ORIGINAL ARTICLE

Copepoda of lowland springs: diversity patterns and integrative taxonomy of *Cyclops*

Sabina Smolska,[1*] Maciej Karpowicz,[2] Magdalena Świsłocka-Cutter,[3] Elżbieta Jekatierynczuk-Rudczyk,[4] Maria Hołyńska[5]

[1]Doctoral School, University of Bialystok; [2]Department of Hydrobiology, Faculty of Biology, University of Bialystok; [3]Department of Zoology and Genetics, Faculty of Biology, University of Bialystok; [4]Department of Environmental Protection, Faculty of Biology, University of Bialystok; [5]Museum and Institute of Zoology, Polish Academy of Sciences, Warszawa, Poland

ABSTRACT

Groundwater copepods in lowland Europe remain insufficiently studied compared to those from mountainous regions. Here, we present the results of a faunistic and ecological survey of lowland springs, conducted in the context of earlier research on groundwater copepods from over 100 wells in the same region. Springs, as natural interfaces between aquifers and surface waters, provide diverse habitats and favorable conditions for groundwater-affiliated fauna. We examined copepod assemblages in 34 lowland springs in northeastern Poland, representing rheocrene, limnocrene, and helocrene types, which were sampled in summer and autumn. We identified a total of 23 species of Copepoda, comprising 13 Cyclopoida and 10 Harpacticoida. Most of these species were associated with hyporheic or groundwater habitats. Cyclopoida dominated in terms of abundance, especially in limnocrene springs, and showed little seasonal variation, while Harpacticoida exhibited markedly higher diversity and abundance in summer. The Cyclopoida assemblage was dominated by *Eucyclops serrulatus*, *Diacyclops bicuspidatus*, and *Cyclops strenuus*, whereas Harpacticoida were represented mainly by *Attheyella crassa*, *Canthocamptus staphylinus*, and by six *Bryocamptus* species. Many of the common copepod species were also found in groundwater (wells) in the same region. Still, overall richness, particularly of Harpacticoida, was higher in springs due to the presence of heterogeneous benthic microhabitats. We identified three *Cyclops* species in the springs that were not recorded in the groundwater (wells) of this region. Conversely, we did not detect *C. furcifer* in the studied springs, despite its presence in nearby wells and temporary puddles. To corroborate morphology-based identifications of the *Cyclops* species, we applied an integrative taxonomic approach combining morphological traits with the amplification and sequencing of 12S rRNA and ITS-1 markers. We confirmed the presence of three distinct species: *C. strenuus*, *C. insignis*, and *C. borealis* (a senior synonym of *C. heberti*), for which we provide descriptions of key morphological traits, along with molecular data. These findings highlight the function of lowland springs as ecotonal habitats fostering distinct copepod assemblages, including taxa characteristic of both groundwater and benthic microhabitats.

*Corresponding author: s.smolska@uwb.edu.pl

Key words: groundwater; hyporheic zone; meiobenthos; Cyclopoida; Harpacticoida; *Cyclops*.

Citation: Smolska S, Karpowicz M, Świsłocka-Cutter M, *et al.* Copepoda of lowland springs: diversity patterns and integrative taxonomy of Cyclops. *J Limnol 2025;84:2242.*

Edited by: Diego Fontaneto, *CNR-IRSA Water Research Institute, Verbania-Pallanza, Italy.*

Contributions: SS, Copepoda identification, field studies, formal analysis, visualization, manuscript original drafting; MK, conceptualization, Copepoda identification, field studies, formal analysis, visualization, supervision, manuscript original drafting; MŚC, genetic analysis, visualization, manuscript original drafting; EJR, field studies, manuscript review and editing; MH, Copepoda identification, supervision, manuscript review and editing. All authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare no competing interests, and all authors confirm accuracy.

Received: 24 July 2025.
Accepted: 8 September 2025.

J. Limnol., 2025; 84:2242
DOI: 10.4081/jlimnol.2025.2242

INTRODUCTION

Copepods are widespread and ecologically significant components of groundwater ecosystems, contributing substantially to the biodiversity of subterranean aquatic habitats. Over 1000 species have been documented from continental groundwater environments, where they play key roles in ecosystem functioning and may serve as indicators of groundwater quality and surface-subsurface hydrological connectivity (Galassi *et al.*, 2009; Castaño-Sánchez *et al.*, 2021; Iannella *et al.*, 2020).

In contrast to the relatively well-studied mountain regions of Europe, such as the Dinaric Alps, the Pyrenees, or the Carpathians, where groundwater fauna often exhibit high levels of endemism (Rouch, 1994; Galassi, 2001; Ferreira *et al.*, 2007; Dumnicka *et al.*, 2020; Iannella *et al.*, 2020), the lowland areas of Northern and Central Europe remain insufficiently investigated (Martin *et al.*, 2009; Smolska *et al.*, 2024). Nevertheless, there is evidence that Pleistocene glaciations impoverished groundwater fauna in Central and Northern Europe (Deharveng *et al.*, 2009; Martin *et al.*, 2009), as well as in North America (Strayer *et al.*, 1995; Gibert and Culver, 2009). Polish data follow this pattern, with most stygobiotic species found in the unglaciated Carpathian region, while records from post-glacial lowlands are sparse (Dumnicka and Galas, 2017; Dumnicka *et al.*, 2020; Pociecha *et al.*, 2021). Our previous research conducted in over 100 groundwater wells in northeastern Poland corroborates these trends (Karpowicz *et al.*, 2021; Smolska *et al.*, 2024). Within the entire copepod assemblage, only a single stygobiont species, *Elaphoidella elaphoides* (Karpowicz, 2016), was recorded, occurring at just one locality. Most of the identified taxa in wells were classified as stygophiles or stygoxenes, reflecting a low degree of groundwater specialization in these postglacial lowland environments. Despite the limited presence of obligate groundwater species, the overall copepod community composition appeared relatively stable, with a consistent set of species repeatedly observed across multiple wells.

While wells provide valuable insights into the structure of groundwater communities, they represent artificial and spatially restricted environments. In contrast, springs function as natural discharge zones and hydrological interfaces between groundwater and surface aquatic systems, offering more diverse and ecologically heterogeneous habitats. These ecotone zones enable the study of copepod assemblages shaped by both subterranean origin and surface influences. The zone surrounding the spring mouth, together with its rivulets and shallow pools, commonly referred to as the "eucrenal" (Brittain and Eikeland, 1988), supports copepod assemblages composed of three ecological groups. These include benthic species living in surface streams (epirhithral fauna), stygobionts emerging from the aquifer and occupying interstitial sediments near the spring source, and crenobionts, which occur exclusively or preferentially in spring environments (Stoch, 2007). Truly spring-restricted copepods are relatively rare, and the faunal composition of springs often reflects a combination of surface and subsurface elements (Stoch, 2007).

Springs vary significantly in their geomorphological and hydrological characteristics, which consequently influence local environmental conditions. Rheocrene springs produce flowing brooks, limnocrene springs form small pools or ponds at the outflow point, and helocrene springs are characterized by diffuse seepage zones, usually surrounded by marshy vegetation. These types differ in substratum composition, flow regime, oxygen availability, thermal conditions, and habitat structure. Such variability influences copepod species richness, community composition, and the proportion of stygobiotic vs. surface-associated taxa (Cerasoli *et al.*, 2023; Zhai *et al.*, 2015).

Comparative studies from different parts of Europe indicate that springs across regions such as Italy, including Sicily, the Czech Republic, and Finland share a surprisingly similar assemblage of core copepod species. Research on karst springs in the Central Apennines has reported 48 copepod species, with nearly half being obligate groundwater dwellers, whereas the remaining taxa exhibit broad ecological tolerance and occur across diverse spring types (Cerasoli *et al.*, 2023; Pendino *et al.*, 2024). In the Western Carpathians, surveys of helocrene spring fens recorded 20 harpacticoid species, whose composition was shaped by environmental variables but remained relatively consistent across sites (Zhai *et al.*, 2015; Výravský *et al.*, 2023). Similarly, in Finland, studies demonstrated that thermal stability promotes richer cope-

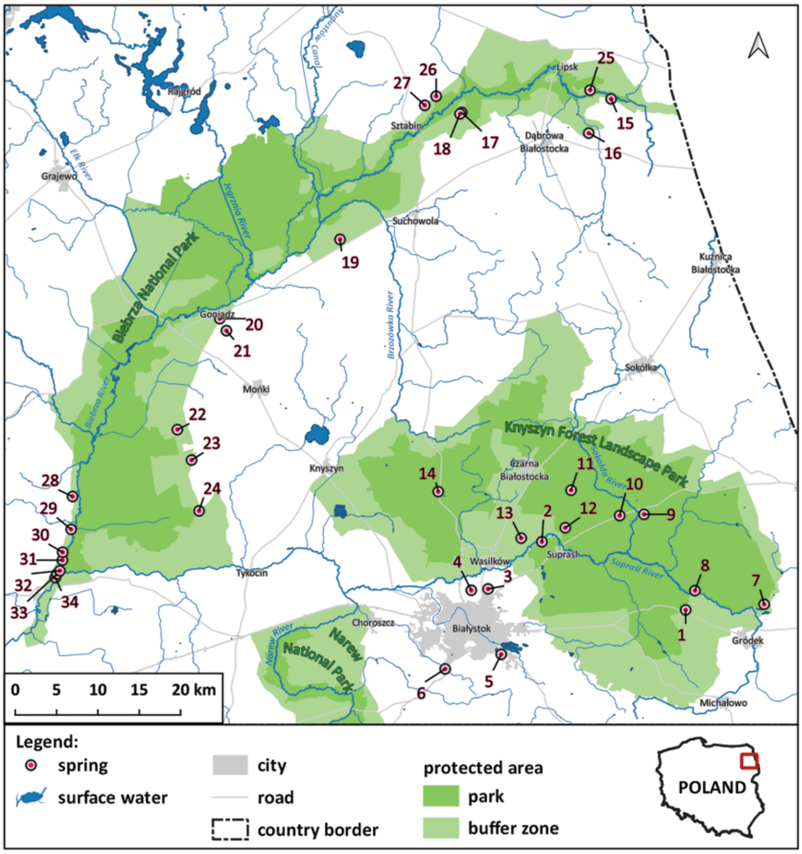

Fig. 1. Map showing the location of the studied springs in NE Poland. Site numbers refer to Tab. 1.

pod and cladoceran communities, and dominant species were frequently shared among multiple spring localities (Särkkä *et al.*, 1997). These observations suggest the existence of a shared group of characteristic copepod species in European spring ecosystems.

