RESEARCH ARTICLE

Citizen science approach reveals groundwater fauna in Switzerland and a new species of *Niphargus* (Amphipoda, Niphargidae)

Roman Alther[1,2], Nicole Bongni[1,2], Špela Borko[3], Cene Fišer[3], Florian Altermatt[1,2]

1 *Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Aquatic Ecology, Überlandstrasse 133, CH-8600, Dübendorf, Switzerland* **2** *University of Zurich, Department of Evolutionary Biology and Environmental Studies, Winterthurerstr. 190, CH-8057, Zürich, Switzerland* **3** *University of Ljubljana, Biotechnical Faculty, Department of Biology, SubBio Lab, Večna pot 111, 1000, Ljubljana, Slovenia*

Corresponding author: Roman Alther (Roman.Alther@eawag.ch)

Abstract

Knowledge on the diversity and distribution of subterranean organisms is still scattered, even in faunistically relatively well-researched countries such as Switzerland. This is mostly due to the restricted access to these subterranean habitats. Better knowledge on these organisms is needed, because they contribute substantially to overall biodiversity of a region, often contain unique elements of biodiversity, and can potentially be indicative of the ecological status of subterranean ecosystems that are providing important ecosystem services such as drinking water. Past research on subterranean organisms has often used highly specialised sampling techniques and expert knowledge. Here, we show that inclusion of non-professionals can be an alternative and highly promising sampling strategy. We retrieved citizen science-based samples from municipal groundwater wells across Switzerland, mainly from the Swiss Plateau. Opportunistic samples from 313 sites revealed a previously undocumented groundwater fauna including organisms from different major invertebrate groups, with a dominance of crustaceans. Here, we studied amphipods of the genus *Niphargus*. Among all 363 individuals sampled, we found in total eight nominal species. Two of them, namely *N. fontanus* and *N. kieferi*, are reported for Switzerland for the first time. We also found four further phylogenetic lineages that are potentially new species to science. One of them is here formally described as *Niphargus arolaensis* **sp. nov.** The description is based on molecular and morphometric data. Our study proves the suitability of citizen science to document subterranean diversity, supports groundwater conservation efforts with data, and raises awareness for the relevance and biodiversity of groundwater amphipods among stakeholders.

Introduction

Groundwater is among the most essential resources for human well-being (Zektser and Everett 2004; Griebler and Avramov 2015). Groundwater is also the largest freshwater habitat on earth (Gibert et al. 1994) and harbours unique and diverse obligate groundwater dwellers (Deharveng et al. 2009), referred to as stygofauna. Very few ecosystems have a comparable history of stable conditions, making groundwater ecosystems evolutionary unique (Culver and Pipan 2009). Subterranean fauna contributes local and unique elements to a region's overall biodiversity (Mammola et al. 2019). However, conservation and management of groundwater is often neglecting its fauna and its role as an ecosystem (Gibert et al. 2005). Given the many anthropogenic threats to groundwater (Burri et al. 2019), this aspect needs to be better considered to understand state and changes of groundwater in the context of climate change, groundwater depletion, or chemical pollution.

For many regions of the world the knowledge about the diversity and distribution of groundwater organisms – or subterranean organisms in general – is mostly lacking (Ficetola et al. 2019). Whereas there exist plethora of monitoring programs and conservation legislations for aboveground biodiversity (Scholes et al. 2012; Proença et al. 2017), their subterranean counterparts are rarely monitored and knowledge is still scattered (Gibert and Culver 2009). This can be largely attributed to both the restricted access to subterranean habitats that requires specialised sampling techniques and the subsequent expert knowledge needed to identify the groundwater organisms (Dole-Olivier et al. 2009). The existing knowledge on subterranean biodiversity therefore stands in stark contrast to its relevance for overall biodiversity.

A key part of groundwater diversity is composed by invertebrates, of which crustaceans, and especially amphipods, are among the most common ones (Sket 1999; Stoch and Galassi 2010). In many countries or biogeographic regions, the diversity and distribution of epigean amphipods is often better documented than their hypogean counterparts. Switzerland can be seen as a typical example thereof (Altermatt et al. 2014, 2019): Only very recently, data about groundwater amphipods were collected and systematically analysed (Fišer et al. 2017, 2018). While half of the known amphipod species in Switzerland live in subterranean habitats (Altermatt et al. 2019), they still contribute to just a tiny fraction of all available records. With four endemic species (*Niphargus luchoffmanni* Fišer et al., 2018; *Niphargus murimali* Fišer et al., 2017, *Niphargus muotae* Fišer et al., 2017, *Niphargus styx* Fišer et al., 2017), however, their proportion of endemics is higher than in any other organismal group in Switzerland (Tschudin et al. 2017), indicating to a potential further undocumented species diversity awaiting its scientific exploration.

Here we addressed this knowledge gap on groundwater organisms, with a focus on the genus *Niphargus* Schiödte 1849, focussing on the Swiss plateau. To cover a fine spatial resolution, we decided to deploy a citizen science approach (Lewenstein 2004), where we built on knowledge and collaboration with local drinking water well managers. About 80% of drinking water in Switzerland originates from groundwater (Freiburghaus 2012), which is collected in wells that are usually maintained and run on a municipal level. Many of these wells present an opportunity to collect and analyse water that is passively collected from the respective aquifer. An intriguing question is whether citizen science could foster biological sampling of these hardly accessible habitats and prompt the study of groundwater fauna. The inclusion of non-professionals in a scientific context, especially in data collection, dates back several hundred years, but gained increased significance in science recently in order to tackle questions at a larger spatial or temporal extent or with a wider scope (Miller-Rushing et al. 2012). Consequently, some of the most extensive datasets in ecology originate from citizen science projects and they can stir interest for a specific topic in a wider public. In our citizen science study we considered well managers as "members of the general public", and sample collection by them required gaining them as participants, distributing sampling kits with detailed instructions and maintain close contact to them. We revealed previously undocumented groundwater diversity for Switzerland, reporting three species of *Niphargus* for the first time for Switzerland, one of which is new to science and we provide a detailed description. We provide genetic barcodes for all found *Niphargus* species and discuss what we perceived as key elements for establishing and maintaining participation by citizen scientists.

Materials and methods

Sampling procedure

We focused on the Swiss plateau, a region where only very little previous data on groundwater amphipods were available (Altermatt et al. 2019), particularly on four cantons (Aargau, Basel-Landschaft, Solothurn, Zürich) in the Northern part of the Swiss Plateau (Fig. 1, grey shading). Within the study area of these four cantons (4,441 km²), we contacted the municipal drinking water well managers (hereafter referred to as well managers) to explain the project and to ask for participation in our study. We did so by sending out standard letters (Suppl. material 2) and making complementing phone calls in March 2019. Additionally, we presented the study plans and aims during the annual meeting of drinking water providers of Switzerland in April 2019. These efforts resulted in a few hundred informed well managers, also from some municipalities outside the four target cantons (Fig. 1).

The sampling of groundwater wells by the well managers followed a predefined protocol, fostering comparability. We sent the sampling material, instructions, and
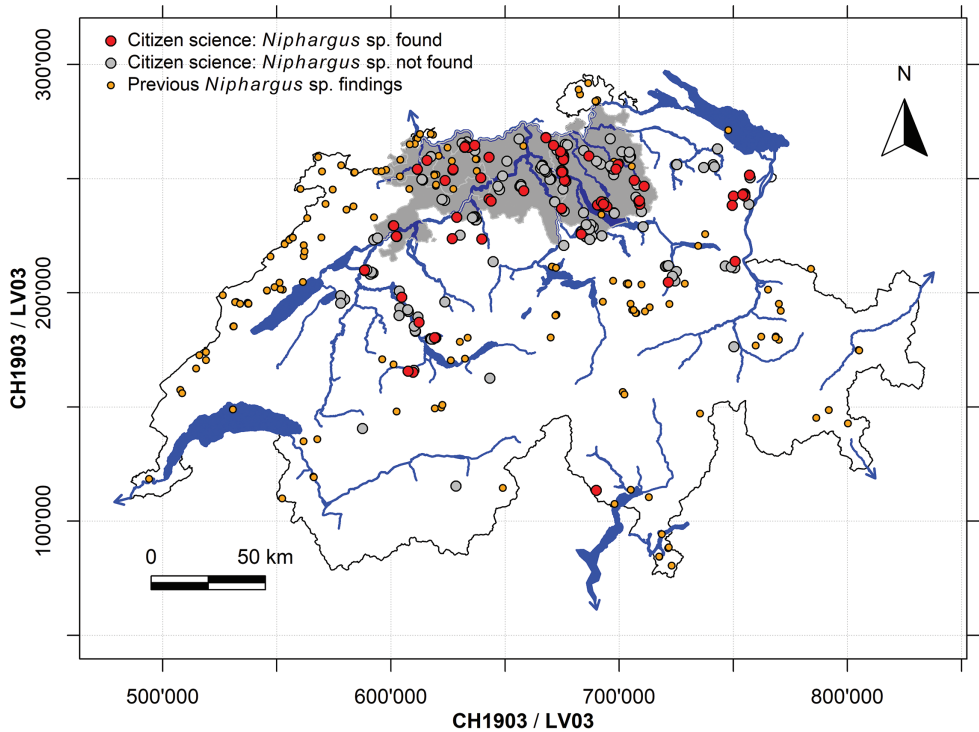
Figure 1. The study area (Switzerland) with the four focus cantons highlighted in grey. The map shows all sites sampled within the current citizen science project in which *Niphargus* sp. were either found (red circles) or not found (grey circles). All previously known findings of *Niphargus* sp. from Switzerland are indicated as orange circles. Geodata from Federal Office of Topography.

