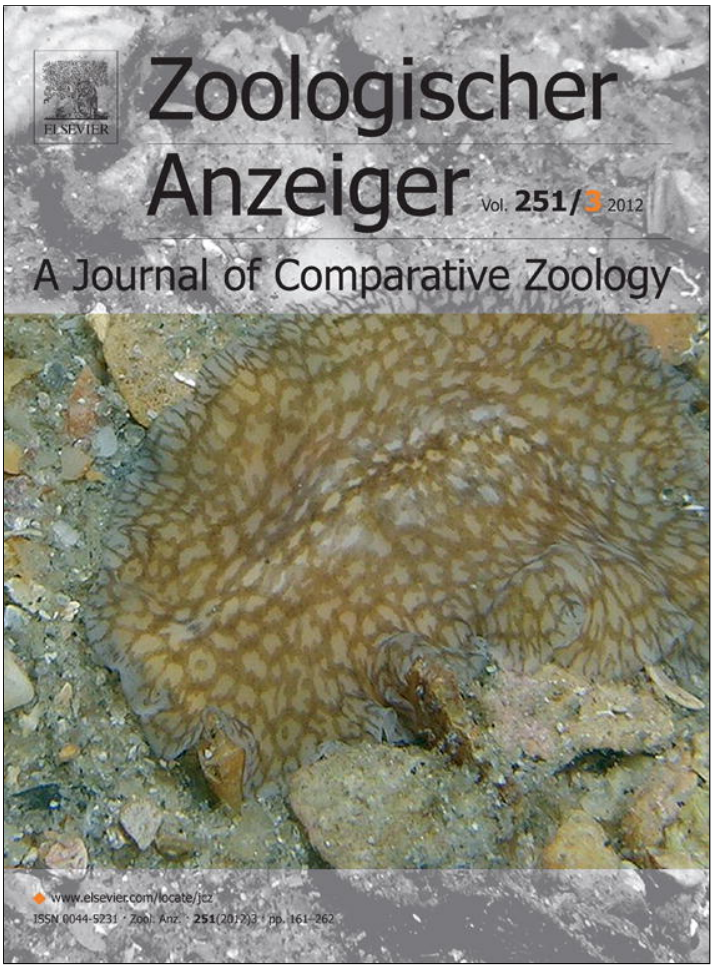

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Morphology and ultrastructure of definitive males of *Arcticotantulus pertzovi* and *Microdajus tchesunovi* (Crustacea: Tantulocarida)

Alexandra S. Petrunina [a], Gregory A. Kolbasov [b,*]

[a] Department of Invertebrate Zoology, Faculty of Biology, Moscow State University, Moscow 119992, Russia
[b] Department of Invertebrate Zoology, White Sea Biological Station, Faculty of, Biology, Moscow State University, Moscow 119992, Russia

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ABSTRACT

Free-swimming definitive males of two species of Tantulocarida (Crustacea), *Arcticotantulus pertzovi* (Basipodellidae) and *Microdajus tchesunovi* (Microdajidae), were reared for the first time. Their morphology and ultrastructure were studied using scanning electron and light microscopy. A detailed analysis of the morphological characters of all currently described species revealed several features typical for most known male tantulocaridans, such as the presence of eight aesthetascs, seven pairs of multifid sensilla on the carapace, paired brush setae on the protopods of the thoracopods, and three furcal setae. The monophyly of the families Microdajidae and Doryphallophoridae is corroborated, while the families Deoterthridae and Basipodellidae are more likely paraphyletic.

1. Introduction

The class Tantulocarida was proposed in 1983 by Boxshall and Lincoln. Since then about 35 species belonging to five families have been described. They are known to inhabit a wide range of depths from shallow waters to the abyssal plain, and from the tropics to boreal and polar regions. Tantulocaridans utilize other benthic crustaceans as hosts (Copepoda, Isopoda, Cumacea, Tanaidacea, Amphipoda, Ostracoda) and lack typical crustacean moults. These enigmatic, parasitic crustaceans have a complex dual life cycle, comprising a parthenogenetic and a sexual phases (Huys et al., 1993). The free-swimming tantulus larva is the infective stage which, upon location of a suitable host, attaches itself to the host cuticle by means of an oral disk at the anterior end of the cephalon. The tantulus larva lacks cephalic appendages but has an unpaired internal stylet of unknown origin. Tantulus larvae (or tantuli) can metamorphose in two different ways: (1) a trunk sac is produced from a swelling appearing behind the cephalon or between the thoracic somites; thus, both thorax (with its thoracopods) and abdomen are not shed off and remain on the body; in this case a male is developed inside this trunk sac; (2) when both thoracic and abdominal somites are shed off as a result of trunk sac formation it indicates that either a sexual female or parthenogenetic stage will be produced. In the sexual cycle, mating occurs between the free-swimming males and sexual females and probably results in the production of nauplii (pers. comm. of Prof. Pedro Martínez Arbizu). In the case of the parthenogenetic cycle, numerous eggs develop inside a trunk sac, with a new generation of infective tantuli emerging from these eggs.

Our knowledge of the Tantulocarida is still incomplete. The life cycle was reconstructed from several different species, and it is likely that some of the stages in the sexual phase (i.e. nauplii) have not yet been described. Descriptions of other stages often lack details on ultrastructure. Sexual females were recorded only twice (Huys et al., 1993; Ohtsuka and Boxshall, 1998) but both individuals were immature.

Male tantulocaridans are known for 14 species in five families, however only for 9 species more or less detailed descriptions exist (Table 1). It is necessary to mention that all previously studied male individuals were immature and described after being extracted from trunk sacs of the preceding stage, meaning that fully developed male tantulocaridans have yet to be studied. The definitive males of two species, *Arcticotantulus pertzovi* Kornev, Tchesunov & Rybnikov, 2004 (family Basipodellidae) and *Microdajus tchesunovi* Kolbasov & Savchenko, 2010 (family Microdajidae) were reared for the first time. In this paper we describe their morphology, including ultrastructural details, using scanning electron microscopy.

2. Material and methods

The material on *M. tchesunovi* and *A. pertzovi* was collected off the White Sea Biological Station of Moscow State University (Velikaya Salma Strait, Kandalaksha Bay) (66°31′41″N, 33°11′08″E)

* Corresponding author.
E-mail addresses: a-sergevna@mail.ru (A.S. Petrunina), gakolbasov@gmail.com (G.A. Kolbasov).

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Table 1
Main morphological characters of tantulocaridan males of families: Microdajidae (M), Basipodellidae (B), Deoterthridae (D) and Doryphallophoridae (Dor). *Abbreviations*: ? – no information, * – doubt information.

Species	Length (μm)	Cephalic dorsal lamellae	Head pores with sensilla	Paired cephalic anterodorsal processes/lobes	thp 1 en–ex setation	thp 2 en–ex setation	Cuticular fold on thp 1–2	thp 3 protopod
M. tchesunovi (M)	355	Longitudinal	7 pairs/multifid tips	Absent	5–6	5–6	Absent	Unsegmented.
M. pectinatus (M)	308	Longitudinal	7 pairs/bifid tips*	Absent	5–6	5–6	Absent	Two-segmented*
M. aporosus (M)	260	?	8 pairs*	Absent	?	?	Absent	?
M. langi (M)	200	?	?	Absent	5–6	5–6	Absent	Unsegmented
S. stocki (B)	400	Longitudinal	?	Absent	5–6	5–6	Absent	Unsegmented
A. pertzovi (B)	260	Longitudinal and transverse	7 pairs/multifid tips	Absent	5–6	5–6	Present	Unsegmented
C. coomansi (D)	320	Longitudinal and transverse	7 pairs/bifid tips	Absent	4–7	4–6	Absent	Unsegmented
D. harrisoni (Dor)	460	Undeveloped	6 pairs/miltifid and single tips*	Present	5–6	5–6	Absent	Unsegmented
P. inusitata (Dor)	620	Longitudinal and transverse	6 pairs/bifid tips*	Present	5–6	5–6	Absent	Two-segmented*