Despite this growing body of knowledge, lowland spring habitats in Central and Northern Europe remain understudied, and detailed faunistic and ecological assessments of their copepod communities are still scarce. The main objectives of this study were: i) to characterize the diversity and community structure of Copepoda, which in our samples were represented solely by Cyclopoida and Harpacticoida, in lowland springs; ii) to assess the influence of different hydromorphological types of springs (limnocrene, helocrene, rheocrene) and seasonal variation (autumn vs. summer) on Copepoda assemblages; and iii) to investigate the species diversity of the genus *Cyclops*, known for the slight morphological divergence among some of its representatives, using an integrative taxonomic approach that served both to confirm morphology-based identifications and to explore phylogenetic relationships among species.

METHODS

Study sites and sampling

Sampling was conducted in early summer and mid autumn, resulting in a total of 36 samples from 34 lowland springs located in northeastern Poland, as two springs (no. 3 and 4) were sampled in both seasons (Tab. 1). Two springs were situated in the vicinity of the city of Białystok, 10 were located within the Knyszyn Forest Landscape Park, and 22 were in the Biebrza National Park (Fig. 1). Among these, twenty-eight springs were

Tab. 1. List of analyzed springs with location and basic environmental characteristics. EC, electrical conductivity. Artificial limnocrenes are marked with asterisk (*).

No.	Name	Latitude	Longitude	Date	Sample volume (L)	Type of spring	Discharge (L s^{-1})	Temp. (°C)	EC (µS cm^{-1})	O_2 (mgL^{-1})
1	Pieszczaniki	53.137508	23.552297	28.06.2021	60	Rheocrene	30.0	11.6	312	8.0
2	Pólko	53.221046	23.300367	28.06.2021	250	Rheocrene	2.8	11.0	425	8.3
3a	Białystok - Jaroszówka	53.173348	23.198068	29.06.2021	30	Rheocrene	5.2	12.5	853	7.7
3b	Białystok - Jaroszówka	53.173348	23.198068	21.11.2023	10	Rheocrene	4.2	5.2	945	9.0
4a	Białystok - Pietrasze	53.173039	23.167676	29.06.2021	40	Rheocrene	5.9	14.9	412	8.9
4b	Białystok - Pietrasze	53.173039	23.167676	21.11.2023	10	Rheocrene	4.3	3.6	365	10.3
5	Białystok - Dojlidy	53.101611	23.214889	21.11.2023	10	Rheocrene	0.7	7.4	469	11.2
6	Turczyn	53.089306	23.112306	21.11.2023	10	Rheocrene	2.4	2.1	391	11.5
7	Piłatowszczyzna	53.138082	23.694512	28.06.2021	10	Rheocrene	1.5	10.4	428	9.7
8	Radunin	53.157751	23.571262	28.06.2021	100	Limnocrene*	1.5	11.8	299	1.1
9	Łaźnie	53.243995	23.488021	28.06.2021	20	Limnocrene	4.5	11.9	334	6.5
10	Budzisk	53.244252	23.443875	28.06.2021	40	Rheocrene	5.2	13.3	386	10.0
11	Pstrągownia	53.275128	23.358767	28.06.2021	60	Rheocrene	4.3	13.2	401	9.4
12	Jałówka	53.234807	23.344209	28.06.2021	30	Rheocrene	2.5	15.1	424	8.5
13	Studzianki Zimny Zdrój	53.226407	23.263346	29.06.2021	30	Rheocrene	2.5	12.1	479	10.0
14	Rezerwat Krzemianka	53.282059	23.118273	29.06.2021	95	Rheocrene	7.8	10.1	448	8.1
15	Koniuszki	53.696306	23.475722	24.11.2021	20	Limnocrene*		4.2	454	5.4
16	Harasimowicze	53.660667	23.430944	24.11.2021	10	Rheocrene		5.7	839	6.4
17	Trzyrzeczki	53.691500	23.201278	24.11.2021	20	Rheocrene	2.2	5.3	719	7.8
18	Trzyrzeczki	53.690083	23.197972	24.11.2021	10	Rheocrene	4.2	5.8	728	10.1
19	Zabiele	53.561306	22.965889	24.11.2021	24	Rheocrene	6.2	6.1	589	9.2
20	Goniądz	53.482389	22.739111	24.11.2021	30	Rheocrene		5.9	584	10.2
21	Wojtówstwo	53.469000	22.750000	24.11.2021	10	Rheocrene	1.5	6.9	598	9.0
22	Chojnowo	53.364250	22.651556	19.11.2021	20	Rheocrene		9.9	687	7.2
23	Nowa Wieś	53.330972	22.674472	19.11.2021	10	Limnocrene*		8.2	918	4.3
24	Krynice	53.275389	22.683472	19.11.2021	20	Rheocrene	1.6	9.7	491	8.9
25	Nowy Rogożyn	53.707222	23.438250	24.11.2021	20	Limnocrene*		4.7	495	3.5
26	Wolne	53.710806	23.156306	24.11.2021	10	Rheocrene	4.5	6.7	489	10.8
27	Krasnoborki	53.701556	23.134528	24.11.2021	10	Rheocrene	0.5	6.2	655	9.5
28	Mocarze	53.297917	22.455472	19.11.2021	100	Rheocrene	8.1	9.1	657	8.9
29	Rutkowskie	53.262306	22.450083	19.11.2021	7	Helocrene		9.5	814	5.9
30	Sieburczyn	53.237889	22.432806	19.11.2021	20	Rheocrene	5.1	10.2	659	9.3
31	Wierciszewo	53.229278	22.431583	19.11.2021	30	Rheocrene	6.2	9.0	835	9.9
32	Ruś (3)	53.217889	22.426139	19.11.2021	30	Rheocrene	4.9	8.9	678	9.3
33	Ruś (2)	53.214528	22.420639	19.11.2021	20	Rheocrene	2.6	8.5	699	10.9
34	Ruś (1)	53.210722	22.417417	19.11.2021	20	Rheocrene	2.7	8.5	799	9.8

classified as rheocrenes, five as limnocrenes (of which four were artificial limnocrenes), and one as a helocrene (Tab. 1). Rheocrene springs are defined by the immediate emergence of water that forms a distinct channel or stream. Limnocrene springs are characterized by the accumulation of water in small, typically shallow standing water bodies such as pools or ponds. Helocrene springs are diffuse, with water seeping over a broad area, saturating marshy ground rather than forming a well-defined outlet (Stoch, 2007; Bottazzi *et al.*, 2011; Jekatierynczuk-Rudczyk and Ejsmont-Karabin, 2023). Example views of the examined spring habitats are presented in Fig. 2.

Water samples from the springs were initially collected using

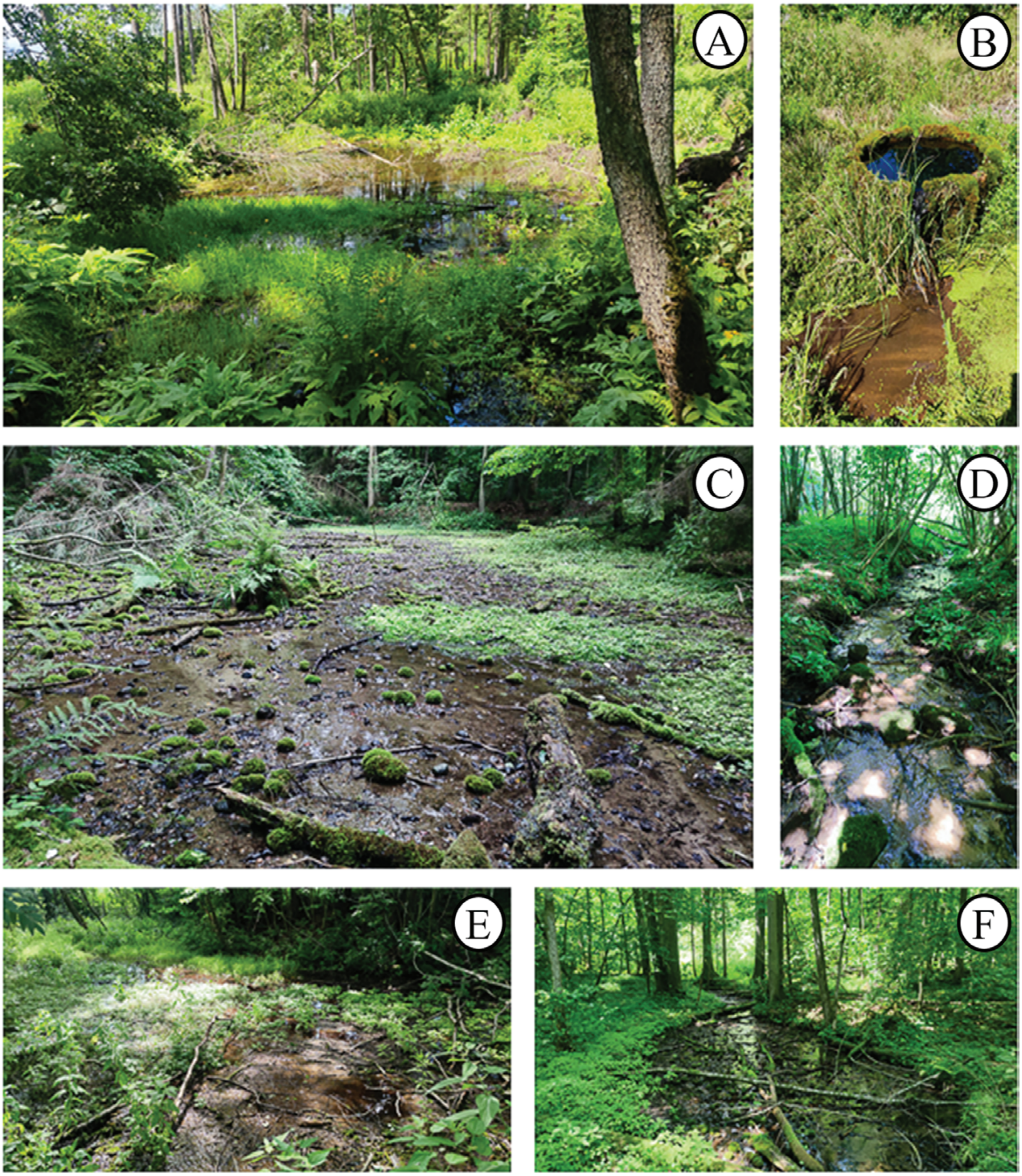

Fig. 2. Example views of the examined spring habitats. A) Limnocrene Łaźnie (no. 9). B) Artificial limnocrene Radunin (no. 8). Rheocrenes: C) Pstrągownia (no. 11). D) Studzianki (no. 13). E) Piłatowszczyzna (no. 7). F) Budzisk (no. 10).

small containers (<250 mL) and subsequently transferred into a calibrated bucket. At each site, we aimed to collect the maximum possible amount of water, resulting in sample volumes ranging from 7 to 250 liters (Tab. 1). The collected water was then filtered through a 50 µm plankton net, and the retained material was preserved in 96% ethanol. The field measurements, including water discharge, temperature, electrical conductivity, and dissolved oxygen, were recorded using an HQ40D Multi Meter (Hach-Lange GmbH, Germany).

