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ORIGINAL ARTICLE

Evaluating the effectiveness of DNA barcoding for genetic identification of Canthocamptidae (Harpacticoida, Copepoda) of Lake Baikal

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Abstract Ancient Lake Baikal (LB) is the prime hotspot of the harpacticoid diversity. The Canthocamptidae harpacticoids in the lake can be considered at least six species flocks. The lacks of unequivocal diagnostic characters in the family species description often lead species identification to an impasse. Recently developed molecular techniques provide a powerful tool to subject copepod taxa to comparative analyses. In order to provide a new base for canthocamptids taxonomy and clarify the systematic in some species flocks, we analyzed the mitochondrial DNA marker COI of 103 harpacticoid specimens from LB belonging to five genera. The materials were obtained from different lake basins from the depths 0.2–1632 m. Using DNA barcoding, we identified 23 genetic species or groups of species, it was almost 1.5 times less than number of morphological species, and divergence of some molecular lineages were not corresponding to morphological species. Results of our study were the discovery of a new species, males of some morphological species, for which only females were previously described, clarification of the level of morphological variability of some species, *etc*. Specifically, it became apparent that morphological and genetic harpacticoid species diversity in LB differ significantly and it has to be detected strictly in complex.

Key words Harpacticoid copepods, species identification, COI mtDNA, molecular analysis, Baikalian endemics, Bayesian analysis.

1 Introduction

Ancient Lake Baikal (LB) is probably the most extensively studied freshwater lake of the world. Scientific interest in this lake remains due to the fact that LB is ideal model systems for evolutionary studies. The origins of LB may lie as far in the past as pre-Miocene times, but it situated in tectonically active and geomorphologically unstable region. In this regard the lake is characterized by a highly diverse and largely fauna, heterogeneous in origin and age, hold hundreds of endemic species (Shön & Martens, 2004; Sherbakov *et al.*, 2016). For harpacticoid copepods, the lake, with about 80 known species, the majority of which are endemic, constitutes prime hotspot of the freshwater biodiversity (Boxshall & Defaye, 2008).

The harpacticoid fauna of LB is represented by eight genera of the family Canthocamptidae and one mono-species genus of the Harpacticidae (Okuneva & Evstigneeva, 2001). Canthocamptid species inhabit all the lake depression from the

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upper littoral to the deepest (about 1,640 m) zones, as well as numerous rivers and streams flowing into LB (Okuneva & Evstigneeva, 2001; Fefilova *et al.*, 2023a, b). The majority of Baikalian endemic canthocamptid species are difficult to identify (especially members of large genera *Bryocamptus* and *Moraria*) due their highly morphologically variable (Alekseeva & Timoshkin, 2023; Alekseeva *et al.*, 2023b; Fefilova *et al.*, 2023a; Novikov *et al.*, 2024) and that at least some of them may represent complexes. It is also obvious, that endemic of LB canthocamptid taxa form so-called species flocks (monophyletic groups of species). According to Boxshall & Evstigneeva (1994), there are six of them, the largest of these comprises the members of the subgenus *Moraria* (*Baikalomoraria*). The most unusual feature of the *M.* (*Baikalomoraria*) is the extremely high level of sexual dimorphism.

It must be clear by now that molecular identification of Baikalian harpacticoid species through the analysis of a small fragment of the genome represents one of additional promising approach for the study of their diversity. Like for other members of the animal kingdom a fragment of subunit I of mitochondrial cytochrome *c* oxidase (COI mtDNA or COI) gene must be used with this approach first (Hebert *et al.*, 2003; Ratnasingham & Hebert, 2007). As experience shows (Voronova *et al.*, 2012), it is impossible a priori to predict the level of COI variability even in sufficiently close taxa, such as families of the same order. Studying the structure of the marker in each specific taxon becomes an important part of phylogenetic research.

Over the last years, DNA barcoding (technique based on the COI determination) has broadly used for freshwater harpacticoid copepods identification (Watson *et al.*, 2015; Kochanova *et al.*, 2018; Kochanova & Gaviria, 2018; Rossel & Martinez Arbizu, 2019; Kochanova *et al.*, 2021; Connolly *et al.*, 2022; Rendoš *et al.*, 2023, and others). However, few amounts of molecular data for harpacticoid species from well-known fauna of LB are available in public repositories (Fefilova *et al.*, 2022, 2023b, c; Kochanova *et al.*, 2024). These data relate to one Harpacticidae species (*Harpacticella inopinata* Sars) and three canthocamptid species: *Canthocamptus* (*Baikalocamptus*) *longifurcatus* Borutzky, *Attheyella* (*Neomrazekiella*) *nordenskioldii* (Lilljeborg), *A.* (*Ryloviella*) *baikalensis* Borutzky.

The primary objective of this study is to provide all for the moment DNA barcodes of Baikalian canthocamptid specimens, and evaluate efficiency identification of harpacticoid taxa of LB using the COI analyses. We planned to solve the following tasks: 1) to refine taxonomic status of close in morphology species or forms, 2) using DNA barcoding to identify males for species which they are not known for, 3) to find potentially new for science species, 4) to compare intraspecific and interspecific genetic distances of Baikalian and non-Baikalian canthocamptids.

2 Materials and methods

2.1 Study region

Lake Baikal is located in Siberia (51°29′–55°46′N and 103°43′–109°58′E), at an elevation of 455 m. The lake stretches from north to southwest for 636 km. It lies in the deep structural depression in the center of Baikal Rift Zone, a SW–NE oriented active tectonic zone aligned along the southern margin of the Siberian Platform. Water in LB is extremely low mineralized, with high concentration of dissolved oxygen from the surface to the maximal depth. The vertical gradient in water temperature is narrow: 3.2–4.4°C (Kozhov, 1963).

2.2 Samples collection

The small meiobenthic copepods, members of the order Harpacticoida and family Canthocamptidae (Figs 1A–C) from LB served as material for our study. The materials were collected in 2017, 2022, and 2023 (Table 1). In 2017, samples were taken from aboard a research vessel by the Limnological Institute, Siberian Branch of the Russian Academy of Sciences by T.Y. Sitnikova and T.V. Naumova (Limnological Institute, Siberian Branch of the Russian Academy of Sciences): pieces of bottom sediments at the depths 43–1,632 m were taken by means of a 0.25 m^2 Ocean grab sampler or by a NIOZ-type box core. In 2022, samples were taken from the shallow-water zone (from depths 10–40 m) of the Southern Baikal basin (Fig. 1D) by a small drag and diver equipment (near Bolshie Koty Village). In 2023, the field study was performed on a research vessel of the Baikal Museum of the Siberian Branch of the Russian Academy of Sciences, the benthos samples were taken from the shallow-water zone around LB (Fig. 1D) using drags and a 0.25 m^2 grab.

In 2017, the sediment (benthos samples) was washed through a net of 30 μm and fixed with alcohol in the field. Then these samples were partly sorted by T.Y. Sitnikova and was given to us. In 2022 and 2023, sediment was sieved through a net of 100 μm, harpacticoids were sorted in the field under a stereo microscopes Fieldmicroscope mini of Nikon (Japan) or

Mikromed (China) and then fixed with non-denatured 96% alcohol. Harpacticoid individuals were preserved at -20°C.

2.3 Morphology study

We studied morphology of harpacticoids in two stages. Before DNA extraction, individuals were classified into taxa groups based on morphology, membership of a genus, species or a morphological form was detected. Development stage (mature, copepodit, eggs) and sex were taken into account. Mature specimens only were identified to species level, but not all. To eliminate mistakes and coordinate the results of molecular and morphological data morphology of skeletons of mature harpacticoid individuals were examined after DNA extraction once again.

Most collected specimens were deposited in the scientific collections of the Institute of Biology, Komi Scientic Centre, Ural Branch of Russian Academy of Sciences.

Harpacticoid taxa were identified using the available literature (Borutzky, 1952; Okuneva, 1989; Fefilova *et al.*, 2022; Alekseeva & Timoshkin, 2023; Alekseeva *et al.*, 2023a, b). For identification we took into account structure of female antennule, female and male caudal rami, thoracic legs (exopods and endopods), anal operculum.

The microscope Leica DM 4000 B was used for dissection and harpacticoid morphology analysis. Images of Baikalian harpacticoids were obtained with using of the ZEISS Axio Imager M2 and a digital camera ASUS ZE520KL Phone.

