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Niphargus entlebuchensis sp. nov., a new subterranean amphipod species (Crustacea, Amphipoda, Niphargidae) restricted to a karst area in Central Switzerland

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Abstract

Groundwater ecosystems are of high interest to both taxonomists and ecologists for their high diversity as well as pronounced biogeographic patterns of occupying organisms persisting across major geological time periods, such as glaciation cycles. Over the last years, coordinated efforts increased the knowledge on the diversity and distribution of stygofauna in Switzerland, particularly the order Amphipoda, revealing several new species with highly localized occurrences. Thereby, citizen science approaches and collaborations with both cavers and drinking water providers have greatly increased the available data. As a result of these activities, we here describe another new species of the genus *Niphargus* Schiödte, 1849, namely *Niphargus entlebuchensis* **sp. nov.** Molecular and morphological species delimitation analyses both clearly support its species status. Multi-marker phylogenetic analyses recover the new species within a clade that comprises several other Swiss species, such as *N. luchoffmanni* Fišer et al., 2018 and *N. muotae* Fišer et al., 2017, while the internal structure of the clade received weak support. So far, the species has only been found in a single karst mountain ridge in Central Switzerland, suggesting that it is a narrow-range endemic.

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Keywords
Biodiversity, citizen science, groundwater, *Niphargus*, stygofauna, taxonomy

Introduction

Switzerland hosts a rich biodiversity across diverse ecosystems (Gattlen and Klaus 2023). Its varying topography and climate zones, spanning lowland to mountain habitats, and its location within Central Europe overlapping with multiple major drainage basins make it a valuable study area for ecological and biogeographic questions. While many ecosystems are well studied and monitored, certain blind spots remain. Among them is groundwater. Groundwater in Switzerland has a volume comparable to that of all Swiss lakes combined and supplies 80% of the country's drinking water (Sinreich et al. 2012). Nevertheless, its biodiversity remains understudied and is consequently largely absent from national monitoring programs and biodiversity reports (Knüsel et al. 2025).

In recent years, and based on previous efforts (e.g., Strinati 1966), substantial work aimed to better understand and document the Swiss subterranean aquatic biodiversity (stygofauna) (Altermatt et al. 2014; Altermatt et al. 2019). Novel sampling approaches were developed and evaluated. Particularly, citizen science programs were established to receive stygofauna samples from cavers (Fišer et al. 2017) and drinking water providers (Alther et al. 2021). These efforts yielded extensive data on Swiss stygofauna with a high spatial resolution (Schneider et al. 2023; Knüsel et al. 2024b). Groundwater amphipods were the most commonly found stygobites, most of them belonging to the genus *Niphargus* Schiödte, 1849 (Schneider et al. 2023), which is a very species-rich genus of groundwater amphipods in the Western Palaearctic (Borko et al. 2021). Using amphipods as a model system enabled better understanding of biogeographic and eco-evolutionary patterns in Switzerland's groundwater ecosystems (Knüsel et al. 2023; Knüsel et al. 2024a; Delić et al. 2025).

The discovery of multiple new groundwater amphipod species highlights that the country's species richness is substantial and was long underestimated (Altermatt et al. 2014; Fišer et al. 2017; Fišer et al. 2018; Altermatt et al. 2019; Alther et al. 2021; Stoch et al. 2024; Delić et al. 2025). Some of these species have small-ranged, endemic distributions, making them an important component of the Swiss natural heritage (e.g., Fišer et al. 2017). Yet, some lineages and clades still require taxonomic revision and formal descriptions (e.g., Knüsel et al. 2023; Delić et al. 2025). Here, we describe a new species of *Niphargus* found exclusively in a karstic area in central Switzerland. In former publications, we had recognized the species status based on molecular differences, and preliminarily referred to it as an undescribed species (*Niphargus* sp. "Schrattenfluh"; Knüsel et al. 2024a, 2024b), yet without a proper morphological and taxonomic description. Here, we formally describe it and provide taxonomic and genetic analyses.

Material and methods

Sampling and origin of specimens

The species studied here was found in one specific karstic mountain ridge (the Schrattenfluh, Fig. 1), which lies within the UNESCO Biosphere reserve Entlebuch in central Switzerland. The mountain ridge is approximately 6 km long and spans an elevation between 1,200 and 2,100 m a.s.l. The first specimens were discovered from samples that were collected as part of a large citizen science study across Switzerland (Knüsel et al. 2024b). These specimens were found in two spring catchment boxes located at the bottom of the western flank of the karstic area. In such spring catchment boxes, groundwater is passively sourced through a horizontal drainage pipe and then used for drinking water supply. The specimens were collected through a filter net (mesh size 0.8 mm, Sefiltec AG, Höri, Switzerland) that was attached to the inlet of the drainage pipe for multiple days. Additional specimens were later collected by speleologists in a cave in the eastern part of the karstic area. In total, 18 specimens were available, all preserved in 80% molecular grade ethanol. The full specimen list including sampling data and GenBank accession numbers is provided in the Suppl. material 1: table S1.

Morphological analyses

For detailed analysis, 2 males and 8 females were partly dissected in glycerol and mounted on slides in glycerol gelatine. The animals were studied under a Zeiss microscope and a stereomicroscope (Leica M165C with a Leica Flexacam C1 camera). Photographs and measurements were made with the LAS X software from Leica using taxonomic characters presented in Fišer et al. (2009) and Premate and Fišer (2024). For the illustrations, we used Adobe Illustrator (v 29.7.1), with photos as background pictures and following digital inking (Coleman 2003).

Molecular work

Genomic DNA was isolated from two to three pereopods using a Chelex/proteinase K solution. We amplified the mitochondrial cytochrome oxidase I (COI) gene for all 18 specimens and three nuclear DNA gene fragments, namely part of the 28S rRNA gene, Histone 3, and ITS, for a subset of three specimens (Suppl. material 1: table S1). For COI we used the primers LCO1490-JJ and HCO2198-JJ (Astrin and Stüben 2008) to amplify a 658 bp long fragment. For H3 we used primers from Colgan et al. (1998) to amplify a 331 bp long fragment and for ITS a 1654 bp long fragment (Flot et al. 2010). Lastly, we amplified a fragment of the 28S rRNA gene (854 bp, herein named 28S-22, Verovnik et al. 2005 with the reverse primer from Zakšek et al. 2007). A detailed description of the laboratory protocol and the primers is provided in Suppl. material 1. Chromatograms were inspected and edited using CodonCode Aligner 11.0.2 (CodonCode Corporation, Centerville, United States).

