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ORIGINAL ARTICLE

SEM studies on first and second gonopod morphology in Mithracidae (Decapoda: Brachyura)

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ABSTRACT

Gonopods are phylogenetically important morphological characters, and in several cases providing diagnostic characters to support taxonomic and phylogenetic studies. Relatively few studies, however, have emphasized in-depth descriptions of gonopods, and the functional morphology of these structures are poorly understood. We use SEM to describe and illustrate the two pairs of gonopods (G1 and G2) of nine species of Mithracidae (Majoidea) and of *Macrocoeloma trispinosum* Latreille, 1825, recently relocated to Epialtidae (Majoidea). The G1 was found to present the typical characteristics of Majoidea: long, with a mesial suture, setae inserted from the base to the distal second third and sensilla in the apical plate; the G2 is always short and conical. The apical region of the G1 was diagnostic for the

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species analyzed, which could provide important characters for differentiating among the majoids; and also for studying the functional, reproductive, and phylogenetic significance of these structures.

Keywords

Majoidea, male reproductive system, scanning electron microscopy (SEM), taxonomy

Introduction

In brachyuran crabs, the gonopods are appendages of pleonal somites 1 and 2 modified as organs for sperm transfer (Guinot *et al.*, 2013). It has long been acknowledged that the morphology of these appendages, especially the first gonopod (G1), has significant taxonomic value in species-specific diagnosis (*e.g.,* Smalley, 1964; Martin and Abele, 1986; Chen *et al.*, 2007; Guinot *et al.*, 2013). Although clearly useful in taxonomy, the morphology of the gonopods is poorly addressed, having undesirable consequences for comparative morphological studies. For instance, depending upon the taxonomic group, cuticular structures such as spines have been named differently, ranging from "tooth-like structures" to "denticles" (Beninger *et al.*, 1991; Rorandelli *et al.*, 2008; Sal Moyano *et al.*, 2011; Kienbaum *et al.*, 2017).

The morphology of the reproductive system in Brachyura has been addressed by many authors, with the focus varying from functional morphology to mating and sperm transference and storage (*e.g.,* Duvernoy, 1850; 1853; Bauer, 1986; Hartnoll, 1969; Guinot, 1979; Tavares and Secretan, 1993; Guinot and Quenette, 2005; Sal Moyano *et al.*, 2011; Becker *et al.*, 2012; Guinot *et al.*, 2013; McLay and Becker, 2015; Becker and Scholtz, 2016; Becker and Bauer, 2020). In Majoidea Samouelle, 1819, a superfamily of Brachyura consisting of approximately 200 genera and 1000 species (Ng *et al.*, 2008; Davie *et al.*, 2015), the available information on the morphology of the G1 is still scarce, and there is even less information available on the morphology of the second gonopod (G2). Most of the literature typically focuses on the gross morphology of the G1, as used for distinguishing between genera and species (*e.g.,* Garth, 1958; Griffin and Tranter, 1986; Neumann, 1996a; 1996b; Wagner, 1990; Tavares and Santana, 2018) and on investigating the reproductive biology of particular species (*e.g.,* Rorandelli *et al.*, 2008; Sal Moyano *et al.*, 2011).

We use SEM (scanning electron microscopy) to study nine mithracid species and *Macrocoeloma trispinosum* Latreille, 1825 to increase knowledge on the cuticular structures associated with the G1 and G2. New information will be useful in taxonomic studies, as well as providing new insights into the reproductive biology of mithracids. The position of *Ma. trispinosum* in the Epialtidae, a crab previously included in Mithracidae, is briefly discussed.

Material and Methods

Adult males of nine mithracid species and *Ma. trispinosum* were manually collected in Brazil (Ubatuba, 23º26'02"S 45º04'16"W) and Venezuela (Isla Margarita, 11°01′04″N 63°55′20″W; and Isla La Tortuga, 10°55′54″N 65°18′29″W). Vulvae of eight adult mithracid female species [females of *Nonala holderi* (Stimpson, 1871) were not available] and *Ma. trispinosum* were also studied. The material is deposited in the collections of the Laboratory of Systematic Zoology (LSZ), Universidade Estadual Paulista (UNESP), and in the carcinological collection of the Museu de Zoologia, Universidade de São Paulo (MZUSP).

The specimens were dissected under a stereomicroscope, Motic K Series, to remove G1 and G2, which were stored in 70 % ethanol. The stereomicroscopy images were obtained on a Leica MZ7s stereomicroscope with Leica IM50 software. For scanning electron microscopy (SEM), after dissection, the gonopods were fixed in 4 % buffered formalin for 48 hours and subsequently submitted to dehydration in an ascending series of alcohol (30–100 %). Subsequently, G1 and G2 were dried in liquid CO_2 in a Balzers Union CPD 020 critical point dryer.

The gonopods were then mounted on aluminum stubs with carbon double-sided tape, and coated with a 10 nm gold layer on a MED 10 Balzers Union sputter coater. The gonopods were analyzed and photographed on a Jeol SEM 5154 scanning electron microscope.

We follow Felgenhauer (1992) in defining spines as a "non-articulated cuticular extension of the exoskeleton that has a base that is generally not as wide as the structure is long". The nomenclature for setae mostly follows Pohle and Telford (1981).

Results

We standardized the terminology used to describe the cuticular structures of the G1 and G2 (Tab. 1), as it is important for species comparisons and homology tests. The term "sensillum" (plural "sensilla") is used to refer to non-articulated structures on the lateral margin of the G1 apex (detail in Figs. 1–10A). Sensilla are characterized by having an infracuticular insertion pore and vary from rounded (detail in Figs. 1A, 2A, 4A–6A, 8A), to conical (detail in Figs. 3A, 9A) and acute (detail in Figs. 7A, 10A). In mithracids, spines vary in size and are commonly found around the aperture of the ejaculatory canal (detail in Figs. 1B–7B, 9B, 10B). Setae are a common cuticular outgrowth in the species we studied, normally present in the proximal half of the G1 and G2. Setae are short or long and vary from simple (detail in Figs. 4A, B, 9A, B) to pappose (detail in Figs. 1A, B–3A, B, 5A, B, 8A, B, 10A, B).

The female vulvae share a similar morphology in all the species studied, with only small differences; mostly in the presence of a small vulvar cover observed in *Pitho laevigata* (A. Milne-Edwards, 1875) and *Ma. trispinosum* (Fig. 11).

Description of G1 and G2 morphology

Amphithrax aculeatus (Herbst, 1790) (Fig. 1).

G1 (Fig. 1A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with pappose setae (Se) from base (Ba) to distal third of shaft; suture (Ss) extending from base to distal margin of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal third gradually tapering, slightly curving towards lateral line, distinct subrounded protuberance (P) on mesial margin; strangled apex (A) formed by apical plate and two lobes - mesial lobe (Ml) rounded and short, lateral lobe (Ll) elongated, subtriangular, ending in an acute tip; lateral margin of sternal surface distally with rounded sensilla (S); apical plate with long, acute spines (Sp) surrounding the ejaculatory canal aperture, with some spines inclined towards internal aperture.

G2 (Fig. 1C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming apical girdle (Ag), mesial process blunt (Mp), medially located.

Table 1. Morphological characters of the two pairs of gonopods (G1 and G2) of the studied species of Mithracidae and Epialtidae, and their main differences.