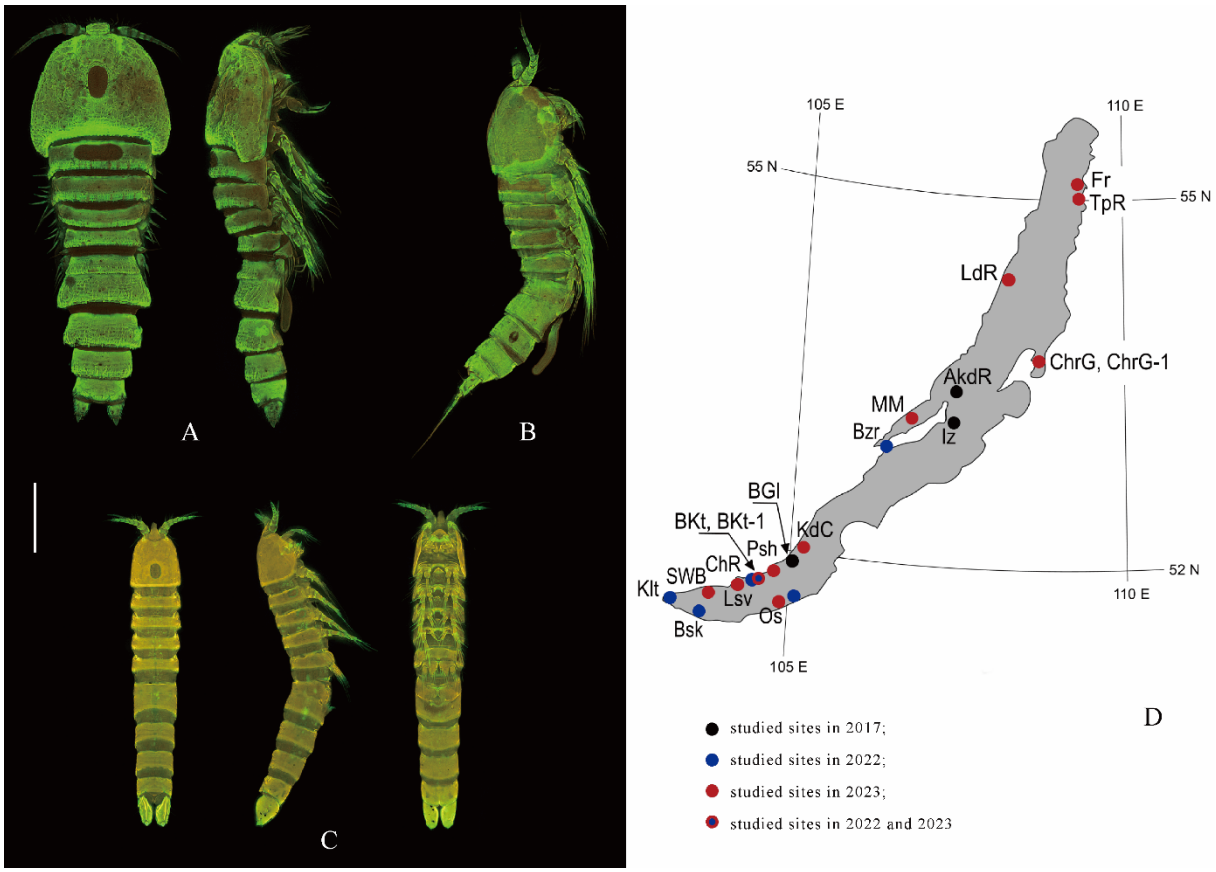

Figure 1. Baikalian endemic harpacticoids and sampling stations. A. Female of *Canthocamptus* (*Baikalocamptus*) *verestschagini* Borutzky, dorsal and lateral views; B. Female of *C.* (*Canthocamptus*) *bulbifer* Borutzky, lateral view; C. Female of *Moraria* (*Baikalomoraria*) sp., dorsal, lateral, and ventral views; D. Map of the locations of sampling stations. Abbreviations see Table 1. Scale bar = 200 μm.

2.4 Molecular analysis

The fragment of COI gene sequence was analyzed for phylogenetic study. DNA was extracted from ethanol-preserved samples as described previously (Kochanova *et al.*, 2018).

The sequence was amplified using several primers (Table 2) in the combinations: LCOI490/HCO2198 (I), ZplankF1_t1/ZplankR1_t1 (II), HCO2198/M1323 (III), and HCO2198/L1384-COI (IV), HCO2612/LCO1384 (V), HCO2612/LCO1490

(VI). For HCO2198/M1323 primer pair, the following PCR cycling conditions were used: 94°C for 9 min, 35 cycles of 94°C for 45 sec, 48°C for 45 sec, 72°C for 60 sec, followed by final elongation at 72°C for 6 min. For all other primer pairs, the following PCR cycling conditions were used: 94°C for 5 min, 5 cycles of 90°C for 30 sec, 45°C for 60 sec, 72°C for 90 sec, followed by 30 cycles of 90°C for 30 sec, 55°C for 45 sec, 72°C for 60 sec, with final elongation at 72°C for 6 min. The "5X ScreenMix-HS" mixture (Evrogen, Russia) was used (3 mM $MgCl_2$, 0.12 mM of each deoxynucleoside triphosphate, 0.3 µM of each primer).

Sequencing was carried out in both directions, using the BigDye Terminator v3.1 (Life Technology) reagent kit in an ABI PRISM 3,500 Genetic Analyzer (Applied Biosystems, Waltham, Massachusetts, USA) in the "Genome" Centre for Collective Use (Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow) or in the "Syntol" company (Moscow). Original nucleotide sequences were deposited at the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/genbank/).

For phylogenetic analyses nucleotide sequences of non-Baikalian harpacticoid species were used (Table 3).

Estimates of Evolutionary Divergence between Sequences were conducted using the Kimura 2-parameter model (Kimura, 1980). This analysis involved 142 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 579 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021).

Table 1. Information regarding investigated samples from LB and Baikal area.

No	Sites	Abbreviations	Date	Coordinates	Depth (m)
1	near Izhimei Cape	Iz	30 June 2017	53.165°N, 107.993°E	1,632
2	Akademicheskiy Ridge	AkdR	8 July 2017	53.415°N, 107.875°E	675
3	near Bolshoe Goloustnoe Village	BGl	3 October 2017	51.583°N, 103.950°E	43–125
4	near Bolshie Koty Village	BKt	25 July 2022	51.902°N, 105.069°E	10–30
5	Chernaya River mouth	ChR	25 July 2022		0.2
6	Gulf Bazarnaya	Bzr	29 July 2022	53.045°N, 106.825°E	6–10
7	near Baikalsk town	Bsk	31 July 2022	51.529°N, 104.147°E	20
8	near Kultuk Village	Klt	31 July 2022	51.712°N, 103.802°E	20
9	near Sukhaya Village	Skh	1 August 2022	°E	14
10	near Listvyanka Village	Lsv	25 May 2023	51.848°N, 104.872°E	0.7
11	near Bolshie Koty Village	B	25 May 2023	51.902°N, 105.069°E	5
12	Maloe More gulf	MM	26 May 2023	53.121°N, 106.999°E	45
13	Chivyrkuysky gulf	ChrG	28 May 2023	53.704°N, 109.062°E	5
14	Chivyrkuysky gulf	ChrG-1	28 May 2023	53.704°N, 109.062°E	20
15	near Frolikha River mouth	Fr	2 June 2023	55.512°N, 109.867°E	11
16	near Tompuda River mouth	TpR	2 June 2023	55.116°N, 109.735°E	30
17	near Peschanaya bay	Psh	25 July 2023	52.240°N, 105.681°E	6–8
18	near Ledyanaya River mouth	LdR	1 August 2023	54.499°N, 108.589°E	5–10
19	South-Western Baikal	SWB	5 August 2023	51.797°N, 104.369°E	5–10
20	near Osinovka Village	Os	6 August 2023	51.505°N, 104.909°E	40
21	near Kadilny Cape	KdC	6 August 2023	51.933°N, 105.258°E	5–7

Table 2. Primers used for the amplification of mtDNA COI gene of harpacticoids from LB.

Primer code	Sequence (5′-3′)	References
H2198-COI	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.*, 1994
L1490-COI	GGTCAACAAATCATAAAGATATTGG	
ZplankF1_t1	TGTAAAACGACGGCCAGTTCTASWAATCATAARGATATTGG	Prosser *et al.*, 2013
ZplankR1_t1	CAGGAAACAGCTATGACTTCAGGRTGRCCRAARAATCA	
L1384-COI	GGTCATGTAATCATAAAGATATTGG	Machida *et al.*, 2004
HCO2612	AGG CCT AGG TGT ATW GGG AAA	
M1323	GAYGAYCARRTTTATAATGT	Karanovic & Cooper, 2012

Table 3. List of non-Baikalian harpacticoid species used for phylogenetic analysis and GenBank accession numbers.

