal planes. Images were edited with Adobe Photoshop CS5 and figures were prepared in CorelDraw X7. The terminologies used were based on Sket & Bruce (2004).

3 RESULTS

Taxonomy.

Suborder: Sphaeromatidea Wägele 1989.

Superfamily: Sphaeromatoidea Latreille 1825

Family: Sphaeromatidae Latreille 1825

Genus*: Bilistra,* Sket & Bruce, 2004

3.1 *Bilistra* sp. nov.

Material examined.

Holotype: MALE; New Zealand, Takaka district, Nelson, Commentary cave (41° 2'13.01"S/172°48'13.57"E); Feb 8. 2020; R.L. Ferreira leg.; ISLA

Paratype: 5 MALES; 1 FEMALE; New Zealand, Takaka district, Nelson, Commentary cave (41° 2'13.01"S/172°48'13.57"E); Feb 8. 2020; R.L. Ferreira leg.; ISLA

Diagnosis: Pleotelson posteroventral margin with a narrow open groove, uropod apices narrowly rounded and eyes reduced.

Description. Males (incl. Holotype). Body 6,72-11,78 mm long and 4,33-6,65 mm wide, width 54-64 % of length, coxal plates slightly tilted. Color (in ethanol 70%), dorsum brownish-yellow

translucent and brown maculated in different patterns. Eyes placed laterally, reduced, ocular region with black inner pigment visible below ocular cuticle and some distinctive ommatidia around 8.

Head slightly rounded anteriorly, dorsal surface smooth, one row of tubercles at the posterior margin of tergites V, VI and VII. First third of epistome thin, larger at the middle, with last third embracing half of the labrum (Fig. 2A). Lower margin of labrum fully setose (Fig. 2A). Antenna I sparsely setose, about 80% as long as antenna II; antennas I connecting above the epistome; peduncular article 1 and flagellum subequal in length, flagellum 87-135% length of peduncular article, articles 2 and 3 together nearly as long as article 1; article 1 widened and long, article 2 widened, article 3 cylindrical, flagellum with 6-10 articles, aesthetascs of flagellum in pairs of two, aesthetascs combined with few setae (Fig. 2B;D). Antenna II robust, about 31-36% as long as body; when in natural position can reach posterior margin of pereonite II; all articles with setae and weak spines, articles 1 and 2 nearly twice as wide as long, 3 and 4 twice as long as wide; flagellum with 8-21 articles (Fig. 2B;C).

Mandible incisor process 4-dentate, lacinia mobilis tridentate; approximately 16 setae at lacinia mobilis and around 15 setae by vestigial lacinea-like scale on right mandible; setae originate at the molar base, long, reach molar's apice; molar with short setae along its circumference. Mandibular palp articles 2-3 with 11 and 22 strong setae, article 3 distal setae is twice as long as others. Palp with article 1-3 decreasing in length. Inferior surface of proximal article with thin setae, article 2 stout, distal article with superior margin concave and inferior margin straight half as wide as long (Fig. 3A;B).

Maxilla I lateral lobe long, more than twice as wide as medial lobe, with six curved spatulate conical-shaped, 5 serrate spines and 2 little spines at the base; medial lobe with 4 large plumose setae apically and simple setae (Fig. 3C;D). Maxilla II outer lobes with approximately 12 long and pectinate spines; inner lobe apical margin with 20 plumose spines, thinner spines all over the external face and 6 short spines in the internal face (Fig. 3E). Maxilliped palp articles 1-4 bearing pairs of setae distolaterally and dense rows of setae distally; 3-4 lobes longer than wide (Fig. 3F).

Pereonite I longest, anterolateral margin not encompassing the head that projects itself forward the coxal plate in dorsal view (Fig. 1A). Pereonite II-VII about equal length. Posterior pleonal tergite with posterior margin reaching 2 curved parallel sutures either side of mid-line (*Sphaeroma*-type pleon) (Fig 1A;7A). Pleotelson, as wide as long in the middle, and with posterior half in a slightly V-shape, at lateral view, pleotelson has a general slight convex shape (Fig 1A;B;8A). Pleotelson with 10 grouping of setae on its surface, possibly mechanoreceptors (Fig.8A-C).

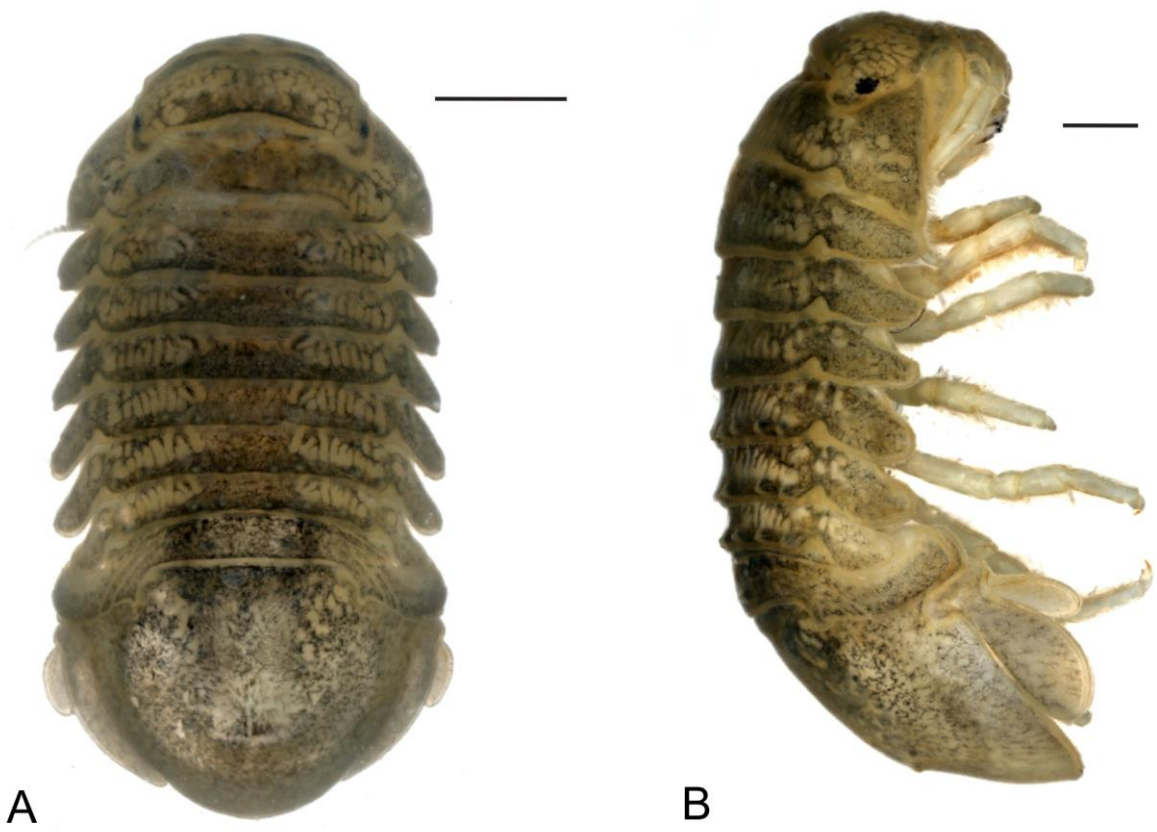

Fig. 1. - *Bilistra* n. sp. A) Male dorsal view; B) Male lateral view. Scale A: 2mm; B: 1mm.

