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FIRST SOUTH AMERICAN PHREATOGAMMARID, WITH COMMENTS ON THE ARRANGEMENT OF COXAL AND STERNAL GILLS, AND ON THE BIRAMOUS CONDITION OF THE SEVENTH PEREIOPOD IN AMPHIPODS

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A B S T R A C T

Ruffia patagonica, a new genus and species of phreatogammarid amphipod, is described from a coastal cave on Madre de Dios Island (Chilean Patagonia). This is the first record of the family outside continental waters of New Zealand and New Caledonia. The new taxon displays some of the more distinctive features of the family, such as gnathopods with the palm margin lined with numerous unicuspid robust setae and with a denticulated inner margin of the dactylus; fifth pereiopod clearly smaller than pereiopod six and seven; ''coxal gill'' (= exopod) absent from the seventh pereiopod; oöstegites broadened; urosomites with posterodorsal robust setae; and third uropod equiramous with an elongated peduncle and with unsegmented cylindrical rami, the latter provided with numerous robust setae. In addition, the sternites of all pereionites, pleonites, and urosomites show a portion of integument delimited by hyaline frill that is presumably osmoregulatory in function, and is equivalent to the pair of sternal gills displayed by other phreatogammarids on several body somites. The new genus is unique among phreatogammarids in displaying sexual dimorphism in the third epimeral plate, in the protopod of the third pleopod, in the armature of the first uropod, and in the endopod of the second uropod. Even though Phreatogammaridae have been suggested to be related to the broadly distributed marine family, Melphidippidae, their restriction to continental waters of former Notogean territories points to vicariance by plate tectonics of a non-marine ancestor rather than to dispersal as the main mechanism leading to their current distribution pattern.

KEY WORDS: Amphipoda, biogeography, Chile, Gondwana, Magellanic region, *Ruffia patagonica*, stygofauna, vicariance

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INTRODUCTION

General Overview

The diversity of amphipod crustaceans in continental waters of South America is scant compared to other landmasses. Aside a handful of groundwater bogidiellids (Koenemann and Holsinger, 1999, and references therein) and two ingolfiellids (Noodt, 1961, 1965), the only amphipods known thus far from that continent belong to the speciose epigean talitroid Nearctic and Neotropical genus *Hyalella* (Smith, 1874), with ca. 44 South American species (González and Watling, 2002, 2003a, 2003b; González et al., 2006), 12 of which are endemic to the high Andean lake Titicaca (González and Watling, 2003a). Barnard and Barnard (1983) were the first to notice this feature; they adduced that both the thermophoby of amphipods as a group, and the remoteness of South America to the rest of Gondwana could explain the continent was left aside by the major waves of colonization of continental waters by amphipod crustaceans. Alternatively they suggested that the most ancient South American freshwater amphipods might have become extinct and were replaced by the recently evolved and successful hyalellids.

In this framework, any new record of continental water amphipods from the region can shed light on the causes of its faunal impoverishment. Since it remains unclear whether bogidiellids are primarily freshwater forms, any finding of other families supposedly of old freshwater origin could be very relevant in that respect. Here we describe a new genus and species of phreatogammarid from southern Chile, a seemingly limnic family known thus far only from continental waters of New Zealand and New Caledonia. The specimens were gathered in a coastal limestone cave during the French-Chilean caving expedition ''Ultima Patagonia 2006'' to Madre de Dios Archipelago (Province Ultima Esperanza; XIIth Region), organised by the Association Centre-Terre and the French Federation of Speleology (Jaillet, 2006a; Bréhier, 2007; see also http://www.centre-terre.fr). Next to the exploration and survey of more than 8 km of underground passages, the discovery of archeological remains and the performance of geomorphological studies (Jaillet, 2006b; Jaillet et al., 2008), the team carried out sampling of cave fauna in both aquatic and terrestrial habitats. The new taxon, completely unpigmented and with partially regressed eyes, is the fourth stygobiont amphipod reported from Chile after the coastal interstitial bogidiellid *Pseudingolfiella chilensis* (Noodt, 1959) and the ingolfiellideans *Ingolfiella manni* Noodt, 1961 and *Ingolfiella uspallatae* Noodt, 1965 (Noodt, 1961, 1965).

Habitat

The animals were gathered in a resurgence placed at the end of one of the branches of Seno Eleuterio (Fig. 1), one

of the main fjords in the western part of Madre de Dios, an island 880 m in elevation placed 250 km NW of Puerto Natales. The island, of ca. 600 km^2, supports a mean annual rainfall of 8000 mm and has its valleys under 400 m in altitude densely covered with Magellanic primary *Notophagus* forest. Nevertheless, the island presumably remained completely glaciated until 11,000 yr BP (Sugden et al., 2005). The geological basement of the western part of the island mostly corresponds to highly karstified limestones Upper Carboniferous to Lower Permian in age (Forsythe and Mpodozis, 1983).

Cave de Fin del Seno is a karstic spring that opens onto the beach a few meters from the shore, although at high tide the sea can reach it. It is a through cave 186 m long and −16 m deep, whose main entrance leads to a sump 78 m long and −13 m deep, which emerges in a subterranean lake opening in the forest. A second sump 57 m long and −9 m deep, with a blind secondary branch reaching −16 m depth, departs from this lake to end in a small room with a sinkhole opening to the exterior, too small for a person to fit through. A violently flowing creek empties into this room from a lake placed outside only 30 m away.

During our first visit, the outflow was estimated at 200 $L \cdot s^{-1}$ and no pycnoclines were noticeable, although the water was clearly brackish. The current was much lower on the next dives, and a sharp pycnocline was present at −10 m depth.

The animals were rare in the cave during episodes of high rainfall when the freshwater outflow was more intense, but they were quite numerous below the sharp halocline developed in the water column during low freshwater outflow periods. That could suggest a preference of the animals for brackish water. Furthermore, they seemed to be closely linked to the substratum. No other caves on the island rendered the species, nor was it found in holes dug out on the seashore (Karaman-Chappuis interstitial sampling method), nor in ordinary marine shallow-water habitats also investigated for invertebrate fauna. Unfortunately, the marine environment in the immediate vicinity of the cave was not surveyed for amphipods, and the possibility remains that the new taxon was an ordinary marine littoral form that had penetrated into the salt-water layer of the cave as a natural extension of its ordinary habitat.

Material and Methods

Sampling was done by means of cave diving techniques. Most specimens were collected using an artificial substratum (a plastic bottle filled with polyamide line, in which animals get entangled) laid in the deepest part of the cave for a month, and with traps baited with meat set nearby for 24 h. Additional specimens where caught directly by sight with a hand net. The animals were fixed in 70% ethanol and, once at the laboratory, internal tissues of several specimens were partially cleared with lactic acid to facilitate study. Drawings were prepared using a camera lucida on an Olympus BH-2 and a Leica DM2500 microscope, both equipped with Nomarski differential interference contrast optics. Body measurements were derived from the sum of the maximum dorsal dimensions of individual somites excluding the telson. Appendages preserved on permanent slides were mounted in lactophenol and the coverslips sealed with nail varnish. Material is deposited in the Crustacea collection of the Zoological Museum Amsterdam [ZMA].

Following Boxshall (2004) a distinction is made in descriptions of the antennule and antenna between articles (corresponding to subdivisions of true segments by the formation of annuli, each lacking intrinsic musculature) and proper segments (displaying intrinsic musculature). The so-called lobes (medial and lateral), and the palp displayed by the amphipod maxillule, are identified as coxal and basal endites and as endopod, respectively, on the basis of comparison with the basic pattern exhibited in malacostracan crustaceans where this limb is biramous and comprises a protopod with coxa and basis, each with a single endite, an up to 3-segmented endopod, and an unsegmented exopod (Boxshall, 1997). The segmentation of the maxilliped is homologised with that of pereiopods, with the so-called palp corresponding to the merus-dactylus portion, whereas the inner and outer lobes are identified as basal and ischial endites, respectively. This precise naming is especially necessary after the recent discovery of amphipods displaying a supplementary, innermost lobe on the maxilliped next to the usual two (Iannilli et al., 2006, and references therein); this additional lobe corresponds to an ordinary coxal endite.

Following Watling (1989), the term ''spine'' in descriptions is restricted for rigid armature elements with a hollow central core that do not articulate basally to the body integument.

Gnathopods I and II, and pereiopods III to VII appear abbreviated elsewhere as G1-G2 and P3-P7, respectively; uropod I-III, as U1-U3.

Systematics

Order Amphipoda Latreille, 1816
Suborder Gammaridea Latreille, 1802
Phreatogammaridae Bousfield, 1982

Emended Diagnosis.—Head lacking rostrum and anteroventral notch, with truncate lateral lobes. Antennule longer than antenna, with 2- to 8-articulate accessory flagellum. Antennules and antennae lacking calceoli. Inner lobes of paragnaths wanting. Medial margin of coxal endite of maxillule lined with setae; endopod 2-segmented. Inner lobe of maxilla with transverse row of setae on anterior surface. Gnathopods subchelate, with palm margin lined with numerous (except in *Caledonietta* Iannilli and Ruffo, 2007, which bears few) unicuspid robust setae; dactylus with denticulated inner margin. Pereiopod V much smaller than P6-P7. Coxal gills present on G2 and P3-P6. Paired sternal gills present on pereionites II to VI (*Caledonietta*); on pereionite VII only (*Phreatogammarus fragilis*; *P. propinquus*); apparently wanting (*P. helmsii*; *P. waipoua*); or displaying an osmoregulatory epithelial field on sternites of all pereionites, pleonites and urosomites (*Ruffia*, n. gen.). Oöstegites present on G2 and P3-P5 (*Phreatogammarus*, *Ruffia*) or on G2 and P3-P4 (*Caledonietta*), and at least some large and broad. Urosomites provided with dorsal robust setae on posterior margin. Uropod III equiramous, with elongated protopod; rami cylindrical, with numerous robust setae; exopod unsegmented. Telson fully or partially cleft, with appressed lobes.

