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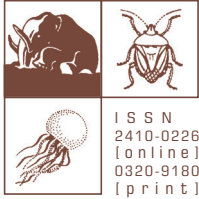
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## Integrative redescription of *Canthocamptus (Baicalocamptus) longifurcatus* (Harpacticoida: Canthocamptidae), an endemic species from Lake Baikal


## Интегративное переописание *Canthocamptus (Baicalocamptus) longifurcatus* (Harpacticoida: Canthocamptidae), эндемичного вида из озера Байкал

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**Abstract.** A redescription of the Lake Baikal endemic species, *Canthocamptus (Baicalocamptus) longifurcatus* Borutzky, 1947 (Harpacticoida: Canthocamptidae), is provided. The illustrated redescription of morphology of the female and male is supplemented by a genetic barcoding based on sequences of the mitochondrial COI marker. *Canthocamptus (B.) longifurcatus* is morphologically very similar to another representative of the subgenus, *C. (B.) verestschagini* (Borutzky, 1931), but can be well distinguished from the latter by the armature of the female furca and the structure of the last pair of swimming legs in the female and male. An analysis of the obtained COI sequences and their comparison with those of other harpacticoid species confirmed that *C. (B.) longifurcatus* belongs to the genus *Canthocamptus* Westwood, 1836: the smallest genetic differences (on average, 29.5%) were observed between the studied Baikal species and *C. staphylinus* Jurine, 1820 from Europe (Lake Geneva, Switzerland).

**Резюме.** Выполнено переописание гарпактикоиды *Canthocamptus (Baicalocamptus) longifurcatus* Borutzky, 1947 (Harpacticoida: Canthocamptidae) – эндемика озера Байкал. Иллюстрированное переописание морфологии самки и самца дополнено данными генетического баркодинга на основе митохондриального маркера COI. По морфологии *C. (B.) longifurcatus* весьма сходен с другим представителем подрода – *C. (B.) verestschagini* (Borutzky, 1931), но хорошо отличается от него вооружением фурки самки и строением последней пары плавательных ног самки и самца. Анализ и сравнение последовательностей COI, полученных для *C. (B.) longifurcatus* и для других видов гарпактикоид, подтвердило принадлежность *C. (B.) longifurcatus* к роду *Canthocamptus* Westwood, 1836: наименьшие генетические различия (в среднем 29.5%) получены между исследованным байкальским видом и *C. staphylinus* Jurine, 1820 из Европы (озеро Женева, Швейцария).

**Key words:** taxonomy, diagnostic characters, morphology, genetic barcoding, redescription, Russian Federation, Harpacticoida, Canthocamptidae, *Canthocamptus*, *Baicalocamptus*

**Ключевые слова:** таксономия, диагностические признаки, морфология, генетический баркодирование, переописание, Россия, Harpacticoida, Canthocamptidae, *Canthocamptus*, *Baicalocamptus*

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## Introduction

Together with the nematodes, harpacticoid copepods are among the dominant groups of the meiobenthic fauna in Lake Baikal. The latter ecological group plays an important role in the cycle of organic matter. Of the 69 identified harpacticoid species living in Lake Baikal, 62 are endemic (Okuneva & Evstigneeva, 2001). According to Okuneva (1989) and Okuneva & Evstigneeva (2001), the morphological features, which are usually used for species diagnostics, are often variable in the Baikal species. Therefore, most likely the number of harpacticoid species in Lake Baikal is much higher than currently known; moreover some taxa can represent groups of cryptic species or subspecies. In order to estimate the taxonomic richness of the harpacticoids in the lake, as well as their diversity and systematic position, integrative studies are needed. Redescription of all species using both morphological and genetic (species barcodes) information is essential in modern taxonomic studies.

According to Okuneva & Evstigneeva (2001), an endemic subgenus *Baicalocamptus* Borutzky, 1931 belonging to the genus *Canthocamptus* Westwood, 1836 is represented by four species living in Lake Baikal, two of which have not yet been described. *Canthocamptus* (*Baicalocamptus*) *verestschagini* (Borutzky, 1931) and *C. (B.) longifurcatus* Borutzky, 1947 were discovered in the first half of the 20th century, but only females of the latter were described in the original publication. Later on, Okuneva (1989) described the male of *C. (B.) longifurcatus* but the morphological diagnosis was too short and the illustrations of caudal rami and legs did not contain scale bars.

The type specimens of *C. (B.) longifurcatus* have apparently been lost. The original description of the species (Borutzky, 1947) does not indicate the depositary of the type specimens. The Zoological Museum of M.V. Lomonosov Moscow State University (MSU), where the Borutzky's collection is stored, and Limnological Institute of the Siberian Branch of the Russian Academy of Sciences (Irkutsk) returned negative answers to our inquiries about the presence of the type specimens of *C. (B.) longifurcatus*. In addition, our personal attempts to find the type specimens in these institutions were unsuccessful.

The aim of this article is to redescribe the female and male of *C. (B.) longifurcatus* from Lake Baikal using an integrative approach, i.e. morphological techniques (light and confocal laser scanning microscopy) combined with genetic barcoding. In addition, a comparison with other species of the genus *Canthocamptus* and other taxa of the family Canthocamptidae Sars, 1906 was performed based on COI mitochondrial DNA analysis.

## Material and methods

### Sampling

The material for the present study was obtained partly from the collection of the identified Baikal harpacticoid species of the Zoological Museum of M.V. Lomonosov Moscow State University. These samples (preserved in formalin) have been collected in 1968 from two localities of the lake: (1) the vicinity of Bol'shie Koty Village and (2) the vicinity of Utulik Village, both in the Irkutsk Province. Additionally, we studied a sample of harpacticoid copepods taken from the lake by T.Ya. Sitnikova (Limnological Institute, Siberian Branch of the Russian Academy of Sciences, Irkutsk). The latter sample collected in 2017 from the vicinity of Bol'shoe Goloustnoe Village, was taken with a dredge from the lake bottom (crushed rocks, gravels and sponges) at 43–125 m. Living organisms including harpacticoids were sorted from bottom sediment at the laboratory, fixed in 96% ethanol and stored at -20 °C. All examined material was obtained from the sites located in the southern part of Lake Baikal.

All examined specimens are now deposited at the Zoological Museum of Institute of Biology, Komi Scientific Centre, Ural Branch of Russian Academy of Sciences (ZMIB).

### Microscopy and imaging

Morphological features were analysed and measurements were done using a Leica DM 4000B microscope. Drawings were performed with a drawing tube assembled to the microscope, with magnification of 200–1000×. The photos were obtained with a digital camera ASUS ZE520KL Phone. The final versions of drawings were made using Adobe Photoshop CS3 Extended and Xara Photo & Graphic Designer 6 software packages.

For confocal laser scanning microscopy (CLSM), the specimens were stained with Congo Red overnight and mounted on slides following the procedure described by Michels & Büntzow (2010). The material was scanned using a Carl Zeiss LSM 710 laser confocal microscope (Zeiss, Germany) with the lenses Plan-Apochromat 20×/0.8 and 63×/1.40 Oil DIC M27, filters 570–670 nm and lasers 561 nm: 3.0%.

### Morphological analysis

For morphological analysis, we used 14 specimens of *C. (B.) longifurcatus* (females and males) from formalin and ethanol samples taken from Lake Baikal. Appendages and body ornamentation were examined.

For comparison of *C. (B.) longifurcatus* with other species of the subgenus *Canthocamptus*, we used our own material of *C. (C.) staphylinus staphylinus* Jurine, 1820 from Lake Geneva, Switzerland, *C. (C.) microstaphylinus* Wolf, 1905 from a pond in the Botanical Garden of Syktyvkar, Russia, and *C. (C.) glacialis* Liljeborg, 1902 from Lake Bolshoy Ngosavey, the Polar Urals, Russia. In addition, the specimens of *C. (C.) robertcokeri* M.S. Wilson, 1958 from Lake Ontario, USA, and the specimens of *C. (C.) cf. vagus* Coker et Morgan, 1940, *C. (C.) staphylinoides staphylinoides* Pearse, 1905, *C. (C.) assimilis* Kiefer, 1931 from different localities of the USA were examined. The specimens of *C. (C.) robertcokeri* were provided by J.K. Connolly (Cornell University, Department of Natural Resources and the Environment, Cornell Biological Field Station, Bridgeport, New York State, USA). Finally, we examined other American specimens of *Canthocamptus* [*C. (C.) cf. vagus*, *C. (C.) staphylinoides staphylinoides*, *C. (C.) assimilis*] from formalin samples in the collection of the Smithsonian Institution, National Museum of Natural History (USNM), Washington, the USA (USNM catalogue numbers: 259602, 28.304, 278179).