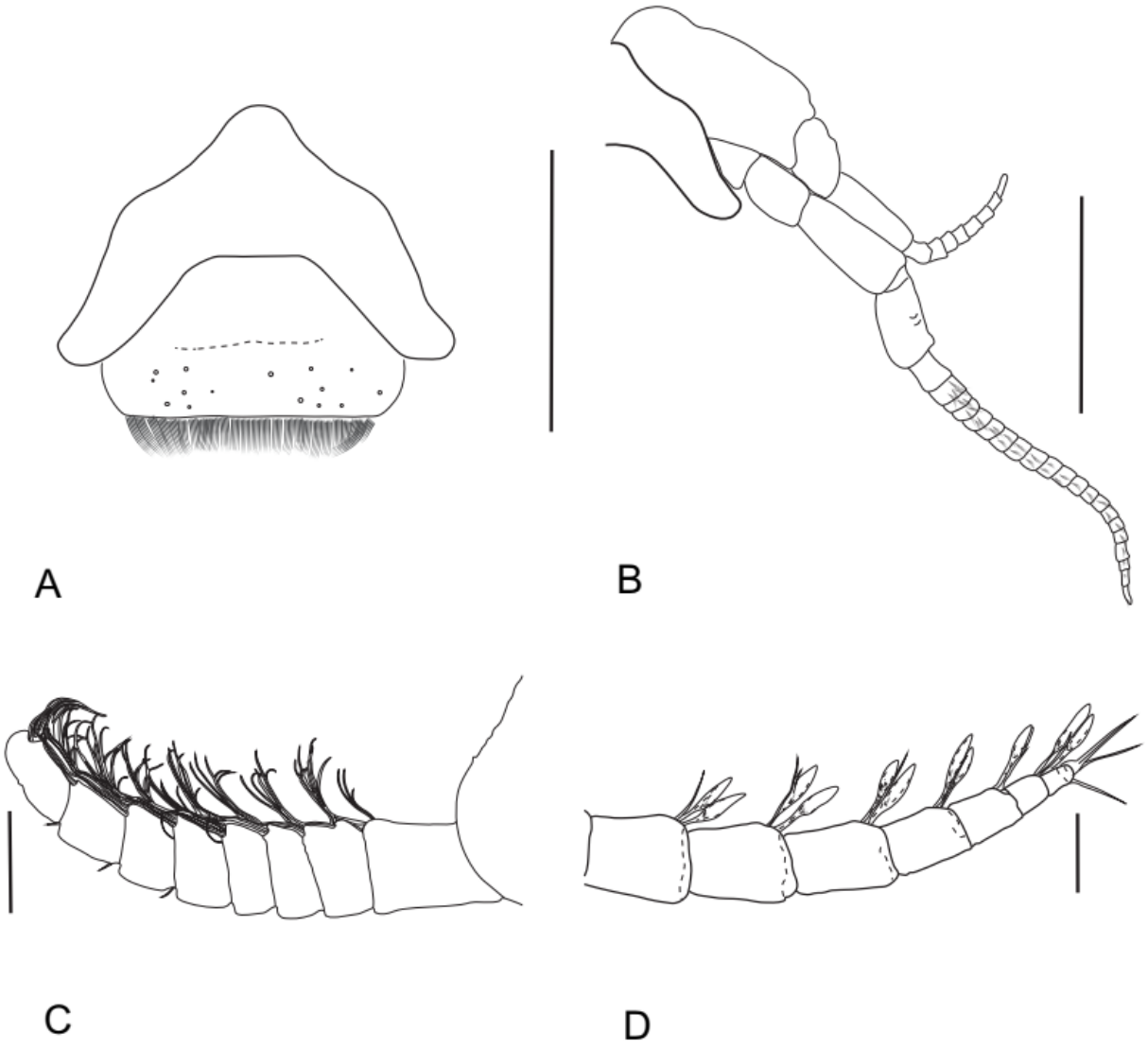

Fig. 2. *Bilistra* n. sp. A) Epistoma and Labrum. B) Antenna I and Antenna II. C) Antenna II details. D) Antenna I apical articles. Scales A-B: 1mm; C: 200µm; D: 100µm.