data sheets (Fig. 2A, Suppl. materials 3–8) to well managers that had agreed to pursue sampling. The sampling material provided included food-safe filter bags, cable ties, a small aquarium net, sample tubes prefilled with 80% molecular grade Ethanol, forceps, and labels. The food-safe filter bags (monofilament, nylon thermo-setting, polypropylene, polyester, PEEK; mesh size 800, diameter 100 or 180 mm; Sefiltec AG, Höri, Switzerland) were fixed by the well managers to the (piezo-)pipe draining groundwater from the aquifer to the drinking water well (Fig. 2B). This sampling method excluded pumped waters and relied on passively collected aquifers. The filter bags collected all material that was washed from the aquifer for a week. The local well managers checked the contents for organisms after this sampling period. They transferred all organisms observed to the provided sample tubes containing Ethanol (molecular grade, 80%) with provided forceps. Additionally, or if sampling procedure was not possible according to the above protocol, the overflow chamber (Fig. 2C) could be sampled with a small aquarium net (Tetra Fish-Net, 10.0 × 8.0 cm; mesh size 0.5 × 1.0 mm; Tetra GmbH, Melle, Germany). We specifically asked to report also if there were no organisms found. Samples and completed data sheets were sent back via postal service.
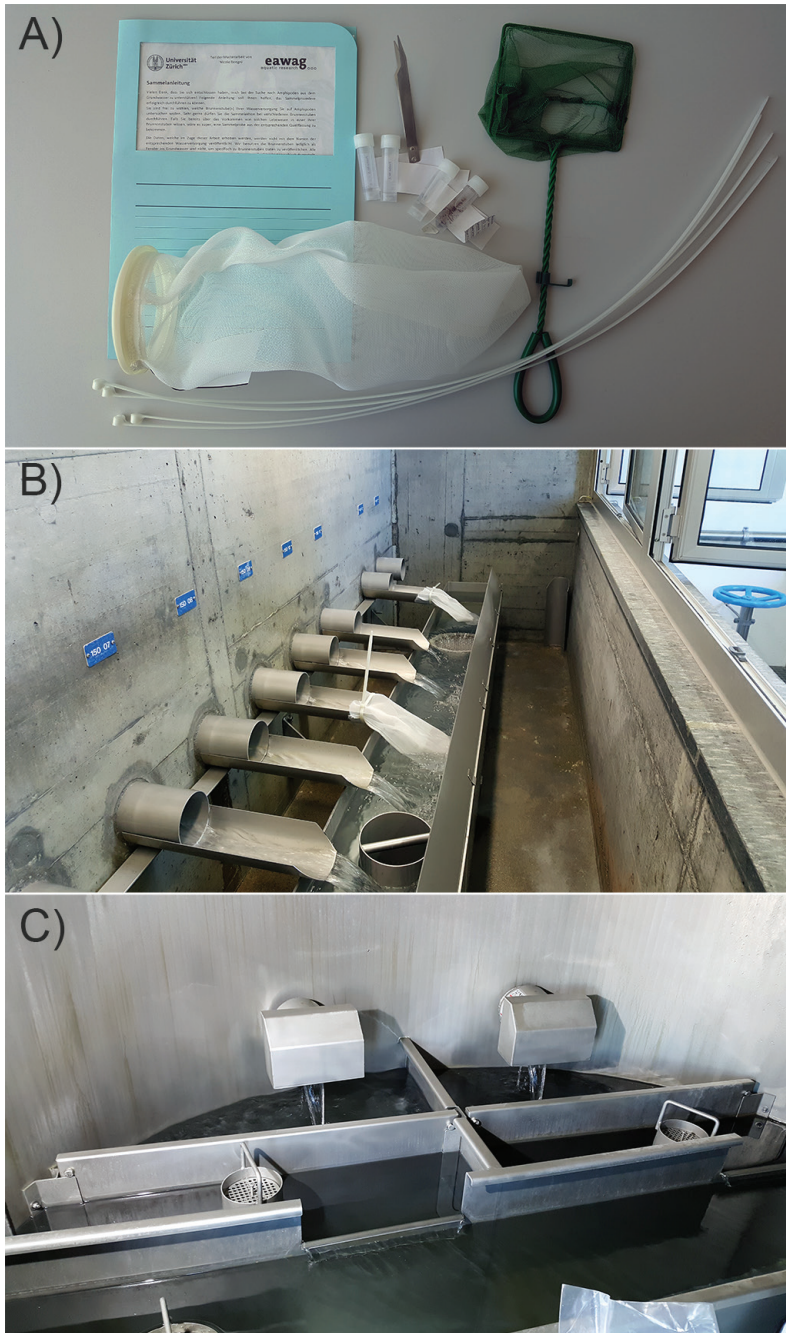
Figure 2. A Sampling material, instructions, and data sheets that were provided to well managers **B** the filter bags were fixed to groundwater draining pipes and collected all washed organisms larger than 0.8 mm **C** the overflow chambers were sampled with a small aquarium net (here: type locality of *Niphargus arolaensis* sp. nov.).

Morphological analysis and identification

After receiving back the samples, we separated all amphipods from organic matter and other macroinvertebrates, using a sorting plate and a stereomicroscope (Nikon SMZ1500, 0.75–11.25×). We identified the *Niphargus* specimens to species level with a stereomicroscope (Olympus SZX9) and a light microscope (Zeiss Primo Star). For detailed analysis, we dissected a few specimens in glycerol, and mounted them on glass slides in glycerol gelatine. We performed morphometric measurements using the program cellSense (Olympus) according to the landmarks detailed in Fišer et al. (2009). We prepared morphological illustrations using digital inking (Coleman 2003, 2009) in Adobe Illustrator 2020. We took template pictures on a Leica M205C with a mounted Canon EOS 5D Mark III. All other invertebrates were identified to relatively coarse taxonomic levels using various determination literature (Freude et al. 1981; Sartori and Landolt 1999; Schminke et al. 2007; Waringer and Graf 2011; Lubini et al. 2012; Bährmann and Müller 2015; Pfeifle and Decker 2019; Stresemann et al. 2019).

Molecular and phylogenetic analysis

For *Niphargus* specimens, we sequenced from each site at least one specimen of each morphologically distinct *Niphargus* species, resulting in 120 specimens from 68 sites. We isolated genomic DNA from one of the pereopods using the GenElute Mammalian Genomic DNA (Sigma-Aldrich, United States). We amplified the two nuclear DNA gene fragments: part of 28S rRNA gene (28S), histone H3 (H3) and the mitochondrial cytochrome oxidase I (COI) gene. We used primers from Colgan et al. (1998) for H3 fragment, primers from Verovnik et al. (2005) for 28S fragment and primers LCO 1490 and HCO 2198 (Folmer et al. 1994) for COI fragment. PCR cycling conditions for 28S and H3 are described in Fišer et al. (2013). For COI we followed the protocol of KAPA2G Robust PCR Kit (Sigma-Aldrich, United States). PCR products were purified using Exonuclease I and FastAP (Thermo Fisher Scientific Inc., United States) according to the manufacturer's instructions. Bidirectional sequencing was performed by Macrogen Europe (Amsterdam, Netherlands), using PCR amplification primers. We assembled and edited chromatograms in Geneious 11.0.3 (Biomatters, New Zealand).

We aligned the sequences with MAFFT 7.388 (Katoh and Standley 2013), using E-INS-I algorithm with scoring matrix 1PAM/k=2 and with the highest gap penalty. For 28S we eliminated poorly aligned positions and divergent regions with Gblocks (Talavera and Castresana 2007). We concatenated and partitioned alignments by codon position for H3 and COI and one partition for 28S.