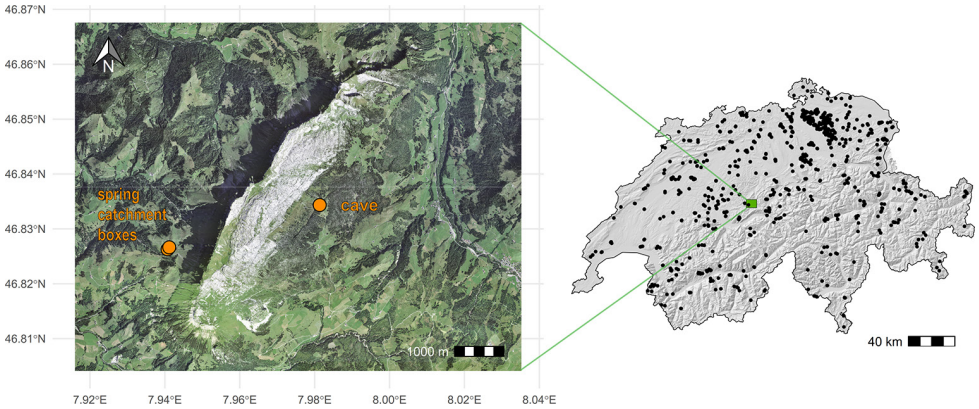

Figure 1. Map of the sampling locations. The karstic mountain ridge "Schrattenfluh" is visible on the left image (geodata: ESRI imagery) with the sampling sites marked in orange. The right image shows the map of Switzerland and all sites sampled for groundwater amphipods by Knüsel et al. (2024b) as a reference of sampling intensity and spatial coverage (geodata: Swisstopo).

Phylogenetic analyses

To assess the phylogenetic position of *Niphargus entlebuchensis* sp. nov. within the genus, we assembled a dataset of sequences based on previous studies (Delić et al. 2025; Rexhepi et al. 2025) aiming to represent major phylogenetic lineages and to include potentially closely related taxa. We included one individual per species and retrieved their available sequences for COI, H3 and 28S from GenBank. The dataset for phylogenetic analyses comprised 145 Niphargidae taxa, with three species from the family Pseudoniphargidae used as outgroup: *Pseudoniphargus spiniferus*, *P. gorbeanus*, and *Microniphargus leruthi* (Weber et al. 2021). The metadata with all GenBank accession numbers is provided in Suppl. material 2.

The sequences were aligned prior to concatenation using MAFFT 7.4 (Katoh and Standley 2013) from the NGPhylogeny.fr webserver (Lemoine et al. 2019) with E-INS-I algorithm, default gap extend and gap opening penalties, and scoring matrix 1PAM/k = 2. For 28S rRNA, poorly aligned positions were eliminated using Gblocks, resulting in an alignment of 664 bp (Talavera and Castresana 2007).

The aligned sequences for the three markers were then concatenated to a 1653 bp matrix (Suppl. material 3) and separate partitions were used for each of the three codon positions for COI and H3 (Suppl. material 1).

Phylogenetic relationships were reconstructed using maximum likelihood (ML) in IQ-TREE 2.4.0 (Minh et al. 2020) and Bayesian inference (BI) in MrBayes 3.2.7 (Ronquist et al. 2012). For the ML analysis, best-fit substitution models were selected using ModelFinder (implemented in IQ-TREE) based on the corrected Akaike information criterion (AICc) (Suppl. material 1). Node support was assessed using the ultrafast bootstrap approximation (1000 replicates) and SH-like approximate likelihood ratio test. For Bayesian inference, we chose the optimal substitution model based on a ModelFinder search from IQ-TREE and AICc, using a restricted testing procedure for

models implementable in MrBayes (Suppl. material 1). Two independent MCMC runs, each with four chains were run for 20 million generations, sampled every 1000th generation. The first 25% of the sampled trees were discarded as burn-in and a 50% majority-rule consensus tree was calculated from the remaining trees. The BI analyses were run on the CIPRES Science Gateway (Miller et al. 2010, accessible at www.phylo.org).

Molecular species delimitation

Based on the COI sequences, we ran ASAP (Assemble Species by Automatic Partitioning, Puillandre et al. 2021) using the Kimura two-parameter substitution model. Furthermore, we used MolD (Fedosov et al. 2022) to identify diagnostic nucleotide positions within the new species' COI sequence compared to the other taxa included in this study. The software for both analyses was accessed through iTaxoTools (Vences et al. 2021). We also calculated the average uncorrected pairwise genetic differences (i.e., p-distances) for COI. The COI alignment is provided in Suppl. material 4.

Results

In total, we collected 18 specimens from two spring catchment boxes and one cave. All specimens were sequenced on COI and shared a single haplotype. Three specimens were sequenced on the nuclear markers 28S rRNA, H3, and ITS and showed no intraspecific variability in either of these (GenBank accession numbers in Suppl. material 1).

Phylogenetic analyses

The phylogenetic analyses indicated that the new species *Niphargus entlebuchensis* sp. nov. is related to a clade comprising also other species known for Switzerland, such as *Niphargus luchoffmanni* (Fišer et al. 2018), *Niphargus muotae* (Fišer et al. 2017) and *Niphargus arolaensis* (Alther et al. 2021) (Figs 2, 3). The exact position of the new species within the clade is ambiguous, which is not uncommon for species in this part of the *Niphargus* phylogeny. Compared to the ML phylogeny, the Bayesian analysis recovered slightly more taxa within that clade, particularly some belonging to the polyphyletic *Niphargus rhenorhodanensis* species complex (Delić et al. 2025). However, both phylogenetic analyses showed weak support values for the given clade, hence the overall phylogenetic position for the clade and *N. entlebuchensis* sp. nov. nested within the clade cannot yet be resolved with certainty.

Molecular species delimitation

The species status of *N. entlebuchensis* sp. nov. was well supported by molecular methods. All ten best-scoring ASAP partitions recognized it as a distinct species (Suppl. material 5). MolD identified two diagnostic nucleotides in the COI alignment of