Environmental characteristics of springs

Discharge from springs ranged from 0.5 to 30 L s^{-1}, with an average of 4.7±5.3 L s^{-1} (Tab. 1). There was no significant difference in discharge between rheocrene and limnocrene springs. The temperature ranged from 2.1 to 15.1°C, with substantial differences between seasons (F=56.3; $p<0.0001$). The mean temperatures were 7.7±2.2°C in autumn and 12.3±1.5°C in summer. The electrical conductivity (EC) ranged from 299 to 945 µS cm^{-1}, with an average of 577±185 µS cm^{-1}. There were no significant differences in EC among the spring types, but significant seasonal variations were observed (F=15.2; $p=0.0004$). The mean EC was 648±158 µS cm^{-1} in autumn and 433±158 µS cm^{-1} in summer. The oxygen concentration ranged from 1.1 to 11.5 mg L^{-1}, with an average of 8.5±2.2 mg L^{-1} (Tab. 1). No significant seasonal variation in oxygen concentration was detected, whereas significant differences were observed among spring types (F=38.9; $p<0.0001$). The mean oxygen concentration in rheocrene was 9.3±1.2 mg L^{-1}, while in limnocrene it was 4.2±1.8 mg L^{-1}.

Zooplankton identification

Copepoda (exclusively represented by Cyclopoida and Harpacticoida) were preliminarily analyzed in a 2 mL plankton chamber using an Olympus BX53 microscope equipped with cellSens imaging software. Individual specimens were carefully isolated from the plankton chamber and prepared on microscope slides for detailed morphological analysis. To examine diagnostic features, copepods were dissected under a stereomicroscope, exposing the diagnostic structures such as the thoracic legs (P1-P5), caudal rami, antennule, and the coxopodite of P4. An alternative approach to species identification involved mounting whole specimens on microscope slides in Hoyer's medium, which clears the tissue and makes the specimens transparent, thereby facilitating faster diagnostic assessment (Karpowicz, 2016; Smolska *et al.*, 2024). The species were identified using monographs (Einsle, 1996a; Janetzky *et al.*, 1996; Błędzki and Rybak, 2016) and taxonomic papers (Einsle, 1996b; Karaytug and Boxshall, 1998; Hołyńska and Dahms, 2004; Karanovic and Krajiček, 2012; Sukhikh and Alekseev, 2015; Novikov and Sharafutdinova, 2022). Species-level identification was performed on adult specimens and copepodites at stages CIV-CV, while earlier copepodite stages (CI-CIII) and nauplii were only counted. Species occurrence data of Copepoda have been deposited in the GBIF repository, and are accessible at https://doi.org/10.15468/qvm8x.

PCR identification of *Cyclops* species

Two individuals each of *Cyclops strenuus* and *C. insignis* from this study, along with two individuals of *C. furcifer* from a previous study (Karpowicz *et al.*, 2021), were selected for genomic DNA isolation, which was performed using the Qiagen Tissue Kit (Qiagen, Hilden, Germany). The procedure was conducted in accordance with the manufacturer's protocol. The isolated DNA was stored at –20°C until further molecular analyses. Subsequently, the polymerase chain reaction (PCR) technique was employed to amplify specific DNA fragments from individual *Cyclops* specimens. To confirm the species identity of the examined *Cyclops* individuals and to test phylogenetic relationships of the haplotypes, two genetic markers were selected for PCR: the mitochondrial 12S ribosomal RNA gene (12S rRNA) and the nuclear internal transcribed spacer 1 (ITS-1). PCR reactions targeting the selected genetic markers were performed in a final volume of 5 µL. The reaction mixture of each sample consisted of 1.7 µL of Taq Master Mix (Qiagen), 1 µL of distilled water (Qiagen), and 0.3 µL of primer mix, containing 0.052 µL of each primer (100 pmol/µL). The primers used for amplification of the 12S gene fragment (L13337-12S and H13845-12S) were obtained from Machida *et al.* (2004), while those for the ITS-1 fragment (SP-1-5'138 and SP-1-3') were sourced from Chu *et al.* (2001). For each reaction, 3 µL of the reaction mixture and 2 µL of previously isolated DNA from *Cyclops* individuals were used. Amplification was performed using a PCR profile, which included an initial denaturation step at 95°C for 15 min, followed by 40 amplification cycles. Each cycle consisted of three steps: denaturation at 94°C for 30 s, primer annealing at 57°C for 90 s (for both primer pairs), and elongation at 72°C for 60 s. A final extension step was conducted at 60°C for 30 min. Following PCR, the products were purified using two enzymes: Exonuclease I (Exo I, derived from *E. coli*) and Shrimp Alkaline Phosphatase (SAP). A mixture containing 0.5 µL of Exo I (20 U/µL) and 1 µL of SAP (1 U/µL) was added to 5 µL of the PCR product.

For the sequencing reaction, a mixture was prepared for each sample consisting of 1 µL of concentrated BDT reaction buffer (Life Technologies), 1 µL of BDT v3.1 reaction mix (containing labeled nucleotides and DNA polymerase, Life Technologies), 1 µL of diluted forward primer (3.2 pmol), 5 µl of distilled water (Qiagen), and 2 µL of the purified PCR product (12S rRNA or ITS-1). To remove unincorporated fluorescently labeled dideoxynucleotides, a commercial ExTerminator kit (A&A Biotechnology) was used. After purification, the samples were subjected to electrophoresis and directly analyzed using a 4-capillary automated sequencer (ABI 3130 Avant, Applied Biosystems). Sequence reading, alignment, and editing were performed using software such as Chromas Lite v.2.01 (Technelysium Pty Ltd., 2005) and BioEdit Sequence Alignment Editor v.7.0.1 (Hall, 1999).

Phylogenetic analysis

The jModelTest program was employed to identify the model that most accurately reflects the evolutionary relationships among the analyzed sequences (Posada, 2008). Phylogenetic trees based on the variability of the 12S rRNA and ITS-1 markers were constructed using the GTR+G model (General Time Reversible + Gamma distribution). To evaluate the phylogenetic relationships among the obtained haplotypes of the 12S rRNA gene and ITS-1, along with relevant sequences retrieved from GenBank, phylogenetic trees were constructed using the maximum likelihood (ML) method implemented in MEGA version 11.0.13 (Tamura *et al.*, 2021), with 1000 bootstrap replicates used to assess node support. Additionally, sequences labeled in GenBank as *Acanthocyclops americanus* (Marsh, 1893) were included as outgroups. As discussed in Hołyńska *et al*. (2025), the name *A. americanus* remains a *nomen dubium* because the designation of the neotype of *A. americanus,* proposed by Miracle *et al*. (2013), contradicts Article 75.3.5 of the International Code of Zoological Nomenclature and is therefore invalid. The sequences in GenBank labeled as *A. americanus* correspond to *Acanthocyclops trajani* Mirabdullayev and Defaye, 2002. For the 12S rRNA analysis, the GenBank accession numbers are KC130347 and KP773075; and for the ITS-1 analysis, the accession numbers are KP773033 and KP773034.

Statistical analysis

To compare the abundance and number of species of Cyclopoida and Harpacticoida among different hydromorphological types of springs (limnocrene, helocrene, rheocrene) and between seasons (autumn vs. summer), a one-way ANOVA was performed, followed by Tukey's HSD (Honestly Significant Difference) test for *post-hoc* comparisons. Descriptive statistics were visualized using box plots (Fig. 3). All statistical analyses were conducted using XLSTAT-Ecology (Addinsoft).

RESULTS

Characteristics of Copepoda (Cyclopoida, Harpacticoida) communities in lowland springs

A total of 23 Copepoda species/taxa were identified across all sites, including 13 Cyclopoida and 10 Harpacticoida. Species richness ranged from 0 to 7 per spring, with a mean of 3.1±2.2 species. Copepoda were absent from three springs (3b, 4b, and 19). In most cases, a higher number of Harpacticoida species was associated with a lower number of Cyclopoida species, and *vice versa*. No significant differences in species richness were observed for either Cyclopoida or Harpacticoida among the different hydromorphological types of springs (Fig. 3A,B). However, Cyclopoida species richness was most variable in limnocrene springs (Fig. 3A), whereas Harpacticoida exhibited the highest variation in rheocrene springs (Fig. 3B).

Harpacticoida abundances ranged from 0 to 11.9 ind. L^{-1}, with a mean of 0.8±2.1 ind. L^{-1}, although values were most frequently below this average. No significant differences in Harpacticoida abundance were observed among the limnocrene and rheocrene springs (Fig. 3D). Cyclopoida abundances were considerably higher (F=201.7; p<0.0001) in limnocrene springs (mean: 21.6±25.0 ind. L^{-1}) compared to rheocrene springs (mean: 1.0±1.9 ind. L^{-1}) (Fig. 3C). Exceptionally high abundances of both Cyclopoida (200.7 ind. L^{-1}) and Harpacticoida (3.6 ind. L^{-1}) were recorded in a single helocrene spring (no. 29). This site was dominated by Cyclopoida, particularly *Diacyclops bicuspidatus* (88.7 ind. L^{-1}) and *Megacyclops viridis* (8.7 ind. L^{-1}), alongside the harpacticoid *Canthocamptus staphylinus* (3.1 ind. L^{-1}).