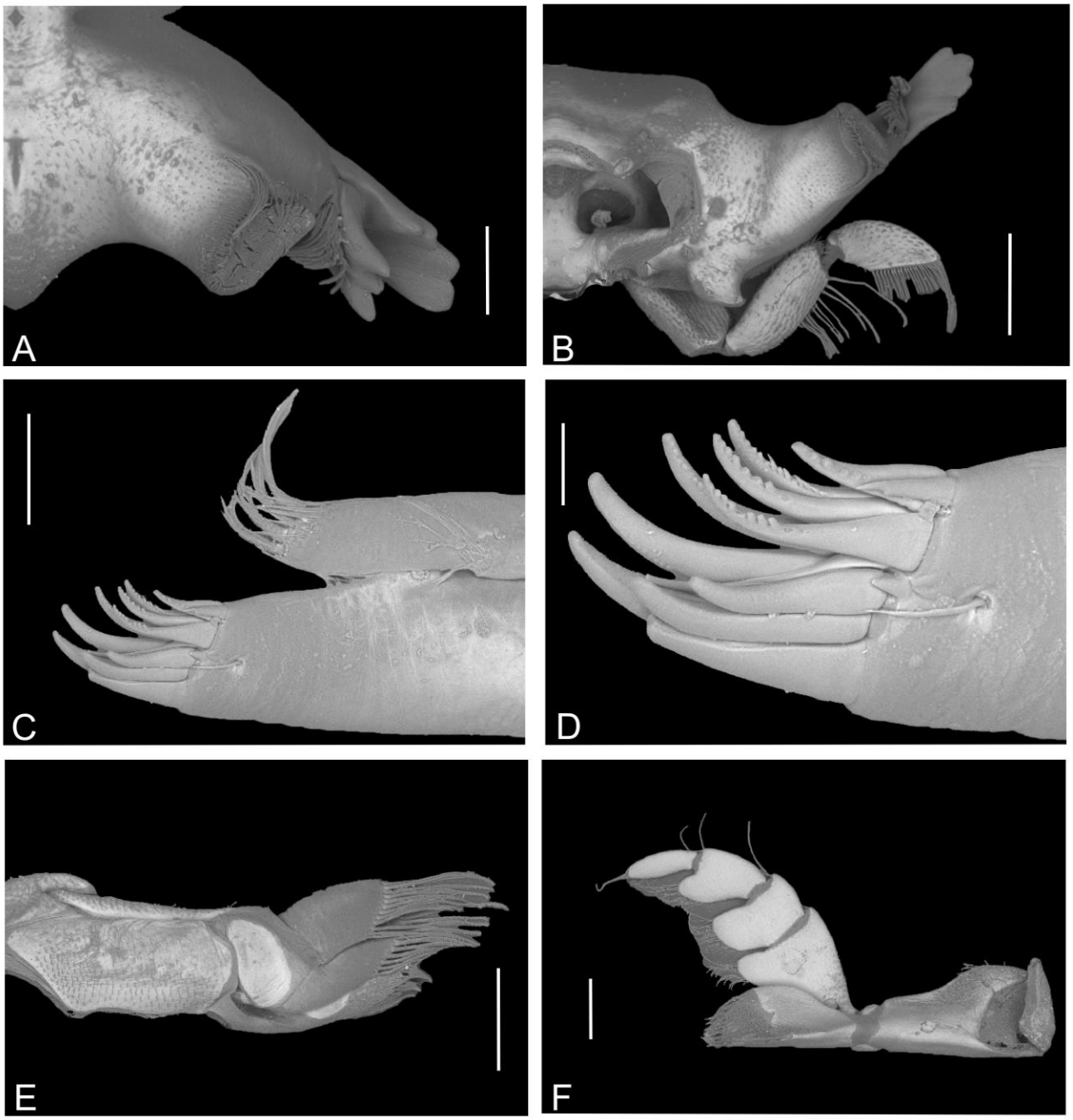

Fig. 3. - *Bilistra* n. sp. A-F) Mouth parts A) Left mandible, to show incisor process, molar process and lacinia mobilis tridentate; B) Right mandible and mandibular palp; C) Maxilla I; D) Maxilla I lateral lobe; E) Maxilla II; F) Maxilliped palp. Scales A:150µm; B:250µm; C:150µm; D:50µm; E-F:250µm.

Lengths of pereopods I-VII increasing to 133-175% in the last. Pereopod VII 54-59% body length. Pereopod VII articles (basis, ischium, merus, carpus, propodus and dactyli) lengths in relation: Male 1- 100 : 102 : 41 : 49 : 57 : 33; male 2- 100 : 91 : 39 : 43 : 51 : 31; male 3- 100 : 91 : 40 : 47 : 60 : 26; male 4- 100 : 92 : 38 : 41 : 32 : x and male 5- 100 : 95 : 38 : 45 : 58 : 32.

Dactyli stout, distal unguis thin, long and curved, approximately 2-4 times as long as conical ventral spine; 1 long setae approximately twice of spine, between unguis and spine, 2 setae

at the base of unguis. Pereopod merus and carpus with long spines distally, with few setae along all articles surfaces. All pereopods with inferior surface of articles 4-6 (merus, carpus, and propodus) covered by densely setulose mat (fringe); sparser setulose fringe covering all other pereopod surfaces (*Bilistra*-type pereopod) (Fig. 5).

Pereopod I with the thichest articles, articles 2 and 4 (basis and merus) robust; propodus less than twice as long as wide, outer margin indistinctly convex and inner margin straight; with 11 serrate setae, mainly in the distal half of its anterior face and with 1serrate long, conical and slightly curvated. Dactylus very stout, with one short spine bellow unguis (Fig. 4).

Pleopods I-III similar to each other, rami progressively increasing in size from I to III, with marginal setosity in exopodite increasing progressively and in endopodite decreasing only at pleopod III (table I). Pleopod II with an ovate exopodite with 35-44 plumose setae that are long at distal and short along exterior margins. Endopodite with 20-28 lateral setae; interior part differentiated, elongated, longer than endopodite, appendix masculina visible (Fig. 6B). Pleopod III exopodite with distal third separated by oblique and slightly curved articulation; protopodites more than 3 times as wide as long, intero-distally each with 2 spines; endopodites with internal vertical margin, and distal margin round; exopodites ovate; rami with plumose marginal setae, endopodites with long setae distally and along half-length of medial margins, very finely setulose around medio-proximal angle and close to plumose setae; exopodites with plumose setae distally and along entire outer margins; only distal setae are long, lateral setae decreasing in length proximally (Fig. 6C); endopodites with approximately 14-28 setae, exopodites with 27-48 setae (Fig. 6A-C; Table I.). Pleopods IV-V. Endopodites and exopodites ovoid, fleshy, with two transverse folds; exopodite IV with 8-11 short setae along proximal part of lateral margin; exopodite V with 2 small scale patches apically (Fig. 7C); endopodite V smaller than exopodite V. Uropodal protopodite approximately one third of uropod, exopodite 43-52% length of endopodite; each ramus nearly half as wide as long, endopodite distally evenly narrowed, exopodite more narrowly ovate; exopodite rounded, endopodite apically rounded (Fig. 7A;A'). Penes 2-3 times as long as wide, conical, apices separated (Fig. 7B).

Female - Oostegits at pereopods II-IV well developed, ventrally positioned.

