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Zoogeography of epigean freshwater Amphipoda (Crustacea) in Romania: fragmented distributions and wide altitudinal variability

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Abstract

Inland epigean freshwater amphipods of Romania are diverse and abundant for this region has a favourable geographical position between the Balkans and the Black Sea. Excluding Ponto-Caspian species originating in brackish waters and freshwater subterranean taxa, there are 11 formally recognized epigean freshwater species recorded from this country. They belong to 3 genera, each representing a different family: *Gammarus* (Gammaridae, 8 species or species complexes), *Niphargus* (Niphargidae, 2 epigean species) and *Synurella* (Crangonyctidae, one species). Their large-scale distribution patterns nevertheless remain obscure due to insufficient data, consequently limiting biogeographical interpretations. We provide extensive new data with high resolution distribution maps, thus improving the knowledge of the ranges of these taxa. *Gammarus* species display substantial altitudinal variability and patchy, fragmented distribution patterns. They occur abundantly, particularly in springs and streams, from lowlands to sub-mountainous and mountainous regions. In the light of recent molecular research, we hypothesize that the complex geomorphological dynamics of the Carpathian region during the Late Tertiary probably contributed to their allopatric distribution pattern. Contrasting with *Gammarus*, the genera *Niphargus* and *Synurella* exhibit low altitudinal variability, broad ecological valences and overlapping distributions, being widespread throughout the lowlands. The current distribution of *N. hrabei* and *N. valachicus* seems to be linked to the extent of the Paratethys during the Early Pliocene or Pleistocene. We further discuss the taxonomic validity of two synonymized and one apparently undescribed taxon, and provide an updated pictorial identification key that includes all taxa and forms discussed in our study. The mosaic distribution of epigean freshwater amphipod species in Romania shows that this region is particularly suitable for phylo- and biogeographical analyses of this group.

Key words: geographic range, distribution patterns, habitat preferences, allopatry, sympatry, *Gammarus*, *Niphargus*, *Synurella*

Introduction

Distribution patterns offer valuable insights towards understanding historical factors that have shaped the contemporary distributions of species (Brown *et al.* 1996). Freshwater amphipod crustaceans are particularly suitable for biogeographical studies because of their restricted dispersal capabilities and the fragmentary nature of freshwater habitats (Väinölä *et al.* 2008; Hou *et al.* 2011). Amphipods are predominantly aquatic benthic animals that do not possess free-swimming larval stages or resistant propagules, and thus are prone to genetic differentiation and isolation (J.L. Barnard & C.M. Barnard 1983). Furthermore, many freshwater taxa display allopatric or discontinuous distributions, frequently presumed to result from vicariant events of geological origin, such as island separation, sea level fluctuations, and continental break up, or that follow ancient drainage patterns (Hogg *et al.* 2006; Finston *et al.* 2007; Bauzà-Ribot *et al.* 2011, 2012).

The European continent is inhabited by a relatively high number of freshwater amphipod species with diversity increasing towards the south-east (Väinölä *et al.* 2008). The diversity of the amphipod fauna of Romania is rich due to the favourable geographical position of the country, being situated at the edge of the Balkan Peninsula and the

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Black Sea, two important centres of European biodiversity. The Carpathian Mountains, which are predominant in Romania, are also considered a hotspot region in terms of biodiversity and endemism (Balint *et al.* 2011). Furthermore, the region also experienced dynamic geological events during the Late Tertiary (Popov *et al.* 2004) and was an important glacial refuge throughout the Pleistocene glaciations (Schmitt 2007). Coupled with its heterogeneous relief, Romania provides an adequate setting to study amphipod biogeographical patterns at a more focused, finer scale. Therefore, it can bring more insight into processes that are responsible for shaping geographical distributions of freshwater benthic crustaceans.

In this study, we focus only on epigean freshwater amphipod species, i.e., species that complete their life cycle in surface freshwaters and occasionally occur in subterranean waters; therefore we exclude the Ponto-Caspian brackish/freshwater taxa and typically stygobiotic (subterranean) species. The inland surface freshwaters of Romania are inhabited by 11 formally recognized native amphipod taxa that fulfil the above criteria. They belong to three genera, each representing a different family: *Gammarus* Fabricius, 1775 (Gammaridae, 8 species/species complexes), *Niphargus* Schiödte, 1849 (Niphargidae, 2 species) and *Synurella* Wrzesniowski, 1877 (Crangonyctidae, 1 species) (Cărăuşu *et al.* 1955; G. Karaman & Pinkster 1977a; Papp *et al.* 2008; Copilaş-Ciocianu 2013).