Composition.—Thus far, the family comprises three genera and six species. *Phreatogammarus* Stebbing, 1899 (type genus) is endemic to New Zealand and embraces four species: *P. fragilis* (Chilton, 1882) (type species) is the only stygobiont, whereas *P. propinquus* Chilton, 1907, *P. helmsii* Chilton, 1918 and *P. waipoua* Chapman, 2003 are all epigean forms (Chapman, 2003, 2004). *Caledonietta maryae* Ruffo and Iannilli, 2007 (stygobiont) is a monotypic genus recently described from groundwaters in New Caledonia (Ruffo and Iannilli, 2007). *Ruffia patagonica*, n. gen., n. sp., described herein, is endemic to the subterranean waters of Chilean Patagonia.

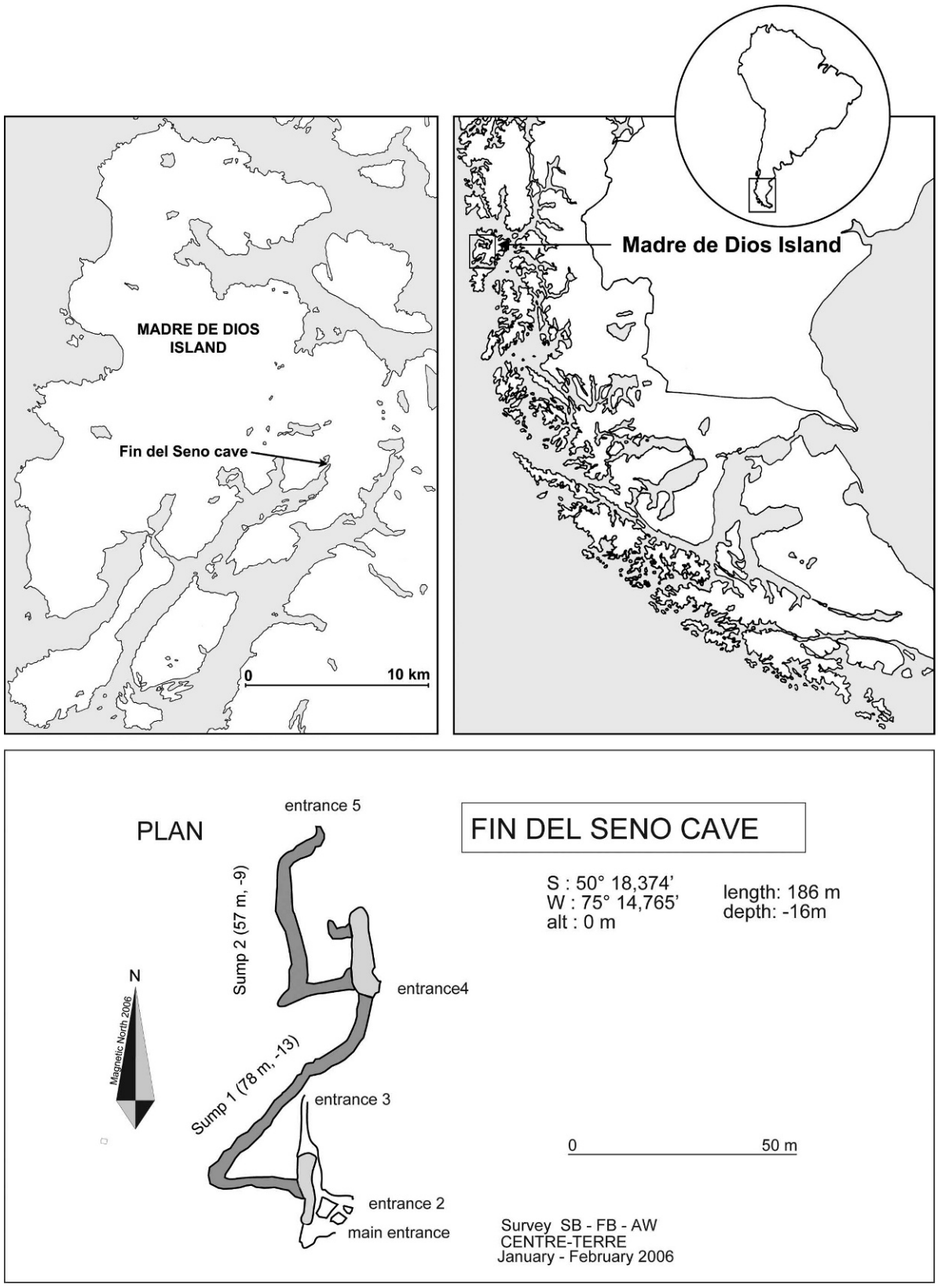

Fig. 1. Location and topography of Fin del Seno cave on Madre de Dios Island, SW Chile. Dark grey shading on cave topography denotes completely submerged passages, light grey shading, subterranean lakes; portions in white correspond to terrestrial passages.

Ruffia n. gen.

Diagnosis.—Antennular accessory flagellum 2-articulate. Antennal peduncle segment 1 swollen, protruding laterally. Mandible molar process with modified, distally expanded setulose spade-like robust seta on distal margin. Coxal endite of maxillule fully setose medially; basal endite with 11 rake-like robust setae. Gnathopods subsimilar in appearance although G1 slightly smaller than G2; both with elongate carpus and subchelate propodus, latter with subparallel margins and with oblique palm furnished with numerous, evenly spaced flagellate robust setae. Oöstegites present on G2 and P3-P5; those on G2 and P3 spatulate, that on G2 largest and widest, those on P4-P5 slender, normal. Coxae of P5-P7 discontiguous; anterior lobe of coxa VI vestigial; lobe wanting on coxa VII. Basis of P5-P7 slender. Third epimeral plate strongly sexually dimorphic. Protopod of pleopod III sexually dimorphic, displaying finger-like anterolateral process in male. Urosomites I-III each with pair of posterodorsal robust setae; ecdysial robust seta present on urosomite I anterior of the insertion of uropod I. Uropod I protopod with basofacial robust seta; exopod with modified armature in male. Endopod of uropod II sexually dimorphic, expanded and with modified armature in male. Telson wider than long, cleft until midway, with evenly rounded lobes. Ordinary sternal gills absent, but an area of permeable epithelium delimited by hyaline frill is present on sternites of all pereionites, pleonites and urosomites.

Etymology.—Genus named after Prof. Sandro Ruffo (Verona), in recognition of his lasting contribution to the knowledge of amphipod taxonomy and biogeography.

Type Species.—*Ruffia patagonica* n. sp. by original designation.

Ruffia patagonica n. sp.
Figs. 2-11

Material Examined.—Fin del Seno cave, Isla Madre de Dios, Ultima Esperanza, XIIth Region, Chile. Coordinates: 50°18,374′S; 75°14,765′W. Holotype: Female (oöstegites developed, setose) 6.62 mm, completely dissected and mounted on a single slide [ZMA Amph. 206064]. Paratypes: One female 5.22 mm and 7 preparatory females (oöstegites non-setose) 4,99, 4.83, 5.17, 4.49, 5.32, 5.28 and 5.80 mm, all in one vial, 70% ethanol [ZMA Amph. 206065]; six males 4.78, 4.70, 4.90, 5.12, 4.19, 3.72 mm in one vial, 70% ethanol [ZMA Amph. 206066]; foregoing male paratype 4.78 mm partially dissected, with G1-G2, P3, third epimeral plates with corresponding pleopods, plus uropods I-III and telson separately preserved and mounted on a single slide. Collected by F. Bréhier, 22 February 2006.

Diagnosis.—As for the genus.

Description of Female.—Body unpigmented. Head with small, rounded ordinary faceted eyes (Fig. 2A, B). Urosomite I with pair of long simple setae anterior of ecdysial robust seta (Figs. 2A and 11A; not represented in Fig. 10A). Epimeral plates (Fig. 2C) evenly rounded, with 0-1-3 flagellate robust setae on distal margin, respectively; posterior margin of each plate with three simple setae.

Antennule (Fig. 3A) with peduncle segments progressively shorter towards distal, relative lengths 100: 82: 44; main flagellum up to 19-articulate, each article with single reduced aesthetasc except proximal six; accessory flagellum slightly surpassing distally proximal article of main flagellum.

Antenna (Fig. 3B) with slender gland cone; segments 4-5 of peduncle about equal in length; flagellum up to 11-articulate, about same length as peduncle.

Labrum with coarse tiny setules posterodistally (Fig. 3D). Paragnaths with two different types of coarse spinules distally on each lobe (Fig. 3E).