Species	G1						G2
	Curvature	Setal types	Protuberance	Lobe	Sensilla	Spines	Mesial process
Mithracidae							
Amphithrax aculeatus	Basal	Pappose	Subrounded	Two	Rounded	Long	Medially
Mithraculus coryphe	Basal	Pappose	Subrounded	Two	Rounded	Long	Medially
Mithraculus forceps	Basal	Pappose	Subrounded	Two	Conical	Short	Medially
Mithraculus sculptus	Basal	Simple	Subrounded	Two	Rounded	Long	Medially
Mithrax hispidus	Basal	Pappose	Subrounded	Two	Rounded	Long	Laterally
Mithrax tortugae	Basal	Pappose	Subrounded	Two	Rounded	Short	Medially
Nonala holderi	Central	Pappose	Absent	Absent	Acute	Short	Medially
Pitho laevigata	Distal	Pappose	Absent	Absent	Rounded	Absent	Medially
Epialtidae							
Teleophrys ruber	Basal	Simple	Subtriangular	Two	Conical	Long	Laterally
Macrocoeloma trispinosum	Basal	Pappose	Subtriangular	One	Acute	Short	Laterally

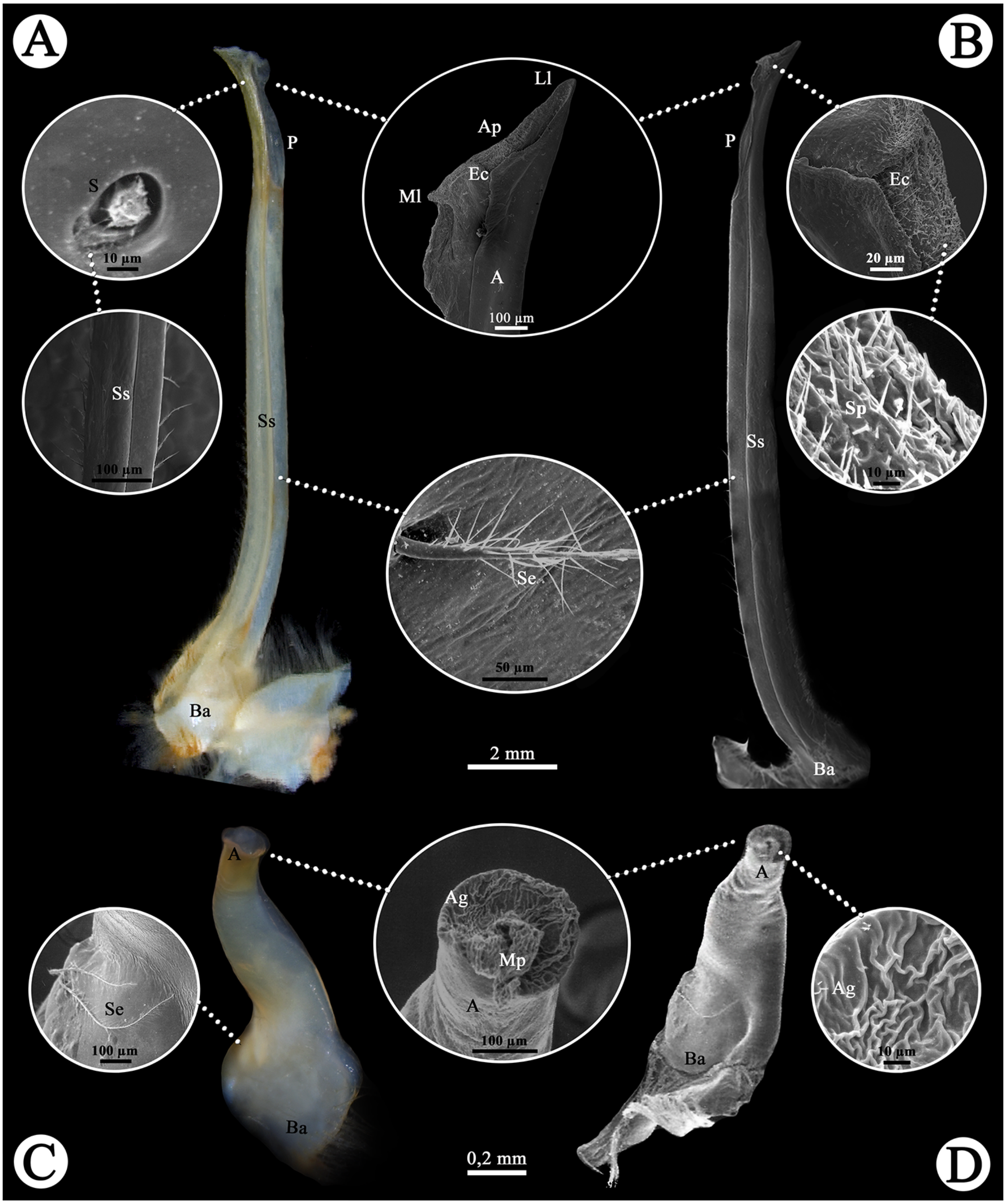

Figure 1. Gonopods of *Amphithrax aculeatus* (Herbst, 1790) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex. Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; L1, lateral lobe; M1, mesial lobe; Mp, mesial process; P, protuberance; S, sensillum; Se, setae; Ss, suture; Sp, spine.

Mithraculus coryphe (Herbst, 1801) (Fig. 2).

G1 (Fig. 2A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with pappose setae (Se) from base (Ba) to distal second third of shaft; suture (Ss) extending from base to distal end of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal fourth gently tapering, slightly curved towards lateral line, subrounded protuberance (P) on mesial margin; strangled apex (A) formed by apical plate and two distinct lobes - mesial lobe (M1) smaller, subtriangular, ending in blunted tip, lateral lobe (L1) larger, subtriangular, border rounded, distal slightly ending in acute tip; lateral margin of sternal surface distally with rows of small rounded sensilla (S); apical plate with long, acute spines (Sp) surrounding ejaculatory canal aperture, with some spines inclined towards internal aperture.

G2 (Fig. 2C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming the apical girdle (Ag), mesial process blunt (Mp) medially located; few small spines (Sp) bordering apical girdle.

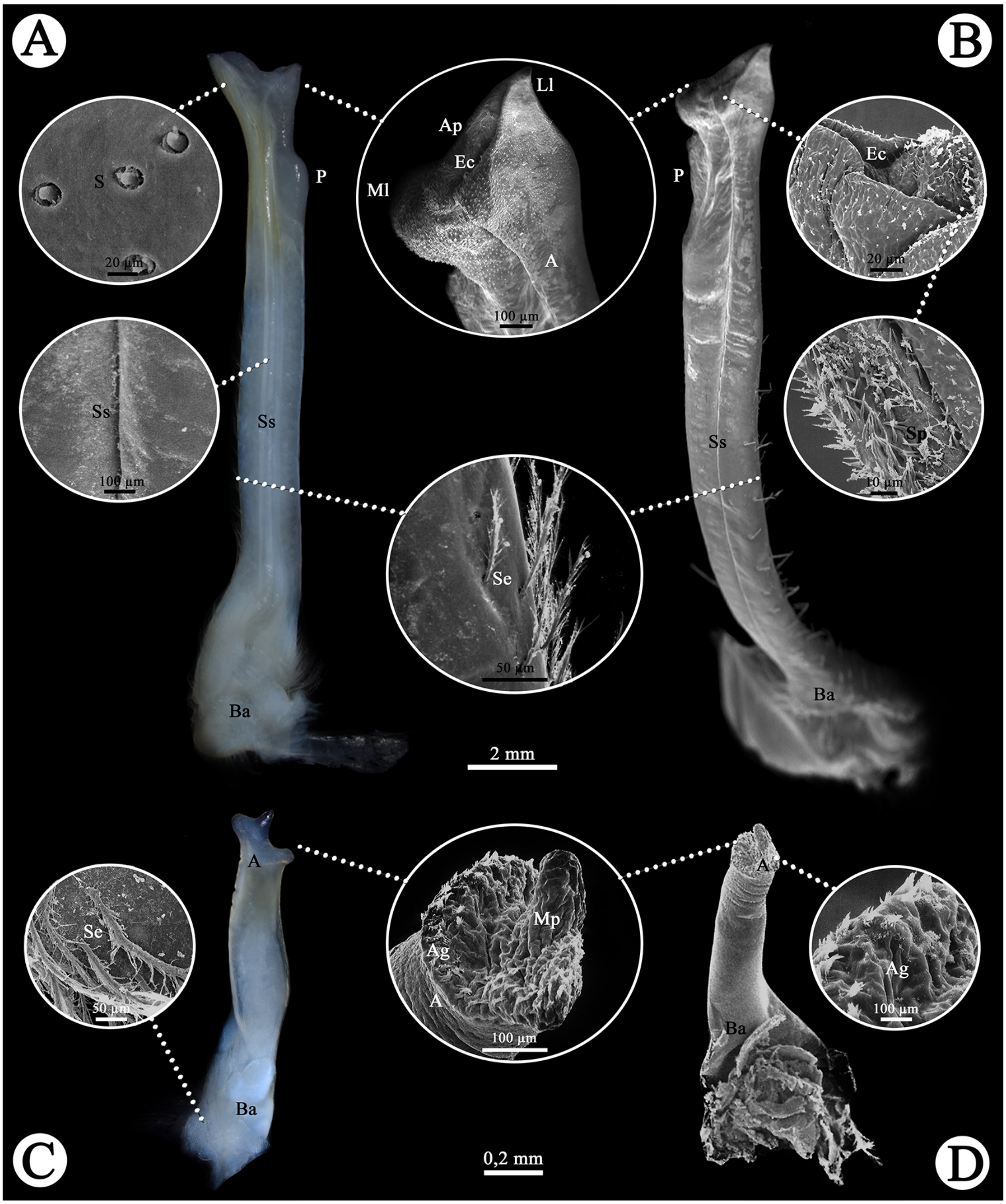

Figure 2. Gonopods of *Mithraculus coryphe* (Herbst, 1801) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; L1, lateral lobe; M1, mesial lobe; Mp, mesial process; P, protuberance; S, sensilla; Se, setae; Ss, suture; Sp, spine.