Species	thp 3 en–ex setation	thp 4–5 protopod	thp 6 setation	thps with brush setae	Abdominal lamellae	Penis, length, structure	Furcal rami, length, form	Furcal setae	Reference
M. tchesunovi (M)	5–6	Two-segmented	6	1–5	Reduced	60 μm, curved, with terminal opening	47 μm, cylindrical, slender	3 (2 terminal, 1 subterminal)	Kolbasov and Savchenko (2010) and herein
M. pectinatus (M)	5–6	Two-segmented	5*	1–5	Reduced	55 μm, curved, with terminal opening	15 μm, cylindrical, slender	3 (terminal)*	Boxshall et al. (1989)
M. aporosus (M)	?	?	?	?	?	?	14 μm, cylindrical, slender	3 (2 terminal, 1 subterminal)	Grygier and Sieg (1988)
M. langi (M)	5–6	Two-segmented	5*	1?–5	?	70 μm, curved, with terminal opening	27 μm, cylindrical, slender	2 (terminal*)	Boxshall and Lincoln (1987)
S. stocki (B)	5–6	Unsegmented (4),* two-segmented (5)	5*	1–6	Developed	48 μm, curved, with terminal opening	17 μm, cylindrical, slender	3 (terminal)	Boxshall and Huys (1989)
A. pertzovi (B)	5–6	Two-segmented	6	2*–6	Developed	22 μm, curved, with dorsal subterminal opening	15 μm, short, lancete-shaped	3 (subterminal)	Kolbasov et al. (2008), herein
C. coomansi (D)	4–6	Two-segmented	6	1–5	Developed	45 μm, curved, with terminal opening	32 μm, tapering, slender	3 (subterminal), with ventral spinous process	Huys, 1990
D. harrisoni (Dor)	5–6	Two-segmented	6	1–6	Reduced	120 μm, straight, with terminal opening	Reduced to single seta*	1*	Boxshall and Lincoln (1987)
P. inusitata (Dor)	5–6	Two-segmented	5*	1–6	Developed	153 μm, straight, terminal opening	74 μm, strongly elongated, slender	3 (1 terminal, 1 subterminal, 1 middle)	Ohtsuka and Boxshall (1998)

in July–August, 2007–2009. Sediment samples were obtained with a hyperbenthic Ockerman dredge from depths of 20–50 m and rinsed through a 50-μm sieve. Host specimens of the harpacticoid copepods *Bradya typica* Boeck, 1873, *Pseudobradya acuta* Sars, 1904 (for *A. pertzovi*) and the tanaidacean *Typhlotanais* sp. (for *M. tchesunovi*), infested with tantuli containing males at a late stage of development, were kept separately in small dishes in the refrigerator at +4 °C for several days (less than two weeks) until adult free-swimming males appeared. Males were fixed in glutaraldehyde less than 24 h after hatching. Two males of both species were mounted in glycerol on glass slides and examined using a WILD light microscope. Line drawings were made using oil immersion objectives on an Olympus BX 51 microscope equipped with Nomarski differential interference contrast. Several male individuals were selected for scanning electron microscopy. This material was postfixed in 2% OsO_4, then dehydrated in a graded ethanol series and acetone, and critical point dried in CO_2. The specimens were then sputter-coated with platinum-palladium alloy and examined in a JEOL JSM-6380LA microscope at operating voltages of 15–20 kV.

TAXONOMY

Class Tantulocarida Boxshall & Lincoln, 1983

Family Basipodellidae Boxshall & Lincoln, 1983

Genus *Arcticotantulus* Kornev, Tchesunov & Rybnikov, 2004

A. pertzovi Kornev, Tchesunov & Rybnikov, 2004

(Figs. 1–5)

Material examined. Five fully developed males of *A. pertzovi* were examined using either scanning electron microscopy (three specimens) or light microscopy (two specimens). Locality: White Sea (66°31′41″N, 33°11′08″E), depths 20–50 m, pelite silt.

Description of adult male. Body consisting of cephalothorax incorporating first and second thoracic somites, four free thoracic somites and two-segmented abdomen terminating in paired, unsegmented furcal rami. Thoracic somites each with a pair of natatory thoracopods. Total body length from anterior margin of cephalothorax to end of furcal rami (not including furcal setae) about 260 μm.

Cephalothorax about 110 μm long. Dorsal shield (carapace) with conspicuous ornamentation consisting of strong longitudinal and transverse ridges (Figs. 1A and 2A, B); tiny lamellae forming two symmetrical lateral, striated zones (Figs. 1A, 2A and 3E); rest of shield covered by small, irregular cuticular outgrowths making surface look granulated (Fig. 3B, D, E). Rostrum small, tapering towards anterior margin of cephalothorax (Fig. 3A). Posterolateral angles rounded (Figs. 1A and 2A). Anterior ventral surface of cephalothorax granulated and with tiny striations; cuticular ornamentation (Fig. 2D) represented by one medial and two lateral longitudinal ridges. Pit corresponding to entrance site of umbilical cord about 1.7 μm in diameter lacking any associated structures, located 30 μm from anterior margin of cephalothorax (Fig. 2D, F).

Anterior margin of cephalothorax with paired depressions, each containing tuft of four aesthetascs (Fig. 2C and D), with longest aesthetasc situated anterior to cluster of remaining three (Fig. 3C). Aesthetascs approximately 23 μm long (longest one), with rounded tips; microvillar surface formed by numerous, densely arranged, sharp cuticular protrusions (Fig. 2E).

Cephalothorax (carapace) with seven pairs of dorsal sensillate pores (Figs. 1A and 2A, B) and one pair of tiny anterior pores without sensilla (Fig. 3A). Each pore surrounding only one sensillum with short basis and multiple filiform tips (Fig. 3B); number of filiform tips ranging from two to five (Fig. 3A–F). Four anterior (A_{1-4}) pores arranged in two pairs on each side of carapace (Fig. 2A, B). First pair of pores (A_1, A_2) positioned anteriorly, about 10 μm and 14 μm from tip of rostrum, respectively, and slightly displaced laterally (Fig. 3A–C); containing sensilla with two and three filiform tips, respectively. Second pair of anterior pores (A_3 and A_4) more laterally positioned (Figs. 1A and 3C–E); sensillum of pore A_4 with highest number of filliform tips (five) (Figs. 1A and 3E), that of A_3 with only three tips. Posterior pores P_1 (with two sensillar tips) and P_2 (with four sensillar tips) at 43 μm and 22 μm from the posterior margin, respectively (Figs. 1A and 3F). In some paratypes a reduced number of sensillar tips was observed (Fig. 3B, D), but this could be attributable to the imperfect condition of the material (sensillar tips frequently stick together hampering correct observation of their number).

Cephalothorax with two pairs of natatory thoracopods (1–2). Four free pedigerous thoracic somites, each with pair of thoracopods (3–6) and well developed tergites ornamented with cuticular ridges arranged in rows of polygonal cells (Figs. 1A and 4A). Successive tergites gradually decreasing in width.

All thoracopods with unsegmented rami. Thoracopods 1–5 biramous. Thoracopod 1 (Figs. 1B and 4B) with exopod being larger than endopod; exopod armed with five short, thick terminal setae and endopod with four slender terminal setae; endopodal segment almost entirely covered by broad membranous cuticular extension; articulation between endopod and protopod not clearly discernible; exopod with outer cuticular fold displaying fringed margin (Fig. 1B). Thoracopod 2 with cuticular membranes both along distal margin of protopod and inner margin of endopod (Figs. 1C and 4C); exopod larger than endopod, armed with six terminal setae; endopod with five terminal setae. Segmentation and setation of thoracopods 3–5 similar to those in thoracopod 2; difference in size between exopods and endopods less pronounced than in thoracopod 2 and they lack membranous expansions. Thoracopods 4–5 with two-segmented protopods, large coxa and small unarmed basis. Thoracopods 2–6 with two inner brush setae arising from proximal part of protopods (Figs. 1C–G and 4D–F); length of setae about 10 μm, not exceeding size of protopods, and slightly decreasing in length from second to sixth thoracopods. Distal third of setae brush-like, carrying numerous spinules (Fig. 4F). Brush setae on thoracopod 1 not observed (Fig. 4B), but probably concealed by membranous extension of endopod.

Thoracopod 6 (Figs. 1G and 5C) uniramous and three-segmented; long coxa with pair of brush setae; basis short, unarmed; exopod with six terminal setae, outermost seta shortest, others almost equally long.

Protopods of all thoracic limbs ornamented with dense cuticular spinules covering anterior and lateral surfaces (Figs. 4A and 5C). Protopods of thoracopods 2–3 with small serrate scales on anterior surface (Fig. 4C (inset) and D).

Abdomen two-segmented, comprising genital first somite with penis and second somite with furcal rami. Genital somite about 16 μm long, with prominent ventral protrusion almost extending to posterior margin of abdominal somite (Figs. 1I and 5C). Penis slender, curved towards ventral side in its central part, with straight tapering distal portion about 25 μm long measured from junction with ventral protrusion (Figs. 1I and 5D). Articulation between protrusion and penis with thin membranous integument, giving flexibility to penis (Fig. 5F). Sperm ducts opening located subterminally on dorsal side of penis (Fig. 5F). Second abdominal somite (eighth trunk somite) elongate, about 26 μm long, dorsal surface with pattern of cuticular ornamentation (Fig. 5B, C). Furcal rami short, lancet-shaped, about 15 μm long and with three equal subterminal, serrate setae arising from depressions on dorsal surface of ramus (Figs. 1H, I and 5B, D, E); ventral surface smooth (Fig. 5A, F).