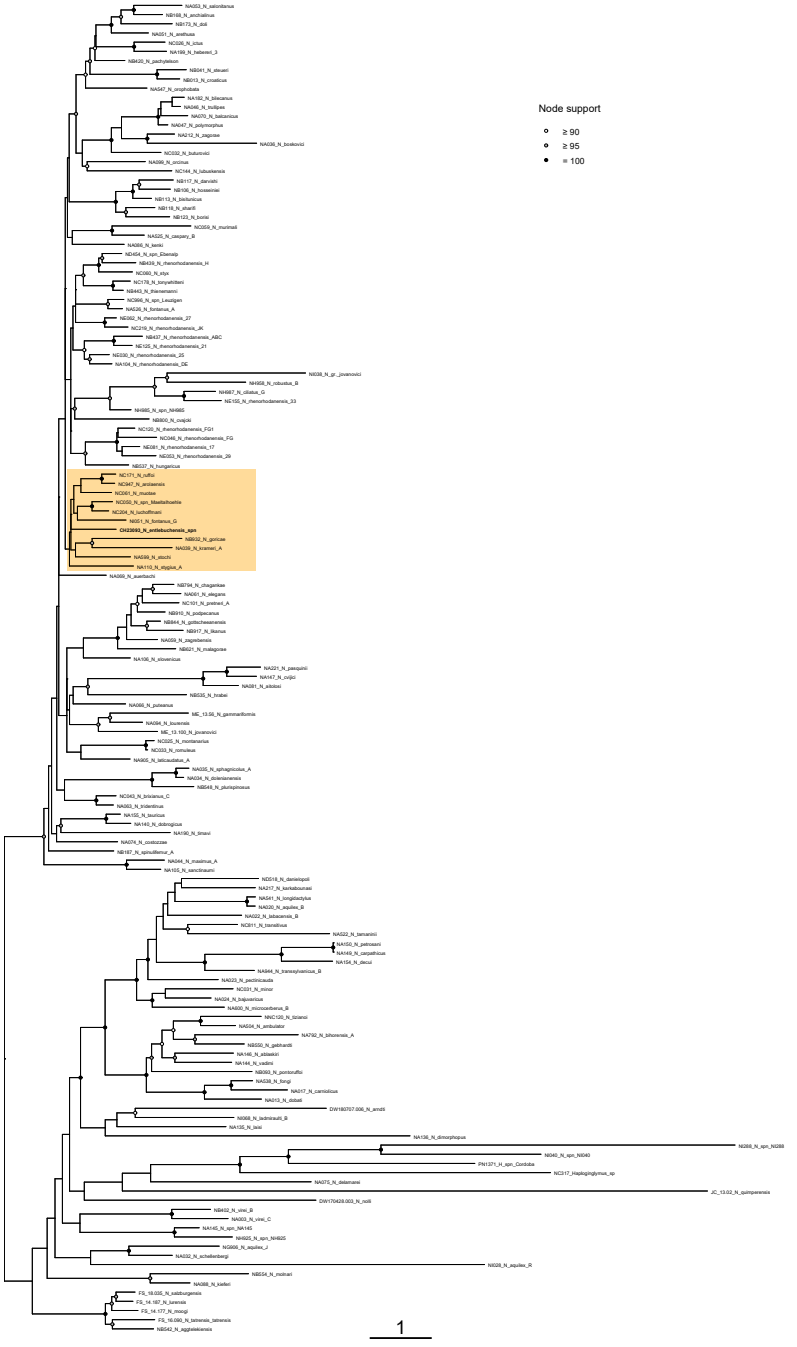

Figure 2. Phylogenetic relationships of *Niphargus* species focusing on the new taxa from Switzerland, inferred using Maximum Likelihood (IQ-TREE) based on concatenated COI, 28S rRNA and H3 gene sequences. The clade that comprises *N. entlebuchensis* sp. nov. is highlighted in orange. The tree was rooted using *Microniphargus leruthi* and two species of *Pseudoniphargus* (not shown). Node support values (UF-Boot) are color coded, the tree with full node support values is provided in the Suppl. material 1.

N. entlebuchensis sp. nov.: 'C' at position 456 and 'G' at position 586 (Suppl. material 4), with an rDNC support of 100%. The lowest interspecific uncorrected p-distance observed in COI was 10.9% between *N. entlebuchensis* sp. nov. and *Niphargus arolaensis* (Suppl. material 1: table S3).

Species description

Notes on terminology and labelling: We follow standard amphipod terminology. Setal groups on distal article of the mandibular palp are named as in Karaman (1993). Landmarks, measures and characters are presented in Fišer et al. (2009). Appendages and somites are labelled with Roman numbers increasing in antero-posterior direction, whereas articles of individual appendages are labelled with Arabic numbers, increasing in proximal-distal direction.

Niphargus species are generally armed with setae, non-articulated structures attached to cuticle within a pit, forming a simple joint. However, to simplify the description, we use the term "setae" for thin and flexible structures, "spines" for thick setae, and plumose setae for long setae with lateral hair. In the illustrations, not all setae are drawn in densely setose parts of the body unless they provide diagnostic information.

We report numeric characters in the description as intervals, acquired from two males and two females. Sexually dimorphic traits are indicated separately, in the brackets.

Class Malacostraca Latreille, 1802
Order Amphipoda Latreille, 1816
Family Niphargidae Bousfield, 1977
Genus *Niphargus* Schiödte, 1849

***Niphargus entlebuchensis* sp. nov.**
https://zoobank.org/2B886E58-39C9-4453-AD76-F786B1484F3C

Type locality. Schrattenfluh karstic mountain ridge in central Switzerland. Holotype collected from a cave (Silwängenhöhle, WGS84 46.83435, 7.98135, 1567 m a.s.l., approx. 100–150 m within the cave, –25 m depth, in small and permanently flowing seepage water, Martin Trüssel leg.). Paratypes collected from drinking water provisioning wells (Imbrig Quellen, Wasserversorgungs-Genossenschaft Marbach, WGS84 46.82628, 7.94073, 1433 m a.s.l., Bruno Renggli leg.).

Material examined. *Holotype*: • Male (id 28279), 18.5 mm long. Dissected, body appendages mounted on slides. Stored in the collection of Naturéum, Muséum cantonal des sciences naturelles, Département de Zoologie, Lausanne, Switzerland, under voucher numbers GBIFCH01224687, GBIFCH01224688, and GBIFCH10031611.

***Paratypes*:** • One male (id 22873) and two females (id 22871, id 22874) of respective lengths 13.4, 12.6, and 13.2 mm. Dissected, body appendages mounted on slides. Stored in the collection of Naturéum, Muséum cantonal des sciences naturelles,

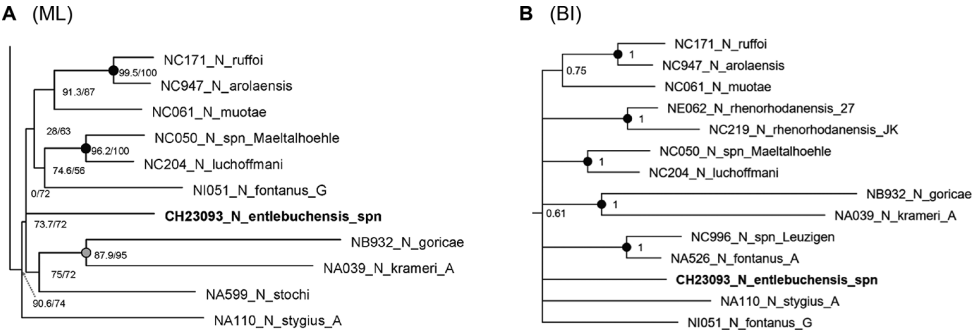

Figure 3. Phylogenetic relationships within the clade containing *N. entlebuchensis* sp. nov. shown as a pruned subtree from the full phylogenetic trees constructed using **A.** Maximum Likelihood (ML, IQ-TREE, clade shown here corresponds to highlighted orange part in Fig. 2); **B.** Bayesian inference (BI, MrBayes). Node support is given as approximate likelihood ratio test (SH-aLRT, %) / ultrafast bootstrap support (UFBoot, %) for ML and probabilities for BI. The full trees are provided in Suppl. material 1.