We used terminology of Huys & Boxshall (1991), with some modifications. Abbreviations used in the text are as follows: A1, antennule; A2, antenna; Ae, aesthetasc; Endp (Endps), endopod (endopods); Endp-1 – Endp-3, first to third endopod segments; Exp (Exps), exopod (exopods); Exp-1 – Exp-3, first to third exopod segments; P1–P6, first to sixth thoracopod.

### Molecular genetic analysis

For molecular genetic analysis, we used five specimens of *C. (B.) longifurcatus* from the ethanol sample taken in the vicinity of Bol'shoe Goloustnoe. A gene fragment of the first subunit of mitochondrial DNA cytochrome oxidase (COI mtDNA or COI) was analysed. The DNA extraction and sequencing were performed at the Limnological Institute in Irkutsk and at the University of Helsinki, using different protocols.

Two specimens were sequenced using the following protocol. Genomic DNA was extracted using the Chelex protocol outlined in Walsh et al. (1991) and described in Kochanova et al. (2018). In order to amplify the COI mtDNA gene, we used L1384-COI (GGT CAT GTA ATC ATA AAG ATA TTG G) and H2612-COI (AGG CCT AGG AAA TGT ATM GGG AAA) primers (Machida et al., 2004). The PCR mixes and amplification protocols for the mitochondrial COI gene are described in Kochanova & Gaviria (2018). PCR products were visualised by electrophoresis in 2% agarose gel and purified with the ExoSap-IT PCR Product Clean-Up kit (Applied Biosystems, Waltham, Massachusetts, USA). Sequencing was carried out in both directions, using the BigDye Terminator v3.1 (Life Technology) reagent kit in an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) in the “Genome” Centre for Collective Use (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow).

Three other specimens were analysed in a slightly different way. Total DNA for molecular genetic analysis was isolated from somatic tissue using proteinase K according to the protocol described by Mayor et al. (2010). For PCR, we used universal primers LCO-1490 and HCO-2198 (Folmer et al., 1994). Amplification was carried out in a T100 thermal cycler (BioRad, USA) using PCR reagents from Evrogen (Russia). The PCR mixes and amplification protocols for the mitochondrial COI gene are described in Mayor et al. (2019). The separation and isolation of amplicons for sequencing from the agarose gel was performed according to the protocol described previously (Mayor et al., 2010). The nucleotide sequences of the target fragments were determined in an ABI 3500 8-capillary genetic analyser (Thermo Fisher



Scientific, USA) using the ABI PRISM BigDye Terminator v. 3.1 sequencing kit.

The obtained nucleotide sequences were deposited in the GenBank database (<https://www.ncbi.nlm.nih.gov/genbank/>) under the numbers: MH824144, MH824145, MH824146, MZ169062, and MZ169063.

The five obtained nucleotide sequences were aligned using the ClustalW algorithm. Phylogeny was reconstructed using Maximum Likelihood analysis in IQ-TREE software (Minh et al., 2020). Additional sequences of species in the family Canthocamptidae (Electronic supplementary material 1; see the section “Addenda”) were included to illustrate the position of *C. (B.) longifurcatus* among other taxa. The best-fitting model of nucleotide substitution was selected in jModel-Test v. 2.1.7 based on the likelihood scores for 88 different models and under the Akaike Information Criterion (AICc) (Posada, 2008). The selected model was GTR+G+I (general time reversible model with inter-site rate variation modelled with a gamma distribution). Mean genetic distances between species were calculated in MEGA X software (Kumar et al., 2018).

## Results

Subclass **Copepoda** Milne Edwards, 1840

Superorder **Podoplea** Giesbrecht, 1882

Order **Harpacticoida** Sars, 1903

Family **Canthocamptidae** Brady, 1880

Subfamily **Canthocamptinae** Brady, 1880

Genus ***Canthocamptus*** Westwood, 1836

Subgenus ***Baicalocamptus*** Borutzky, 1931

***Canthocamptus* (*Baicalocamptus*) *longifurcatus*** Borutzky, 1947

*Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947: 1826 (female); Okuneva, 1989: 19 (female, male).

*Canthocamptus* (*Baicalocamptus*) *longifurcatus*: Borutzky, 1952: 154 (female); Okuneva & Evstigneeva, 2001: 470 (in checklist).

*Canthocamptus longifurcatus*: Novikov & Sharafutdinova, 2022: 59 (in key and in generic diagnosis; revision of genus).

(Figs 1–8)

**Material examined.** **Russia, Irkutsk Prov.**, Lake Baikal nr. Utulik Vill., approx. 51°35'31"N 103°57'43"E, 9 June 1968, 6 females on slides, 1 male on slide (ZMIB: nno. HRP-1, HRP-4, HRP-7(2), HRP-11); Lake Baikal nr. Bol'shoe Goloustnoe Vill., 52°00'05.5"N 105°21'22.1"E, 3 Oct. 2017, T. Sitnikova leg., 2 males in ethanol samples (ZMIB: nno. HRP-2, HRP-3(1)); Lake Baikal nr. Bol'shye Koty Vill., approx. 51°54'11"N 105°4'28"E, 15 March 1968, 2 females on slides (ZMIB: nno. HRP-5, HRP-6).

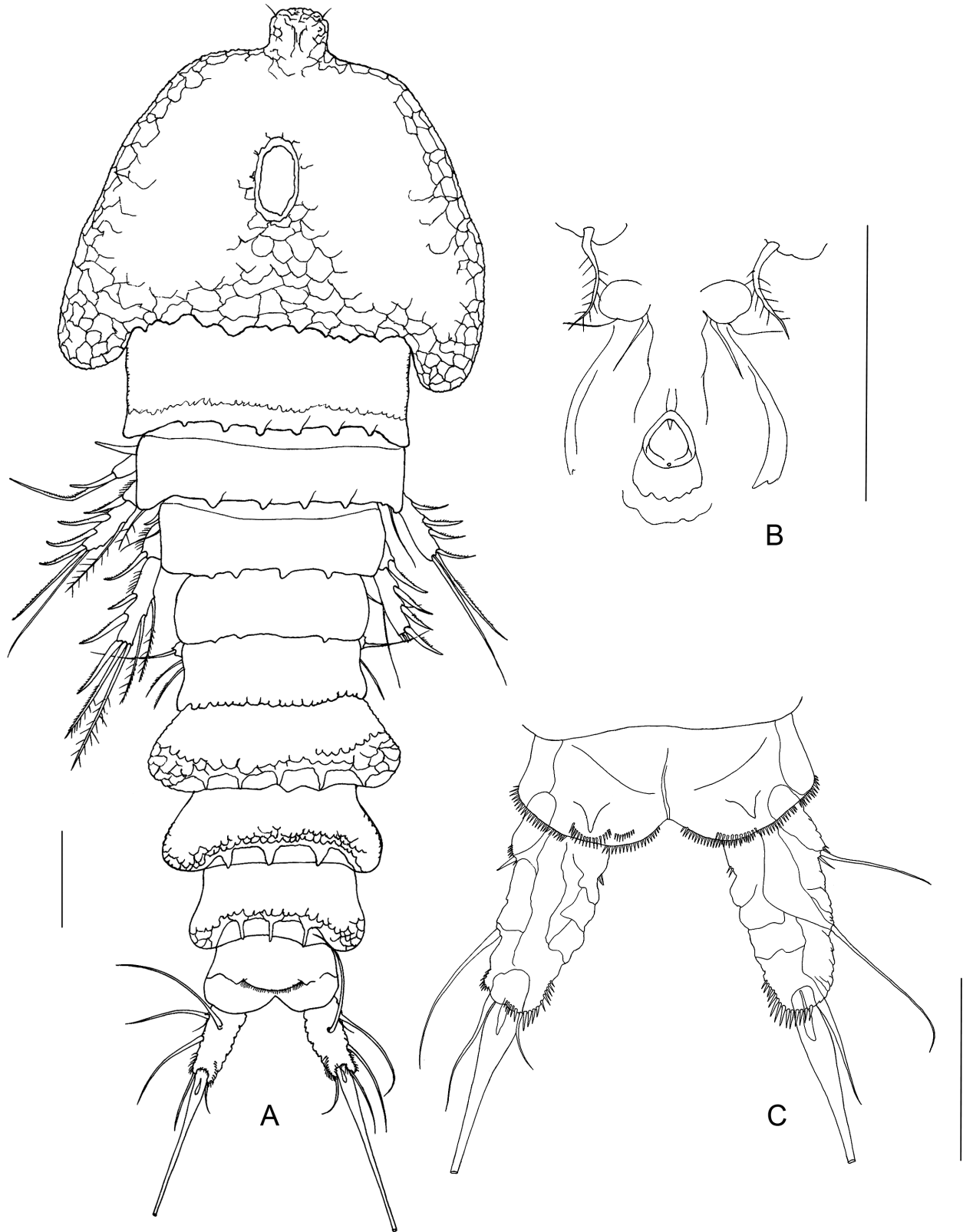
**Morphological redescription.** **Female** (Figs 1–3, 7a, c). Total length (measured from tip of rostrum to posterior margin of caudal rami) 1150–1400 µm (n = 8).