Left mandible with 6-denticulate incisor and 4-denticulate lacinia, latter articulated via proximo-lateral condyle to incisor (Fig. 4A, B). Spine row comprising four elements, each pappose along one side only (Fig. 4A). Molar process columnar with complex arrangement of plates and spinules on grinding surface, and with short pappose molar seta on proximal margin (Fig. 4A); distal margin with cluster of pappose slender setae aside modified spade-like robust seta (Fig. 4D); mandibular palp (Fig. 4A) 3-segmented, relative length of segments 46: 100: 65; proximal segment naked; second segment with about nine unequal setae along distal half of medial margin; distal segment with medial margin expanded about midway, armature comprising three E-setae, about 10 D-setae, and isolated B-seta; ornamentation of setae as figured; patch of short lanceolate spinules (Fig. 4B) present on lateral surface of segment.

Right mandible as left counterpart except for 4-denticulate incisor and bifid lacinia (Fig. 4F), both branches of latter lined distally with numerous rounded denticles; anterodistal margin of lacinia with patch of spinules (Fig. 4E).

Maxillule (Fig. 5A) coxal endite with ca. 17 plumose setae along medial margin plus isolated shorter subdistal seta on posterior surface; distal margin of endite evenly rounded. Endopod distal segment with six flattened robust setae distally and three subdistal setae on posterior surface; isolated pore opening on anterior surface of segment as figured; distal margin of segment with row of five short triangular spiniform processes.

Maxilla (Fig. 4G) inner lobe with heterogeneous array of setae (viz. simple, bicuspidate, tricuspidate, plumose or serrulate; Fig. 4G, H) along distal and medial margins; oblique row of setae considerably shorter than plumose setae on anterior surface; outer lobe with subdistal row of unipinnate setae on posterior surface plus a cluster of more slender, shorter simple setae arranged on distal margin as figured.

Maxilliped (Fig. 5B) basal endite subrectangular, with three flattened robust setae subdistally close to distomedial angle, plus row of long plumose setae and long slender robust seta subdistally on medial margin. Ischial endite (Fig. 5C) reaching distal end of carpus, with ca. 15 lanceolate robust setae along straight medial margin; lateral margin evenly convex.

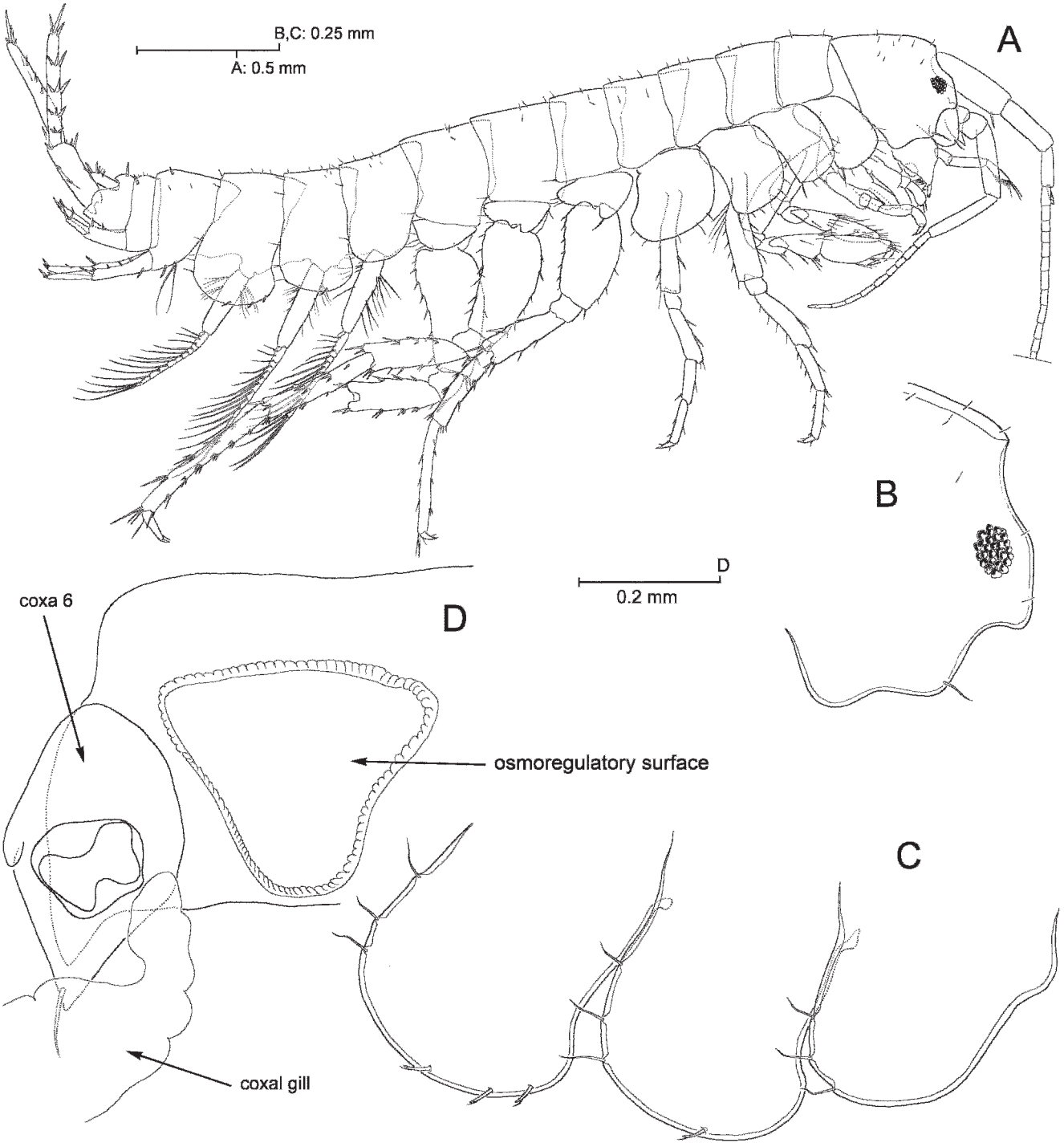

Fig. 2. *Ruffia patagonica*, n. gen., n. sp. preparatory female paratype 4.99 mm. A, habitus, lateral; B, detail of head; C, right epimeral plates; D, sternite of sixth pereionite showing putative osmoregulatory surface, ventral.

Coxal gills (Figs 2D; 7A; 8A, C; 9A, B) present from G2 to P6, ovate, smooth and stalked. Oöstegites (Figs 8A, C; 9A) spatulate with plumose margins. Osmoregulatory field on body sternites inverted trapezoid in outline on pereionites and pleonites (Fig. 2D), and ellipsoid with main axis perpendicular to longitudinal body axis on urosomites.

Coxal plates I-IV not reduced, about similar in length (Figs 2A; 6A; 7A; 8A, C), each with anterior margin partially overlapping one in front, with sparsely set simple setae along distal margin and variably concave posterior margin. Coxa V (Fig. 9A) with well developed anterodistal lobe bearing single distal seta; single seta subdistally on posterior lobe. Coxa VI (Fig. 9B) with single simple seta