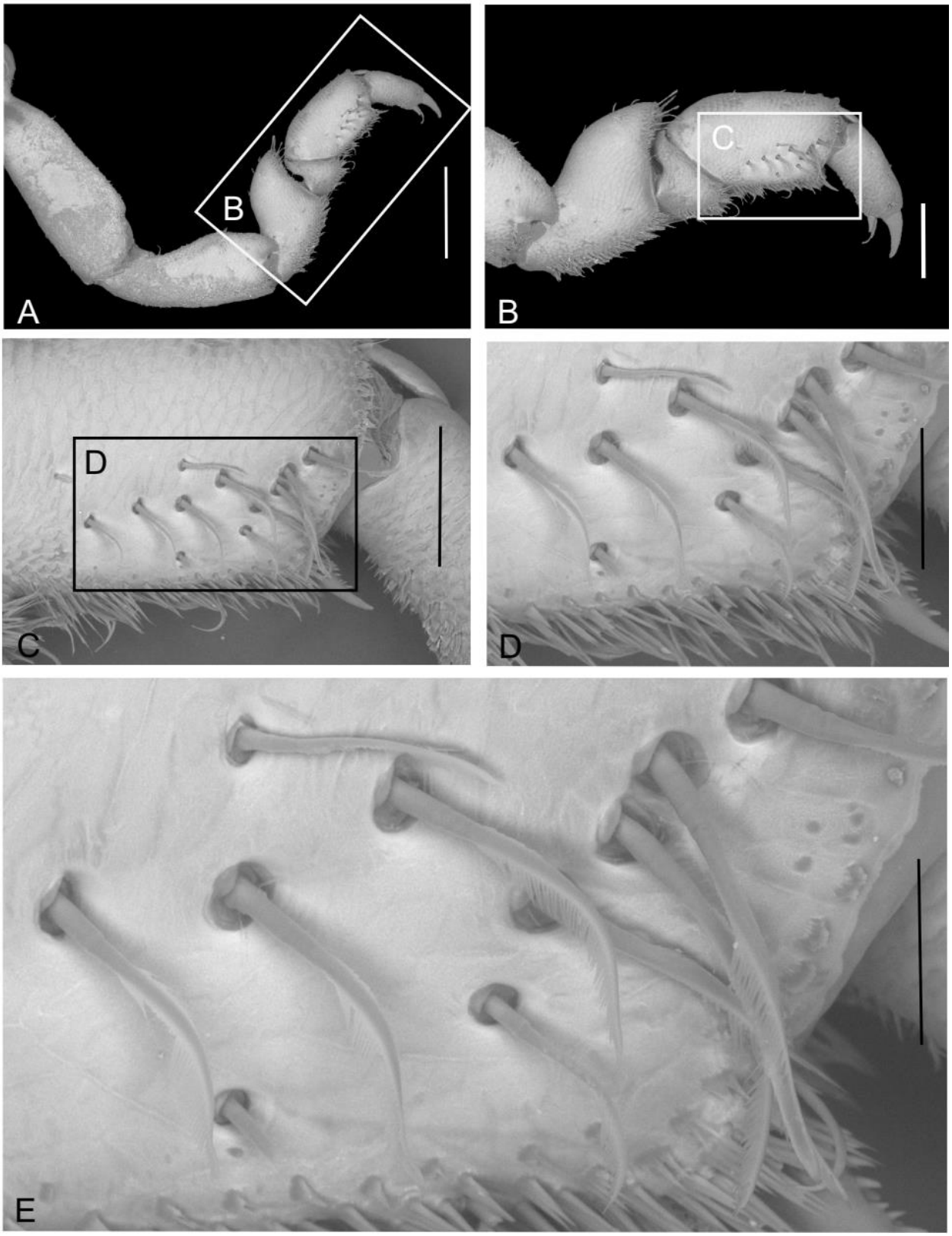

Fig. 4. *Bilistra* n. sp. A-E) Pereopod I, to show details. E) Serrate setae. Scale A:500µm B:250µm C:100µm D-E:50µm.

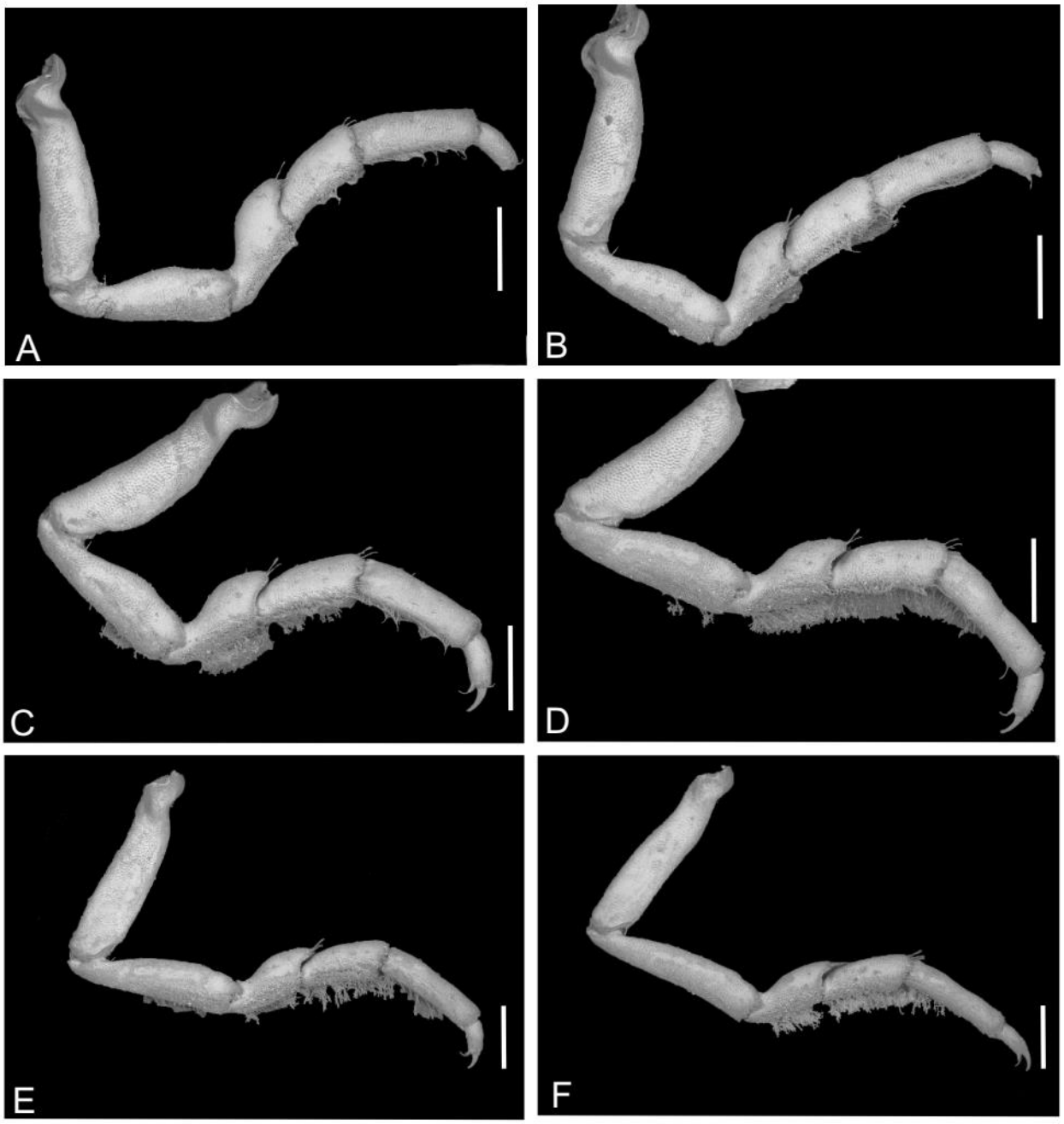

Fig. 5. *Bilistra* n. sp. A-F) Pereopods II-VII. Scale A-F:500µm.