Département de Zoologie, Lausanne, Switzerland, under voucher numbers GBIFCH01224689–GBIFCH01224692 and GBIFCH10031612–GBIFCH10031614.

Etymology. The name "*entlebuchensis*" is derived from the region Entlebuch (and the UNESCO Biosphäre Entlebuch), where the species was discovered. The region itself is named after the stream Entle, whose name is probably of Celtic origin and is thought to mean "to spring forth" or "to emerge", as water from a spring (Greule 2014).

Morphological diagnosis. Slender and mid to large sized *Niphargus*. Maxilla I outer lobe with 9–11 bifurcate spines, the inner-most spine may have an additional denticle. Propodi of gnathopods mid-to large, palm oblique. Pereopod dactyli III–VII with a single spine accompanied with a tiny seta at the base of the nail. Uropod I rami sexually moderately dimorphic; uropod III sexually dimorphic and elongated in males. At the base of uropod I 2, rarely 1 spine(s).

Molecular diagnosis. COI: 'C' at position 456 and 'G' at position 586, rDNC support 100%. The alignment is available in Suppl. material 4.

Description. ***Body length.*** Up to 18.5 mm.

Head, pereonites, pleonites and urosomites (Fig. 4). Head approximately 10% of body length; rostrum absent. Pereonites I–VI without setae; pereonite VII with 1 postero-ventral seta.

Pleonites I–III with up to 3 slender posterodorsal setae. Urosomite I 1 postero-dorso-lateral thin spine; urosomite II 2–3 postero-dorso-lateral 3 spines; and posterior margin of urosomite III without spines.

At the base of uropod I 2, rarely 1 spine(s).

Epimeral plates. Epimeral plate I with pointed postero-ventral corner and angular convex posterior margin with 4 spines/setae. Epimeral plate II with approximately perpendicular, pointed postero-ventral corner and slightly to distinctly convex posterior and ventral margins; ventral and posterior margin with 2–3 spines and 3–5 setae,

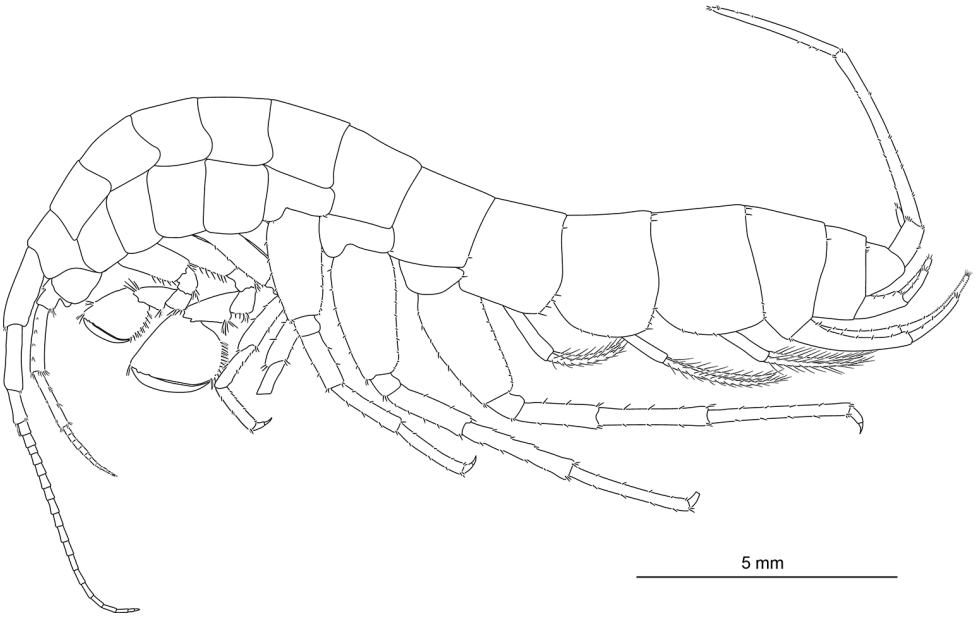

Figure 4. *Niphargus entlebuchensis* sp. nov., male holotype (18.5 mm). Appendages were rearranged digitally after drawing. Telson is not drawn (but see Fig. 8E).

respectively. Epimeral plate III with slightly yet distinctly inclined, pointed posteroventral corner; concave to sinusoid posterior margin and slightly convex ventral margins; ventral and posterior margin with 3 setae and 4–6 setae, respectively.

Antennae (Fig. 5). Antenna I (Fig. 5A) 49–51% of body length. Flagellum of antenna I with up to 22–24 articles; each article with 1 aesthetasc. Peduncle articles lengths progressively shorter. Proximal article of peduncle dorso-distally slightly produced. Accessory flagellum biarticulated; distal article shorter than half of proximal article length.

Antenna I : antenna II length 1 : 0.44–0.48. Flagellum of antenna II (Fig. 5B) with 10–11 articles; aesthetascs absent. Peduncle article 4 slightly shorter than peduncle article 5; flagellum slightly shorter than length of peduncle articles (4 + 5).

Mouth parts (Fig. 6). Labrum (Fig. 6A) of roughly triangular shape, typical of the genus. Labium (Fig. 6B) bilobate, inner lobes slightly exceeding half of the length of the outer lobes.

Left mandible (Fig. 6C, D). Incisor with 5 teeth, lacinia mobilis with 4 teeth; a long row of serrated setae between lacinia mobilis and molar process. A long seta at the base of the molar process. Right mandible (Fig. 6E, F). Incisor process with 4 teeth, lacinia mobilis bifurcate with 9–13 small denticles, a row of denticulate setae and molar process with a long basal seta. Second mandibular palp (Fig. 6C, E) article : distal article length 1 : 1.15–1.18. Proximal palp article without setae; second article with 15–18 setae; distal article with 1–2 groups of A setae with totally 6–10 setae; 3–4 B groups with totally 9–12 setae; 18–30 D setae; 6–8 E setae.

