



Six new species of *Diacyclops* (Copepoda: Cyclopoida) from Australia

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Abstract

Nine species and one subspecies of the freshwater copepod genus *Diacyclops* Kiefer, 1927 have been reported so far from Australia. Two of them are nearly cosmopolitan and frequently found in disturbed surface waters, one is endemic to eastern Australia and found predominantly in springs, while all others are endemic to the Pilbara region of Western Australia and found only in subterranean waters. Here I describe another six endemics: one from a spring in South Australia (*D. leijsi* **sp. nov.**), one from two caves in the south-western corner of Western Australia (*D. eberhardi* **sp. nov.**), one from several bores in the Pilbara region of Western Australia (*D. ballaballensis* **sp. nov.**), and three from several bores in New South Wales (*D. hancocki* **sp. nov.**, *D. tomlinsonae* **sp. nov.**, and *D. huntervalleyensis* **sp. nov.**). All six new species are easily distinguishable from each other by a unique shape and armature of the caudal rami and a different armature formula of the swimming legs. All, except one pair, also have a different armature formula of the antenna. Interestingly, all nine Western Australian endemics have a 12-segmented antennula (and other characters of the *alticola*-group), while the four endemics from eastern Australia have this appendage further reduced. Because of this, and previous molecular work that suggested a polyphyly of the Western Australian species, I performed a cladistic analysis of all 16 Australian taxa based on 21 morphological characters. As with many groups that are dominated by subterranean species, the resulting cladograms suggest numerous homoplasies. The group of species from eastern Australia is certainly polyphyletic, while the Western Australian group appears to be paraphyletic. A key to all Australian species is also included.

Key words: biodiversity, biogeography, cladistics, crustaceans, phylogeny, taxonomy

Introduction

Diacyclops is the largest genus of the copepod family Cyclopidae Rafinesque, 1815 (see Boxshall & Halsey 2004; Dussart & Defaye 2006; Walter & Boxshall 2023), and it is found in various freshwater habitats on all continents, including Antarctica (Karanovic *et al.* 2014). Its centre of diversity is in the temperate Palaearctic region (Dussart & Defaye 2006; Karanovic 2006), but this might be a sampling bias. This genus has a long history of taxonomic problems though (Stoch 2001), and it was speculated to be polyphyletic or at least paraphyletic by many researchers (Monchenko & von Vaupel Klein 1999; Monchenko 2000; Karanovic 2005, 2006). Although the genus name is valid, and its type species is surrounded by a flock of closely related congeners, ever since its initial erection by Kiefer (1927), as a subgenus of *Cyclops* Müller, 1785, it has had the misfortune of having assigned to it all cyclopoids with even superficially similar fifth legs (Karanovic 2005). From the start Kiefer (1927) recognised two distinct groups of species within *Diacyclops*: one containing *D. bicuspidatus* (Claus, 1857) [type species], *D. bisetosus* (Rehberg, 1880), *D. crassicaudis* (Sars, 1863), and some others; and the other group including species with a higher degree of appendage oligomerization, such as *D. languidus* (Sars, 1863), *D. languidoides* (Lilljeborg, 1901), and *D. stygius* (Chappuis, 1924). Reid & Strayer (1994) noted that the diagnosis of this genus is so broad that it is effectively based solely on the structure of the fifth leg, a character considered probably plesiomorphic by Karanovic (2005, 2006).

Another part of the troubled taxonomic history of *Diacyclops* is the similarity of its fifth leg to that typical for *Acanthocyclops* Kiefer, 1927; many species have been transferred back and forth between these two genera multiple times (see Morton 1985; Pesce 1996; Pandourski 1997; Monchenko & von Vaupel Klein 1999; Dussart & Defaye 2006; Karanovic *et al.* 2012a). Ferrari (1991) even suggested that *Diacyclops* and *Acanthocyclops* might belong to two different monophyletic groups of cyclopoids, as he grouped *Acanthocyclops* with *Eucyclops* Claus, 1893 and

Ectocylops Brady, 1904, which belong to a different subfamily. However, the subfamilial division of Cyclopidae is well supported both by morphological and molecular evidence (see, for example, Karanovic & Krajcicek 2012a).

A revision of the *Diacyclops/Acanthocyclops* group would require redescriptions of nearly 200 species. In addition, evidence for cryptic speciation in some of the more widely distributed taxa (Monchenko 2000; Karanovic & Krajcicek 2012b), a common phenomenon in freshwater cyclopoids (Bláha et al 2010; Karanovic & Krajcicek 2012a; Karanovic & Blaha 2019), must be taken into account. Unfortunately, most of the species of *Diacyclops* have been described from subterranean habitats, from very few specimens, and for some the types are no longer extant or are impossible to trace. All of this makes a comprehensive revision more difficult to accomplish. Some initial attempts have been made, however, to separate obviously unrelated species into newly established genera (Lescher-Moutoué 1976; Reid *et al.* 1999; Reid & Ishida 2000; Karanovic 2000, 2005; Karanovic *et al.* 2012a). The general agreement among taxonomists seems to be that the genus must be split into several monophyletic lineages, many of which are recognised as species groups today (Reid & Strayer 1994; Pesce 1996), but also that it must be revised together with the closely related genus *Acanthocyclops*.

So far only ten species and subspecies of *Diacyclops* have been reported from Australia. The nearly cosmopolitan *D. bisetosus* was found commonly in surface waters of Tasmania and Victoria by Morton (1985). He also recognised that *D. crassicaudoides* (Kiefer, 1928), which was described from New Zealand (Kiefer 1928) and subsequently reported from Tasmania (Brehm 1953), is a junior synonym of *D. bisetosus*. Morton (1985) also described the first Australian endemic species: *D. cryonastes* Morton, 1985. He noted that this species has a wide altitude range in Tasmania, while in Victoria and southern New South Wales it could only be found in high alpine areas of the Great Dividing Range. All sampling locations reported by Morton (1985) were surface water habitats. Karanovic *et al.* (2014) reported *D. cryonastes* from subterranean waters of central New South Wales, which also extended its known range more than 500 km northwards. Pesce & De Laurentiis (1996) described the first subterranean species from Western Australia: *D. humphreysi* Pesce & De Laurentiis, 1996. De Laurentiis *et al.* (1999) amended the description of *D. humphreysi* based on newly collected material and described two other subterranean species from Western Australia: *D. einslei* De Laurentiis, Pesce & Humphreys, 1999 and *D. reidae* De Laurentiis, Pesce & Humphreys, 1999. Karanovic (2006) redescribed *D. einslei* and *D. humphreysi* based on newly collected material and described four new subterranean taxa from Western Australia: *D. cockingi* Karanovic, 2006; *D. humphreysi unispinosus* Karanovic, 2006; *D. scanloni* Karanovic, 2006; and *D. sobeprolatus* Karanovic, 2006. Interestingly, all Western Australian species were described from the Pilbara region (NW corner of Western Australia). *Diacyclops humphreysi unispinosus* was limited to Barrow Island, which lies some 50 km off the Pilbara coast, and owing to eustatic changes would have been a part of the mainland for most of the previous several million years before about 8000 BP (Humphreys 1991; McNamara & Kendrick 1994). Karanovic (2006) also found interbreeding populations between *D. humphreysi unispinosus* and the nominotypical subspecies in the coastal zone of the Pilbara region. Finally, Karanovic & Krajcicek (2012b) reported the nearly cosmopolitan *D. bicuspidatus* from New South Wales and speculated that its presence in Australia could be a result of anthropogenic translocation associated with early shipping activities, as proven for some other widely distributed surface water cyclopoids (Karanovic & Krajcicek 2012a). Karanovic & Krajcicek (2012b) also tested the monophyly of Western Australian congeners using one mitochondrial and one nuclear marker.

Material and methods

I did not collect any of the six new species described here, neither had I had a chance to visit their type localities subsequently, so I have no information to provide on their habitats other than that supplied on labels by the people who collected or donated them. Responsibility for the accuracy of coordinates, which are given here as decimal degrees, also lies with the collectors. I also have no information on sampling methods and material fixation; all specimens were sent to me in ethanol. Specimens of *Diacyclops leijsi* were collected in 2003 from mound springs in South Australia by Dr. Remko Leijts (South Australian Museum, Adelaide); those on microscope slides or in alcohol are deposited in the South Australian Museum, Adelaide. Specimens of *D. eberhardi* were collected in 2002 from two caves in the Margaret River Region of Western Australia by Dr. Stefan M. Eberhard (University of New South Wales, Sydney); those on microscope slides or in alcohol are deposited in the Western Australian Museum, Perth. Specimens of *D. ballaballaensis* were collected in 2003 from groundwater bores in the Pilbara Region of Western

Australia by Dr. Jim Cocking and Dr Mike Scanlon, and sent to me by Dr. Stuart Halse (all from Bennelongia environmental consultancy, Perth); additional specimens were collected in 2007 and 2008 from the same region by Dr. Erin Thomas (Outback Ecology environmental consultancy; subsequently acquired by MWH Global); those on microscope slides or in alcohol are deposited in the Western Australian Museum, Perth. Specimens of *D. hancocki*, *D. tomlinsonae*, and *D. huntervalleyensis* were collected from 2005 to 2007 from subterranean bores in two regions of New South Wales by Dr. Peter J. Hancock (Water Resources Group, Adelaide) and Dr. Moya Tomlinson (Australian Rivers Institute, Griffith University); they are all deposited in the Australian Museum, Sydney. Specimens of all six species that are mounted on a single scanning electron microscope (SEM) stub are also deposited in the Australian Museum, Sydney. Specimen numbers and relevant locality data are given in species descriptions below.

Female habitus was examined in a drop of glycerol on a cavity-well microscope slide without a coverslip, to avoid any distortion. The female urosome was also examined in glycerol but on a flat microscope slide with a coverslip, so two human hairs of appropriate thickness were mounted between the slide and coverslip to minimize compression. Specimens were subsequently dissected and mounted on flat microscope slides in Faure's medium (prepared according to Stock & von Vaupel Klein 1996) with a coverslip, so all appendages and male urosomes were compressed. During and after the examination the water slowly evaporated at room temperature and the appendages eventually remained in a completely dry medium, ready for a long-term storage. All line drawings were prepared using a drawing tube attached to a Leica DMLS brightfield compound microscope, equipped with C-planachromatic objectives up to 100x oil. Specimens that were not drawn were examined in a drop of glycerol on a cavity-well microscope slide with objectives of up to 63x dry, and after examination were preserved in absolute ethanol. Specimens for scanning electron micrography were transferred from absolute ethanol to pure isoamyl-acetate, critical-point dried, mounted on a single stub, coated in gold, and observed under a Hitachi S-4700 SEM on the in-lens detector, with an accelerating voltage of 10 kV and working distance of 13 mm; micrographs were taken with a digital camera.

The terminology for morphological characters follows Huys and Boxshall (1991), except for the numbering of setae on the caudal rami (their terminology is here based on their relative position) and small differences in the spelling of some appendages (antennula, mandibula, maxillula instead of antennule, mandible, maxillule), as in other crustacean groups (see Karanovic 2020, 2021). Only the first presented species is described in full, while all subsequent descriptions are shortened by making them comparative. They are listed by the descending number of antennular segments, which usually also correlates with reductions in other (but not all) appendages.

To estimate the phylogenetic relationships between the new species, and among other Australian congeners, characters for morphological cladistic analysis were coded and optimized using the computer program WinClada, version 1.00.08 (Nixon, 1999), and then analysed using NONA, version 2 (Goloboff, 1999). No assumption of apomorphy or plesiomorphy was assigned to character states a priori. All analyses were performed with unweighted characters, using heuristic searches and default software parameters. Because the original description of *D. cryonastes* was based on a limited set of morphological characters (Morton 1985), 3 males and 10 females were examined from New South Wales, Hunter Valley, bore 80437, sample no. 2HD32, 32.04835°S 150.81944°E, 4 July 2005, collected by Dr. Peter J. Hancock; they are also deposited in the Australian Museum.

Taxonomy

Diacyclops leijsi sp. nov.

(Figs. 1A, 2–7)

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Type locality. South Australia, Wabma Kadarbu Mound Springs, sample no. TC17049, 29.453111°S 136.858°E, 15 March 2003, collected by R. Leijs.

Holotype. Adult female dissected on one microscope slide.

Paratypes. One male and 1 female from type locality dissected on 1 microscope slide each; 2 males and 3 females from type locality on 1 SEM stub (row no. 6), together with 5 other species described here; 4 males, 7 females, and 2 copepodids from type locality in 1 alcohol vial.

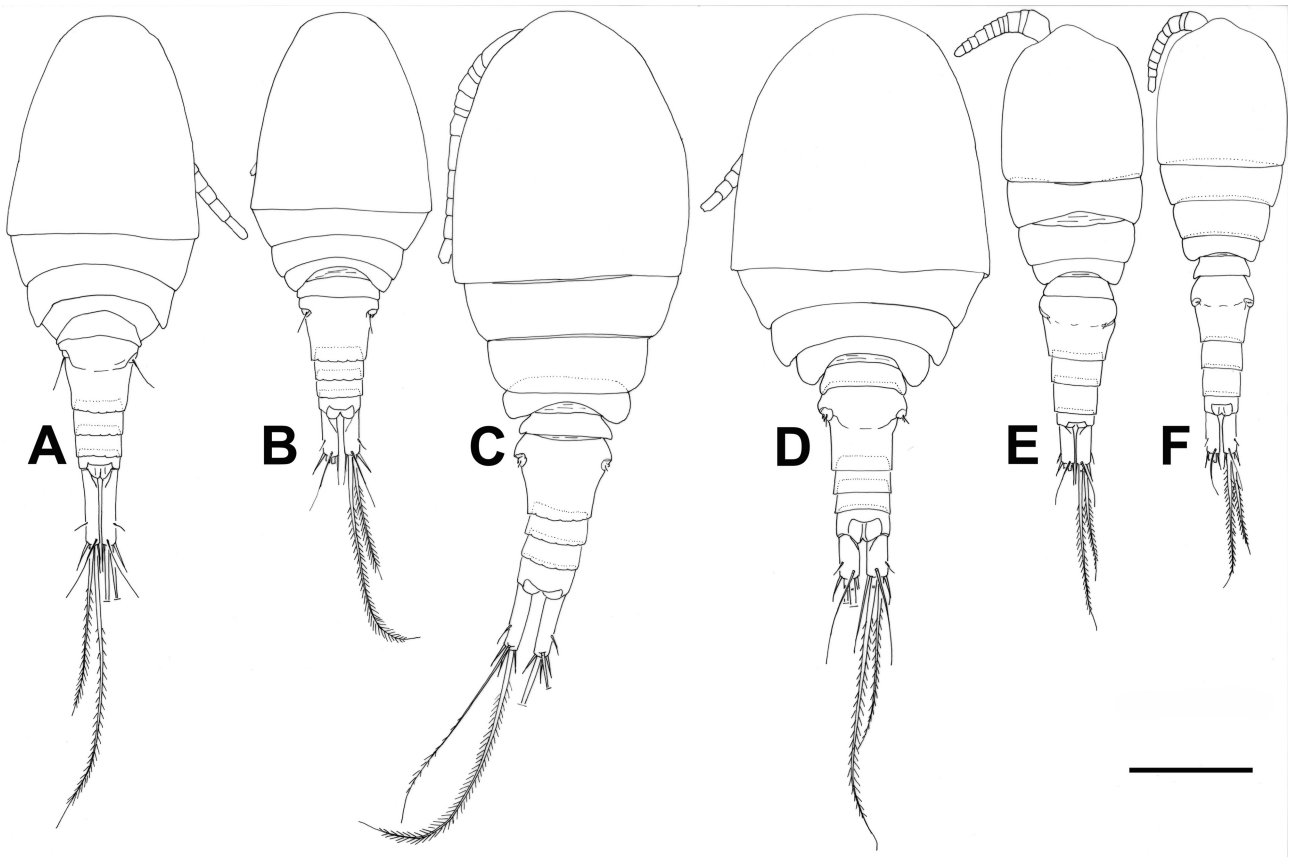


FIGURE 1. Habitus in dorsal view of holotype females of all six new species: A, *Diacyclops leijsi* sp. nov.; B, *D. eberhardi* sp. nov.; C, *D. ballaballaensis* sp. nov.; D, *D. hancocki* sp. nov.; E, *D. tomlinsonae* sp. nov.; F, *D. huntervalleyensis* sp. nov. Scale bar 100 μ m.

Etymology. The species is named after Dr. Remko Leijs, South Australian Museum, Adelaide, who collected this species and entrusted it to me for identification. The name is a noun in the genitive singular.

Diagnosis. Female. Body length, excluding caudal setae, from 440 to 485 μ m. Habitus (Figs. 1A, 6A) spindle-shaped, nearly 2.8 times as long as wide, with prosome/urosome ratio of 1.25, and posterior end of cephalothorax about 2.5 times as wide as genital double-somite in dorsal view. Integument on all somites thin and wrinkled, with dense bacterial cover, spinules only on anal somite and caudal rami, cuticular pores on all somites, and sensilla on all but penultimate somite. Hyaline fringes of prosomites smooth, while those of genital double-somite and 2 subsequent urosomites coarsely serrated (Figs. 1A, 6B). Genital double-somite (Fig. 2A) slightly wider than long in ventral view, widest at first fifth of its length and gradually tapering posteriorly, widest part about 1.5 times as wide as posterior margin; seminal receptacle large and shaped like a mature mushroom; copulatory pore relatively large; copulatory duct short, narrow, and well-sclerotized. Anal somite (Fig. 2A) with small spinules along ventral margin. Caudal rami (Fig. 2A) slender, narrowly spaced, nearly 5 times as long as wide and 3 times as long as anal somite; principal terminal setae without breaking planes, inner one about 1.2 times as long as entire urosome and 1.6 times as long as outer one; dorsal seta about as long as caudal ramus, 1.5 times as long as innermost terminal seta, and twice as long as outermost terminal seta. Antennula (Figs. 2B, 6E) 17-segmented, slightly longer than cephalothorax, with single aesthetasc on twelfth segment and setae formula 8.4.2.6.3.2.2.1.1.0.1.1.0.1.2.2.8; ultimate segment about 2.4 times as long as wide. Antenna (Fig. 3A) 5-segmented, without exopodal seta, with setae formula 0.2.1.8.7; second and fifth segment of similar length. Labrum (Fig. 3B) trapezoidal, with 2 diagonal rows of 10 slender spinules each on anterior surface; cutting edge nearly straight, with 14 sharp teeth between pointed lateral corners. Mandibula (Fig. 3C, D) slender, with 2 long and 1 short seta on 1-segmented palp; cutting edge with 4 slender spinules on anterior surface, 8 apical teeth, and dorsalmost unipinnate seta; ventralmost tooth strongest and quadricuspidate, second, fourth, and seventh teeth from ventral side bicuspidate, all other teeth unicuspidate. Maxillula (Figs. 3E, 6C)

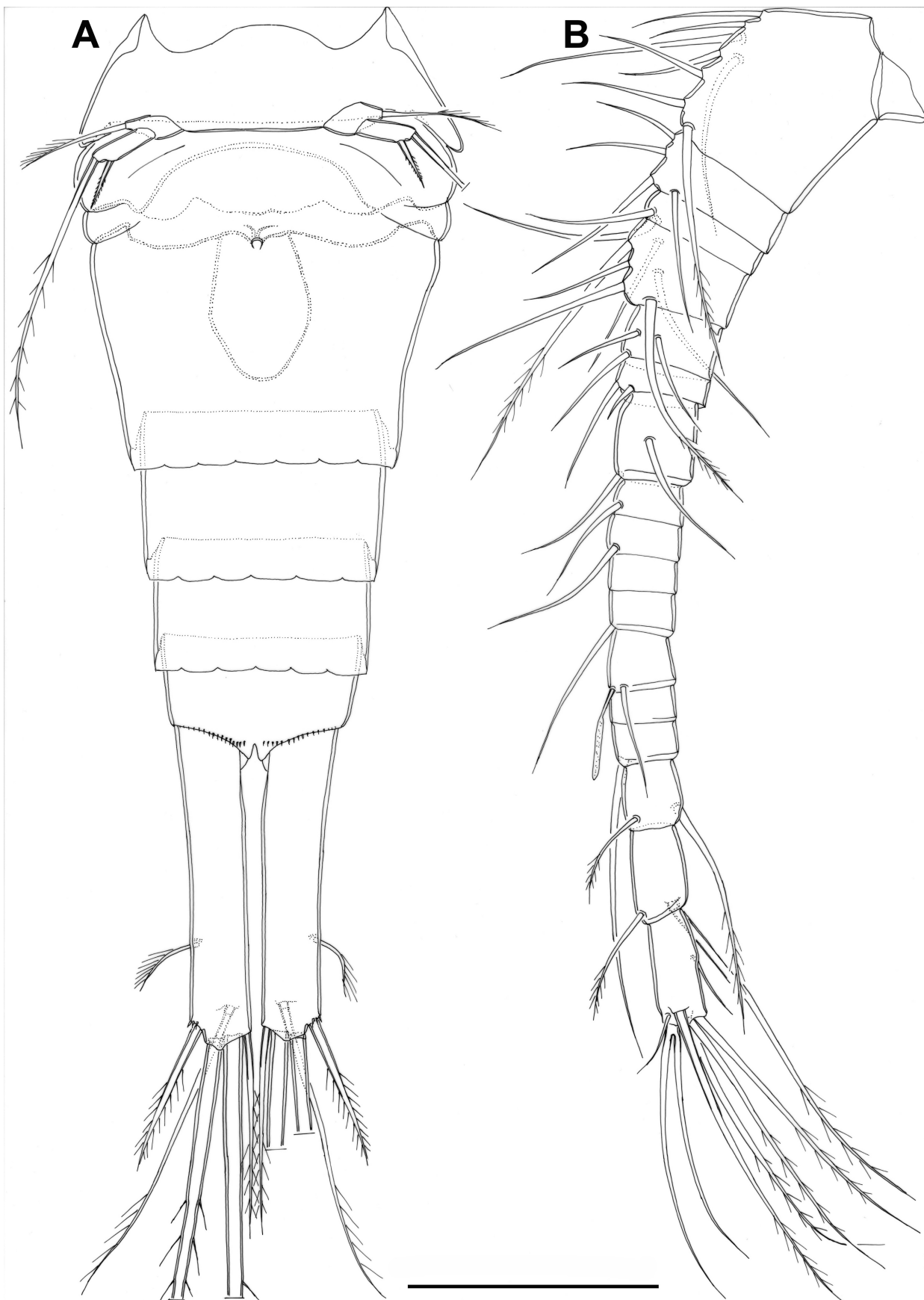


FIGURE 2. *Diacyclops leijsi* sp. nov., line drawings, holotype female: A, urosome, ventral view; B, antennula. Scale bar 50 μ m.

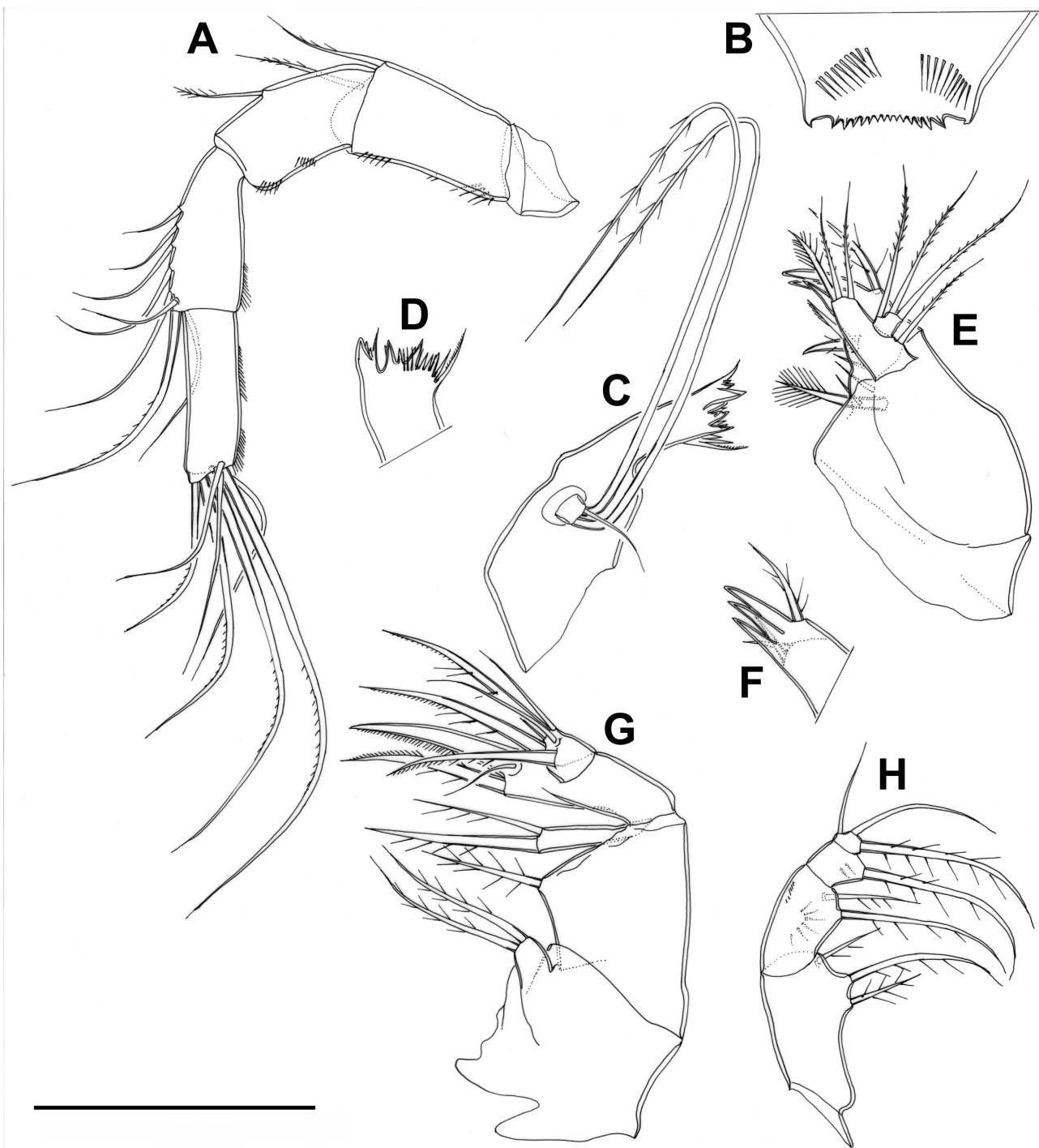


FIGURE 3. *Diacyclops lejisi* sp. nov., line drawings, holotype female: A, antenna; B, labrum; C, mandibula; D, cutting edge of mandibula; E, maxillula; F, praecoxal arthrite of maxillula; G, maxilla; H, maxilliped. Scale bar 50 μ m.