Body strongly flattened (Figs 1a, 7a), raspberry pink in colour. Ornamentation of integument with large tile cells and folds. Body somites wide, at least twice as wide as long.

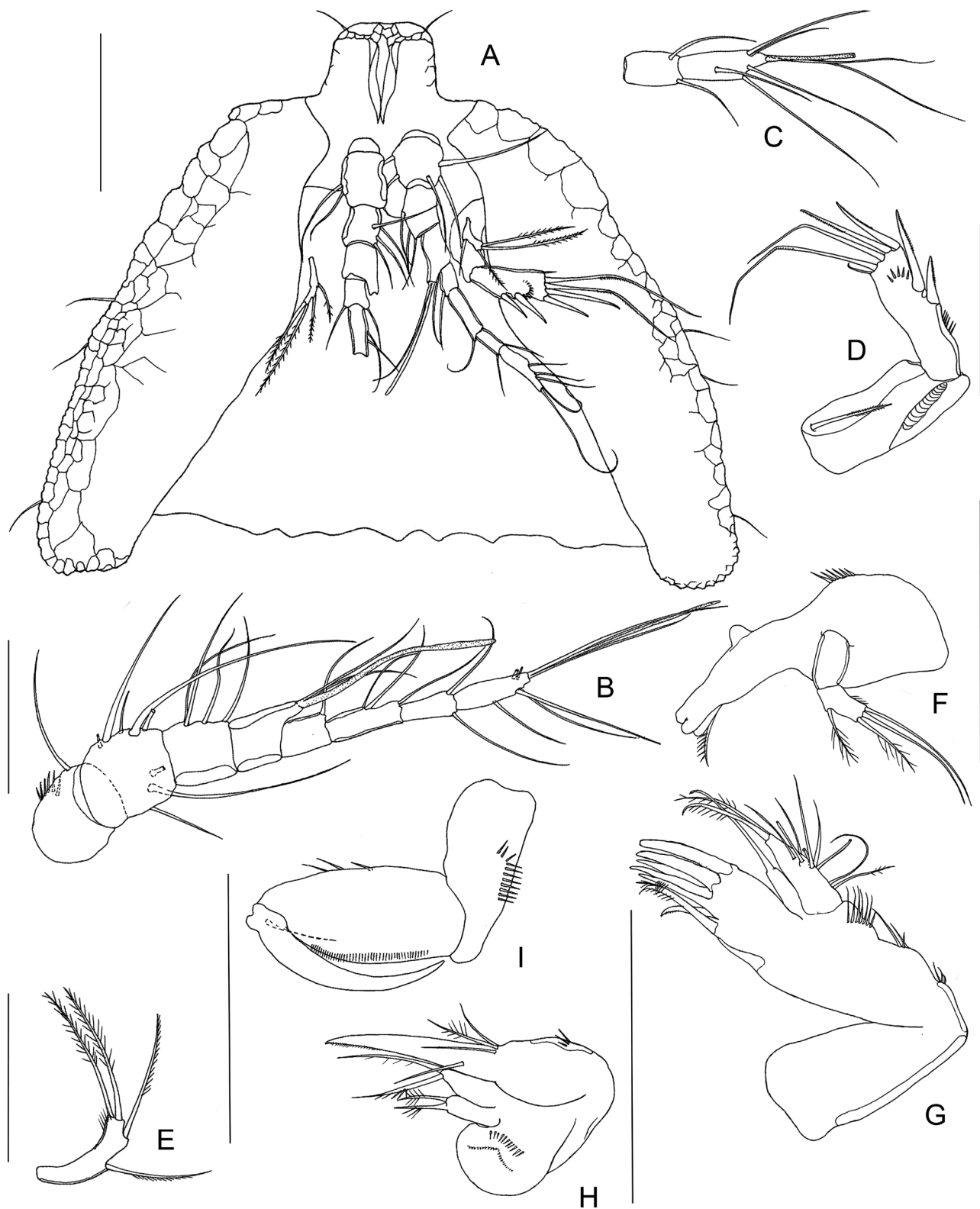
Cephalothorax (Figs 1a, 2a) with integumental dorsal window, wider than thoracal somites, with posterior parts considerably projecting laterally; lateral margins of cephalothorax exposed to ventral side (Fig. 2a). Naupliar eye absent. Rostrum large (length 60 µm, width 90 µm), fused with cephalothorax, subquadrate, with a pair of noticeable sensilla at its anterior apex. Thorax evenly tapering from first somite to abdomen; abdominal somites (except anal somite) wider than thoracic ones, their hind angles laterally protruding as rounded lobes. Posterior margins of thoracic and abdominal somites without spinules; posterior margins of somites dorsally strongly serrate, forming coarse folds looking as sparse blunt teeth. Posterior margins of abdominal somites with discontinuous rows of ventral spinules. Ventral surface of abdominal somites with thin short capilliform spinules.

Genital field (Fig. 1b) located near anterior margin of genital somite, with rather large copulatory pore located in median depression. Anal somite with large anal operculum. Anal operculum rounded, with numerous short thin spinules. Ventral surface of anal somite with a pair of short triangular projections, covered with thin short capilliform spinules (Fig. 1c).

Caudal rami conical (Fig. 1a, c), about 2.2 times as long as wide (length 105 µm, width 47 µm). Integument of caudal rami with longitudinal and transverse folds, similar to that of body somites. Distal part of caudal rami with numerous small



**Fig. 1.** *Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947, female (specimen on slide HRP-1). A, habitus, dorsal view; B, genital field; C, anal somite and caudal rami, dorsal view. Scale bars: 100  $\mu$ m.



**Fig. 2.** *Canthocamptus (Baicalocamptus) longifurcatus* Borutzky, 1947, female (A, specimen on slide HRP-3; B, E–H, specimen on slide HRP-1; C, D, specimen on slide HRP-4; I, specimen on slide HRP-11). A, cephalosome, ventral view; B, A1; C, two distal segments of A1; D, Endp of A2; E, Exp of A2; F, mandible; G, maxillule; H, maxilla; I, maxilliped. Scale bars: 100  $\mu$ m.

spinules at inner and outer margins; proximal part with small group of spinules at inner and outer margins. Each ramus with seven setae (I–VII). Inner terminal seta (seta V) the thickest and longest (in all examined specimens, its apex broken), with basal section expanded. Outer terminal seta (seta III) and inner terminal seta (seta VI) short and thin; outer seta about 0.75 times as long as caudal ramus, inner seta half as long as outer seta. Two lateral setae (setae I and II) and dorsal seta (seta VII) present. Dorsal seta inserted in proximal part of caudal rami.

Antennule relatively short, eight-segmented, far not reaching posterior margin of cephalothorax (Fig. 2b, c). Antennules often hidden under cephalothorax at ventral side (Fig. 2a). Aesthetasc of fourth segment cylindrical with rounded tip, almost reaching apex of antennule. Setal formula: 1, 8, 4, 2+Ae, 1, 3, 2, 7+Ae.

Antenna with allobasis, one-segmented Exp (Fig. 2d) and Endp (Fig. 2e). Allobasis with one seta. Exp with two apical, one subapical and one lateral setae. Last segment of Endp with two lateral spines, two distal spines accompanied by two geniculated setae and one short thin spine at outer corner.

Mandible (Fig. 2f) robust; gnathobase with several teeth and pinnate seta at dorsal corner twice as long as each tooth. Mandibular palp two-segmented, with four setae: proximal segment (basis) without seta, distal Endp segment with four setae (one inserted laterally, three apically).

Maxillule (Fig. 2g) composed of robust praecoxa, coxa and basis. Praecoxal arthrite with seven strong apical spines. Coxa with cylindrical endite and distal long seta. Basis with two distal pinnate setae and several setae proximally. Endp and Exp represented by setae located near base of basis.

Maxilla (Fig. 2h) two-segmented, composed of proximal syncoxa and basis. Syncoxa with rows of outer spinules and two endites, each with two terminal pinnate setae. Basis drawn out into a strong serrate claw with one accompanying seta. Endp represented by three setae.