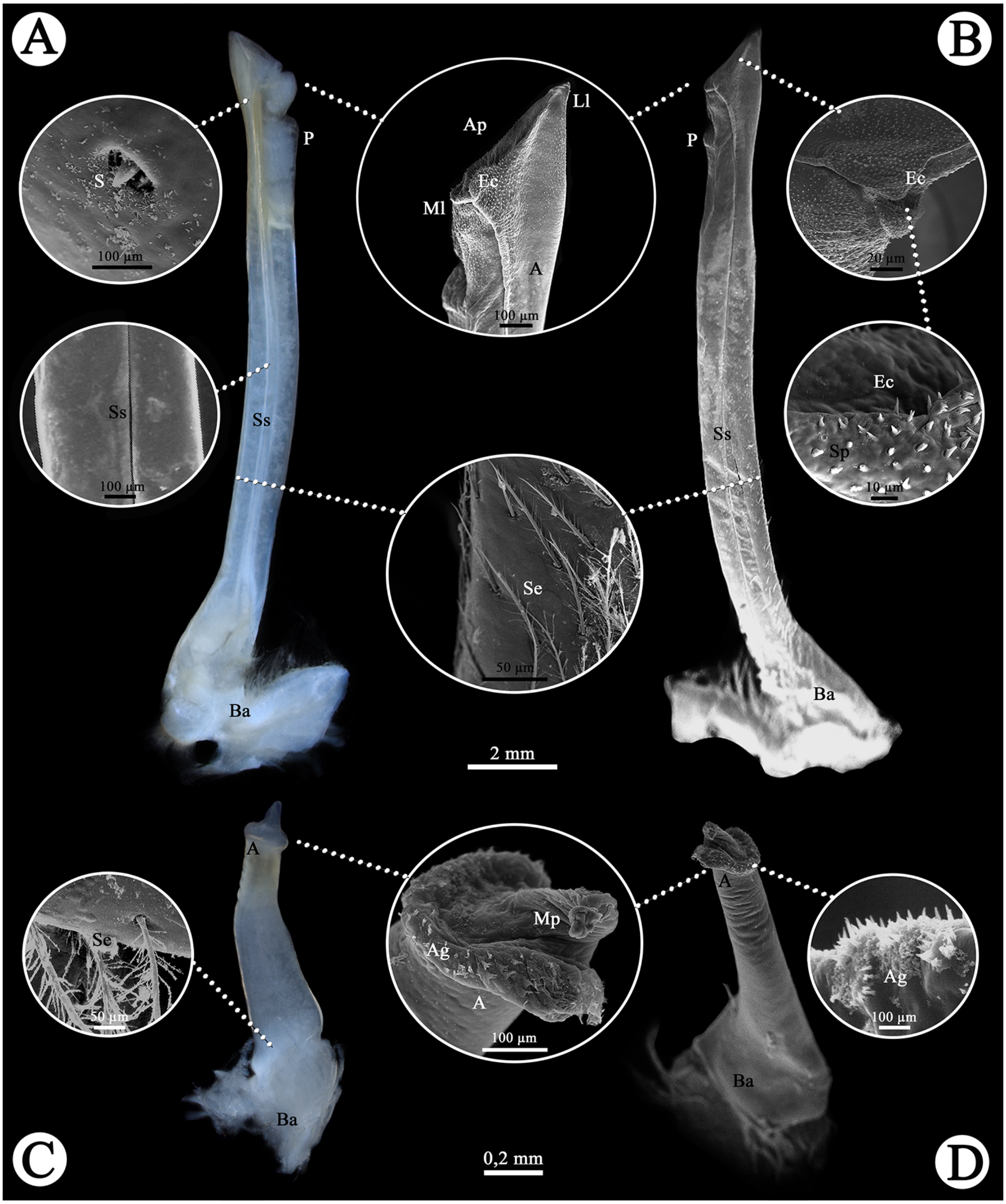

Figure 3. Gonopods of *Mithraculus forceps* A. Milne-Edwards, 1875 in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; L1, lateral lobe; M1, mesial lobe; Mp, mesial process; P, protuberance; S, sensillum; Se, setae; Ss, suture; Sp, spine.

Mithraculus forceps A. Milne-Edwards, 1875 (Fig. 3).

G1 (Fig. 3A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with pappose setae (Se) from base (Ba) to distal third of shaft; suture (Ss) extending from base to distal margin of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal third gently tapering, slightly curved towards lateral line, subrounded protuberance (P) on mesial margin; strangled apex (A) formed by apical plate and two lobes - mesial lobe (M1) smaller and rounded; lateral lobe (L1) subtriangular, ending in an acute tip; lateral margin of sternal surface distally with conical sensilla (S); apical plate with short, acute spines (Sp) surrounding the ejaculatory canal aperture, with some spines inclined to internal aperture.

G2 (Fig. 3C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming the apical girdle (Ag), mesial process blunt (Mp) medially located. Few small spines (Sp) bordering apical girdle.

Mithraculus sculptus (Lamarck, 1818) (Fig. 4).

G1 (Fig. 4A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with simple setae (Se) from base (Ba) to distal third of shaft; suture (Ss) extending from base to distal margin of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal third gradually tapering, slightly curved towards lateral line, distinct subrounded protuberance (P) on mesial margin; strangled apex (A) formed by apical plate and two lobes - mesial lobe (Ml) rounded and short; lateral lobe (Ll) elongated, subtriangular, ending in acute tip; lateral margin of sternal surface distally with round sensilla (S); apical plate with long, acute spines (Sp) surrounding ejaculatory canal aperture, with some spines inclined towards internal aperture.

G2 (Fig. 4C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming apical girdle (Ag), mesial process blunt (Mp) medially located.

Mithrax hispidus (Herbst, 1790) (Fig. 5).

G1 (Fig. 5A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with pappose setae (Se) from base (Ba) to distal third of shaft; suture (Ss) extending from base to distal margin of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal third gradually tapering, slightly curved towards lateral line, distinct subrounded protuberance (P) on mesial margin; strangled apex (A) formed by apical plate and two lobes - mesial lobe (Ml) rounded, short; lateral lobe (Ll) elongated, subtriangular, ending in acute tip; lateral margin of sternal surface distally with rounded sensilla (S); apical plate with long, acute spines (Sp) surrounding ejaculatory canal aperture, with some spines inclined towards internal aperture.

G2 (Fig. 5C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming apical girdle (Ag) with few and small spines (Sp) bordering apical girdle; mesial process (Mp) large, conical, blunt, laterally located.

Mithrax tortugae Rathbun, 1920 (Fig. 6).

G1 (Fig. 6A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with pappose setae (Se) from base (Ba) to distal third of shaft; suture (Ss) extending from base to distal margin of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal third gradually tapering, slightly curved towards lateral line, distinct subrounded protuberance (P) on mesial margin; strangled apex (A) formed by apical plate and two lobes - mesial lobe (Ml) short subtriangular ending in acute tip; lateral lobe (Ll) elongated, subtriangular, ending in acute tip; lateral margin of sternal surface distally with rounded sensilla (S); apical plate with short, acute spines (Sp) surrounding ejaculatory canal aperture, with some spines inclined towards internal aperture.

G2 (Fig. 6C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming apical girdle (Ag) with few and small spines (Sp) bordering apical girdle; mesial process (Mp) large, conical and blunt, medially located.

Nonala holderi (Stimpson, 1871) (Fig. 7).