Family Microdajidae Boxshall & Lincoln, 1983

Genus *Microdajus* Greve, 1965

M. tchesunovi Kolbasov & Savchenko, 2010

(Figs. 6–9)

Material examined. Four fully developed males of *M. tchesunovi* were examined using either scanning electron (three specimens)

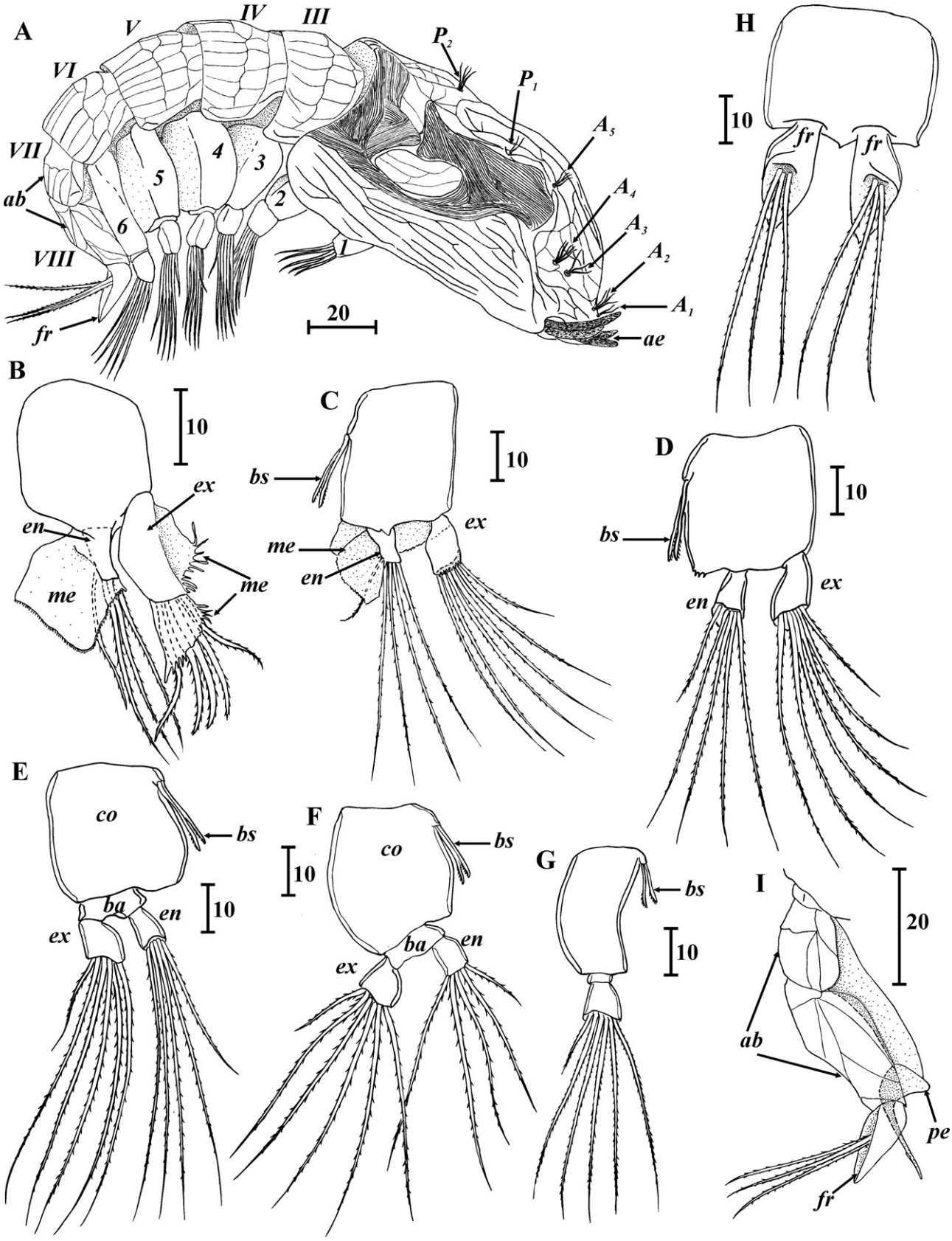

Fig. 1. General view and appendages of definitive male *Arcticotantulus pertzovi*. (A) Habitus, lateral (trunk somites numbered in Roman numerals, thoracopods in Arabic numerals); (B–G) anterior view of thoracopods 1–6, respectively; (H) second abdominal somite with furcal rami, dorsal; (I) abdomen with penis, lateral. Abbreviations: *ab* – abdomen, *ae* – aesthetascs, A_1, A_2, A_3, A_4, A_5, P_1, P_2 – sensillate pores on carapace, *ba* – basis, *bs* – brush setae, *co* – coxa, *en* – endopod, *ex* – exopod, *fr* – furcal rami, *me* – cuticular membrane extenions of thoracopods 1–2, *pe* – penis. Scale bars in μm.

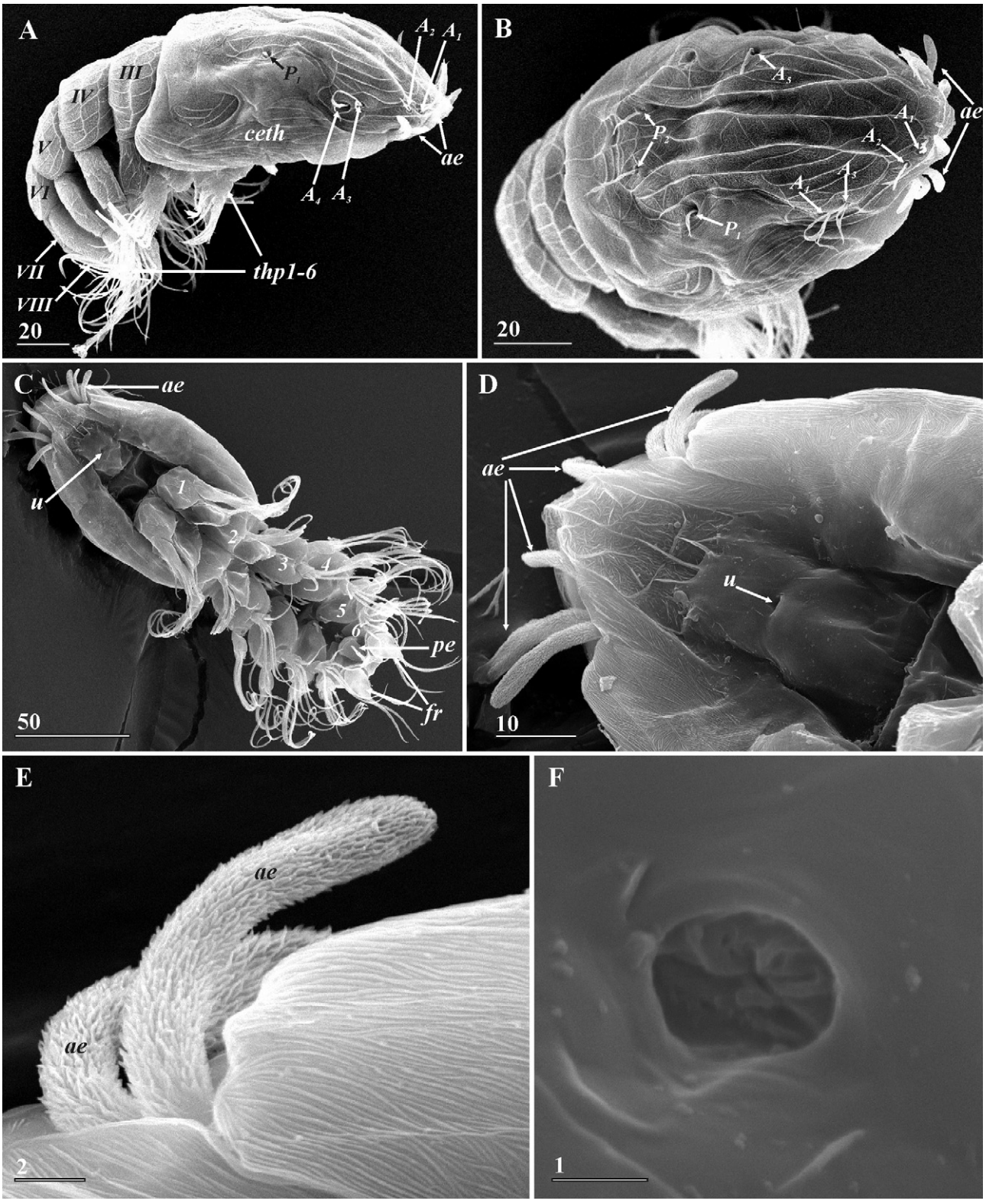

Fig. 2. SEM micrographs. General view and cephalothorax of male *Arcticotantulus pertzovi* (trunk somites numbered in Roman numerals, thoracopods in Arabic numerals). (A) Habitus, lateral; (B) cephalothorax, dorsal; (C) habitus, ventral; (D) anterior part of cephalothorax, ventral; (E) aesthetascs, ventral; (F) site of entrance of umbilical cord. Abbreviations: *ae* – aesthetascs, A_1–A_5 – anterior sensillate pores on carapace; *ceth* – cephalothorax, *fr* – furcal rami, P_1–P_2 – posterior sensillate pores on carapace, *pe* – penis, *thp 1–6* – thoracopods 1–6, *u* – site of entrance of unmbilical cord. Scale bars in μm.

or light microscopy (one specimen); one developing male dissected from a trunk sac was examined using light microscopy only. Locality: White Sea (66°31′41″N, 33°11′08″E), depths 20–50 m, pelite silt.