Species	Sites	Barcode index numbers	References
Canthocamptus (*Canthocamptus*) *staphylinus* Jurine	Lake Geneva, Switzerland	MG209735.1–MG209737.1	Kochanova *et al.*, 2018
Pesceus reductus (Wilson)	Lena River delta, Russia	OQ389562–OQ389564	our data, unpublished
Bryocamptus (*Bryocamptus*) *vejdovskyi* (Mrazek)	Lena River delta, Russia	OP970219, OP970220	Fefilova *et al.*, 2023d
	Putorana Plateau, Russia	OP970217	Fefilova *et al.*, 2023d
B. (*Echinocamptus*) *nivalis* (Willey)	Lake Ontario, USA	ZOOPS293-19, ZOOPS294-19	Connolly *et al.*, 2022
Attheyella (*Neomrazekiella*) *dentata* (Poggenpol)	Putorana Plateau, Russia	ON661332–ON661334	Fefilova *et al.*, 2023d
Moraria (*Moraria*) *mrazeki* Scott	Western Sayan Mountains, Russia	OP093567–OP093569	Fefilova *et al.*, 2023d

Table 4. Summary of the number of Baikalian harpacticoid species, individuals and GenBank accession numbers.

No	Species or morphological form	Sites (see Table 1)	No of individuals subjected to DNA barcoding	No of sequences (primers combination: I-VI)	Barcode accession numbers
1	*Canthocamptus* (*Baikalocamptus*) *longifurcatus* Borutzky	BGl	5	5 (I)*	MH824144–MH824146, MZ169062, MZ169063
	—″—	BKt	2	2 (I)	OP963383, OP963384
2	*C.* (*B.*) *verestschagini*	KdC	4	3 (I) + 2 (II)	OR682133–OR682137
3	*C.* (*Canthocamptus*) *latus* Borutzky	BKt	2	1 (II) + 2 (IV)	OR570291, OR570292, OR591462
	—″—	Psh	1	1 (I)	OR672827
	—″—	SWB	3	3 (II)	OR672828–OR672830
	—″—	KdC	2	3 (II)	OR689321, OR689322, OR672828
4	*C.* (*C.*) *bulbifer* Borutzky	KdC	3	3 (II)	OR689318–OR689320
	—″—	SWB	1	1 (II)	OR689323
5	*C.* (*C.*) *baikalensis* Borutzky	Klt	2	1 (V) + 1 (VI)	OR596580, OR613461
6	*Pesceus baikalensis* Borutzky	BKt	1	1 (I)	OR528782
	—″—	Fr	1	1 (II)	OR528783
7	*Bryocamptus* (*Bryocamptus*) *abyssicola* Borutzky & Okuneva	Fr	2	2 (II) + 1 (I)	OR637881–OR637883
	—″—	MM	1	1 (I)	OR724725
8	*B.* (*B.*) sp. 1	Fr	3	2 (I) + 3 (II)	OR636304–OR636308
9	*B.* (*B.*) sp. 2	ChrG-1	2	2 (I) + 1 (II)	OR578791, OR578793, OR578795
10	*B.* (*B.*) sp. 3	Fr	1	1 (I) + 1 (II)	OR575133, OR578792
11	*B.* (*B.*) sp. 4	Os	1	1 (I) + 1 (II)	OR724951, OR724952
12	*B.* (*B.*) *longifurcatus* Borutzky	LdR	8	8 (I)	OR678212–OR678219
13	*B.* (*B.*) *cokeri* Borutzky	Fr	1	1 (I) + 1 (II)	OR575134, OR575135
14	*B.* (*Rheocamptus*) *littoralis* Borutzky & Okuneva	Lsv	1	1 (I)	OR726433

Table 4 (continued)

No	Species or morphological form	Sites (see Table 1)	No of individuals subjected to DNA barcoding	No of sequences (primers combination: I-VI)	Barcode accession numbers
15	*B.* (*R.*) cf. *cristatus* Borutzky & Okuneva	ChrG-1	1	1 (I) + 1 (II)	OR575131, OR575132
16	*B.* (*R.*) sp. 1	Bzr	1	1 (I) + 1 (II) + 1 (III)	OR575128–OR575130
17	*B.* (*Echinocamptus*) *werestschagini* Borutzky	ChrG-1	3	2 (I) + 1 (II)	OR528785–OR528787
	—″—	ChrG	1	1 (II)	OR528784
	—″—	BKt	1	1 (I)	OQ436440
18	*B.* (*E.*) *smirnovi* Borutzky	Bzr	1	1 (II)	OR570290
	—″—	AkdR	1	1 (IV)	OR758769
19	*B.* (*E.*) cf. *parvus* Borutzky	MM	1	1 (I)	OR724950
20	*B.* (*E.*) sp. 1	MM	1	1 (I)	OR578794
21	*Bryocamptus* sp. 1	Iz	1	1 (I)	OP970173
22	*B.* sp. 2	Klt	1	1 (II)	OR570283
23	*B.* sp. 3	Iz	1	1 (I)	OP970174
24	*Attheyella* (*Neomrazekiella*) *nordenskioldii* Lilljeborg	ChR	4	4 (I)**	OQ401014–OQ401016, OR528775
	—″—	Skh	1	1 (I)**	OP903365
25	*A.* (*Ryloviella*) *baikalensis* Borutzky	Skh	4	4 (I)**	OP970221–OP970224
	—″—	BKt	8	4 (I) + 3 (II) + 1 (III)	OR528766–OR528772, OR535313
	—″—	TpR	2	1 (I) + 1 (II)	OR528773, OR528774
	—″—	Bsk	3		OR535308–OR535310
	—″—	MM	2	2 (I)	OR535311, OR535312
	—″—	Os	1	1 (I)	OR674851
26	*Moraria* (*Baikalomoraria*) *brevicauda* Borutzky	BKt	1	1 (I)	OR637880
27	*M.* (*B.*) *longicauda* Borutzky	BKt	1	1 (I)	OQ436439
28	*M.* (*B.*) *werestschagini* Borutzky	Skh	2	2 (I)	OQ436441, OQ436442
29	*M.* (*B.*) cf. *laticauda* Borutzky	Klt	1	1 (II)	OR570289
30	*M.* (*B.*) cf. *spinulosa* Borutzky & Okuneva	Klt			OR570284, OR570285, OR570282, OR570288
31	—″—	BKt	8	4 (I) + 8 (II)	OR608216–OR608227
32	*M.* (*B.*) sp. 1	Bzr	1	1 (I) + 1 (II)	OR570280, OR570286
33	*M.* (*B.*) sp. 2	Klt	1	1 (I) + 1 (II)	OR570281, OR570287

*Published in Fefilova *et al.* (2022); **Published in Fefilova *et al.* (2023b).

Bayesian estimation search was performed using MrBayes (Ronquist *et al.*, 2011) on the CIPRES Science Gateway v.3.1 portal with 5 parallel runs of 50,000,000 generations each, sampling every 1,000 generations. Partitions for the entire molecular dataset were checked using MrModeltest v.2.3 software (Nylander, 2004). The analyses were performed with 0.1 temperature setting. The Bayesian inference analysis of the molecular dataset terminated the search at a MrBayes average standard deviation of split frequencies (ASDSF) that was < 0.01 in all cases. MrBayes consensus phylogram was converted into a graphical tree in iTOL v6 (https://itol.embl.de/). To add Bayesian support values to delimited species on the input tree a Bayesian implementation of the PTP model (Zhang *et al.*, 2013) and GMYC method (Michonneau, 2016) were used.

3 Results

3.1 DNA sequences

Consensus sequences of COI fragments of 103 harpacticoid individuals from the family Canthocamptidae sampled from 21 stations in LB (Fig. 1, Table 1) were analyzed. The 123 DNA barcodes representing morphospecies from five genera and eight subgenera were generated. Most from these barcodes (108) presenting taxonomic units not previously available in GenBank or BOLDSYSTEMS (Table 4).

The primers combinations LCOI490/HCO2198 and ZplankF1_t1/ZplankR1_t1 were the most effective for COI amplification: 59% of sequences was obtained using the LCOI490/HCO2198 combination and 36% using the ZplankF1_t1/ ZplankR1_t1 combination of primers (Table 4). Only 5% of sequences were obtained using other four primer combination. So, the highest sequencing success rate was obtained with one-two primers combinations, but they proved unsuitable for DNA amplification of the members of some species.