Maxilliped (Fig. 2i) three-segmented, composed of coxa, basis and one-segmented Endp. Coxa with pinnate seta and several spinules at inner margin. Basis 2.5 times as long as wide, with small outer

spinules equal in length. Endp drawn out into a strong acutely curved claw with seta at base.

P1–P3 with three-segmented Exps and three-segmented Endps (Figs 3a–c).

P1 (Fig. 3a): basis with robust inner and outer spines. Endp-1 longer than two first Exp segments, with setae on inner margin. Endp-1 more than three times as long as Endp-2 and Endp-3, with pinnate seta on inner margin. Endp-3 with two distal armatures: curved strong unipinnate outer spine and thin seta being twice as long as spine. Exp-1, Exp-2 and Exp-3 with strong unipinnate outer spine. Exp-2 with unipinnate inner seta. Exp-2 and Exp-3 without inner armature.

P2 (Fig. 3b): Endp with one seta on inner margin in each of two first segments and two setae on inner margin of terminal segment. Endp-3 with two pinnate setae on inner margin, two pinnate setae on distal margin, and one long unipinnate subapical spine on outer margin. Exp-1 without armature on inner margin and one pinnate spine on outer margin. Exp-2 with one pinnate seta on inner margin and one pinnate spine on outer margin. Exp-3 with one bare seta on inner margin, three pinnate spines on outer margin, and one pinnate seta and one long unipinnate spine on distal margin. Exp-2 segment with inner seta about 1.7 times as long as Exp-3.

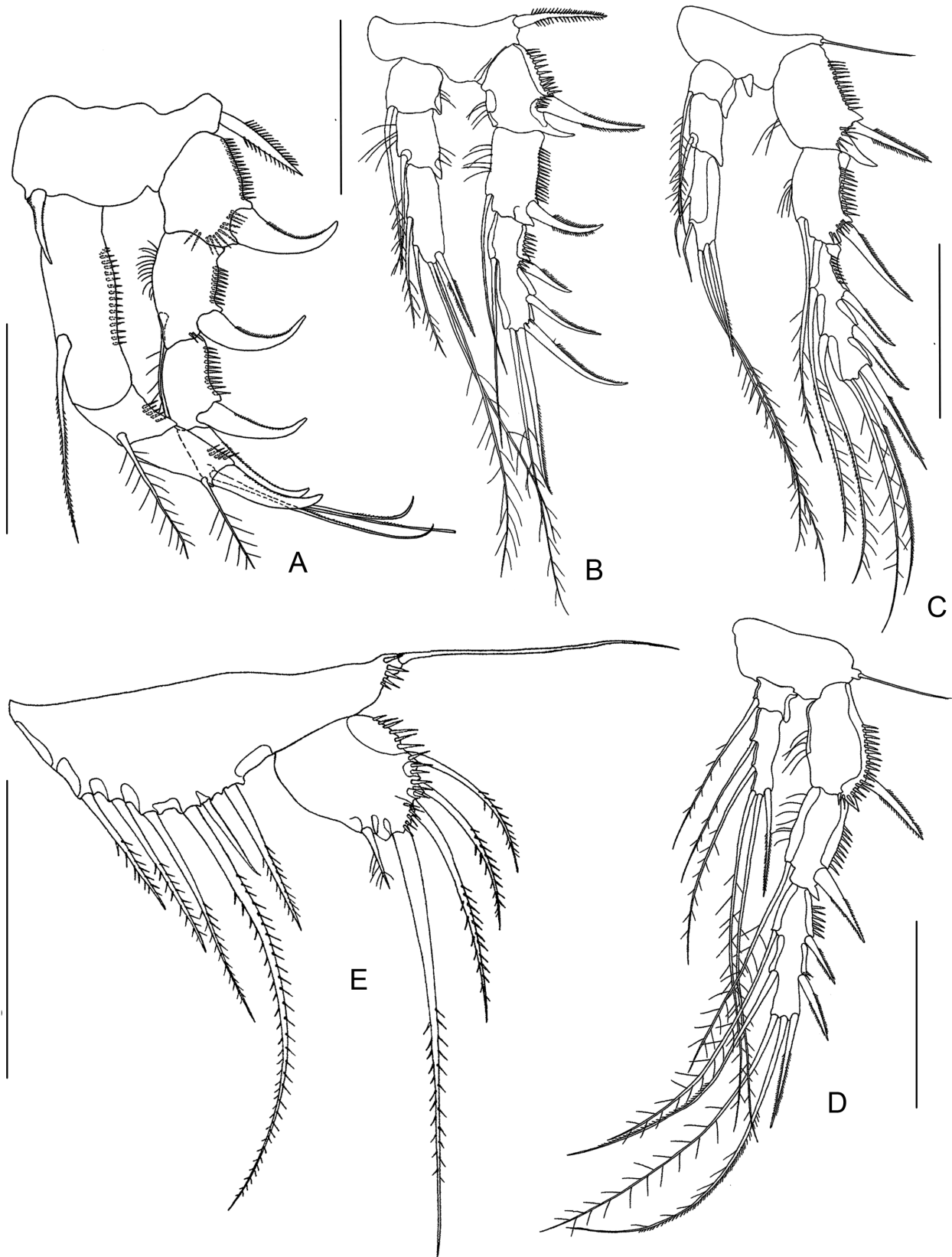
P3 (Fig. 3c) with chaetotaxy alike P2 except following details: basis with a strong short spine on margin between Exp and Endp, setae of inner margin of Endp 3 naked (in P2, pinnate) and distal outer armature transformed into a pinnate seta (in P2, this armature represented by long unipinnate spine).

P4 (Fig. 3d) with three-segmented Exp and two-segmented Endp. Endp-1 with one inner seta, Endp-2 with two inner setae, two long distal setae and one inner-distal spinule being about 0.3 times as long as terminal setae. Exp-2 segment with inner seta being about 1.7 times as long as Exp-3.

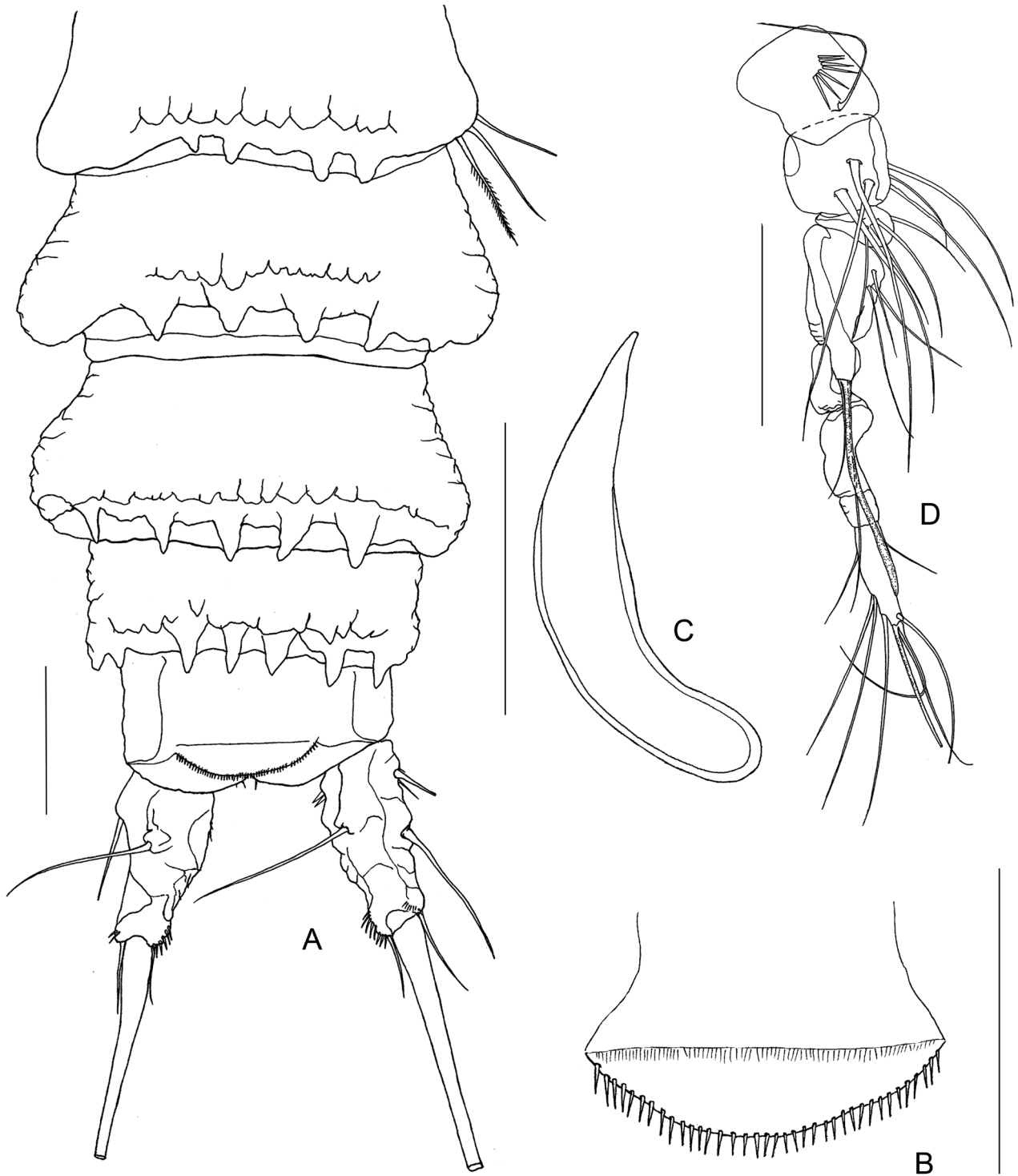
Armature formula (Lang system) of Exps of P2–P4: 3, 3, 1; 3, 2, 1; 3, 2, 2.