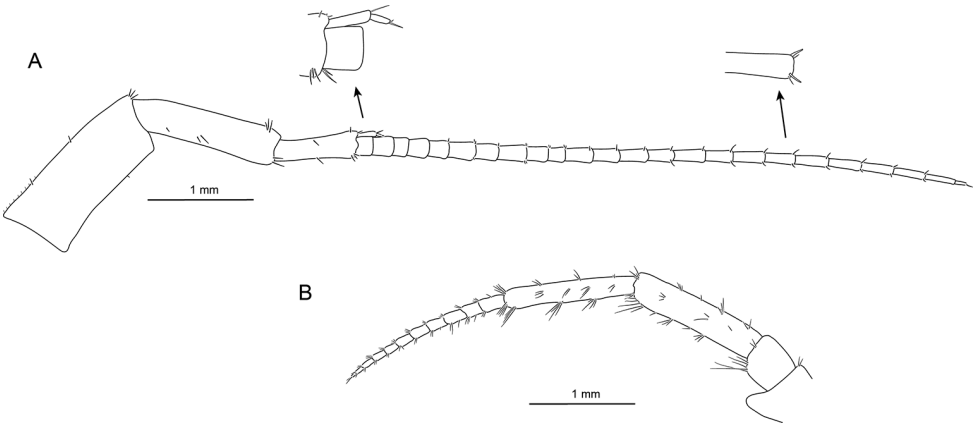

Figure 5. *Niphargus entlebuchensis* sp. nov., male holotype. **A.** Antenna I; **B.** Antenna II.

Maxilla I (Fig. 6G) palp biarticulated; distal article with 5–7 apical setae. Outer lobe of maxilla I with 9–11 spines; inner spine with 2–3 denticles, the rest bifurcate; inner lobe with 2–3 setae.

Maxilla II (Fig. 6H) bilobed; inner lobe slightly smaller than outer lobe; both of them setose apically.

Maxilliped (Fig. 6I) palp article 2 with 8–11 rows of setae along inner margin; distal article with a pair of dorsal setae and 2 small setae at the base of the nail; outer lobe with 12–14 flattened spines and 4–7 serrated setae; inner lobe with 4 flattened apical spines, 1 subapical spine and 6–8 denticulated setae.

Gnathopods (Fig. 7). Gnathopod I (Fig. 7A) coxal plate of rhomboid shape with rounded corners; anterior and ventral margins of coxa I with 9–10 setae. Basis armed with long setae at anterior and posterior margins. Ischium with posterodistal row of 9–14 setae. Carpus : propodus length 1 : 0.73–0.94. Anterior margin of carpus with one distal group of setae; carpus posteriorly with transverse rows of setae proximally, a row of lateral setae, a short row of setae at the base medially and submarginal setae groups (not all setae illustrated in detail); posterior enlargement wide, inserted medially. Propodus length : width 1 : 1.17–1.30; diagonal : length 1 : 0.76–0.88; diagonal : width 1 : 0.87–0.93. Along posterior margin 6–9 rows of 3 to 6 denticulate setae. Anterior margin and sub-margin with 2–4 setae groups, totally with 7–20 setae; antero-distal group of setae with 8–13 setae. A group of 4–5 long thin setae proximally to palmar spine; small groups of surface setae present (not illustrated). Palmar corner with strong palmar spine, single short supporting spine on inner side and 3–4 denticulate spines on outer side. Total dactylus length : nail length 1 : 0.2–0.25; along anterior margin 7–10 setae (single or in groups); short setae along inner margin present.

Gnathopod II (Fig. 7B) coxal plate width : depth 1 : 0.9–1; anterior and ventral margin with 8–13 setae. Carpus : propodus length 1 : 1.14–1.37. Anterior margin of carpus only with one distal group of setae; posterior margin densely setose, not deviating from the commonly observed setal patterns, a row of lateral setae, a short row

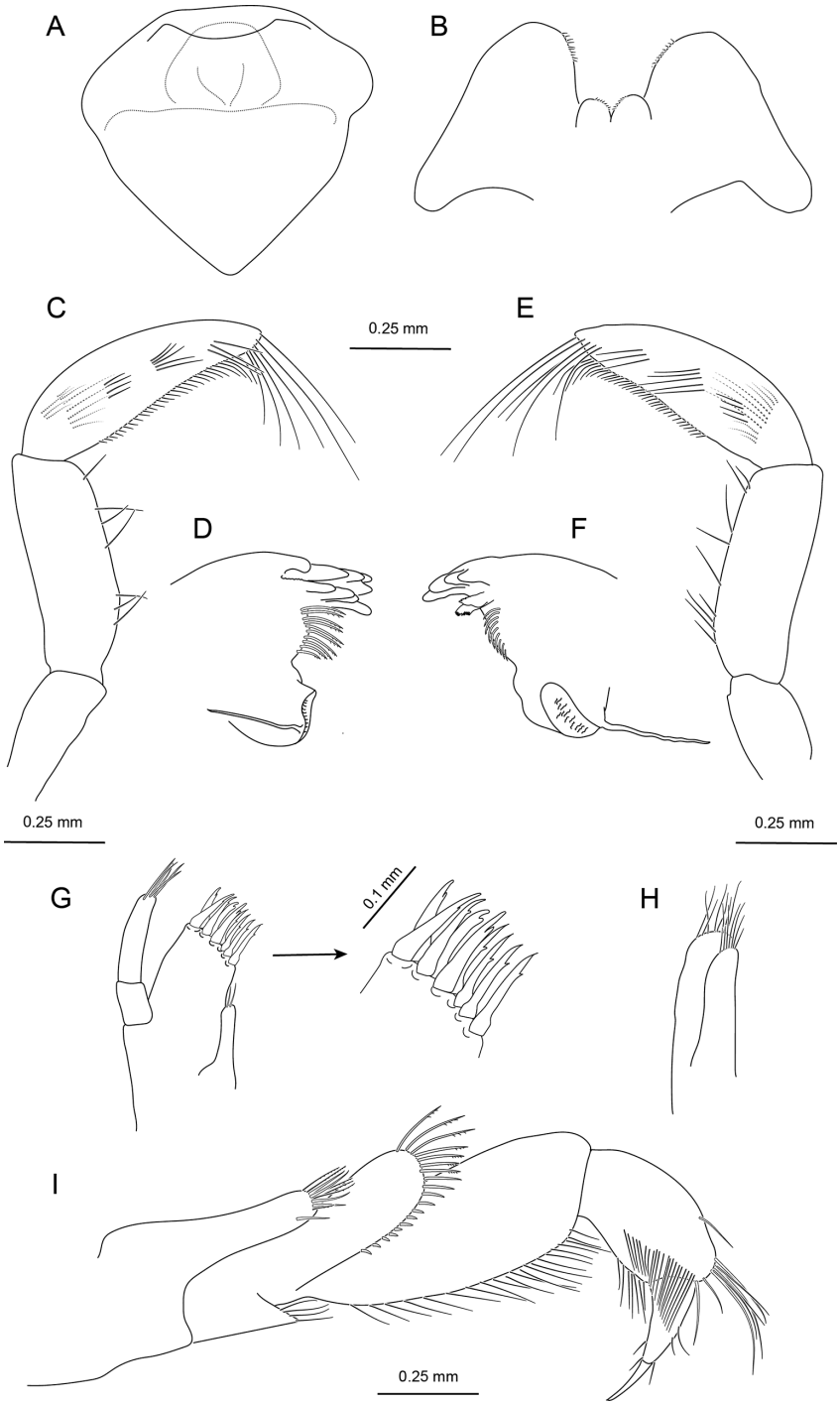

Figure 6. *Niphargus entlebuchensis* sp. nov., male holotype mouthparts. **A.** Labrum; **B.** Labium; **C, D.** Left mandible including mandibular palp; **E, F.** Right mandible including mandibular palp; **G.** Maxilla I; **H.** Maxilla II; **I.** Maxilliped.