The length of three sequences was < 500 bp: the length of the OP963383 (*C.* (*B.*) *longifurcatus*) was 284 bp, the OQ436441 (*M.* (*B.*) *werestschagini*) 358 bp, the OR596580 (*C.* (*C.*) *baikalensis*) 417 bp. The length of other DNA barcodes of the 100 Baikalian harpacticoids specimens ranged between 540 and 671 bp.

3.2 Morphological identification

Members of five genera and nine subgenera of the harpacticoid Canthocamptidae family from LB were analyzed based on morphology. Twenty-one taxa of the species level were detected (Table 4), of which 26 individuals were attributed to five *Canthocamptus* species, 2 to one *Pesceus* species, 22 to eight *Bryocamptus* species, 25 to two *Attheyella* species, and 14 to five *Moraria* species. So we identified on morphological characters 15 (14.6 %) individuals to genus or subgenus level only (Table 4). The reason for the incomplete identification was the presence in individuals of feature inconsistent with the descriptions of known species or poor preservation, underdevelopment of some characters (in copepodites). Unfortunately, some rare individuals from the deep-water zone of LB (*B.* sp. 2, *B.* sp. 3) belonged to this group too. All identified canthocamptids belong to endemic of LB species except *A.* (*N.*) *nordenskioldii*.

All five recoded for LB *Canthocamptus* species were found: two members of the subgenus *Baikalocamptus* and tree members of the nominative subgenus (*Canthocamptus*) (Table 4). Members of these two subgenera diverge from each other in the body shape (Figs 1A–B), integument structure, caudal rami and male endopods structure. There is a problem of separating the two Baikalian *Canthocamptus* species: *C.* (*C.*) *latus* and *C.* (*C.*) *bulbifer*. The main characteristic distinguishing females of them is structure of apical caudal setae. We have identified individuals within one or another steppe curved outer apical seta on each caudal ramus as *C.* (*C.*) *bulbifer*, and individuals with absolutely straight that seta as *C.* (*C.*) *latus* (Fig. S1A). Male of *C.* (*C.*) *bulbifer* is unknown, meanwhile, three male individuals (barcode index numbers in Table 4: OR689321, OR689322, OR672828) with straight apical setae were involved in the analysis. They were attributed to *C.* (*C.*) *latus*.

Bryocamptus are perhaps the most difficult to identify due the smallest size of individuals (0.4–0.6 mm), the largest species diversity in LB and the most individual variability. So after morphological examination we detected 17 species or morphological forms of genus *Bryocamptus* (Table 4). From six of them belonging to the nominative subgenus, only three morphological forms were identified to species level. Not identified *B.* (*B.*) sp. 1, *B.* (*B.*) sp. 2, and *B.* (*B.*) sp. 3 were close to *B.* (*B.*) *abyssicola*, but differed from it in the armament of anal operculum and presence/ absence setae on the first segment of endopods of thoracic legs.

From subgenus *Rheocamptus* we identified in LB two species, and one individual was determined to subgenus level only (Table 4). Members of this *Bryocamptus* subgenus characterized by the two-segmented endopod of the first pair of the

thoracic legs.

Four Baikalian members of the subgenus *Echinocamptus* were analyzed (Table 4). From them *B.* (*E.*) sp. 1 was not identified. It was close to *B.* (*E.*) *parvus*, but had the less number of setae on thoracic legs endopods. It should be noted that *B.* (*E.*) *parvus* and *B.* (*E.*) *smirnovi* are similar in the majority of the morphological characteristics and different mainly in the structure of endopod of the first pair of the thoracic legs. We attributed individuals of this *Echinocamptus* group to *B.* (*E.*) *parvus* group if they had two-segmented endopod of the first pair of the thoracic legs, and to *B.* (*E.*) *smirnovi* if they have that endopod three-segmented.

We primarily paid attention to the structure of the caudal rami for identification of the *Moraria* (*Baikalomoraria*) individuals to species level. Ten individuals of females and males were belonged to the species *M.* (*B.*) *spinulosa* (Table 4). They were with thick, weakly tapering to the distal edge caudal rami and the swollen at the base apical setae of the same length as caudal rami (Fig. S1G, H). At the same sample (near Bolshie Koty Village), we distinguished two morphological groups of *M.* (*B.*) cf. *spinulosa*: with spinules on the inner side of the caudal rami (Fig. S1H) and without them (Fig. S1G). At least females (sometimes males) of other analyzed *Moraria* species: *M.* (*B.*) *werestschagini* (Fig. S1D), *M.* (*B.*) *brevicauda* (Fig. S1E), *M.* (*B.*) *longicauda* (Fig. S1F), and *M.* (*B.*) cf. *laticauda* (Fig. S1I) were different well from each other and *M.* (*B.*) cf. *spinulosa* in structure of the caudal rami and apical setae.

We did not have problem with identification of members of the mono-species genera *Pesceus* and *Attheyella* by morphological features. Twenty specimens of *A.* (*R.*) *baikalensis* from the six locations of the Southern and Northern Basins of LB were similar in the main morphological characteristics. The females of this species from the places near Sukhaya Village and near the Tompuda River mouth have the caudal rami twice as long as other individuals.

3.3 Molecular identification and phylogenetic analysis

All obtained sequences of harpacticoids from LB were analyzed together with sequences of the non-Baikalian canthocamptid species from the common with them genera: *Canthocamptus*, *Pesceus*, *Bryocamptus*, *Attheyella*, and *Moraria*. These sequences were used for Bayesian molecular phylogenetic tree construction (Fig. 2). Two or three sequences for one individual obtained using different primers were also presented on the tree, most often the distances between them were absent, but sometimes amounted to a small value. As expected, these sequences (of different lengths) combined on the tree and thus confirmed each other. The COI of the Baikalian individuals in this study were clustered in most cases according to named species, subgenera and genera. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Although parts of the trees were not well supported (posterior probabilities < 70), but the monophyly of the single species or species groups was supported (posterior probabilities > 95).

All Baikalian and non-Baikalian members of the genus *Canthocamptus* formed a separate genetic group on the phylogenetic tree (Fig. 2). Within this group, the two species of the LB endemic subgenus *Baikalocamptus* were separated as the monophyletic group I. Genetic distances between this group and group II (*Canthocamptus* (*Canthocamptus*)) account more than 20%. Interspecific distances between the sequences of *C.* (*B.*) *verestschagini* were zero, whereas intraspecific distances between the sequences of two *Baikalocamptus* morphological species were reaching 4.6% and were close to the interspecific distances for *C.* (*B.*) *longifurcatus*: 0–3.2% (Table S1).

Species of the subgenus *Canthocamptus* separated from each other on the phylogenetic tree with the exception of *C.* (*C.*) *latus* and *C.* (*C.*) *bulbifer*. These two species formed a common molecular-genetic group with the distances between sequences 0–8.8% (Table S2). It was revealing that distances between sequences of the females of *C.* (*C.*) *latus* (Fig. 1SA) and *C.* (*C.*) *bulbifer* both identified by morphology (Figs 1SB, C) were close to the distances between sequences of the females of one morphological species: 0.2–8.0% (*C.* (*C.*) *latus* and *C.* (*C.*) *bulbifer*), 0.2–8.4% (*C.* (*C.*) *latus* and *C.* (*C.*) *latus*), 0.2–0.7% (*C.* (*C.*) *bulbifer* and *C.* (*C.*) *bulbifer*). It is remarkable that males identified by morphology as *C.* (*C.*) *latus* were very close according genetic distances (0.2–0.9%) to the *C.* (*C.*) *bulbifer* females (Table S2). That means the unknown male of *C.* (*C.*) *bulbifer* was identified using DNA barcoding, it looks like the male *C.* (*C.*) *latus*, and these mentioned *Canthocamptus* morphological taxa with a high probability are one species variable in the female caudal rami structure.

The monophyletic group IV in the phylogenetic three was formed by twenty-six sequences of Baikalian *Bryocamptus* (*Bryocamptus*) members and not identified *Bryocamptus* sp. 1, *B.* sp. 2, and *B.* sp. 3 (Fig. 2). Half of all these sequences have been united into common group with identified species *B.* (*B.*) *abyssicola*. Individuals of this species were differed in 0–0.9% in the COI structure. The position on the phylogenetic tree and the genetic distances values (Table S3) makes it possible to attribute not identified members of *Bryocamptus* subgenera (*B* (*B.*) sp. 1 and *B.* (*B.*) sp. 3) to the species *B.* (*B.*) *abyssicola*. But sequences of *B.* (*B.*) sp. 2 formed a separate group on the three and distances between its sequences and neighboring *B.* (*B.*) *abyssicola* were slightly larger (4.6–5.2%). Probably it indicates that *B.* (*B.*) sp. 2 is a new for science species, it has some morphological distinct characteristics too.