G1 (Fig. 7A, B): long, robust, slightly flattened dorsoventrally; center slightly curved mesially towards mesial line; lateral margin with pappose setae (Se) from base (Ba) to distal third of shaft; suture (Ss) from base to distal end of tip, acute ejaculatory canal aperture (Ec) on pleonal surface margin; distal end markedly tapered, devoid of lobe; distal torsion pronounced, few short spines (Sp) surrounding ejaculatory canal aperture; lateral margin of pleonal surface distally with short, acute sensilla (S).

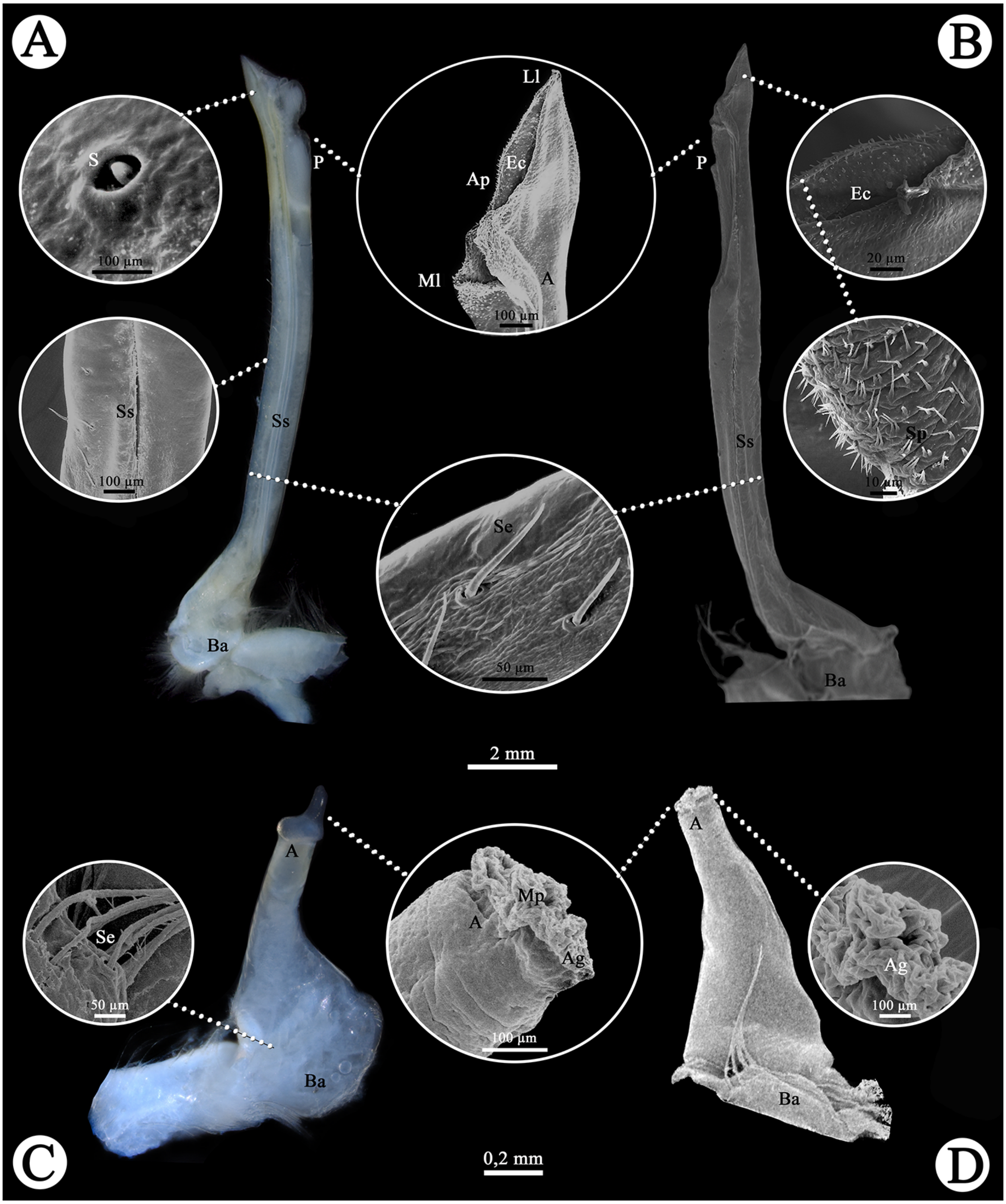

Figure 4. Gonopods of *Mithraculus sculptus* (Lamarck, 1818) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; L1, lateral lobe; M1, mesial lobe; Mp, mesial process; P, protuberance; S, sensillum; Se, setae; Ss, suture; Sp, spine.

G2 (Fig. 7C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming the apical girdle (Ag), mesial process blunt (Mp), medially located.

Pitho laevigata (A. Milne-Edwards, 1875) (Fig. 8).

G1 (Fig. 8A, B): long, robust, rounded; distal third slightly curved towards mesial line; lateral margin with few pappose setae (Se) from base (Ba) to distal third of shaft, simple setae on lateral side of apex; suture

(Ss) extending from base to distal end (De) of tip, acute ejaculatory canal aperture on pleonal surface margin; distal end tapering abruptly, forming large process, mesially curved, spine on ejaculatory canal absent; lateral margin of sternal surface distally with rounded sensilla (S).

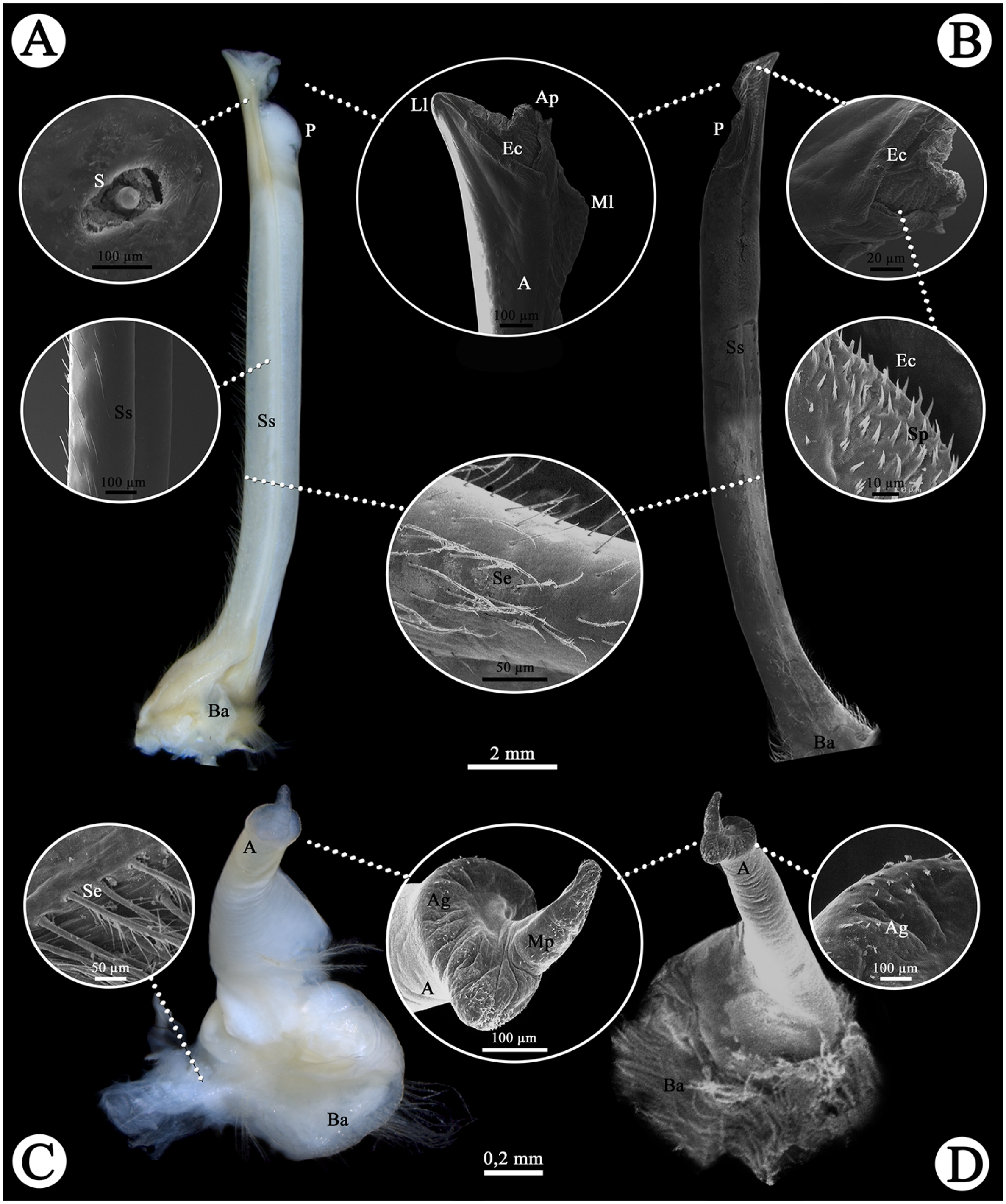

Figure 5. Gonopods of *Mithrax hispidus* (Herbst, 1790) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; Ll, lateral margin; Ml, mesial margin; Mp, mesial process; P, protuberance; S, sensillum; Se, setae; Ss, suture; Sp, spine.