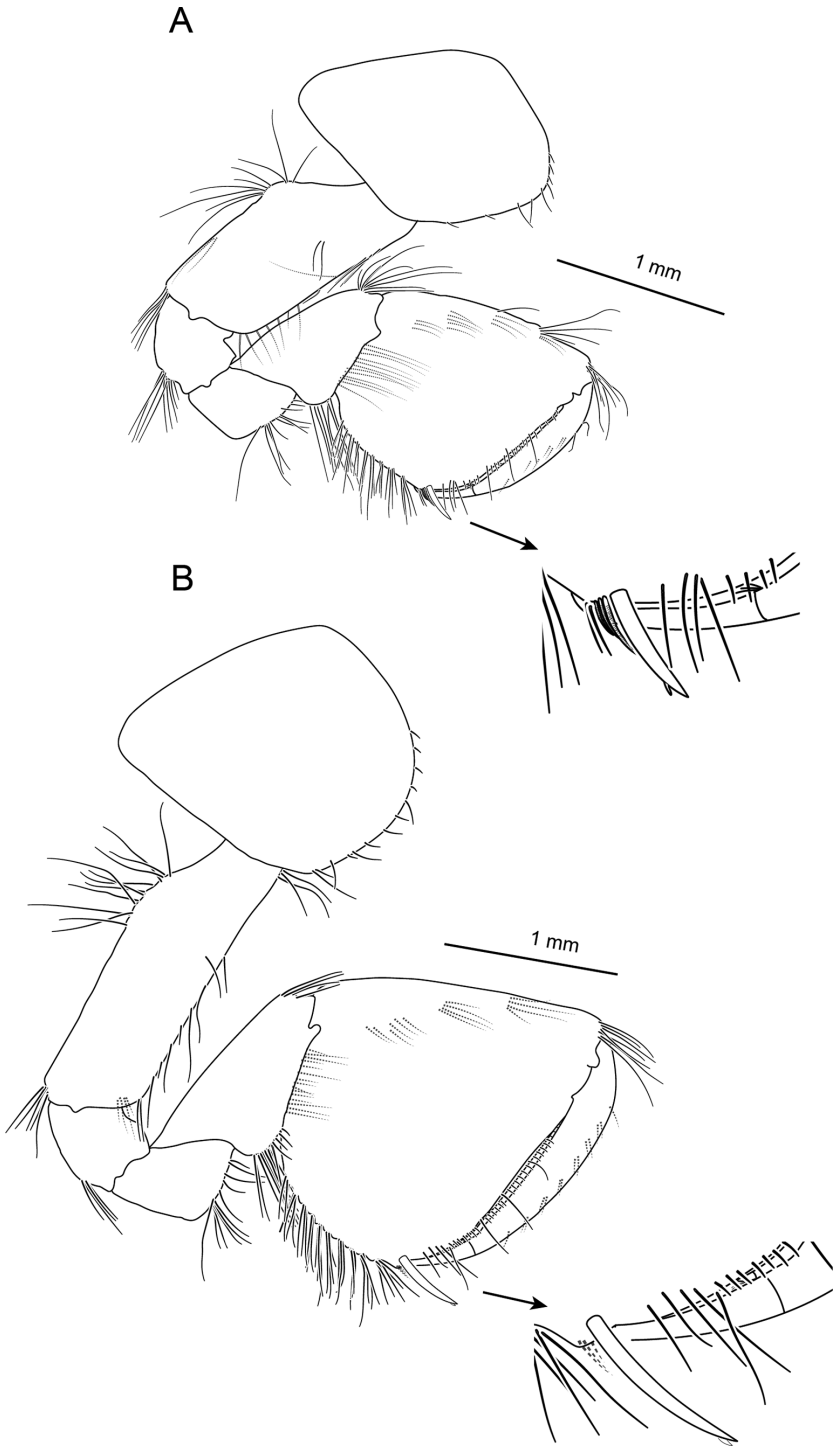

Figure 7. *Niphargus entlebuchensis* sp. nov., male holotype gnathopods. **A.** Gnathopod I; **B.** Gnathopod II. Not all setae are drawn in densely setose parts unless they provide diagnostic information.

of setae at the base medially and submarginal setae groups (not all setae illustrated in detail); posterior enlargement wide, inserted medially. Gnathopod II propodus size (length + width + diagonal) 0.25–0.27 body length; the length of gnathopod I propodus : the length of gnathopod II propodus 1 : 1.17–1.45. Propodus length : width 1 : 1.00–1.06; diagonal : length 1 : 0.87–0.99; diagonal : width 1 : 0.87–0.99. Posterior margin with 8–10 rows of denticulate setae. Anterior margin and sub-margin with 1–4 setae groups, totally with 2–14 setae; anterodistal group of setae with 7–13 setae. A group of 4–5 long thin setae proximally to palmar spine; small groups of surface setae present (not illustrated). Palmar corner with strong palmar spine, single short supporting spine on inner side and 1–3 denticulate spines on outer side. Total dactylus length : nail length 1 : 0.20–0.25. Along anterior margin 12–14 setae (single and in groups); short setae along inner margin present.

Pereopods (Fig. 8). Pereopods III–IV (Fig. 8A, B) of subequal lengths, in ratio 1.0 : 0.95–1.00.

Coxal plate III width : depth 1 : 0.9–1.0; along antero-ventral margin 9–12 setae. Dactylus III with dorsal plumose seta; at the base of nail a spine and a seta.

Coxal plate IV width : depth 1 : 0.8–0.9; posteriorly slightly concave; along antero-ventral margin 9–13 setae. Propodus : dactylus lengths 1 : 0.45–0.48; total dactylus length : nail length 1 : 0.45–0.55. Dactylus IV with dorsal plumose seta; at the base of nail a spine and a seta.

Pereopods V–VII (Fig. 8C–E). Pereopod V length : VI length : VII length 1 : 1.30–1.40 : 1.30–1.37. Pereopod VII length 50–60% body length.