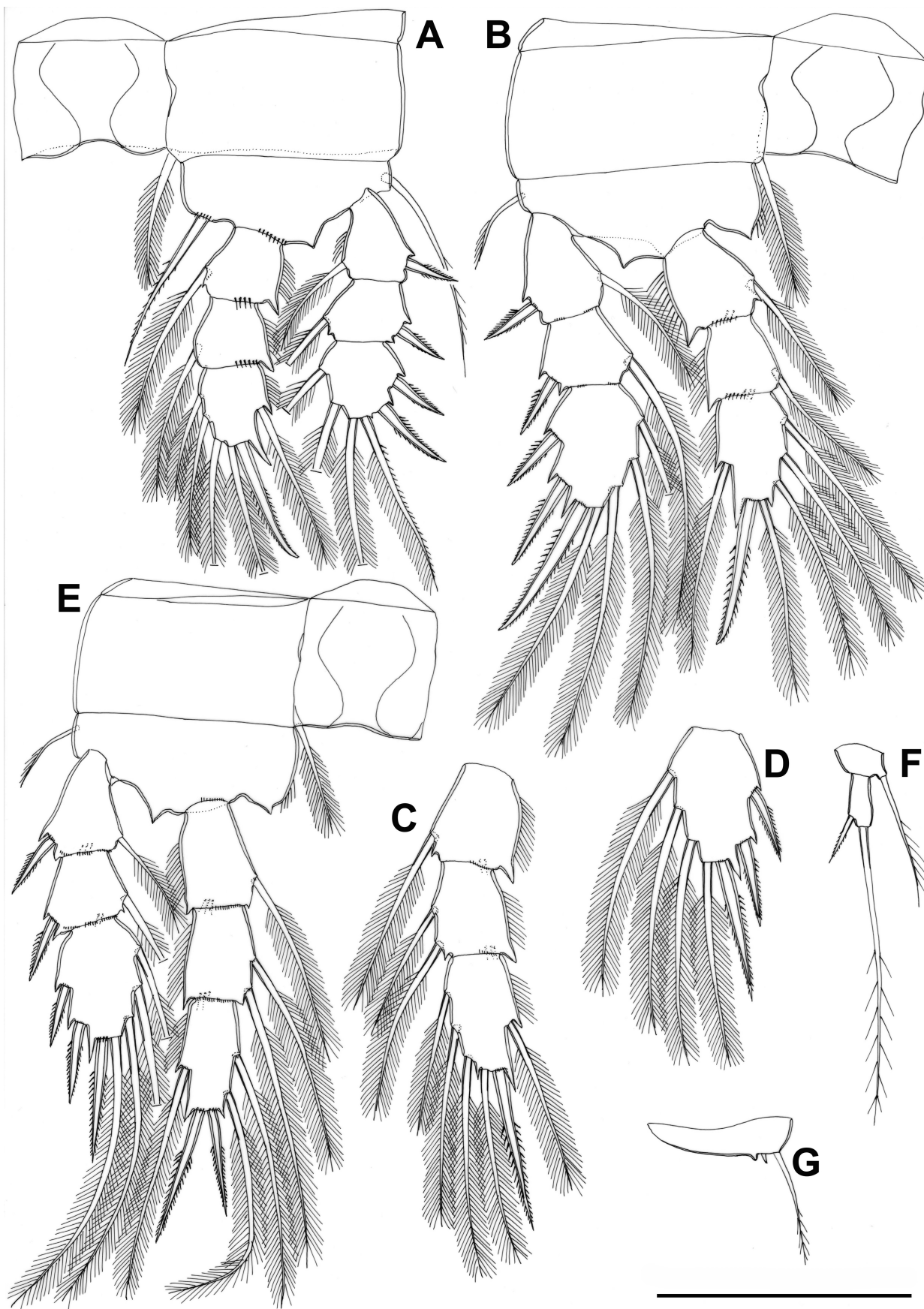


FIGURE 4. *Diacyclops leijsi* sp. nov., line drawings, holotype female: A, first swimming leg; B, second swimming leg; C, endopod of third swimming leg; D, third exopodal segment of third swimming leg; E, fourth swimming leg; F, fifth leg; G, sixth leg. Scale bar 50 μ m.

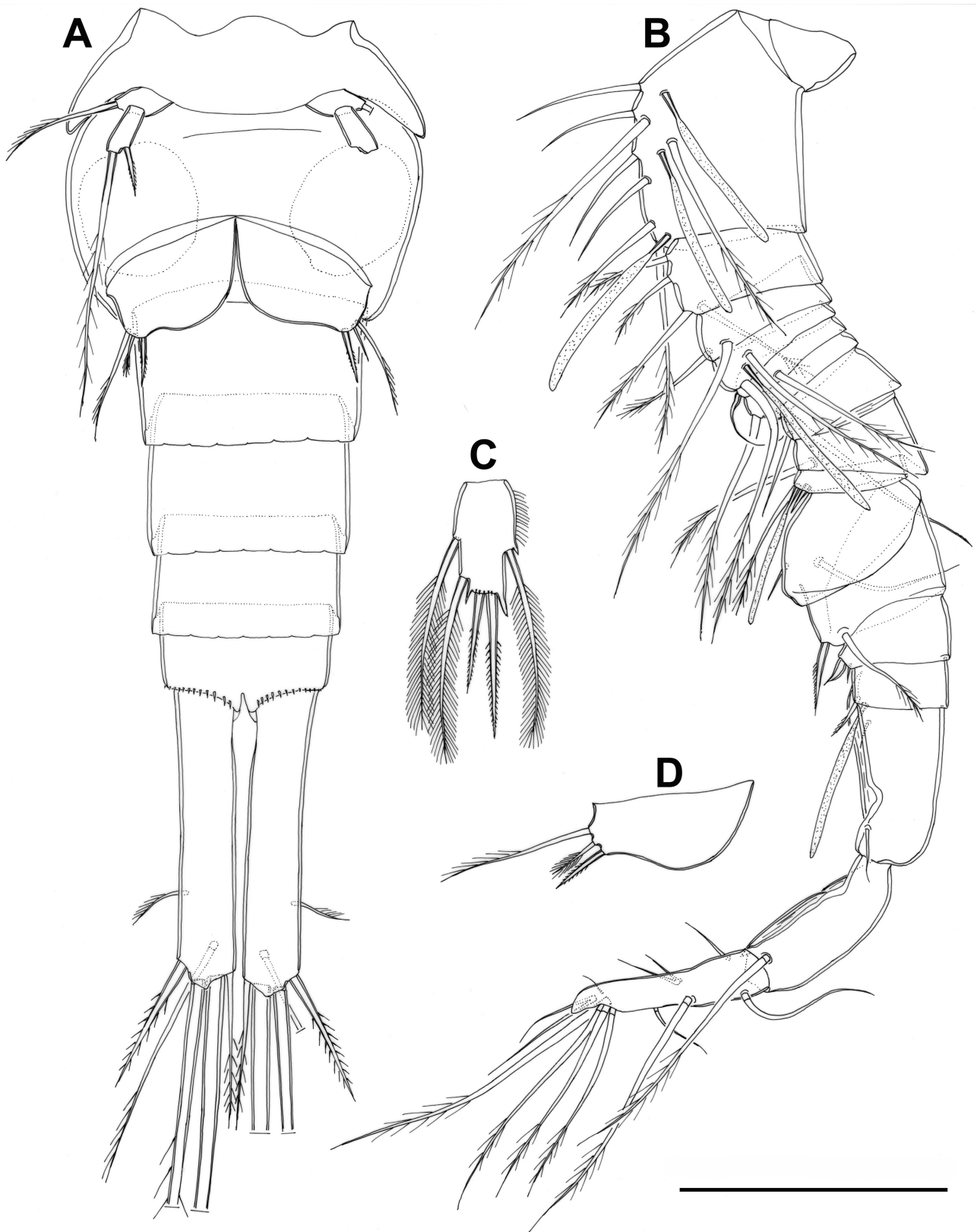


FIGURE 5. *Diacyclops leijsi* **sp. nov.**, line drawings, paratype male 1: A, urosome, ventral; B, antennula; C, third endopodal segment of fourth swimming leg; D, sixth leg. Scale bar 50 μ m.

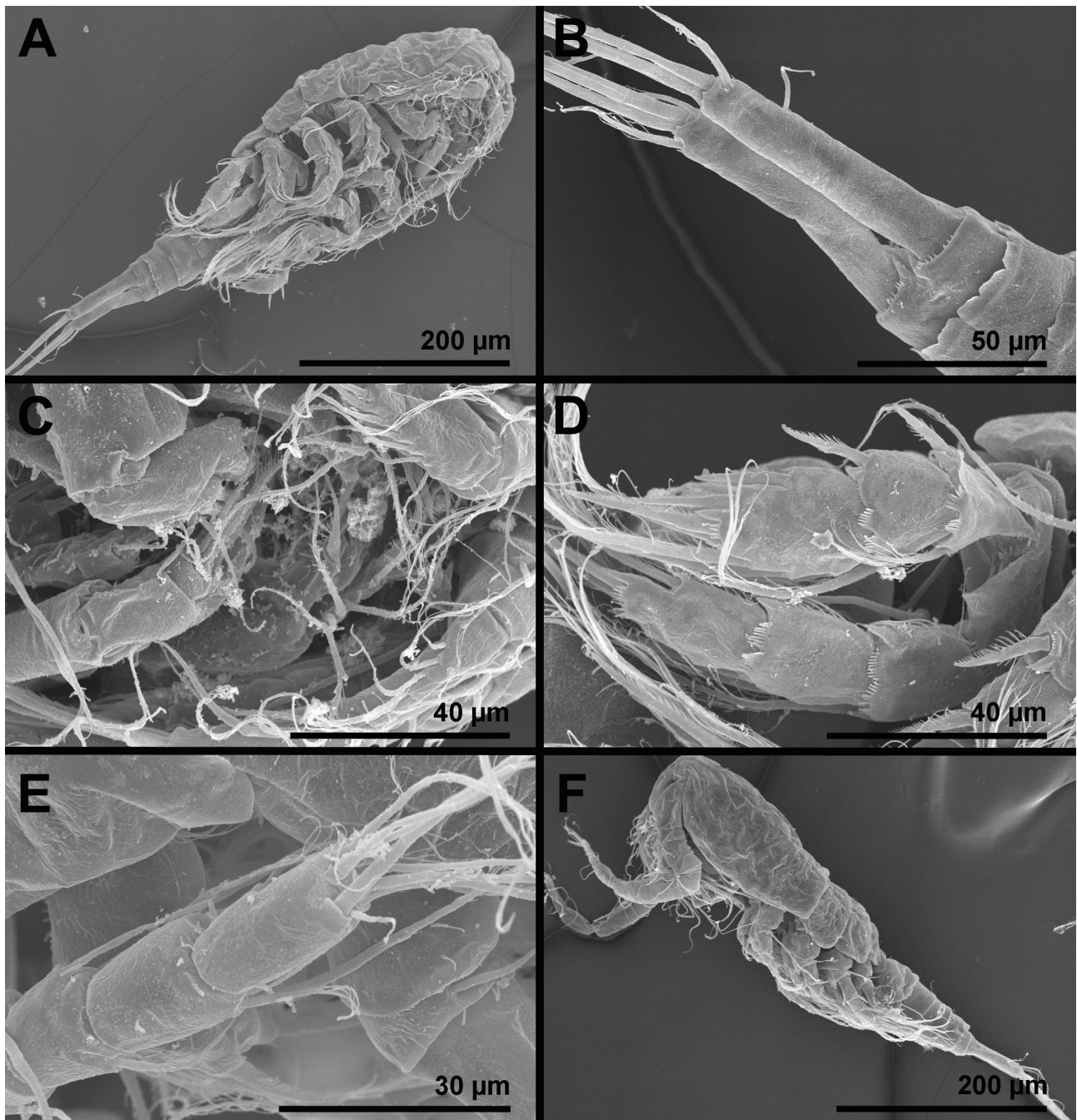


FIGURE 6. *Diacyclops leijsi* sp. nov., scanning electron micrographs, A–D, paratype female 1, ventral; E, paratype female 2, lateral; F, paratype male 2, lateral: A, habitus; B, caudal rami; C, mouth appendages; D, fourth swimming leg; E, distal part of antennula; F, habitus.

composed of praecoxa and 2-segmented palp, unornamented; praecoxal arthrite bearing 4 strong distal spines and 6 medial elements; palp with slender exopodal seta, 3 slender setae on endopod, and 1 strong and 2 slender setae on inner margin of coxobasis. Maxilla (Figs. 3G, 6C) 4-segmented, with 2 slender setae on praecoxa, 1 slender seta on proximal endite of coxa, 1 strong and 1 slender seta on highly mobile distal endite of coxa, basis expanded into robust claw with 1 strong and 1 very slender seta, and endopod with 3 strong and 2 slender setae. Maxilliped (Figs. 3H, 6C) 4-segmented, with long spinules on second and third segment and setae formula 3.2.1.3. All swimming legs (Figs. 4A, B, C, D, E, 6D) large and wide, with smooth intercoxal sclerite with slightly concave distal margin, each leg composed of minute and triangular praecoxa, large and rectangular coxa, short basis, 3-segmented exopod and 3-segmented endopod; basis of first leg with long outer seta and long inner spine, latter slightly longer than

first 2 endopodal segments combined; basis of second to fourth legs with shorter outer seta and inner distal corner pointed; all first and second exopodal segments, all first endopodal segments, and second endopodal segment of first and second legs with single inner seta; second endopodal segment of third and fourth legs with 2 inner setae; third exopodal segments spine formula 2.3.3.3 and setae formula 4.4.4.4; third endopodal segments of first to third leg with 3 inner setae, 1 apical seta, 1 apical spine, and 1 outer seta; third endopodal segment of fourth leg nearly twice as long as wide, with 2 inner setae, 2 apical spines, and 1 outer seta; its outer spine nearly 1.3 times as long as segment or inner spine. Fifth leg (Figs. 2A, 4F) small and slender, 2-segmented, with single outer seta on first segment and apical seta and subapical inner spine on second segment; second segment about 2.2 times as long as wide and only slightly longer than its spine. Sixth leg (Fig. 4G) small semicircular plate, with long seta and 2 minute spines; seta almost as long as plate width.

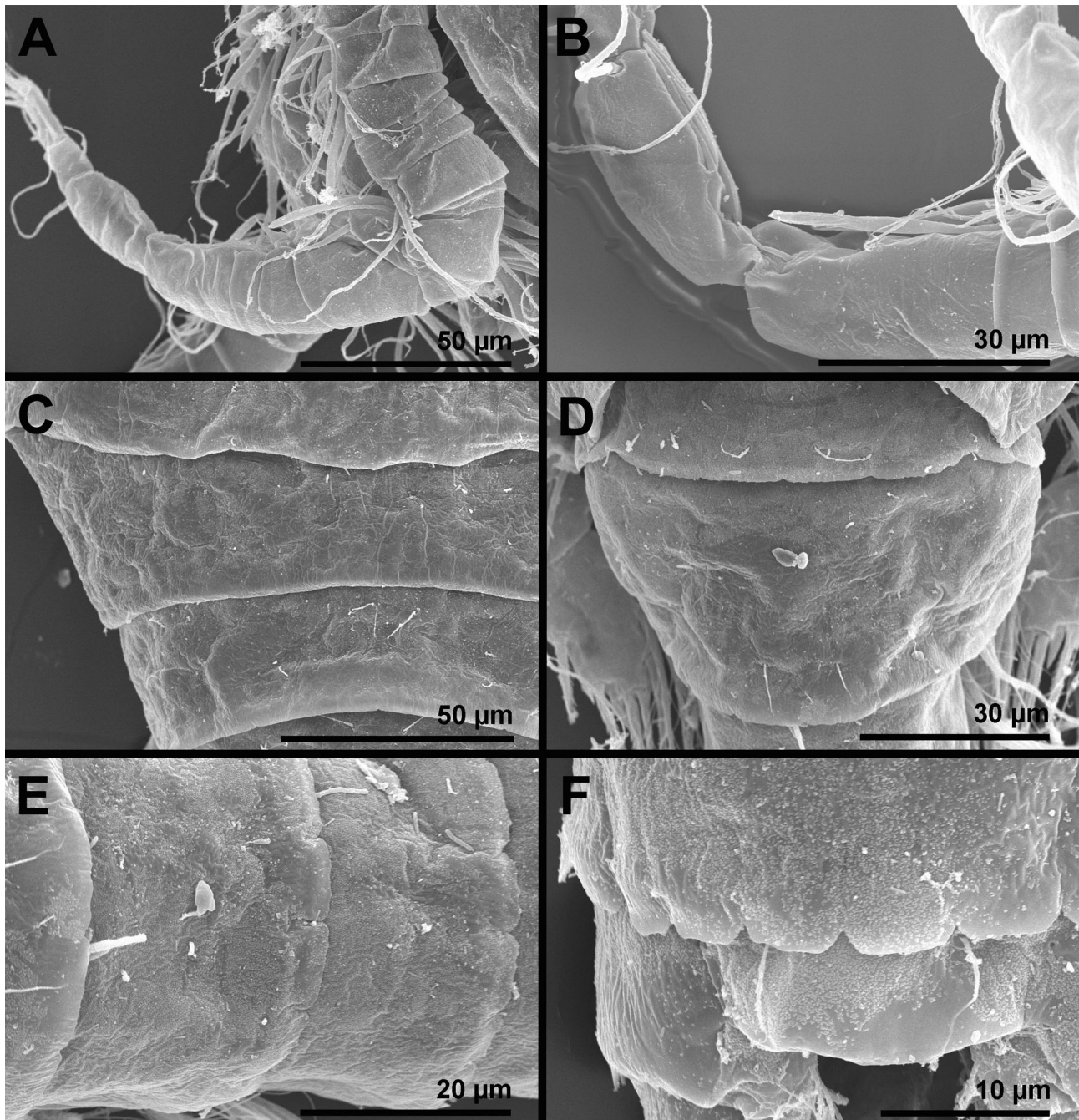


FIGURE 7. *Diacyclops leijsi* sp. nov., scanning electron micrographs, A & B, paratype male 2, lateral; C–F, paratype male 3, dorsal: A, left antennula; B, detail of right antennula; C, free prosomites; D, first two urosomites; E, third and fourth urosomites; F, last two urosomites.

Male. Body length from 435 to 465 μm . Habitus (Fig. 6F) and urosome (Fig. 5A) slightly slenderer than in female; free genital somite (Figs. 5A, 7D) more than 1.5 times as wide as subsequent urosomite, with relatively small ovoid spermatophores. Ornamentation of cephalothorax (Fig. 6F), free prosomites (Figs. 6F, 7C), and urosomites (Fig. 7D, E, F) as in female. Caudal rami (Figs. 5A, 6F) slightly slenderer than in female, with slightly shorter dorsal and innermost terminal setae. Antennula (Figs. 5B, 7A, B) slender, strongly prehensile and digeniculate, 16-segmented, with anvil-shaped cuticular ridges on anterior margin of fourteenth and fifteenth segments (distal geniculation); 3 aesthetascs present on first segment, while fourth, ninth, and thirteenth segments carry 1 aesthetasc each; setae formula 8.4.2.2.2.1.1.2.2.2.1.2.1.13; several setae minute and slender, 1 on each ninth, eleventh, and twelfth segment spiniform, and 2 pairs of setae on ultimate segment fused basally; penultimate segment about 2.5 times as long as wide. Antenna, labrum, mandibula, maxillula, maxilla, maxilliped, all swimming legs (Fig. 5C), and fifth leg (Fig. 5A) as in female. Sixth leg (Fig. 5A, D) longer than in female, with better developed innermost spine and with central slender seta; outermost seta about 2.7 times as long as spine or central seta.

***Diacyclops eberhardi* sp. nov.**

(Figs. 1B, 8–12)

urn:lsid:zoobank.org:act:66BA5A5F-161F-46F8-B638-614CC67D06CB

Type locality. Western Australia, Margaret River, Easter Cave, 34.274367°S 115.099297°E, 12 March 2002, collected by S. Eberhard.

Holotype. Adult female dissected on 1 microscope slide.

Paratypes. One male from type locality dissected on 1 microscope slide; 2 males and 2 females from type locality on 1 SEM stub (row no. 5), together with 5 other species described here; 3 males and 11 copepodids from type locality in 1 alcohol vial; 3 males in 1 alcohol vial from Jewel Cave, Margaret River, Western Australia, 34.2738°S 115.098413°E, 6 March 2002, collected by S. Eberhard.

Etymology. The species is named after Dr. Stefan M. Eberhard, University of New South Wales, Sydney, who collected this species and entrusted it to me for identification. The name is a noun in the genitive singular.