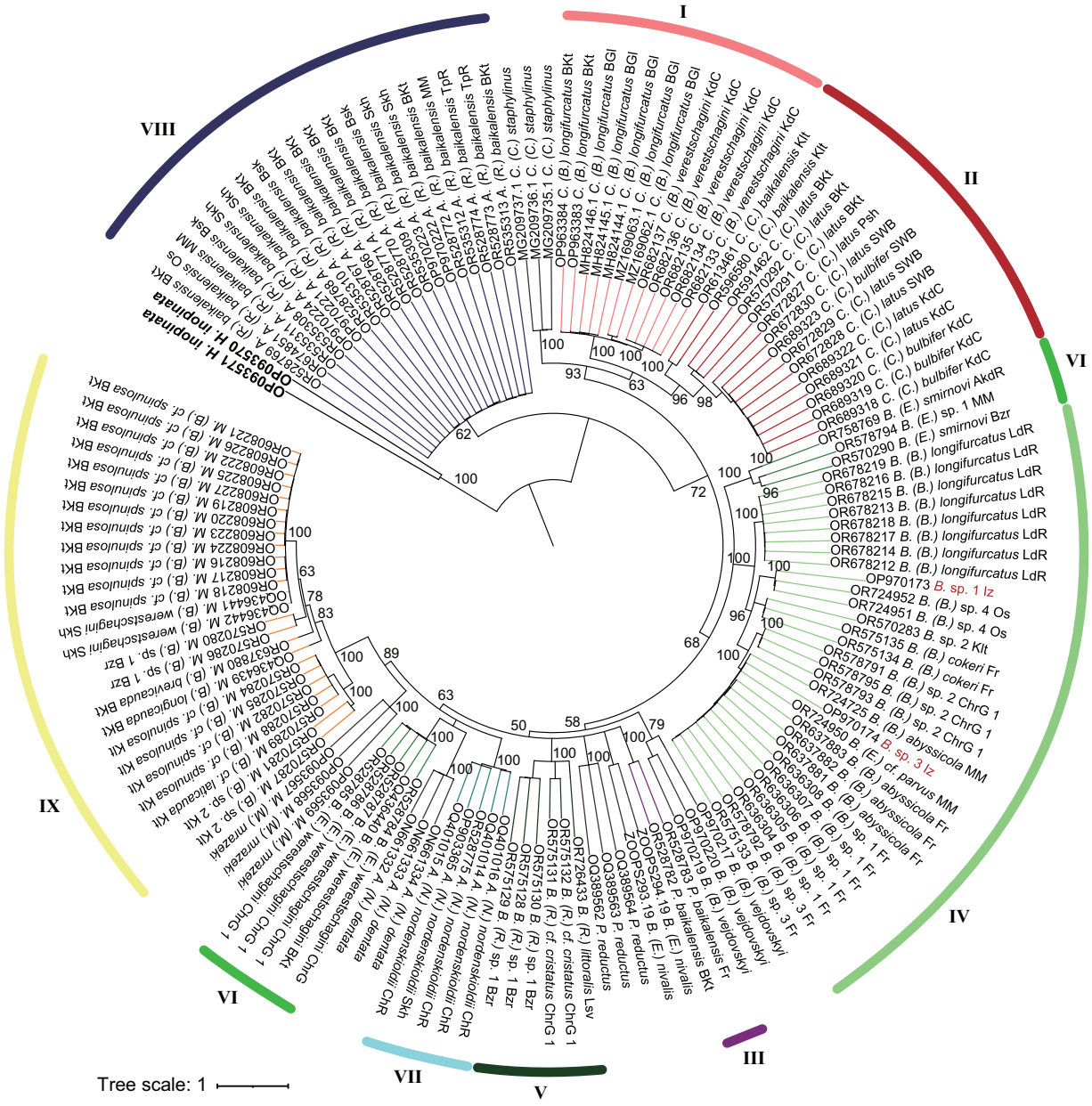

Figure 2. Bayesian molecular phylogenetic tree based on the COI gene. Baikalian harpacticoid sequences are colors highlighted: I. *Canthocamptus* (*Baikalocamptus*); II. *C.* (*Canthocamptus*); III. *Pesceus*; IV. *Bryocamptus* (*Bryocamptus*); V. *B.* (*Rheocamptus*); VI. *B.* (*Echinocamptus*); VII. *Attheyella* (*Neomrazekiella*); VIII. *A.* (*Rhyloviella*); IX. *Moraria* (*Baikalomoraria*). The tree is rooted with *Harpacticella inopinata* Sars (fam. Harpacticidae) haplotypes. Numbers at the nodes are supports values.

Molecular-genetic detection of taxonomical status of the rare harpacticoid specimens from the deep-water zone of LB was the important result of the study. One of these specimens (*B.* sp. 3) from the location near Izhimei Cape at the depth 1,632 m has been identified without morphology testing as *B.* (*B.*) *abyssicola*: was recognized the minimal value of genetic distance 0.4 % (Table S4) between sequences of *B.* sp. 3 and others and it was between this one and the sequences of *B.* (*B.*) *abyssicola* and *B.* (*E.*) cf. *parvus* from the shallow site at the Maloe More gulf (depth 45 m). We also have to admit that most likely the identification by morphology as *B.* (*E.*) cf. *parvus* of an individual from the Maloe More gulf was incorrect. Other analyzed members of the deep-water harpacticoid fauna of LB also have been joined the *Bryocamptus* genetic groups in the phylogenetic tree (Fig. 2). So, *B.* sp. 1 from the depth 1,632 m united with *B.* (*B.*) sp. 4 from the site near Osinovka Village (depth 40 m) to common group: genetic distance between them was 0.6%. Sequence of *B.* (*E.*) *smirnovi* from the site on Akademicheskiy Ridge (depth 675 m) was very close on the tree to the sequences of the same species individual from Gulf Bazarnaya (depth 6–10 m) and *B.* (*E.*) sp. 1 from the Maloe More gulf. But, distances between sequences in the group *B.* (*E.*) *smirnovi*—*B.* (*E.*) sp. 1 were from 5.3 up to 12.0%. It may be indicated presence of high molecular variety in the species *B.*

(*E.*) *smirnovi* in LB or really different close species.

One small group V on the phylogenetic tree was formed by sequences of members of the subgenera *Rheocamptus*. In this group each from three identified and not identified species was well separated from its neighboring species with intraspecific distances 14.3–15.8% (Table S4).

Disjunctive group VI on the phylogenetic tree corresponded to all *Bryocamptus* (*Echinocamptus*) species of which five sequences of four individuals from three samples attributed to *B.* (*E.*) *werestschagini* (Table 4, Fig. 2). Distances (0.3–1.4%) between *B.* (*E.*) *werestschagini* sequences (Table S5) were smaller than in the group *B.* (*E.*) *smirnovi*—*B.* (*E.*) sp. 1 or the group *C.* (*C.*) *latus*—*C.* (*C.*) *bulbifer*, were larger than in *C.* (*B.*) *verestschagini*, and *B.* (*B.*) *longifurcatus* groups of sequences: 0–0.4%.

Phylogenetic analysis of *Moraria* (*Baikalomoraria*) showed presence six genetic subgroups into common for the subgenera group, IX on the tree (Fig. 2). Sequences of identified by morphology *M.* (*B.*) *brevicauda*, *M.* (*B.*) *longicauda* and not identified *M.* (*B.*) sp. 1, *M.* (*B.*) sp. 2 were clustered separately and differed from other *Baikalomoraria* in 5.4–16.9% (Table S6). Other 19 sequences formed two groups with distance into each group <1.1% (Table S6). That indicates unexpected result: well differenced in structure of the female caudal rami *M.* (*B.*) *werestschagini* and *M.* (*B.*) cf. *spinulosa* (from the one of two groups) were nearly identical in COI, and evolutionary divergence between sequences of these morphological species was absent. At the same time, according to the results obtained *M.* (*B.*) cf. *laticauda* and *M.* (*B.*) cf. *spinulosa* from the other group belong to another "genetic species".

Two groups each represented by one *Attheyella* species were very clearly separated on the tree (Fig. 2). Distances between *A.* (*N.*) *nordenskioldii* COI-sequences were 0–2.2%; sequences of *A.* (*R.*) *baikalensis* did not differ or differed by a maximum of 1.9%. So, intraspecific distances of the sequences of Baikalian Canthocamptidae were generally less than 2–3%, but the maximum intraspecific distance existed in *C.* (*C.*) *latus* (up to 8.8%), *C.* (*B.*) *longifurcatus* (up to 3.2%).