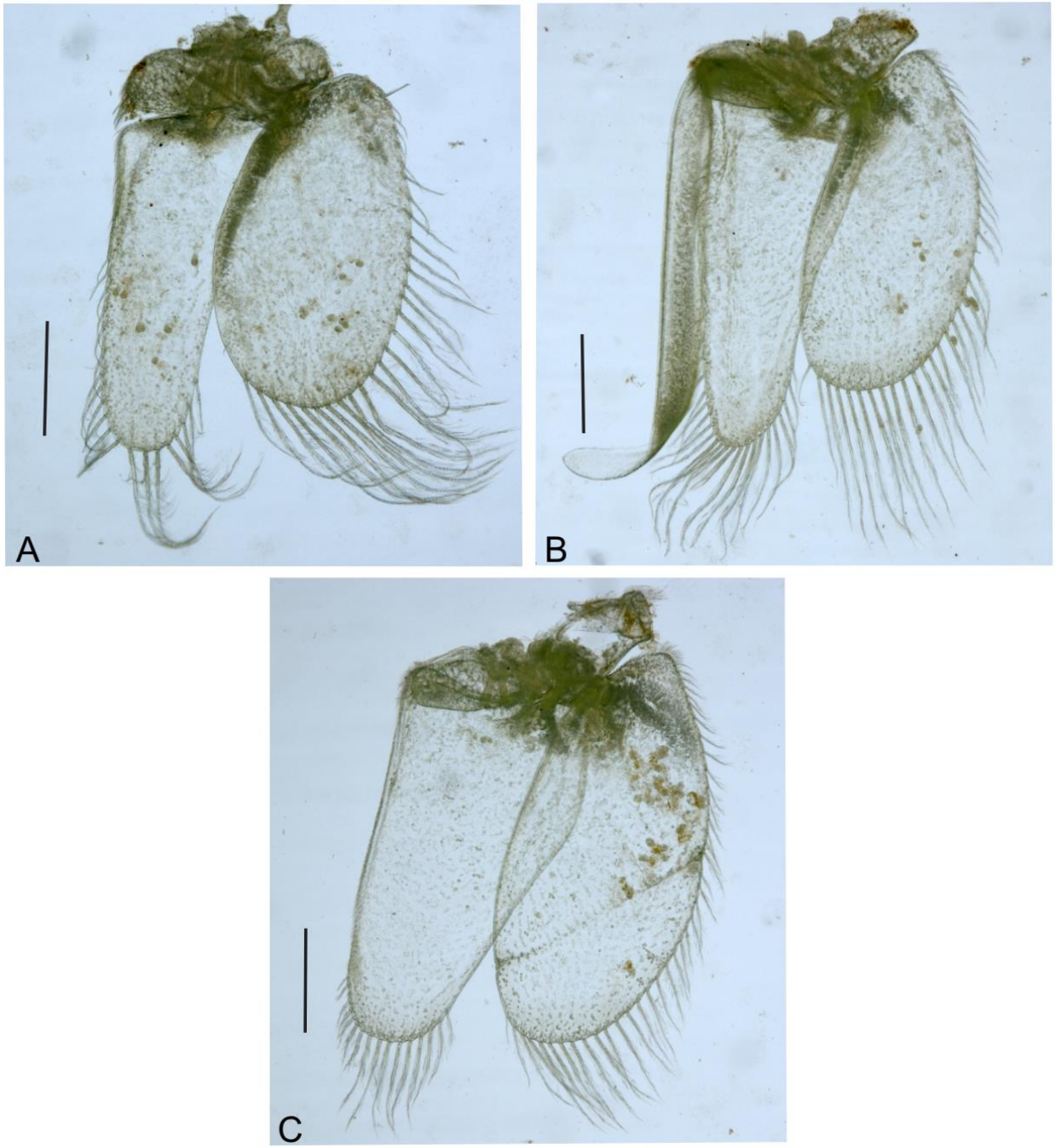

Fig. 6. - *Bilistra* n. sp. A-C Male; A) Pleopod I; B) Pleopod II, appendix masculina visible; C) Pleopod III, exopodite with articulation. Scale A-C:250µm.

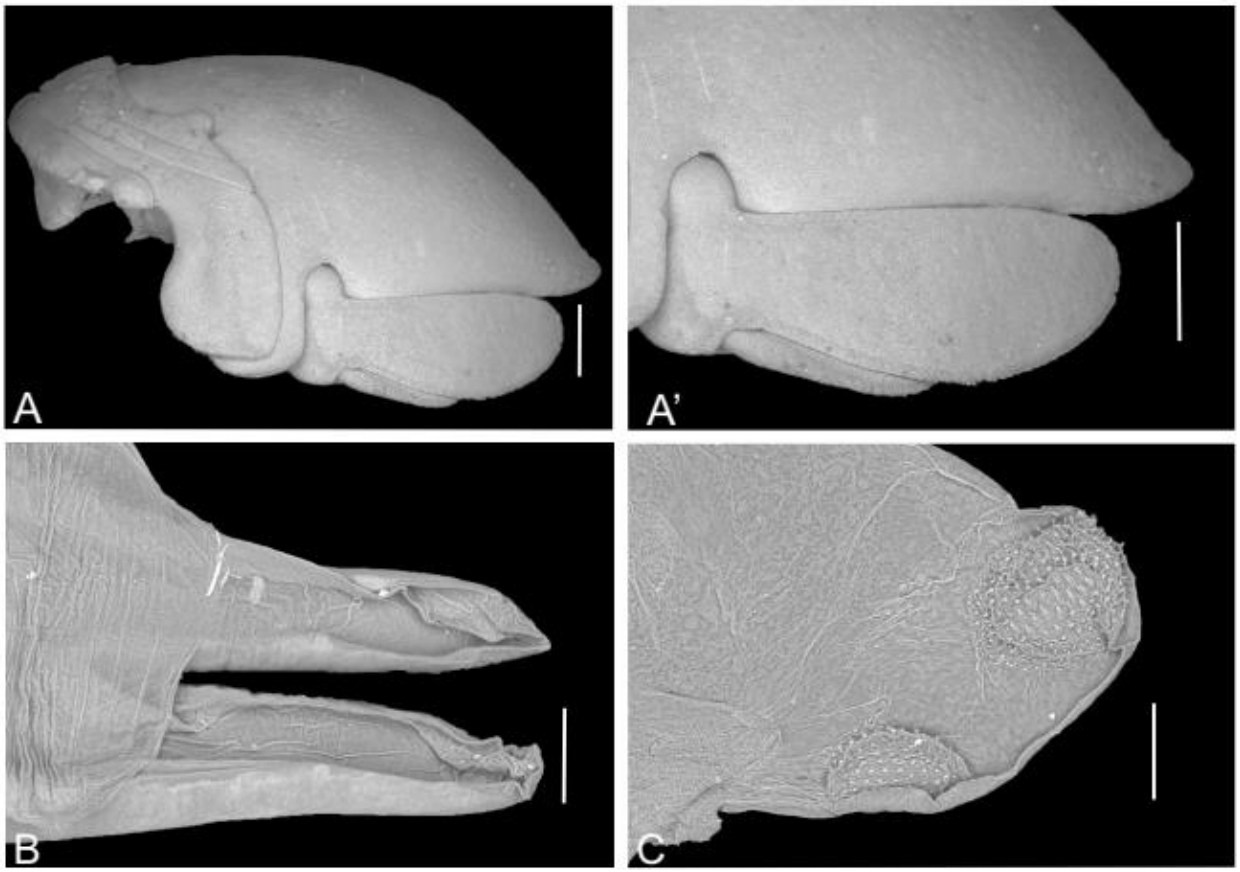

Fig. 7. – *Bilistra* n. sp. A) Female pleonite I, pleotelson, uropod lateral view; A') Female uropod details; B) Penes; C) Male Pleopod V, to show scale patches. Scales A-A'500µm; B-C:100µm.