Diagnosis. Female. Body length from 360 to 470 μm . Habitus (Figs. 1B, 11A) spindle-shaped, about 2.5 times as long as wide, with prosome/urosome ratio of nearly 1.5, and posterior end of cephalothorax about 2.3 times as wide as genital double-somite in dorsal view. Integument on all somites (Fig. 11B, C, D, E) thin and smooth, with light bacterial cover; general distribution of spinules and cuticular pores on somites as in *D. leijsi*, but their number differs and differences in distribution prevent homologisation (especially on cephalothorax). Hyaline fringes of prosomites (Fig. 11C) and first urosomite (Fig. 11D) smooth, those of genital double-somite and 2 subsequent urosomites (Fig. 11E) slightly wavy. Genital double-somite (Fig. 8A) slightly wider than long in ventral view, widest at first fifth of its length and gradually tapering posteriorly, widest part about 1.5 times as wide as posterior margin; seminal receptacle large and shaped like a young mushroom; copulatory pore large; copulatory duct wide, long, and well-sclerotized. Anal somite (Figs. 8A, 11E) with large spinules along ventral margin. Caudal rami (Figs. 8A, 11F) stubby, spaced about a half or ramus' width, nearly 3 times as long as wide and about twice as long as anal somite; principal terminal setae with breaking planes, inner one about 1.3 times as long as entire urosome and 1.75 times as long as outer one; dorsal seta about 1.5 times as long as caudal ramus, 2.2 times as long as innermost terminal seta, and nearly 2.5 times as long as outermost terminal seta. Antennula (Fig. 8B) 12-segmented, slightly shorter than cephalothorax, with 1 aesthetasc on ninth and eleventh segments each and setae formula 8.4.2.6.2.2.3.2.2.2.8; ultimate segment about 1.6 times as long as wide. Antenna (Fig. 8C) 5-segmented, without exopodal seta, with setae formula 0.2.1.6.7; second segment nearly 1.3 times as long as fifth segment. Labrum, mandibula (Fig. 8D), maxillula (Fig. 8E), maxilla (Fig. 8F), and maxilliped as in *D. leijsi*. Segmentation of all swimming legs (Fig. 9A, B, C, D, E) as in *D. leijsi*, but they are all wider, and especially endopod of fourth leg; basis of first leg with long outer seta and short inner spine, latter slightly longer than first endopodal segment; basis of second to fourth legs with shorter outer seta and inner distal corner blunt; all first exopodal segments without inner seta; all second exopodal segments, all first endopodal segments, and second endopodal segment of first to third legs with single inner seta; second endopodal segment of fourth leg with 2 inner setae; third exopodal segments spine formula 2.3.3.3 and setae formula 4.4.4.4; third endopodal segments of first to third leg with 2 inner setae, 1 apical seta, 1 apical spine, and 1 outer seta; third endopodal segment of fourth leg 1.36 times as long as wide, with 2 inner setae, 2 apical spines, and 1 outer seta; its outer spine nearly 1.4 times as long as segment and twice as long as inner spine. Fifth leg (Figs. 8A,

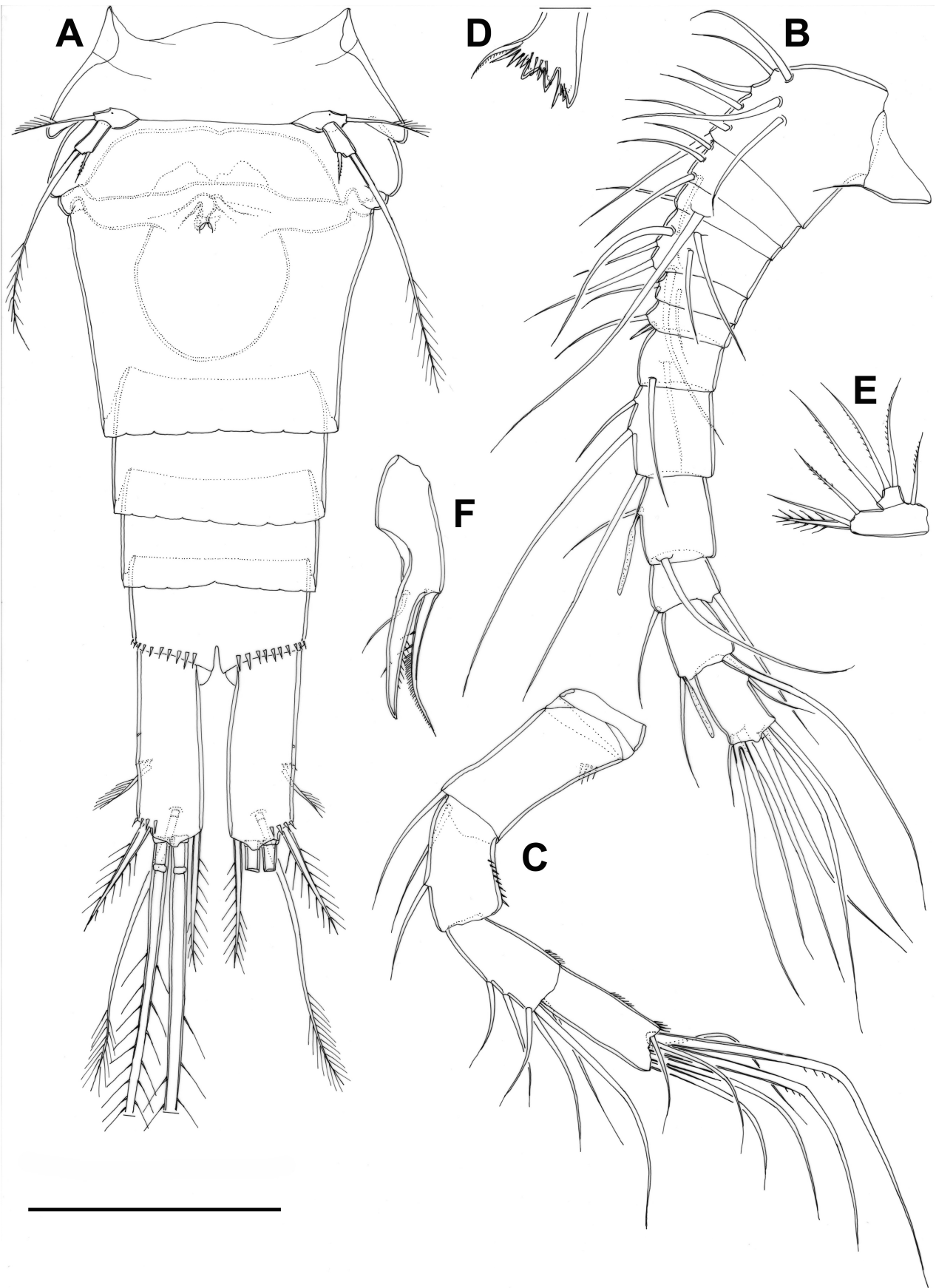


FIGURE 8. *Diacyclops eberhardi* sp. nov., line drawings, holotype female: A, urosome, ventral; B, antennula; C, antenna; D, cutting edge of mandibula; E, maxillular palp; F, basis of maxilla. Scale bar 50 μ m.

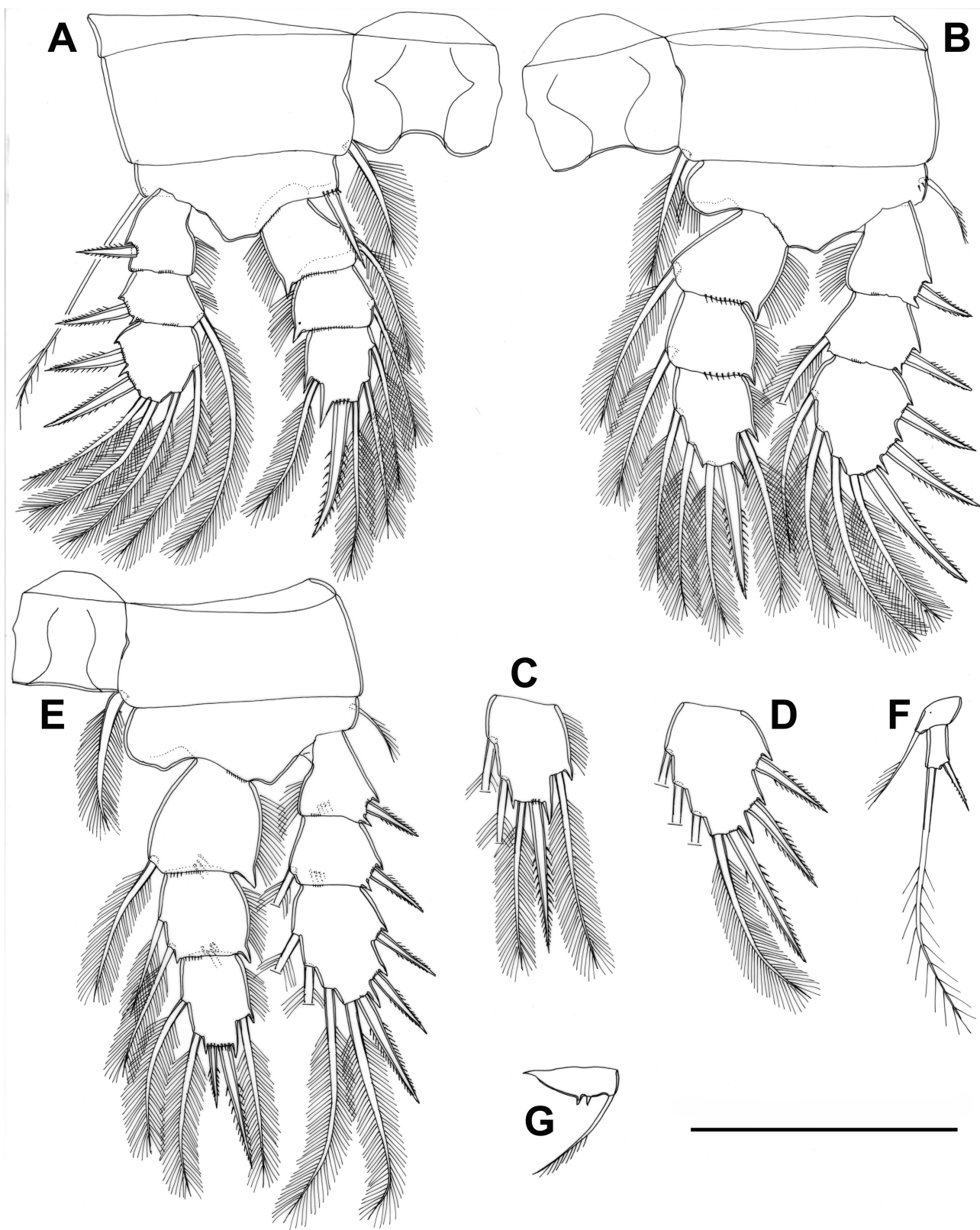


FIGURE 9. *Diacyclops eberhardi* sp. nov., line drawings, holotype female: A, first swimming leg; B, second swimming leg; C, third endopodal segment of third swimming leg; D, third exopodal segment of third swimming leg; E, fourth swimming leg; F, fifth leg; G, sixth leg. Scale bar 50 μ m.

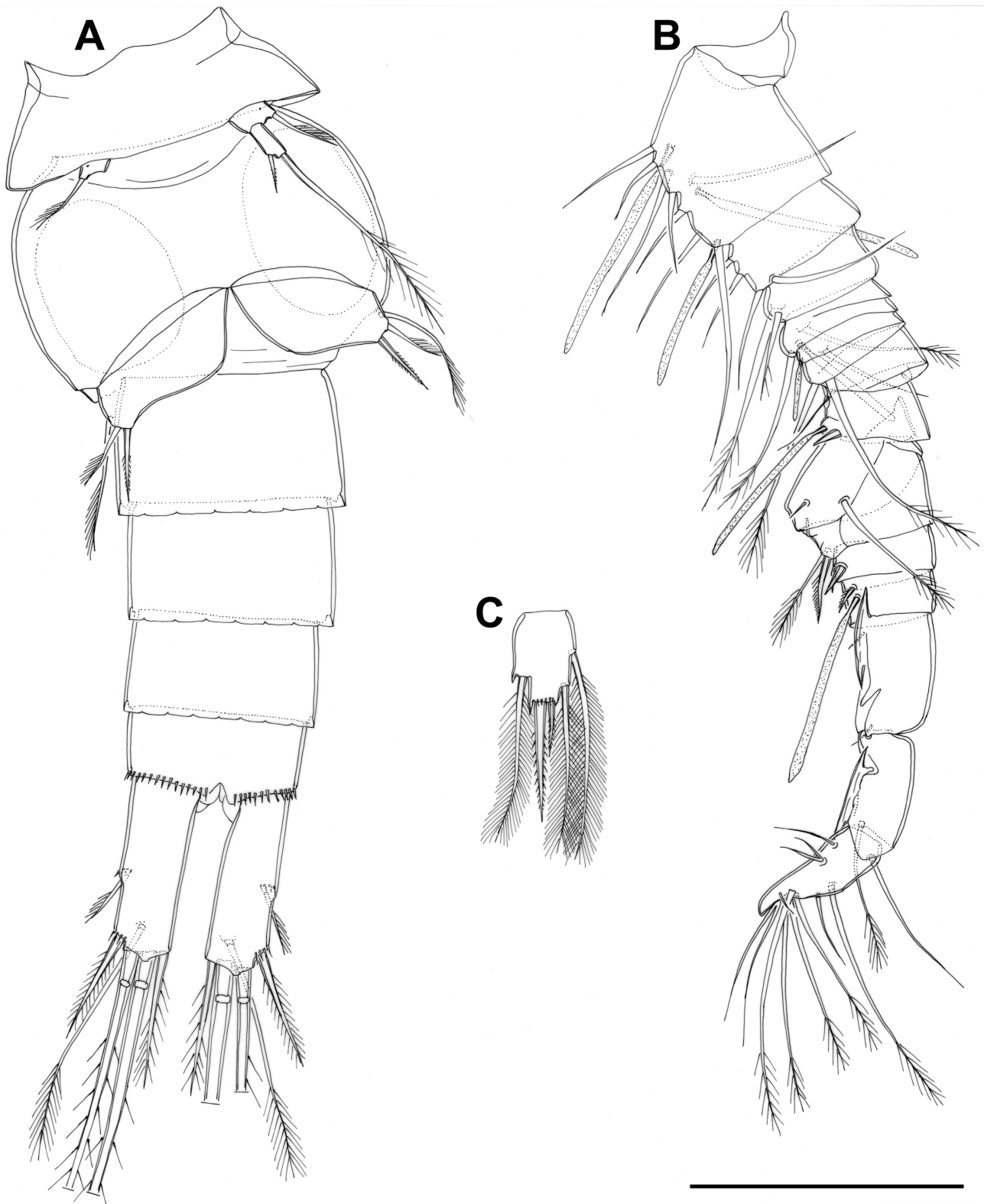


FIGURE 10. *Diacyclops eberhardi* sp. nov., line drawings, paratype male 1: A, urosome, ventral; B, antennula; C, third endopodal segment of fourth swimming leg. Scale bar 50 μ m.

9F) shape and segmentation as in *D. leijsi*; second segment about 1.8 times as long as wide and slightly shorter than its spine. Sixth leg (Fig. 9G) narrower than in *D. leijsi*, but with little difference in armature; seta slightly longer than plate width.

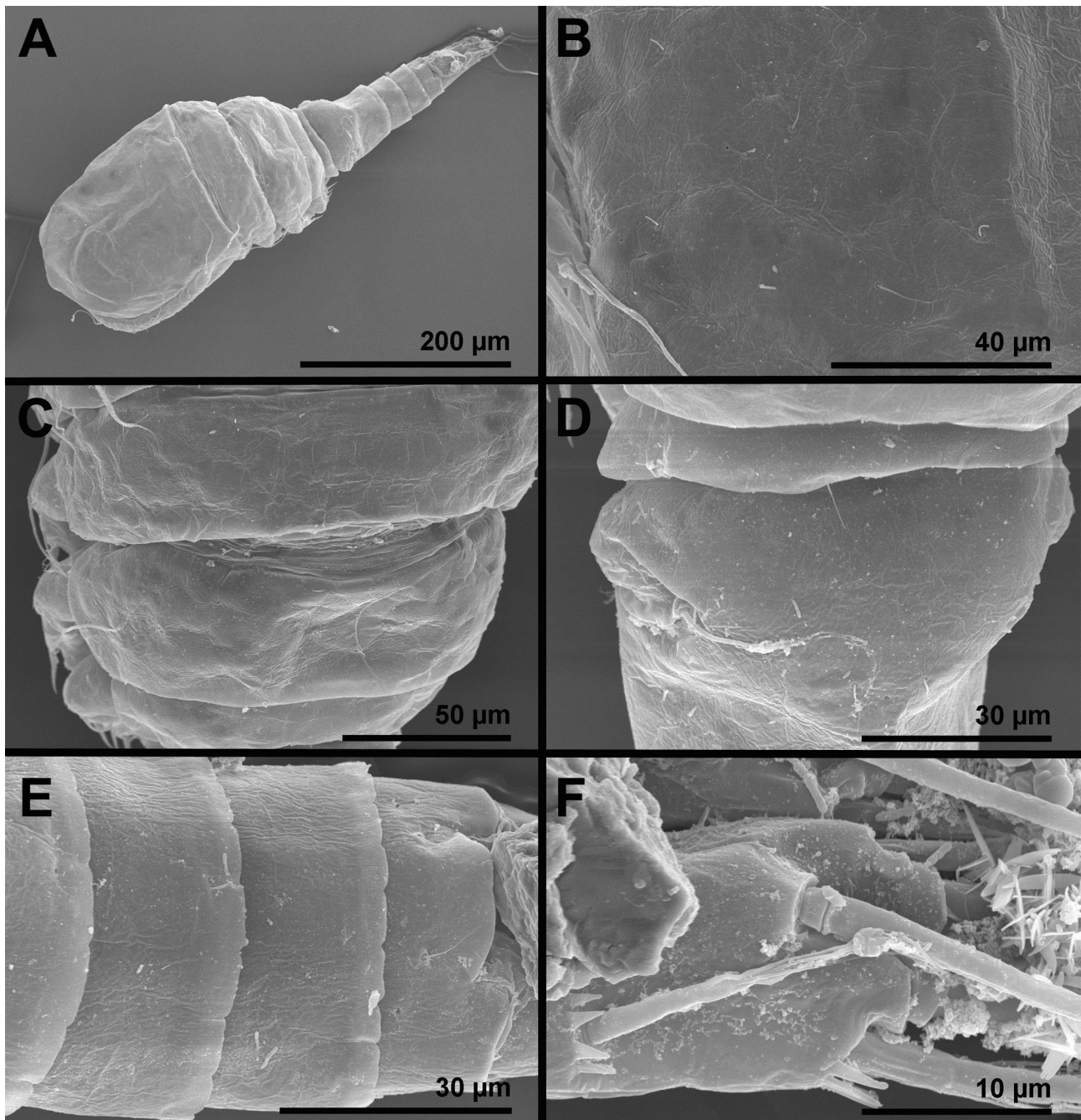


FIGURE 11. *Diacyclops eberhardi* sp. nov., scanning electron micrographs, paratype female 1, dorsal: A, habitus; B, central part of cephalothoracic shield; C, first and second free prosomites; D, first urosomite and anterior half of genital double-somite; E, last three urosomites; F, posterior part of left caudal ramus.

Male. Body length from 360 to 450 μm . Habitus (Fig. 12A) and urosome (Fig. 10A) slightly slenderer than in female; free genital somite (Fig. 10A) about 1.6 times as wide as subsequent urosomite, with relatively large ovoid spermatophores. Ornamentation of cephalothorax (Fig. 12B), free prosomites (Fig. 12C), and urosomites (Figs. 10A, 12A, D) as in female. Anal somite (Figs. 10A, 12A) shorter than in female and with smaller ventral spinules. Caudal rami (Figs. 10A, 12A) slightly slenderer than in female, with shorter dorsal seta. Antennula (Figs. 10B, 12E) very similar to that in *D. leijsi*, but shorter, fourth and fifth segments fused on anterior surface, and ninth segment almost completely fused with eighth; armature and ornamentation of first 3 and last 7 segments as in *D. leijsi*; armature of fourth to ninth segments slightly misplaced because of segment fusion, but also 2 setae missing on fused eighth and ninth segment, and 1 additional small aesthetasc present on sixth segment (maybe homologous to

one seta in *D. leijsi*); penultimate segment about 1.8 times as long as wide. Antenna (Fig. 12F), labrum, mandibula, maxillula (Fig. 12F), maxilla (Fig. 12F), maxilliped (Fig. 12F), all swimming legs (Fig. 10C), and fifth leg (Fig. 10A) as in female. Sixth leg (Fig. 10A) as in *D. leijsi*.

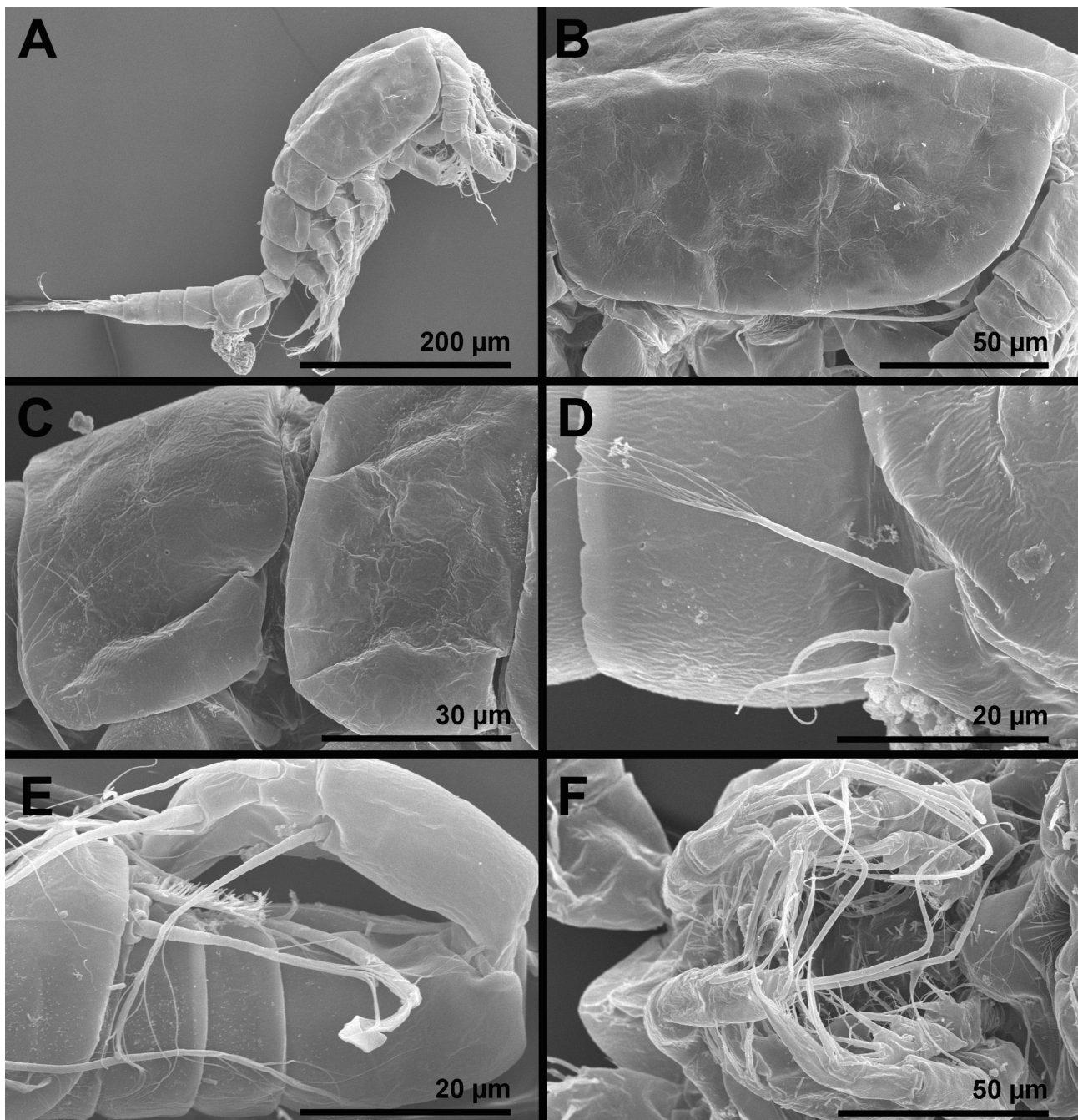


FIGURE 12. *Diacyclops eberhardi* sp. nov., scanning electron micrographs, A–D, paratype male 2, lateral; E & F, paratype male 3, ventral: A, habitus; B, cephalothoracic shield; C, second and third free prosomites; D, sixth leg; E, antenna; F, mouth appendages.

***Diacyclops ballaballaensis* sp. nov.**

(Figs. 1C, 13–16)

urn:lsid:zoobank.org:act:22E70096-E81F-4238-9576-FC7E40509A05

Type locality. Western Australia, Pilbara Region, Balla Balla Station, bore BBR052, sample no. FBN111, 20.764256°S 117.750643°E, 5 August 2008, collected by E. Thomas.

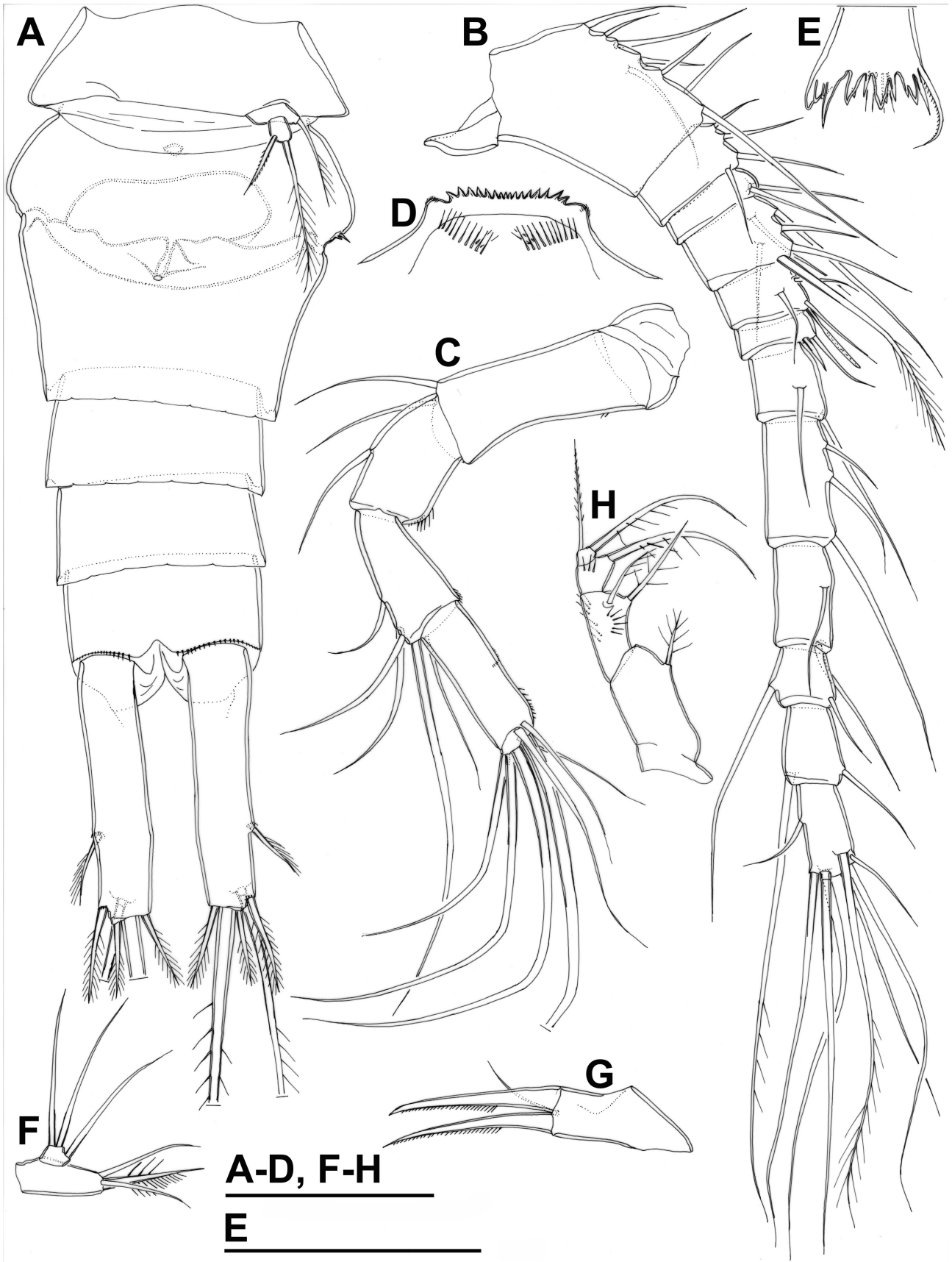


FIGURE 13. *Diacyclops ballaballaensis* sp. nov., line drawings, holotype female: A, urosome, ventral; B, antennula; C, antenna; D, labrum; E, cutting edge of mandibula; F, maxillular palp; G, basis of maxilla; H, maxilliped. Scale bars 50 μ m.