Description of adult male. Body comprising cephalothorax incorporating first and second pedigerous thoracic somites, four free thoracic somites and two-segmented abdomen terminating in paired, unsegmented furcal rami. Thoracic somites each with

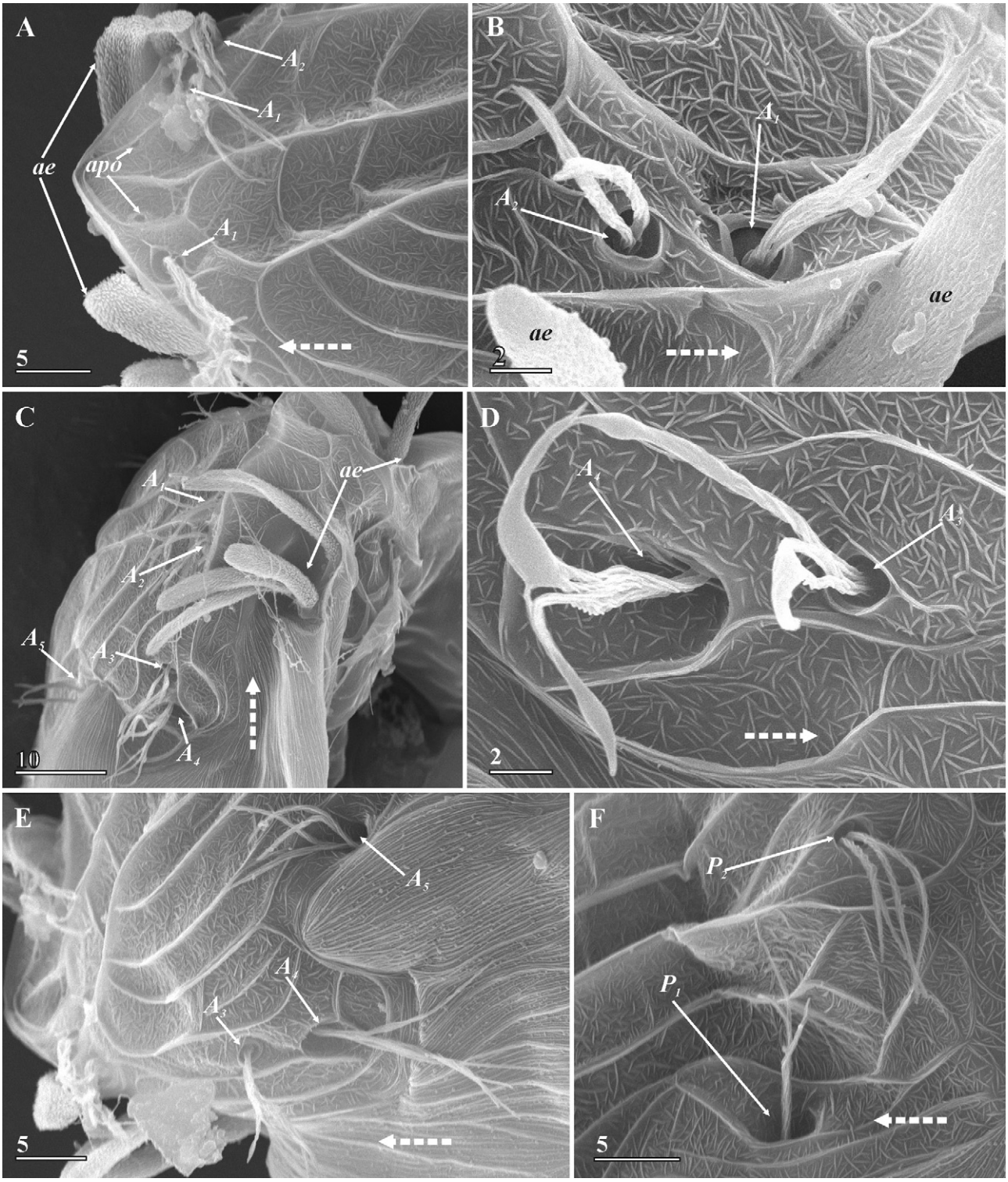

Fig. 3. SEM micrographs. Sensory structures on carapace of male *Arcticotantulus pertzovi*, anterior orientation indicated by dotted arrow. (A) Anterior part of cephalothorax, dorsal; (B) anterior sensillate pores (A_1–A_2); (C) aesthetascs, anteriolateral; (D) anterior sensillate pores (A_3–A_4); (E) anterior sensillate pores (A_3, A_4, A_5); (F) posterior sensillate pores (P_1–P_2). Abbreviations: A_1–A_5 – anterior sensillate pores; *ae* – aesthetascs, *apo* – small anterior pores without sensilla; P_1–P_2 – posterior sensillate pores. Scale bars in μm.

a pair of natatory thoracopods. Total body length from anterior tip of cephalothorax to end of furcal rami (not including furcal setae) approximately 355 μm.

Cephalothorax about 133 μm long, with two tufts of four aesthetascs in anterior part (Figs. 6A, B and 7B); three aesthetascs originating from one site posterior to slightly displaced anterior one (Fig. 7D, E). Aesthetascs about 30 μm long, flattened, have truncated tips with lacerated margins (Fig. 7C–G). Aesthetascs with microvillar surface, consisting of numerous small pointed protrusions (Fig. 7C). Carapace slightly compressed laterally in anterior part (Fig. 7B); without swollen areas or lateral expansions; with sparse dorsal surface ornamentation, including five distinct

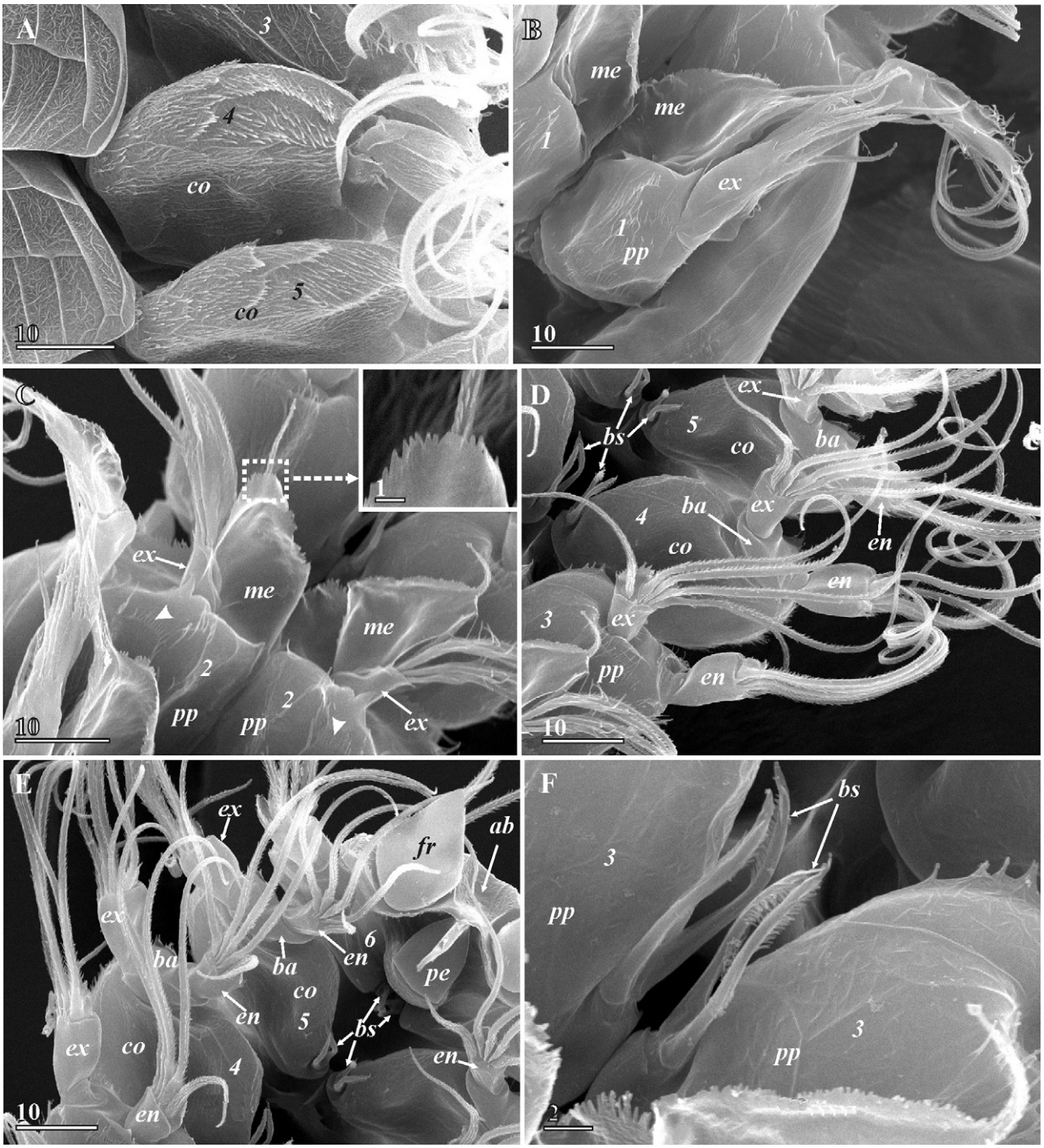

Fig. 4. SEM micrographs. Structure of thoracopods of male *Arcticotantulus pertzovi* (thoracopods numbered in Arabic numerals). (A) protopods of thoracopods 3–5, lateral; (B) thoracopod 1, anterior; (C) thoracopod 2, anterior, margin of cuticular membranous extension enlarged in inset, multifid scales on protopod indicated with arrowheads; (D) thoracopods 3–5, anterioventral; (E) posterior part of hind body with thoracopods 4–5, abdomen with penis and furcal rami, ventral; (F) brush setae of thoracopods 3, anterior. Abbreviations: *ab* – abdomen, *ba* – basis, *bs* – brush setae, *co* – coxa, *en* – endopod, *ex* – exopod, *fr* – furcal rami, *me* – cuticular membranous extensions of thoracopods 1–2, *pe* – penis, *pp* – protopod. Scale bars in μm.