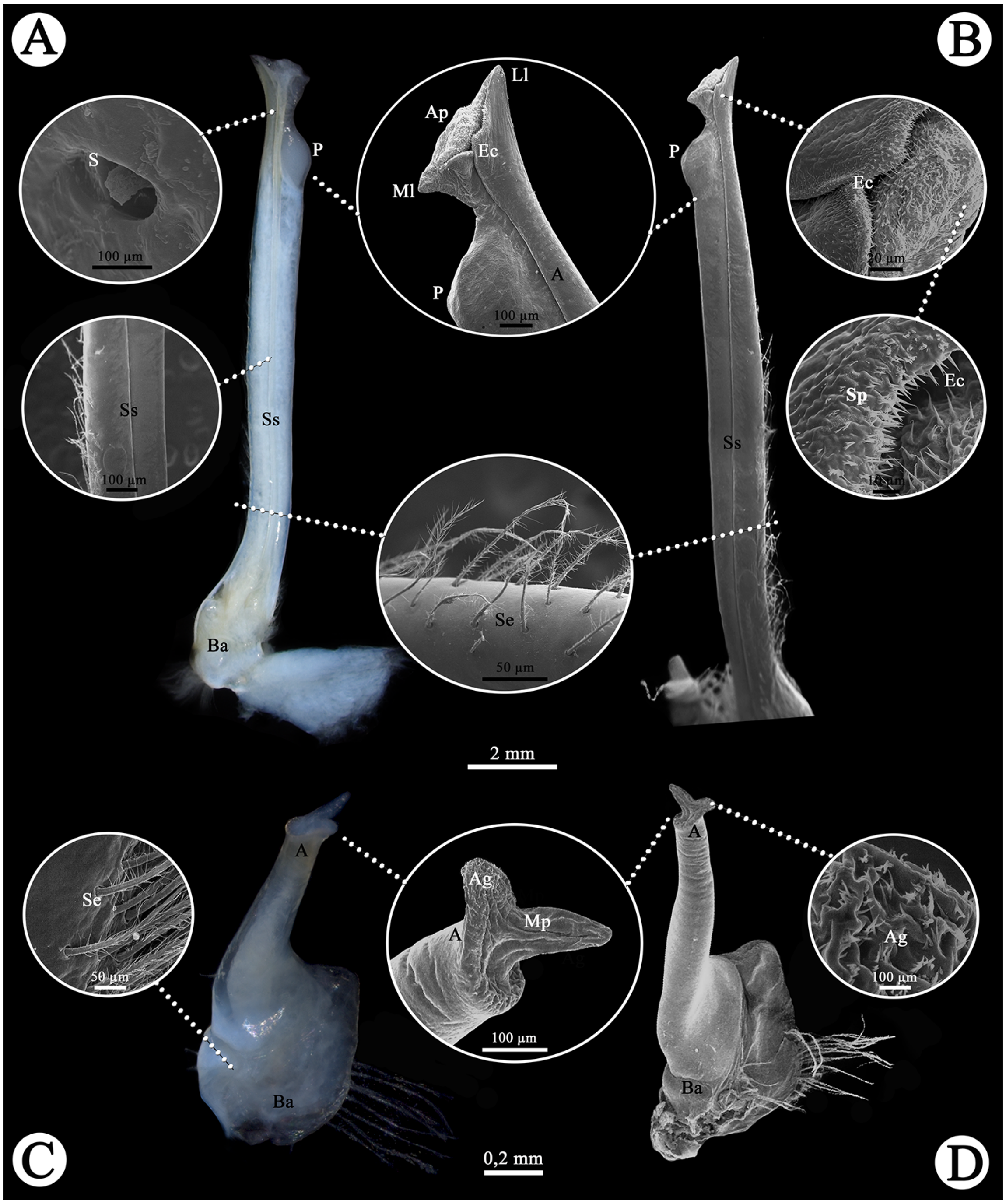

Figure 6. Gonopods of *Mithrax tortugae* Rathbun, 1920 in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; Ll, lateral margin; Ml, mesial margin; Mp, mesial process; P, protuberance; S, sensillum; Se, setae; Ss, suture; Sp, spine.

G2 (Fig. 8C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming apical girdle (Ag), large mesial process (Mp) blunt, medially located.

Teleophrys ruber (Stimpson, 1871) (Fig. 9).

G1 (Fig. 9A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with simple setae (Se) from base (Ba) to distal second third of shaft; suture

(Ss) extending from base to distal end of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal fourth gently tapering and slightly curved towards lateral line, with subtriangular protuberance (P) on mesial margin; strangled apex (A) formed by apical plate and two distinct lobes – mesial lobe (M1) small, oval; lateral lobe (L1) larger, subtriangular, border rounded distally, ending in blunt tip; lateral margin of sternal surface distally with small conical sensilla (S); apical plate with long, acute spines (Sp) surrounding ejaculatory canal aperture, with some spines inclined towards internal aperture.

Figure 7. Gonopods of *Nonala holderi* (Stimpson, 1871) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ba, basis; Ec, ejaculatory canal aperture; Mp, mesial process; S, sensillum; Se, setae; Ss, suture; Sp, spine.