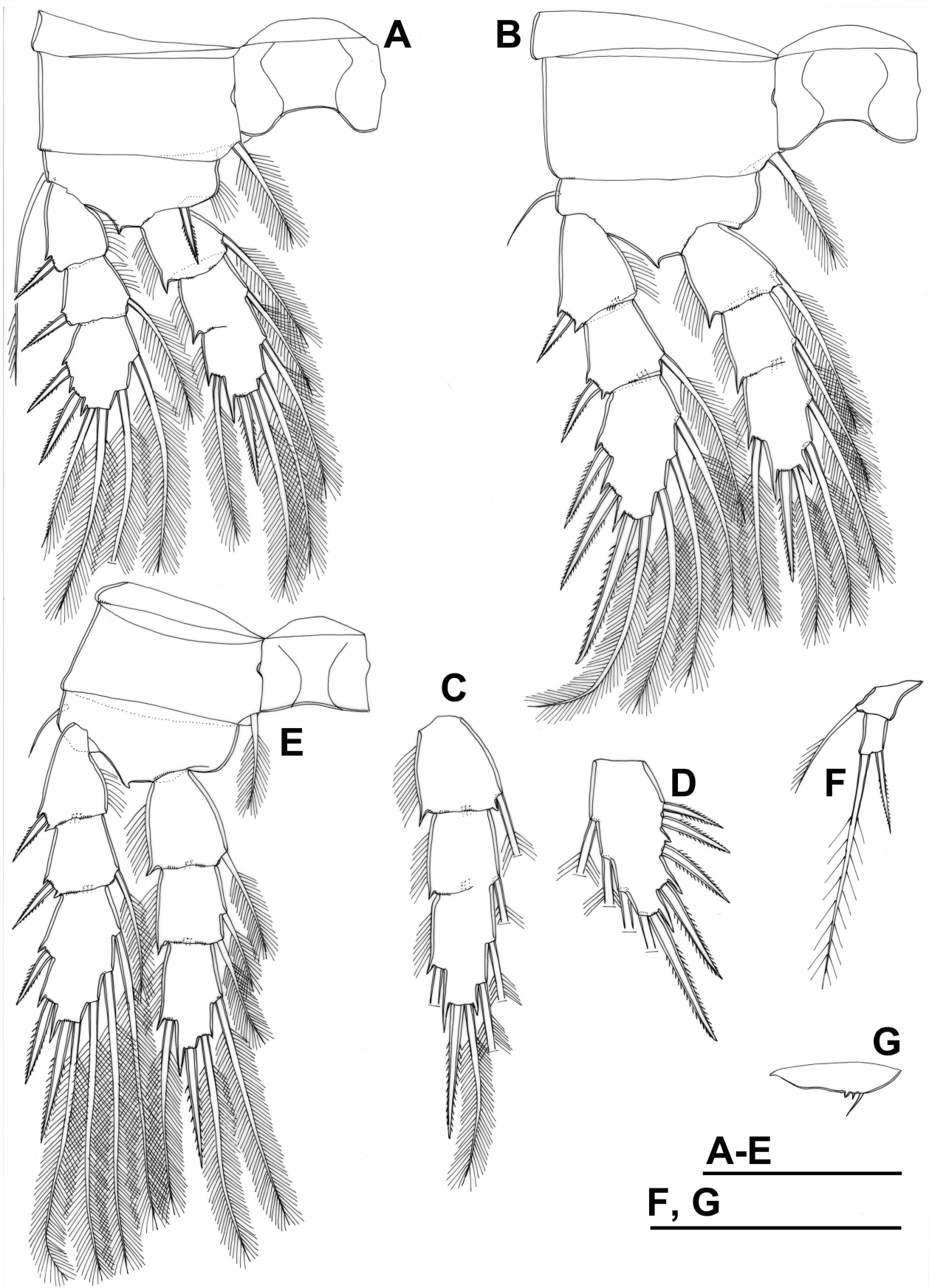


FIGURE 14. *Diacyclops ballaballaensis* sp. nov., line drawings, holotype female: A, first swimming leg; B, second swimming leg; C, endopod of third swimming leg; D, aberrant third exopodal segment of third swimming leg; E, fourth swimming leg; F, fifth leg; G, sixth leg. Scale bars 50 μ m.

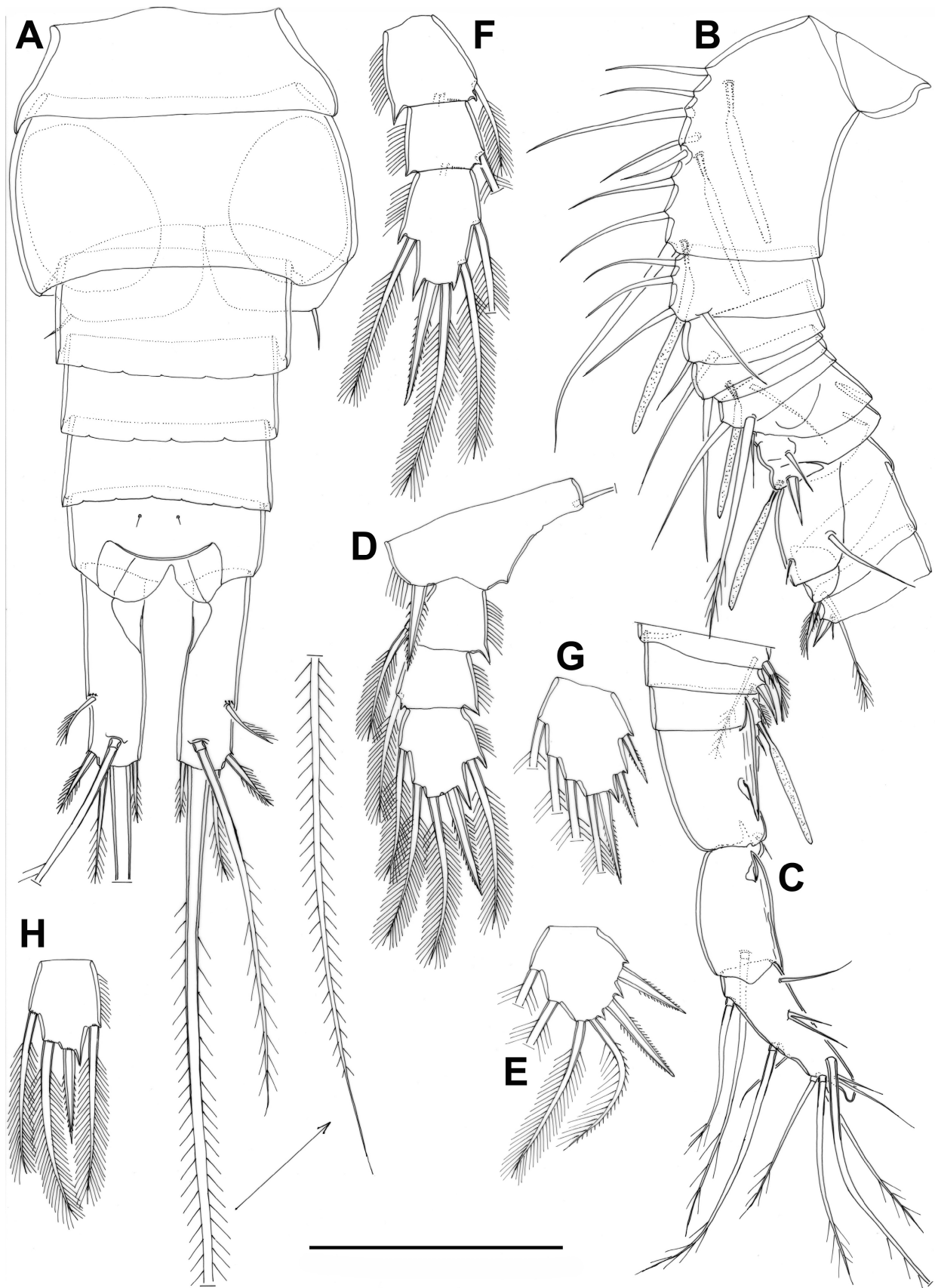


FIGURE 15. *Diacyclops ballaballaensis* sp. nov., line drawings, paratype male 1: A, urosome, dorsal; B, proximal part of antennula; C, distal part of antennula; D, basis and endopod of first swimming leg; E, third exopodal segment of first swimming leg; F, endopod of third swimming leg; G, third exopodal segment of fourth swimming leg; H, third endopodal segment of fourth leg. Scale bar 50 μ m.

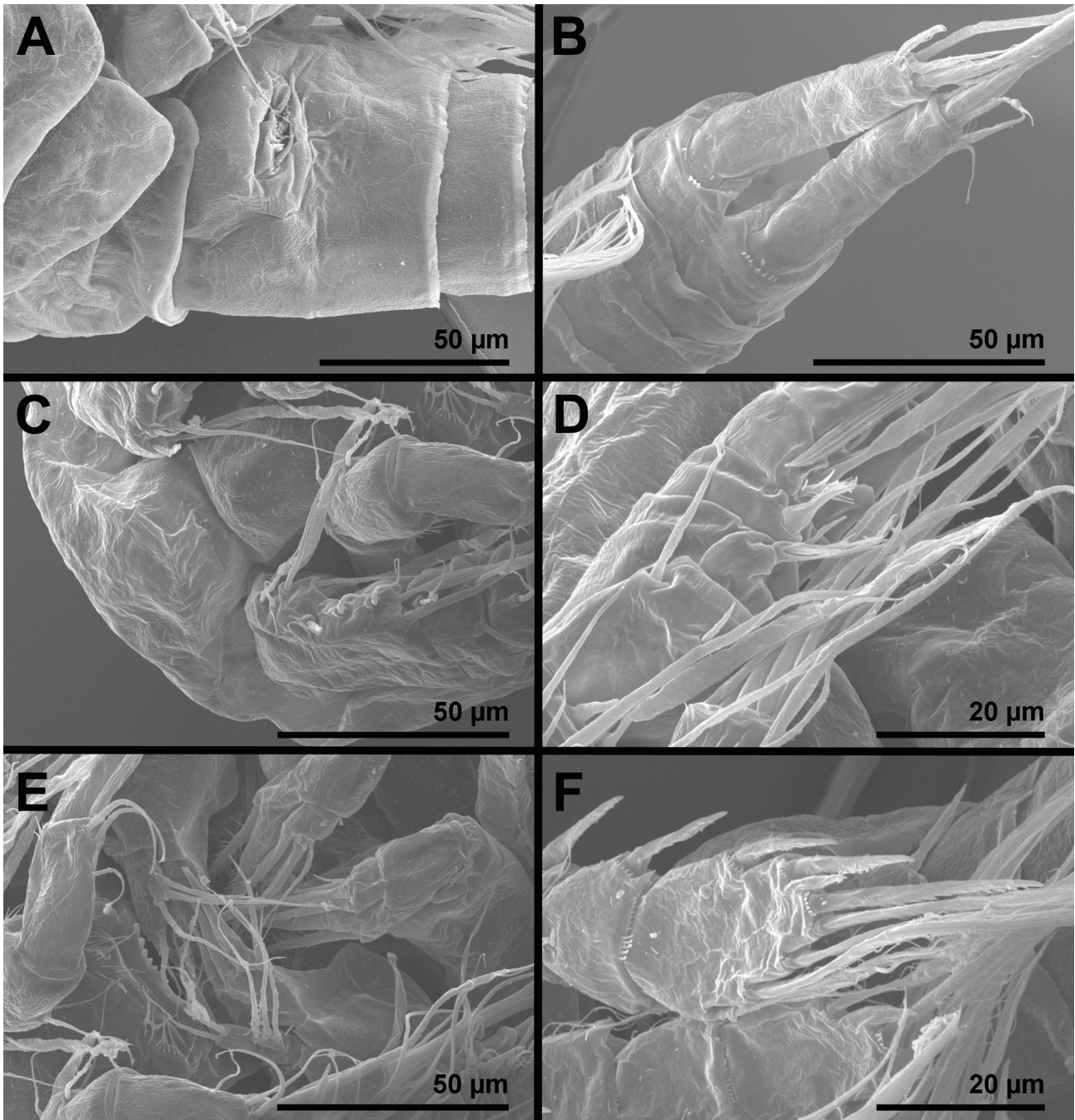


FIGURE 16. *Diacyclops ballaballaensis* sp. nov., scanning electron micrographs, A, paratype female 1, lateral; B–F, faratype male 2, ventral: A, genital double-somite; B, posterior part of urosome and caudal rami; C, rostrum and first segments of antennula and antenna; D, central part of antennula; E, mouth appendages; F, distal part of fourth swimming leg.

Holotype. Adult female dissected on 1 microscope slide.

Paratypes. One female from type locality dissected on 1 microscope slide; 1 female and 1 copepod from type locality in 1 alcohol vial; 1 male dissected on 1 microscope slide from bore BBWP015, Balla Balla Station, Pilbara Region, Western Australia, 20.805288°S 117.758197°E, sample no. FBN102, 8 June 2008, collected by E. Thomas; 1 male and 1 female from bore BBWP015 (see above) and 1 female from Harding Dam, Pilbara Region, Western Australia, 20.970278°S 117.090278°E, sample no. HD3/00, 3 April 2003, collected by J. Cocking and M. Scanlon, together on 1 SEM stub (row no. 1), together with 5 other species described here; 1 copepodid from bore BBMB007, Balla Balla Station, Pilbara Region, Western Australia, 20.749846°S 117.778707°E, sample no. FBN012, 4 December 2007, collected by E. Thomas, in 1 alcohol vial.

Etymology. The species name refers to its type locality, Balla Balla Station, which is a pastoral lease and cattle station about 140 km east of Karratha in Western Australia. It is an adjective for place, made with the Latin suffix “-ensis”.

Diagnosis. Female. Body length from 520 to 540 μm . Habitus (Fig. 1C) robust, 2.75 times as long as wide, with prosome/urosoma ratio of more than 1.6, and cephalothorax nearly 2.5 times as wide as genital double-somite in dorsal view. Integument on all somites (Fig. 16A, B) thin and wrinkled, with light bacterial cover; general distribution of spinules and cuticular pores on somites as in *D. leijsi*. Hyaline fringes of prosomites (Fig. 16A) and first urosoma (Fig. 16A) smooth, those of genital double-somite and 2 subsequent urosomites (Figs. 13A, 16B) very slightly wavy. Genital double-somite (Figs. 13A, 16A) wider than long in ventral view, widest at first third of its length and gradually tapering posteriorly, widest part about 1.5 times as wide as posterior margin; seminal receptacle smaller than in previous 2 species, anterior part less dome-like, posterior part short and broad; copulatory pore small and located more posteriorly than in previous 2 species; copulatory duct wide, long, and well-sclerotized. Anal somite (Figs. 13A, 16B) more cleft than in previous 2 species, with minute spinules along ventral margin. Caudal rami (Figs. 13A, 16B) long and slender, widely spaced, nearly 4.6 times as long as wide and about 2.7 times as long as anal somite; principal terminal setae without breaking planes, inner one strong and slightly longer than entire urosoma, outer one greatly reduced and even shorter than innermost terminal seta; dorsal seta extremely long, about 3 times as long as caudal ramus, nearly 10 times as long as innermost terminal seta, and about 9 times as long as outermost terminal seta. Antennula (Fig. 13B) 12-segmented, slightly shorter than cephalothorax, with single aesthetasc on fifth segment and setae formula 8.4.2.6.3.2.2.3.2.2.2.8; ultimate segment about 1.9 times as long as wide. Antenna (Fig. 13C) 5-segmented, without exopodal seta, with setae formula 0.2.1.5.7; second segment about 1.4 times as long as fifth segment. Labrum (Fig. 13D) with 2 diagonal rows of 12–13 slender spinules each on anterior surface, and short rows of minute spinules in blunt lateral corners; cutting edge nearly straight, with 16 sharp teeth. Mandibula (Fig. 13E) similar to that in *D. leijsi*, but with 1 less unicuspidate tooth on cutting edge. Maxillula (Fig. 13F) also similar to that in *D. leijsi*, but without exopodal seta on its palp. Maxilla (Fig. 13G) similar to that in *D. leijsi*, but basal spine less robust and partly articulated basally. Maxilliped slenderer than in previous 2 species, with setae formula 1.2.1.2. Shape and segmentation of all swimming legs (Fig. 14A, B, C, E) as in *D. leijsi*, but second and third endopodal segment of first to third leg partly fused towards inner margin, while endopod of fourth leg clearly 3-segmented; basis of first leg with long outer seta and short inner spine, latter slightly shorter than first endopodal segment; basis of second to fourth legs with shorter outer seta and inner distal corner blunt; all first exopodal segments without inner seta; all second exopodal segments, all first endopodal segments, and second endopodal segment of first to third legs with single inner seta; second endopodal segment of fourth leg with 2 inner setae; third exopodal segments spine formula 2.3.3.3 and setae formula 4.4.4.4; third endopodal segments of first to third leg with 2 inner setae, 1 apical seta, 1 apical spine, and 1 outer seta; third endopodal segment of fourth leg about 1.7 times as long as wide, with 2 inner setae, 1 apical spine, and 1 outer seta; its apical spine slightly longer than segment. Fifth leg (Figs. 13A, 14F) shape and segmentation as in *D. leijsi*; second segment about 1.7 times as long as wide and only half as long as its spine. Sixth leg (Figs. 14G, 16A) minute, with very short outer seta.

Male. Body length from 515 to 530 μm . Habitus and urosoma (Fig. 15A) slightly slenderer than in female; free genital somite (Fig. 15A) about 1.5 times as wide as subsequent urosoma, with relatively large ovoid spermatophores. Ornamentation of cephalothorax, free prosomites, and last 3 urosomites (Fig. 15A) as in female. Caudal rami (Fig. 15A) significantly shorter than in female, only about 3.5 times as long as wide in ventral view; dorsal seta also considerably shorter in female, only about 1.9 times as long as ramus; reduced outer principal terminal seta longer than in female, about 1.9 times about as long as innermost terminal seta. Antennula (Figs. 15B, C, 16C, D) very similar to that in *D. leijsi*, but shorter, fifth segment reduced in size, and ninth segment almost completely fused with eighth; armature and ornamentation of first 4 and last 7 segments as in *D. leijsi*, except ultimate segment missing smallest seta in distal cluster; fifth segment unarmed (two setae missing), ninth segment missing one seta, and seta on eighth segment smaller when compared to *D. leijsi*; penultimate segment about 1.7 times as long as wide. Antenna (Fig. 16C, E), labrum (Fig. 16E), mandibula (Fig. 16E), maxillula (Fig. 16E), maxilla (Fig. 16E), and maxilliped as in female. All swimming legs (Figs. 15D, E, F, G, H, 16F) with completely divided second and third endopodal segments, but armature and ornamentation as in female; third exopodal segments of first (Fig. 15E) to fourth (Fig. 15G) legs, as well as third endopodal segment of fourth leg (Fig. 15H), shorter than in female. Fifth leg as in female. Sixth leg (Fig. 15A) with single short spine.

Variability. One third swimming leg of the holotype female showed an aberrant third exopodal segment (Fig. 14D), with 5 spines. The other third leg was normal, with three spines, as in the second swimming leg (Fig. 14B).

***Diacyclops hancocki* sp. nov.**

(Figs. 1D, 17–21)

urn:lsid:zoobank.org:act:9413A572-A2CE-41A3-8BAC-DC692D7625B0

Type locality. Australia, New South Wales, Tamworth, bore 30150, sample no. 3T30P0-100, 31.08221°S 150.91257°E, 20 July 2006, collected by P. Hancock.

Holotype. Adult female dissected on 1 microscope slide.

Paratypes. One male and 1 copepodid from type locality dissected on 1 microscope slide each; 2 males and 4 females from type locality on 1 SEM stub (row no. 4), together with 5 other species described here; 3 females and 1 copepodid from type locality in 1 alcohol vial; 2 males, 6 females, and 3 copepodids from type locality in 1 alcohol vial; 2 males from bore 93024, Tamworth, New South Wales, 31.36435°S 151.20272°E, sample no. 7T26P0-100, 17 July 2007, collected by M. Tomlinson; 1 female from bore 93028, Tamworth, New South Wales, 31.30358°S 151.1456°E, sample no. 8T44P200-300, 23 Oct 2007, collected by M. Tomlinson.

Etymology. The species is named after Dr. Peter J. Hancock, Water Resources Group, Adelaide, who collected this species and entrusted it to me for identification. The name is a noun in the genitive singular.

Diagnosis. Female. Body length from 455 to 540 µm. Habitus (Fig. 1D) very robust, 2.2 times as long as wide, with prosome/urosome ratio of about 1.6, and cephalothorax nearly 2.9 times as wide as genital double-somite in dorsal view. Integument on all somites (Fig. 20A, B) thin and smooth, without bacterial cover; general distribution of spinules and cuticular pores on somites as in *D. leijsi*. Hyaline fringes of prosomites (Fig. 20A) and first urosomite (Fig. 20A) smooth, those of genital double-somite and 2 subsequent urosomites (Figs. 17A, 20B) very slightly serrated. Genital double-somite (Figs. 17A, 20A) only slightly wider than long in ventral view, widest at first third of its length, abruptly tapering towards midlength, and nearly cylindrical in posterior half, widest part about 1.4 times as wide as posterior margin; seminal receptacle narrower than in previous 3 species, anterior part ovoid, about 2.3 times as wide as long, and about 1.3 times as wide as posterior heart-shaped part; copulatory pore very small and located at about 2/5 of somite length; copulatory duct shorter and narrower than in *D. ballaballaensis*, also less sclerotized, but with similarly inflated first half. Anal somite (Figs. 17A, 20) with long and slender spinules along ventral margin; anal operculum shortest of all species described here. Caudal rami (Figs. 17A) short and stout, widely spaced but not as much as in *D. ballaballaensis*, about twice as long as wide and less than 1.5 times as long as anal somite; principal terminal setae with breaking planes, inner one about 1.3 times as long as entire urosome and 1.6 times as long as outer one; dorsal seta about 2.3 times as long as caudal ramus, only slightly longer than innermost terminal seta, and about 3.2 times as long as outermost terminal seta. Antennula (Fig. 17B) 11-segmented, somewhat shorter than cephalothorax, with 1 aesthetasc on fourth, eighth, and tenth segments each, and setae formula 8.4.8.3.2.2.2.2.2.8; ultimate segment about 1.8 times as long as wide. Antenna (Fig. 17C) 5-segmented, slenderer than in previous 3 species, with well-developed exopodal seta, total setae formula 0.3.1.7.7; second segment about 1.4 times as long as fifth segment. Labrum (Fig. 20C) with 2 diagonal rows of 12–13 slender spinules each on anterior surface; cutting edge slightly concave, with 17–19 sharp teeth between blunt and smooth lateral corners. Mandibula (Figs. 17D, 20C) similar to that in *Diacyclops leijsi* sp. nov., but with 7 slender spinules on anterior surface. Maxillula (Figs. 17E, 20D) also similar to that in *D. leijsi*, but apical spine fused basally to inner margin of coxobasis. Maxilla (Figs. 17F) similar to that in *D. leijsi*, but basal claw with several additional spinules and endopod visibly 2-segmented. Maxilliped (Figs. 17G, 20F) similar to that in *D. ballaballaensis*, but second segment with fewer spinules and setae formula 2.1.1.2. Segmentation of all swimming legs (Fig. 18A, B, C, D, E) as in *D. leijsi*, but they are slightly wider, and intercoxal sclerite of first leg with two short arched rows of minute spinules on anterior surface; basis of first leg with short outer seta and very strong inner spine, latter slightly longer than first 2 endopodal segments combined; basis of fourth legs with less sharp inner distal corner than those of second and third legs; first exopodal segment of first and fourth legs without inner seta, while that segment of second and third leg with inner seta; all second exopodal segments, all first endopodal segments, and second endopodal segment of first to third legs with single inner seta; second endopodal segment of fourth leg with 2 inner setae; third exopodal segments spine formula 3.4.4.3 and setae formula 5.5.5.5; third endopodal segment of first leg with 2 inner setae, 1 apical seta, 1 apical spine, and 1 outer seta; third endopodal segment of second and third legs with 3 inner setae, 1 apical seta, 1 apical spine, and 1 outer seta, but apical spine on second leg much longer than that on third leg; third endopodal segment of fourth leg 1.6 times as long as wide, with 2 inner setae, 2 apical spines, and 1 outer seta; its outer spine very slightly shorter than inner spine and only about half as long as segment. Fifth

leg (Fig. 17A) shape, segmentation, and proportions of segments and armature as in *D. leijsi*, except inner margin of second segment somewhat convex. Sixth leg (Figs. 18F) not larger than in *D. leijsi*, but with much larger spines than in any other species described here; outer seta less than half as long as plate width.