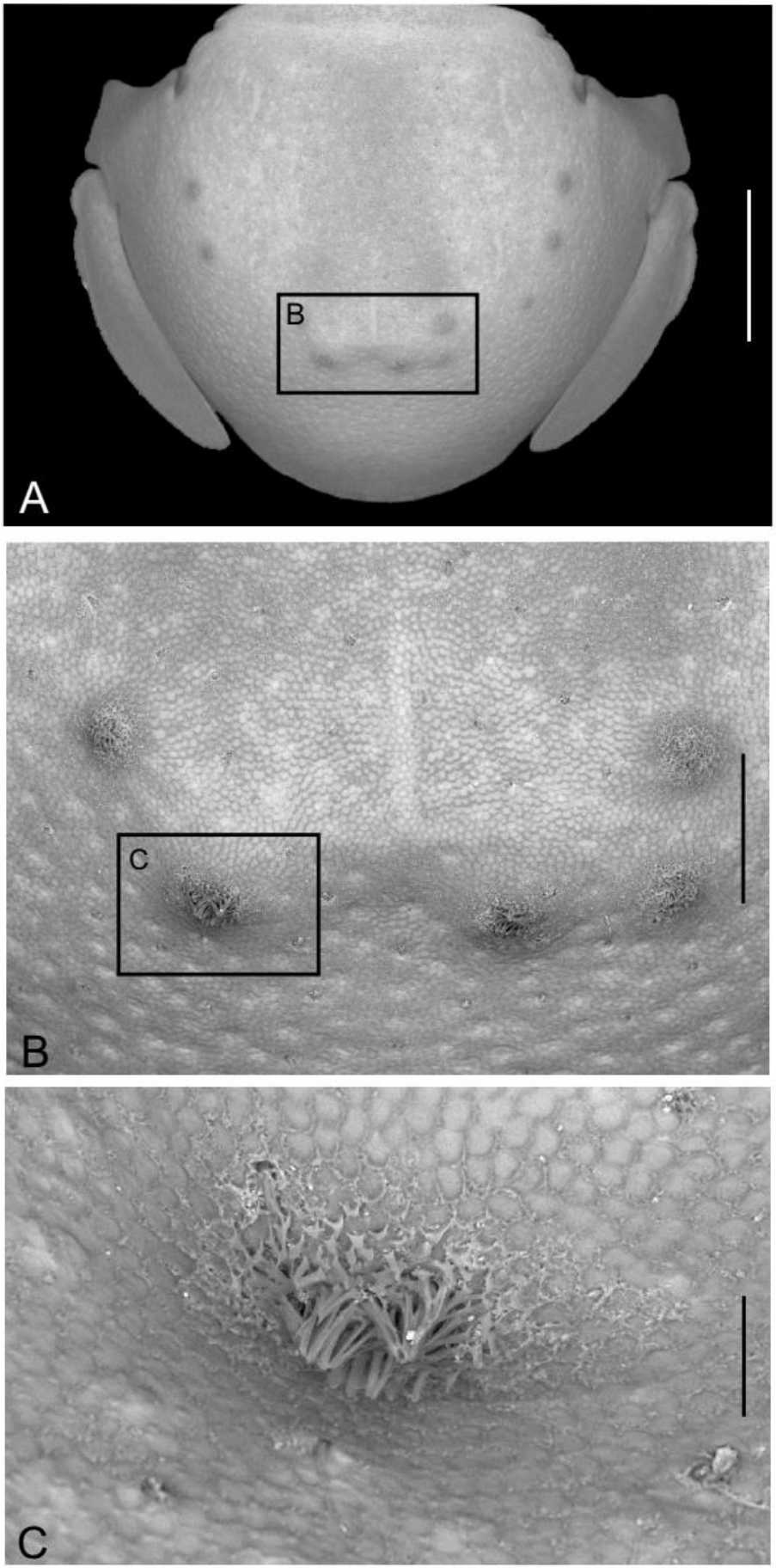

Fig. 8. - *Bilistra* n. sp. A-C) Male Pleotelson; C) grouping of setae details. Scales A: 1mm; B: 250µm; C: 50µm.

Table I. Some characteristic numbers and relative measures in *Bilistra* n. sp.

Metrics	**Female 1**	**Male 1**	**Male 2**	**Male 3**	**Male 4**	**Male 5**
Body length	9,63mm	8,72mm	8,80mm	11,78mm	6,72mm	7,69mm
A I (% A II)/ Number articles in flagellum	83,26/7	82,39/10	85,35/7	75,32/10	88/6	78,37/6
A II (% body length)/ Number articles in flagellum	36,56/18	34,51/18	30,80/14	33,98/21	34,12/16	34,92/17
Length of articles in flagellum in A I	100/38,40/82,97/108,39	100/40,74/73,80/114,15	100/57,91/97,30/134,68	100/41,36/59,50/86,66	100/34,64/59,46/91,30	100/39,24/70,88/92,68
Length of articles in flagellum in A II	100/72,32/132,89/143,60/474,93	100/188,19/344,72/331,67/960,24	100/111,6/225,6/166,4/576,6	100/213,36/335,14/362,87/1067,82	100/91,32/239,88/236,99/728,90	100/93,54/172,98/128,22/597,98
Lengths radios pereopods I/ II/ VII	100/109,99/148,11	100/125,74/154,54	100/128,85/160,48	100/125,91/158,03	100/126,65//133,03	100/147,30/175,30
Length radios of articles in pereopod VII	100/82,11/36,87/36,69/48,92/21,94	100/101,68/40,76/49,42/57,24/33,10	100/91,27/38,92/43,40/50,73/31,23	100/91,18/39,85/46,94/59,83/26,39	100/92,14/37,67/41,01/32,24/x	100/95,47/37,96/44,66/57,91/31,58
Relative length of pereopods II and VII in % of body length	40,22/54,16	46,60/57,27	44,24/55,10	47,22/59,26	51,42/54,02	49,70/59,14
Number of marginal setae on pleopod I (endopodite+ exopodite)	16+32	21+31	20+29	22+37	15+27	19+27
Number of marginal setae on pleopod II (endopodite+ exopodite)	20+38	26+36	23+35	28+44	21+34	24+37
Number of marginal setae on pleopod III (endopodite+ exopodite)	19/44	18+40	16+40	22+48	16+38	14+35
Uropodal protopodite	0,93mm	0,86mm	0,87mm	1,37mm	0,66mm	0,68mm
Uropodal endopodite	2,00mm	1,77mm	1,82mm	2,63mm	1,55mm	1,45mm
Uropodal protopodite (%uropodal endopodite)	46,25	48,7	47,42	51,97	42,64	47,13

Habitat and ecological remarks

Specimens of *Bilistra* n. sp. are only known from the Commentary cave (41° 2'13.01"S/172°48'13.57"E). This cave is located in the Takaka region, in northern South Island (Fig. 9A). This limestone cave has a single accessible entrance (Fig. 9B), located on the slope of a hill, which borders an alluvial plain lying to the upper Takaka (Figure 9C). It is inserted in the Motupipi limestone, which continues beneath the land surface to the west, probably for a considerable distance, but well below the local water table. The hill in which the cave entrance is located presents several small sinkholes, thus indicating an intense capture of surface water that is directed underground.