longitudinal cuticular ridges, several longitudinal lamellae laterally and one transverse lamella near posterior margin (Fig. 7B). Posterolateral angles of carapace not protruding, blunt (Fig. 6A). Anterior margin rounded, without distinct rostrum (Fig. 6B). Ventral surface of cephalothorax without cuticular ornamentation; simple pit representing entrance site of umbilical cord, located 40 μm from anterior margin. Dorsal surface of carapace (Figs. 6A, B and 7B–H) with seven pairs of pores (3–4 μm in diameter) and containing sensilla with numerous filiform tips (from two to eight). Position of pores similar to those in *A. pertzovi*. Four pairs of anterior (A_{1-4}) pores arranged in two sets on each side (Figs. 6B and 7B, D): A_{1-2} being close together, about 18 μm from anterior margin of carapace (Figs. 6B and 7D, E) and A_{3-4} being more lateral, about 15 μm posterior to A_2 (Fig. 7D, F). A_5 and P_1 (Figs. 6A and 7B, G, H) equally displaced laterally, about 26 μm from median line. Posterior pores (P_{1-2}) located 38 μm and 30 μm from posterior margin of carapace,

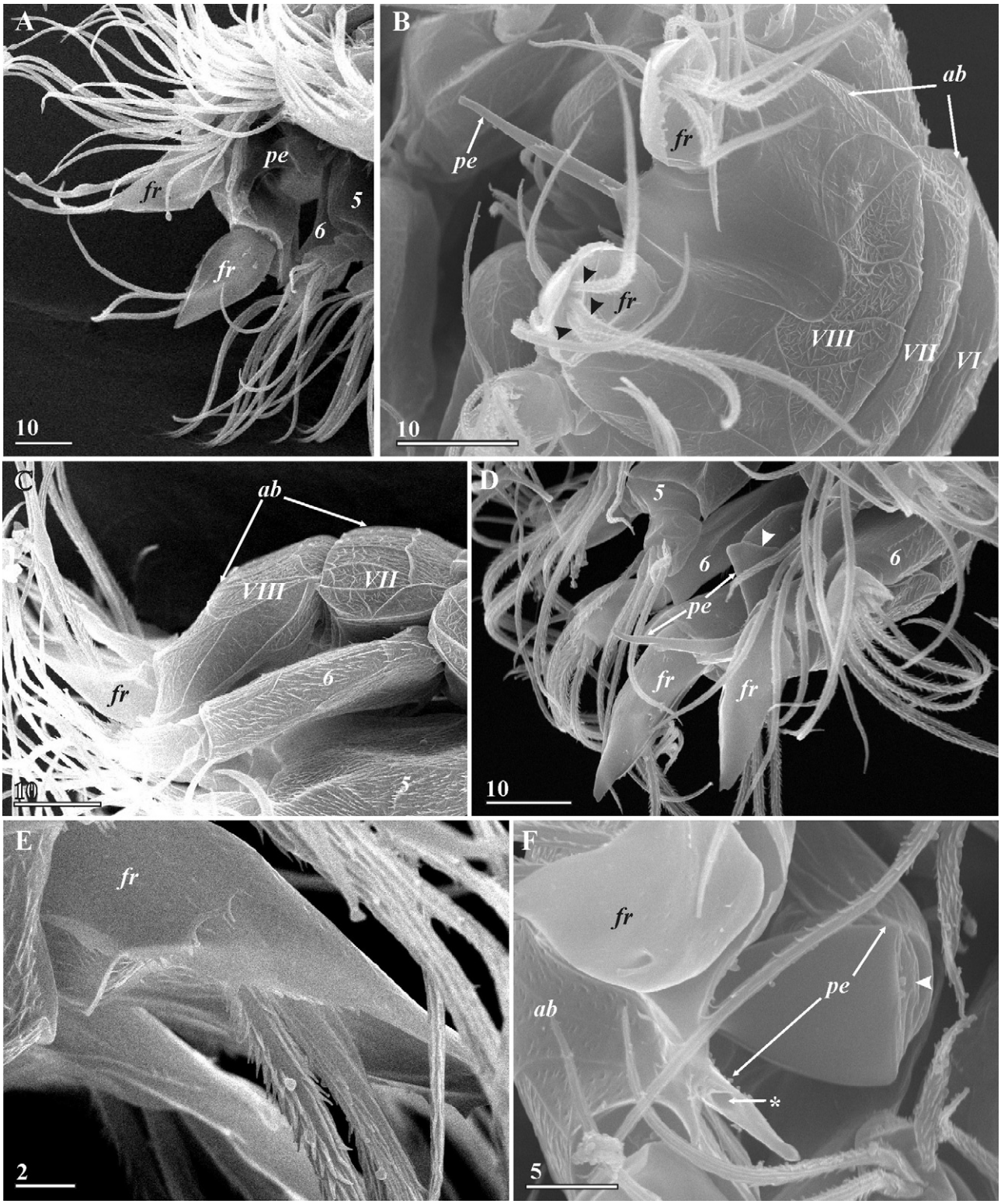

Fig. 5. SEM micrographs. Abdomen, furcal rami and penis of male *Arcticotantulus pertzovi* (trunk somites numbered in Roman numerals, thoracopods in Arabic numerals). (A) Furcal rami and penis, ventrolateral; (B) abdomen with furcal rami, posteriodorsal, furcal setae indicated by arrowheads; (C) abdomen, lateral; (D) furcal rami and penis, ventrolateral, articulation between penis and ventral projection of 1st abdominal (genital) somite indicated by arrowhead; (E) furcal ramus, lateral; (F) penis, ventrolateral, opening of penis marked by asterisk, articulation between penis and ventral extension of 1st abdominal (genital) somite indicated by arrowhead. Abbreviations: *ab* – abdomen, *fr* – furcal rami, *pe* – penis. Scale bars in μm.