P5 (Figs 3e, 6c) with separate Exp and baseoendopod. Baseoendopod wide (width 126  $\mu\text{m}$ ), endopodal lobe almost not projecting upon basal margin of Exp, its maximum length 40  $\mu\text{m}$ . Endopodal lobe with five pinnate spiniform setae and one inner short bare seta, half as long as outer-





**Fig. 3.** *Canthocamptus (Baicalocamptus) longifurcatus* Borutzky, 1947, female (A, C, D, specimen on slide HRP-1; B, E, specimen on slide HRP-4). A, P1; B, P2; C, P3; D, P4; E, P5. Scale bars: 100  $\mu$ m.



**Fig. 4.** *Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947, male (specimen on slide HRP-2). **A**, abdomen, dorsal view; **B**, anal operculum; **C**, spermatophore; **D**, A1. Scale bars: 100  $\mu\text{m}$  (A, C, D), 50  $\mu\text{m}$  (B).

most pinnate seta. Third inner seta of endopodal lobe the longest, about twice as long as fourth one. Outer endopodal seta long, bare. Exp oval, longer

than endopodal lobe, its length 43  $\mu\text{m}$ , width 40  $\mu\text{m}$ . Outer margin of Exp with numerous small spinules, dorsal and ventral surfaces smooth, with-

out armatures. Exp with five pinnate spiniform setae on outer margin, inner seta tiny, second inner seta the longest, more than twice as long as Exp segment. Three outer setae half as long as second inner seta. Two very short bare spines inserted near margin between shortest and longest setae.

P6 (Fig. 1b) fused, small, with short robust pinnate seta on each side of copulatory pore.

*Male* (Figs 4–6, 7b, c, 8). Total length (measured from tip of rostrum to posterior margin of caudal rami) 1150–1300  $\mu\text{m}$  ( $n = 6$ ).

Habitus similar to that of female. Ornamentation of cephalothorax, thoracic somites, abdominal somites and caudal rami similar to those of female (Figs 4a, 5a, 7b, 8a–c). Caudal rami (Figs 4a, 5a) about 2.1 times as long as wide (length 100  $\mu\text{m}$ , width 48  $\mu\text{m}$ ). Anal operculum (Fig. 4b), antenna, mouthparts, P1 (Figs 5b, 7b) and Exps of P2–P3 (Figs 5c, 6a, b) similar to those of female. Spermatophore (Fig. 4c) wide, sacciform. Antennule (Fig. 4d) eight-segmented, geniculate. Aesthetasc on segment 4 not reaching distal margin of segment 8.

Endps of P2–P4 two-segmented.

P2 (Fig. 5c): Endp-1 with one inner seta, one thin long inner setula and outer setulae. Endp-2 three times as long as Endp-1, with one short inner seta, spiniform inner armature, two apical setae and one apical spine; innermost apical seta the shortest, outermost spine the longest.

P3 (Fig. 6a): Endp three-segmented, barely longer than two first Exp segments (Exp-1 and Exp-2) combined. Endp-1 with a long pinnate seta. Endp-2 with a long inner apophysis being as long as all segments of Endp combined. Apophysis finely serrate at inner distal apex. Endp-3 oval, smooth.

P4 (Fig. 6b): Endp two-segmented, shorter than two first Exp segments combined. Endp-1 with one inner seta, Endp-2 with two inner pinnate setae, two apical setae and apical spine; short outer apical spine not modified.

P5 (Fig. 6c) two-segmented, with Exp and baseoendopod. Baseoendopod lobe with outer seta and two pinnate spines, inner about 2.5 times as long as outer one. Baseoendopod lobe with group of several small spinules near base of outer seta. Exp conical with 4–5 spines and small spinules on outer margin. Exp length 40  $\mu\text{m}$ , width up to

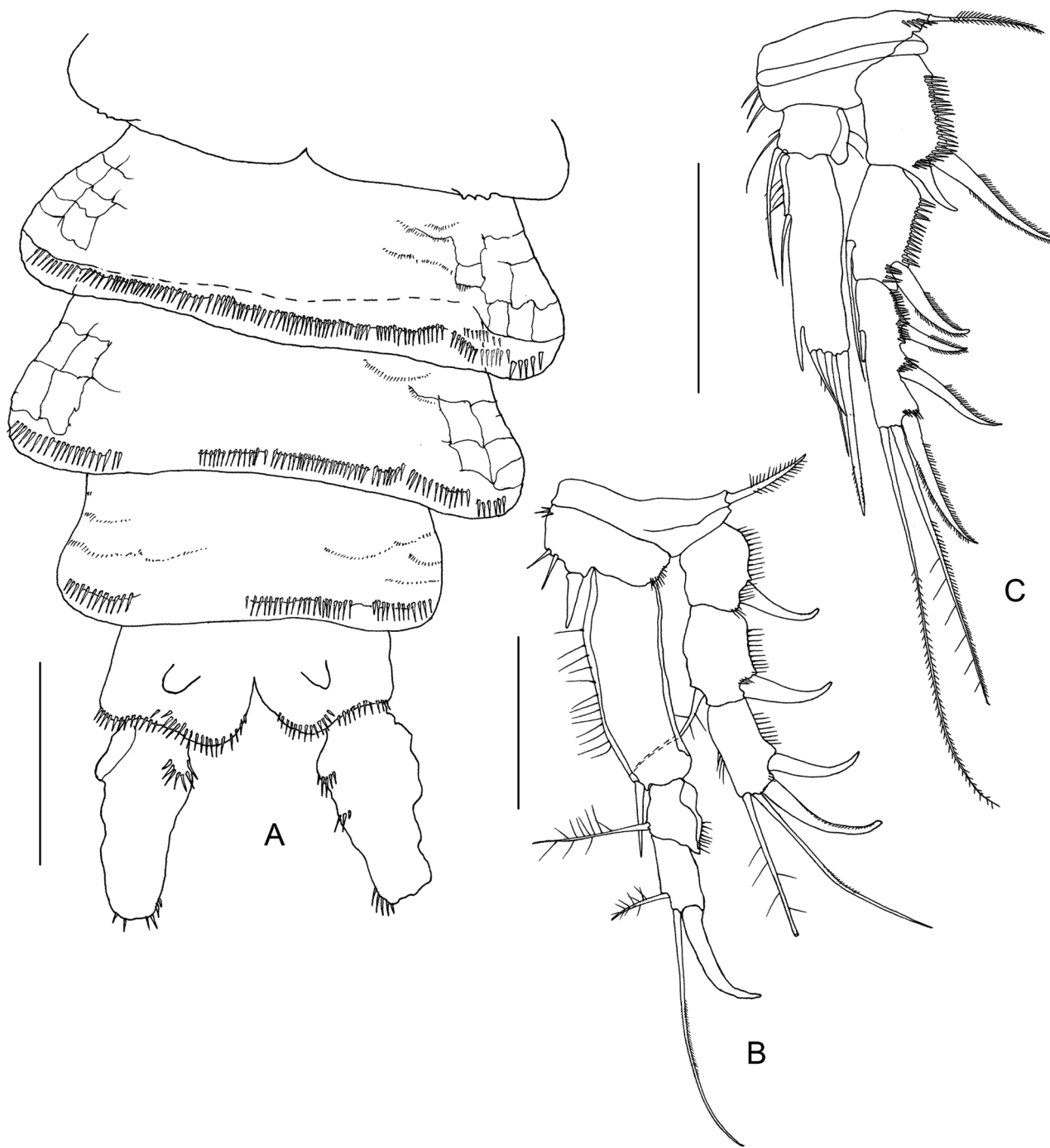
24  $\mu\text{m}$ . Inner apical spine of Exp the longest, about twice as long as outer apical spine; short inner bare spine present on right leg and absent on left one.