We ran molecular phylogenetic analyses to assess the phylogenetic position of new *Niphargus* species within the genus. The dataset comprised six specimens of newly described species and 163 *Niphargus* taxa from different phylogenetic lineages with emphasis on potentially closely related species, each represented by one specimen. We used *Microniphargus leruthi* Schellenberg, 1934 and two species from genus *Pseudoniphargus* Chevreux, 1901 as an outgroup. We used available sequences from previous studies (Altermatt et al. 2014; Esmaeili-Rineh et al. 2015; Fišer et al. 2017, 2018,

2019, and references therein) and newly obtained sequences for seven specimens. The list of studied species, the origin of samples, and GenBank accession numbers are available in Suppl. material 9: Table S1 and Suppl. material 10: Table S2.

We reconstructed the phylogenetic relationships with Bayesian inference (BA) in MrBayes v3.2.6 (Ronquist et al. 2012) and maximum likelihood (ML) in IQ-TREE 1.6.6 (Nguyen et al. 2015). For BA we chose the optimal substitution model using Partition Finder 2 (Guindon et al. 2010; Lanfear et al. 2017), under corrected Akaike information criterion (AICc) (Suppl. material 11: Table S3). We ran two simultaneous independent runs with four chains each for 20 million generations, sampled every 1000th generation. Convergence was assessed through average standard deviation of split frequencies, LnL trace plots and PSRF, and the effective sample size. We analysed results in Tracer 1.7 (Rambaut et al. 2018). We discarded the first 25% of trees and calculated the 50% majority rule consensus tree. In ML analysis we used an option to simultaneously determine the best-fit substitution model (Suppl. material 11: Table S3) and run phylogenetic inference analysis, with ultrafast bootstrap approximation (UFBoot) and SH-like approximate likelihood ratio test (SH-aLRT) (Guindon et al. 2010; Minh et al. 2013). Phylogenetic analyses were run on the CIPRES Science Gateway (Miller et al. 2010) and IQ-TREE web server (Trifinopoulos et al. 2016). The corresponding NEXUS files are available on Zenodo (10.5281/zenodo.4770187). Finally, we calculated the average uncorrected pairwise genetic differences (e.g. p-distance) for the COI fragment between the *Niphargus arolaensis* sp. nov. and all other species using Geneious 11.0.3.

Results

Our citizen science approach proofed successful and had high return rates. First response rate to an initial letter asking for participation was 21% (40 out of the 191 contacted well managers). Subsequent talking to the well managers in person at the annual meeting resulted in more than two thirds of positive feedback. Some of the participants that initially received the letter volunteered only after meeting in person. We sent the sampling kit (Fig. 2A) and instructions to 130 well managers. 82 of those well managers participated in our study during spring and summer 2019.

The well managers provided either samples or information about null findings. Many well managers sampled multiple sites, resulting in 313 unique sites sampled (pipes draining different aquifers but collected in the same water well were considered separate sites). Additionally, some sites were sampled repeatedly, resulting in 491 samples that were sent back to our lab. 56% (274) of the samples contained organisms, totalling to over 1,900 specimens. The samples contained overall 18 different orders of macroinvertebrates. These were: Amphipoda (Crustacea, Malacostraca), Araneae (Arachnida), Chordeumatida (Diplopoda), Coleoptera (Insecta), Diptera (Insecta), Entomobryomorpha (Entognatha, Collembola), Ephemeroptera (Insecta), Hemiptera (Insecta), Hymenoptera (Insecta), Isopoda (Crustacea, Malacostraca), Julida (Diplopoda), Littorinimorpha (Gastropoda), Plecoptera (Insecta), Poduromorpha (Entognatha, Collembola), Polydesmida (Diplopoda), Pseudoscorpiones (Arachnida), Opiliones (Arachnida), and
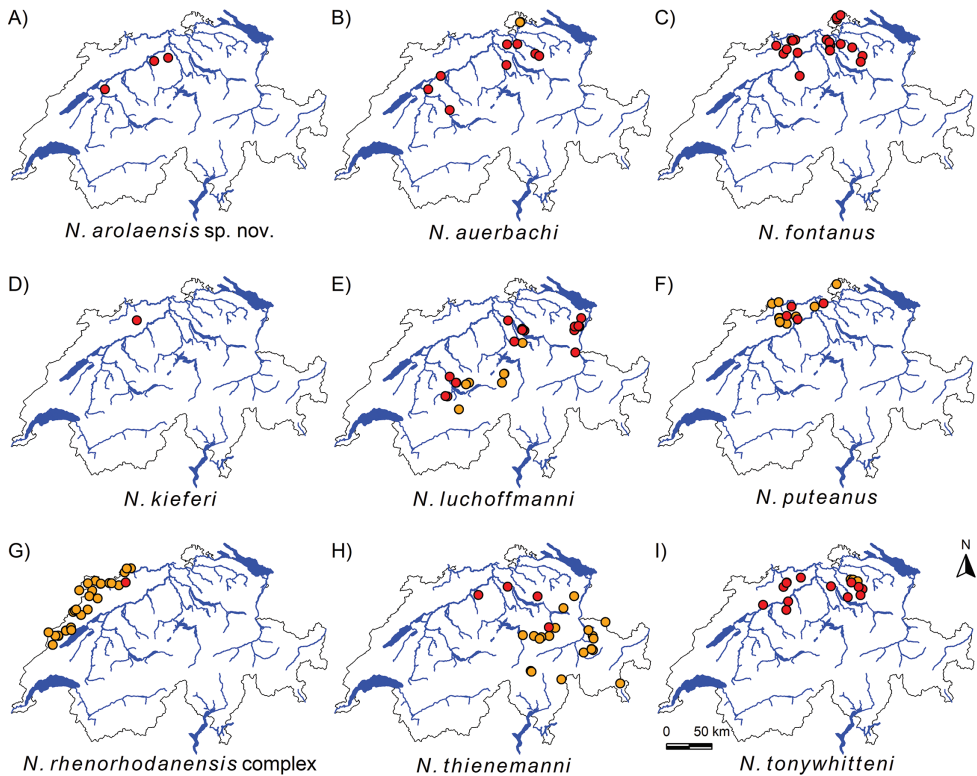
Figure 3. Maps depicting all *Niphargus* sp. findings and their respective taxonomic assignment from the current citizen science project (red circles) as well as all previously known findings of the respective species (orange circles). One species is new to science (**A**) and two species are reported for the first time for Switzerland (**C, D**). Six species (**B, E–I**) were previously reported from Switzerland. Map **G** shows all cryptic *N. rhenorhodanensis* species, including the newly found specimen of lineage H in red. Geodata from Federal Office of Topography.

Trichoptera (Insecta). Some of these organisms were not groundwater inhabitants, but may have been washed in from surface waters, or even of terrestrial origin. Amphipods were the most common and most widespread groundwater organisms in the samples, with in total 424 individuals collected from 74 sites. The majority of those (363 individuals from 63 sites) belonged to the genus *Niphargus*, while the remaining were epigean *Gammarus fossarum* that either had been washed from surface waters or colonised the water-wells from downstream sites. Here, we only focus on *Niphargus* species.

Species identity determination using the COI fragment and subsequent alignment to existing barcodes revealed 13 different phylogenetic lineages of *Niphargus*. Nine of them could be ascribed to eight nominal species. These were: *Niphargus auerbachi* Schellenberg 1934, *Niphargus fontanus* Spence Bate, 1859 (belonging to the clade A sensu McInerney et al. 2014), *Niphargus kieferi* Schellenberg, 1936, *Niphargus luchoffmanni* Fišer et al., 2018 (2018), *Niphargus puteanus* (Koch, 1836), *Niphargus rhenorhodanensis* Schellenberg, 1937 (lineages H and JK sensu Lefébure et al. 2007; lineage JK was found far outside its known range and will not be treated here further because of an ongoing revision of this species complex), *Niphargus thienemanni* Schellenberg, 1934, and *Niphargus tonywhitteni*

Fišer et al., 2018 (Fig. 3, Suppl. material 10: Table S2). We recognized four further lineages as potentially new species to science. One of them we here describe as *Niphargus arolaensis* sp. nov. (see section "Species description" below). For few *Niphargus* specimens found in nine samples, we do not yet draw further taxonomic conclusions, either due to immature stages/low number of individuals only, and/or inconclusive results from the sequencing, and we here treat them only at the genus level (*Niphargus* sp.).

Six of the found *Niphargus* species had been previously reported from Switzerland (Fig. 3A–F), namely *N. auerbachi*, *N. luchoffmanni*, *N. puteanus*, *N. rhenorhodanensis*, *N. thienemanni*, and *N. tonywhitteni* (Altermatt et al. 2014, 2019; Fišer et al. 2017, 2018). However, for *N. auerbachi* (Fig. 3F) the last records date back to the 1930s (Schellenberg 1934a; Altermatt et al. 2019).