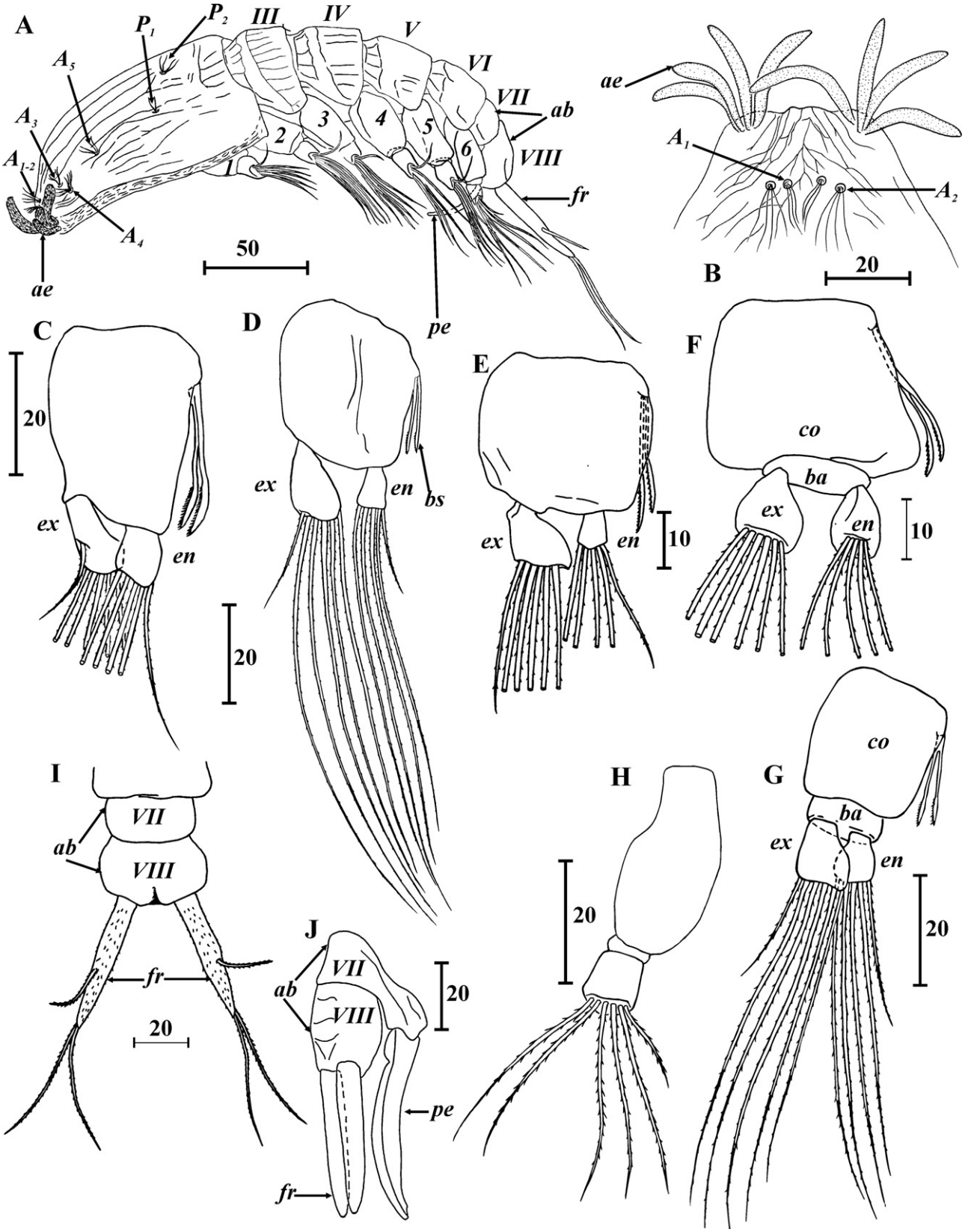

Fig. 6. General view and appendages of definitive male of *Microdajus tchesunovi* (trunk somites numbered in Roman numerals, thoracopods in Arabic numerals). (A) Habitus, lateral; (B) anterior part of cephalothorax, dorsal; (C–E) thoracopods 1–3, respectively, anterior; (F–H) thoracopods 4–6, respectively, posterior; (I) abdomen with furcal rami, dorsal; (J) abdomen of undeveloped male with penis and furcal rami (setae omitted), lateral. Abbreviations: *ab* – abdomen, *ae* – aesthetascs, A_1–A_5 – anterior sensillate pores on carapace; *ba* – basis, *bs* – brush setae, *co* – coxa, *en* – endopod, *ex* – exopod, *fr* – furcal rami, P_1–P_2 – posterior sensillate pores on carapace, *pe* – penis. Scale bars in μm.

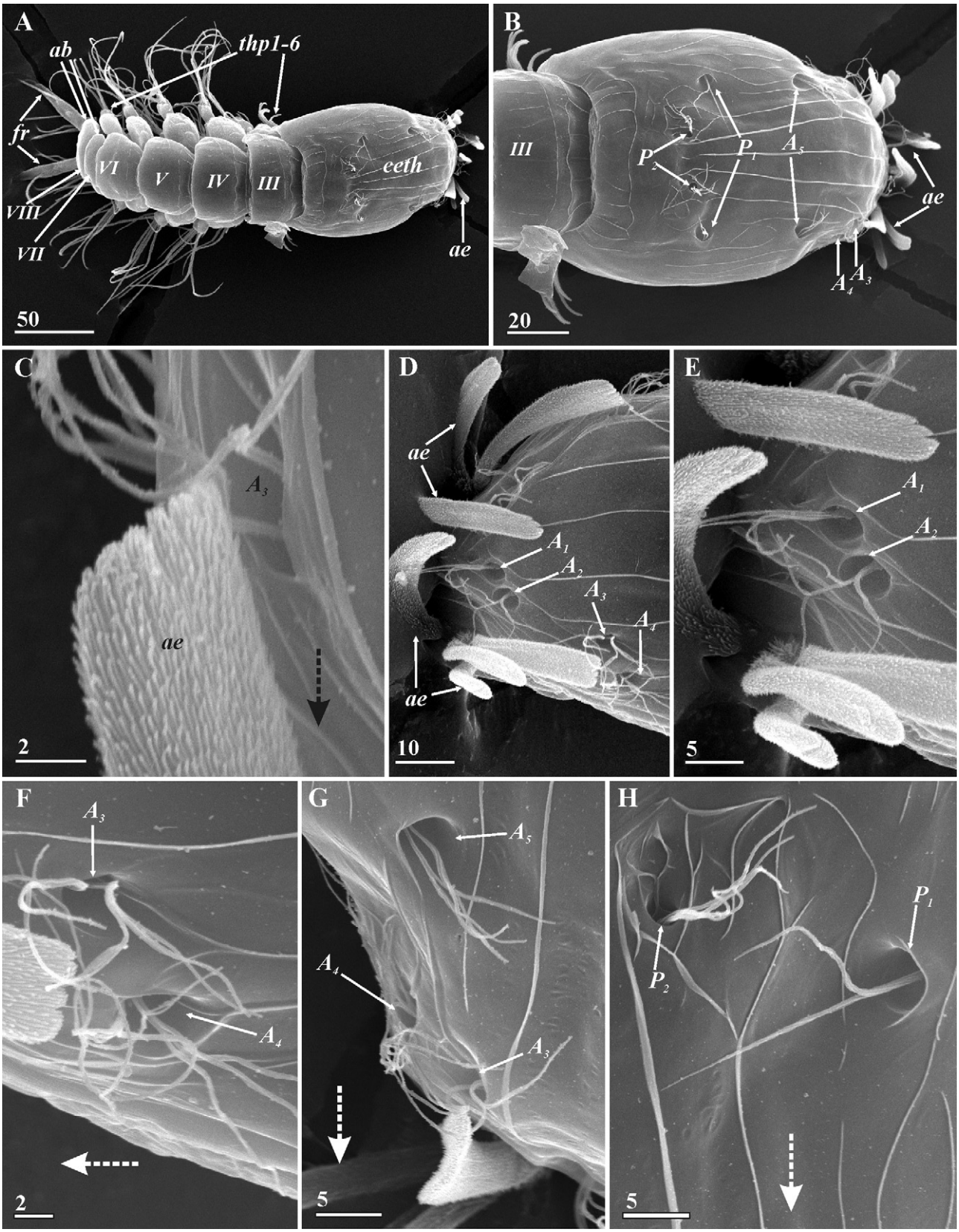

Fig. 7. SEM micrographs. General view and sensory structures of carapace of male *Microdajus tchesunovi* (anterior orientation is marked by dotted arrow, trunk somites numbered in Roman numerals). (A) Habitus, dorsal; (B) cephalothorax, dorsal; (C) tip of aestetasc; (D) anterior part of cephalothorax with aesthetascs, dorsolateral; (E) anterior sensillate pores (A_1–A_2); (F) anterior sensillate pores (A_3–A_4); G–anterior sensillate pores (A_3–A_5); (H) posterior sensillate pores (P_1–P_2). Abbreviations: A_1–A_5 – anterior sensillate pores; *ab* – abdomen, *ae* – aesthetascs, *ceth* – cephalothorax, *fr* – furcal rami, P_1–P_2 – posterior sensillate pores, *thp 1–6* – thoracopods 1–6. Scale bars in μm.

respectively (Figs. 6A and 7B). P_1 pore with bifid sensillum (Fig. 7H), sensillum of A_4 pore with at least eight tips (Fig. 7F, G), other sensilla with four filiform tips.

Posterior part of cephalothorax with two pairs (1–2) of well developed natatory thoracopods (Fig. 6A). Four free thoracic pedigerous somites with tergites increasing in length from third to sixth, but becoming slightly narrower; with distinct posterolateral angles (Figs. 6A and 7A). Tergites ornamented with a certain pattern of tiny transverse and longitudinal lamellae (Figs. 6A and 8A).

Both protopods and rami of thoracopods 1–3 unsegmented (Fig. 6C–E). Exopods armed with five long terminal setae and shorter outer seta, except for thoracopod 1 with smallest seta being positioned subterminally (Fig. 6C). Endopods with four long and one shorter inner terminal setae (Fig. 6C–E). All setae serrated, covered with fine denticles (Fig. 8C, F, H). Rami of thoracopods 4–5 with similar segmentation and setation as in thoracopods 1–3 except for protopods being divided into large coxa and small unarmed basis (Figs. 6F, G and 8D, E). Ornamentation of rami consisting of small regular denticles and tiny cuticular ridges on lateral surfaces (Fig. 8B, F, H).

Protopods (coxae) of thoracopods 1–5 with two brush setae arising from proximal inner margin (Figs. 6C–G and 8D, E, G) and sharing very short basis (Figs. 6G and 8G); distal third of setae brush-like (Figs. 6C–G and 8G). Brush setae almost as (in thoracopods 1–2 and 5) or slightly longer (3–4) than protopods. Protopods of thoracopods 1–4 with small cuticular denticles laterally (Fig. 8A and B).

Thoracopod 6 uniramous, with two-segmented smooth protopod; both coxa and basis unarmed (Figs. 6H and 8H), brush setae absent. Ramus with five equally long terminal setae and small outer one.

Abdomen two-segmented, comprising first genital somite with penis and second somite terminating with furcal rami; both somites lacking distinct ornamentation (Figs. 6A, I and 9A). Genital somite almost rectangular (Figs. 6I and 9A) and more than twice as wide as long (length about 14.7 μm). Penis slightly curved in its proximal part, about 60 μm long (measured from tip to articulation with medial projection of somite), with two lateral furrows in distal half (Figs. 6J and 9D, E) and two sperm ducts (about 1.3 μm in diameter each) opening apically with single gonopore (Fig. 9F). Second abdominal somite wider than long (width 37.6, length 21.7 μm), expanding towards distal part, with median incision posteriorly (Figs. 6I and 9A).