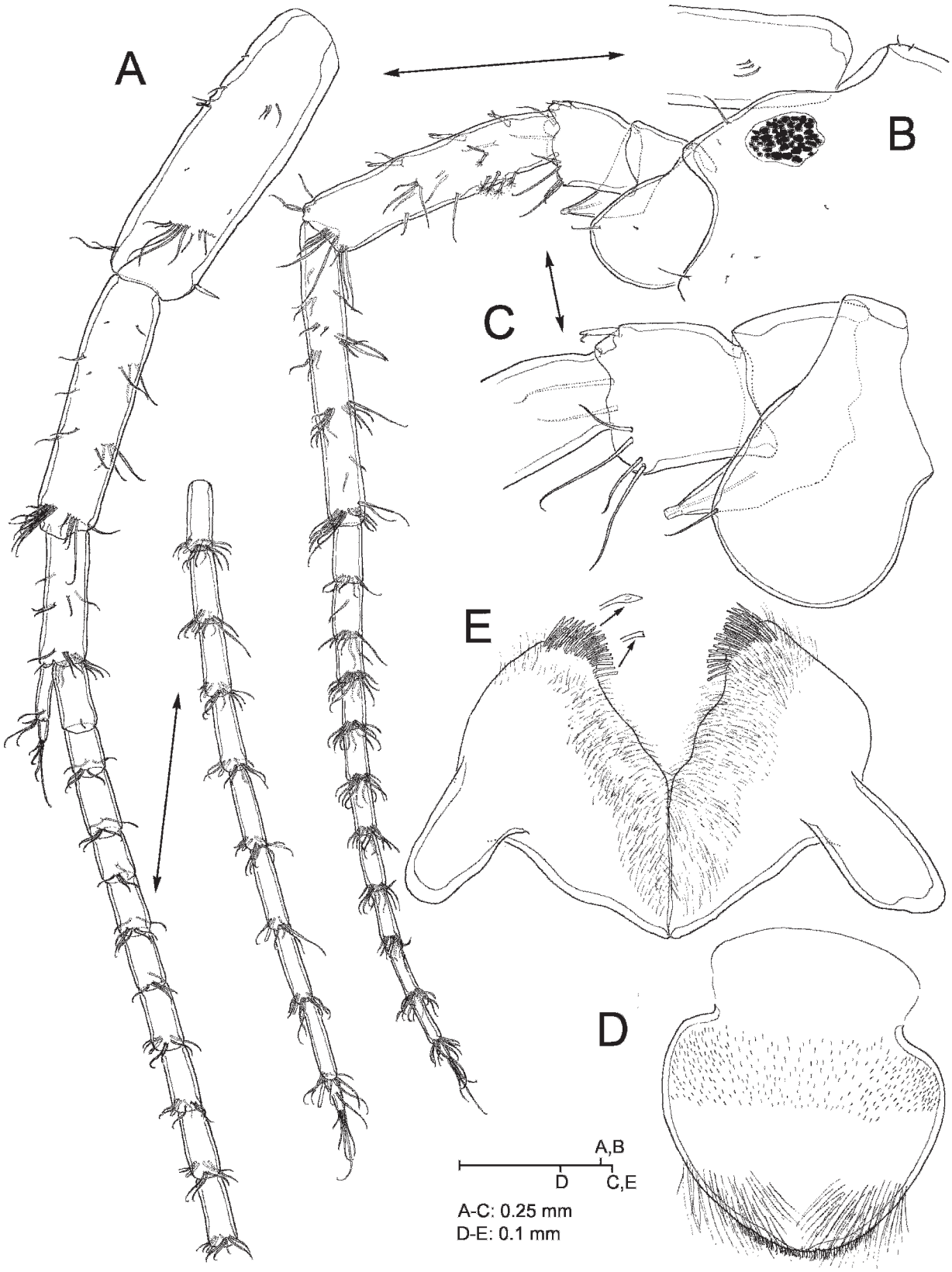

Fig. 3. *Ruffia patagonica*, n. gen., n. sp. brooding female paratype 6.62 mm. A, left antennule, lateral; B, head with left antenna attached, lateral; C, detail of three proximal segments of left antennary peduncle, lateral; D, labrum, anterior; E, paragnaths.

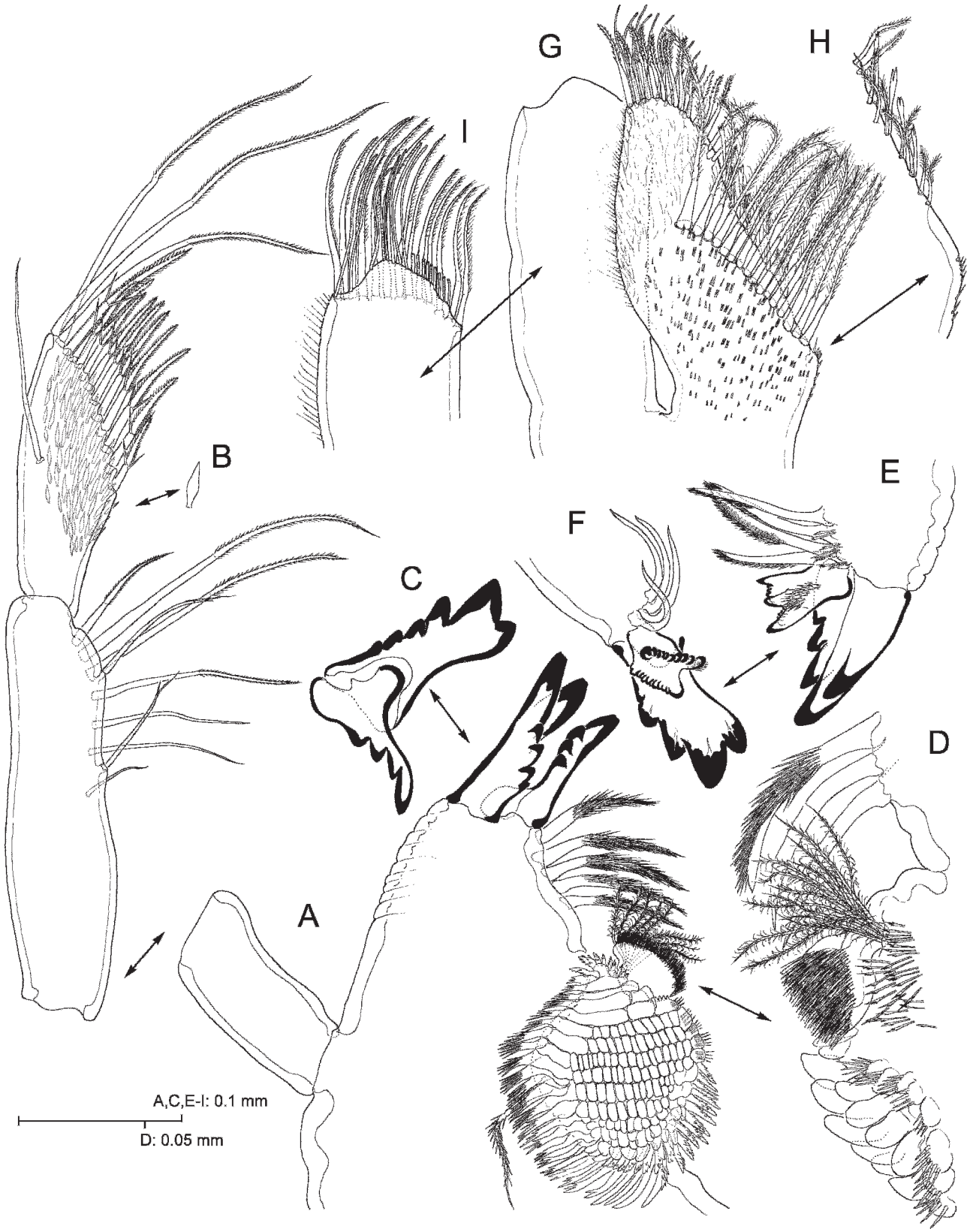

Fig. 4. *Ruffia patagonica*, n. gen., n. sp. brooding female paratype 6.62 mm. A, left mandible with corresponding palp disarticulated, medial; B, detail of lanceolate spinule on lateral surface of distal segment of palp; C incisor and lacinia; D, anterior margin of molar process, lateral; E, distal portion of right mandible, lateral; F, same, medial; G, left maxilla with outer lobe outlined only, anterior; H, detail of armature along medial margin of inner lobe; I, outer lobe with some setae on distal margin partially omitted, anterior.

Fig. 5. *Ruffia patagonica* n. gen., n. sp. brooding female paratype 6.62 mm. A, right maxillule, anterior; B, left maxilliped with armature of ischial endite omitted, posterior; C, detail of ischial endite, posterior.

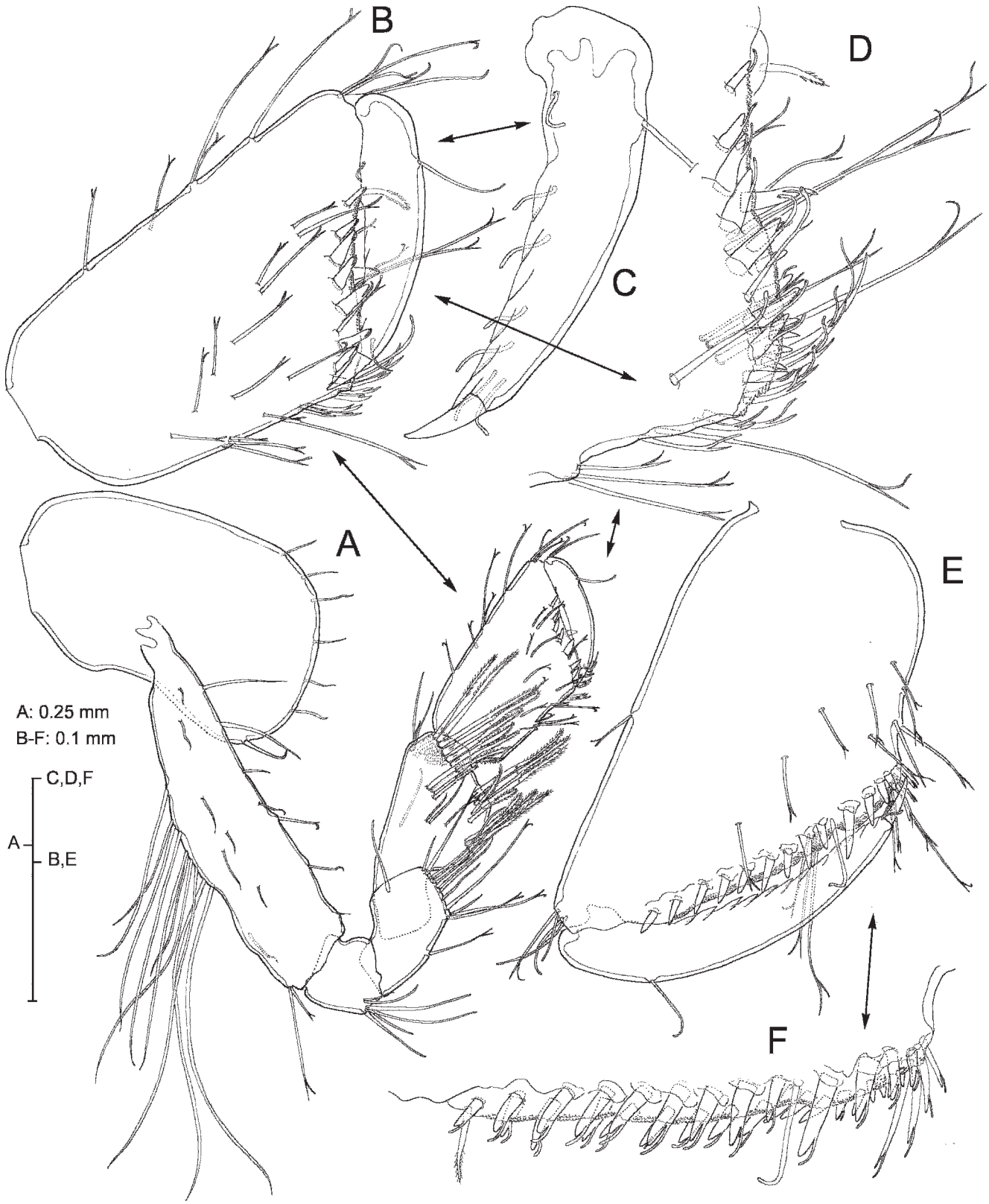

Fig. 6. *Ruffia patagonica*, n. gen., n. sp. A, left G1 of brooding female paratype 6.62 mm, medial; B, detail of propodus and dactylus, with armature on lateral surface of palm margin partially omitted, medial; C, detail of dactylus-unguis, medial; D, detail of palm margin and palm angle armature of propodus, medial; E, right G1 propodus of male paratype 4.78 mm with armature on lateral surface of palm margin partially omitted, medial; F, detail of armature along palm margin and angle, medial.