Two species are herewith reported from Switzerland for the first time, namely *Niphargus fontanus* and *Niphargus kieferi*. We found *N. fontanus* in 19 sites in the Aare drainage area and the Rhine drainage area (Fig. 3G). Our data show that it is a widely distributed and common species in Switzerland. *Niphargus kieferi* we only retrieved from one site near Oberdorf in the canton of Baselland (Fig. 3H).

Twenty-five specimens sampled from three water wells in the cantons of Aargau and Bern, all in the Aare drainage area (Fig. 3J), belong to a new species that we here formally describe as *Niphargus arolaensis* sp. nov. (see section "Species description" below). The species appeared as a unique monophyletic lineage on the multilocus phylogeny (Fig. 4 and Suppl. material 1: Fig. S1), with p-distance on COI of at least 6% to its closest relatives, namely a lineage of probably two species, labelled *Niphargus* cf. *thienemanni* in previous publications (Fišer et al. 2017, 2018). On the other hand, all specimens of *N. arolaensis* sp. nov. closely resemble each other, with p-distances less than 1%. These results support its species status (Lagrue et al. 2014). The molecular data of the six barcoded specimens are deposited on GenBank (Accession numbers are in Suppl. material 9: Table S1 and Suppl. material 10: Table S2).

Both newly constructed phylogenetic trees showed a congruent topology. Swiss amphipods classify into few well-defined lineages. The relationship between these lineages is incompletely resolved. While ML recovered a relatively well supported clade that comprised most species reported from Switzerland (Fig. 4), BA analyses recovered parts of this clade (Suppl. material 1: Fig. S1). In either case, the newly discovered species falls into a well-defined clade comprised of Swiss species.

We submitted all newly generated COI sequences of *Niphargus* species to GenBank. All accession numbers are listed in Suppl. material 9: Table S1. The sampling localities are listed in Suppl. material 10: Table S2.

Species description

Niphargus arolaensis sp. nov.
http://zoobank.org/DCC92744-5C5C-4540-B48F-BF6083A6C57B

Type material. *Holotype* (Figs 5–9): Female, 7.8 mm (tip of rostrum to tip of third urosomite). The sample is deposited in the collection of the Musée de

Figure 4. IQ-Tree: Phylogenetic hypothesis from Maximum Likelihood. Nodes are labelled with ultra-fast bootstrap support (UFBoot)/approximate likelihood ratio test (SH-aLRT) when values are higher than 95/80 respectively. Species that occur in Switzerland are in bold.
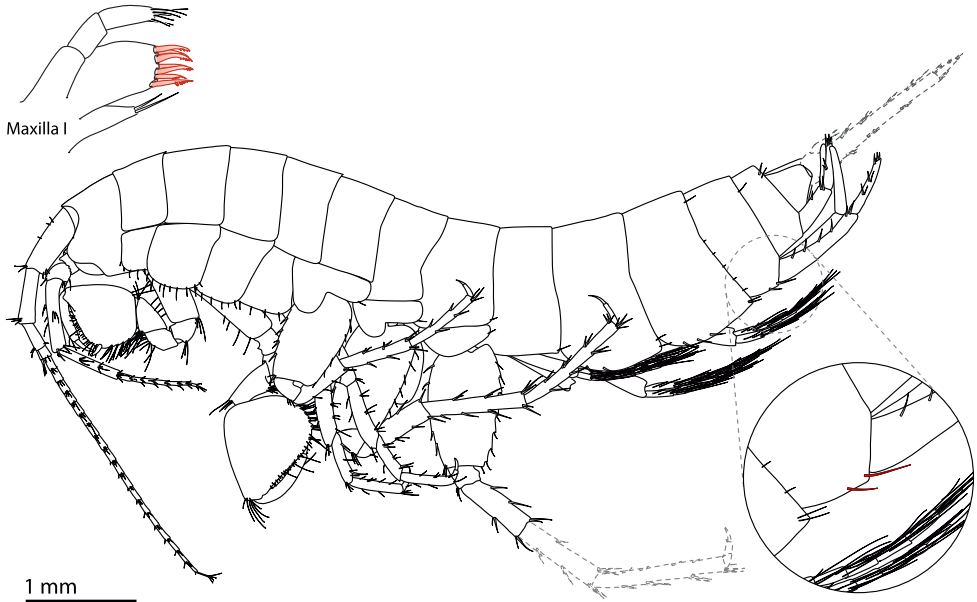
Figure 5. A new *Niphargus* species from Switzerland. *Niphargus arolaensis* sp. nov. (holotype, female 7.8 mm). The two diagnostic features (seven comb-like spiniform setae on the outer lobe of maxilla I and two spiniform setae on the lower distal part of the first urosomite near the insertion of uropod 1) are highlighted on the figure.

Zoologie, Lausanne, Switzerland under voucher number GBIFCH00602901 and GBIFCH00602902.

Paratypes: One male and three females of respective lengths 7.7, 7.8, 8.7 and 9.5 mm; specimens are partially dissected and mounted on slides with voucher numbers GBIFCH00602903, GBIFCH00602904, GBIFCH00602905, GBIFCH00602906, GBIFCH00602907.

Type locality. Stedliquelle (left inflow), Aarberg, Switzerland. CH1903: 588'518, 209'959 (WGS84: 47.04056°N, 7.28756°E), 478 m a.s.l.

Habitat and distribution. Only known from three drinking water wells: Stedtliquelle close to Aarberg, Stöckhof close to Egliswil and Lätzloch close to Kölliken, all in Switzerland.

Etymology. The name "*arolaensis*" is derived from the Latin name of the river Aare (Arola), since all findings were located in the drainage basin of the river Aare.

Diagnosis. Small and slender *Niphargus*, defined by combination of two traits. Two spiniform setae are located on the lower distal part of the first urosomite near the insertion of uropod I (Fig. 5). The outer lobe of maxilla I is armed with seven comb-like spiniform setae (Fig. 5).

Description. *Head and trunk* (Fig. 5): Body length up to 9.5 mm. Head length 6.6–7.4% of body length; rostrum absent. Pereonites I–VI without setae, pereonite VII with tiny seta close to ventro-posterior corner.

Pleonites I–III with up to three setae along the entire dorso-posterior margins. Epimeral plate II roughly perpendicular, posterior and ventral margins convex; ventro-postero-distal corner distinct; along ventral and posterior margins three spiniform and four to five thin setae, respectively. Epimeral plate III inclined, posterior and ventral margin slightly-distinctly concave and slightly convex, respectively; ventro-postero-distal corner distinct but not produced. Along ventral and posterior margin 3–4 spiniform seta; along posterior margin five thin setae.

Urosomite I postero-dorso-laterally with one slender, flexible seta; urosomite II postero-dorso-laterally with 2–3 strong setae among which at least one is strong and stout; urosomite III without seta. Ventrally on urosomite I, at the base of uropod I, are two strong spiniform setae in a row.

Telson (Fig. 9E) length : width ratio is 1 : [0.81–0.85]; cleft measures 0.61–0.75 of telson length; telson lobes margins straight and narrowing apically. Telson armature (per lobe, left-right lobe asymmetry commonly observed): 2–4 apical, 0–1 mesial, 1–2 lateral and no dorsal spiniform setae. Apical spiniform setae as long as 0.50–0.63 of telson length. Pairs of plumose setae inserted medially, along lateral margins.

Antennae (Fig. 6): Antenna I (A) measures 0.40–0.45 of body length. Flagellum with 18–22 articles; each article with one aesthetasc. Peduncle articles in ratio 1 : [0.70–0.82] : [0.35–0.40]. Accessory flagellum biarticulated, proximal : distal article in ratio 1 : [0.25–0.33].

Ratio of lengths antenna I : antenna II as 1 : [0.48–0.52]. Flagellum of antenna II (B) with 7–8 articles; each article with setae and elongated, thick sensilla of unknown function. Peduncle articles lengths 4 : 5 in ratio 1 : [0.89–0.95]; flagellum 0.57–0.66 of length of peduncle articles 4+5.

Mouthparts (Fig. 7): Labrum (A) and labium (B) typical of the genus; inner lobes of labium well visible.

Left mandible (C and D): incisor with five teeth, lacinia mobilis with four teeth; between lacinia and molar a row of serrated setae, molar triturative, at the base of molar long seta. Right mandible (E and F): incisor processus with four teeth, lacinia mobilis with several small teeth, between lacinia and molar a row of thick serrated setae, molar triturative. Mandibular palp article 3 articulated. Ratio of mandibular palp article 2 (middle) : article 3 (distal) is 1 : [1.2–1.37]. Proximal palp article without setae; the middle article with 7–8 setae; distal article with 3–5 A setae in a row; 3–4 B setae; 15–18 D setae and four E setae.