From the entrance hall, the conduits tend to descend until reaching a large gallery crossed

by a stream. In one region of the cave, the main drainage receives the contribution of a small tributary, with a very low flow (Fig. 9D). This small tributary arises from blocks on the side of the conduit. Most specimens of *B.* n. sp. (Fig. 9E) were observed in this small tributary, although few individuals were also found in the main drainage. Specimens were observed both alone and in small groups. The groups were found in small ponds with vegetal debris, probably attracted to such areas due to the presence of this food resource. In fact, one dissected specimen analyzed under scanning electron microscope presented vegetal parts within in the digestive tract (Figure 10), thus indicating that such materials may represent their main food resource. Specimens of *B.* n. sp. share their habitat with troglomorphic amphipods as observed for other *Bilistra* species (Sket & Bruce, 2004), though no interaction among the species could be seen during the collections.

It is important to mention that the ecological-evolutionary status of *B.* n. sp. remains doubtful. While it was only found in the Commentary cave, thus apparently being restricted to this cave (thus, representing a troglobitic species) it does not present strong troglomorphic traits. However, its body pigmentation is reduced (Fig. 9E), especially compared to *B. millari*, which occurs in surface waters. Furthermore, the number of ommatidia also differed considerably among such species*: B. millari* presents 30 ommatidia, while *B.* n. sp. presents 8, thus indicating a considerable eye reduction in the later. For *B. cavernicola* and *B. mollicopulans* the number of ommatidia was not informed on the original description (Sket & Bruce, 2004). Considering the above, although *B.* n. sp. is not highly troglomorphic, its morphological modifications (reduction in tegumentary pigment and ommatidia number) suggest that this species is restricted to caves (troglobitic), thus, deserving efforts for its conservation.

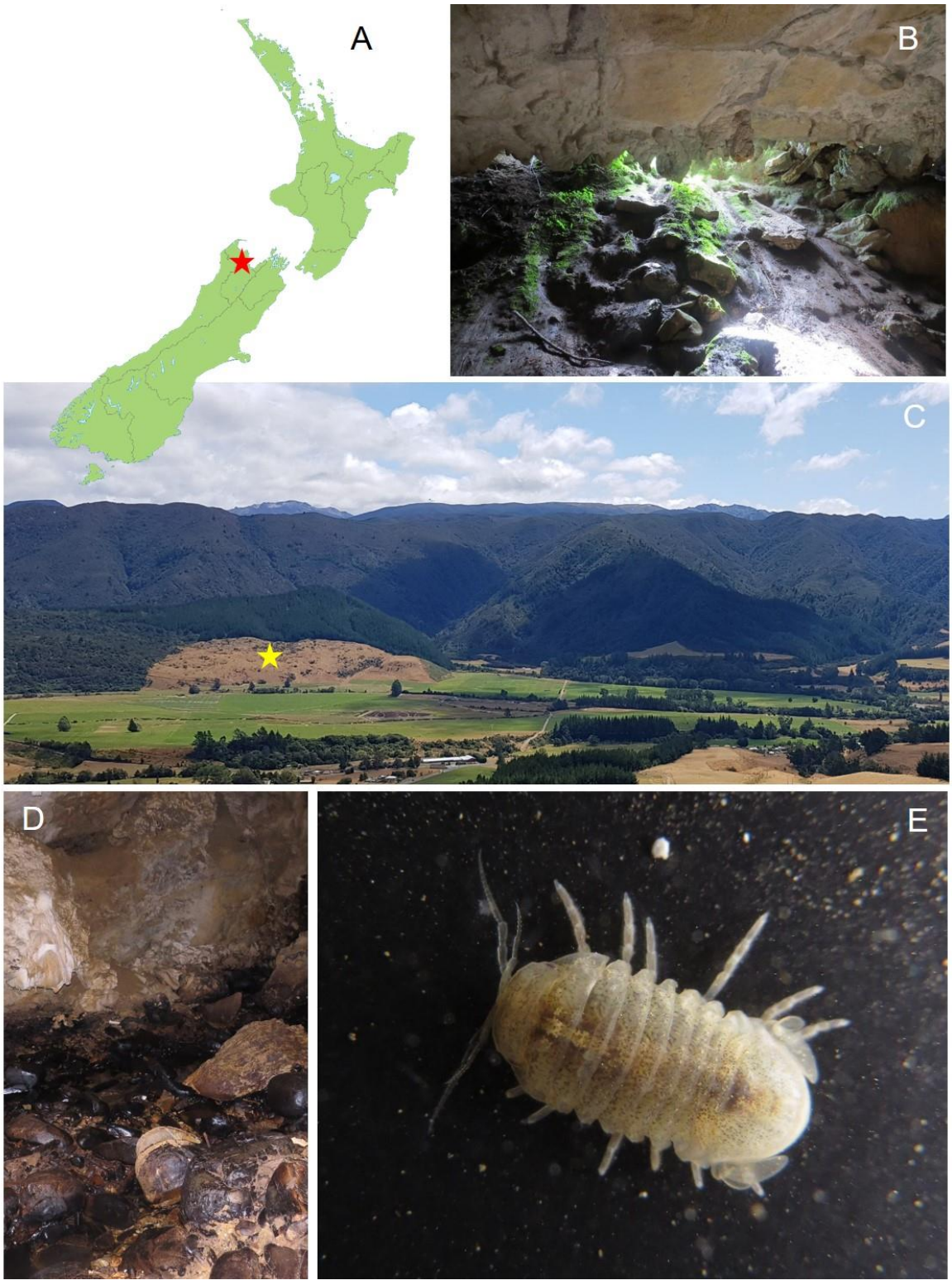

Fig. 9. - *Bilistra* n. sp. habitat. A) Location of Commentary cave on the South Island of New Zealand (red star); B) Commentary cave entrance; C) general aspect of the karst area around Commentary cave (cave entrance location indicated with a yellow star); D) Cave interior, showing a small drainage on which specimens of *B.* n. sp. can be found; E) Living specimen.