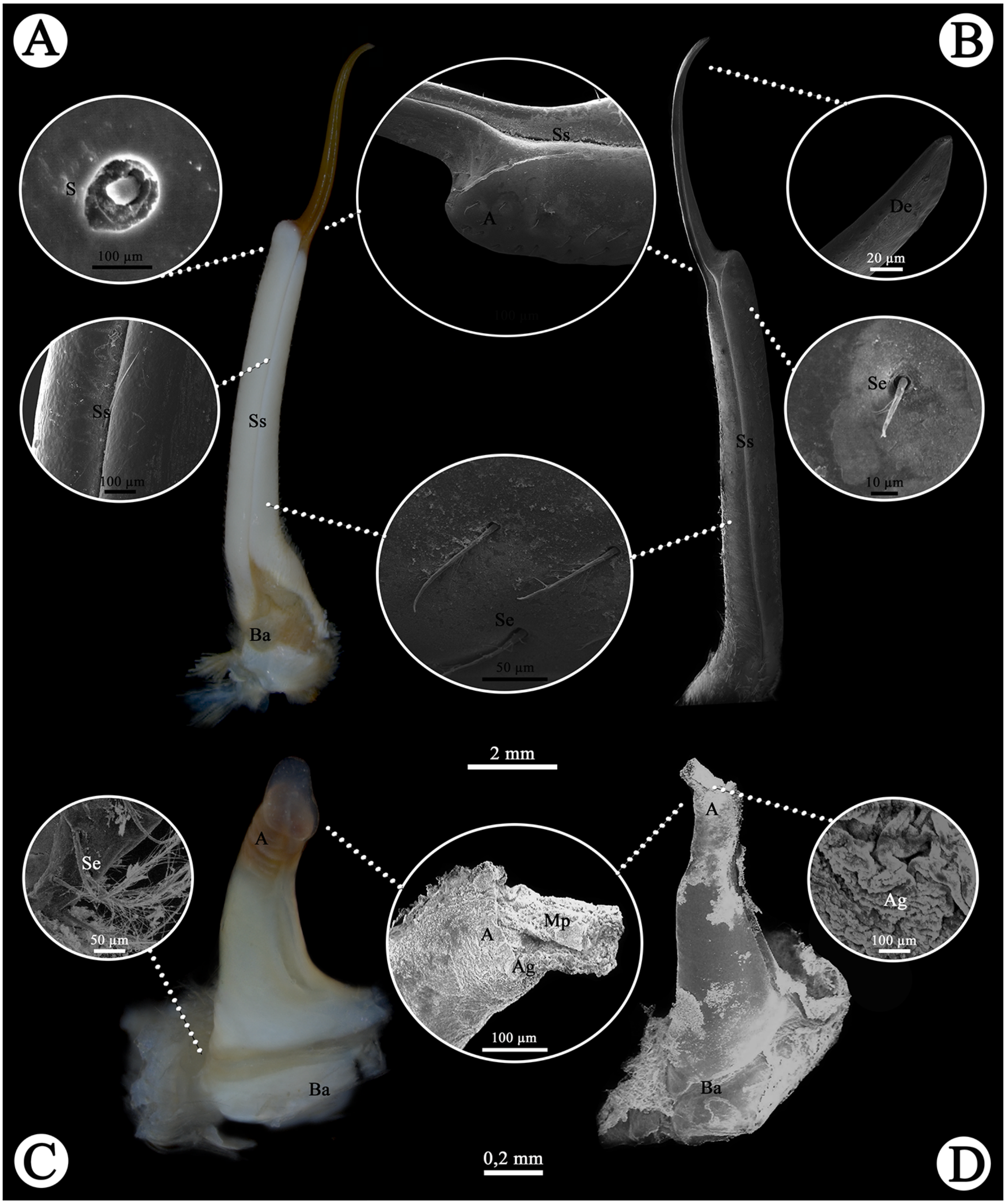

Figure 8. Gonopods of *Pitho laevigata* (A. Milne-Edwards, 1875) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ba, basis; De, distal end; Mp, mesial process; S, sensillum; Se, setae; Ss, suture.

G2 (Fig. 9C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming apical girdle (Ag), large mesial process (Mp) blunt, laterally located.

Macrocoeloma trispinosum (Latreille, 1825) (Fig. 10).

G1 (Fig. 10A, B): long, robust, slightly flattened dorsoventrally; proximal third slightly curved towards mesial line; lateral margin with few simple setae (Se) from base (Ba) to distal second third of shaft; suture

(Ss) extending from base to distal end of apical plate (Ap), ejaculatory canal aperture (Ec) on pleonal surface margin; distal end gently tapering slightly curved towards lateral line; strangled apex (A) formed by apical plate and one distinct lobe - mesial margin (M) small, rounded with piriform aperture; lateral margin (L) larger, subtriangular, border rounded, ending distally in acute tip; lateral margin of sternal surface distally with small, acute sensilla (S); apical plate with short, acute spines (Sp) surrounding ejaculatory canal aperture, with some spines directed towards internal aperture.

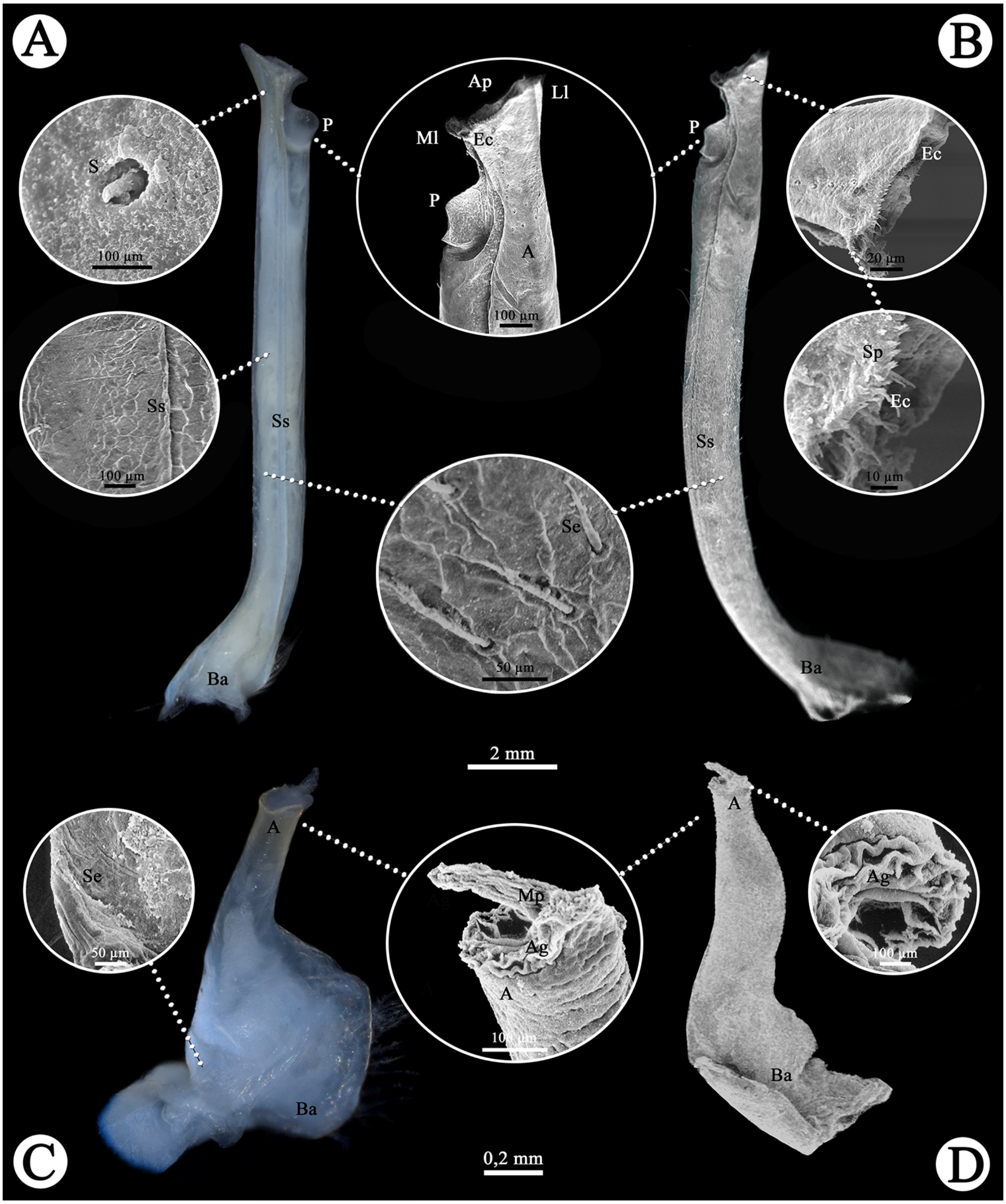

Figure 9. Gonopods of *Teleophrys ruber* (Stimpson, 1871) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; L1, lateral lobe; M1, mesial lobe; Mp, mesial process; P, protuberance; S, sensillum; Se, setae; Ss, suture; Sp, spine.