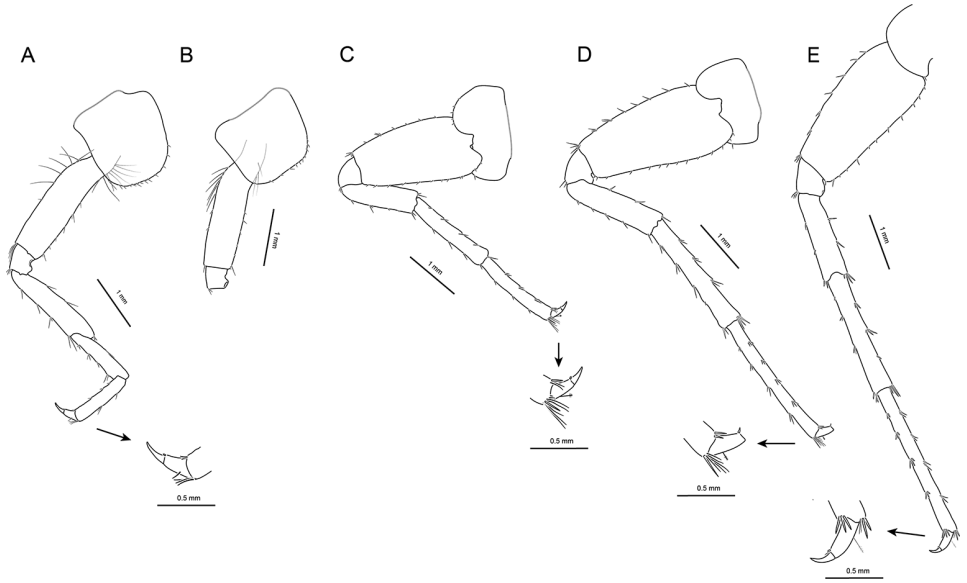

Figure 8. *Niphargus entlebuchensis* sp. nov., male holotype pereopods. **A.** Pereopod III; **B.** Pereopod IV (distal segments missing, could not be illustrated, but measurements taken from the other individuals); **C.** Pereopod V; **D.** Pereopod VI (nail of dactylus missing); **E.** Pereopod VII.

Coxal plate V. Anterior lobe with 5 setae and posterior lobe with 1 seta posteriorly. Basis of pereopod V length : width 1 : 0.51–0.55; posterior margin slightly convex, with weak postero-distal lobe; 11–14 setae posteriorly and anteriorly 7 groups of multiple or single spines and/or setae, thereof 6 at the anterior margin and 1 group at the antero-distal corner. Dactylus of pereopod V with dorsal plumose seta; at the base of nail a spine and a seta.

Coxal plate VI. Posterior lobe with 1 posterior seta. Basis of pereopod VI length : width 1 : 0.50–0.55; posterior margin slightly convex, with weak postero-distal lobe; 14 setae posteriorly and anteriorly 8 groups of multiple or single slender spines and/or setae, thereof 7 at the anterior margin and 1 group at the antero-distal corner. Dactylus of pereopod VI with dorsal plumose seta; at the base of nail a spine and a seta.

Coxal plate VII semiovoid with 1 seta posteriorly. Basis of pereopod VII length : width 1 : 0.50–0.53; posterior margin slightly convex, with weak postero-distal lobe; 12–15 setae posteriorly and anteriorly 7 groups of multiple or single spines and/or setae, thereof 6 at the anterior margin and 1 group at the antero-distal corner. Propodus length : dactylus length 1 : 0.20–0.25. Dactylus of pereopod VII with dorsal plumose seta; at the base of nail a spine and a seta.

Gills II–VI large, irregularly ovoid (data not shown in figure).

Pleopods (Fig. 9A). Pleopods I–III each with 2 hooked retinacles. Pleopod II rami of unequal length, 11–15 and 14–17 articles per inner and outer ramus, respectively.

Uropods. Uropod I (Fig. 9B). Basis with 7–8 dorsolateral spines; 2–3 dorso-medial spines. Exo- : endopodite 1 : 1.00–1.43; rami straight. Endopodite with 3 single spines medially and 4 groups of 1–4 setae/spines laterally; apically 5 spines; exopodite with 3 single spines medially and 4 groups of 1–4 setae/spines laterally; apically 4–5 spines.

Uropod II (Fig. 9C). Exopodite: endopodite length 1 : 1.2–1.25. Basal article with 4 groups of 1–2 spines each. Exo- and endopodite both with 4 spines laterally and 4 spines apically.

Uropod III (Fig. 9D). Uropod III 15–20% and 20–35% of body length in females and males, respectively. Basal article of uropod III with 2–4 lateral short spines and 8–10 apical setae. Basal article length : endopodite length 1 : 0.4; endopodite apically with 1–2 spines and 1–2 setae, in total up to 3 setal elements; laterally 1 spine. Exopodite of uropod III, distal article 0.20–0.25 and 0.20–0.30 of the proximal article. Proximal article with 5–8 groups of setae, plumose setae and spines along inner margin; 4–5 spines and setae groups along outer margin. Distal article with 2–5 groups of 2–4 setae laterally on outer margin, small setae on inner margin and 2–6 apical setae.

Telson (Fig. 9E, F). Length roughly similar to its width, deeply cleft (0.6–0.7 of its length); plumose setae inserted laterally, in the middle part. Telson with 3–4 apical spines per lobe; the longest spine approximately as long as 0.25 of telson length; rarely 1 spine in cleft, 1 lateral spine and 2 dorsal spines.

Variability. Overall, larger individuals have more spines and setae. Appendages of smaller individuals are comparatively shorter than in larger individuals.