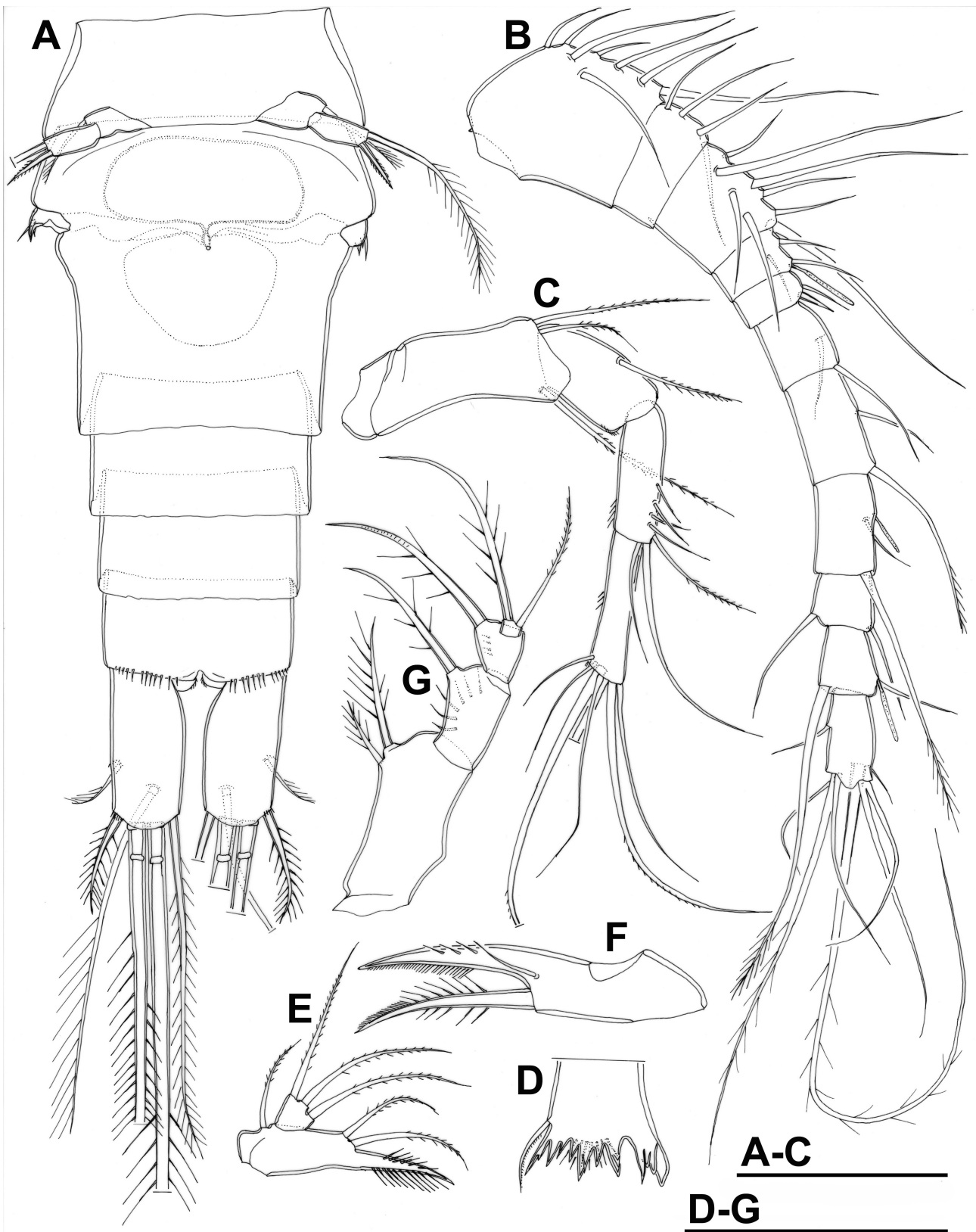


FIGURE 17. *Diacyclops hancocki* sp. nov., line drawings, holotype female: A, urosome, ventral; B, antennula; C, antenna; D, cutting edge of mandibula; F, basis of maxilla; G, maxilliped. Scale bars 50 μ m.

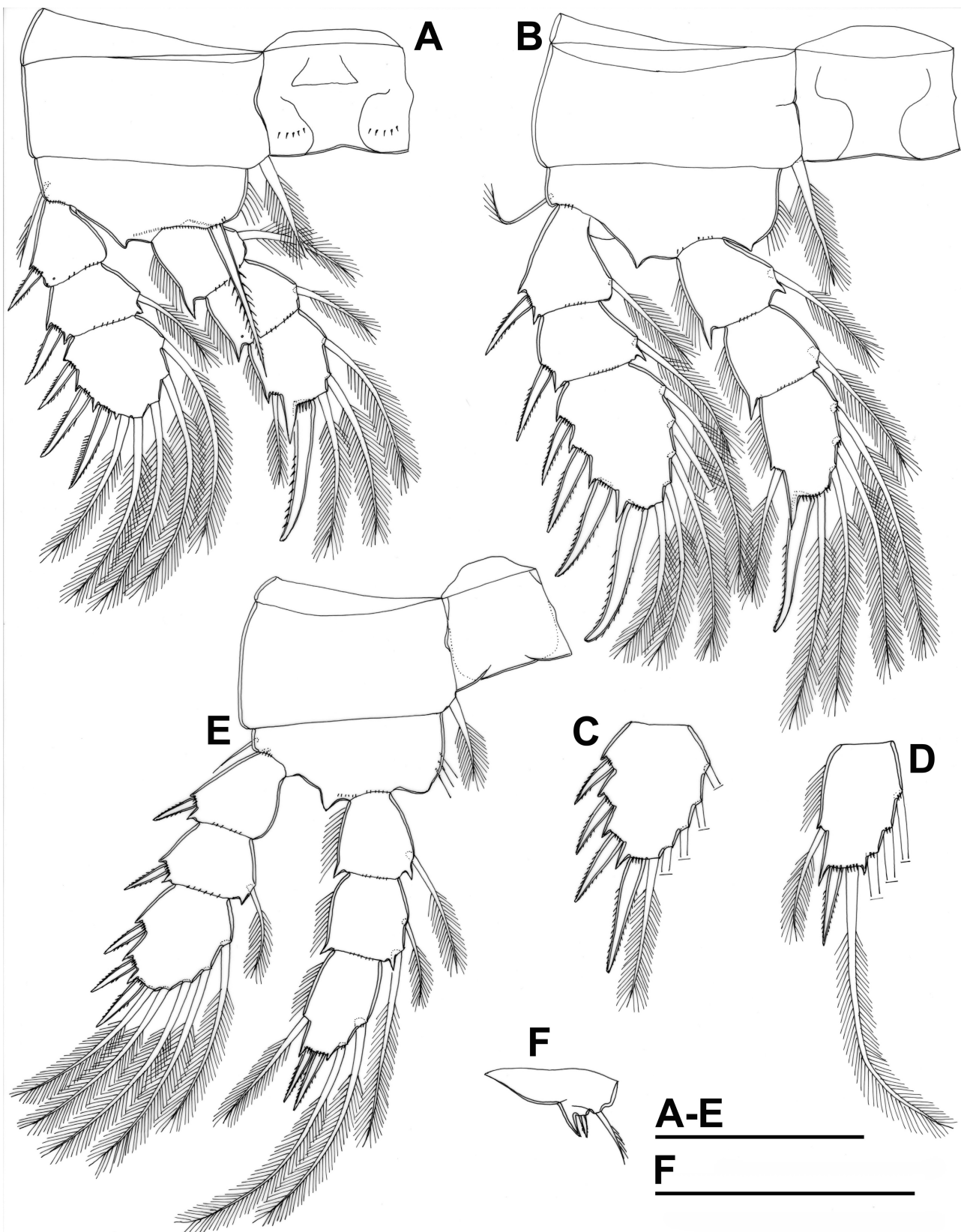


FIGURE 18. *Diacyclops hancocki* sp. nov., line drawings, holotype female: A, first swimming leg; B, second swimming leg; C, third exopodal segment of third swimming leg; D, third endopodal segment of third swimming leg; E, fourth swimming leg; F, sixth leg. Scale bars 50 μ m.

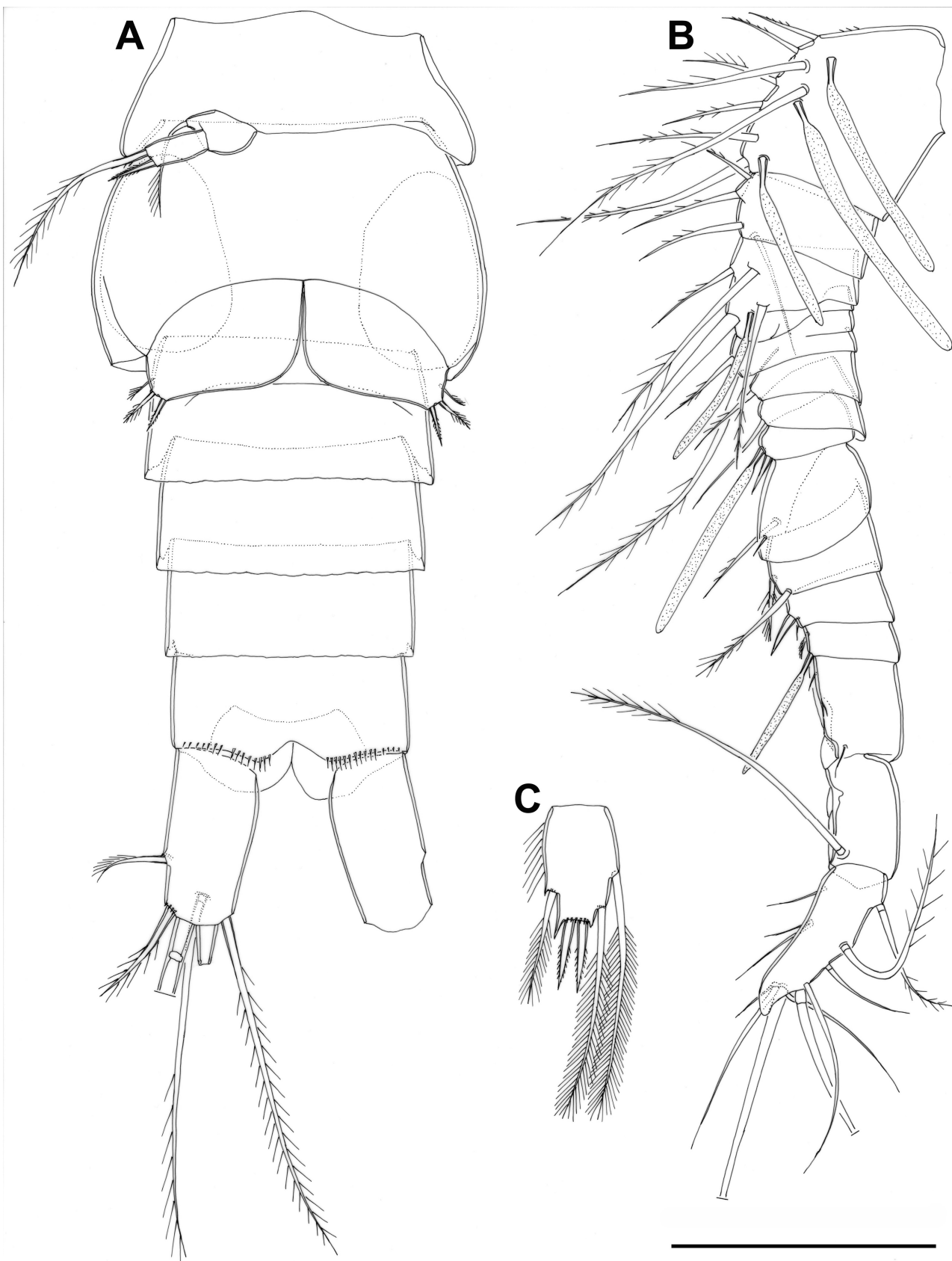


FIGURE 19. *Diacyclops hancocki* sp. nov., line drawings, paratype male 1: A, urosome, ventral; B, antennula; C, third endopodal segment of fourth swimming leg. Scale bar 50 μ m.

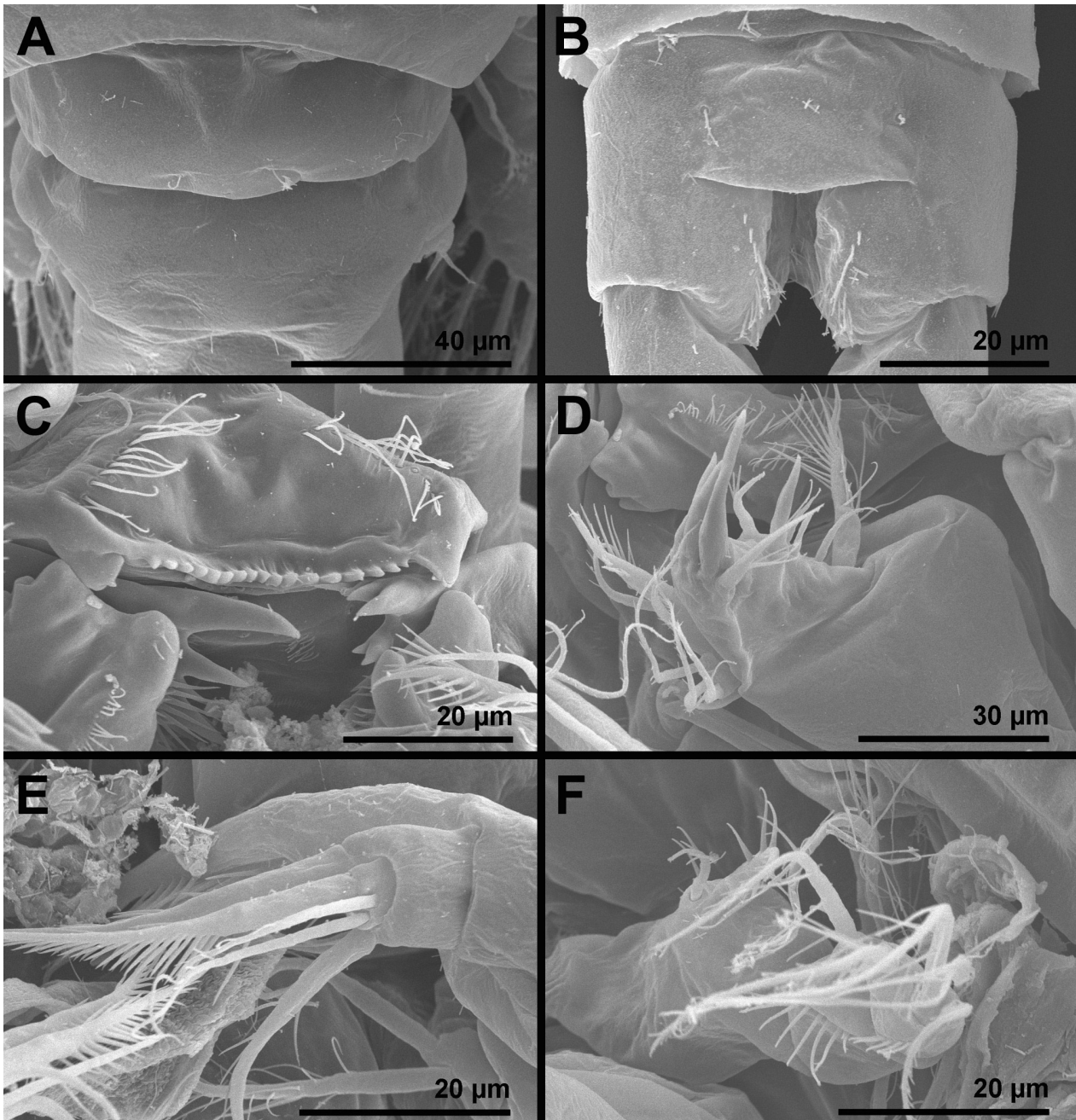


FIGURE 20. *Diacyclops hancocki* sp. nov., scanning electron micrographs, A & B, paratype female 1, dorsal, C–F, paratype female 2, ventral: A, first urosomite and anterior half of genital double-somite; B, anal somite; C, labrum, paragnaths, and cutting edges of mandibulae; D, maxillula; E, maxilla; F, maxilliped.

Male. Body length from 450 to 490 µm. Habitus (Fig. 21A) and urosome (Fig. 19A) slightly slenderer than in female; free genital somite (Fig. 19A) less than 1.4 times as wide as subsequent urosomite, with relatively large ovoid spermatophores. Ornamentation of cephalothorax, free prosomites, and last 3 urosomites (Figs. 19A, 21B) as in female. Caudal rami (Figs. 19A, 21B) also as in female. Antennula (Figs. 19B, 21C) very similar to that in *D. ballaballaensis*, except that third and fourth segments are fused completely on anterior surface, as well as fifth and sixth segments, although remnants of their original segmentation remain visible on posterior margin; all armature and ornamentation as in *D. ballaballaensis*; penultimate segment also about 1.7 times as long as wide. Antenna (Fig. 21C), labrum (Fig. 21C), mandibula, maxillula, maxilla, maxilliped, all swimming legs (Figs. 19C, 21E), and fifth leg (19A, 21F) as in female. Sixth leg (Figs. 19A, 21F) as in *D. leijsi*, with 1 spine and 2 setae, but all 3 elements of similar length.

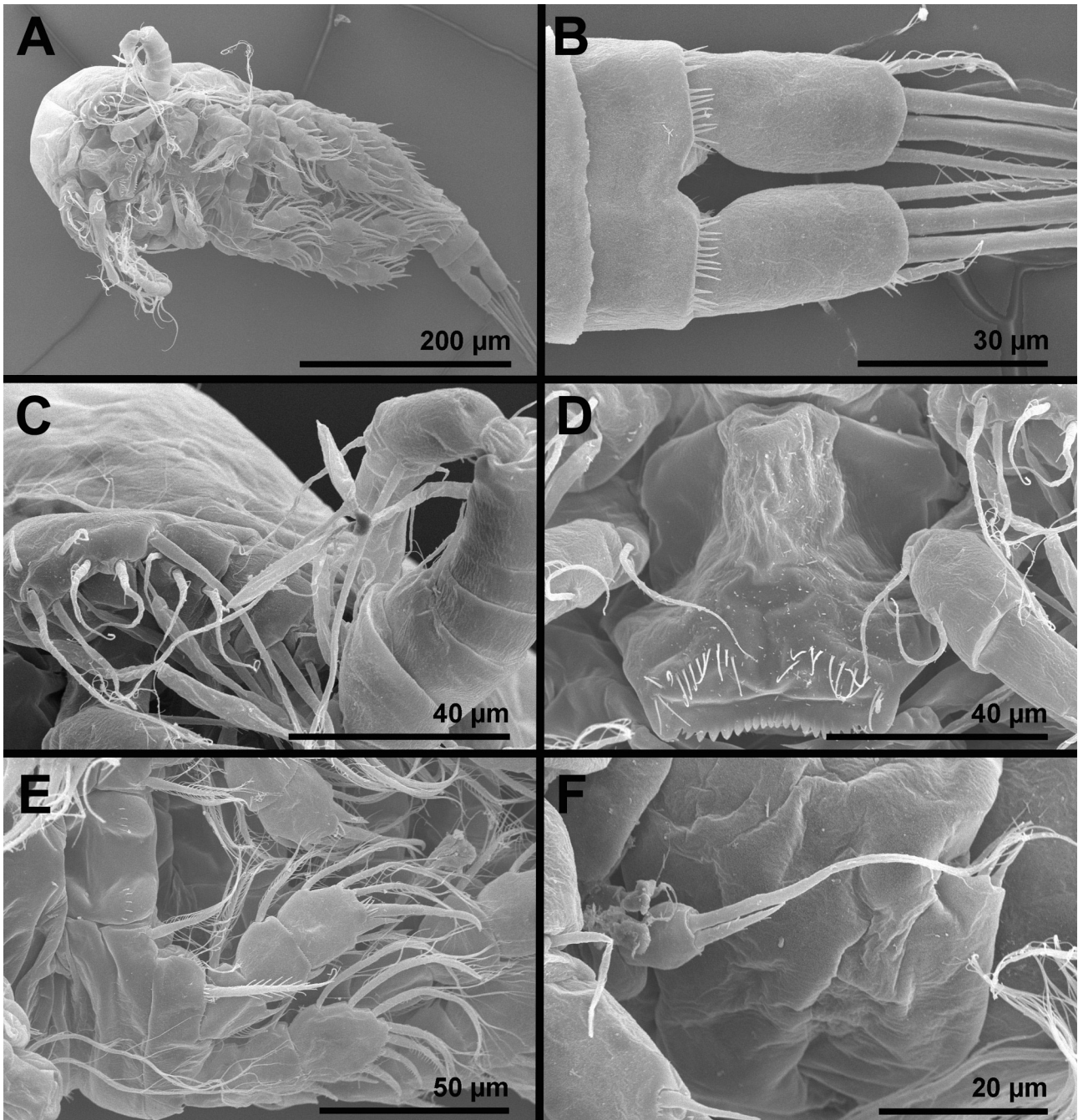


FIGURE 21. *Diacyclops hancocki* sp. nov., scanning electron micrographs, paratype male 2, ventral: A, habitus; B, anal somite and caudal rami; C, antennula; D, labrum; E, first swimming leg; F, fifth and sixth legs.

***Diacyclops tomlinsonae* sp. nov.**

(Figs. 1E, 22–25)

urn:lsid:zoobank.org:act:FB1207A7-C319-489E-AA46-D6F85B4D0AEB

Type locality. Australia, New South Wales, Tamworth, bore 93024, sample no. 7T27P100-200, 31.26435°S 151.20272°E, 17 July 2007, collected by M. Tomlinson.

Holotype. Adult female dissected on 1 microscope slide.

Paratypes. One male, 1 female, and 1 copepodid from type locality dissected on 1 microscope slide each; 3 males and 3 females from type locality on 1 SEM stub (row no. 3), together with 5 other species described here; 16 males, 5 females, and 4 copepodids from type locality in 1 alcohol vial; 10 males, 3 females, and 1 copepodid

from type locality in 1 alcohol vial; 1 female from type locality, but collected on 30 January 2007 by M. Tomlinson, dissected on 1 microscope slide; 1 male and 3 females from type locality, but collected on 30 January 2007 by M. Tomlinson, in 1 alcohol vial.

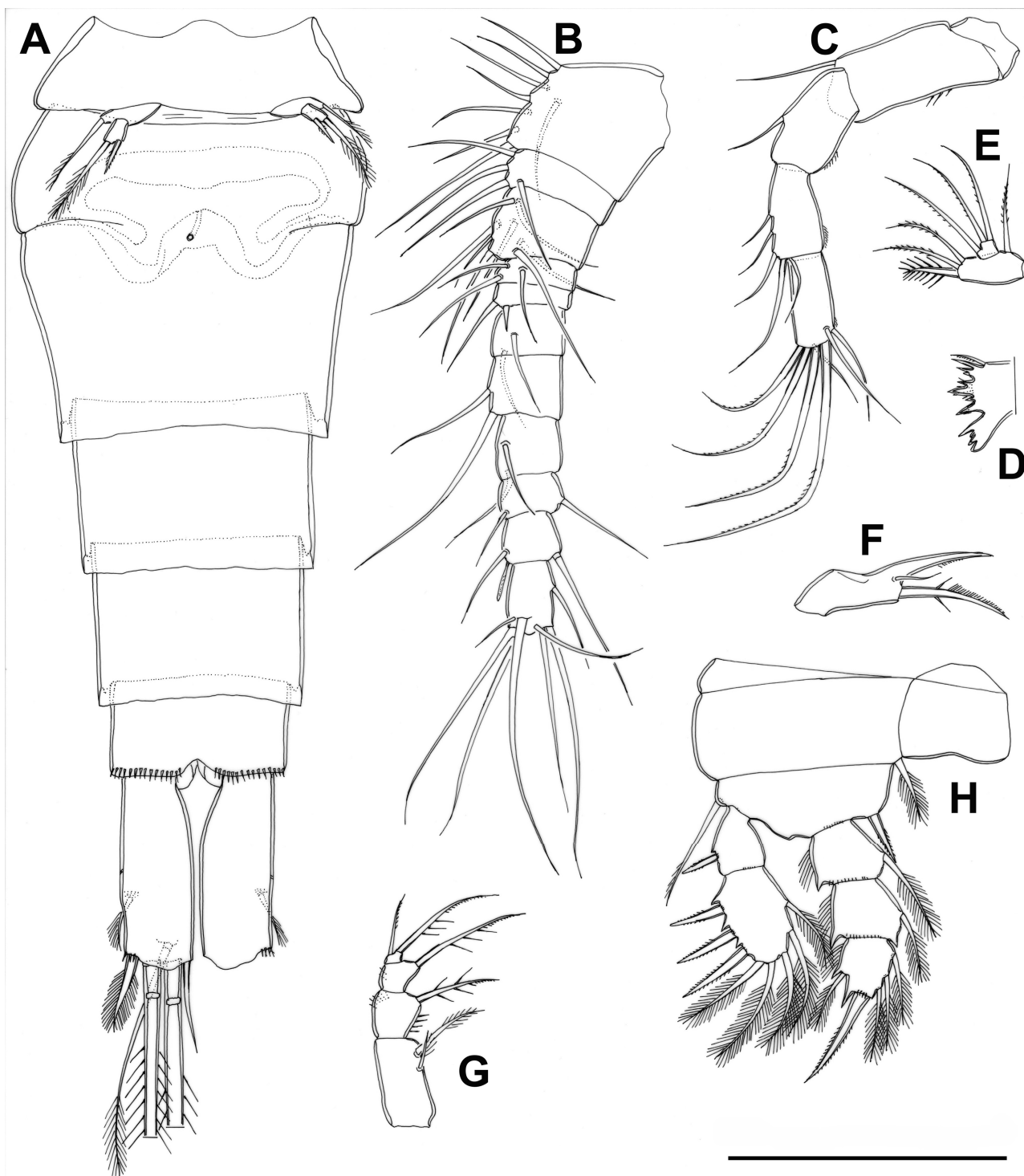


FIGURE 22. *Diacyclops tomlinsonae* sp. nov., line drawings, holotype female: A, urosome, ventral; B, antennula; C, antenna; D, cutting edge of mandibula; E, maxillilar palp; F, basis of maxilla; G, maxilliped; H, first swimming leg. Scale bar 50 μ m.