No substantial seasonal differences were observed in Cyclopoida species richness or abundance between summer and autumn (Fig. 3E,G). In contrast, Harpacticoida exhibited significant seasonal variation in both abundance (F=6.7; p<0.01) and species richness (F=115.9; p<0.0001). In autumn, the mean Harpacticoida species richness was 0.4±0.5 species, and abundance was 0.2±0.7 ind. L^{-1}; whereas in summer, these values increased markedly to 3.8±1.3 species and 2.0±3.1 ind. L^{-1}, respectively (Fig. 3F,H).

Within the cyclopoid genera, *Cyclops* exhibited the highest species richness (three species), followed by *Megacyclops* (two species) and *Paracyclops* (two species). Among all Cyclopoida, *Eucyclops serrulatus* was the most frequent species, present in 44.4% of springs, followed by *Diacyclops bicuspidatus* (30.6%),

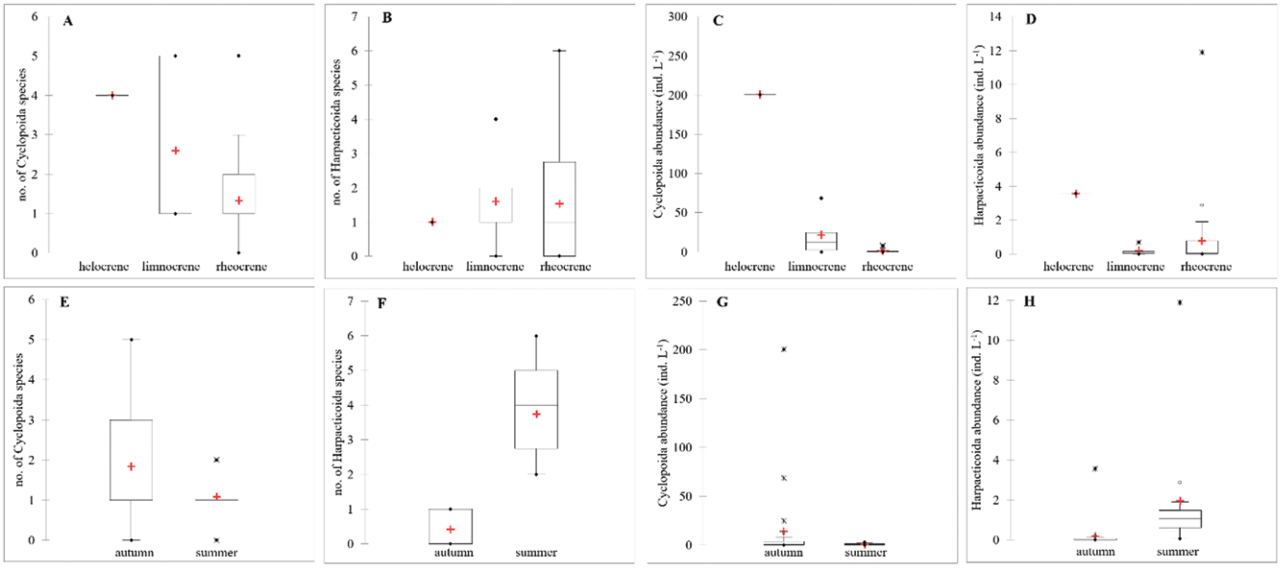

Fig. 3. Abundance and number of Cyclopoida and Harpacticoida species among different hydromorphological types of springs (A-D) and seasons (E-H). The lower and upper limits of the boxes are the first and third quartiles, respectively. The central horizontal line within each box indicates the median, while the crosses denote the mean values. Whiskers extend to the minimum and maximum values, excluding outliers, which are displayed as individual points.

Cyclops strenuus (25.0%), and *Acanthocyclops vernalis* (13.9%). Other *Cyclops* species, such as *C. insignis* and *C. borealis*, were observed only occasionally. Detailed morphological and genetic characteristics of the *Cyclops* species found in the studied springs are presented below (Figs. 4 to 7). Several other species, including *Ectocyclops phaleratus*, *Macrocyclops albidus*, *Megacyclops gigas*, *M. viridis*, *Paracyclops fimbriatus*, *P. imminutus*, and *Thermocyclops crassus*, were each found at fewer than 10% of the sampled sites (Tab. 2).

The Harpacticoida community exhibited high species richness within the genus *Bryocamptus*, represented by six species. The most frequent were *Bryocamptus zschokkei* (36.1%), *Bryocamptus echinatus* (25.0%), and *Bryocamptus (Arcticocamptus)* sp. (25.0%). In addition, *Attheyella crassa* (27.8%) and *Canthocamptus staphylinus* (13.9%) were relatively common. Sporadically occurring taxa included *Bryocamptus cuspidatus* (8,3%), and *Bryocamptus minutus*, *Bryocamptus pygmaeus*, *Maraenobiotus brucei,* and *Parastenocaris* sp., each recorded in fewer than 6% of the springs (Tab. 2). Photography documentation of *B. zschokkei*, *B. echinatus*, *B. cuspidatus*, *B. minutus*, and *B. pygmaeus*, along with their distinguishing features, is presented in *Supplementary Materials (Figs. S1 to S5)*.

These results highlight the high species richness of Copepoda in lowland springs, with many genera comprising both widespread and rare species. Most of the recorded taxa are common components of hyporheic zones and groundwater habitats, reflecting the specific environmental conditions of spring ecosystems.

Unveiling *Cyclops* diversity through integrative taxonomy

Among the identified Cyclopoida, the genus *Cyclops* was represented by the largest number of species. In this study, we present the characteristics of *Cyclops* species, including *Cyclops strenuus*, *Cyclops borealis*, and *Cyclops insignis*, using an integrative taxonomic approach that combines morphological description with genetic analyses. Short morphological diagnoses of the species are provided below.

Cyclops strenuus Fischer, 1851

Cyclops strenuus was found in nine springs (15, 16, 17, 18, 25, 26, 28, 29, and 34), with a particularly high abundance observed in spring no. 15. The body of the analyzed adult female measured 1581 µm (Fig. 4A). The antennule was composed of 17 segments (Fig. 4B). The whole antenna and the posterior surface ornamentation of the antennal coxobasipodite are shown in Fig. 4C and 4D, respectively. The rami of the swimming legs were three-segmented, with a spine formula of 3.4.3.3 (Fig. 4E,H-J). The medial spine of the P1 basipodite was slender and bearing short setules (Fig. 4F). Long spinules arranged in an arc were present on the anterior surface of the P1 basipodite near the insertion of the exopodite and endopodite (Fig. 4G). The last segment of the P4 endopodite was almost three times as long as wide; the inner apical spine was as long as the segment, and the outer spine measured one-third the length of the inner one (Fig. 4J). The ornamentation of the P4 coxopodite (posterior surface) consisted of

Tab. 2. Cyclopoida and Harpacticoida species with occurrence frequencies and sampling locations. The spring numbers correspond to Tab. 1 and Fig. 1.

Species	Freq. (%)	Spring no.
Cyclopoida		
Cyclopidae Rafinesque 1815		
Acanthocyclops vernalis (Fischer, 1853)	13.9	11, 12, 13, 21, 24
Cyclops borealis Lindberg, 1956	2.8	25
Cyclops insignis Claus, 1857	5.6	15, 16
Cyclops strenuus Fischer, 1851	25.0	15, 16, 17, 18, 25, 26, 28, 29, 34
Diacyclops bicuspidatus (Claus, 1857)	30.6	3a, 15, 16, 21, 22, 23, 24, 25, 28, 29, 30
Ectocyclops phaleratus (Koch, 1838)	2.8	25
Eucyclops serrulatus (Fischer, 1851)	44.4	1, 6, 7, 9, 12, 14, 20, 21, 22, 25, 26, 27, 28, 31, 32, 34
Macrocyclops albidus (Jurine, 1820)	5.6	8, 28
Megacyclops gigas (Claus, 1857)	2.8	15
Megacyclops viridis (Jurine, 1820)	8.3	15, 28, 29
Paracyclops fimbriatus (Fischer, 1853)	8.3	1, 4a, 29
Paracyclops imminutus Kiefer, 1929	5.6	10, 31
Thermocyclops crassus (Fischer, 1853)	2.8	26
Harpacticoida		
Canthocamptidae Brady 1880		
Attheyella crassa (Sars G.O., 1863)	27.8	3a, 4a, 7, 9, 10, 11, 12, 13, 14, 31
Bryocamptus (*Arcticocamptus*) sp.	25.0	1, 2, 3a, 7, 9, 10, 11, 12, 14
Bryocamptus (*Arcticocamptus*) *cuspidatus* (Schmeil, 1893)	8.3	1, 10, 14
Bryocamptus (*Limocamptus*) *echinatus* (Mrázek, 1893)	25.0	1, 3a, 7, 9, 10, 12, 13, 14, 30
Bryocamptus (*Bryocamptus*) *minutus* (Claus, 1863)	5.6	8, 33
Bryocamptus (*Rheocamptus*) *pygmaeus* (Sars G.O., 1863)	5.6	8, 14
Bryocamptus (*Rheocamptus*) *zschokkei* (Schmeil, 1893)	36.1	1, 2, 3a, 4a, 7, 9, 10, 11, 12, 13, 14, 26, 34
Canthocamptus staphylinus (Jurine, 1820)	13.9	15, 16, 25, 28, 29
Maraenobiotus brucei (Richard, 1898)	2.8	1
Parastenocarididae Chappuis, 1940		
Parastenocaris sp.	2.8	3a