Using DNA barcoding data, we identified 23 genetic groups on the phylogenetic tree. It was almost one and a half times less than number of morphological species (Table 4). This nonconformance was primarily related to several not identified by morphological features individuals (*B.* (*B.*) sp. 1, *B.* (*B.*) sp. 3, *B.* (*E.*) sp. 1, *B.* sp. 2, *B.* sp. 3) have been defined as *B.* (*B.*) *abyssicola*, *B.* (*E.*) *smirnovi*, *B.* (*B.*) *cokeri* (Fig. 2). It turned out that *B.* sp. 1 belongs to one species with *B.* (*B.*) sp. 4. The result of phylogenetic analysis numbers of molecular-genetic subgroups into groups II and IX on the tree (*Canthocamptus* and *Baikalomoraria*) have been decreased regarding the predicted one. But according to additional species delimination methods (GMYC and bPTP) the Baikalian members of the *Canthocamptus* genera combined into eight species of which *C.* (*B.*) *longifurcatus* and *C.* (*C.*) *baikalensis* divided to two genetic species each, and the *C.* (*C.*) *latus*, *C.* (*C.*) *bulbifer* group to three genetic species. Three different species in the *B.* (*E.*) *smirnovi* complex were detected by the GMYC and bPTP methods: *B.* (*E.*) *smirnovi* from Gulf Bazarnaya, the same morphological species from Akademicheskiy Ridge, and *B.* (*E.*) sp. 1 from Maloe More gulf. This is not surprising given the differences in genetic distances between sequences in these species groups.

4 Discussion

The need to apply a molecular-genetic approach to the study of the species diversity of Harpacticoida in LB became obvious almost at the same time as it began to be used as an additional taxonomy method (Okuneva & Evstigneeva, 2001). By this point, it was clear that forming by adaptive radiation the taxa and morphological diversity of the Baikalian harpacticoids of the Canthocamptidae family is disproportionate large. So, at the time of the last revision (Okuneva & Evstigneeva, 2001), the number of canthocamptid species known for LB was six times more than for example in Lake Ladoga (Kurashov, 1994) and three–five times more than in the Siberian regions: the Lena River delta (Novikov *et al.*, 2021, 2023), Putorana Plateau (Middle Siberia) (Chertoprud *et al.*, 2022).

The interspecific distances calculated by us for Baikalian endemic canthocamptids were comparable in general to within-species distances for members of this family from geographically disconnected European (Kochanova *et al.*, 2018, 2021) or Siberian (Fefilova *et al.*, 2023d) populations. It is generally known that the distances values correlate with the geographical distances between populations, including in relation to vertical connectivity within deep-sea and shallow-water populations at the oceanic scale (Easton & Thistle, 2016; Taylor & Roterman, 2017). We showed that this dependence should be taken into account in the phylogenetic analysis of harpacticoids inhabit the large (the maximal length 636 km) and the deepest freshwater lake: obtained values of the distances between specimens of one morphological species from the littoral and abyssal zones of LB were sometimes bigger than medium level. And it was previously observed (Fefilova *et al.*, 2023a) that the fauna of Baikalian canthocamptids of deep-water habitats was peculiar: several species from the abyssal zone of LB

could not be identified by morphological characteristics.

One of the explanations why ancient lakes are hot spots of biodiversity is organisms' taxa have a large ecological specialisation through divergent natural selection in condition of a large gradient of ecological niches divergence (Shön & Martens, 2004). So the high molecular diversity of widespread species of Baikalian harpacticoids may be due to the high heterogeneity of bottom habitats in the lake, including the presence of in the abyssal zone sites with gas- and oil-bearing fluids (Fefilova *et al.*, 2023a). At the same time, genetic variation within a single habitat species can have extended phenotype effects on associated ecological communities in a complex and highly diverse natural ecosystem (Zytynska *et al.*, 2011). In this regard, there are studies (Eberl *et al.*, 2007) that the pelagic existence of a marine harpacticoid species has led to less phylogenetic structuring than found in other members of the order in the one geographic scale. Although by the yardstick of genetic divergence in mt DNA genes there is rather poor equivalency of taxonomic rank across copepods in general (Baek *et al.*, 2016), according to the median of many estimates within-species distances for the Harpacticoida order (1.6%) less than for the majority of copepod orders, but between-species mean distance (49.6%) for them is large.

The largest within-species distances values for Baikalian harpacticoids (0.5–24%) were calculated for the sub-endemic member of the Harpacticidae family *H. inopinata* (Fefilova *et al.*, 2023c; Kochanova *et al.*, 2024). Just as the Baikalian calanoid *Epischura baikalensis* Sars, this species probably belongs to the oldest representatives of the fauna of LB, and the estimated time of divergence of *E. baikalensis* and another freshwater non-Baikalian *Epischura* species (from Transbaikalia) is 13.7 million years ago (Zaidykov *et al.*, 2019). Relative to this calanoid species, Baikalian endemic canthocamptids are probably an evolutionarily young group of invertebrates with a fast morphological diversification. It has been long recognized that as a result of adaptive radiation novel phenotypic characteristics and new species emerge at an unusually high rate (Karanovic & Cooper, 2012; Parins-Fukuchi *et al.*, 2021). As shown by our study, phylogenetic assumptions based on analysis of morphological traits of Baikalian cantocamptids (Boxshall & Evstigneeva, 1994) are generally supported by molecular data. Four from six known species flocks formed good supported monophyletic groups on the Bayesian molecular phylogenetic tree: *Canthocamptus* (*Canthocamptus*) + *C.* (*Baikalocamptus*) group, *Bryocamptus* (*Bryocamptus*) group, *B.* (*Rheocamptus*) group, and *Moraria* (*Baikalomoraria*) group. In addition, one flock in LB is formed by three *Morariopsis* species (Karanovic & Abe, 2010). But *Morariopsis* individuals have been not found by us and accordingly were not included in the analysis. There are not any molecular-genetic data on this genus in the GenBank and BOLDSYSTEMS databases. There is not enough data for molecular justification of the *Bryocamptus* (*Echinocamptus*) species flock, on the tree this group looks like polyphelitic. Currently, four *Echinocamptus* species in LB (Okuneva & Evstigneeva, 2001) are known, two of these were identified using DNA-barcoding in this work. But previous (Fefilova *et al.*, 2023a) and present studies have shown that at least in the deep-water zone of the lake it is possible to find new for the science species of this subgenus.

With respect to the *Canthocamptus* group we detected an phylogenomic conflict. On the one hand the data we obtained did not confirm the presence of polyphyly in the genus, which was detected using analysis of morphological features (Novikov & Sharafutdinova, 2022). On the another hand, members of the *Baikalocamtus* and *Canthocamptus* subgenera were grouped on the tree in such a way that it contradicts them taxonomic position. We detected four molecular lines for five species. On the plus side, genetic separation of *A.* (*N.*) *nordenskioldii* within Baikalian harpacticoid species has been confirmed again. At the time this species was known as a *Canthocamptus* species in LB (Okuneva & Evstigneeva, 2001), but recently it was identified as a widespread *Attheyella* species (Novikov & Sharafutdinova, 2022; Fefilova *et al.*, 2023b). It is interesting in the context of assessing the molecular genetic structure of a widespread species that distances between sequences of *A.* (*N.*) *nordenskioldii* from the Baikal area including LB and from the Western Sayan Mountains were 0.2–3.4% (Fefilova *et al.*, 2023b).

The COI marker does not often contain enough phylogenetic signal for taxonomical levels higher than species or subspecies (Voronova *et al.*, 2012; Baek *et al.*, 2016), and some of our results reflect that. So in the most cases, the tree obtained did not show phylogenetic relationships within the genera represented by both Baikalian and non-Baikalian species. And although the phylogenetic species diversity patterns obtained well consistent with morphological diversity patterns, we see problems for some taxa groups.