Maxilla I (G and H), distal palp article with 6–7 apical setae. Outer lobe of maxilla I with a row of 7 stout spiniform setae, each with many (>4) denticles (comb-like); inner lobe with two setae along medial and apical margin.

Maxilla II (I and K) inner lobe slightly smaller than outer lobe; both lobes setose apically and medially.

Maxilliped (L) inner lobe with three stout flattened and tooth-like setae apically and 6–11 setae along latero-apical margins; outer lobe with 7–11 stout and flattened, tooth-like setae mesially-subapically and 5–7 thick rounded and hairy setae apically. Maxilliped palp article 2 with 8–10 rows of setae along inner margin; dactylus with a dorsal seta, and few tiny setae at the socket.
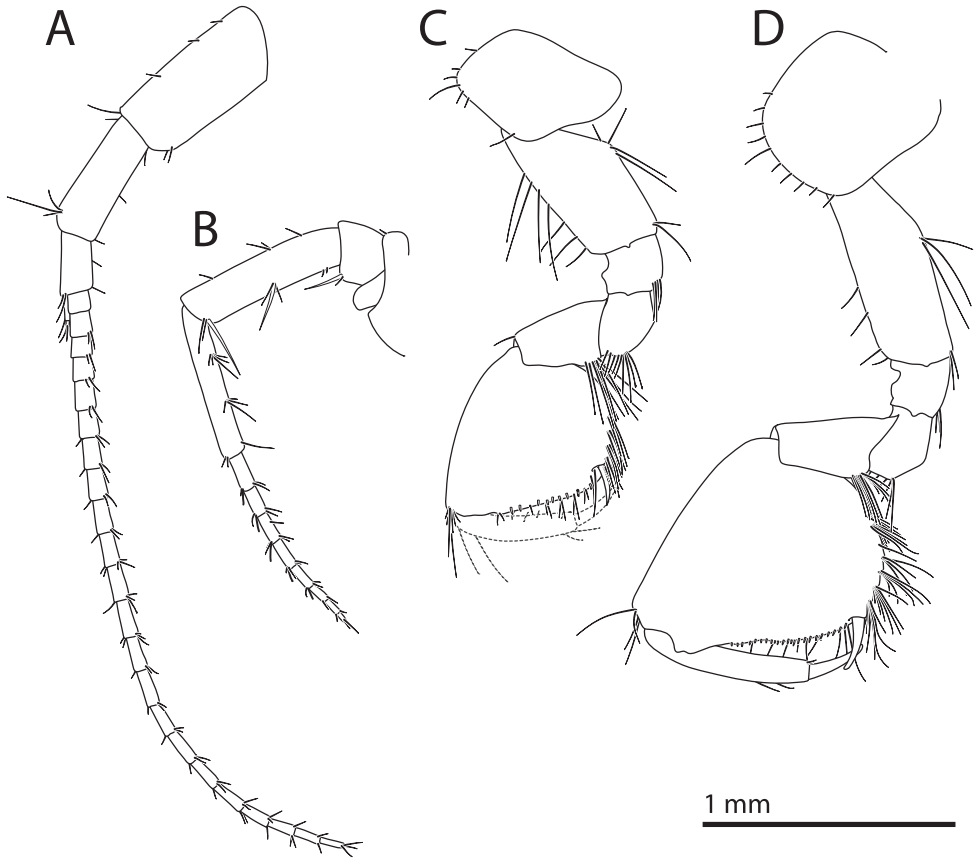
Figure 6. *Niphargus arolaensis* sp. nov. **A** antenna I **B** antenna II **C** gnathopod I **D** gnathopod II. The dactylus on gnathopod I (grey dashed) is added from the other body side.

Coxal plates, and gills (Figs 6 and 8): Coxal plate I in shape of flattened parallelogram; anterior and ventral margin of coxa I with 5–6 setae. Coxal plates II–IV width : depth ratios as [0.90–1.10] : 1, [0.85–0.94] : 1 and [1.00–1.13]: 1, respectively; anterior and ventral margins with 8–9, 6–11 and 6–7 setae. Coxal plate IV posteriorly shallowly concave. Coxal plates V–VI with well-developed anterior lobe, posterior coxal margin with one seta. Coxal plate VII half-circular with one posterior seta. Gills II–VI narrowly ovoid.

Gnathopod I (Fig. 6C): Ischium with up to 5 postero-distal setae in a single row. Carpus 0.70–0.73 of propodus length; broadened proximally. Carpus with only one distal group anteriorly, transverse rows of setae on a posterior bulk and a row of setae postero-laterally. Propodus quadratic with moderately inclined palm. Along posterior margin 4–5 rows of setae. Anterior margin with antero-distal group counting 5–10 setae and additional 8–10 setae in three groups. On the inner surface are several pairs of short setae. Palmar corner armed with one strong and stout palmar spine, a group of three long thin and simple setae anteriorly to palmar spine, one strong short and smooth "supporting" spine on the inner surface and three serrated spines
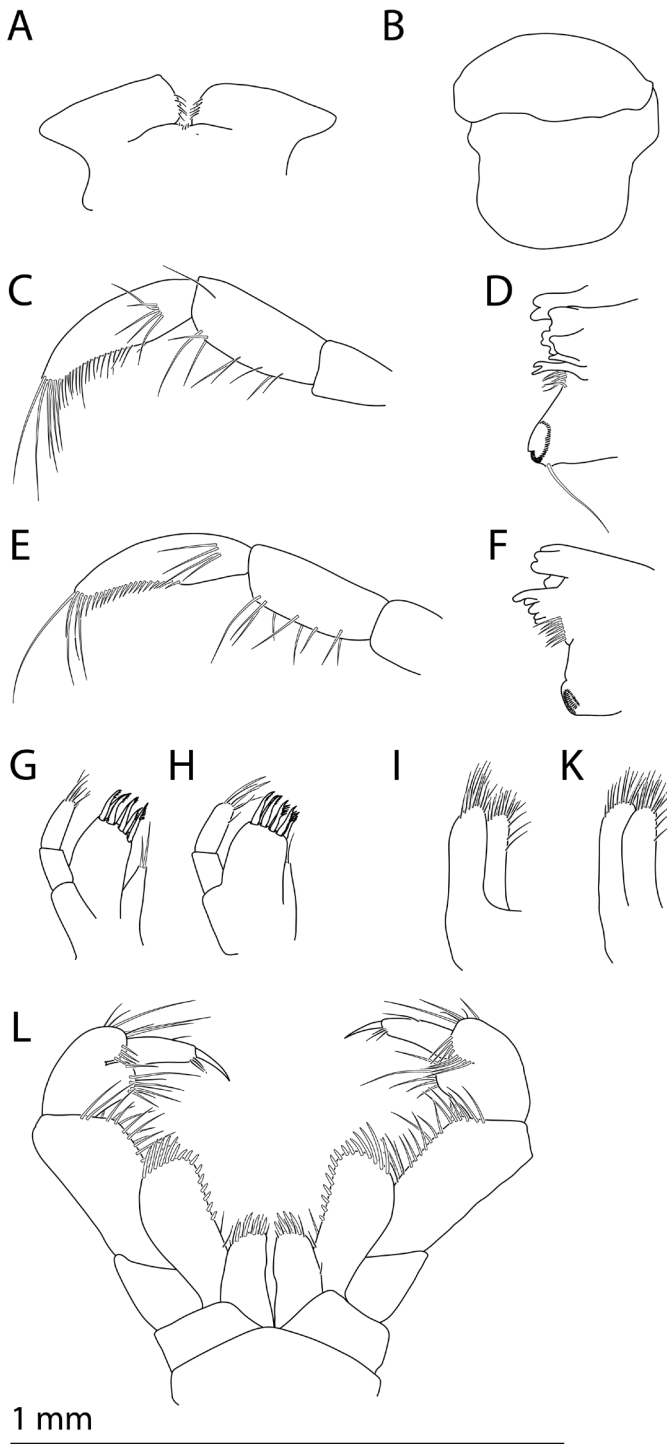
Figure 7. *Niphargus arolaensis* sp. nov., mouthparts **A** labrum **B** labium **C** left mandibular palp **D** left mandible **E** right mandibular palp **F** right mandible **G, H** maxilla I **I, K** maxilla II **L** maxillipeds.
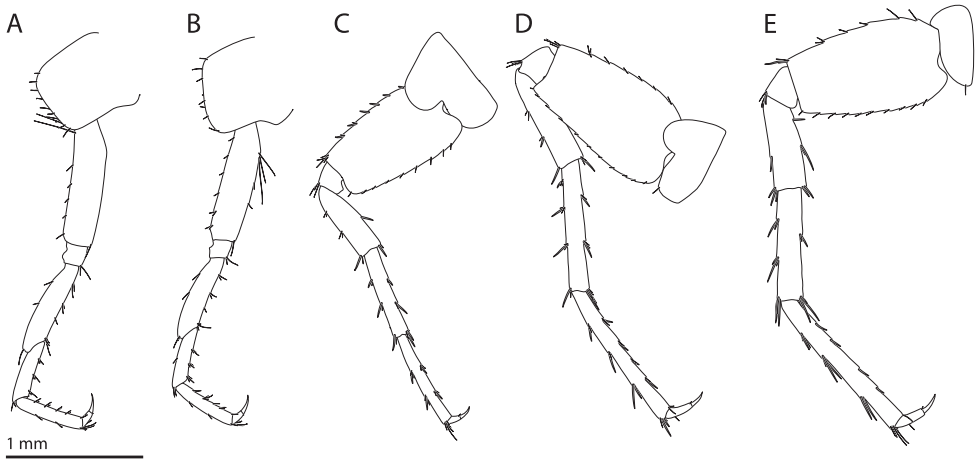
Figure 8. *Niphargus arolaensis* sp. nov. **A–E** pereopods III–VII.