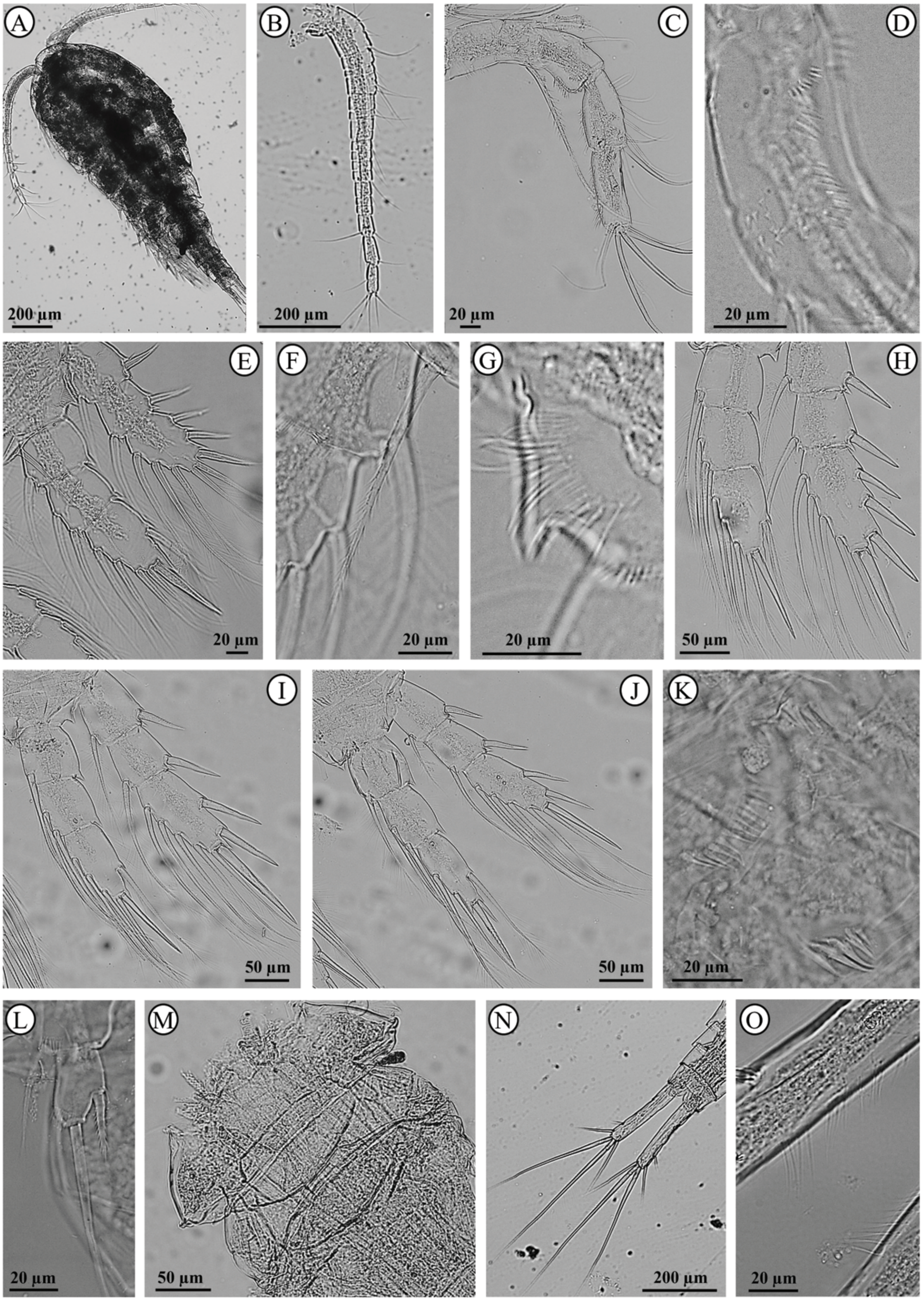

Fig. 4. *Cyclops strenuus*. A) Habitus. B) Antennule (A1). C) Antenna (A2). D) Posterior surface ornamentation of the antennal coxobasipodite. E) First leg (P1). F) Medial spine of the P1 basipodite. G) Long spinules on the anterior surface of P1 basipodite, near the insertions of endopodite and exopodite. H) Second leg (P2), note four spines on the third exopodite segment. I) Third leg (P3). J) Fourth leg (P4). K) Coxopodite of P4 (posterior view). L) Fifth leg (P5). M) Pediger 5. N) Caudal rami. O) Hairy inner margin of the caudal rami.

a proximal row of spinules and a few spinules along the distal margin (Fig. 4K). P5 had two segments (Fig. 4L). The first segment had a short lateral seta and a row of spinules at the insertion of the seta; the second segment had an apical seta and a medial spine, and slender spinules at the insertions of both structures (Fig. 4L). Pediger 5 was slightly wider than the genital double-somite and had blunt lateral ends (Fig. 4M). Caudal rami were approximately 5.0 times as long as wide, and had a hairy inner margin (Fig. 4N,O). *Cyclops strenuus* can be distinguished from other species of the genus by the following characteristics: pediger 4 with maximal width at its midpoint (Fig. 4A); bluntly rounded pediger 5 (Fig. 4M); and a relatively short outer spine on the last segment of P4 endopodite, measuring one-third the length of the inner one (Fig. 4J).

Cyclops borealis Lindberg, 1956

Cyclops heberti Einsle, 1996 is considered a junior synonym of *C. borealis* Lindberg, 1956 (Hołyńska and Wyngaard, 2019).

Only one specimen of this species was found in spring no. 25. The body length of the studied adult female was 1545 µm. The antennule was composed of 17 segments (Fig. 5A). The antenna habitus and the posterior surface ornamentation of the antennal coxobasipodite are shown in Fig. 5B and 5C, respectively. The rami of the swimming legs were three-segmented, with a spine formula of 3.4.3.3 (Fig. 5D,G-I). The medial spine of the P1 basipodite was slender and bearing short setules (Fig. 5E). Long spinules arranged in an arc were present on the anterior surface of the P1 basipodite near the insertion of the exopodite and endopodite (Fig. 5F). The last segment of the P4 endopodite was more than twice as long as wide; the inner apical spine was as long as the article, and the outer spine was half the length of the inner one (Fig. 5I). The ornamentation on the P4 coxopodite (posterior surface) consisted of a proximal row of spinules and two conspicuously robust spinules near the distal margin (Fig. 5J). P5 was two-segmented (Fig. 5K). Segmentation and setation of P5 were the same as in *C. strenuus*, yet spinules were only present at the insertions of the medial spine and the apical seta on the second segment. (Fig. 5K). Pediger 5 had lateral lobes (Fig. 5L). Caudal rami were relatively long, with a length-to-width proportion of 6:1 and a hairy inner margin (Fig. 5M,N). *Cyclops borealis* can be distinguished from other species in the genus by the following characteristics: pediger 5 with lateral lobes (Fig. 5L); length proportion of the inner and outer apical spines on the last segment of the P4 endopodite is about 2.0 (Fig. 5I); few distinctly robust spinules are present near the distal margin of P4 coxopodite (posterior surface) (Fig. 5J); lack of spinules at the insertion of the lateral seta on the first segment of P5 (Fig. 5K).

Cyclops insignis Claus, 1857

Cyclops insignis was found in low abundance in springs no. 15 and 16. The body length of the examined adult female was 2134 µm (Fig. 6A). The antennule consisted of 14 segments (Fig. 6B), which distinguishes this species from other species within the genus. The whole antenna and the posterior surface ornamentation of the antennal coxobasipodite are shown in Fig. 6C and Fig. 6D, respectively. The rami of the swimming legs were three-segmented with a spine formula of 2.3.3.3 (Fig. 6E,H-J). The medial spine of the P1 basipodite was stout and bearing stout setules (Fig. 6F). Long spinules arranged in an arc were absent on the anterior surface of the P1 basipodite near the insertion of the exopodite and endopodite (Fig. 6G). The last segment of the P4 endopodite was twice as long as wide; the inner apical spine was almost as long as the segment itself, and the outer spine measured approximately two-thirds the length of the inner one (Fig. 6J). The ornamentation on the P4 coxopodite (posterior surface) was composed of groups of spinules near the proximal margin, and a long row of fine spinules along the distal margin (Fig. 6K). P5 segmentation and setation were the same as in *C. strenuus* (Fig. 6L). A row of small spinules was present at the insertion of the lateral seta on the first segment, and the insertion of the medial spine and apical seta on the second segment (Fig. 6L). The second segment of P5 was slender, with a robust medial spine as long as the segment itself (Fig. 6L). Pediger 5 was slightly wider than the genital double-somite and pointed posteriorly (Fig. 6M). Caudal rami were elongated and slim, with a length-to-width proportion of 7:1 and a hairy inner margin (Fig. 6N,O).

Molecular analyses

Analysis of the mitochondrial 12S ribosomal RNA gene fragments in three species of *Cyclops* revealed the presence of one haplotype in each species: *C. strenuus*, 400 bp, GenBank accession number PV827566; *C. insignis*, 404 bp, GenBank accession number PV827567; and *C. furcifer*, 401 bp, GenBank accession number PV827568. Concerning the ITS-1 marker, we identified one haplotype for *C. strenuus* (369 bp), which was identical to the haplotype described by Hołyńska and Wyngaard (2019) (GenBank accession number MK329399), one haplotype for *C. insignis* (391 bp, GenBank accession number PV826712), and one haplotype for *C. furcifer* (384 bp), which was identical to the *C. strenuus* haplotype provided by Krajíček *et al.* (2016) (GenBank accession number KP773051). The source data (geographical coordinates and location) available for sequence KP773051 in GenBank, however, show that the specimen in question was collected at a site where *C. furcifer* was recorded by the authors, but *C. strenuus* was not (Supplementary Information, Table S1 in Krajíček *et al*. 2016), which clearly indicates an unintentional labeling error for sequence KP773051.

The conspecific morphotypes consistently grouped in the maximum likelihood trees inferred from the mitochondrial 12S rRNA and the nuclear ITS-1 markers (Fig. 7A,B). The only exceptions are the clades in which *C. furcifer* grouped with some "*C. strenuus*" haplotypes (KP773090 in the 12S tree, and KP773051 and KP773005 in the ITS-1 tree). The three "*C. strenuus*" sequences, however, came from an ephemeral pool in Tchořovice (Czech Republic), which has been listed by Krajíček *et al.* (2016) in Table 1 of the Supplementary Information as the collection site of *C. furcifer* rather than *C. strenuus*, indicating unintentional mislabeling for these sequences in GenBank.

In terms of the interspecific relationships, both the 12S and ITS-1 trees appear to support "Clade 1" (Fig. 7A,B), although this clade is represented by fewer sampled taxa in the ITS-1 tree. The monophyly of the *C. vicinus*–*C. kikuchii* group ("Clade 3") received strong support in both trees. In contrast, the phylogenetic relationships of *C. strenuus, C. furcifer*, *C. insignis,* and *C. scutifer*, all of which are members of "Clade 2" as defined by Krajíček *et al*. (2016), differ between the 12S and ITS-1 trees (Fig. 7A,B). The more basal nodes show low support in both phylogenies, indicating that the relationships among main clades remain unresolved.