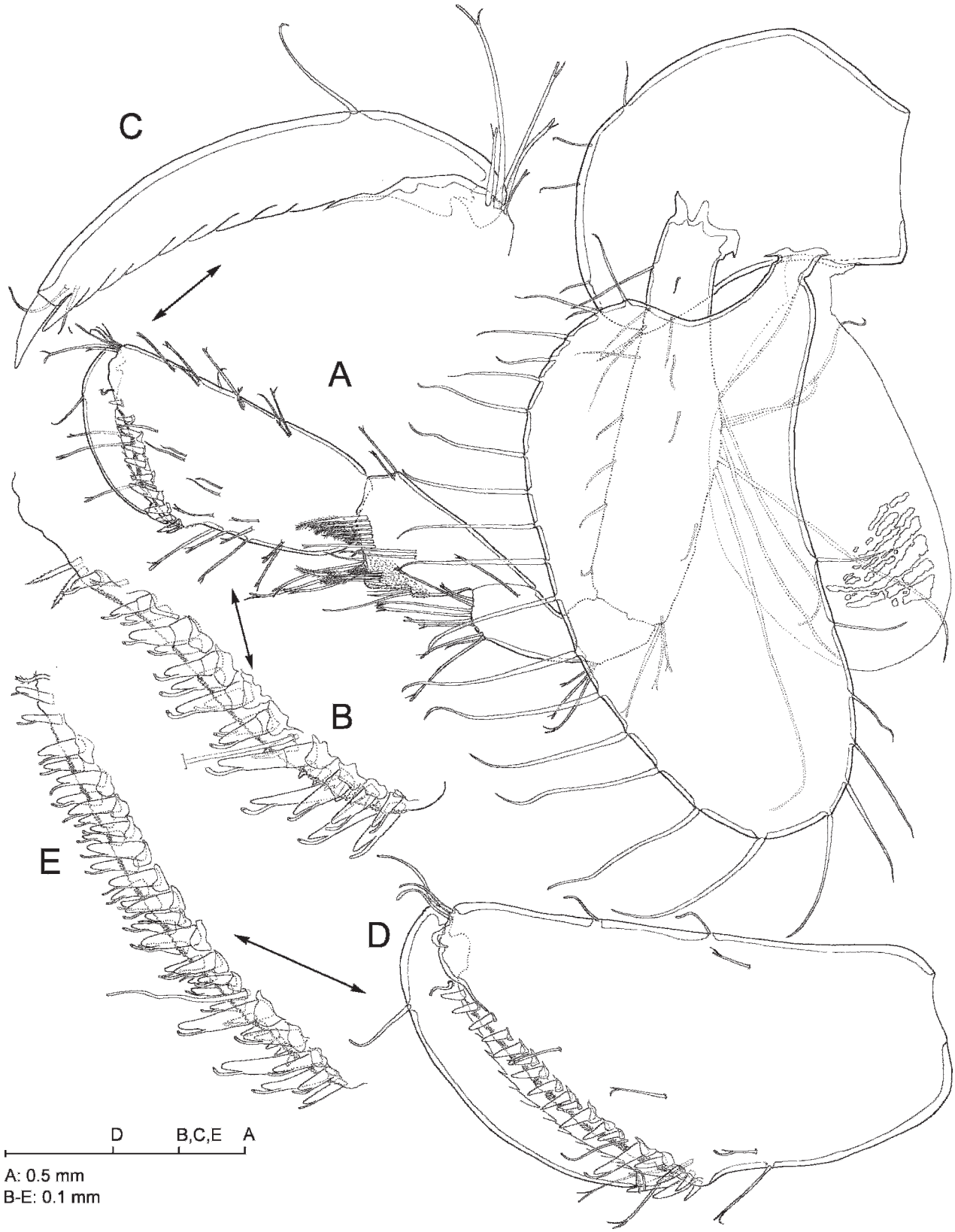

Fig. 7. *Ruffia patagonica*, n. gen., n. sp. A, right G2 of brooding female paratype 6.62 mm, medial; B, detail of armature on palm margin and angle, medial; C, detail of dactylus-unguis, medial; D, propodus-dactylus of right G2 of male paratype 4.78 mm, medial; E, detail of armature on palm margin and angle, medial.

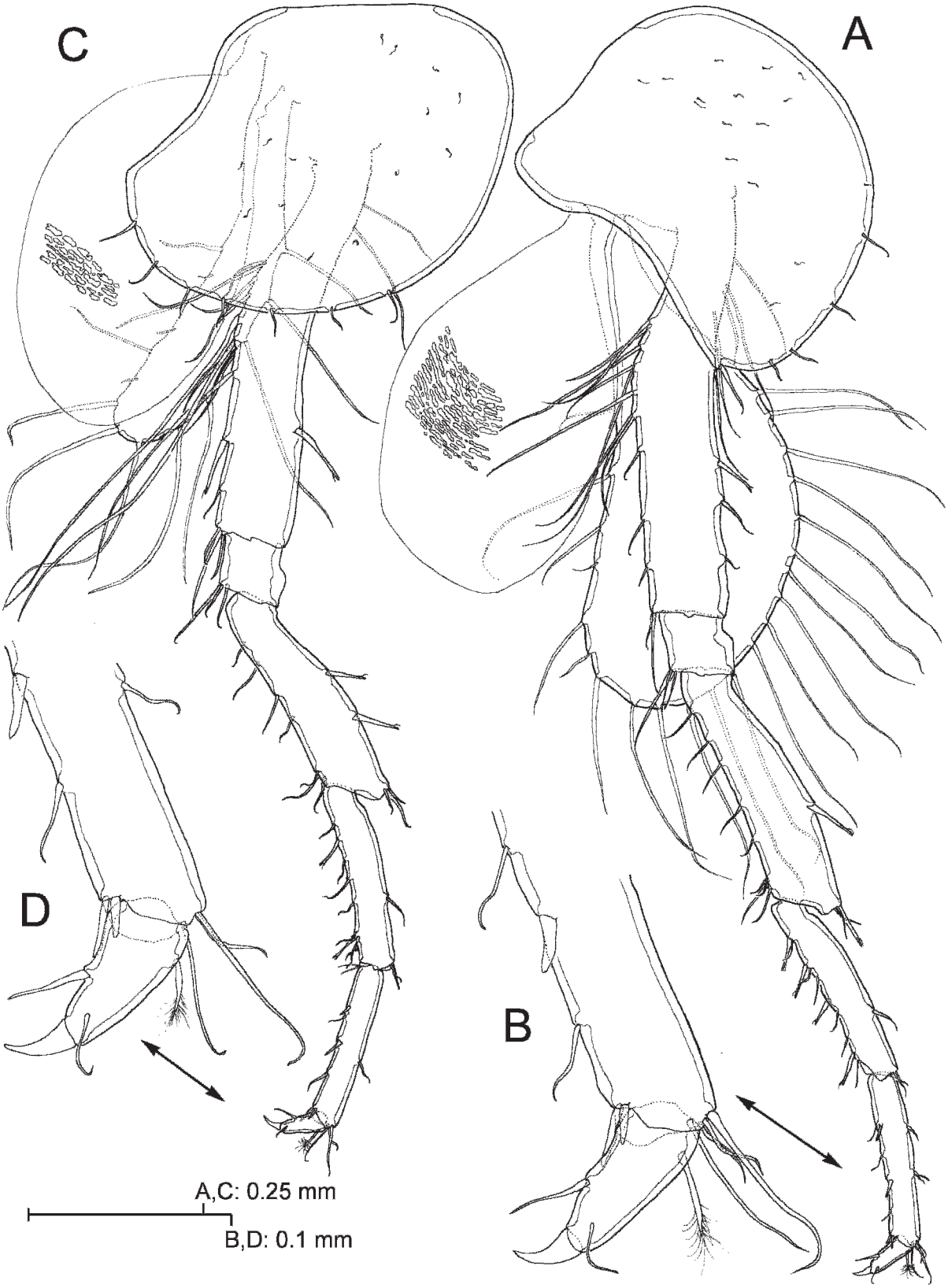

Fig. 8. *Ruffia patagonica*, n. gen., n. sp. brooding female paratype 6.62 mm. A, right P3, lateral; B, detail of distal portion of latter, lateral; C, right P4, lateral; D, detail of distal portion of latter, lateral.