behind the palmar spine. Palm with a row of short setae. Nail length 0.30–0.34 of total dactylus length; along anterior margin 2–4 single seta; along inner margin a row of short setae.

Gnathopod II (Fig. 6D): Ischium with 1–3 postero-distal setae in a single row. Carpus 0.66–0.84 of propodus length, proximally broadened. Carpus with a single groups of distal setae anteriorly; some transverse rows of setae on a posterior bulk and a row of setae postero-laterally. Propodus hoof-shaped with strongly inclined palm and large. Circumference measures up to 0.19–0.23 of body length; ratio between propodus I and II lenghts is [0.74–0.95] : 1. Along posterior margin six rows of setae. Anterior margin with antero-distal group counting 6–8 setae and additional 4–5 setae in 2–3 groups. On the inner surface are several pairs of short setae. Palmar corner armed with one strong and stout palmar spine, a group of 2–3 long thin and simple setae anteriorly to palmar spine, one strong short and smooth spine on the inner surface and 1–2 serrated spines behind the palmar spine. Palm with a row of short setae. Nail length 0.30–0.34 of total dactylus length; along anterior margin 2–4 single seta; along inner margin a row of short setae.

Pereopods III–IV (Fig. 8A and 8B): Lengths of pereopods III : IV as [0.90–0.97] : 1. Dactyli III–IV long and slender, dactylus IV measures 0.42–0.46 of propodus IV; nail length 0.56–0.65 of total dactylus length. Dactyli III–IV with 1 dorsal plumose seta; at the base of nail 1 tiny seta and one tiny spiniform seta.

Pereopods V–VII (Fig. 8C–8E): Lengths of pereopods V : VI : VII is 1 : [1.34–1.41] : [1.31–1.41]; pereopod VII measures 0.46–0.47 of body length.

Bases V–VII slender, respective length : width ratios as 1 : [0.57–0.64], 1 : [0.57–0.64] and 1 : [0.58–0.64]; posterior margins straight or slightly convex, distally ending with small to moderate-sized lobes; posterior margins armed with 8–10, 9–10 and 8–10 setae, respectively; anterior margins armed with 6–7, 6–7 and 5–6 groups of stouter setae, respectively. Dactyli V–VII with one dorsal plumose seta; at the base of
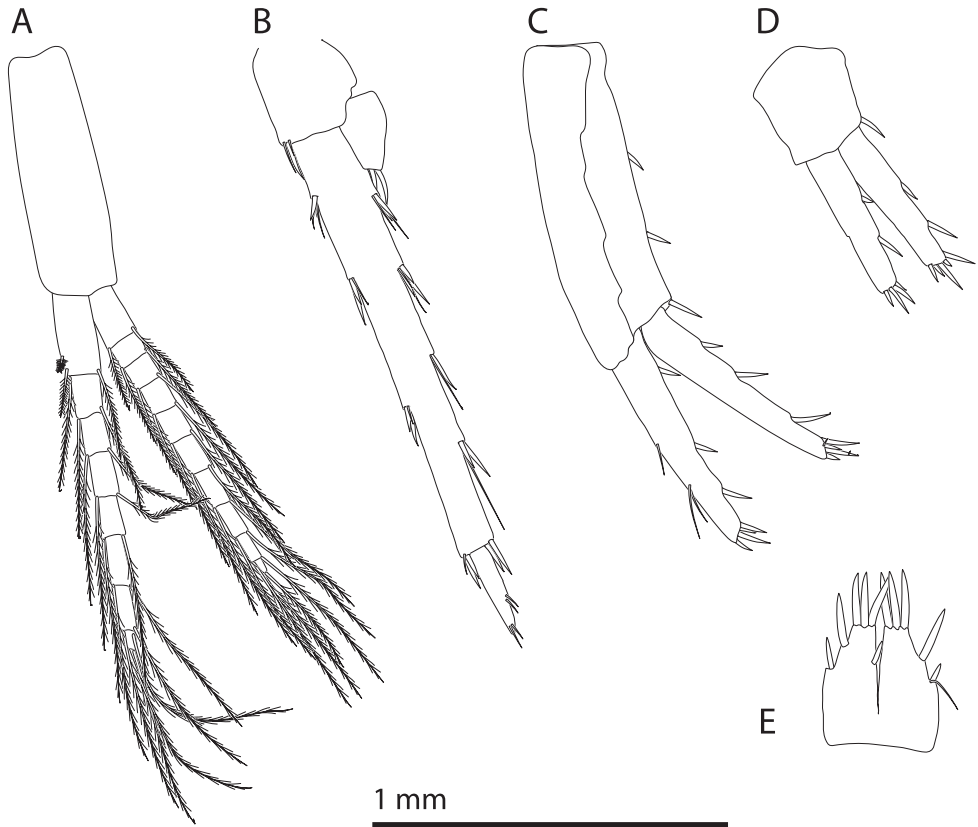
Figure 9. *Niphargus arolaensis* sp. nov. **A** pleopod II **B–D** uropods I–III **E** telson.

nail one tiny setae and one spiniform seta. Dactylus VII long and slender, its length measures 0.28–0.32 of propodus length; nail long, measuring 0.34–0.38 of total dactylus length.

　　Pleopods and uropods (Fig. 9): Pleopods I–III (A) with two hooked retinacles. Pleopod II inner and outer rami with 6–7 and 8–9 articles, respectively.

　　Uropod I (B) protopodite with six dorso-lateral spiniform setae and 2–3 dorso-medial spiniform setae. The ratio exopodite : endopodite lengths is 1 : [0.98–1.06]; rami straight. Endopodite with four individual spiniform setae laterally, rarely accompanied with a slender and flexible seta, and four spiniform setae apically. Exopodite with 2–6 spiniform setae alone or in groups; apically 4–6 spiniform setae.

　　Uropod II (C) exopodite : endopodite lengths ratio is 1 : [1.00–1.05].

　　Uropod III (D) rod-shaped, measuring 0.20–0.22 of body length. Protopodite elongated, sometimes with a single weak lateral seta and with 5–7 apical spiniform setae. Endopodite short, measures approximately 0.56–0.63 of protopodite length; laterally armed with 0–1 spiniform setae, apically armed with 3–4 spiniform setae, of which 1–2 are strong and spiniform. Exopodite of uropod III rod-shaped, distal article 0.16–0.22 of the proximal article length. Proximal article with five groups of spiniform

and plumose setae along inner margin and 4–5 groups of spiniform setae along outer margin. Distal article with 0–2 setae laterally and 1–4 setae apically.

Variability. We found no sexual dimorphism in proportions, females had oostegites on pereopods II–IV. Number of setae vary, smaller specimens had fewer setae.