Furcal rami (Figs. 6I and 9A, B) about 47.5 μm long, slender, tapering slightly distally, surface ornamented with fine denticles (Fig. 9C). Each ramus armed with two subequal terminal setae and one outer seta (Figs. 6I and 9B).

3. Discussion

Males are known for 14 tantulocaridan species belonging to all five currently recognized families (Boxshall and Lincoln, 1987; Boxshall, 1988; Grygier and Sieg, 1988; Boxshall and Huys, 1989; Boxshall et al., 1989; Huys, 1990; Huys et al., 1992, 1997; Ohtsuka and Boxshall, 1998; Kolbasov et al., 2008; Knudsen et al., 2009; Kolbasov and Savchenko, 2010). Importantly, all previous male descriptions, except that of *A. pertzovi* (Kolbasov et al., 2008), were based on potentially undeveloped (immature) individuals since they were dissected from the trunk sac formed by the tantulus larva. This implies that body length, size and setation of thoracopods and furcal rami could be different from that of the definitive adult. In some cases descriptions do not yeild enough details for them to be included in morphological analisys. Thus, only nine descriptions contain information sufficient for comparison: *A. pertzovi*, *Coralliotantulus coomansi* Huys, 1990, *Doryphallophora harrisoni* (Boxshall & Lincoln, 1987), *Microdajus aporosus* Grygier & Sieg, 1988, *M. langi* Greve, 1965, *M. pectinatus* Boxshall, Huys & Lincoln, 1989, *M. tchesunovi*, *Paradoryphallophora inusitata* Ohtsuka & Boxshall, 1998, and *Stygotantulus stocki* Boxshall & Huys, 1989 (Table 1). Thorough comparison of these males was conducted based on their main morphological characters (Table 1) and several common features and differences were revealed. It is suggested here that these characters should be used in future studies of tantulocarid male morphology and phylogenetic reconstruction of the group.

Species of the family Doryphallophoridae have the largest males within the Tantulocarida – 620 μm for *P. inusitata*, and 460 μm for *D. harrisoni*. Size of males in other tantulocaridan families range from 200 μm (Microdajidae, *Microdajus langi*) to 400 μm (Basipodellidae, *S. stocki*).

A. pertzovi has one of the smallest males known within the Tantulocarida. The total length of the fully developed male is about 260 μm. Previously described males which approach this species in size (*M. aporosus*) or are even smaller (*M. langi*) were based on undeveloped specimens that were still connected with the tantulus cephalon via an umbilical cord (Boxshall and Lincoln, 1987; Grygier and Sieg, 1988). The definitive male of the only other known species of the genus *Microdajus* (*M. tchesunovi*) significantly exceeds (355 μm) the length of definitive male of *A. pertzovi*.

The number of aesthetascs – two clusters of four on each side seems to be the typical condition for all tantulocaridans. However, Huys (1990) described one bifid and two normal aesthetascs on each side for the male of *C. coomansi* (Deoterthridae). This could either be interpreted as an advanced condition caused by basal fusion of two anterior aesthetascs, or represent an underdeveloped state in the immature male.

The sensory apparatus of tantulocaridan males includes numerous sensilla arising from pores or pits distributed over the dorsal surface of the carapace. The number of pores was reported for only seven species and ranges from six to eight. However, the predominanting number of pores is seven which is considered to be the ancestral condition (Huys, 1990). The presence of eight pairs of these sensilla in *M. aporosus* (Grygier & Sieg, 1988) requires confirmation since other species of this genus (*M. pectinatus* and *M. tchesunovi*) have only seven pairs of sensillate pores (Grygier and Sieg, 1988; Kolbasov and Savchenko, 2010; Table 1 herein). The reported presence of six pairs of sensillate pores in both males of the family Doryphallophoridae, *D. harrisoni* and *P. inusitata* (Boxshall and Lincoln, 1987; Ohtsuka and Boxshall, 1998), with the probable absence of the P_1 pore, is a potential apomorphic condition, provided the observations were correct. Although previous descriptions showed that pores may contain several sensilla, our studies demonstrated that each pore more likely has only one sensillum that could have bifid or multifid filiform tips originating from a common short base (unpublished data). The number of these tips in each sensillum is specific for each pore and could be different between tantulocaridan species ranging from two to eight (Table 1).

Thoracopods of tantulocaridan males show a remarkable consistency in their segmentation and setation of the rami (Table 1). Protopods 1–3 are typically unsegmented with the endopods and exopods armed with five and six setae, respectively. The presence of a two-segmented protopod in thoracopod 3 of *Microdajus pectinatus* (Boxshall et al., 1989) appears to be a misinterpretation, because the other two species of the genus, *M. langi* and *M. tchesunovi*, have an unsegmented protopod (Boxshall and Lincoln, 1987; herein). The setation formula of thoracopods 1–3 is consistent in the Tantulocarida except for *C. coomansi* (Table 1); this condition appears to be an apomorphic state for this genus and potentially also for the family Deoterthridae. Thoracopods 4–5 of tantulocarid males are characterized by two-segmented protopods, and endopods and exopods armed with five and six setae, respectively (Table 1). The

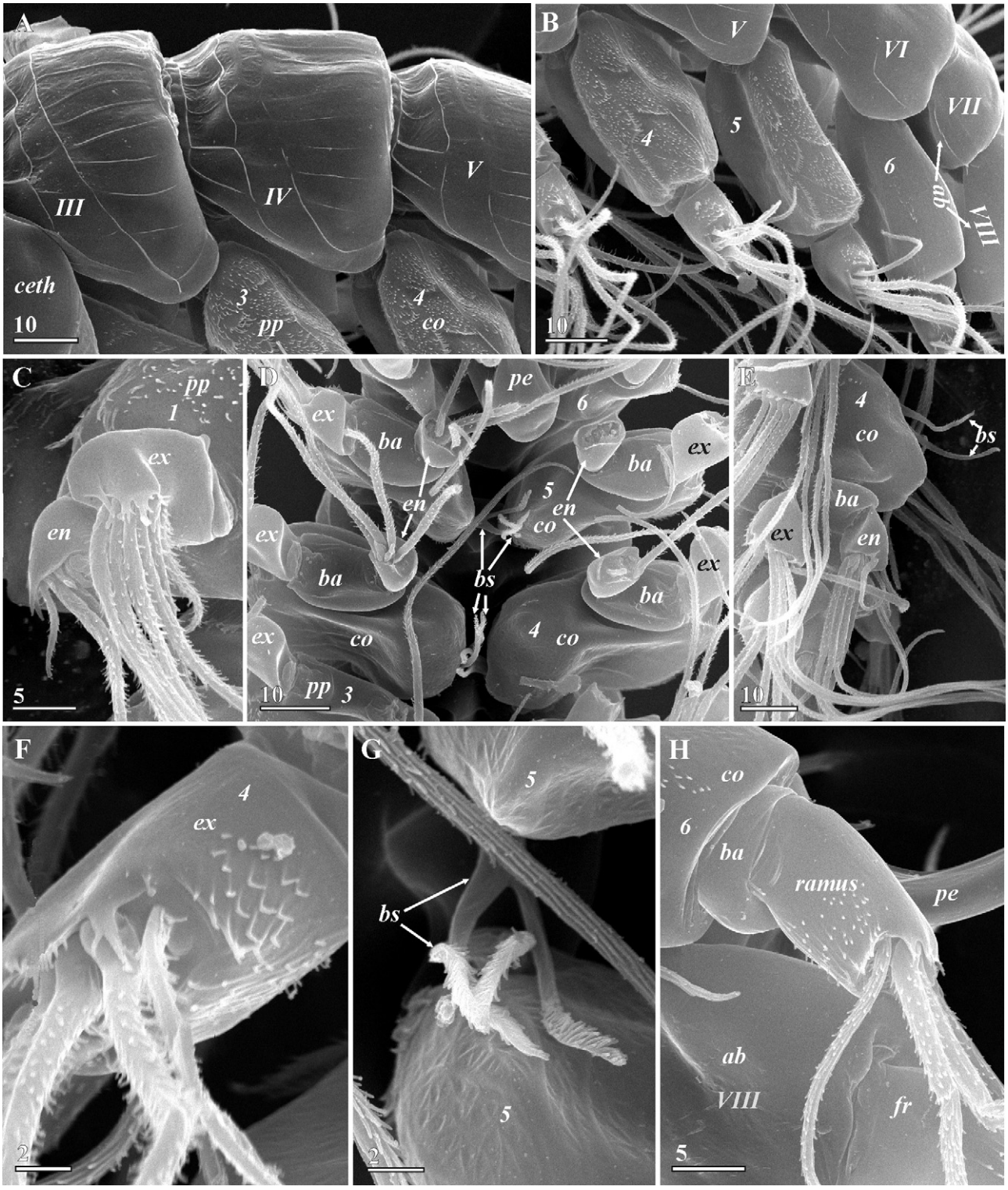

Fig. 8. SEM micrographs. Ornamentation of thoracic tergites and thoracopods of male *Microdajus tchesunovi* (trunk somites numbered in Roman numerals, thoracopods in Arabic numerals). (A) Tergites of thoracic somites 3–5, lateral; (B) thoracopods 4–6, lateral; (C) thoracopod 1, ventral; (D) thoracopods 4–5, ventral; (E) thoracopod 4, anterior; (F) exopod of thoracopod 4, ventral; (G) interlocked brush setae of thoracopods 5, ventral; (H) thoracopod 6, lateral. Abbreviations. *ab* – abdomen, *ba* – basis, *bs* – brush setae, *ceth* – cephalothorax, *co* – coxa, *en* – endopod, *ex* – exopod, *fr* – furcal rami, *pe* – penis, *pp* – protopod. Scale bars in μm.

presence of an unsegmented protopod 4 in *S. stocki* (Boxshall & Huys, 1989) probably requires confirmation before it can be interpreted as an apomorphic state.