An unexpected result was found as integrative analysis of the *Moraria* (*Baikalomoraria*) species flock. Eleven from 22 determined in LB and endemic for the lake morphological species of this subgenus have variations between sexes on the shape and armature of the caudal rami (Okuneva & Evstigneeva, 1989). Herewith females have the caudal rami untypical for the *Moraria* genus shape and the middle apical caudal seta reduced or transformed, while the males caudal rami are typical conical with the normal developed middle apical seta: its length is the same or more than the length of the caudal ramus. Males of two *Baikalomoraria* species from this morphological group (*M.* (*B.*) *coronata* Borutzky and *M.* (*B.*) *laticauda*) are unknown. And species identification of *Baikalomoraria* is generally rely upon the female caudal rami structure (Borutzky, 1952; Okuneva, 1989; Alekseeva & Timoshkin, 2023; Alekseeva *et al.*, 2023b). The same kind of sexual

dimorphism was found in a few members of other genera or subgenera in LB: *Moraria* (*Moraria*) *gracilipes* Borutzky & Okuneva, *C.* (*B.*) *verestschagini*, and in *Maraenobiotus slovenicus* Brancelj & Karanovic from temporary karstic spring in central Slovenia (Brancelj & Karanovic, 2015), and *Attheyella* (*Attheyella*) *tahoensis* Bang, Baguley & Moon from Lake Tahoe (North America) (Bang *et al.*, 2015). First, there is the morphological-ecological conclusion that such structure of the caudal rami in different harpacticoid taxa connect with ecological condition in the habitats, namely a consistently low water temperature. In respect of sexual dimorphism, it has evolutionary significance as the female caudal rami structure is important functionally in canthocamptids mating, and that play a role in sexual segregation of species due to sympatric distribution (Boxshall & Evstigneeva, 1994; Ishida, 1994; Novikov *et al.*, 2024). It is remarkable that in none of the samples from LB did at least one couple of mating *Moraria* (*Baikalomoraria*) or *C.* (*B.*) *verestschagini* have not been found. In practical terms this made it very difficult to identify species by morphology. And results of using the DNA barcoding for the genetic identification of members of the *Baikalomoraria* subgenus have been unsatisfactory unfortunately. We do not rule out gaps in the molecular data can be the reason for that. But at least two explanations can be proposed too. First, some morphological Baikalian species of *Baikalomoraria* may be abnormal forms of other species as was estimated in relation to *A.* (*A.*) *tahoensis* (Bang *et al.*, 2015). Second, it cannot be excluded these abnormal females may be hybrid forms. It has been shown (Ronco *et al.*, 2021) that interspecific hybridization plays a certain role in species diversification in adaptive radiation: episodic crossing between members of diverging genetic and morphological lines can further accelerate the emergence of new combinations of features. At the same time, the using of mtDNA analysis is ineffective in identification of interspecific hybrids (Voronova *et al.*, 2012). And it must be said that no such findings of intraspecific hybrids of the Canthocamptidae have been reliably identified, and even among copepods only a couple of similar cases are known (Parent *et al.* 2012; Sukhikh & Fefilova, 2024).

5 Conclusion

As a result of our studies, they were first obtained and deposited to GenBank information on 108 DNA barcodes of canthocamptid harpacticoids of the genera *Canthocamptus*, *Pesceus*, *Bryocamptus*, *Attheyella*, and *Moraria*, of which the majority are endemics of LB. The obtained DNA sequences were used to estimate genetic diversities and phylogenetic relationships at the intra- and supraspecific level of morphologically identified species of this ancient lake, a hot spot of canthocamptids diversity. Certainly general phylogenetic patterns presented are preliminary: it is necessary to use several mitochondrial and nuclear genetic markers to its refine. However, it has been shown that the DNA barcoding is effective approach for finding at least among members of the *Bryocamptus* genus new for science species, a better understanding of Baikalian and non-Baikalian harpacticoid taxonomy, species flocks recognition, and ascertain perspectives of study of harpacticoid fauna diversity in the lake. Specifically, it became apparent that morphological and genetic harpacticoid species diversity in LB differ significantly and it has to be detected strictly in complex.

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Table S1. Genetic distances between the sequences of *C.* (*B.*) *verestschagini* (*C.v.*) and *C.* (*B.*) *longifurcatus* (*C.lg.*) from LB.

Species	Code		1	2	3	4	5	6	7	8	9	10	11
C.v.	OR682133	1											
C.v.	OR682134	2	0.000										
C.v.	OR682135	3	0.000	0.000									
C.v.	OR682136	4	0.000	0.000	0.000								
C.v.	OR682137	5	0.000	0.000	0.000	0.000							
C.lg.	MH824144	6	0.032	0.032	0.032	0.032	0.032						
C.lg.	MH824145	7	0.032	0.032	0.032	0.032	0.032	0.000					
C.lg.	MH824146	8	0.032	0.032	0.032	0.032	0.032	0.000	0.000				
C.lg.	MZ169062	9	0.046	0.045	0.045	0.045	0.045	0.032	0.032	0.032			
C.lg.	MZ169063	10	0.040	0.039	0.040	0.039	0.039	0.030	0.030	0.030	0.009		
C.lg.	OP963383	11	0.033	0.033	0.033	0.033	0.033	0.007	0.007	0.007	0.033	0.033	
C.lg.	OP963384	12	0.035	0.034	0.034	0.034	0.034	0.014	0.014	0.014	0.030	0.029	0.018

Table S2. Genetic distances between the sequences of *C.* (*C.*) *latus* (*C.l.*) and *C.* (*C.*) *bulbifer* (*C.b.*) from LB.

Species	Code													
C.l. ♀	OR570291	1												
C.l. ♀	OR570292	2	0.002											
C.l. ♀	OR591462	3	0.002	0.004										
C.l. ♀	OR672827	4	0.076	0.078	0.072									
C.l. ♂	OR672828	5	0.082	0.084	0.078	0.029								
C.l. ♀	OR672829	6	0.078	0.080	0.074	0.029	0.004							
C.l. ♀	OR672830	7	0.082	0.084	0.078	0.029	0.004	0.004						
C.l. ♀	OR689318	8	0.080	0.082	0.076	0.027	0.009	0.009	0.009					
C.b. ♀	OR689319	9	0.078	0.080	0.074	0.025	0.007	0.007	0.007	0.002				
C.b. ♀	OR689320	10	0.078	0.080	0.074	0.025	0.007	0.007	0.007	0.002	0.000			
C.b. ♀	OR689323	11	0.080	0.082	0.076	0.027	0.002	0.002	0.002	0.007	0.005	0.005		
C.l. ♂	OR689321	12	0.086	0.088	0.082	0.033	0.014	0.014	0.014	0.009	0.007	0.007	0.011	
C.l. ♂	OR689322		0.082	0.084	0.078	0.029	0.011	0.011	0.011	0.005	0.004	0.004	0.009	0.013

Table S3. Genetic distances between the sequences of members of the subgenus *Bryocamptus* (*Bryocamptus*) from LB.

Species*	code													
B.(B.)a.	OR637881	1												
B.(B.)a.	OR637882	2	0.000											
B.(B.)a.	OR724725	3	0.009	0.009										
B.(B.)1	OR636304	4	0.005	0.005	0.007									
B.(B.)1	OR636306	5	0.012	0.012	0.012	0.006								
B.(B.)1	OR636308	6	0.005	0.005	0.007	0.000	0.006							
B.(B.)2	OR578791	7	0.046	0.046	0.046	0.046	0.052	0.046						
B.(B.)2	OR578795	8	0.046	0.046	0.046	0.046	0.052	0.046	0.000					
B.(B.)3	OR575133	9	0.010	0.010	0.013	0.005	0.010	0.005	0.055	0.055				
B.(B.)4	OR724951	10	0.138	0.138	0.133	0.138	0.144	0.138	0.154	0.154	0.140			
B.(B.)lg.	OR678212	11	0.134	0.134	0.127	0.132	0.139	0.132	0.135	0.135	0.140	0.150		
B.(B.)lg.	OR678213	12	0.132	0.132	0.125	0.130	0.137	0.130	0.133	0.133	0.137	0.148	0.002	
B.(B.)ck.	OR575134	13	0.122	0.122	0.121	0.125	0.134	0.125	0.125	0.125	0.127	0.129	0.135	0.133

*Abbreviations: *B.*(*B.*)*a.*—*B.* (*B.*) *abyssicola*; *B.*(*B.*)1—*B.* (*B.*) sp. 1; *B.*(*B.*)2—*B.* (*B.*) sp. 2; *B.*(*B.*)3—*B.* (*B.*) sp. 3; *B.*(*B.*)4—*B.* (*B.*) sp. 4; *B.*(*B.*)*lg.*—*B. (B.) longifurcatus*; *B.*(*B.*)*ck.*—*B.* (*B.*) *cokeri*.

Table S4. Genetic distances between the sequences of members of the subgenus *Bryocamptus* (*Rheocamptus*), not identified to subgenus level members of the *Bryocamptus* genus and *B.* (*B.*) *abyssicola* from LB.