Remarks and affiliation. The diagnosis is a combination two unambiguous traits. Two strong spiniform setae at the base of uropod I is a rare character, hitherto found only in *Niphargus bodoni* G. Karaman, 1985 (Italy, Karaman 1985), *Niphargus lindbergi* S. Karaman, 1956 (Borko et al. 2019; Greece, Karaman 2018), *Niphargus sertaci* Fišer, Çamur-Elipek & Özbek, 2009 (Western Turkey, Fišer et al. 2009), *Niphargus turcicus* Andreev & Kenderov, 2012 (Eastern Turkey, Andreev Kenderov 2012) and *Niphargus borisi* Esmaeili-Rineh, Sari & Fišer, 2015 (Iran, Esmaeili-Rineh et al. 2015). However, all these species have a different spiniform setae on outer lobe of maxilla I, i.e., the inner seta is multidenticulate and the remaining six setae have 1–3 denticles. By contrast, the herein described *N. arolaensis* sp. nov. has all these spiniform setae on outer lobe of maxilla I multidentate. To ease its identification in Europe, it is noteworthy that the species remarkably differs from *N. bodoni* in shape of its gnathopods. The Italian species has much smaller and more quadratic propods of gnathopods I–II, while the herein described species from Switzerland has relatively large propodi with a strongly inclined palm. Finally, it is worthy to warn that the newly described *N. arolaensis* sp. nov. superficially resembles *Niphargus forelii* Humbert, 1876 from the Alpine region. It is small, of relatively slender body, with large gnathopods, long and slender dactyli, a telson with no dorsal spiniform setae, but very long apical and marginal spiniform setae. Besides the diagnostic combination, the newly described species differs from *N. forelii* as its males apparently do not have an elongated uropod III (Karaman and Ruffo 1990).

Discussion

Unlike for the Swiss cave fauna (Strinati 1966), there is yet no general overview published about groundwater fauna for Switzerland. Here, we provide the means of tapping into this knowledge gap by applying a citizen science approach, with a focus on amphipods of the genus *Niphargus*. The opportunistic sampling campaign revealed organisms from 18 different orders. An important fraction of all individuals belonged to the genus *Niphargus*. We present a conclusive overview for those species across the Swiss Plateau, reporting 13 lineages belonging to eight nominal species, of which two are for the first time reported for Switzerland and one is even new to science. The results confirmed that a collaboration with local drinking water well managers could successfully generate data about groundwater fauna, data that would be hard to collect in a different manner (Thornhill et al. 2019) but is very valuable for biodiversity research and conservation (Theobald et al. 2015). The fraction of further subterranean species (other than *Niphargus* sp.) will be analysed and treated in a separate study.

The collaboration with well managers significantly increased the current knowledge about Swiss *Niphargus* species, raising the number of known sites of *Niphargus*

occurrence by about 22% (288, compared to 45 in Altermatt et al. 2013 and 225 in Altermatt et al. 2019). We also raised the known number of amphipod species from Switzerland to 43 species (Altermatt et al. 2019; https://www.amphipod.ch/en/resources/checklist/), adding three species to the Swiss Amphipoda checklist, namely *N. fontanus*, *N. kieferi* and *N. arolaensis* sp. nov.

The most spectacular finding of this citizen science project was the finding of a species new to science, here formally described as *N. arolaensis* sp. nov. (Figs 3J, 6–9). A total of 25 specimens were retrieved from three water wells in Aarberg (canton of Bern), Egliswil, and Kölliken (both canton of Aargau). These findings were all close to the Aare river, and fit into a biogeographic region that has been shaped by the Aare glacier (in the Chibanian). Morphologically, *N. arolaensis* sp. nov. is not very distinctive, and it is hard to align it with other ecologically distinct species. Being small, it resembles other groundwater inhabiting *Niphargus* species. Interestingly, while the gnathopods indicate that *N. arolaensis* sp. nov. might be a predator, the comb-like maxillar spines suggest that the species might feed on small particles.

While the adjacent mountainous regions (Jura Mountains and Alps) have been more intensely studied with respect to subterranean amphipods, these studies almost exclusively focussed on karstic regions (especially caves) or on interstitial habitats and less on inaccessible alluvial aquifers. Cave habitats are almost absent in the Swiss Plateau and many interstitial habitats, especially of the larger rivers, have been heavily modified by humans by river regulations and dams. Our study now shows that the groundwater habitats in the Swiss Plateau, geologically largely dominated by alluvial habitats shaped by glaciers, is (next to karstic caves and interstitial) another important habitat of *Niphargus* in Switzerland, encompassing a surprisingly high diversity of *Niphargus* species.

Swiss amphipods classify into few well-defined clades with different phylogenetic origin within *Niphargus* (Fig. 4 and Suppl. material 1: Fig. S1). However, most of the Swiss species aggregate into one phylogenetic lineage. This pattern emerged only after including new samples, obtained by this study. These new samples bring new views on the historical biogeography of species, indicating the putative presence of local radiations in groundwater. It is expected that future sampling of groundwater will reveal additional *Niphargus* species, will clarify the status of this potential Swiss radiation and enable us to explore its biogeographical and evolutionary history.

Next to *Niphargus arolaensis* sp. nov., we also report two additional species new to the Swiss fauna. The first one belongs to the *Niphargus fontanus* species complex, originally described from the United Kingdom, but also found in continental Europe. Its lineages may not be told apart based on morphology alone, and formal revision of the complex is pending. Our specimens found belong to the lineage *N. fontanus* A, that was reported from France, Belgium, Germany and parts of Austria (Hartke et al. 2011; McInerney et al. 2014). *Niphargus fontanus* A had been sampled in our pilot study (Fig. 3G) in the canton of Schaffhausen in 2018 (Rodrigues, unpublished). Subsequent sampling across the Swiss Plateau in 2019 added many more findings over an area covering a few thousand km². *Niphargus fontanus* turned out to be a widely

distributed and frequently found species, with findings scattered across the Swiss Plateau, both within the Rhine drainage area as well as within the Aare drainage area. The fact that this seemingly widely distributed species had not been found before is highlighting the need for further investigations of the groundwater fauna in Switzerland.

The second species reported for the first time for Switzerland is *Niphargus kieferi* (Fig. 3H). This species was first described as subspecies *Niphargus jovanovici kieferi* by Schellenberg from a well near Gündlingen (Germany) in the Upper Rhine plain (Schellenberg 1936), but later raised to full species by Karaman (Karaman 1980). The species is distributed in the Upper Rhine plain in France and Germany. In 2001 and 2002, the species was reported from several sites in Baden-Württemberg (Fuchs 2007), relatively close to Switzerland. We found a single specimen near Oberdorf (500 m a.s.l.) in the canton of Baselland within the Rhine drainage area and the finding fits well into the previously known distribution.

We also increased the knowledge on the distribution of six *Niphargus* species hitherto already known from Switzerland, mostly for the Swiss Plateau, but also beyond. Specifically, for the recently described species *N. luchoffmanni* (Fig. 3A), previous findings were restricted to the Central Alps in Switzerland (Fišer et al. 2018; Altermatt et al. 2019). We here report more findings around Lake Thun (Aare drainage area), in the Alpine Rhine valley in Eastern Switzerland (Rhine drainage rea), and also around Lake Zurich (Limmat catchment). This suggest that *N. luchoffmanni* may be representative for prealpine regions or lower elevations of the Swiss northern alpine regions. *Niphargus thienemanni* (Fig. 3D) was hitherto only reported from springs and groundwater habitats in alpine regions above 1000 m a.s.l., and up to 2560 m a.s.l., specifically the Eastern Alps (Altermatt et al. 2019; Austria, Germany, Switzerland; Schellenberg 1942). Here we show that it also occurs at elevations around 500 m a.s.l., which is interesting from an ecological point of view, and indicating a wide ecological (elevational) niche. *Niphargus tonywhitteni* (Fig. 3E), in its original description, was only reported from the Töss catchment in North-eastern Switzerland, and exclusively from interstitial samples (Fišer et al. 2018) as well as from a few sites in Austria and South-western Germany (Fišer et al. 2018). Here, we expand the known distribution to the whole Swiss Plateau, showing that the species is much more widespread and likely more common than initially thought (Altermatt et al. 2019). It is now also reported from the Aare drainage area, the High Rhine drainage area, and the Limmat catchment. *Niphargus puteanus* and *N. rhenorhodanensis* (represented with two phylogenetic lineages, namely H and JK sensu Lefébure et al. 2007) (Fig. 3B) were the only species in our study without a major increase in their known distribution. All findings matched well with the previously known distribution in northern and western Switzerland, respectively (Altermatt et al. 2019). Finally, we confirmed the presence of *N. auerbachi* (Fig. 3F) in Switzerland after almost a century without records. After its original description based on samples from Schaffhausen, Northern Switzerland (Schellenberg 1934a) in the 1930ies, it had never been found again (Altermatt et al. 2019). A putative finding from the Hölloch cave by Moeschler (1989) was classified as a misidentification (Fišer et al. 2017). We rediscovered *Niphargus auerbachi* during a

pilot study in Schaffhausen in 2018 (Rodrigues, unpublished), using the same citizen science approach, which was then complemented with various additional findings of the species in the greater Zurich area (Rhine and Limmat catchment) and in the Aare catchment around Bern, indicating that *N. auerbachi* is much more widespread across the Swiss plateau than initially thought (Fig. 3F).