P6 (Fig. 6c) consisting of two strong pinnate spines and a short thin bare outer seta. Inner spine the longest.

*Morphological comparison and variability.* Morphology of the examined females agrees with that of *C. (B.) longifurcatus* as described by Borutzky (1947, 1952), in the body shape, structure of A1, Exp of A2, P1–P5, caudal rami, and anal operculum. Morphological characters of the males under study conform to those of *C. (B.) longifurcatus* as described by Okuneva (1989), in the body shape, structure of P1, P3–P5, caudal rami, and anal operculum. The males examined by us differ from the Okuneva's description in the structure of Endp-2: Okuneva (1989) described spiniform inner armature as a short spine whereas we describe it a finger-like armature of the segment (Fig. 5c).

All females and males of *C. (B.) longifurcatus* examined in our study were very similar to each other in the important diagnostic characters. In addition to the variability of Endp-2 in males, we also observed a variability in the number of spines on Exp of male P5. One of the examined males has four spines on Exp of one leg 5 and five on the other, whereas all other 5 males have five spines. According to the description of *C. (B.) longifurcatus* by Okuneva (1989), the males have five spines on Exp of P5 as well.

*Canthocamptus (B.) longifurcatus* can be easily differentiated from *C. (B.) verestschagini* by the armature of the female caudal rami, as well as by the structure of P5 in female and male (Borutzky, 1931, 1952; Okuneva, 1989). According to the description of *C. (B.) verestschagini* in Borutzky (1931, 1952) and Okuneva (1989), the female of the latter species has the middle apical seta of the caudal rami transformed into a short conical spine or tubercle in contrast to a long strong seta which is present in *C. (B.) longifurcatus*. Its Exp of P5 is wider than in *C. (B.) longifurcatus* and covered by the rows of spinules [in *C. (B.) longifurcatus*, the rows of spinules are located on the margin of Exp]. As distinct from *C. (B.) longifurcatus*, Exp of P5 in the male of *C. (B.) verestschagini* is covered by the rows of spinules, same as in the female



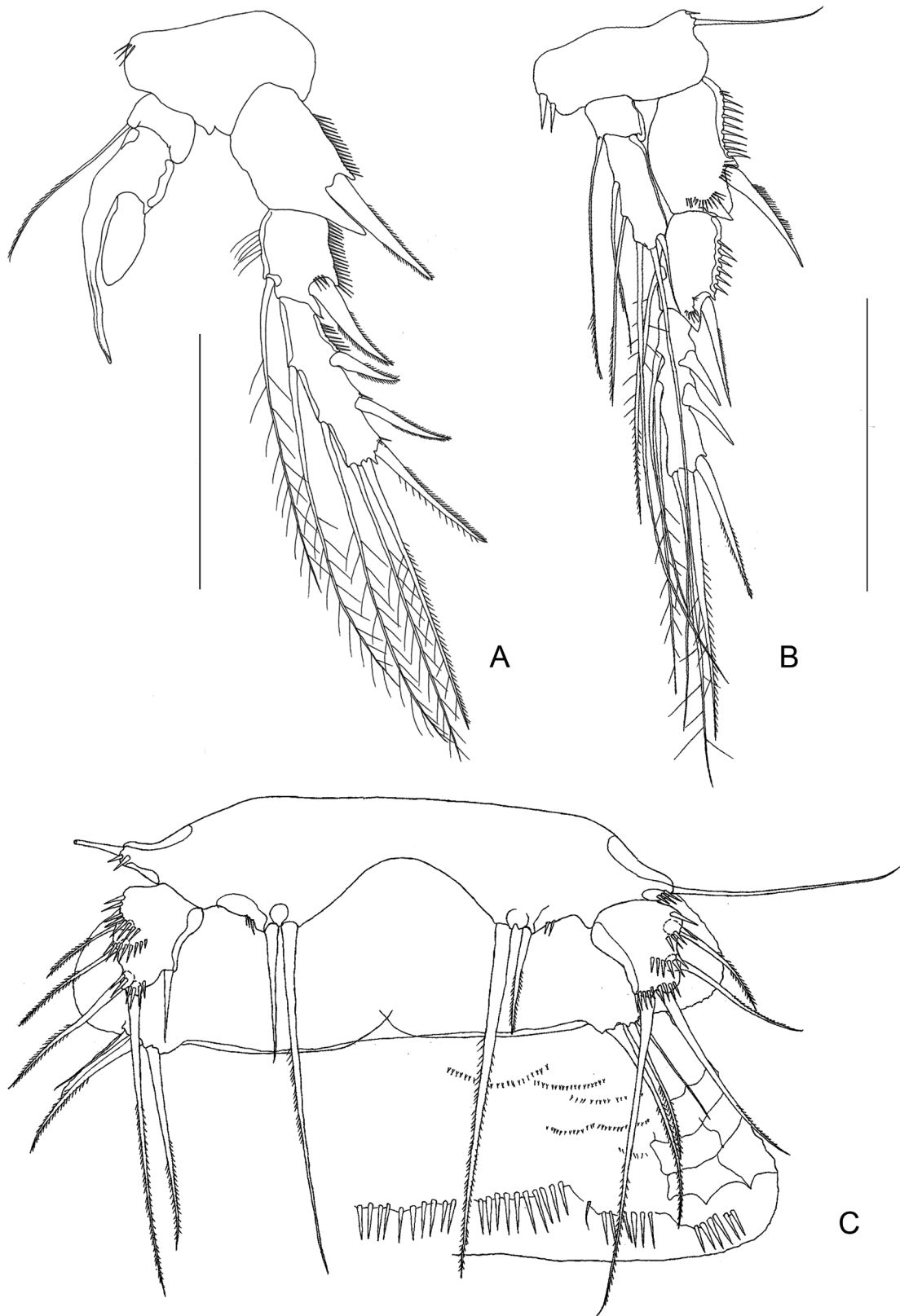
**Fig. 5.** *Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947, male (specimen on slide HRP-2). **A**, abdomen, ventral view; **B**, P1; **C**, P2. Scale bars: 100  $\mu$ m.

and armed by six spines [vs. five in *C. (B.) longifurcatus*].

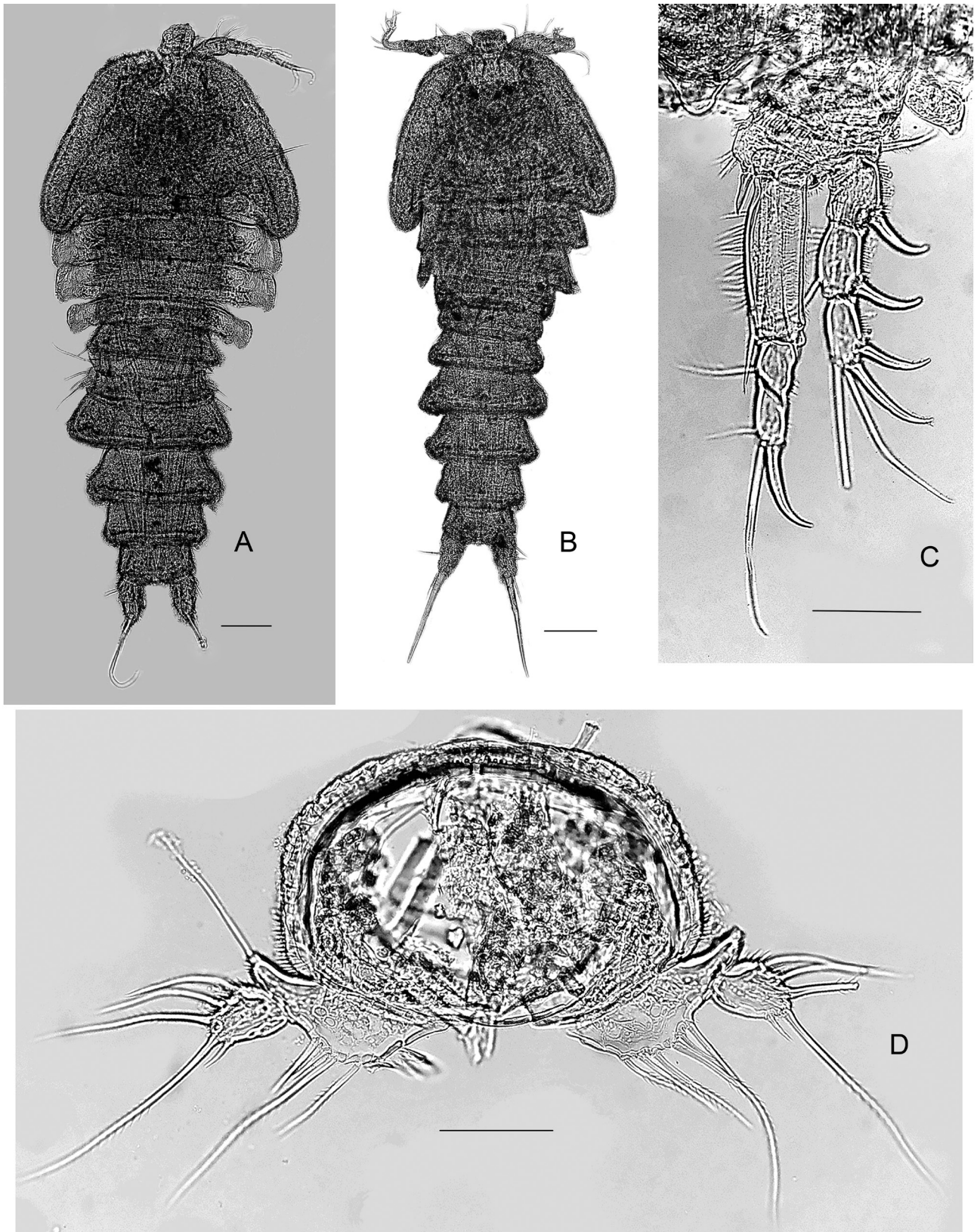
Our analysis of the morphology of the females and males of *C. (B.) longifurcatus* and other non-Baikal species of the genus *Canthocamptus* from Europe, the Urals and North America [*C. (C.)*