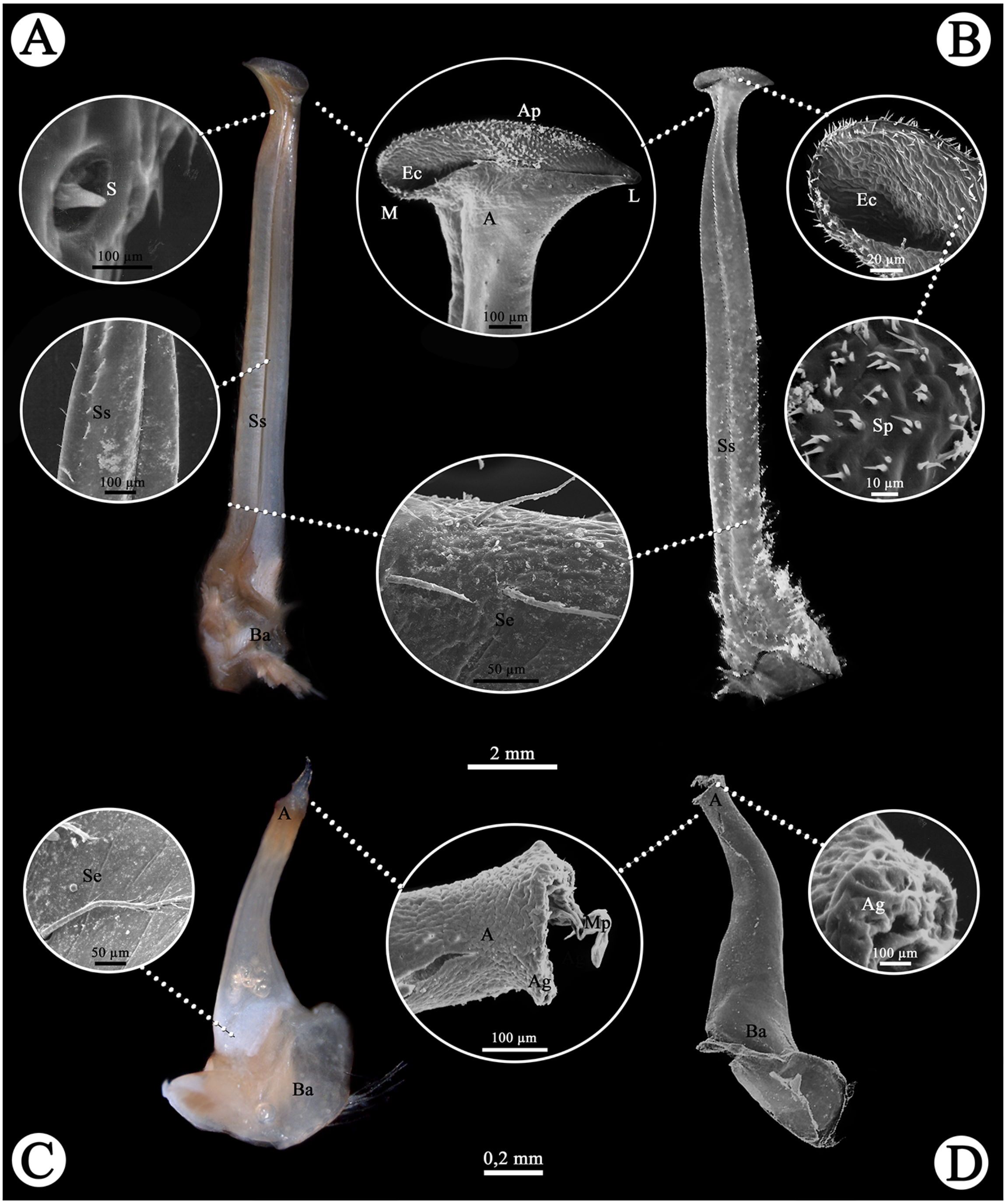

Figure 10. Gonopods of *Macrocoeloma trispinosum* (Latreille, 1825) in pleonal view. (**A**) left first gonopod in stereomicroscopy; (**B**) right first gonopod in scanning electron microscopy; (**C**) left second gonopod in stereomicroscopy; (**D**) right second gonopod in scanning electron microscopy; A, apex; Ag, apical girdle; Ap, apical plate; Ba, basis; Ec, ejaculatory canal aperture; L, lateral margin; M, mesial margin; Mp, mesial process; S, sensillum; Se, setae; Ss, suture; Sp, spine.

G2 (Fig. 10C, D): short, conical, tapering distally; basally enlarged with few sparse plumodenticulate setae (Se); apex (A) with wrinkles forming the apical girdle (Ag), mesial process (Mp) blunt, laterally located; Few small spines (Sp) bordering apical girdle.

Discussion

General morphology of majoid gonopods

The function of the cuticular structures of the gonopods during copulation remains unknown in most majoids, as well as in many other groups of Brachyura. Furthermore, the terminology used in the literature is confusing. The overall morphology of G1 and G2 presents a pattern that so far appears to be observed in all species of this superfamily, where G1 is generally much longer than G2 and characterized by having a tubular cuticle that forms the ejaculatory canal; whose suture is well visible, with the aperture placed at the apex of G1.

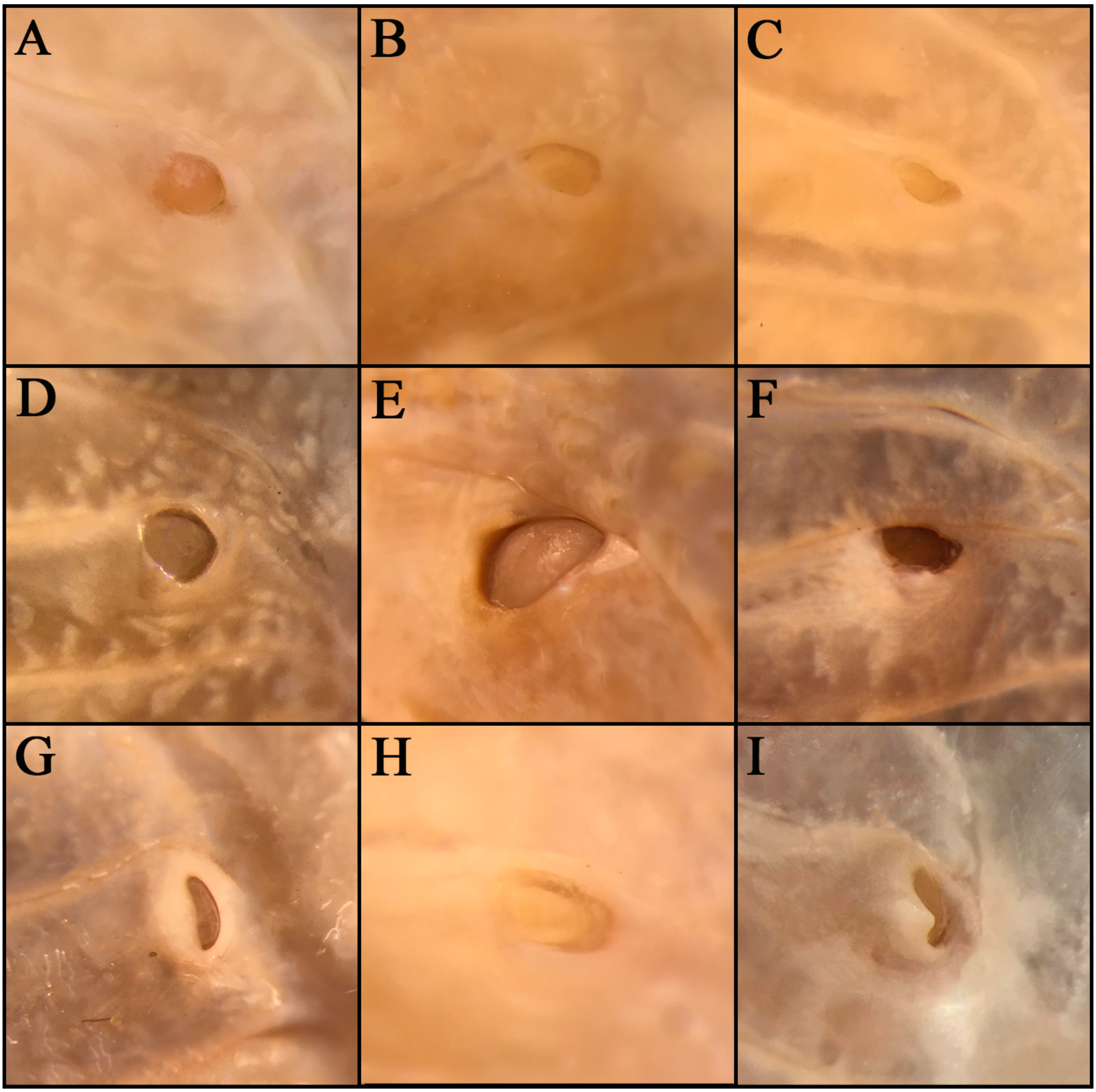

Figure 11. Left vulva of: (**A**) *Amphithrax aculeatus* (Herbst, 1790) cw 48.9mm (LSZ012). (**B**) *Mithraculus coryphe* (Herbst, 1801) cw 19.0mm (LSZ001). (**C**) *Mithraculus forceps* A. Milne-Edwards, 1875 cw 17.2mm (LSZ002). (**D**) *Mithraculus sculptus* (Lamarck, 1818) cw 14.7mm (LSZ006). (**E**) *Mithrax hispidus* (Herbst, 1790) cw 88.1mm (MZUSP18874). (**F**) *Mithrax tortugae* Rathbun, 1920 cw 39.6mm (MZUSP8064). (**G**) *Pitho laevigata* (A. Milne-Edwards, 1875) cw 30.6mm (LSZ009). (**H**) *Teleophrys ruber* (Stimpson, 1871) cw 12.5mm (LSZ003). (**I**) *Macrocoeloma trispinosum* (Latreille, 1825) cw 22.6mm (LSZ007).