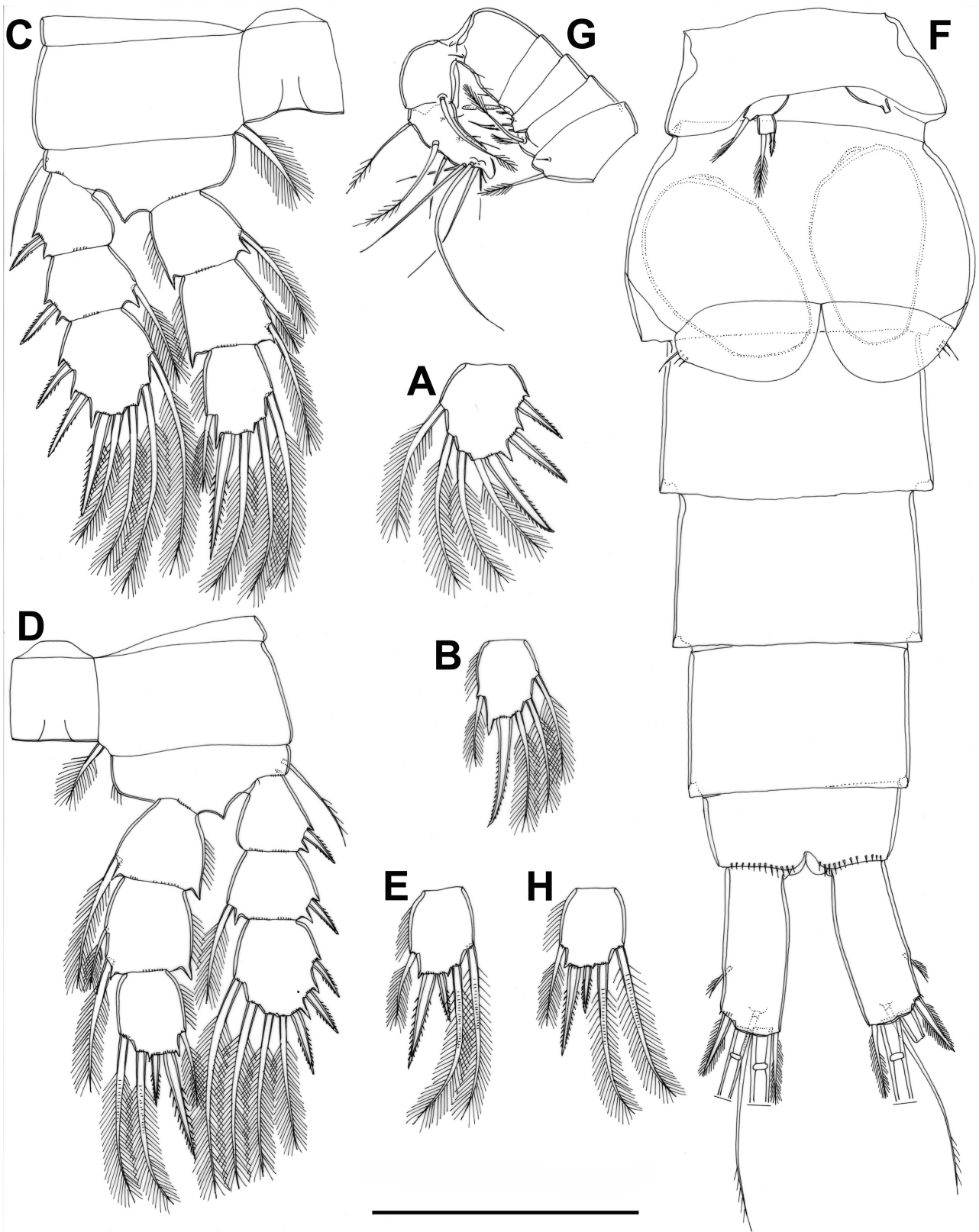


FIGURE 23. *Diacyclops tomlinsonae* sp. nov., line drawings, A–D, holotype female; E, paratype female 1; F–H, paratype male 1: A, third exopodal segment of second swimming leg; B, third endopodal segment of second swimming leg; C, third swimming leg; D, fourth swimming leg; E, third endopodal segment of fourth swimming leg; F, urosome, ventral; G, distal part of antennula; H, third endopodal segment of fourth swimming leg. Scale bar 50 μ m.

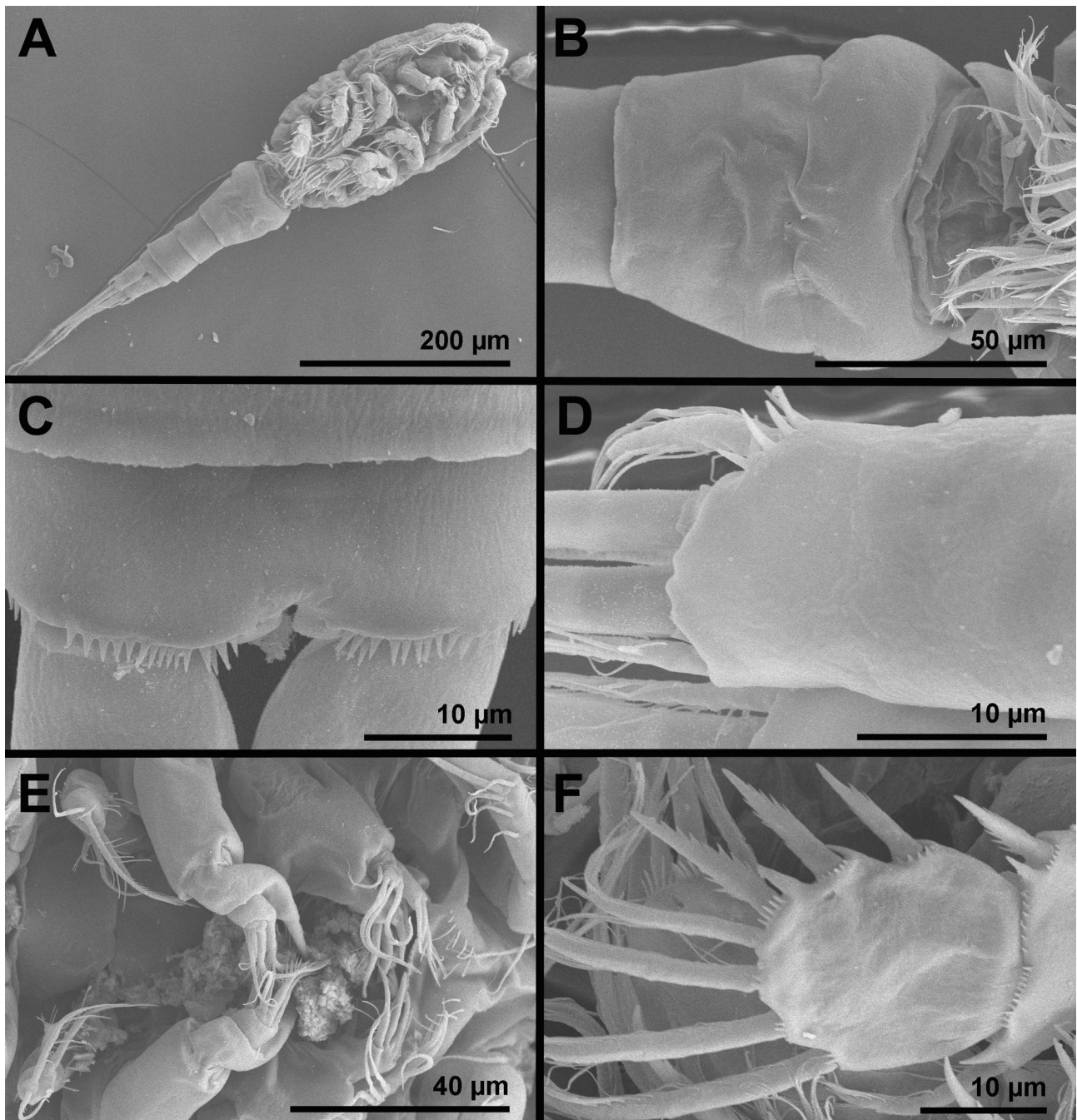


FIGURE 24. *Diacyclops tomlinsonae* sp. nov., scanning electron micrographs, paratype female 2, ventral: A, habitus; B, genital double-somite; C, anal somite and anterior part of caudal rami; D, posterior part of right caudal ramus; E, mouth appendages; F, third exopodal segment of fourth swimming leg.

Etymology. The species is named after Dr. Moya Tomlinson, Australian Rivers Institute, Griffith University, Nathan, who collected this species and entrusted it to me for identification. The name is a noun in the genitive singular.

Diagnosis. Female. Body length from 355 to 420 µm. Habitus (Figs. 1E, 24A) slender, more than 3.1 times as long as wide, with prosome/urosome ratio of about 1.35, and cephalothorax 1.83 times as wide as genital double-somite in dorsal view. Integument on all somites (Fig. 24A, B, C) thin and smooth, with almost no bacterial cover; general distribution of spinules and cuticular pores on somites as in *D. leijsi*. Hyaline fringes of prosomites (Fig. 24A) and urosomites (Figs. 22A, 24B, C) smooth. Genital double-somite (Figs. 22A, 24B) about as long as wide, widest at first quarter of its length and gradually tapering posteriorly, widest part about 1.5 times as wide as posterior margin;

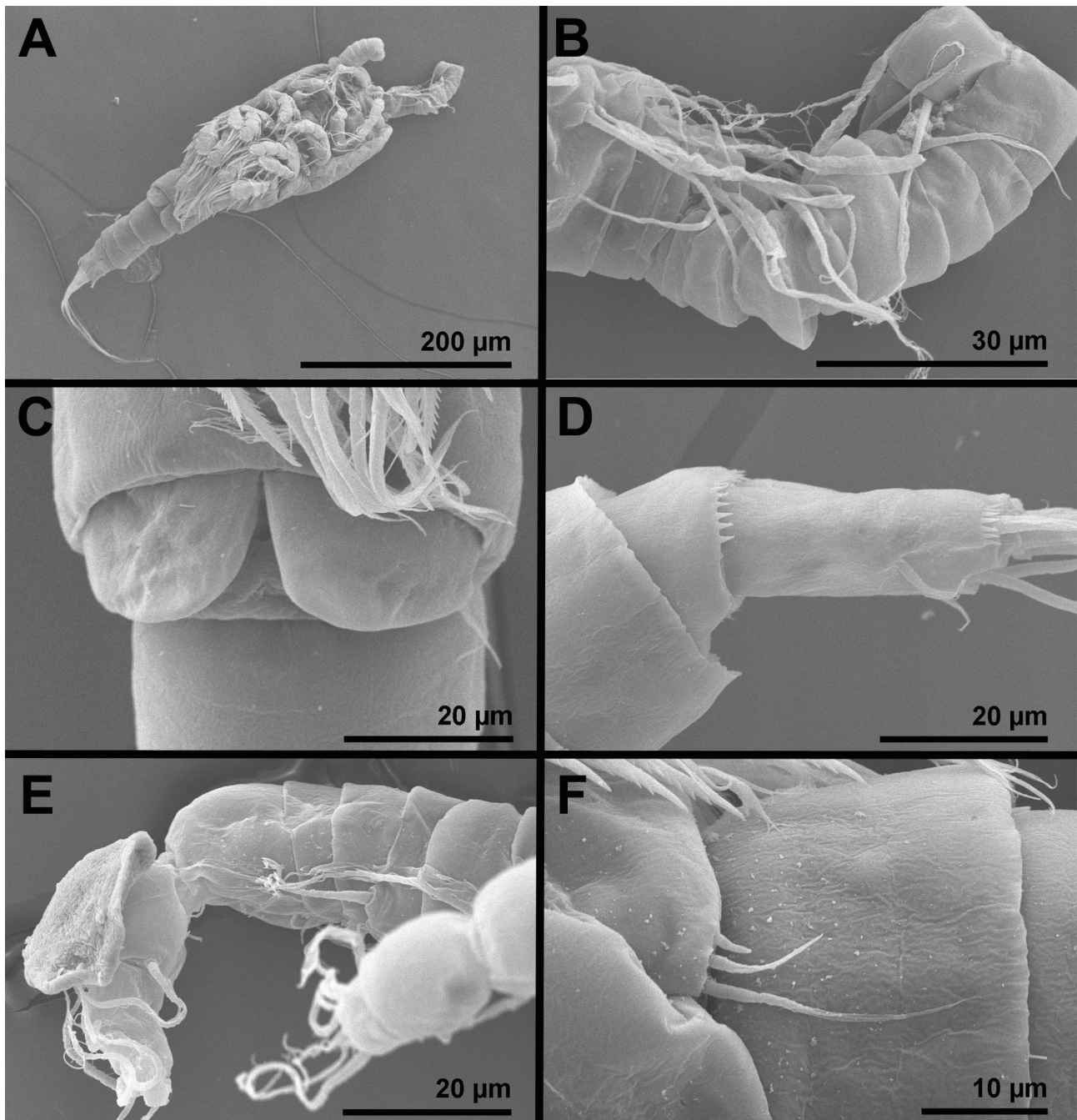


FIGURE 25. *Diacyclops tomlinsonae* sp. nov., scanning electron micrographs, A–C, paratype male 2, ventral; D–F, paratype male 3, lateral: A, habitus; B, antennula; C, sixth legs; D, last two urosomites and caudal rami; E, antennula; F, sixth leg.

seminal receptacle small and butterfly-shaped, posterior part greatly reduced; copulatory pore small and located at about 2 fifths of somite length; copulatory duct narrow, long, and weakly sclerotized. Anal somite (Figs. 22A, 24C) with large spinules along ventral and lateral margins. Caudal rami (Figs. 22A, 24D) of medium length and stout, narrowly spaced, about 2.75 times as long as wide and about twice as long as anal somite; principal terminal setae with breaking planes, inner one about 0.9 times as long as entire urosome and 1.45 times as long as outer one; dorsal seta about 1.2 times as long as caudal ramus, 2.6 times as long as innermost terminal seta, and 3 times as long as outermost terminal seta. Antennula (Fig. 22B) 11-segmented, half as long as cephalothorax, with single aesthetasc on penultimate segment and setae formula 8.4.8.3.2.2.2.2.2.8; ultimate segment about 1.4 times as long as wide. Antenna (Figs. 22C, 24E) 5-segmented, without exopodal seta, with setae formula 0.1.1.5.7; second segment about 1.6 times as long as fifth segment. Labrum (Fig. 24E) with 2 diagonal rows of 8 slender spinules each on anterior

surface; cutting edge slightly concave, with 16 sharp teeth between blunt and smooth lateral corners. Mandibula (Fig. 22D) similar to that in *D. leijsi*, but with much shorter dorsalmost seta on cutting edge. Maxillula (Figs. 22E, 24E) also similar to that in *D. leijsi*, but with shorter and slightly inflated coxobasis. Maxilla (Figs. 22F, 24E) similar to that in *D. leijsi*, but with shorter basis; basal claw shorter than strong basal seta. Maxilliped (Figs. 22G, 24E) small, slender, with setae formula 2.1.1.2. Shape and segmentation of swimming legs (Figs. 22H, 23A, B, C, D, E; 24F) as in *D. leijsi*, except exopod of first leg 2-segmented and all legs much shorter; basis of first leg with long outer seta and short inner spine, latter slightly shorter than first endopodal segment; basis of second to fourth legs with slightly shorter outer seta and inner distal corner blunt; all first exopodal segments without inner seta; second exopodal segment of second to fourth legs, all first endopodal segments, and second endopodal segment of first and second legs with single inner seta; second endopodal segment of third and fourth legs with 2 inner setae; ultimate exopodal segments spine formula 3.3.3.3 and setae formula 5.4.4.4; third endopodal segments of first to third leg with 2 inner setae, 1 subapical seta, 1 apical spine, and 1 outer seta; third endopodal segment of fourth leg 1.2 times as long as wide, with 2 inner setae, 2 apical spines, and 1 outer seta; its outer spine about 1.3 times as long as segment and slightly more than twice as long as inner spine. Fifth leg (Fig. 22A) shape and segmentation as in *D. leijsi*, but second segment only 1.4 times as long as wide and about 0.7 times as long as its spine. Sixth leg minute, but with two well-articulated spines; outermost seta half as long as plate width and about 4 times as long as spines.

Male. Body length from 330 to 370 μm . Habitus (Fig. 25A) and urosome (Fig. 23F) slightly slenderer than in female; free genital somite (Fig. 23F) less than 1.3 times as wide as subsequent urosomite, with large ovoid spermatophores filling almost entire somite. Ornamentation of cephalothorax, free prosomites, and last 3 urosomites (Fig. 23F, 25C, D, F) as in female. Caudal rami (Figs. 23F, 25D) slightly slenderer than in female, but proportion of setae without any significant difference. Antennula (Figs. 23G, 25B, E) segmentation and armature same as in *D. hancocki*, but all segments much shorter; penultimate segment only slightly longer than wide. Antenna, labrum, mandibula, maxillula, maxilla, maxilliped, all swimming legs (Fig. 23H), and fifth leg (Fig. 23F) as in female. Sixth leg (Figs. 23F, 25C, F) almost perfectly semicircular, outermost seta about 2.2 times as long as central seta, which in turn about 3.3 times as long as minute innermost spine.

Variability. One paratype female from bore 93024, collected on 30 January 2007, had a slightly shorter outer apical spine on the third endopodal segment of the fourth swimming leg (Fig. 23E).

***Diacyclops huntervalleyensis* sp. nov.**

(Figs. 1F, 26, 27)

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Type locality. Australia, New South Wales, Hunter Valley, bore 80071, sample no. 2HD45, 32.006601°S 150.86074°E, 5 July 2005, collected by P. Hancock.

Holotype. Adult female dissected on one microscope slide.

Paratypes. One male and 2 females from type locality dissected on 1 microscope slide each; 5 males, 10 females, and 2 copepodids from type locality in 1 alcohol vial; 1 male and 3 females from bore 80439, Hunter Valley, New South Wales, 32.04921°S 150.94307°E, sample no. 2HD52, 6 July 2005, collected by P. Hancock, on 1 SEM stub (row no. 2), together with five other species described here; 8 males, 13 females, and 2 copepodids from bore 80439 (see above) in 1 alcohol vial; 72 males, 104 females, and 61 copepodids from bore 80439 (see above), but collected on 3 April 2005 by P. hancock, in 1 alcohol vial.

Etymology. The species name refers to its type locality, Hunter Valley, which was named after Hunter River, which was in turn named in 1797 after Captain John Hunter, who was at that time Governor of the British colony in New South Wales. It is an adjective for place, made with the Latin suffix “-ensis”.

Diagnosis. Female. Body length from 340 to 360 μm . Habitus (Figs. 1F, 27A) slender, 3.4 times as long as wide, with prosome/urosome ratio of 1.2, and cephalothorax twice as wide as genital double-somite in dorsal view. Integument on all somites (Fig. 27A, C, F) thin and smooth, with light bacterial cover; general distribution of spinules and cuticular pores on somites as in *D. leijsi*. Hyaline fringes of prosomites (Fig. 27A) and urosomites (Fig. 26A) smooth. Genital double-somite (Figs. 26A, 27A) slightly wider than long in ventral view, widest at first third of its length and gradually tapering posteriorly, widest part about 1.5 times as wide as posterior margin; seminal receptacle smallest of all species described here, almost without posterior part, in shape most similar to that in *D. tomlinsonae*; copulatory pore small and located at about 2 fifths of somite length; copulatory duct wide, long, and

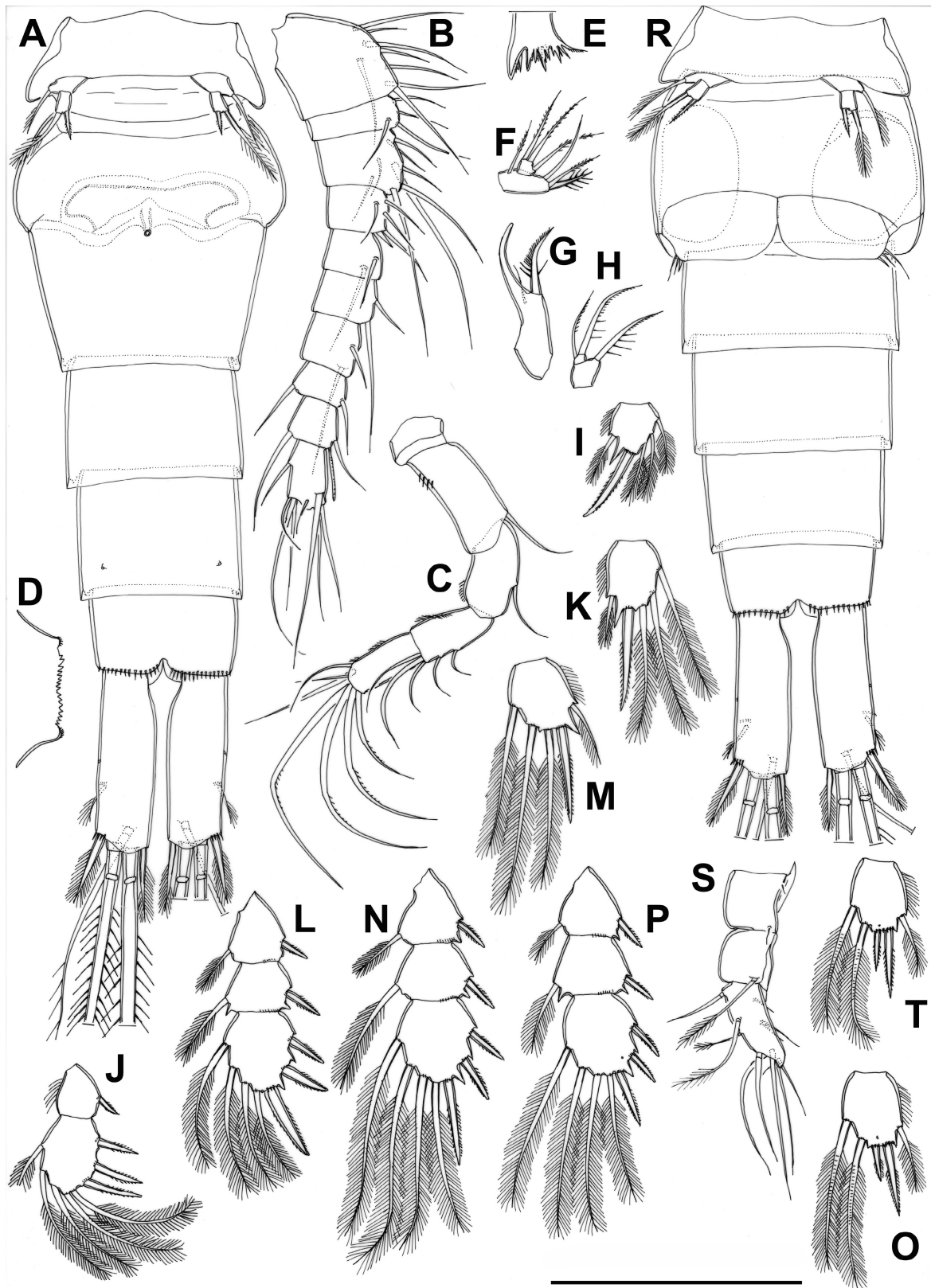


FIGURE 26. *Diacyclops huntervalleyensis* sp. nov., line drawings, A–P, holotype female; R–T, paratype male 1: A, urosome, ventral; B, antennula; C, antenna; D, labrum; E, cutting edge of mandibula; F, maxillilar palp; G, basis of maxilla; H, distal part of maxilliped; I, third endopodal segment of first swimming leg; J, exopod of first swimming leg; K, third endopodal segment of second swimming leg; L, exopod of second swimming leg; M, third endopodal segment of third swimming leg; N, exopod of third swimming leg; O, third endopodal segment of fourth swimming leg; P, exopod of fourth swimming leg; R, urosome, ventral; S, distal part of antennula; T, third endopodal segment of fourth swimming leg. Scale bar 50 μ m.