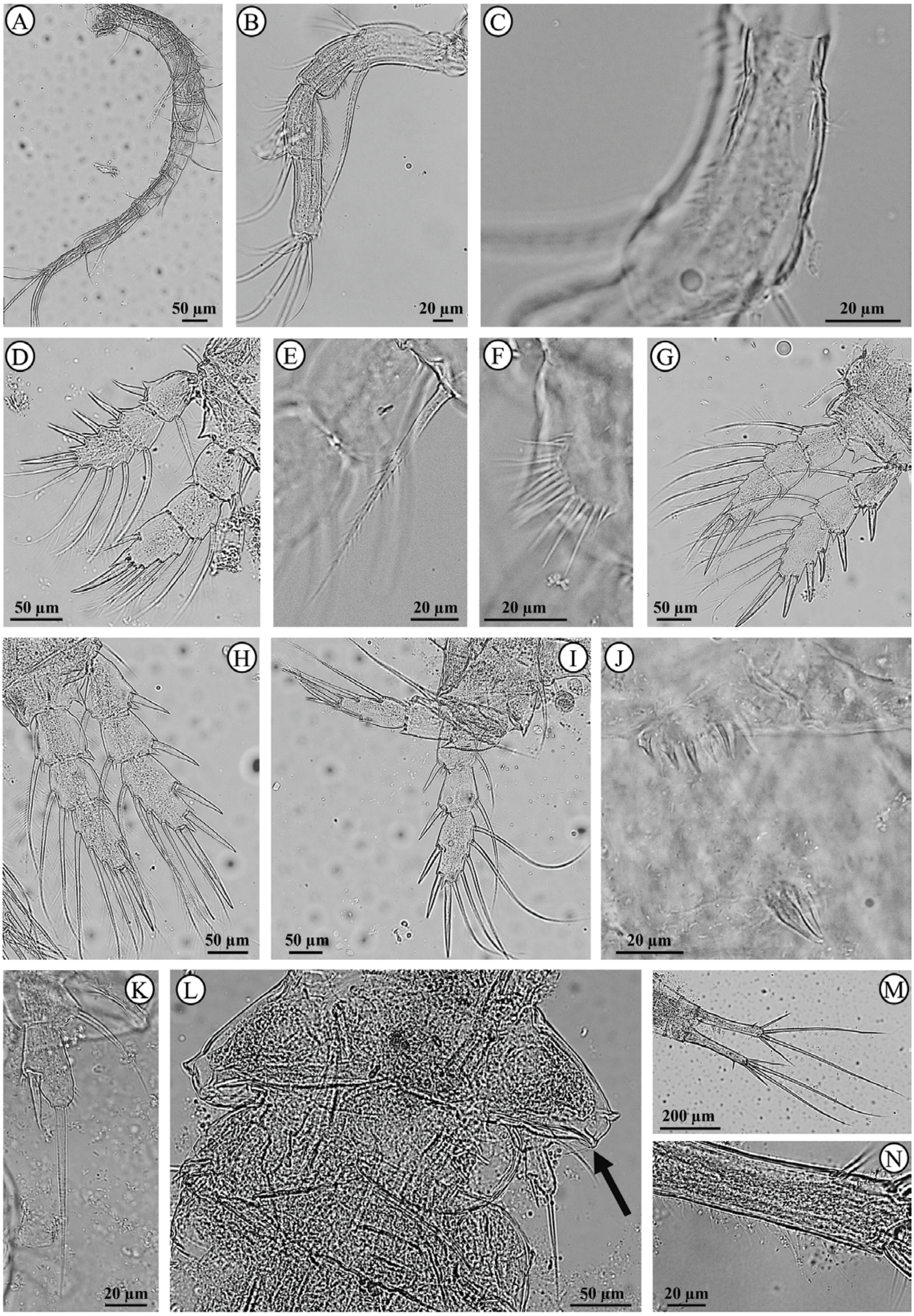

Fig. 5. *Cyclops borealis*. A) Antennule (A1). B) Antenna (A2). C) Posterior surface ornamentation on the antennal coxobasipodite. D) First leg (P1). E) Medial spine of the P1 basipodite. F) Long spinules on the anterior surface of the P1 basipodite, near the insertions of endopodite and exopodite. G) Second leg (P2). H) Third leg (P3). I) Fourth leg (P4). J) Coxopodite of P4 (posterior view), note a few robust spinules next to the distal margin. K) Fifth leg (P5). L) Pediger 5, lateral lobe is arrowed. M) Caudal rami. N) Hairy inner margin of the caudal ramus.

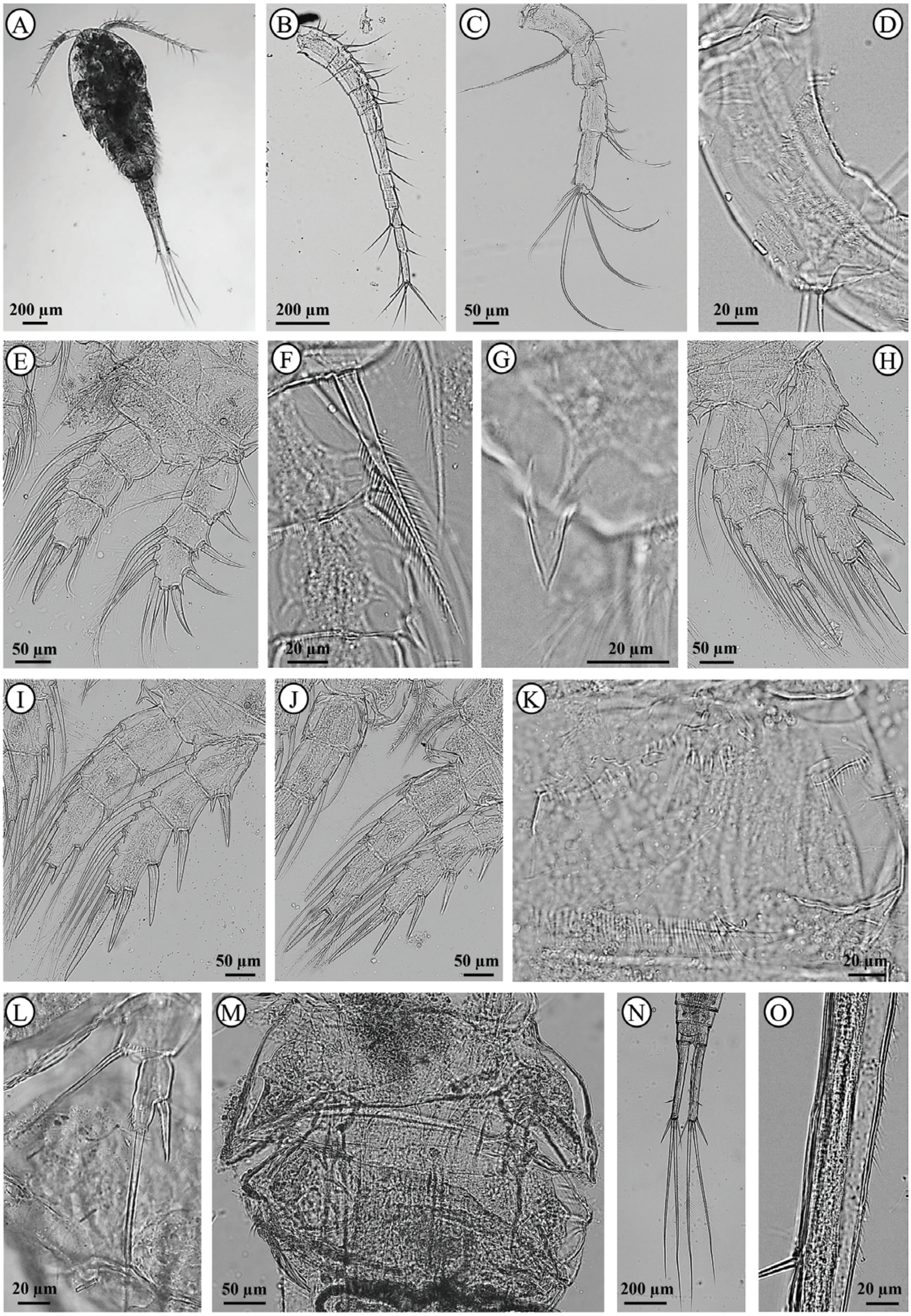

Fig. 6. *Cyclops insignis*. A) Habitus. B) Antennule (A1). C) Antenna (A2). D) Posterior surface ornamentation on the antennal coxobasipodite. E) First leg (P1). F) Medial spine of the P1 basipodite. G) Long spinules are absent on the anterior surface of the P1 basipodite, near the insertions of endopodite and exopodite. H) Second leg (P2), note only three spines on the third exopodite segment. I) Third leg (P3). J) Fourth pair of swimming legs (P4), note robust coxopodite setae. K) Coxopodite of P4 (posterior view), note many long and slender spinules next to the distal margin. L) Fifth leg (P5). M) Pediger 5. N) Caudal rami. O) Hairy inner margin of the caudal ramus.