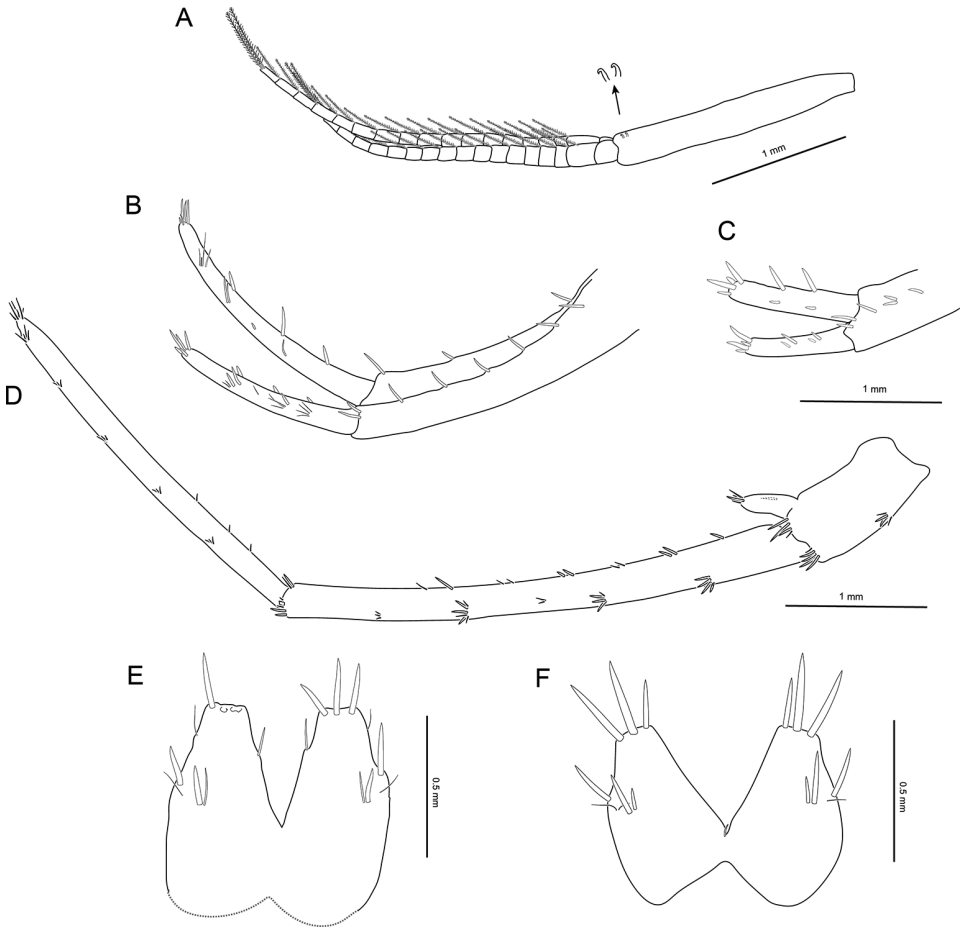

Figure 9. *Niphargus entlebuchensis* sp. nov., male holotype (**A–E**) and male paratype (**F**). **A.** Pleopod II; **B.** Uropod I; **C.** Uropod II; **D.** Uropod III; **E.** Telson (dorsal view, holotype); **F.** Telson (dorsal view, paratype, male, id 22873).

Uropod I is moderately sexually dimorphic, with slightly longer endopodite than exopodite in males. Uropod III is sexually dimorphic, i.e., distal article of exopodite is longer in males than in females.

Comparison with other species. *Niphargus entlebuchensis* sp. nov. in general appearance resembles species like *N. stygius* Schiödte, 1847 and *N. costozzae* Schellenberg, 1935, i.e., species of mid to large size, slender appearance, large gnathopod propodi, sexually little dimorphic uropod I and sexually dimorphic uropod III. From these species it can be unambiguously distinguished by large number (>7) of bifurcated spines on outer lobe of maxilla I. We checked several individuals and this character seems stable. A large majority of *Niphargus* species has only 7 spines on the outer lobe of maxilla I. There are few species (e.g., *N. orophobata* Sket, 1981,

N. caspary Pratz, 1866, *N. pasquinii* Vigna-Taglianti, 1966, *N. lunaris* Karaman, 1985, *N. nadarini* Alouf, 1972, *N. sibillianus* Karaman, 1984, *N. sodalis* Karaman, 1984) that have more. Some of these have multidenticulate, rather bifurcate spines (e.g., *N. orophobata*, *N. caspary*, *N. pasquinii*, *N. sodalis*) or have pectinate pereopods V–VII (*N. nadarini*, *N. sibillianus*) sexually non-dimorphic uropod III (*N. lunaris*). The combination of large number of spines (>7) with simple denticulation, simple dactyli and sexually dimorphic uropod III seems to be unique.

The second character we included into the diagnosis, a pair of spines growing from the same point at the basis of uropod is not stable. Although present in most of the observed individuals, some individuals had only one such spine, or they had left-right asymmetry in double-spine presence-absence. This character is generally rare in *Niphargus*, so far observed in *N. vinodolensis* Fišer, Sket & Stoch, 2006, which differs from herein described species by strongly sexually dimorphic uropod I and small propodi of gnathopods.

Discussion

We found strong molecular and morphological support for the species status of *N. entlebuchensis* sp. nov. Molecular delimitation methods consistently delimit *N. entlebuchensis* sp. nov. as a distinct lineage, yet its precise phylogenetic placement within the genus remains ambiguous. Morphologically, the large number of bifurcated spines on the outer lobe of maxilla I represents an unambiguous diagnostic character separating this species from morphologically similar taxa. The species is characterized by a comparatively large body size relative to other Swiss representatives of the genus, a trait that is likely associated with its occurrence in the karstic aquifer (Borko et al. 2021). An intermediate length of appendages may suggest that this species lives in running water but does not enter the system of crevices (Trontelj et al. 2012).

The species was exclusively recorded from a single mountain ridge in central Switzerland. Given the relatively dense sampling of *Niphargus* sp. across Switzerland, and absence in all of these other sampling sites (Fig. 1), this indicates a relatively high level of endemism. This restricted distribution pattern is comparable to that reported for species from the Hölloch cave system (Fišer et al. 2017) and further highlights the importance of isolated karst systems for endemic subterranean biodiversity (see also Couton et al. 2025). Interestingly, Pierre Strinati, who extensively documented the cave fauna of Switzerland in the 1960s, also visited a large cave in this area (Neuenburgerhöhle), but did not report any *Niphargus* specimens (Strinati 1966). Our recent work, based on a citizen science approach in collaboration with drinking water providers, has enabled data collection with a very high spatial resolution across Switzerland, including previously undersampled regions. This approach has not only enabled us to document *Niphargus* diversity and distribution patterns at a Swiss-wide scale (Knüsel et al. 2024b), but also to detect previously unknown, narrowly distributed species such as the one described here.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Artificial Intelligence (AI) use

The authors accept full responsibility for the content of the manuscript, including the disclosure of any use of AI.
Regarding the use of AI in the preparation of this manuscript, the authors declare the following: GPT.
Used for: Language, style and writing, Other.
AI (ChatGPT) was used for programming in R, as well as for grammar check and writing style assistance.

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Data availability

Information on the origin of samples, the studied specimens and GenBank accession codes are provided in the Supplementary Material.

Supplementary material 1

Specimen list, laboratory protocol, phylogenetic analyses, p-distances
Authors: Mara Knüsel, Roman Alther, Valerija Zakšek, Cene Fišer, Florian Altermatt
Data type: pdf
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Link: https://doi.org/10.3897/subtbiol.58.209466.suppl1

Supplementary material 2

Metadata for phylogenetic analyses

Authors: Mara Knüsel, Roman Alther, Valerija Zakšek, Cene Fišer, Florian Altermatt

Data type: xlsx

Explanation note: Multi-marker *Niphargus* specimen list with GenBank accession numbers.

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Link: https://doi.org/10.3897/subtbiol.58.209466.suppl2

Supplementary material 3

Concatenated sequences

Authors: Mara Knüsel, Roman Alther, Valerija Zakšek, Cene Fišer, Florian Altermatt

Data type: fasta

Explanation note: Concatenated sequences based on COI, H3, and 28S used for phylogenetic analyses.

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Supplementary material 4

COI alignment used for molecular species delimitation

Authors: Mara Knüsel, Roman Alther, Valerija Zakšek, Cene Fišer, Florian Altermatt

Data type: fasta

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Supplementary material 5

ASAP results

Authors: Mara Knüsel, Roman Alther, Valerija Zakšek, Cene Fišer, Florian Altermatt

Data type: pdf

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