Altogether, our study reveals that the *Niphargus* fauna of Switzerland has distinct patterns of biodiversity and distribution. A community of species inhabiting karstic areas (especially caves) is predominantly found in the Jura mountains in (North)Western Switzerland (*N. puteanus*, *N. rhenorhodanensis*, but also *N. virei*). Another community of species is predominantly inhabiting the Northern (pre)Alps, in a wide range of habitats such as caves, interstitial and groundwater, including *N. luchoffmanni*, *N. muotae*, *N. murimali*, *N. styx*, and *N. thienemanni*. Geographically in between, in the Swiss Plateau, we now report a third community cluster of species predominantly inhabiting interstitial and (alluvial) groundwater habitats, including *N. auerbachi*, *N. fontanus*, *N. kieferi*, and *N. tonywhitteni*. Further research is needed especially in the Southern and Western part of Switzerland, especially those falling into the Rhone, Ticino and Adda drainage basins.

Next to the increase in faunistic knowledge on amphipods in Switzerland, our study also showed how a generalizable citizen science approach targeting well managers could be exceptionally fruitful for gaining access to an otherwise hardly accessible ecosystem. There are debates what qualifies to be considered a citizen science project (Heigl et al. 2019), but the potential of these approaches is considerable (Thornhill et al. 2019). A key aspect of our success was that the citizen science approach targeted a well-defined group of people who access groundwater ecosystems for their use and provisioning of drinking water. While the well-defined group of people contacted may have contributed to high response rates, establishing a collaboration between local stakeholders still required a few key elements to be considered also for other similar projects. Firstly, direct personal contact and interaction with the well managers was a main factor for successfully starting and maintaining our collaboration (Evans et al. 2005). Whereas the first contact was a letter, participation rate massively increased after a direct contact (from 25% to 60% of contacted people responding positively). This required many phone calls and meetings in person. The additional time effort to do so, however, paid back in gaining further participants and samples. However, even in our short sampling scheme that required a one-time investment from the volunteers, some well managers initially agreed to take a sample but never provided any data. There are several explanations why people drop out (Marsh and Cosentino 2019). We did not investigate specifically why this was the case in our study, but a targeted community management that takes care of the volunteers might dampen some of the dropouts (Rotman et al. 2012). Secondly, being a native speaker helped a lot in fostering a common basis for collaboration, especially when explaining the goals and implications of the project concisely. Being able to show value for groundwater protection and the benefit for science could be a main motivator (Domroese and Johnson 2017). Thirdly, providing the necessary sampling kit with easy-to-follow guidelines lowered the threshold to participate and guaranteed some standardization among participants. Fourthly,

we were relatively flexible with respect to the implementation of the sampling protocol, which seemed to be an important aspect motivating well managers to participate. The local well managers are experts on their drinking water well and they often already knew about the presence of groundwater fauna and where or how to sample it best. Tapping into this knowledge, and not prescribing too strict sampling protocols likely contributed to our high rate of success. Allowing for some flexibility does not automatically increase noise in the data but may improve them (Schmeller et al. 2009). Fifthly, after the samples were sent back, providing feedback was an imperative (Rotman et al. 2012). In our case, the benefit of participating in the study was the information about the local fauna that was returned to each volunteer. Additionally, the results were published in a stakeholder oriented journal (Alther et al. 2020). Regular updates about the project, e.g., using a web blog or newsletter, asking for feedback on the scientific results, or involvement in the data analysis typically increase identification with the project (Heigl et al. 2019). The present study had an exploratory character, making these additional participatory measures hard to be implemented in time. Finally, we assured and communicated data protection from start, for example clarifying which data and how they would be published. This lowered the threshold to participate, as some drinking water managers expressed concerns about a possible release of names and specific localities of their drinking water wells. We therefore agreed that the data collected are only published in specific scientific journals, without highlighting single well managers or municipalities (or only after consultation with the respective well manager).

Overall, our approach proved highly successful. However, there are still some possible limitations associated to the approach and methods chosen. Since the groundwater was sampled in a passive way and not pumped, most retrieved samples were in a good state. However, the collected organisms may not be representative of the overall diversity in the respective localities, since some types of organisms might get washed out more easily than others. The discharge differed considerably between the sampled localities and could change depending on the surface conditions (personal communication by the well managers). Additionally, only organisms bigger than 0.8 mm were collected due to the chosen mesh size. All these circumstances make the approach a rather qualitative assessment, likely to underestimate the true diversity of groundwater fauna, highlighting the need of further and more intense sampling. This should not only cover different seasons, but all biogeographic regions of Switzerland. The herein described citizen science approach offers the potential of sampling an extended timescale and to capture potential seasonal patterns (Dickinson et al. 2010; Gouraguine et al. 2019). This is especially needed since data series or seasonal data about groundwater fauna are generally very scarce and temporal dynamics only poorly understood (Mammola et al. 2020). Consequently, little is known about the ecosystem services provided by these organisms (Griebler and Avramov 2015), such as drinking water provisioning, and if groundwater communities could be indicative of the ecological status of subterranean ecosystems (Griebler et al. 2014; Mammola et al. 2020). We thus expect that citizen science approaches may be generally valid and useful for gaining access to an unprecedented number of samples for hitherto largely understudied ecosystems such as groundwater.

Conclusion

Our study showed the feasibility of a citizen science approach in collecting data on groundwater fauna on a large spatial scale. This concept hasn't been applied at this extent to study groundwater fauna. Collaboration with local well managers resulted in groundwater samples from 313 sites, mainly across the Swiss Plateau. They included different major invertebrate groups, mainly crustaceans. We focused on the genus *Niphargus*, with 363 individuals the most common taxa in the available samples. We report eight nominal species (*N. auerbachi*, *N. luchoffmanni*, *N. puteanus*, *N. rhenorhodanensis*, *N. thienemanni*, *N. tonywhitteni*, *N. fontanus* and *N. kieferi*), with the latter two being reported for Switzerland for the first time. Additionally, we discovered four phylogenetic lineages that are potentially new species to science. One of them we describe as *Niphargus arolaensis* sp. nov. Our study is a proof-of-concept, showing that a citizen science approach could increase spatial coverage substantially, but could also raise awareness about groundwater biodiversity among stakeholders.

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Supplementary material 1

Figure S1

Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt

Data type: image (.tiff file)

Explanation note: MrBayes: Phylogenetic hypothesis from Bayesian inference. Nodes are labelled with posterior probabilities, when higher than 0.80. Lower values are reported in light-grey for focal Swiss clade. Species that occur in Switzerland are in bold.

Link: https://doi.org/10.3897/subtbiol.39.66755.suppl1

Supplementary material 2

Supporting file 1

Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt

Data type: pdf document

Explanation note: Standardized information letter to well managers, in German.

Link: https://doi.org/10.3897/subtbiol.39.66755.suppl2

Supplementary material 3

Supporting file 2
Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt
Data type: pdf document
Explanation note: Sampling instructions to well managers, in German.
Link: https://doi.org/10.3897/subtbiol.39.66755.suppl3

Supplementary material 4

Supporting file 3
Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt
Data type: pdf document
Explanation note: Sampling instructions to well managers, in French.
Link: https://doi.org/10.3897/subtbiol.39.66755.suppl4

Supplementary material 5

Supporting file 4
Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt
Data type: pdf document
Explanation note: Sampling protocol to well managers, in German.
Link: https://doi.org/10.3897/subtbiol.39.66755.suppl5

Supplementary material 6

Supporting file 5
Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt
Data type: pdf document
Explanation note: Sampling protocol to well managers, in French.
Link: https://doi.org/10.3897/subtbiol.39.66755.suppl6

Supplementary material 7

Supporting file 6
Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt
Data type: pdf document
Explanation note: Sampling labels to well managers, in German.
Link: https://doi.org/10.3897/subtbiol.39.66755.suppl7

Supplementary material 8

Supporting file 7
Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt
Data type: pdf document
Explanation note: Sampling labels to well managers, in French.
Link: https://doi.org/10.3897/subtbiol.39.66755.suppl8

Supplementary material 9

Table S1

Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt

Data type: excel table

Explanation note: List of species used for phylogenetic analyses, with the origin of samples, and GenBank accession numbers.

Link: https://doi.org/10.3897/subtbiol.39.66755.suppl9

Supplementary material 10

Table S2

Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt

Data type: excel table

Explanation note: List of discovered Niphargus individuals from the citizen science approach with GenBank accession numbers.

Link: https://doi.org/10.3897/subtbiol.39.66755.suppl10

Supplementary material 11

Table S3

Authors: Roman Alther, Nicole Bongni, Špela Borko, Cene Fišer, Florian Altermatt

Data type: table (docx. file)

Explanation note: Results of substitution model selection for Bayesian inference (Partition Finder 2) and maximum likelihood (IQ-TREE) analyses.

Link: https://doi.org/10.3897/subtbiol.39.66755.suppl11