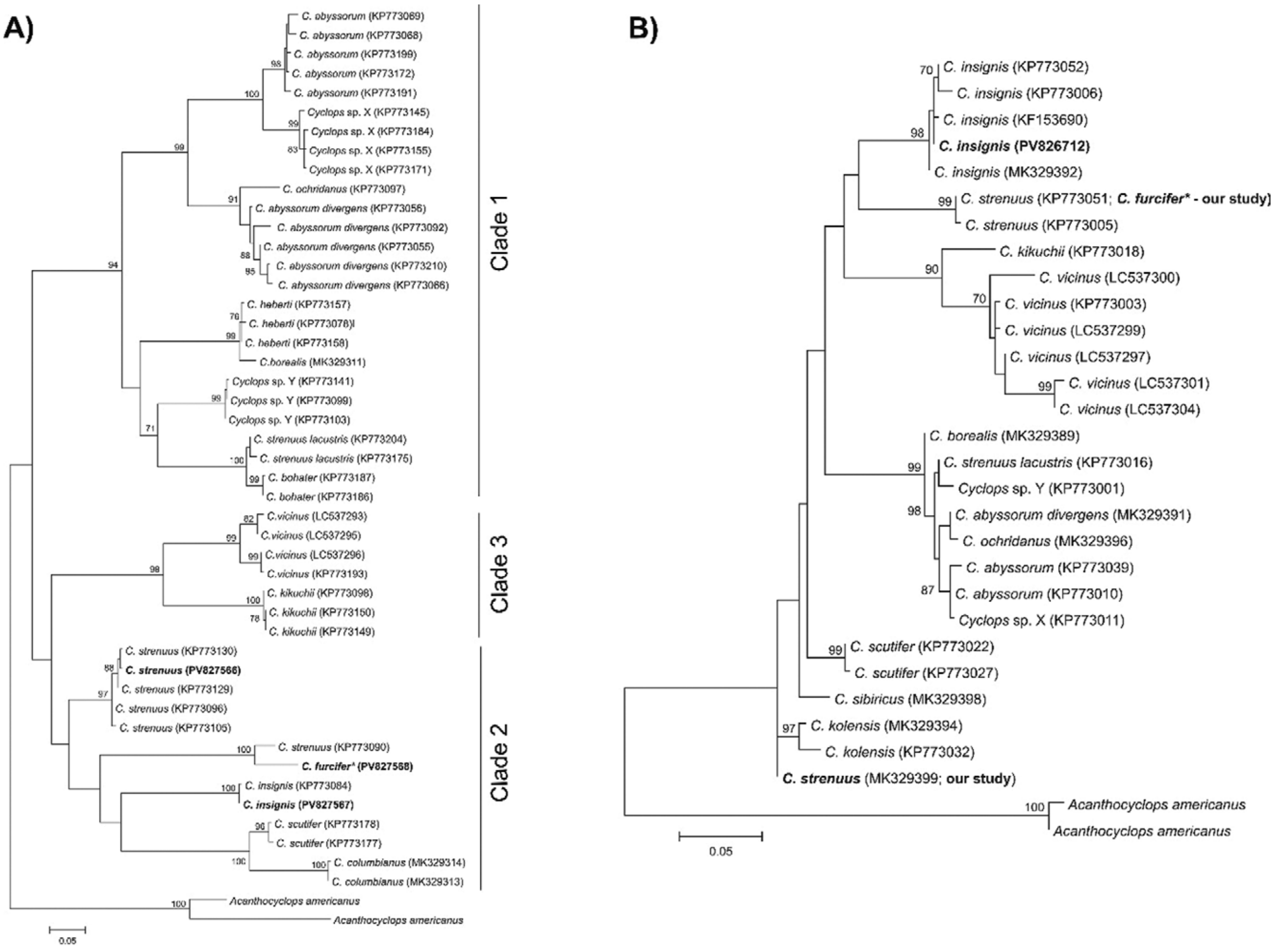

Fig. 7. Maximum likelihood (ML) trees showing the phylogenetic relationships among *Cyclops* haplotypes based on (A) the mitochondrial 12S ribosomal RNA gene and (B) the nuclear internal transcribed spacer 1. Haplotypes obtained in this study are shown in bold. Numbers following species names represent accession numbers of sequences retrieved from GenBank. ML trees were constructed using the GTR+G model of molecular evolution. Numbers at nodes indicate bootstrap support values based on 1,000 replicates. Scale bars represent p-distances. The species names displayed in the figure reflect those deposited in GenBank, which in some cases do not correspond to the currently accepted taxonomy. *Cyclops strenuus lacustris*, as used in GenBank, is recognized by contemporary taxonomists as a distinct species, *Cyclops lacustris* Sars G.O., 1863. Similarly, *Cyclops abyssorum divergens* is currently treated as a separate species, *Cyclops divergens* Lindberg, 1936. *Cyclops heberti* Einsle, 1996 is considered a junior synonym of *Cyclops borealis* Lindberg, 1956 (see Hołyńska and Wyngaard, 2019). *Acanthocyclops americanus* (Marsh, 1893) is regarded as a *nomen dubium* (Mirabdullayev and Defaye, 2004; Hołyńska *et al.*, 2025). For more details, see the METHODS section. The sequences labeled as *A. americanus* in GenBank correspond to *Acanthocyclops trajani* Mirabdullayev and Defaye, 2002. Mislabeling issues for some haplotypes of *C. strenuus* (KP773090, KP773051, KP773005) are discussed in the RESULTS section.

DISCUSSION

Our study expands the current understanding of copepod assemblages in lowland spring ecosystems by documenting a total of 23 species, including 13 Cyclopoida and 10 Harpacticoida. This taxonomic resolution is unprecedented for this region, particularly in the case of harpacticoids, which are rarely identified to the species level in Polish studies and are often reported collectively (Karpowicz, 2016; Ejsmont-Karabin *et al.*, 2020). The frequent occurrence of *Canthocamptus staphylinus* and several *Bryocamptus* species further underscores the importance of springs as unique habitats that support diverse meiofaunal communities, often overlooked in routine biomonitoring programs. Notably, Harpacticoida were virtually absent from over 100 wells investigated in the same region, except for *Canthocamptus staphylinus* (Smolska *et al.*, 2024), a species frequently found in the water column (Novikov and Sharafutdinova, 2022). In contrast, most harpacticoid taxa are typically associated with benthic substrates or related microhabitats, such as moss mats or sediment interstices (Boxshall and Defaye, 2008). This further emphasizes the unique ecological conditions of springs that facilitate the persistence of these benthic-associated taxa, which are otherwise rare or undetectable in adjacent aquifer environments.

Most of the recorded taxa are commonly associated with groundwater or hyporheic environments and were also reported from more than 100 wells in the same region (Karpowicz *et al.*, 2021; Smolska *et al.*, 2024). The most frequently encountered species included *Eucyclops serrulatus*, *Diacyclops bicuspidatus*, *Acanthocyclops vernalis*, *Paracyclops fimbriatus*, and *Macrocyclops albidus*, all of which have also been reported from spring habitats across Central and Northern Europe, including the Czech Republic (Zhai *et al.*, 2015), Belgium (Martin *et al.*, 2009), Finland (Särkkä *et al.*, 1997, 1998), and Italy (Stoch, 2007). Their widespread distribution across contrasting geographic and climatic regions suggests that these taxa form the core of the European spring-dwelling copepod fauna, regardless of topography.

Despite the ecological continuity with groundwater environments, no true stygobionts were detected in the studied springs. This finding aligns with previous work in postglacial lowlands, where stygobiotic species are generally rare (Deharveng *et al.*, 2009; Martin *et al.*, 2009). The recorded assemblages were predominantly composed of stygophiles, which exhibit affinity for subterranean habitats but are not strictly confined to them. We considered *Thermocyclops crassus* the only accidental species in groundwater habitats, as it was represented by a single individual, and is otherwise common in surface waters of the region (Ejsmont-Karabin *et al.*, 2020; Karpowicz and Ejsmont-Karabin, 2021). *Thermocyclops crassus*, a species typical of tropical waterbodies, also occurs in the summer plankton in the northern temperate region, but may exhibit limited tolerance to low temperatures (Nowakowski and Sługocki, 2024).

The significantly higher species richness and abundance of Harpacticoida recorded in summer are likely driven by elevated temperatures during this season, despite the generally thermally stable conditions of springs. This seasonal pattern suggests that even moderate increases in temperature may lead to a richer benthic microbial and algal food base, which enhances reproductive activity or developmental rates in benthic harpacticoids (Cerasoli *et al.*, 2023; Výravský *et al.*, 2023). In contrast, no seasonal differences were observed for Cyclopoida, which may reflect their broader thermal tolerance and potentially more stable population dynamics in groundwaters, as well as their predominantly planktonic mode of life, in contrast to the benthic lifestyle of harpacticoids that directly benefit from the richer benthic microbiome in summer.

Unexpectedly high diversity was observed within the genus *Cyclops*, with three species recorded: *C. strenuus*, *C. insignis*, and *C. borealis*. The latter is treated here as a senior synonym of *C. heberti* based on morphological congruence and the priority of description (Lindberg, 1956; Einsle, 1996b; Hołyńska and Wyngaard, 2019). The absence of *C. furcifer* in the sampled springs is noteworthy as the species was abundant in nearby wells and temporary puddles (Karpowicz and Smolska, 2024; Smolska *et al.*, 2024), and may reflect microhabitat specificity or interspecific interactions within spring habitats.

Morphology and molecule-based (mitochondrial 12S ribosomal RNA and nuclear ITS-1 markers) species identification yielded congruent results, which demonstrates the usefulness of both approaches in *Cyclops* taxonomy. Apparent discrepancies in the identification of *C. furcifer* using morphological vs. sequence data (*C. furcifer* grouped with some haplotypes labeled as *C. strenuus* in GenBank in the 12S and ITS-1 trees; Fig. 7A,B) have been clarified by the comparisons of the "source" information available in GenBank to the species collection data provided in the original publication by Krajíček *et al.*, 2016. Unintentional labeling errors can be inferred from the "source" data in all three haplotypes. This case perfectly illustrates the importance of providing detailed information about the collection sites in both the original publication and in Genbank (Features - Source).

Since the molecular markers, taxon sampling, and our outgroup choice largely fit those used by Krajíček *et al.*, 2016, we could not expect a substantially new phylogeny for the genus *Cyclops*. The phylogenies inferred from the mitochondrial 12S and nuclear ITS-1 markers support monophyly of a species-rich group, Clade 1 as defined by Krajíček *et al.* (2016), and sister relationships of *C. vicinus* and *C. kikuchii*, but the basal relationships remain unresolved in *Cyclops*. A phylogenetic analysis (Hołyńska and Wyngaard, 2019), which combined both morphological and molecular characters, reached the same conclusion and mentioned several issues (e.g., challenges in identifying appropriate outgroups, rapid speciation during early evolution of *Cyclops*, limited taxon sampling in the molecule-based analyses) that may hinder a better understanding of the evolution of the genus.

CONCLUSIONS

This study provides novel insights into the copepod fauna of lowland springs, revealing that these ecotonal habitats sustain a rich and distinctive assemblage of both groundwater and benthic species. The high species richness, particularly of harpacticoids, underscores the ecological heterogeneity of springs and their capacity to support taxa not commonly detected in adjacent aquifers. Our findings also demonstrate clear seasonal patterns in harpacticoid diversity and abundance, likely linked to temperature fluctuations, and highlight the contrasting dynamics of Cyclopoida and Harpacticoida in spring environments. The integration of morphological and molecular data further elucidated the taxonomy of *Cyclops* species, contributing to a better understanding of their diversity. Overall, these results suggest that springs deserve more attention in biodiversity studies, as they form unique interfaces between surface and subsurface ecosystems and support a mix of common and less frequent copepod species.