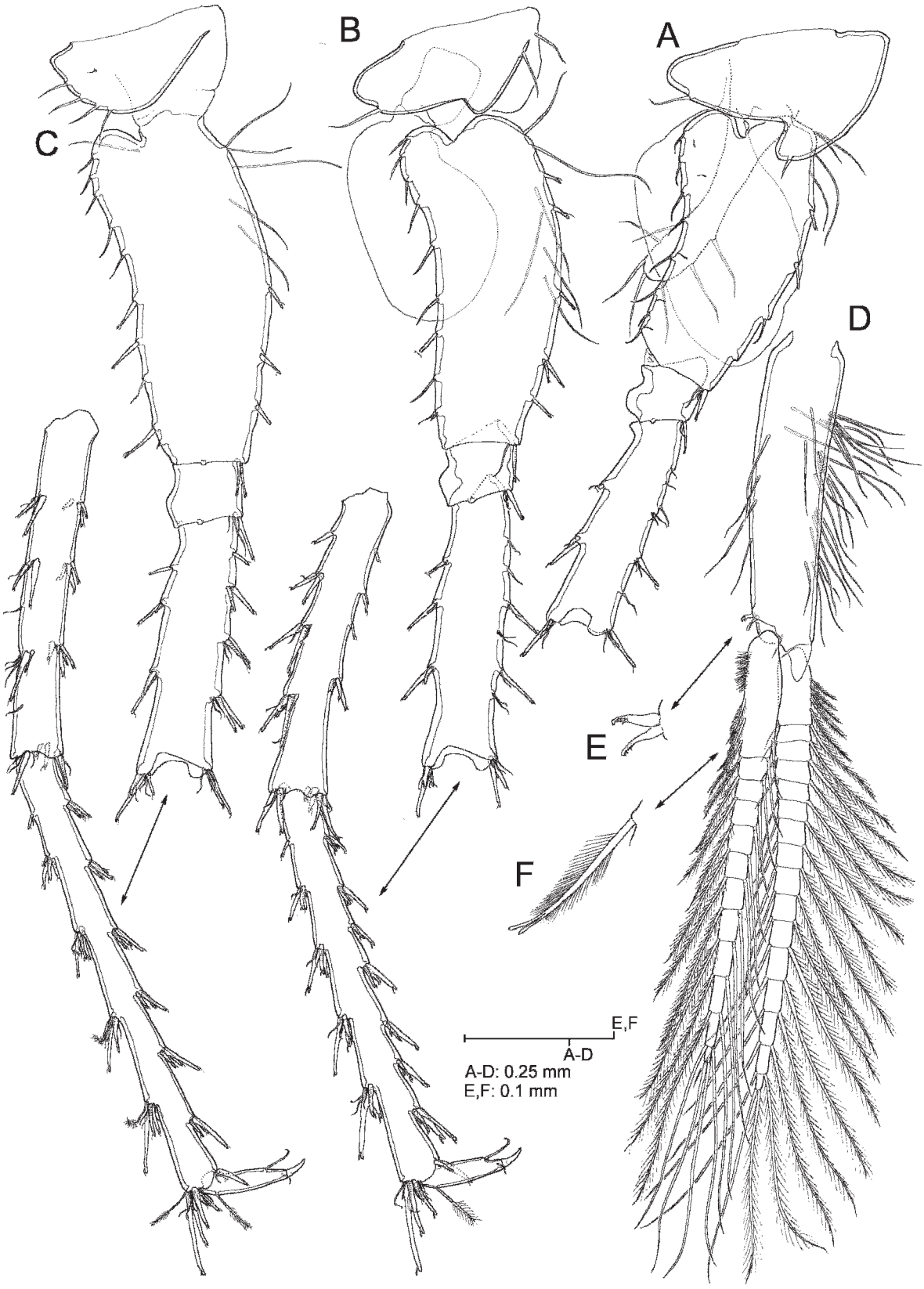

Fig. 9. *Ruffia patagonica*, n. gen., n. sp. brooding female paratype 6.62 mm. A, proximal portion of right P5, lateral; B, right P6, lateral; C, right P7, lateral; D, right pleopod I, posterior; E, detail of retinacles; F, detail of proximal seta on medial margin of proximal article of endopod.

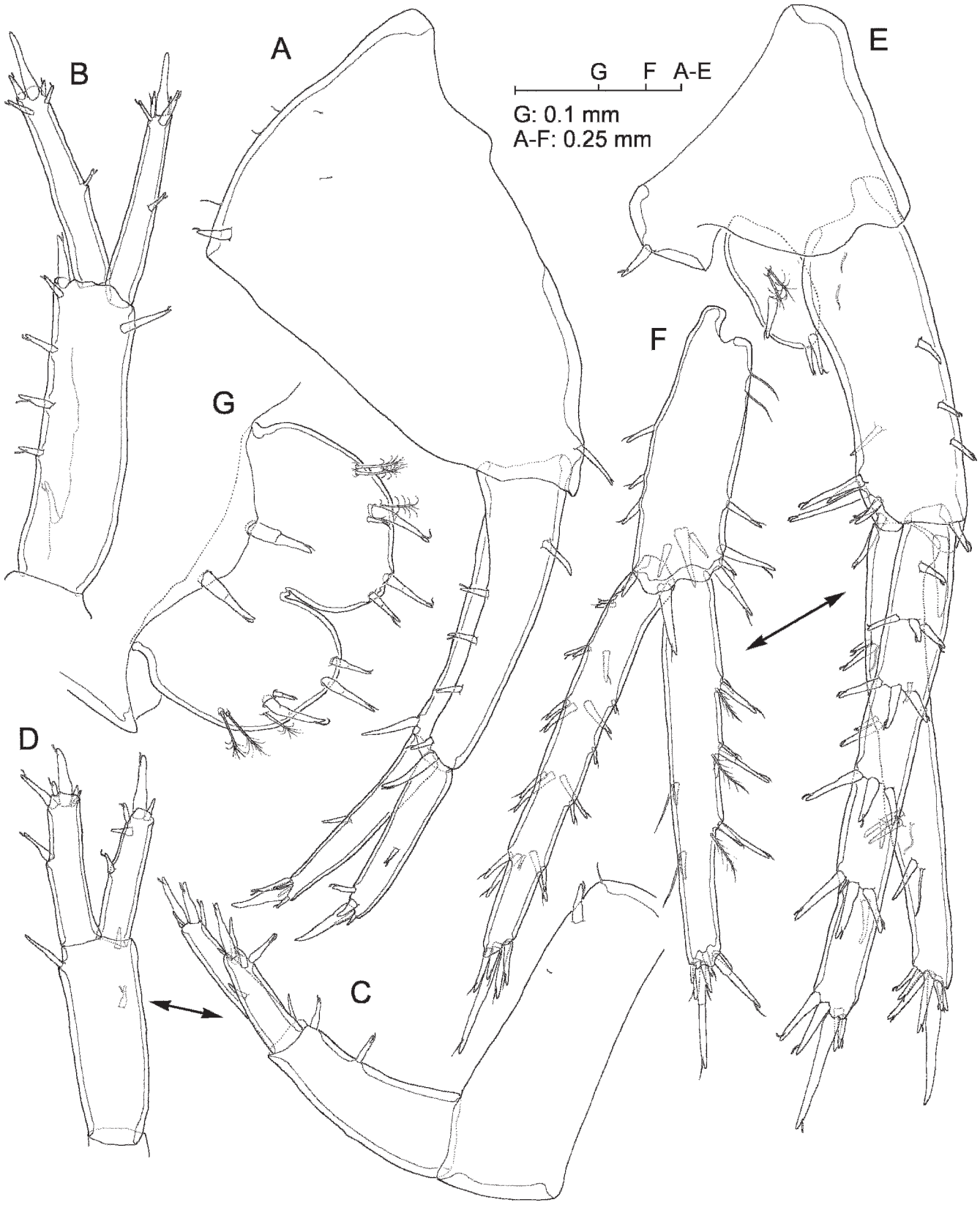

Fig. 10. *Ruffia patagonica*, n. gen., n. sp. brooding female paratype 6.62 mm. A, urosomite I with right U1 attached, lateral (two long setae placed anterior to ecdysial robust seta omitted); B, right U2, posterior; C, urosomite II with right U2 attached, lateral; D, right U2, anterior; E, urosomite III with right U3 and telson attached, lateral; F, right U3, anterior; G, telson and dorsodistal portion of pleonite III, dorsal.