Species*	Code		1	2	3	4	5	6	7	8	9
B.(R.)l.	OR726433	1									
B.(R.)cr.	OR575131	2	0.146								
B.(R.)cr.	OR575132	3	0.146	0.000							
B.(R.) sp.1	OR575128	4	0.158	0.148	0.148						
B.(R.) sp.1	OR575129	5	0.158	0.148	0.148	0.000					
B.(R.) sp.1	OR575130	6	0.158	0.143	0.143	0.008	0.008				
B. sp.1	OP970173	7	0.245	0.267	0.267	0.281	0.281	0.276			
B. sp.2	OR570283	8	0.244	0.254	0.254	0.254	0.254	0.240	0.139		
B. sp.3	OP970174	9	0.249	0.260	0.260	0.259	0.259	0.244	0.126	0.113	
B.(B.)a.	OR724725	10	0.262	0.263	0.263	0.264	0.264	0.258	0.135	0.117	0.004

*Abbreviations: *B.(R.)l.—B. (R.) littoralis*; *B.(R.)cr.—B. (R.) cristatus*; *B.(B.)a.—B. (B.) abyssicola*.

Table S5. Genetic distances between the sequences of members of the subgenus *Bryocamptus* (*Echinocamptus*) and not identified to subgenus level members of the *Bryocamptus* genus from LB.

Species*	Code											
B.(E.)w.	OR528784	1										
B.(E.)w.	OR528785	2	0.014									
B.(E.)w.	OR528786	3	0.010	0.003								
B.(E.)w.	OQ436440	4	0.010	0.014	0.011							
B.(E.)s.	OR570290	5	0.256	0.252	0.251	0.247						
B.(E.)s.	OR758769	6	0.241	0.242	0.236	0.239	0.116					
B.(E.)p.	OR724950	7	0.252	0.252	0.252	0.243	0.265	0.246				
B. (E.) sp.1	OR578794	8	0.266	0.261	0.261	0.255	0.053	0.120	0.277			
B. sp.1	OP970173	9	0.251	0.256	0.253	0.251	0.239	0.242	0.125	0.262		
B. sp.2	OR570283	10	0.253	0.263	0.257	0.250	0.258	0.270	0.116	0.268	0.139	
B. sp.3	OP970174	11	0.252	0.252	0.252	0.246	0.262	0.246	0.004	0.278	0.126	0.112

*Abbreviations: *B.(E.)w.—B. (E.) werestschagini*; *B.(E.)s.—B. (E.) smirnovi*; *B.(E.)p.—B. (E.)* cf. *parvus*.

Table S6. Genetic distances between the sequences of some Baikalian members of the *Moraria* (*Baikalomoraria*) subgenus from LB.

Species*	Code												
M.b. ♀	OR637880	1											
M.lg. ♂	OQ436439	2	0.144										
M.w. ♀	OQ436441	3	0.144	0.111									
M.w. ♀	OQ436442	4	0.130	0.125	0.008								
M.lt. ♀	OR570289	5	0.161	0.144	0.134	0.158							
M.s. ♀	OR570284	6	0.161	0.159	0.134	0.158	0.000						
M.s. ♂	OR570282	7	0.161	0.159	0.134	0.158	0.002	0.002					
M.s. ♂	OR608218	8	0.137	0.124	0.011	0.006	0.156	0.156	0.156				
M.s. ♂	OR608219	9	0.134	0.123	0.006	0.008	0.150	0.150	0.150	0.010			
M.s. ♀	OR608223	10	0.131	0.123	0.006	0.006	0.150	0.150	0.150	0.008	0.002		
M.(B.) sp.1	OR570280	11	0.130	0.116	0.101	0.108	0.179	0.179	0.179	0.108	0.112	0.109	
M.(B.) sp.2	OR570281	12	0.149	0.143	0.138	0.150	0.054	0.055	0.054	0.149	0.148	0.147	0.169

*Abbreviations: *M.b.—M. (B.) brevicauda*; *M. lg.—M. (B.) longicauda*; *M.w.—M. (B.) werestschagini*; *M.lt.—M. (B.)* cf. *laticauda*; *M.s.—M. (B.)* cf. *spinulosa*.

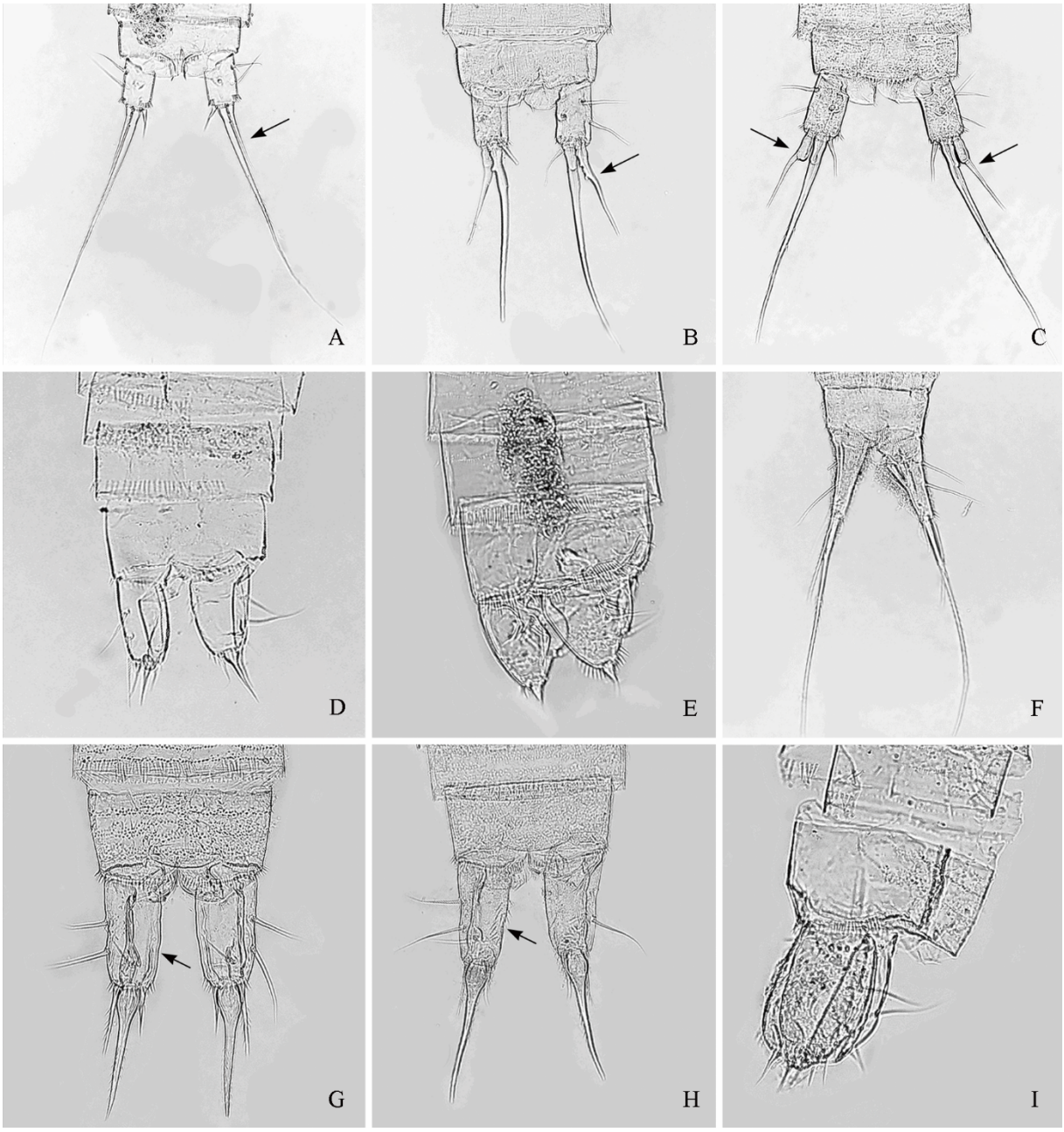

Figure S1. Photo of caudal rami of canthocamptid females and male (*M.* (*B.*) *longicauda*) from LB stored in the slides after DNA analysis. A. *C.* (*C.*) *latus* (OR672830); B–C. *C.* (*C.*) *bulbifer* (OR689319, OR689318); D. *M.* (*B.*) *werestschagini* (OQ436441); E. *M.* (*B.*) *brevicauda* (OR637880); F. *M.* (*B.*) *longicauda* (OQ436439); G. *M.* (*B.*) cf. *spinulosa* without spinules on inner sides of caudal rami (OR608219, OR608220); H. *M.* (*B.*) cf. *spinulosa* with spinules on inner sides of caudal rami (OR608223, OR608224); I. *M.* (*B.*) cf. *laticauda* (OR570289). Barcode accession numbers in brackets. Some features shown by arrows.