Different types of setae are distributed throughout the G1, and very short spines are usually only found near the aperture of the ejaculatory canal. The setae are hypothesized to be sensory and/or mechanical receptors during copulation (Beninger *et al.*, 1991; Rorandelli *et al.*, 2008), and the spines of the ejaculatory canal aperture may have a mechanical role in the rupture of the spermatophores, transferring free sperm cells to the females for immediate fertilization or storage (Rorandelli *et al.*, 2008; Antunes *et al.*, 2018). The G1 apical morphology and the type and distribution of the setae on the G1 vary considerably between species [see Beninger *et al.* (1991) for *Chionoecetes opilio* (O. Fabricius, 1788), Oregoniidae; Neumann (1996a) for *Maja crispata* Risso, 1827, *Maja squinado* (Herbst, 1788), and *Neomaja goltziana* (d'Oliveira, 1889), Majidae; Rorandelli *et al.* (2008) for *Inachus phalangium* (Fabricius, 1775), Inachidae; Sal Moyano *et al.* (2011) for *Libinia spinosa* Guérin, 1832, Epialtidae; and Kienbaum *et al.* (2017) for *Ms. sculptus,* Mithracidae and *Stenorhynchus seticornis* (Herbst, 1788), Inachoididae]. The sensilla present in the apical region are other structures with a possibly mechanoreceptive function (Rorandelli *et al.*, 2008), assisting in G1 positioning during copulation (Beninger *et al.,* 1991). Nevertheless, these cuticular structures have been named differently by several authors, such as "short setae", "tooth-like" structures or "denticles" (*e.g.,* Beninger *et al.*, 1991; Rorandelli *et al.*, 2008; Sal Moyano *et al.*, 2011; Kienbaum *et al.*, 2017).

The G2 is inserted into the mesial foramen of G1, whereas the penis is inserted into the lateral foramen of G1. The G2 is small and tubular, and only one-fifth of the length of the G1, with the apical region consisting of an apical girdle with a central protuberance. It is morphologically much more conservative than the G1 and shows very small differences among studied majoids (Beninger *et al.*, 1991; Neumann, 1996a; Sal Moyano *et al.*, 2011; Kienbaum *et al.*, 2017). The main variation in the G2, among the species studied, is in the position and size of the mesial process. This process is medial in position in *A. aculeatus*, *Ms. coryphe*, *Ms. forceps*, *Ms. sculptus*, *Mx. tortugae*, *N. holderi* and *P. laevigata* (Figs. 1C, D, 4C, D, 6C, D, 8C, D) and lateral in *Mx. hispidus*, *T. ruber,* and *Ma. trispinosum* (Figs. 5C, D, 9C, D, 10C, D). *Pitho laevigata* (Fig. 8C, D) has the largest mesial process, while it is more conical in *Mx. hispidus* (Fig. 5C, D) and *Mx. tortugae* (Fig. 6C, D) and blunt in the other species. The role of a long G2 in sperm transfer is interpreted as a plunger or piston that pumps within the G1 (Bauer, 1986; Beninger *et al.*, 1991; Becker *et al.*, 2012; Guinot *et al.*, 2013; Kienbaum *et al.*, 2017). In Majoidea, however, all species have a short G2, and according to Becker *et al.* (2012), the ultrastructure and histology suggest a certain capacity for the G2 to become inflated, sealing the G1 ejaculatory canal and minimizing water influx and sperm loss.

General morphology of G1 and vulvae of Mithracidae

In most mithracids the G1 is long, robust and has a tubular structure, slightly flattened dorsoventrally. The G1 apical plate, for most mithracids, consists of two lobes and a protuberance on the mesial margin of the distal region (Figs. 1A, B, 6A, B, 9A, B). Short spines surround the aperture of the ejaculatory canal, and the lateral margin of the G1 apex has a row of sensilla (Figs. 1A–10A). These short spines around the ejaculatory canal aperture are observed in all studied species, except for *P. laevigata*. Similarly, all studied species have sensilla in the lateral margin of the G1 apex, mainly on the sternal surface, ranging from a rounded tip (detail in Figs. 1A, 2A, 4A, 5A, 6A, 8A), to conical (detail in Figs. 3A, 9A) and acute (detail in Figs. 7A, 10A). Sensilla have been previously reported in other majoids as "small and short setae inside a pore" in *I. phalangium* (see Rorandelli *et al.*, 2008), "very short setae" in *Ms. sculptus* and *S. seticornis* (see Kienbaum *et al.*, 2017), "setae of type 2" in *L. spinosa* (see Sal Moyano *et al.*, 2011) or "setae of type III" in *C. opilio* (see Beninger *et al.,* 1991). The spines have been termed "tooth-like" in *I. phalangium* (see Rorandelli *et al.*, 2008), "denticles" in *Ms. sculptus* and *S. seticornis* (see Kienbaum *et al.*, 2017), and "short setae" in *C. opilio* and *L. spinosa* (see Beninger *et al.*, 1991; Sal Moyano *et al.*, 2011). Here, we consider all these structures to be homologous.

The G1 is provided with numerous setae, mostly pappose, whose position varies along the shaft. In *A. aculeatus*, *Ms. forceps*, *Mx. hispidus*, *Mx. tortugae* and, *N. holderi,* the pappose setae are arranged along the lateral margin of the G1, from the base to the distal third of the shaft, whereas in *Ms. coryphe,* pappose setae are found from the base to the distal second third of the shaft. *Mithraculus sculptus* differs from both congeners and superficially resembles *T. ruber* in having simple setae from the base to the distal third of the shaft. *Pitho laevigata* has fewer pappose setae from the base to the distal third of the shaft and simple setae on the lateral surface of the apex of G1.

Although the G1 differs significantly in shape among the studied species, their vulvae do not differ fundamentally from one another (Fig. 11), and only small differences were observed in the vulvar cover of *P. laevigata* and *Ma. trispinosum*. This suggests that the vulvae and G1 do not require exact matching to each other for mechanical coupling.

Apart from the consistent morphology found in *Mithrax* Latreille, 1816 and *Mithraculus* White, 1847 species, *N. holderi* and *P. laevigata* have the most dissimilar G1 (Figs. 7, 8), with the apex without lobes and protuberances in the mesial margin of the distal region. The G1 shaft of *P. laevigata* is tubular, with an extremely long apex and pronounced apical process, and the aperture of the ejaculatory canal is distinctly reduced and located distally, without spines or sensilla around it. Despite the obvious morphological differences, when compared to other mithracids, Windsor and Felder (2014) included *Pitho* Bell, 1836 in the well-supported Mithracidae clade; an action supported by previous works (Hultgren and Stachowicz, 2008; Hultgren *et al.*, 2009; Santana *et al.*, 2016). Similarly, in *N. holderi,* the gonopod is completely different from all other mithracids, with the G1 ending in an abrupt distal taper, without lobes and with a distinct apical torsion. These strong differences in the G1 morphology support the placement of this species in a separate genus (previously *Mithrax holderi*), although Windsor and Felder (2014) did not include a discussion about the gonopod for *Nonala* Windsor and Felder, 2014. Gonopod morphology has been frequently used to tell species apart, but much less frequently to regroup species (Garth, 1958; Griffin and Tranter, 1986; Wagner, 1990).

The position of *Pitho* and *Nonala* in the tree topology of Windsor and Felder (2014) has led to different interpretations of gonopod morphological evolution. The basal position of *Pitho* may be indicative of a tubular G1 being a plesiomorphic character, whereas a dorsoventrally flattened G1 should be considered synapomorphic among mithracids. On the other hand, the distal torsion of G1 in *Nonala* is a very distinct autapomorphy of the genus since, apart from distal torsion, most of its G1 morphology is similar to other mithracids.

The position of Macrocoeloma Miers, 1879

Historically, *Macrocoeloma*, has been regarded as a Mithracidae (Rathbun, 1925; Williams, 1984; Melo, 1996). The genus, however, was recently transferred to Epialtidae by Colavite *et al.* (2020). Indeed, in *Ma. trispinosum*, the G1 differs greatly from the typical mithracid in having a unilobed apex characterized by a piriform ejaculatory canal aperture located at the mesial margin, and in having the lateral margin ending in a distinct acute tip (Fig. 10A, B). Generally, the G1 of *Ma. trispinosum* resembles more closely that of other epialtids, such as *L. spinosa*, *Stenocionops furcatus* (Olivier, 1791), and *Notolopas mexicanus* Garth, 1940. Thus, the G1 morphology firmly supports the placement of *Macrocoeloma* in Epialtidae.

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