ACKNOWLEDGMENTS

This article received financial support from the Polish Ministry of Science and Higher Education through a subsidy to maintain the research potential of the Faculty of Biology, University of Bialystok. The authors are grateful to Adam Więcko for preparing the map of the study area and to Dana Klímová Hřívová for her valuable consultations on the identification of Harpacticoida.

REFERENCES

Błędzki LA, Rybak JI, 2016. Freshwater Crustacean Zooplankton of Europe: Cladocera & Copepoda (Calanoida, Cyclopoida). Key to Species Identification, with Notes on Ecology, Distribution, Methods and Introduction to Data Analysis. Springer, Berlin.

Bottazzi E, Bruno MC, Pieri V, Di Sabatino A, Silveri L, Carolli M, Rossetti G, 2011. Spatial and seasonal distribution of invertebrates in Northern Apennine rheocrene spring. J Limnol 70:77-92.

Boxshall GA, Defaye D, 2008. Global diversity of copepods (Crustacea: Copepoda) in freshwater. Hydrobiologia 595:195-207.

Brittain JE, Eikeland TJ, 1988. Invertebrate drift - A review. Hydrobiologia 166:77-93.

Castaño-Sanchez A, Pereira JL, Goncalves FJM, Reboleira ASPS, 2021. Sensitivity of a widespread groundwater copepod to different contaminants. Chemosphere 274:129911.

Cerasoli F, Di Lorenzo T, Di Cicco M, Di Giuseppe D, Mancini E, Di Sabatino A, 2023. Assessing spatial and temporal changes in diversity of copepod crustaceans: a key step for biodiversity conservation in groundwater-fed springs. Front Environ Sci 11:1051295.

Chu KH, Li CP, Ho HY, 2001. The first internal transcribed spacer (ITS-1) of ribosomal DNA as a molecular marker for phylogenetic and population analyses in crustacea. Mar Biotechnol (NY) 3:355-361.

Deharveng L, Stoch F, Gibert J, Bedos A, Galassi D, Zagmajster M, et al. 2009. Groundwater biodiversity in Europe. Freshwater Biol 54:709-726.

Dumnicka E, Galas J, 2017. An overview of stygobiontic invertebrates of Poland based on published data. Subterr Biol 23:1-18.

Dumnicka E, Galas J, Nejberek K, Urban J, 2020. The influence of Pleistocene glaciations on the distribution of obligate aquatic subterranean invertebrate fauna in Poland. Zool Anz 286:90-99.

Einsle U, 1996a. Copepoda: Cyclopoida. Genera *Cyclops*, *Megacyclops*, *Acanthocyclops*, p. 1-82. In: HJF Dumont (ed.), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World. SPB Academic Publishing.

Einsle U, 1996b. *Cyclops heberti* n.sp. and *Cyclops singularis* n.sp., two new species within the genus *Cyclops* ('*strenuus*-subgroup') (Crust. Copepoda) from ephemeral ponds in southern Germany. Hydrobiologia 319:167-177.

Ejsmont-Karabin J, Kalinowska K, Karpowicz M, 2020. Structure of Ciliate, Rotifer, and Crustacean Communities in lake systems of Northeastern Poland, p. 77.101. In: E Korzeniewska and M Harnisz (eds.), Polish River Basins and Lakes – Part II. The Handbook of Environmental Chemistry 87. Springer.

Ferreira D, Malard F, Dole-Olivier M-J, Gibert J, 2007. Obligate groundwater fauna of France: diversity patterns and conservation implications. Biodiv Conserv 16:567-596.

Galassi DMP, 2001. Groundwater copepods: diversity patterns over ecological and evolutionary scales. Hydrobiologia 453:227-253.

Galassi DMP, Huys R, Reid JW, 2009. Diversity, ecology and evolution of groundwater copepods. Freshwater Biol 54:691-708.

Gibert J, Culver DC, 2009. Assessing and conserving groundwater biodiversity: an introduction. Freshwater Biol 54:639-648.

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98.

Hołyńska M, Dahms HU, 2004. New diagnostic microcharacters of the cephalothoracic appendages in *Cyclops* O. F. Müller, 1776 (Crustacea, Copepoda, Cyclopoida). Zoosystema 26: 175-198.

Hołyńska M, Wyngaard GA, 2019. Towards a phylogeny of *Cyclops* (Copepoda):(in)congruences among morphology, molecules and zoogeography. Zool Scr 48:376-398.

Hołyńska M, Świsłocka-Cutter M, López C, Karpowicz M, 2025. Freshwater cyclopids (Crustacea: Copepoda) first recorded in South America - arguments for and against their non-native status. Eur Zool J 92:631-659.

Iannella M, Fiasca B, Di Lorenzo T, Biondi M, Di Cicco M, Galassi DMP, 2020. Spatial distribution of stygobitic crustacean harpacticoids at the boundaries of groundwater habitat types in Europe. Sci Rep 10:19043.

Janetzky W, Enderle R, Noodt W, 1996. Crustacea: Copepoda: Gelyelloida und Harpacticoida. Süßwasserfauna von Mitteleuropa 8. Gustav Fischer Verlag.

Jekatierynczuk-Rudczyk E, Ejsmont-Karabin J, 2023. Rotifers of inter-forest springs. Diversity 15:153.

Karanovic T, Krajiček M, 2012. 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.

Karaytug S, Boxshall GA, 1998. The *Paracyclops fimbriatus*-complex (Copepoda, Cyclopoida): a revision. Zoosystema 20:563-602.

Karpowicz M, 2016. New data to the knowledge on the Harpacticoida (Crustacea, Copepoda) fauna in Poland. Fragm Faun 59:87-98.

Karpowicz M, Ejsmont-Karabin J, 2021. Diversity and structure of pelagic zooplankton (Crustacea, Rotifera) in NE Poland. Water 13:456.

Karpowicz M, Smolska S, Świsłocka M, Moroz J, 2021. First insight into groundwater copepods of the Polish lowland. Water 13:2086.

Karpowicz M, Smolska S, 2024. Ephemeral puddles - potential sites for feeding and reproduction of hyporheic Copepoda. Water 16:1068.

Krajíček M, Fott J, Miracle MR, Ventura M, Sommaruga R, Kirschner P, Černý M, 2016. The genus *Cyclops* (Copepoda, Cyclopoida) in Europe. Zool Scr 45:671-682.

Lindberg K, 1956. Courtes diagnoses de quelques membres nouveaux ou peu connus du genre *Cyclops* s. str. Boll Soc Entomol Ital 86:112-117.

Machida RJ, Miya MU, Nishida M, Nishida S, 2004. Large-scale gene rearrangements in the mitochondrial genomes of two calanoid copepods *Eucalanus bungii* and *Neocalanus cristatus* (Crustacea), with notes on new versatile primers for the srRNA and COI genes. Gene 332:71-78.

Martin P, De Broyer C, Fiers F, Michel G, Sablon R, Wouters K, 2009. Biodiversity of Belgian groundwater fauna in relation to environmental conditions. Freshwater Biol 54:814-829.

Mirabdullayev IM, Defaye D, 2004. On the taxonomy of the *Acanthocyclops robustus* species complex (Copepoda, Cyclopidae): *Acanthocyclops brevispinosus* and *A. einslei* sp. n. Vestn Zool 38:27-37.

Novikov A, Sharafutdinova D, 2022. Revision of the genus *Canthocamptus* (Copepoda: harpacticoida) with a description of a new species from the Lena River Delta (Northeastern Siberia). Eur J Taxon 826:33-63.

Nowakowski K, Sługocki Ł, 2024. Contrasting responses of *Thermocyclops crassus* and *T. oithonoides* (Crustacea, Copepoda) to thermal stress. Sci Rep 14:7660.

Pendino V, Vecchioni L, Stoch F, Marrone F, 2024. Checklist and distribution of the groundwater crustacean fauna from Sicily, Italy. J Limnol 83:2199.

Pociecha A, Karpowicz M, Namiotko T, Dumnicka E, Galas J, 2021. Diversity of groundwater crustaceans in wells in various geologic formations of southern Poland. Water 13:2193.

Posada D, 2008. jModelTest: phylogenetic model averaging. Mol Biol Evol 25:1253-1256.

Rouch R, 1994. Copepoda, p. 105-111. In: C Juberthie and V Decu (eds.), Encyclopaedia Biospeologica. International Society for Subterranean Biology.

Särkkä J, Levonen L, Mäkelä J, 1997. Meiofauna of springs in Finland in relation to environmental factors. Hydrobiologia 347:139-150.

Särkkä J, Levonen L, Mäkelä J, 1998. Harpacticoid and cyclopoid fauna of groundwater and springs in southern Finland. J Mar Syst 15:155-161.

Smolska S, Karpowicz M, Świsłocka M, Jekatierynczuk-Rudczyk E, Więcko A, Tarasewicz K, 2024. The patchy distribution of groundwater copepods in the lowland river valley. Ecohydrol Hydrobiol 24:773-784.

Stoch F, 2007. Copepods colonising Italian springs. In: M Cantonati, E Bertuzzi and D Spitale (eds.), The spring habitat: biota and sampling methods. Monografie del Museo Tridentino di Scienze Naturali 4:217-235.

Strayer DL, May SE, Nielsen P, Wollheim W, Hausam S, 1995. An endemic groundwater fauna in unglaciated eastern North America. Can J Zool 73:502-508.

Sukhikh N, Alekseev V, 2015. Genetic and morphological heterogeneity within *Eucyclops serrulatus* (Fischer, 1851) (Crustacea: Copepoda: Cyclopidae). J Nat Hist 49:2929-2953.

Tamura K, Stecher G, Kumar S, 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol Biol Evol 38: 3022-3027.

Výravský D, Hřívová DK, Bojková J, Horsák M, Zhai M, 2023. Effects of thermal stability on microcrustacean assemblages in spring fens. Inland Waters 13:86-100.

Zhai M, Hřívová D, Peterka T, 2015. The harpacticoid assemblages (Copepoda: Harpacticoida) in the Western Carpathian spring fens in relation to environmental variable and habitat age. Limnologica 53:84-89.

Online supplementary material:

Fig. S1. Bryocamptus zschokkei.

Fig. S2. Bryocamptus echinatus.

Fig. S3. Bryocamptus cuspidatus.

Fig. S4. Bryocamptus minutus.

Fig. S5. Bryocamptus pygmaeus.