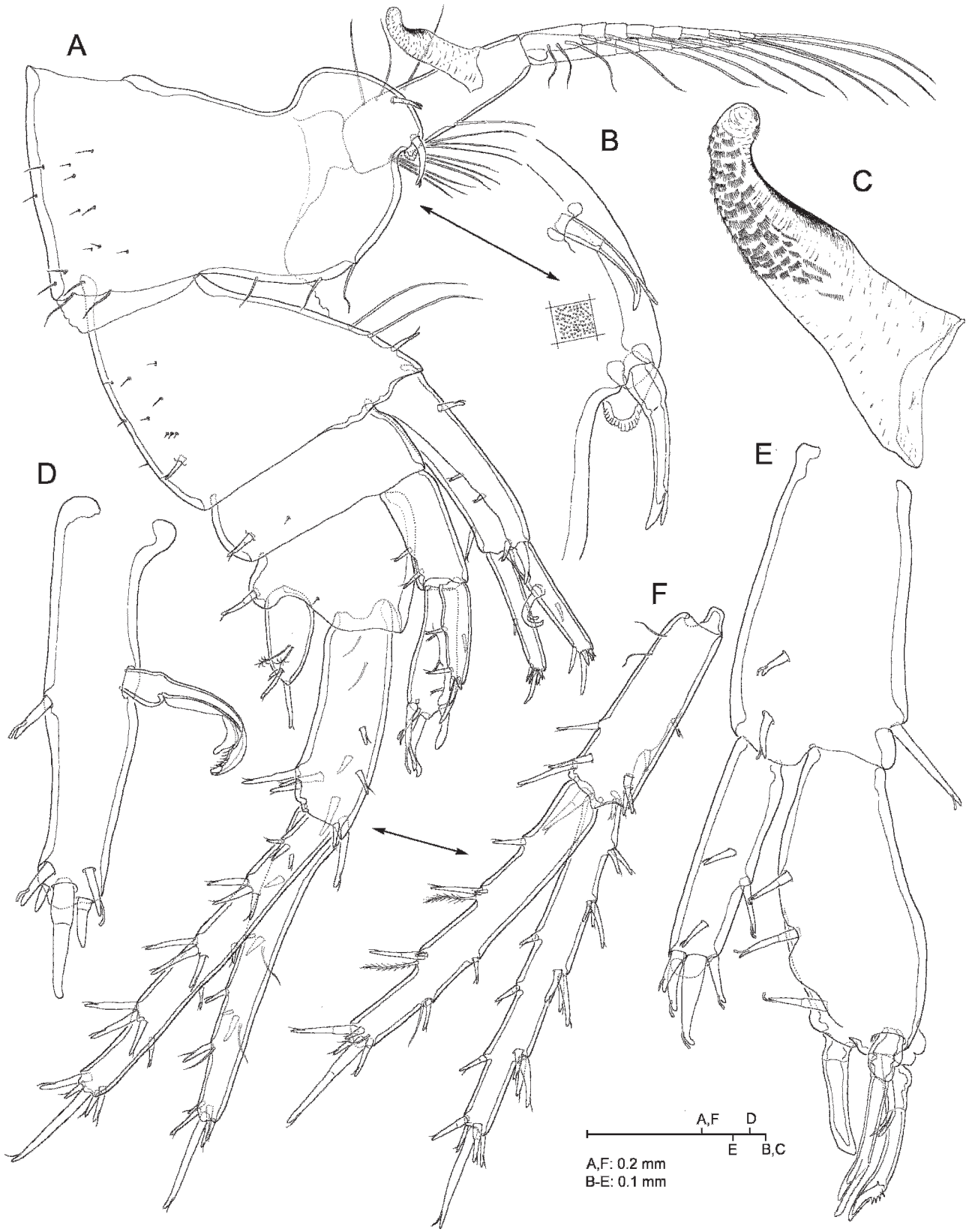

Fig. 11. *Ruffia patagonica*, n. gen., n. sp. male paratype 4.78 mm. A, pleonite III, urosome and telson with corresponding right limbs attached, lateral; B, detail of armature on distal margin of epimeral plate III; C, detail of anterolateral finger-like process on protopod of pleopod III; D, detail of exopod of right U1 with modified robust seta on outer margin, posterior; E, left U2, posterior; F, right U3, posterior.

proximally on anterior margin of hardly developed anterodistal lobe; posterior lobe with subdistal seta. Coxa VII (Fig. 9C) posterior lobe with three marginal setae.

Both gnathopods and P3-P4 each provided with tuft of long simple setae on posterior margin of basis. Gnathopod I (Fig. 6A) with carpus elongate, slightly longer than propodus; latter 1.7 times as long as broad, with palm angle placed at 62% of maximum (= dorsal) length of segment (Fig. 6B); palm margin lined with sparsely set (about eight at each side) short and stout flagellate robust setae (Fig. 6D); lateral side of palm margin lined with additional row (partially overlapping row of ordinary flagellate robust setae) of seven long and slender curved flagellate robust setae close to palm angle, setae progressively longer towards dorsal (= lateral; Fig. 6D); Dactylus with ca. six broad, flattened denticles along medial margin (Fig. 6C).

Gnathopod II (Fig. 7A) with propodus 1.9 times as long as broad and 1.4 times as long as carpus, with palm angle placed at 60% of maximum length of segment. Palm margin (Fig. 7B) lateral side lined with 12 flagellate robust setae, of which six more proximal to palm angle reduced, plus cluster of three strong flagellate robust setae close to palm angle partially overlapping former row; medial side of palm margin lined with ca. 10 strong flagellate robust setae. Dactylus with about eight broad, flattened denticles along medial margin (Fig. 7C).

Pereiopods III-IV (Fig. 8A, C) similar, slender, each with 1-2 slender robust setae on lateral (= anterior) margin of merus, and several on medial (= posterior) margin of carpus and propodus. Dactylus with stout simple seta about midway of medial margin; unguis short and curved, attaining about 40% (P3) or 43% (P4) length of corresponding dactylus (Fig. 8B, D).

Pereiopods V-VII (Fig. 9A-C) with numerous robust setae, especially on distal segments; P7 longest due to proportionally longer carpus and propodus (cf. Fig. 8B and C). Basis of each limb with convex anterior margin and concave posterior margin, latter produced postero-proximally into lobe. Dactylus slightly longer in P7 than in P6, each with single simple seta subdistally on medial margin. Unguis attaining about 32% (P6) or 27% (P7) length of corresponding dactylus.

Pleopods (Figs 2A; 9D) with elongate protopod (about four times as long as broad), with pair of ordinary retinacles (Fig. 9E) and setose anterolateral surface. Rami about 1.5 times as long as protopod, multiarticulate; proximal article of endopod with patch of setules proximally on medial margin and with medial armature elements modified as short, plumose flagellate robust setae (Fig. 9F).

Uropod I (Fig. 10A, B) protopod longer than rami, with four flagellate robust setae along lateral margin, unarmed medial margin, and with one strong flagellate robust seta at posterolateral and posteromedial angle of segment; exopod slightly shorter than endopod, both rami with one short flagellate robust seta about midway of medial margin plus one ordinary robust seta with two shorter flagellate robust setae at each side terminally on segment.

Uropod II (Fig. 10C, D) shorter than uropod I, also with protopod longer than rami and with exopod slightly shorter than endopod; armature of protopod comprising single flagellate robust seta on posterolateral margin and one robust seta at each posterodistal angle, inner robust seta longest; each ramus with one flagellate robust seta on medial margin and four (exopod) and five (endopod) terminal robust setae.

Uropod III (Fig. 10E, F) longest uropod, with rami about 1.4 times as long as protopod; protopod armature comprising three flagellate robust setae along lateral margin and short flagellate robust seta on distolateral angle, two short simple setae proximally plus single flagellate robust seta on medial margin, and three unequal flagellate robust setae around distomedial angle of segment; stout flagellate robust seta placed midway of distal margin on anterior surface of segment, and three flagellate robust setae displayed subdistally on posterior surface. Both rami provided with numerous flagellate robust setae, with endopod bearing also several short plumose setae (see Fig. 10F).

Telson (Fig. 10G) broader than long, armature comprising 2 + 1 penicillate setae and two subterminal and two terminal flagellate robust setae on each lobe.

Description of Male.—As female except for propodus and dactylus of both gnathopods, epimeral plate III, pleopod III, and uropods I and II. Gnathopod I (Fig. 6E) propodus about 1.5 times as long as broad, with palm angle placed about midway of maximum length of segment (vs. 1.7 times as long as broad with palm angle at 62% of maximum length of segment in female). Armature of palm margin comprising 13 flagellate robust setae along medial side and 17 along lateral side (Fig. 6F; vs. only eight at each side in female). Dactylus with 12 denticles along medial margin (vs. only six in female).

Gnathopod II propodus with palm more oblique than in female (Fig. 7D; palm angle placed at 46% of maximum length of segment compared to at 60% in female); palm margin armature comprising 17 medial and 19 lateral flagellate robust setae (Fig. 7E; vs. 10 and 12 in female, respectively). Dactylus with 12 medial denticles (vs. eight in female).

Epimeral plate III distal margin with strong excavation about midway and with two submarginal flagellate robust setae (Fig. 11A); rounded process with corrugated integument placed adjacent to excavation (Fig. 11B). Protopod of pleopod III with proximal S-shaped finger-like process anterolaterally (Fig. 11A); process partially covered with comb-like setulose cuticular scales dorsodistally (Fig. 11C).

Uropod I exopod with lateral robust seta modified, bifid, curved and ornamented as in Fig. 11D. Endopod of uropod II roughly spatulate, with three small ordinary flagellate robust setae along lateral margin and four heterogeneous, transformed robust setae distally (Fig. 11E). Uropod III (Fig. 11F) as in female.

Etymology.—Species name refers to the geographical region were it dwells in, namely Chilean Patagonia.