The single ramus of thoracopod 6 is typically armed with six setae, while four species (*P. inusitata*, *M. langi*, *M. pectinatus*, *S. stocki*) have only five (Table 1). Since *M. tchesunovi* was studied thoroughly with both SEM and light microscopy, we assume the presence of six setae on thoracopod 6 to be diagnostic for the genus *Microdajus*. The reported presence of five setae in *M. langi* and *M. pectinatus* could therefore be considered the result of imperfect observation. Such scenario could also be applied to *P. inusitata*, since the only other known doryphallophorid male (*D. harrisoni*), has six

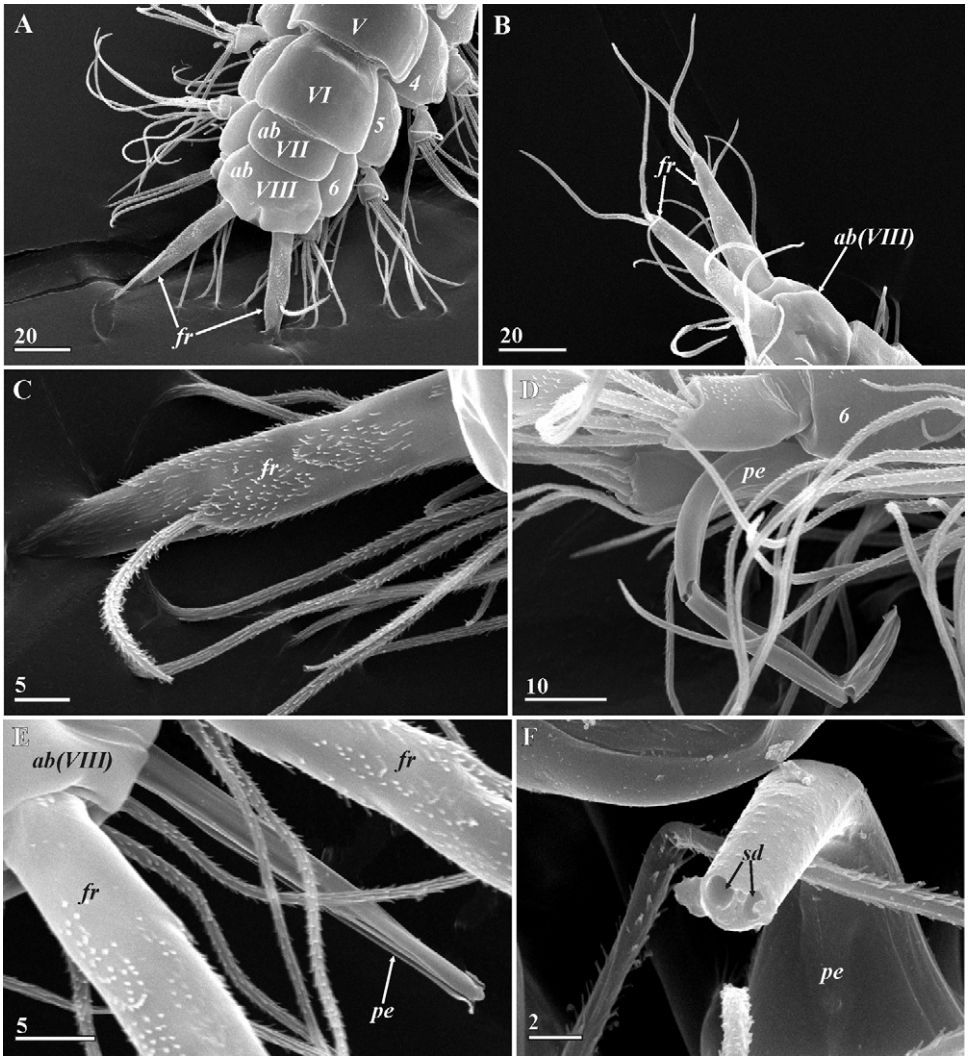

Fig. 9. SEM micrographs. Abdomen, furcal rami and penis of male *Microdajus tchesunovi* (trunk somites numbered in Roman numerals, thoracopods in Arabic numerals). (A) Posterior part of hind body, dorsal; (B) furcal rami, lateral; (C) furcal ramus, dorsal; (D) penis (partially broken in two sites), lateral; (E) furcal rami and penis, dorsal; (F) penis, anterior, showing two seminal ducts (distal part broken). Abbreviations. *ab* – abdomen, *fr* – furcal rami, *pe* – penis, *sd* – seminal ducts. Scale bars in μm.

setae on thoracopod 6. The presence of six setae on the single ramus of thoracopod 6 may indicate its exopodal origin, since the exopods of other thoracopods have also typically six setae.

All known tantulocaridan males possess paired brush setae arising from the inner proximal margin of the protopods (Table 1). Males of microdajids and *C. coomansi* have these brush setae on thoracopods 1–5. The other four species belonging to the families Doryphallophoridae and Basipodellidae have these setae also on the sixth thoracopods. We cannot confirm absence or presence of these setae on thoracopod 1 in *A. pertzovi*, because this area of protopod is hidden with membranous fold.

Furcal rami of male tantulocaridans are typically armed with three setae (Table 1), except for two reports: *D. harrisoni* has only one seta on each ramus, and *M. langi* has two apical setae (Boxshall and Lincoln, 1987). Other doryphallophorid (*P. inusitata*) and microdajid (*M. aporosus*, *M. pectinatus* and *M. tchesunovi*) species typically have three furcal setae (Grygier and Sieg, 1988; Boxshall et al., 1989; Ohtsuka and Boxshall, 1998; Kolbasov and Savchenko, 2010; Table 1 herein), providing additional credence those previous reports of either one or two setae in these families should be misinterpretations.

The shape of male furcal rami varies significantly between tantulocaridan families, but are typically similar between species of the same family (Table 1). Males of the genus *Microdajus* (Microdajidae) are characterized by cylindrical and slender furcal rami. The furcal rami of members of the Doryphallophoridae are distinctly elongate and slender. The single known deoterthrid male (*C. coomansi*) has tapering and slender furcal rami. In known males of basipodellid species the furcal rami are either slender and cylindrical (*S. stocki*) or short and lancet-shaped (*A. pertzovi*).

Kolbasov et al. (2008) contructed a matrix of larval morphological characters and suggested that the families Basipodellidae and Deothertridae are paraphyletic. Recently, Knudsen et al. (2009) proposed to remove *Arcticotantulus* from the Basipodellidae to the Deoterthridae, although morphological analysis indicated that only three deoterthrid genera (*Amphitantulus* Boxshall & Vader, 1993, *Cumoniscus* Bonnier, 1903 and *Deoterthron* Bradford & Hewitt, 1980) may form a monophyletic clade (Kolbasov et al., 2008). On the one hand the differences in morphology of the males of *A. pertzovi*, *S. stocki* indicate the paraphyly of the Basipodellidae, but on the other hand the differences between males of *A. pertzovi* and *C. coomansi* suggest that the former cannot belong to deoterthrid clade.

Thus the generalized (putative plesiomorphic) characters for the tantulocaridan males are (i) eight aesthetascs of simple elongated shape; (ii) transverse and longitudinal lamellae/ridges on dorsal surface of the cephalothorax and hindbody; (iii) seven pairs of cephalic pores with multifid sensilla; (iv) five setae on endopods

and six setae on exopods of thoracopods 1–5 and six setae on the single ramus of thoracopod 6; (v) brush setae on protopods of thoracopods 1–6; (vi) anteriorly curved penis with terminal opening, and (vii) three furcal setae.

Members of the family Microdajidae possess the following male apomorphic characters: reduced dorsal lamellae, especially on abdominal and seventh trunk somites and reduced brush setae on thoracopod 6. Males of the family Doryphallophoridae are characterized by the presence of paired cephalic anterodorsal processes/lobes, strongly elongated, slender furcal rami; a straight, posteriorly directed penis and, probably, six pairs of sensillate pores on the carapace.

Males of *A. pertzovi* differ from other tantulocaridans in having an unusal cuticular membrane-like fold on thoracopods 1–2, a subterminal opening of the penis, short lancet-shaped furcal rami, and a probable reduction of the brush setae on thoracopod 1. The presence of an unsegmented protopod on thoracopod 4 may distinguish the male of *S. stocki* from other tantulocaridan males but this needs confirmation. The male of *C. coomansi* has a different setation formula for thoracopods 1–5, reduced brush setae on thoracopod 6 and a ventral spinous process on the furcal rami.

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