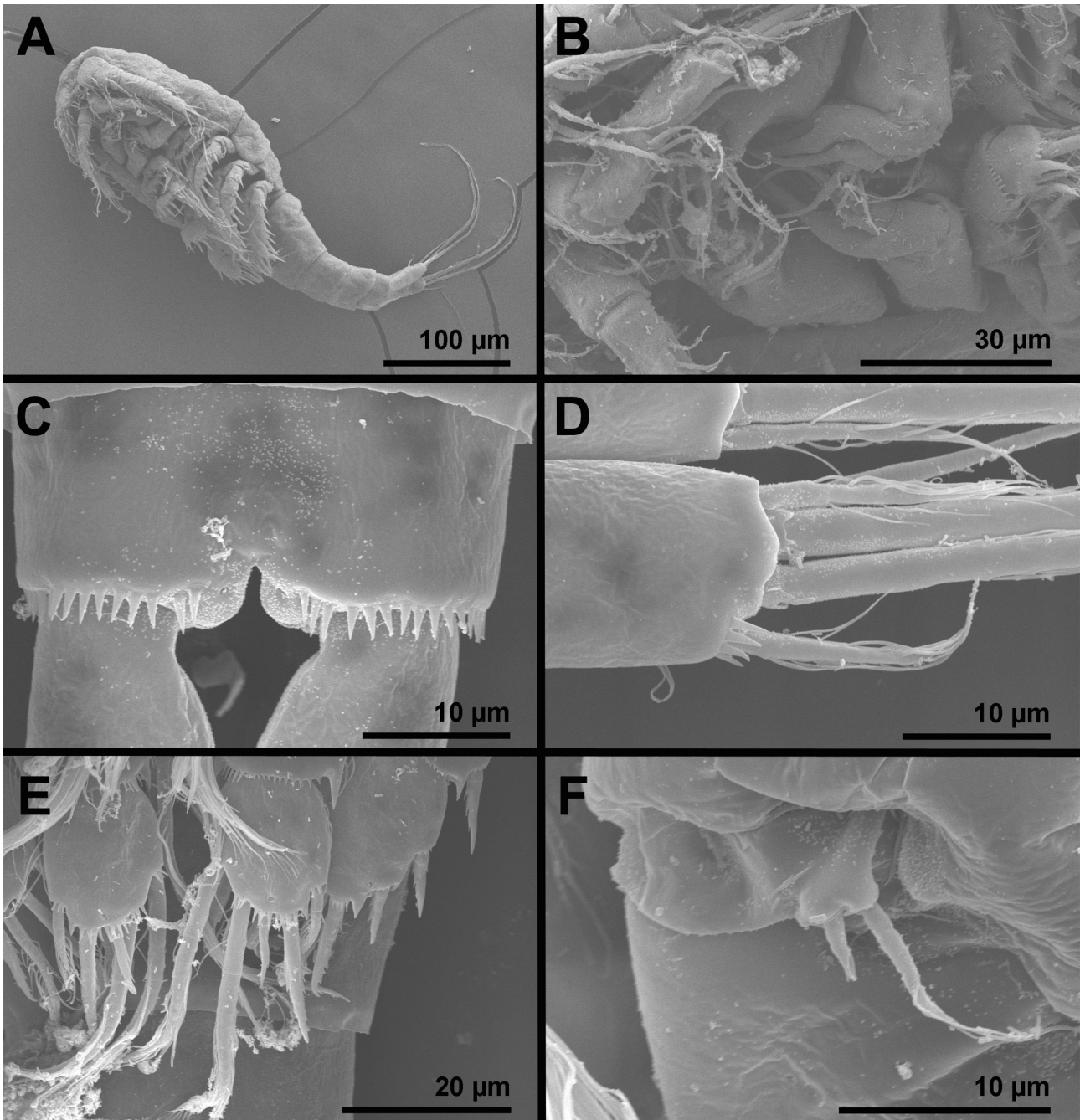


FIGURE 27. *Diacyclops huntervalleyensis* sp. nov., scanning electron micrographs, A & B, paratype female 1, ventral; C–E, paratype female 2, ventral; F, paratype female 3, lateral: A, habitus; B, mouth parts; C, anal somite and anterior part of caudal rami; D, posterior part of caudal rami; E, distal part of fourth swimming leg; G, sixth leg.

well-sclerotized. Anal somite (Figs. 26A, 27C) with large spinules along ventral and lateral margins. Caudal rami (Figs. 26A, 27D) of medium length and stout, narrowly spaced, about 3 times as long as wide and about twice as long as anal somite; principal terminal setae with breaking planes, inner one about 0.7 times as long as entire urosome and 1.35 times as long as outer one; dorsal seta about 1.25 times as long as caudal ramus, 3.1 times as long as innermost terminal seta, and 3.4 times as long as outermost terminal seta. Antennula (Fig. 26B) 10-segmented, half as long as cephalothorax, with single aesthetasc on penultimate segment and setae formula 8.4.8.5.2.2.2.2.2.8; ultimate segment about 1.3 times as long as wide. Antenna (Figs. 26C, 27B) 5-segmented, without exopodal seta, with setae formula 0.1.1.5.7; second segment about 1.6 times as long as fifth segment. Labrum (Fig. 24E) with 2 diagonal rows of 8 slender spinules each on anterior surface and row of minute spinules on blunt lateral corners;

cutting edge slightly concave, with 15 sharp teeth. Mandibula (Fig. 26E), maxillula (Figs. 26F, 27B), maxilla (Figs. 26G, 27B), and maxilliped (Figs. 26H, 27B) as in *D. tomlinsonae*. Shape, segmentation, and armature of swimming legs (Figs. 26I, J, K, L, M, N, O, P, 27B, E) as in *D. tomlinsonae*, except first exopodal segment of second to fourth legs with inner seta; third endopodal segment of fourth leg 1.3 times as long as wide; its outer spine about as long as segment and twice as long as inner spine. Fifth leg (Fig. 26A) and sixth leg (Fig. 27F) as in *D. tomlinsonae*.

Male. Body length from 330 to 355 μm . Habitus and urosome (Fig. 26R) slightly slenderer than in female; free genital somite (Fig. 26R) less than 1.3 times as wide as subsequent urosomite, with small ovoid spermatophores. Ornamentation of cephalothorax, free prosomites, and last 3 urosomites as in female. Caudal rami (Fig. 26R) slightly slenderer than in female, but proportion of setae without any significant difference. Antennula (Fig. 26S) segmentation, armature, and proportions as in *D. tomlinsonae*; penultimate segment about as long as wide. Antenna, labrum, mandibula, maxillula, maxilla, maxilliped, all swimming legs (Fig. 26T), and fifth leg (Fig. 26R) as in female. Sixth leg (Fig. 26R) similar to that in *D. tomlinsonae*, but cuticular plate shorter and with nearly straight posterior margin.

Cladistics

Despite numerous morphological differences between the six newly described species, many characters could not be used for a cladistic analysis because of their autapomorphic nature among Australian congeners. The following 21 informative characters were selected, with character states in parentheses (note: NONA requires characters to start from zero, rather than one):

0. Caudal rami, principal terminal setae, breaking planes: present (0); absent (1).
1. Caudal rami, dorsal seta: shorter than innermost seta (0); longer than innermost seta, but shorter than outer principal terminal seta (1); as long as outer principal terminal seta (2).
2. Seminal receptacle, posterior part: present (0); reduced (1).
3. Antennula: 17-segmented (0); segments 8–11 and 12–14 fused (1).
4. Antennula, segments 3 & 4: articulated (0); fused (1).
5. Antenna, exopodal seta: present (0); absent (1).
6. Antenna, number of inner basal setae: 2 (0); 1 (1).
7. Antenna, second endopodal segment, number of setae: 7 or more (0); 6 (1); 5 (2).
8. Maxilliped, first segment, number of setae: 3 (0); 2 (1); 1 (2).
9. Maxilliped, second segment, number of setae: 2 (0); 1 (1).
10. Maxilliped, fourth segment, number of setae: 3 (0); 2 (1).
11. First swimming leg, basis, inner spine: about as long as first endopodal segment (0); as long as or longer than first two endopodal segments combined (1).
12. First swimming leg, exopod: 3-segmented (0); 2-segmented (1).
13. First swimming leg, first exopodal segment, inner seta: present (0); absent (1).
14. First swimming leg, third endopodal segment, number of setae: 5 (0); 4 (1).
15. Second and third swimming legs, first exopodal segment, inner seta: present (0); absent (1).
16. Second and third swimming legs, third endopodal segment, number of setae: 5 (0); 4 (1).
17. Third swimming leg, second endopodal segment, number of setae: 2 (0); 1 (1).
18. Fourth swimming leg, first exopodal segment, inner seta: present (0); absent (1).
19. Fourth swimming leg, third endopodal segment, number of spines: 2 (0); 1 (1).
20. Fourth swimming leg, third endopodal segment: outer spine longer than inner spine (0); outer spine shorter than inner spine (1).

TABLE 1. Character states scored for all 16 Australian representatives of the genus *Diacyclops*. Dashes denote missing characters in two taxa. NONA requires characters to be numbered starting from zero. See text for the explanation of characters and character states.

Taxa \ Characters	0	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	20
<i>D. ballaballaensis</i>	1	2	0	1	0	1	0	2	2	0	1	0	0	1	1	1	1	1	1	1	-
<i>D. bicuspidatus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>D. bisetosus</i>	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
<i>D. cockingi</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0
<i>D. cryonastes</i>	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
<i>D. eberhardi</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0
<i>D. einslei</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0
<i>D. h. humphreysi</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0
<i>D. h. unispinosus</i>	0	1	0	1	0	1	0	1	1	0	0	0	0	1	1	1	1	1	1	1	-
<i>D. hancocki</i>	0	1	0	1	1	0	0	0	1	1	1	1	0	1	1	0	0	1	1	0	1
<i>D. huntervalleyensis</i>	0	1	1	1	1	1	1	2	1	1	1	0	1	1	1	0	1	0	0	0	0
<i>D. leijsi</i>	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>D. reidae</i>	0	1	0	1	0	1	0	1	1	0	0	0	0	0	1	0	1	0	0	0	0
<i>D. scanloni</i>	0	2	0	1	0	1	0	1	1	0	0	1	0	0	1	0	1	1	0	0	0
<i>D. sobeprolatus</i>	0	2	0	1	0	1	0	1	0	0	0	0	0	1	1	1	1	1	1	0	0
<i>D. tomlinsonae</i>	0	1	1	1	1	1	1	2	1	1	1	0	1	1	1	1	1	0	1	0	0

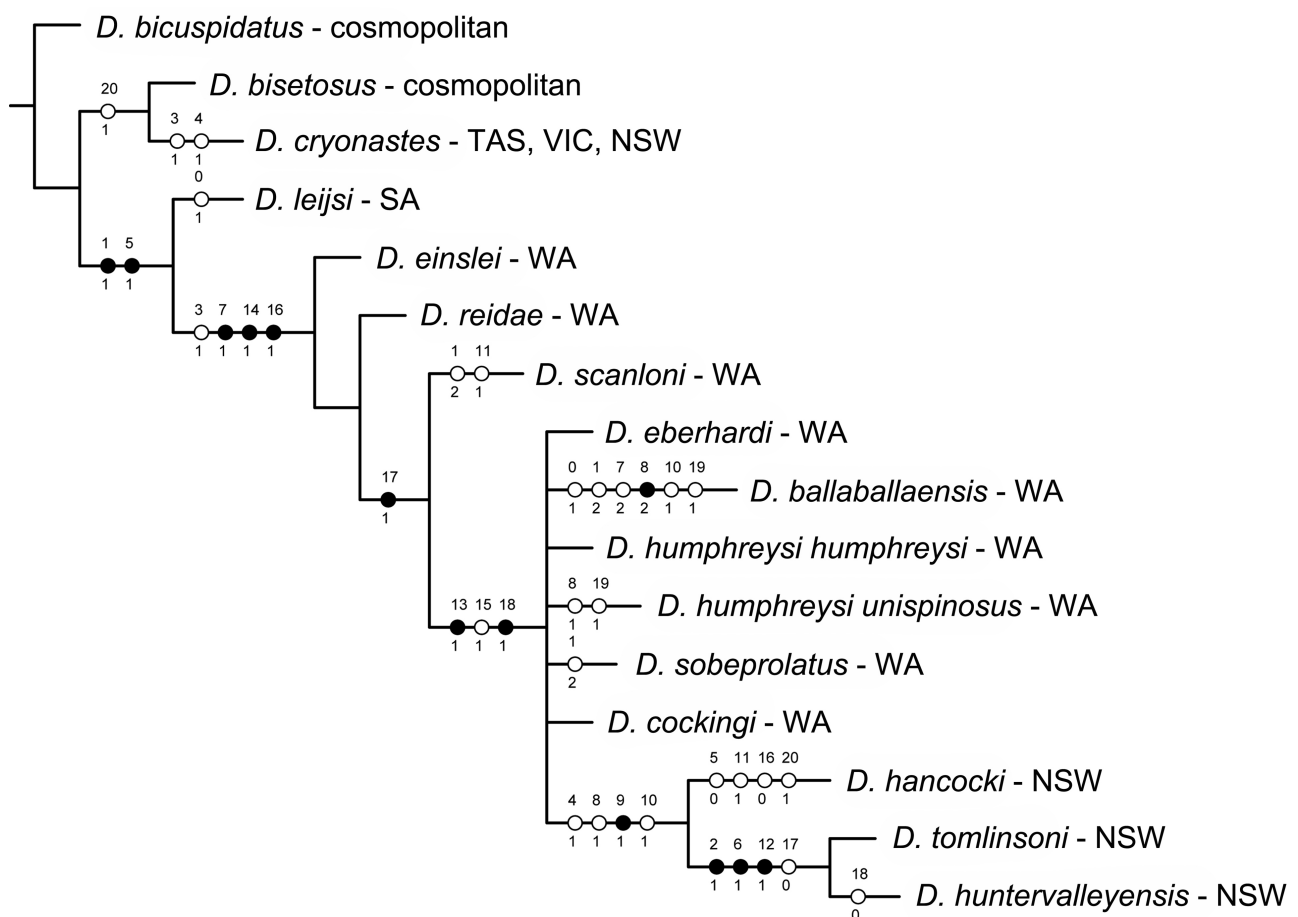


FIGURE 28. Strict consensus of three equally parsimonious cladograms resulting from an analysis of 21 morphological characters (Table 1), scored for 16 Australian representatives of the genus *Diacyclops*. The tree was constructed using Winclada/ NONA and heuristic search method, with default parameters and all characters equally weighted. Full circles represent presumed synapomorphies, empty circles presumed plesiomorphies or homoplasies, numbers above circles characters, and numbers below circles character states. Standard ISO abbreviations for Australian states are used to denote distributions of species: NSW, New South Wales; SA, South Australia; TAS, Tasmania; VIC, Victoria; WA, Western Australia.

A character matrix was constructed (Table 1) to contain all character states for all species, except for the last character that could not be scored for two taxa, because they have a single spine on the third endopodal segment of the fourth leg. A cladistic analysis with all characters unweighted resulted in three equally parsimonious trees (not shown), with a length of 42 steps, a consistency index (Ci) of 0.56, and a retention index (Ri) of 0.75. Their consensus (Fig. 28) resulted in five collapsed nodes, was four steps longer, had a Ci of 0.52, and an Ri of 0.69. The analysis suggests that the group of species from eastern Australia with 11-segmented (*D. cryonastes*, *D. hancocki*, *D. tomlinsonae*) or 10-segmented (*D. huntervalleyensis*) antennulae is polyphyletic, although the three new subterranean species from New South Wales appear to be more closely related than their widely different habitus, antenna, and swimming legs would suggest intuitively. It is apparent that *D. tomlinsonae* and *D. huntervalleyensis* are sister species, and it is also worth noting that they are allopatric. Contrary to previous evidence from limited molecular studies, the Western Australian endemics are not polyphyletic; in this cladistic analysis the *alticola*-group appears to be paraphyletic. It is also worth noting that *D. eberhardi* shares all 21 scored characters with *D. h. humphreysi* and *D. cockingi*, despite their highly disjunct distributions. The only South Australian endemic (*D. leijsi*) has a basal and rather isolated position among Australian congeners. Most of the highly unusual characters of *D. ballaballaensis* among other members of the *alticola*-group in the Pilbara region seem to be homoplasies.

Discussion

Affinities of the new species to Australian congeners. All six new species could be easily distinguished from each other even before any dissection, by a combination of habitus shape, caudal rami shape and armature, and segmentation of the antennula (Fig. 1). They also all have a different armature formula of the swimming legs (Figs. 4, 9, 14, 18, 22, 23, 24), the smallest difference being that between *D. eberhardi* and *D. ballaballaensis* (one or two apical spines on the fourth leg endopod), and then between *D. tomlinsonae* and *D. huntervalleyensis* (first exopodal segment of second to fourth legs with or without inner seta). All new species, except *D. tomlinsonae* and *D. huntervalleyensis*, also differ in the armature formula of the antenna. As can be seen from the cladistic analysis (Fig. 28) the latter two are more closely related to each other than to any other Australian congener, and they are the only Australian species with a two-segmented exopod of the first leg. They share additionally the shape of habitus, seminal receptacle, and caudal rami (although these are slightly slenderer in *D. huntervalleyensis*), but can be easily distinguished by the segmentation of antennula (11-segmented in *D. tomlinsonae*, 10-segmented in *D. huntervalleyensis*) and the abovementioned armature of the first exopodal segment of second to fourth legs. These two species share the armature formula of the maxilliped with the third new species from New South Wales, *D. hancocki*, but the latter has the third exopodal segment of the second and third legs with four spines and five setae, which is an autapomorphy among Australian congeners (and therefore not used in the cladistic analysis), and also a very plesiomorphic armature of the antenna. Therefore *D. hancocki* seems to be only distantly related to the other two species. It is also worth noting that *D. tomlinsonae* and *D. huntervalleyensis* are allopatric, while *D. hancocki* and *D. tomlinsonae* are sympatric. Life in sympatry is presumably much easier when congeners are only distantly related, and it is a pattern commonly observed among Australian subterranean copepods (Karanovic & Cooper 2011; 2012). As suggested by the phylogenetic analysis above (Fig. 28), the South Australian *D. leijsi* has no close relatives among Australian congeners; it shares most morphological characters with the two cosmopolitan representatives (*D. bicuspidatus* and *D. bisetosus*), except for the absence of exopodal seta on the antenna, absence of breaking planes on the principal terminal caudal setae, much longer dorsal caudal seta, and some smaller differences in proportions of segments and armature. Both Western Australian new species (*D. eberhardi* and *D. ballaballaensis*) fit comfortably into the *alticola*-group, as defined in Karanovic (2006), even though they differ markedly in their habitus shape (Fig. 1B, C), caudal rami shape and armature (Figs. 8A, 13A), armature of the antenna (Figs. 8C, 13C), armature of the maxillula (Figs. 8E, 13F), armature of the third endopodal segment of the fourth leg (Figs. 9E, 14E), length of the subapical spine on the fifth leg (Fig. 9F, 14F), and proportions of many segments and armature elements. Most of these differences, however, are oddities of *D. ballaballaensis*, which also include principal caudal setae without breaking planes and outer principal caudal seta extremely reduced. The latter is an autapomorphy of this species among Australian congeners (Fig. 13A). On the other hand, *D. eberhardi* is so similar to other Australian members of the *alticola*-group, that it has all 21 characters scored in the cladistic analysis exactly the same as in *D. h.*

humphreysi and *D. cockingi* (Table 1). It differs from both by a larger and differently shaped seminal receptacle, and from *D. cockingi* additionally by shorter innermost terminal caudal setae. Additional small differences between *D. eberhardi* and *D. h. humphreysi* include proportions of several segments, and they are surprisingly small considering that these taxa are separated by nearly 2000 km of land with rich fauna of subterranean copepods (Karanovic 2004), but without any *Diacyclops* representatives. These disjunct distributions are probably a consequence of the aridification of the Australian continent, as was shown for other copepods (Karanovic 2006; Karanovic & Tang 2009) and various plants and animals (Martin 2006; Owen *et al.* 2017). Even though Karanovic & Krajicek (2012b) postulated that the *alticola*-group could be polyphyletic, it should be noted that their conclusion was based on single gene analyses and that their clades had a relatively low support. Hopefully in the future a wider taxon and marker sampling will shed more light on the phylogeny of the Western Australian *Diacyclops*.

Affinities of the new species to other members of *Diacyclops*. As mentioned above both *D. eberhardi* and *D. ballaballaensis* belong to the *alticola*-group of species (Karanovic 2006), which besides the nine Western Australian species and subspecies additionally includes only *D. alticola* Kiefer, 1935 and *D. longifurcus* Shen and Sung, 1963, from India and China respectively (see Shen *et al.* 1979). Both Asian species could be easily distinguished from all Australian members of this group by much longer caudal rami, but their descriptions are not detailed enough to allow further comparisons. Extremely reduced outer principal caudal setae, as in *D. ballaballaensis*, could be found only in the French subterranean *Kieferiella delamarei* (Lescher-Moutoué, 1971), which shows some connections with the genera *Diacyclops* and *Acanthocyclops* (see Lescher-Moutoué 1971, 1976). This species, however, differs from *D. ballaballaensis* by many morphological characters, including a 17-segmented antennula, so the reduction of the caudal seta has to be convergent. The Antarctic *D. walkeri* Karanovic, Gibson, Hawes, Andersen & Stevens, 2014 has somewhat reduced outer principal caudal setae, but not to the same extent as in the former two species (see Karanovic *et al.*, 2014). Another rare feature of *D. ballaballaensis* is a single apical spine on the third endopodal segment of the fourth leg, which it shares with another Western Australian member of the *alticola*-group: *D. humphreysi unispinosus* (see Karanovic 2006). This character was so far reported in the genus as a species character for the North American *D. albus* Reid, 1991, and only as an anomaly for the Antarctic *D. walkeri* and the Majorcan *D. balearicus* Gourbault & Lescher-Moutoué, 1979 (see Reid 1991; Gourbault & Lescher-Moutoué 1979; Karanovic *et al.* 2014). The former two are members of the *languidoides*-group (see Karanovic 2006), while the latter is a member of the *michaelseni*-group (see Morton 1985; Karanovic *et al.* 2014), and therefore only remotely related to the members of the *alticola*-group. The cladistic analysis above suggests that this character is homoplastic.

Diacyclops leijsi belongs to the *bicuspidatus*-group, based on the 17-segmented antennula, all swimming legs with 3-segmented exopods and endopods, spine formula of the third exopodal segment of the swimming legs 2.3.3.3, and the third endopodal segment of the fourth leg with longer outer apical spine. However, all members of this group have two setae on the second endopodal segment of the first and second swimming legs (see Monchenko 1974; Kiefer, 1978; Stoch 1987; Chang 2009; Suárez-Morales *et al.* 2013), so it seems that *D. leijsi* has no close relatives in the genus as a whole and not just among Australian congeners.

Diacyclops hancocki differs from all congeners by the spine formula of the third exopodal segment of the swimming legs (3.4.4.3). Only three species have been described so far in this genus with four spines on the second and third legs: *D. andinus* Locascio de Mitrovich & Menu-Marque, 2001 from Argentina; *D. salisae* Reid, 2004 from Indiana; and *D. nikolasarburni* Suárez-Morales, Mercado-Salas & Barlow, 2013 from New Mexico. However, all these species also have four spines on the fourth leg and differ from *D. hancocki* by many other characters (Locascio de Mitrovich & Menu-Marque 2001; Reid 2004; Suárez-Morales *et al.* 2013). Interestingly, the latter authors reported the spine formula for *D. nikolasarburni* as very variable, and including ten different permutations, but never 3.4.4.3. However, this exact formula is prevalent in a number of completely unrelated cyclopoid genera, such as *Halicyclops* Norman, 1903; *Macrocyclops* Claus, 1893; *Eucyclops*; *Tropocyclops* Kiefer, 1927; *Paracyclops* Claus, 1893; *Ectocyclops*; etc. (see Monchenko 1974). Obviously, it is a plesiomorphic character that tells us nothing about the phylogenetic relationships of *D. hancocki* to other congeners. Three of the four characters that support its sister relationship with *D. tomlinsonae* and *D. huntervalleyensis* are either plesiomorphies or homoplasies (Fig. 28), so it seems that they are also only distantly related. It could be that *D. hancocki* is an Australian relic from the time of the origin of the genus *Diacyclops*.