Remarks.—The new taxon displays some of the more distinctive features of Phreatogammaridae, such as: 1) dactylus of gnathopods with medial margin denticulated; 2) palm margin of gnathopods lined with numerous unicuspid

robust setae; 3) broadened oöstegites; 4) urosomites with posterodorsal robust setae; 5) uropod III equiramous with elongated peduncle and with unsegmented cylindrical rami, the latter provided with numerous robust setae; 6) pereiopod V much smaller than pereiopods VI-VII; and 7) "coxal gill" (= exopod) wanting on pereiopod VII. In addition, the sternites of all pereionites, pleonites, and urosomites show a portion of integument surrounded by hyaline frill presumably osmoregulatory in function, equivalent to the pair of sternal gills displayed by other phreatogammarids on several body somites. The new genus is also unique among phreatogammarids in displaying sexual dimorphism on the epimeral plate III, the protopod of pleopod III, the armature of uropod I, and the endopod of uropod II.

Discussion

Coxal and Sternal Gills in Phreatogammarids and Other Amphipods

The presence and precise arrangement of sternal gills in phreatogammarids has not been resolved completely. Whereas *Ruffia* lacks such structures and *C. maryae* displays them on pereionites II-VI, the information derived from the published descriptions of the four species of *Phreatogammarus* is confusing. Chapman (2003) did not report them to occur in *P. helmsii* and *P. waipoua*, but she showed a presumed coxal gill on P7 and a pair of coxal gills on G2 of both species. In her follow-up paper redescribing *P. fragilis* and *P. propinquus*, Chapman (2004) did not report the occurrence of a coxal gill on P7 of these species but of a sternal gill instead; in addition, the G2 of both species was described as showing a single, simple coxal gill instead of a pair of gills. It is worth mentioning that cases of doubled coxal gills, i.e., a pair of coxal gills per pereiopod, have never been reported in amphipods, whereas bilobed coxal gills are relatively common (Steele and Steele, 1991). In addition, the putative coxal gill present on the P7 of several amphipod groups is not homologous to the rest of the coxal gills, since it is implanted on the basis of this limb and not on the coxa (Steele and Steele, 1991) and consequently should be considered as an exopod.

In order to resolve the arrangement of coxal and sternal gills in *Phreatogammarus*, we have examined four specimens of *P. fragilis* from Eyreton (New Zealand's South Island) preserved at the Crustacea collection of The Natural History Museum, London [BMNH reg. nos. 1928.12.1.2283-2285]. In this taxon, brooding females display simple, ovoid, pedunculate, coxal gills from G2 to P6, broad oöstegites with sparsely setose margin from G2 to P5 (that on P5 somewhat reduced, much smaller than the corresponding coxal gill), and large, ovoid, coxal gill-like sternal gills provided with a short peduncle on pereionites II-VII. Unfortunately, no material of the two species, *P. helmsii* and *P. waipoua*, is currently available for re-examination, and the issue of the paired coxal gills present on G2 of these species awaits confirmation. Nevertheless, the presence of a sternal gill on G2 of *P. fragilis*, and the coxal gill-like appearance of sternal gills in this species suggest that one of the presumed coxal gills on G2 of *P. helmsii* and *P. waipoua* could correspond to a sternal gill.

As mentioned above, *Ruffia* does not display ordinary sternal gills, but bears instead an area of presumed permeable epithelium on the sternite of all pereionites, pleonites, and urosomites (Fig. 2D). Similar structures have been reported to occur on the sternites of pereionites II-VII of some euryhaline aroids [*Grandidierella japonica* Stephensen, 1938] and melitids [*Melita setiflagella* Yamato, 1988], and of a freshwater eusirid [*Sternomoera japonica* (Ueno, 1933)], and these were proven to be osmoregulatory in function (Kikuchi and Matsumasa, 1997). The condition in *Ruffia* differs from these taxa only in that these structures are also displayed on pereionite I and on all pleonites and urosomites. Kikuchi et al. (1993) demonstrated that these structures are equivalent to sternal gills in *S. japonica*, where the sternal gill walls are an extension of the sternal osmoregulatory epithelium. The presence of these ventral epithelial fields on the pleonites and urosomites of *Ruffia* reinforces the sternal gill condition of the digitiform ventral structures present on the pleonite I of some crangonyctids (Holsinger, 1977), and especially of the large subovate processes present in the same position in some freshwater melitids (Sawicki et al., 2005).

Phreatogammarid Biogeography

Phreatogammaridae appear to be restricted to continental waters of New Zealand (four species in genus *Phreatogammarus* Stebbing, 1899; see Chapman, 2003; 2004), New Caledonia (*C. maryae* see Iannilli and Ruffo, 2007), and the Chilean Patagonia (*R. patagonica*, described above). Vonk (2000) reported presumed phreatogammarids in Japan, but these correspond instead to members of the recently described Lucioblividae Tomikawa, 2007 (Tomikawa et al., 2007; personal observation).

According to Barnard and Barnard (1983) and Barnard and Karaman (1983) Phreatogammaridae has an entirely freshwater history and represents one of the older lineages of extant gammaroideans, but other authors (Bousfield, 1982; Iannilli and Ruffo, 2007) relate this group to the broadly distributed marine family Melphidippidae Stebbing, 1899 and advocate implicitly their direct marine origin. Indeed, Hurley (1954) cited phreatogammarids in brackish waters of New Zealand, although Chapman (2003: 646; 2004: 76) has shown that these records correspond to locations close to the brackish zone of coastal freshwater streams, but not the brackish zone proper. Sutherland (2006) sampled over 400 sites throughout New Zealand and reported three species of *Phreatogammarus* in 17 sites, most of them very near the coast. Although no salinity measures are given, the author suggests coastal dispersal. Bousfield (1982) also reported the occurrence of phreatogammarids in oligohaline brackish estuaries and salt marshes of the same islands, although these taxa still remain to be described and their habitat preferences determined in precise detail.

The new taxon from Patagonia was not recorded at any other stations further inland despite intensive sampling. In addition, and as previously mentioned, sternal osmoregu-

latory epithelial fields are known only to occur in marine euryhaline amphipods, or in exceptional freshwater taxa belonging to typical marine families. Thus, it cannot be ruled out that the new taxon was an ordinary shallow-water marine form that had penetrated into the cave during episodes of strong intrusion of seawater concomitant with low freshwater outflow. Unfortunately, the immediate marine shallow-water habitat around the cave was not surveyed for the presence of the amphipod, which could have resolved this issue. Nevertheless, it is worth mentioning that nothing similar to a phreatogammarid (or a melphidippid) has been reported thus far from marine waters of the Magellanic region (De Broyer and Rauschert, 1999; Chiesa and Alonso, 2007; Chiesa et al., 2005; González et al., 2008), and therefore the possibility that the amphipod was a primary freshwater form forced to occupy the brackish coastal rim during past episodes of glaciation cannot be ruled out. In fact, the ties of the species described herein to coastal subterranean brackish water would explain how it managed to overcome the glacial period ending 11,000 yr BP, when all inland freshwater habitats on Madre de Dios had most probably vanished (Sugden et al., 2005).

Regardless of whether *R. patagonica* is primarily marine or freshwater in origin, linked to coastal marine habitats or to inland freshwaters, phreatogammarids as a whole are currently limited to southern continents. The fact that they cannot be considered unambiguously as a primarily freshwater group reduces their biogeographical value as indicators of past terrestrial connections. If phreatogammarids are primarily freshwater taxa, then their current distribution most probably is the result of a series of vicariant events associated with the break-up of Gondwana, and a ''terminus ante quem'' for the origin of the family could be derived from the age of the last land connection established between all the former territories of a supercontinent that currently harbour phreatogammarids. This date falls within the Late Jurassic, around 150 Ma ago, when the severing of South America from Antarctica began according to palaeomagnetic evidence (Veevers et al., 1980; Hallam, 1994). New Zealand and New Caledonia would not separate from Austro-Antarctica until much later, about 82 Ma ago, when a continental fragment including the North and South Islands, plus Norfolk Island and Lord Howe Island became detached from the main continental mass. This fragment was further subdivided, with New Caledonia drifting away from the Northern Island of New Zealand more than 20 Ma ago (Weissel et al., 1977; Lawver et al., 1992; Coleman, 1980; Hall, 2002). Nevertheless, it is likely that the isolation by sea of these islands from Austro-Antarctica took place somewhat earlier than the 82 Ma suggested by paleomagnetic data, and 110 Ma could be a better estimate provided the effect of the generalized mid-Cretaceous sea level rise (Stevens, 1980; Hallam, 1994).

In this scenario, the putative absence of phreatogammarids from Antarctica and, especially, from Australia is odd since a continuous link between South America and these land masses existed during the Late Mesozoic. Australia and Antarctica began to drift apart in the Late Jurassic, with the final separation and onset of deep-water circulation between both land masses at about 38 Ma ago (Cande and Mutter, 1982; Lawver et al., 1992; Woodburne and Zinsmeister, 1984). Whereas the absence of phreatogammarids from Antarctica could be related to the formation of ice sheets on that continent, their absence from Australia, if not an artefact produced by sampling uneveness, suggests that the family was originally restricted to a narrow strip of Gondwana connecting Tierra de Fuego to New Caledonia and New Zealand via Marie Byrd Land and the Campbell Plateau (Woodburne and Zinsmeister, 1984; Hallam, 1994).

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