*assimilis*, *C. (C.) glacialis*, *C. (C.) microstaphylinus*, *C. (C.) robertcokeri*, *C. (C.) staphylinoides*, *staphylinoides*, *C. (C.) staphylinus staphylinus*, and *C. (C.) cf. vagus*] generally confirmed the presence of a number of common features important for the diagnostics of both subgenera, *Baicalocamptus*





**Fig. 6.** *Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947, male (specimen on slide HRP-2). A, P3; B, P4; C, first abdominal somite with P5 and P6. Scale bars: 100  $\mu$ m.



**Fig. 7.** *Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947, female (D), male (A, B, C) (A, C, specimen on slide HRP-5; B, specimen from ethanol sample; D, specimen on slide HRP-1). A, B, habitus, dorsal view; C, P1; D, P5. Scale bars: 100  $\mu$ m.



and *Canthocamptus* s. str. These characters are the number of segments of the Exps and Endps of P1–P4 in females and males, the armature and morphology of Endp of P2 in males. The shape and size proportions of caudal rami in the studied species were similar. Number and length ratio of the spines on Exps and basoendopods of P5 in females and males were similar as well.

Firstly, our attention is drawn to the difference in structure of P4 Endp in males: in all non-Baikal *Canthocamptus* an inner spine of P4 Endp is modified into a thin finger-like appendage. However, according to Borutzky (1931) this spine is not modified in *C. (B.) longifurcatus* and *C. (B.) verestschagini*. There are some differences in P3 Endp of males: most of non-Baikal species of *Canthocamptus* have long apical setae on the distal segment of P3 Endp, but this segment is smooth in both species of *Baicalocamptus* and in *C. (C.) microstaphylinus*.

**DNA sequences.** Nucleotide sequences of 636 bp COI mtDNA gene region were obtained from five specimens of *C. (B.) longifurcatus*. On the phylogenetic tree, all five sequences formed a single clade located within a larger clade that included other *Canthocamptus* species. The closest relative species to *C. (B.) longifurcatus* was *C. (C.) staphylinus*, with the genetic distance of 29.5% between them, while the genetic distances between the specimens of *C. (B.) longifurcatus* were 2%. (The matrix of genetic distances is provided in Electronic supplementary material 2; see the section “Addenda”).

## Discussion

The representatives of the subgenus *Baicalocamptus* are harpacticoids that have perhaps the most curious phenotype among the endemic Canthocamptidae of Lake Baikal. Based on some features of the body shape (thoracic and abdominal somites of unequal width, strongly protruding lateral edges of the somites forming lobes) and structure of the integument (rough folds and tooth-like formations on the posterior edges of the body segments), *Baicalocamptus* was for a short time regarded as a monotypic genus belonging to a monogeneric family, Baicalocamptidae (Borutzky, 1931). After the description of *C. (B.) longifurcatus*, the status of *Baicalocamptus* was changed:

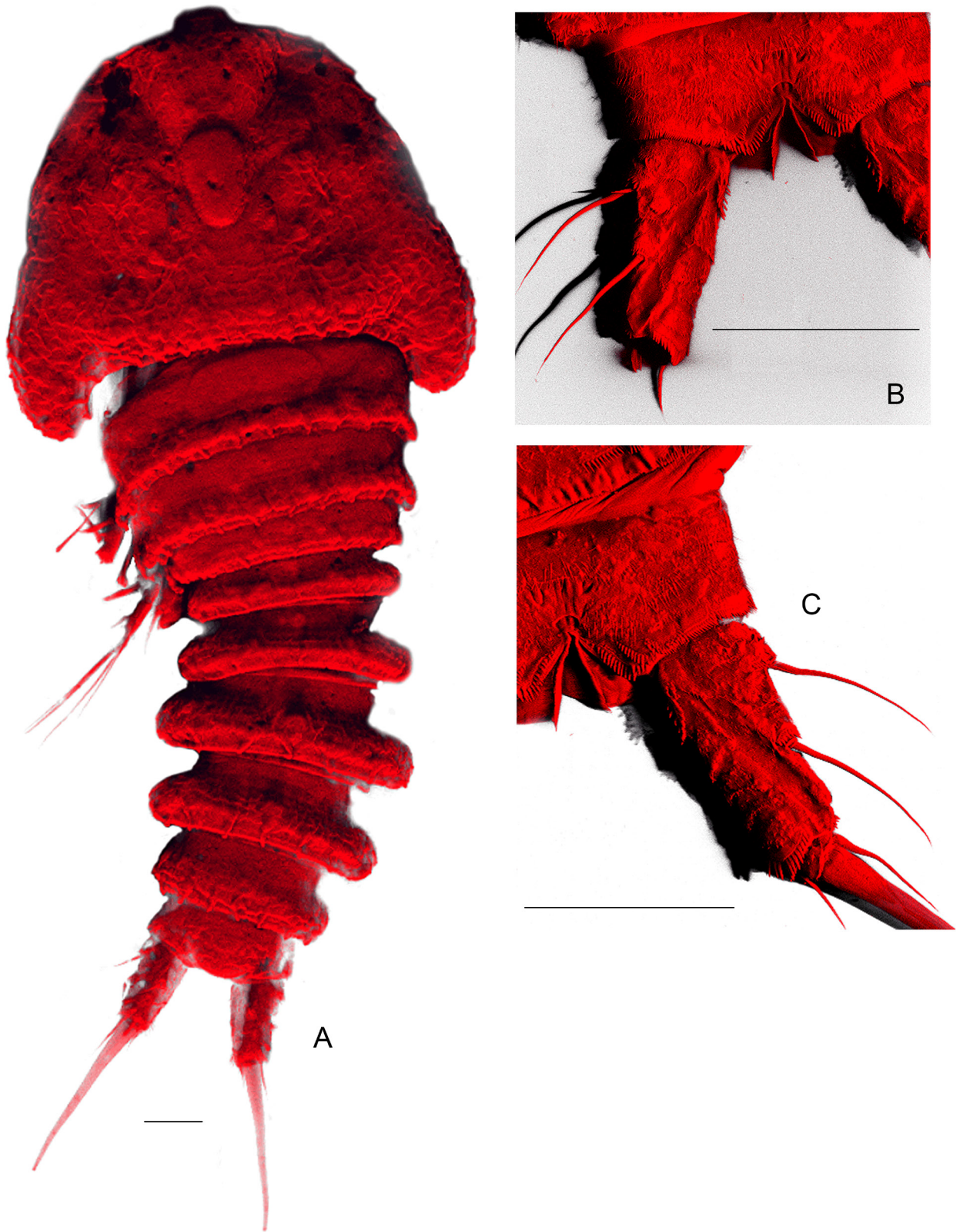
based on an additional analysis of representatives of *Canthocamptus* from Lake Baikal and a high similarity in structure of the genital field of females and P2–P4 of males, a high similarity of *Baicalocamptus* and *Canthocamptus* was confirmed, and the former was accepted as a subgenus of the latter (Borutzky, 1947). The peculiarities of the body shape of its representatives were explained as an adaptation to inhabiting the great depths, although the specimens of *C. (B.) longifurcatus* used in the original description were collected from a depth of only 26 m. According to Okuneva (1989) and Okuneva & Evstigneeva (2001), *Baicalocamptus* is rarely found in Baikal at a depth of less than 5 m.