Diacyclops tomlinsonae and *D. huntervalleyensis* also have a unique position in the genus, and differ from all known members by the segmentation of the swimming legs (with exopod of the first leg two-segmented and all other exopods and endopods three-segmented). This is surprising, given the wide range of swimming legs

segmentation in this genus (Monchenko & Von Vaupel Klein, 1999). In fact, this segmentation has been reported so far only once in the entire family: in the endemic and monospecific Western Australian genus *Orbuscyclops* Karanovic, 2006. They additionally share the armature of the antenna, but differ in the fifth leg, antennula, and armature formula of the swimming legs (Karanovic 2006). Perhaps the two subterranean species from New South Wales warrant a separate generic status, as did two genera from New Zealand with a unique segmentation of the swimming legs that could have fit under a broad *Diacyclops* umbrella (see Karanovic 2005). However, I refrain from formally erecting a new genus for these two species, because it might be beneficial to test their relationships with the genus *Orbuscyclops* and Australian members of *Diacyclops* with molecular markers first. *Diacyclops huntervalleyensis* has a ten-segmented antennula, which is also a rare character in the genus, reported so far only for *D. abyssicola* (Lilljeborg, 1901) (see Monchenko & Von Vaupel Klein 1999). However, the latter is a member of the *languidoides*-group, and therefore only remotely related. As mentioned elsewhere (Karanovic 2005), this group also probably warrants a separate generic status.

Zoogeographic connections of the Australian *Diacyclops*. Two nearly cosmopolitan species that are present in Australia (*D. bicuspidatus* and *D. bisetosus*) are probably introduced by anthropogenic activities, as was shown for some other cosmopolitan cyclopoids that live in disturbed surface-water habitats here (Karanovic & Krajicek 2012a). *Diacyclops leijsi* seems to be endemic to South Australian mound springs, and without any close relatives. Most characters it shares with the cosmopolitan *D. bicuspidatus* are plesiomorphies and therefore without value for estimating phylogenetic relationships. The only other Australian congener that lives in surface waters (*D. cryonastes*) is a member of the circum-Antarctic *michaelseni*-group (sensu Borutzky & Vinogradov 1957; Morton 1985), which additionally includes four species from Antarctica, one from Tierra del Fuego and Falkland Islands, and one from Patagonia (Mrázek 1901; Ekman 1905; Scott 1914; Lindberg 1949; Borutzky & Vinogradov 1957; Borutzky 1962; Dartnall 1995; Dartnall & Hollwedel 2007; Karanovic *et al.* 2014). It is obvious that members of this group prefer cold waters, which is apparent also in the habitat preferences of the Australian *D. cryonastes*: in the colder climate of Tasmania it lives in a variety of surface waters at all altitudes (Morton 1985), while in the warmer New South Wales it was only found in one high alpine lake (Morton 1985) and in subterranean waters (Karanovic *et al.* 2014; material examined here). The *michaelseni*-group shows a clear Southern Gondwana connection, which is common for many Australian groups of plants and animals (Giribet & Edgecombe 2006; Kodandaramaiah *et al.* 2018). All other Australian *Diacyclops* live exclusively in subterranean waters and have more interesting zoogeographic connections. All Western Australian species (Fig. 28) are members of the *alticola*-group, with additional members known only from India and Tibet in China (Kiefer 1935, 1939; Shen & Sung 1963; Shen *et al.* 1979). This is also a Gondwana connection, as India shared a boarder with Western Australia for as long as Early Cretaceous (Fitzgerlad 2002; Zhang *et al.* 2015). *Diacyclops hancocki* seems to be a relic in the Australian Fauna without any clear connections to other congeners; I would not be surprised if future molecular studies reveal its basal position in the entire *Diacyclops/Acanthocyclops* group. Finally, the two sister species from New South Wales (*D. tomlinsonae* and *D. huntervalleyensis*) are unique enough in the genus to deserve their own species group. I am confident that this group will expand significantly once more subterranean waters are surveyed in eastern Australia. The *tomlinsonae*-group might have some connections with the Western Australian monospecific genus *Orbuscyclops*, as discussed above, but maybe also with the New Zealand monospecific genus *Abdiacyclops* Karanovic, 2005. The latter is, unfortunately, still known only after females (Karanovic 2005). All these hypotheses remain to be tested with molecular tools, which would also have to be used in any comprehensive revision of the *Diacyclops/Acanthocyclops* group. The areas studied in Australia, with a high cyclopine endemism, has resemblance to other geographical zones in which the copepod diversity has been surveyed, like the Yucatan Peninsula, where speciation can occur in a relatively limited area with a dynamic geomorphology (Fiere *et al.* 1996, 2000).

Key to Australian species of *Diacyclops*:

1. Female antennula of less than 17 segments 4
- Female antennula 17-segmented 2
2. Third endopodal segment of fourth leg with inner spine longer than outer spine *D. bisetosus* (Rehberg, 1880)
- This segment with inner spine shorter than outer spine 3
3. Principal caudal setae with breaking planes; dorsal caudal seta shorter than innermost caudal seta
..... *D. bicuspidatus* (Claus, 1857)
- Principal caudal setae without breaking planes; dorsal caudal seta longer than innermost caudal seta *D. leijsi* sp. nov.

4.	Female antennula 10-segmented	<i>D. huntervalleyensis</i> sp. nov.
-	Female antennula of more than 10 segments	5
5.	Female antennula 11-segmented	6
-	Female antennula 12-segmented	8
6.	Exopod of first leg 2-segmented.	<i>D. tomlinsonae</i> sp. nov.
-	Exopod of first leg 3-segmented.	7
7.	Third exopodal segment of first to fourth leg spine formula 2.3.3.3	<i>D. cryonastes</i> Morton, 1985
-	This segment spine formula 3.4.4.3	<i>D. hancocki</i> sp. nov.
8.	Outer principal caudal seta strongly reduced	<i>D. ballaballaensis</i> sp. nov.
-	Outer principal caudal seta well-developed	9
9.	First exopodal segment of first to fourth legs without inner seta	12
-	This segment with with inner seta	10
10.	Dorsal caudal seta as long as outer principal caudal seta	<i>D. scanloni</i> Karanovic, 2006
-	Dorsal caudal seta shorter than outer principal caudal seta	11
11.	Labrum with very convex cutting edge	<i>D. reidae</i> De Laurentiis, Pesce & Humphreys, 1999
-	Labrum with slightly convex cutting edge	<i>D. einslei</i> De Laurentiis, Pesce & Humphreys, 1999
12.	Dorsal caudal seta as long as outer principal caudal seta	<i>D. sobeprolatus</i> Karanovic, 2006
-	Dorsal caudal seta shorter than outer principal caudal seta	13
13.	Dorsal caudal seta about as long as innermost caudal seta	<i>D. cockingi</i> Karanovic, 2006
-	Dorsal caudal seta much longer than innermost caudal seta	14
14.	Seminal receptacle large and mushroom-like	<i>D. eberhardi</i> sp. nov.
-	Seminal receptacle small and ovoid	15
15.	Third endopodal segment of fourth leg with two apical spines	<i>D. humphreysi humphreysi</i> Pesce & De Laurentiis, 1996
-	This segment with 1 apical spine	<i>D. humphreysi unispinosus</i> Karanovic, 2006

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References

- Bláha, M., Hulák, M., Slouková, J. & Těšitel, J. (2010) Molecular and morphological patterns across *Acanthocyclops vernalis-robustus* species complex (Copepoda, Cyclopoida). *Zoologica Scripta*, 39, 259–268.
<https://doi.org/10.1111/j.1463-6409.2010.00422.x>
- Borutzky, E.V. (1962). New data on the copepod *Acanthocyclops mirnyi* Borutzky & M. Vinogradov from the Antarctic region. *Zoologicheskii Zhurnal*, 41, 1106–1107.
- Borutzky, E.V. & Vinogradov, M.E. (1957) Occurrence of a Cyclopidae (*Acanthocyclops mirnyi* sp. n.) on the Antarctic continent. *Zoologicheskii Zhurnal*, 36, 199–203.
- Boxshall, G.A. & Halsey, S.H. (2004) *An Introduction to Copepod Diversity*. The Ray Society, London, 966 pp.
- Brehm, V. (1953) Contributions to the freshwater microfauna of Tasmania. Part 1. Copepoda. *Papers and Proceedings of the Royal Society of Tasmania*, 87, 33–62.
<https://doi.org/10.26749/TGVE2417>
- Chang, C.Y. (2009) Inland-water Copepoda. *Illustrated Encyclopedia of Fauna and Flora of Korea*, 42, 1–687. [in Korean]
- Dartnall, H.J.G. (1995) Rotifers, and other aquatic invertebrates, from the Larsemann Hills, Antarctica. *Papers and Proceedings of the Royal Society of Tasmania*, 129, 17–23.
<https://doi.org/10.26749/rstpp.129.17>
- Dartnall, H.J.G. & Hollwedel, W. (2007) A limnological reconnaissance of the Falkland Islands; with particular reference to the waterfleas (Arthropoda: Anomopoda). *Journal of Natural History*, 41, 1259–1300.
<https://doi.org/10.1080/00222930701401010>
- De Laurentiis, P., Pesce, G.L. & Humphreys, W.F. (1999) Copepods from ground waters of Western Australia, IV. Cyclopids from basin and craton aquifers (Crustacea: Copepoda: Cyclopidae). *Records of the Western Australian Museum*, 19, 243–257.
- Dussart, B. & Defaye, D. (2006) *World Directory of Crustacea Copepoda of Inland Waters, II – Cyclopiformes*. Backhuys Publishers, Leiden, 352 pp.

- Ekman, S. (1905) Cladoceren und Copepoden aus Antarktischen und subantarktischen Binnengewässern gesammelt von der Schwedischen Antarktischen Expedition 1901–1903. *Wissenschaftliche Ergebnissen der schwedischen Südpolarexpedition 1901–1903*, 5, 1–40.
- Ferrari, F. (1991) Using patterns of appendage development to group taxa of *Labidocera*, Diaptomidae and Cyclopidae (Copepoda). In: Uye, S.I., Nishida, S. & Ho, J.S. (Eds.), Proceedings of the Fourth International Conference on Copepoda. *Bulletin of Plankton Society of Japan*, Special Volume, pp. 115–128.
- Fiers, F., Reid, J.W., Iliffe, T.M. & Suárez-Morales, E. (1996) New hypogean cyclopoid copepods (Crustacea) from the Yucatan Peninsula, Mexico. *Contributions to Zoology*, 66, 65–102.
<https://doi.org/10.1163/26660644-06602001>
- Fiers, F., Ghenne, V. & Suárez-Morales, E. (2000) New species of continental copepods (Crustacea, Cyclopoida) from the Yucatan Peninsula, Mexico. *Studies of Neotropical Fauna and Environment*, 35, 209–251.
<https://doi.org/10.1076/snfe.35.3.209.8862>
- Fitzgerlad, P. (2002) Tectonics and landscape evolution of the Antarctic plate since the breakup of Gondwana, with an emphasis on the West Antarctic Rift System and the Transantarctic Mountains. *Royal Society of New Zealand Bulletin*, 35, 453–469.
- Giribet, G. & Edgecombe, G.D. (2006) The importance of looking at small-scale patterns when inferring Gondwanan biogeography: a case study of the centipede *Paralamyctes* (Chilopoda, Lithobiomorpha, Henicophidae). *Biological Journal of the Linnean Society*, 89, 65–78.
<https://doi.org/10.1111/j.1095-8312.2006.00658.x>
- Goloboff, P. (1999) *NONA (NO NAME). Version 2*. Published by the author, Tucumán. [Computer software]
- Gourbault, N. & Lescher-Moutoué, F. (1979) Faune des eaux souterraines de Majorque. *Endins, Mallorca*, 5/6, 43–54.
- Humphreys, W.F. (1991) Troglobites on Barrow Island, Western Australia. *The Western Caver*, 31, 11–14.
- Huys, R. & Boxshall, G.A. (1991) *Copepod Evolution*. The Ray Society, London, 468 pp.
- Karanovic, T. (2000) On *Reidicyclops*, new genus (Crustacea, Copepoda), with the first description of the male of *Reidicyclops trajani* (Reid & Strayer, 1994), new combination. *Beaufortia*, 50, 79–88.
- Karanovic, T. (2004) Subterranean copepods (Crustacea, Copepoda) from arid Western Australia. *Crustaceana Monographs*, 3, 1–366.
https://doi.org/10.1163/9789047412779_003
- Karanovic, T. (2005) Two new genera and three new species of subterranean cyclopoids (Crustacea, Copepoda) from New Zealand, with redescription of *Goniocyclops silvestris* Harding, 1958. *Contributions to Zoology*, 74, 223–254.
<https://doi.org/10.1163/18759866-0740304002>
- Karanovic, T. (2006) Subterranean copepods (Crustacea, Copepoda) from the Pilbara region in Western Australia. *Records of the Western Australian Museum*, Supplement 70, 1–239.
<https://doi.org/10.18195/issn.0313-122x.70.2006.001-239>
- Karanovic, T. (2020) Four new Cyclopina (Copepoda, Cyclopinidae) from South Korea. *ZooKeys*, 992, 59–104.
<https://doi.org/10.3897/zookeys.992.54856>
- Karanovic, T. (2021) Two new marine cyclopinids (Crustacea: Copepoda: Cyclopoida) from interstitial habitats in Korea. *Zootaxa*, 5051 (1), 319–345.
<https://doi.org/10.11646/zootaxa.5051.1.14>
- Karanovic, T. & Blaha, M. (2019) Taming extreme morphological variability through coupling of molecular phylogeny and quantitative phenotype analysis as a new avenue for taxonomy. *Scientific Reports*, 9 (2429), 1–14.
<https://doi.org/10.1038/s41598-019-38875-2>
- Karanovic, T. & Cooper, S.J.B. (2011) Molecular and morphological evidence for short range endemism in the *Kinneccaris solitaria* complex (Copepoda: Parastenocarididae), with descriptions of seven new species. *Zootaxa*, 3026 (1), 1–64.
<https://doi.org/10.11646/zootaxa.3026.1.1>
- Karanovic, T. & Cooper, S.J.B. (2012) Explosive radiation of the genus *Schizopera* on a small subterranean island in Western Australia (Copepoda: Harpacticoida): unraveling the cases of cryptic speciation, size differentiation and multiple invasions. *Invertebrate Systematics*, 26, 115–192.
<https://doi.org/10.1071/IS11027>
- Karanovic, T., Gibson, J.A.E., Hawes, I., Andersen, D.T. & Stevens, M.I. (2014) *Diacyclops* (Copepoda: Cyclopoida) in Continental Antarctica, including three new species. *Antarctic Science*, 26, 250–260.
<https://doi.org/10.1017/S0954102013000643>
- Karanovic, T. & Krajcicek, M. (2012a) When anthropogenic translocation meets cryptic speciation globalized bouillon originates; molecular variability of the cosmopolitan freshwater cyclopoid *Macrocyclus albidus* (Crustacea: Copepoda). *International Journal of Limnology*, 48, 63–80.
<https://doi.org/10.1051/limn/2011061>
- Karanovic, T. & Krajcicek, M. (2012b) First molecular data on the Western Australian *Diacyclops* (Copepoda, Cyclopoida) confirm morpho-species but question size differentiation and monophyly of the *alticola*-group. *Crustaceana*, 85, 1549–1569.
<https://doi.org/10.1163/156854012X651709>
- Karanovic, T. & Tang, D. (2009) A new species of the copepod genus *Australoencyclops* (Crustacea: Cyclopoida: Eucyclopiniae)

- from Western Australia shows the role of aridity in habitat shift and colonisation of ground water. *Records of the Western Australian Museum*, 25, 247–264.
[https://doi.org/10.18195/issn.0312-3162.25\(3\).2009.247-263](https://doi.org/10.18195/issn.0312-3162.25(3).2009.247-263)
- Karanovic, T., Yoo, H. & Lee, W. (2012a) A new species of cyclopoid copepods from Korean subterranean waters reveals interesting connections with the Central Asian fauna (Crustacea: Copepoda: Cyclopoida). *Journal of Species Research*, 1, 155–173.
<https://doi.org/10.12651/JSR.2012.1.2.156>
- Kiefer, F. (1927) Versus eines Systems der Cyclopiden. *Zoologischer Anzeiger*, 73, 302–308.
- Kiefer, F. (1928) Beiträge zur Copepodenkunde (VIII). 17. Neue Cyclopiden aus Neuseeland. 18. Diaptomiden und Cyclopiden aus Südafrika. *Zoologischer Anzeiger*, 76, 5–18.
- Kiefer, F. (1935) Fünf neue Ruderfusskrebse aus Indien. Aus den Ergebnisse der Yale North India Expedition 1932. *Zoologischer Anzeiger*, 109, 113–121.
- Kiefer, F. (1939) Freilebende Ruderfusskrebse (Crustacea Copepoda) aus Nordwest und Südindien (Pandschab, Kaschmir, Ladak, Nilgirigebirge). *Memoirs of the Indian Museum Calcutta*, 13, 83–203.
- Kiefer, F. (1978) Zur Kenntnis des *Diacyclops thomasi* (S.A. Forbes, 1882) (Copepoda Cyclopoida). *Crustaceana*, 34, 214–216.
<https://doi.org/10.1163/156854078X00745>
- Kodandaramaiah, U., Braby, M.F., Grund, R., Müller, C.J. & Wahlberg, N. (2018) Phylogenetic relationships, biogeography and diversification of *Coenonymphina* butterflies (Nymphalidae: Satyrinae): intercontinental dispersal of a southern Gondwanan group? *Systematic Entomology*, 43, 798–809.
<https://doi.org/10.1111/syen.12303>
- Lescher-Moutoué, F. (1971) Recherches sur les eaux souterraines. 15. Les Cyclopides de la zone noyée d'un karst. II. *Mesocyclops (Thermocyclops) delamarei* n. sp. *Annales de Spéléologie*, 26, 473–479.
- Lescher-Moutoué, F. (1976) Création du genre *Kieferiella* a la suite de nouvelles observations sur l'espèce *delamarei* (Crustacé, Copépode). *Annales de Spéléologie*, 31, 91–98.
- Lindberg, K. (1949) Contribution à l'étude des Cyclopides (Crustacés Copépodes). *Kunglige Fysiografiska Sällskapets i Lund Förhandlingar*, 19, 98–121.
- Locascio de Mitrovich, C. & Menu-Marque, S. (2001) A new *Diacyclops* (Copepoda, Cyclopoida, Cyclopidae) from northwestern Argentina. *Hydrobiologia*, 453/454, 533–538.
https://doi.org/10.1007/0-306-47537-5_46
- Martin, H.A. (2006) Cenozoic climatic change and the development of the arid vegetation in Australia. *Journal of Arid Environments*, 66, 533–563.
<https://doi.org/10.1016/j.jaridenv.2006.01.009>
- McNamara, K.J. & Kendrick, G.W. (1994) Cenozoic molluscs and echinoids of Barrow Island, Western Australia. *Records of the Western Australian Museum*, Supplement 51, 1–50.
- Monchenko, V.I. (1974) Schelepnoroti Ciklopopodibni Ciklopi (Cyclopidae). *Fauna Ukraïni*, 27 (3), 1–452.
- Monchenko, V.I. (2000) Cryptic species in *Diacyclops bicuspidatus* (Copepoda: Cyclopoida): evidence from crossbreeding studies. *Hydrobiologia*, 417, 101–107.
<https://doi.org/10.1023/A:1003811606429>
- Monchenko, V.I. & von Vaupel Klein, J.C. (1999) Oligomerization in Copepoda Cyclopoida as a kind of orthogenetic evolution in the animal kingdom. *Crustaceana*, 72, 241–264.
- Morton, D.W. (1985) Revision of the Australian Cyclopidae (Copepoda: Cyclopoida), I. *Acanthocyclops* Kiefer, *Diacyclops* Kiefer and *Australocyclops*, gen. nov. *Australian Journal of Marine and Freshwater Research*, 36, 615–634.
<https://doi.org/10.1071/MF9850615>
- Mrazek, A. (1901) Süßwasser-Copepoden. Ergebnisse der Hamburger Magalhaensischen Sammelreise 1892/93. *Hamburg Natural History Museum*, 3 (2/22), 1–29.
- Nixon, K. C. (1999) The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics*, 15, 407–414.
<https://doi.org/10.1111/j.1096-0031.1999.tb00277.x>
- Owen, C.L., Marshall, D.C., Hill, K.B.R. & Simon, C. (2017) How the aridification of Australia structured the biogeography and influenced the diversification of a large lineage of Australian cicadas. *Systematic Biology*, 66, 569–589.
<https://doi.org/10.1093/sysbio/syw078>
- Pandourski, I.S. (1997) Composition, origine et formation de la faune cyclopidienne stygobioe de Bulgarie et definition du groupe d'espèces "kieferi" du genre *Acanthocyclops* (Crustacea, Copepoda, Cyclopoida). *Bolletino del Museo Regionale di Scienze Naturali Torino*, 15, 279–297.
- Pesce, G.L. (1996) Towards a revision of Cyclopinae copepods (Crustacea, Cyclopidae). *Fragmenta Entomologica Roma*, 28, 189–200.
- Pesce, G.L. & De Laurentiis, P. (1996) Copepods from ground waters of Western Australia. III. *Diacyclops humphreysi* n. sp., and comments on the *Diacyclops crassicaudis*-complex (Copepoda, Cyclopidae). *Crustaceana*, 69, 524–531.
<https://doi.org/10.1163/156854096X01096>
- Reid, J.W. (1991) *Diacyclops albus* n. sp. and *Parastenocaris palmerae* n. sp. (Crustacea: Copepoda) from the meiofauna of a stream bed in Virginia, U.S.A. *Canadian Journal of Zoology*, 69, 2893–2902.

<https://doi.org/10.1139/z91-408>

- Reid, J.W. (2004) New records and new species of the genus *Diacyclops* (Crustacea; Copepoda) from subterranean habitats in southern Indiana, USA. *Jeffersoniana*, 12, 1–65.
- Reid, J.W. & Ishida, T. (2000) *Itocyclops*, a new genus proposed for *Speocyclops yezoensis* (Copepoda: Cyclopoida: Cyclopidae). *Journal of Crustacean Biology*, 20, 589–596.
<https://doi.org/10.1163/20021975-99990076>
- Reid, J.W. & Strayer, D.L. (1994) *Diacyclops dimorphus*, a new species of copepod from Florida, with comments on morphology of interstitial cyclopine cyclopoids. *Journal of the North American Benthological Society*, 13, 250–265.
<https://doi.org/10.2307/1467243>
- Reid, J.W., Strayer, D.L., McArthur, J.V., Stibbe, S.E. & Lewis, J.J. (1999) *Rheocyclops*, a new genus of copepods from the southeastern and central U.S.A. (Copepoda: Cyclopoida: Cyclopidae). *Journal of Crustacean Biology*, 19, 384–396.
<https://doi.org/10.2307/1549246>
- Scott, T. (1914) Remarks on some Copepoda from the Falkland Islands collected by Mr Rupert Vallentin. *The Annals and Magazine of Natural History*, 8 (13), 1–11.
<https://doi.org/10.1080/00222931408693449>
- Shen, C.J. & Sung, T.H. (1963) Notes on Copepoda collected from Shigatze and Gyantse regions in Tibet, China. *Acta Zoologica Sinica*, 15 (1), 79–97.
- Shen, C.J., Tai, A.Y., Zhang, C.Z., Li, Z.Y., Song, D.X., Song, Y.Z. & Chen, G.X. (1979) *Crustacea: Freshwater Copepoda*. Fauna Sinica (unnumbered), Academia Sinica Research Group of Carcinology Institute of Zoology, Science Press, Peking, 450 pp. [in Chinese]
- Stoch, F. (1987) Cave-dwelling cyclopoids (Crustacea, Copepoda) from Venezia Giulia (northeastern Italy). *Bulletin Zoologisch Museum Universiteit van Amsterdam*, 11 (5), 41–56.
- Stoch, F. (2001) How many species of *Diacyclops*? New taxonomic characters and species richness in a freshwater cyclopid genus (Copepoda, Cyclopoida). *Hydrobiologia*, 453/454, 525–531.
<https://doi.org/10.1023/A:1013191429008>
- Stock, J.K. & von Vaupel Klein, J.C. (1996) Mounting media revisited: the suitability of Reyne's fluid for small crustaceans. *Crustaceana*, 69, 749–798.
<https://doi.org/10.1163/156854096X00826>
- Suárez-Morales, E., Mercado-Salas, N.F. & Barlow, R. (2013) A new stygobiotic species of *Diacyclops* Kiefer, 1927 (Copepoda, Cyclopidae) from caves in New Mexico, United States. *Crustaceana*, 86, 1155–1177.
<https://doi.org/10.1163/15685403-00003223>
- Walter, T.C. & Boxshall, G.A. (2023) *World of Copepods database*. Available online at <http://www.marinespecies.org/copepoda> (accessed 15 November 2023)
- Zhang, C.-L., Santosh, M., Zhu, Q.-B., Chen, X.-Y. & Huang, W.-C. (2015) The Gondwana connection of South China: Evidence from monazite and zircon geochronology in the Cathaysia Block. *Gondwana Research*, 28, 1137–1151.
<https://doi.org/10.1016/j.gr.2014.09.007>