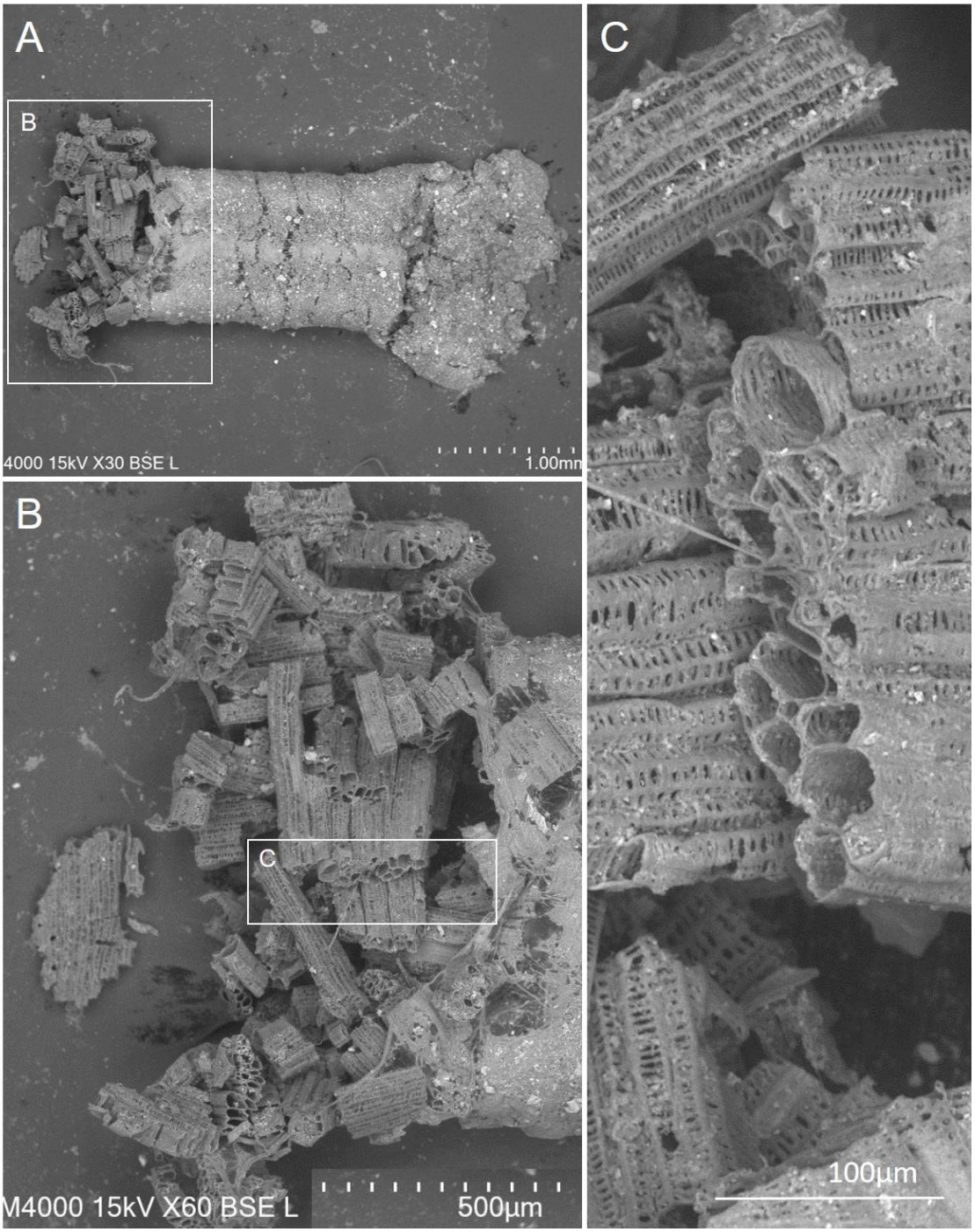

Fig. 10. - *Bilistra* n. sp. dissected intestine. A) A section of the digestive tract (the left part is the "upper"side and the right part represents the "lower" part of this segment; B) Plant material inside the digestive tract; C) Detail of the plant material, showing its fibrous aspect.

4 DISCUSSION

Bilistra n. sp.are similar to *B. mollicopulans* in body size (length and width), reduced eyes with black pigmentation visible deep below feebly faceted ocular region. Moreover, differs from *B. mollicopulans* by the body colour (browish yellow translucent in *B.* n. sp. and whitish translucent in *B. mollicopulans*). Furthermore, *B. mollicopulans* is devoid of tubercles on the pereon, presenting a caudal profile slightly concave while *B.* n. sp. has a general slightly convex

shape. The uropod in *B. mollicopulans* has apices with acute medial point in contrast to *B.* n. sp. with apices narrowly rounded.

Bilistra n. sp. is similar to *B. millari* in width (above 50% of body length), antenna II robust (35% as long as body), pereopods I-VI increasing up to 140% in the last one. And differs from it by body size (*B. millari* with 6,8 – 7,8 mm long and *B.* n. sp. with 6,7 – 11,7 mm long), body colour (*B. millari* is browish green), eyes well developed in *B. millari* (with 30 ommatidia each) and reduced in *B.* n. sp. (8 ommatidia), posterior margin of each tergite with scattered tubercles on pleotelson in *B. millari* and a row of tubercles at the posterior margin V, VI and VII tergites in *B.* n. sp., pleotelson with flattened or scarcely concave caudal surface in *B. millari* and slight convex shape in *B.* n. sp.

Bilistra n. sp. is similar to *B. cavernicola* in body size (length and width), reduced ocular region with black pigment visible below cuticle, maxilla II with about 12 spines on lateral lobes, pereopod VII about 60% of body length and uropod apices narrowly rounded. And differs from it by body colour (browish yellow translucent in *B.* n. sp. and whitish translucent in *B. cavernicola*); presence of a row of tubercles at the posterior margin of IV, V and VI tergites; antenna II robust in *B.* n. sp. and slender in *B. cavernicola*; *B.* n. sp. has some distinctive ommatidia (8) while in *B. cavernicola* the ocular region indistinctly faceted.

B. cavernicola is considered as a troglomorphic species, mostly because of the great eye reduction and lack of pigmentation, besides being found only in cave waters between Motueka and East Takaka. Considering such traits, the new species herein described must also be considered troglomorphic (as already mentioned). Although it has a lesser eye reduction in comparison with *B. cavernicola*, *B.* n. sp. still presents a notable eye reduction and it was only found in Commentary cave.

Conservation issues:

Although the mountains surrounding the Takaka Valley are preserved (being predominantly forested), the hill where the Commentary cave is located has been partially deforested, specifically in the area where the cave entrance is located. Several sinkholes are visible along this slope, and considering that the hill was originally covered by forests, this soil exposure can lead to an increase in the transport of sediment into the cave and the consequent siltation of underground drainages. Additionally, there is an intense land use on the Takaka valey (especially for agriculture) though such alterations are mainly occurring downstream in relation to the cave location. Considering the status of this new species (a cave-restricted organism), it is important to protect it as its habitat. Currently, no *Bilistra* species is listed on the IUCN red list for endangered species, thus it is important that especially the cave species from this genus to be evaluated, in order to verify eventual threats over them.

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