Our morphological comparison of *C. (B.) longifurcatus* with non-Baikal representatives of the subgenus *Canthocamptus* confirmed their undoubted similarity and, consequently, taxonomic relationship. At the same time, we noticed the differences in the structures of the P3 and especially P4 Endps of males between *Baicalocamptus* and non-Baikal *Canthocamptus*. To understand the significance of this feature for the taxonomy of the genus *Canthocamptus* and to clarify the position and status of the subgenus *Baicalocamptus*, a further detailed study of other Baikal *Canthocamptus*, particularly of the subgenus *Canthocamptus*, is necessary.

Recently, Novikov & Sharafutdinova (2022) synonymised *Baicalocamptus* with *Canthocamptus*.

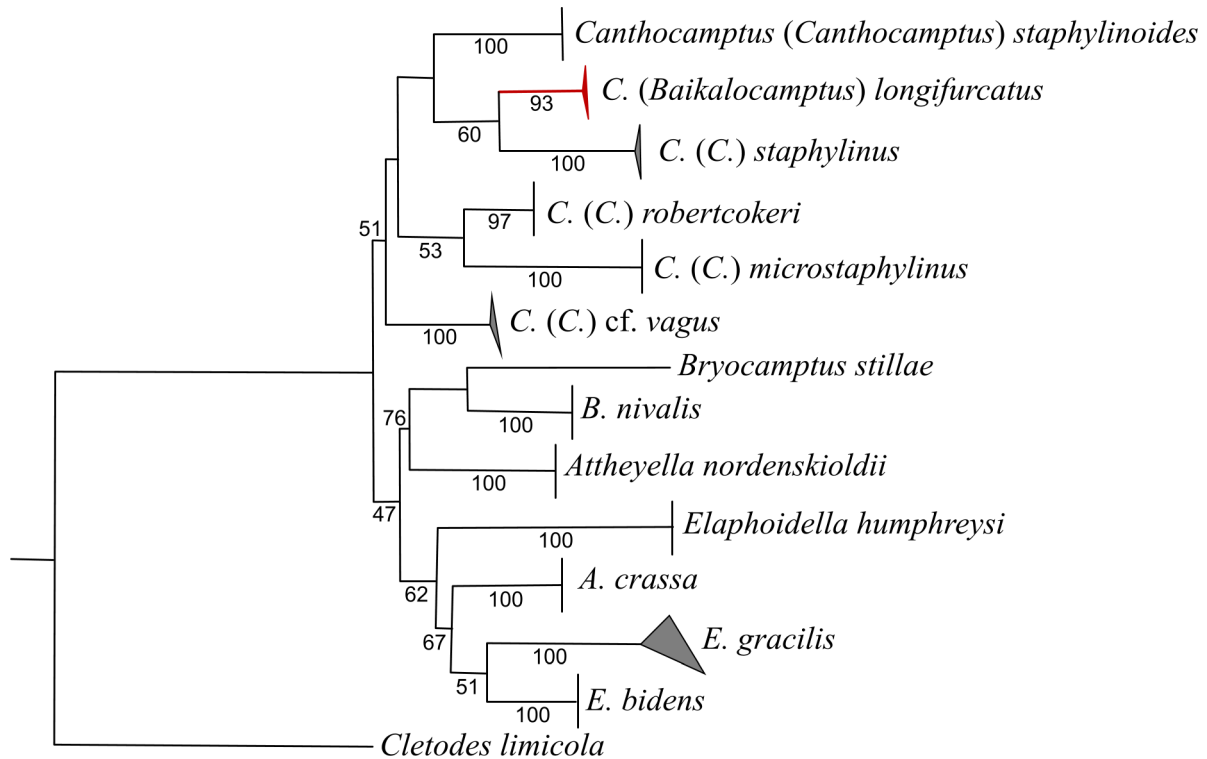
In our opinion, this synonymisation is premature, since investigation of the taxonomic status of *Baicalocamptus* requires a revision of original material of different species in *Canthocamptus* (*Canthocamptus*) and *Baicalocamptus* with “the involvement of a larger number of morphological characters and with the use of molecular data” (Novikov & Sharafutdinova, 2022: 59). Usage of only original descriptions of species, most of which were made in the first half of the 20th century rather than original materials and collection specimens, may lead to erroneous conclusions.

At present, four species of *Canthocamptus* (*Canthocamptus*) are known from Baikal (Okuneva, 1989): *C. (C.) baicalensis* Borutzky, 1947, *C. (C.) bulbifer* Borutzky, 1947, *C. (C.) latus* Borutzky, 1947, and *C. (C.) gibba* Okuneva, 1983,



**Fig. 8.** *Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947, male (CLSM). **A**, habitus, dorsal view; **B, C**, anal somite (partly) and caudal ramus, ventral view. Scale bars: 100  $\mu\text{m}$ .





**Fig. 9.** Maximum likelihood tree based on the sequences of COI mitochondrial DNA gene of *Canthocamptus* (*Baicalocamptus*) *longifurcatus* Borutzky, 1947 and related species. Numbers at the nodes are bootstrap values. As an outgroup, the sequence of *Cletodes limicola* Brady, 1872 (GenBank accession number MH670558) was used. The sequences of the same species were collapsed in one branch. Size of triangles at the end of branches indicates the level of diversity among the sequences.

all being endemic to the lake. However, *C. (C.) gibba* was probably assigned to the genus *Canthocamptus* erroneously: it has a different arrangement of almost all main morphological structures important for identification (Okuneva, 1983), i.e. the caudal rami, female and male P5, and other structures. In Novikov & Sharafutdinova (2022), *C. (C.) gibba* was transferred to the genus *Attheyella* Brady, 1880.

The structural features of Endps of P4 in males of three remaining species of Baikal *Canthocamptus* (*Canthocamptus*) vary between species. In *C. (C.) baicalensis*, the inner spine on the P4 Endp of male is modified into a finger-like appendage (Borutzky, 1952), whereas in two other species it is unmodified. It seems important to study variability of this character in the Baikal *Canthocamptus* (*Canthocamptus*) based on original material, because almost all descriptions of Baikal Canthocamptidae are made at an old (outdated) level and practically are not suitable for adequate compari-

son. It is highly likely that the list of *Canthocamptus* and *Baicalocamptus* species endemic to Baikal can be expanded by describing new representatives, for example, *C. (B.)* sp. 1 and *C. (B.)* sp. 2 (Okuneva & Evstigneeva, 2001) that were found in the northern part of the lake. Descriptions of new species will make it possible to establish clearer boundaries between taxa within the genus.

It is also essential to carry out further genetic barcoding of the harpacticoids of Lake Baikal and to perform a comprehensive analysis based on the comparison of several genetic markers. This study introduces the first molecular genetic data (COI gene of mtDNA) from *C. (B.) longifurcatus* in particular and Lake Baikal harpacticoids in general. The resulting phylogenetic tree should be considered as a draft, since the bootstrap (BS) values of the “intergeneric” branches were relatively low (less than 70), which indicated a generally low reliability of their divergence. Undoubtedly, this is due to the shortcomings of the method (and insuf-

iciency of the data). To be able to better interpret the phylogenetic relationships within the genus and subgenus, genetic data on *C. (B.) longifurcatus* from different parts of Lake Baikal should be included in the analysis. This will allow one to take into account a possible genetic geographical/ecological heterogeneity of the species. It is well known (Timoshkin et al., 2014; Shimaraeva et al., 2018) that the current environmental conditions in the northern and southern parts of Lake Baikal are different due to differences in the level of anthropogenic impact.

## Addenda

*Electronic supplementary material 1.* Sampling localities and GenBank accession numbers of additional sequences used in phylogenetic analysis. File format: PDF. P. s245. Available from: <https://doi.org/10.31610/zsr/2022.31.2.227>

*Electronic supplementary material 2.* Mean COI distances (%) between *Canthocamptus* (*Baicalocamptus*) *longifurcatus* and other *Canthocamptidae* species. File format: PDF. P. s246. Available from: <https://doi.org/10.31610/zsr/2022.31.2.227>

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