The genus *Gammarus* is distributed across the entire European continent and is the most widespread and diverse epigean freshwater amphipod genus, with numerous endemic species exhibiting allopatric distribution patterns (G. Karaman & Pinkster 1977a; 1977b; 1987; Väinölä *et al.* 2008). Moreover, many of the widespread taxa are in fact diverse species complexes comprising cryptic lineages with endemic ranges, especially in south-eastern Europe (Hou *et al.* 2011, 2013; Weiss *et al.* 2013; Mamos *et al.* 2014). In Romania, there are 8 recognized *Gammarus* taxa: *G. arduus* G. Karaman, 1975, *G. balcanicus* Schäferna, 1922, *G. fossarum* Koch, in Panzer, 1836, *G. kischineffensis* Schellenberg, 1937, *G. komareki* Schäferna, 1922, *G. leopoliensis* Jazdzewski & Konopacka, 1989, *G. pulex* Linnaeus, 1758, and *G. roeselii* Gervais, 1835 (Cărăuşu *et al.* 1955; G. Karaman & Pinkster 1977a; Papp *et al.* 2008; Copilaş-Ciocianu 2013). However, most of these might represent lineages distinct from the originally described taxa; evidence for cryptic diversity has not yet been provided for *G. arduu*s, *G. leopoliensis*, and *G. kischineffensis* (but see below).

The second genus, *Niphargus*, although highly diverse in European subterranean waters, is much less represented in surface waters, to which stygobiotic species occasionally penetrate (Meijering *et al.* 1995; Sket 1999). Two epigean species of *Niphargus*, supposedly related (Straškraba 1972) are encountered in central and south-eastern Europe; these are *N. hrabei* S. Karaman, 1932 and *N. valachicus* Dobreanu & Manolache, 1933 (Cărăuşu *et al.* 1955; Straškraba 1972; J.L. Barnard & C.M. Barnard 1983; Nesemann *et al.* 1995; Fišer *et al.* 2009). Both of these species are present in Romania (Cărăuşu *et al.* 1955).

The genus *Synurella* is represented in Europe by only two epigean species (G. Karaman 1974). Among these, *S. ambulans* Müller, 1846 is widely distributed throughout central, eastern and south-eastern Europe (G. Karaman 1974; Sidorov & Palatov 2012), and also occurs in Romania (Cărăuşu *et al.* 1955; Copilaş-Ciocianu & Pârvulescu 2012). The second species, *S. longidactylus* S. Karaman, 1929, is endemic to the ancient Lake Ohrid (G. Karaman 1974).

Although the epigean amphipod fauna of Romania is diverse and widespread, the distribution patterns of these species are largely unknown. Generally, studies dealing with such issues focused mostly on local scales (e.g., Petrescu 1996; Pârvulescu & Hamchevici 2010; Copilaş-Ciocianu & Pârvulescu 2012), and even publications focusing on the country level included very limited data (Cărăuşu *et al.* 1955; Dancău 1972; J.L. Barnard & C.M. Barnard 1983; Petrescu 1994).

We combine new extensive data with a review of relevant literature, and provide updated distribution maps of epigean freshwater amphipod species inhabiting Romania. These data are interpreted in a geological and palaeogeographical context to bring more insights into these biogeographical patterns. We also focus on identity and taxonomic validity of synonymized taxa, and provide an illustrated and updated identification key of epigean freshwater species of the above-mentioned genera that inhabit Romania.

Material and methods

Sampling, data collection and taxa identification. The material used in this study was collected from 455 sampling sites throughout the entire territory of Romania between 2005 and 2013. Specimens were collected using a benthic hand-net with a mesh size of 250 µm and were stored in either 70% or 96% ethanol, or 4% formaldehyde solution. At every sampling locality, we investigated all available microhabitats. A literature review was performed and distribution data were gathered from the relevant studies, including the most recent ones (Cărăuşu *et al.* 1955 and reference therein, Paraschiv *et al.* 2007; Petrescu 1994; 1996; 1997a; 1997b, 1998; 2000; 2009). The material from the studies of Pârvulescu (2008; 2009) was revised and incorporated into this study. Data from both the literature and from this study were taken into consideration for producing the distribution maps. Locality names, geographic coordinates and altitude are provided for each taxon in a table available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.fd8m9 (Copilaş-Ciocianu *et al.* 2014).

Taxa were identified using the morphological delimitation criteria of the following authors: Cărăuşu *et al.* (1955), G. Karaman & Pinkster (1977a; 1977b; 1987) and Jazdzewski & Konopacka (1989). At present it is known that *G. balcanicus*, *G. fossarum*, *G. komareki*, *G. roeselii*, and *G. pulex* are taxonomically challenging poly/paraphyletic cryptic species complexes (indicated by "*s.l.*" below) (Scheepmaker 1990; Müller 1998; Hou *et al.* 2011, 2013; Weiss *et al.* 2013). We are aware that the Romanian populations might represent distinct cryptic lineages, as has been shown for *G. balcanicus* (Hou *et al.* 2011, Mamos *et al.* 2014). Without a detailed insight based on molecular data, we treated the Romanian populations as belonging to the above mentioned morphospecies. However, we took into consideration the three distinct morphs of *G. balcanicus* reported from Romania. Two of them, *G. balcanicus dacicus* Dobreanu & Manolache, 1942 and *G. balcanicus montanus* S. Karaman, 1929, were formally described as subspecies. The third morph resembles *G. balcanicus* from the type locality (Cărăuşu *et al.* 1955), but the results of Hou *et al.* (2011) and Mamos *et al.* (2014) indicate that the Romanian populations are molecularly distinct from those from the type locality and, therefore, we label it *G.* cf. *balcanicus*. Although both above-mentioned subspecies are presently synonymized with *G. balcanicus* (G. Karaman & Pinkster 1987), it seems likely that many such synonymized taxa might actually be distinct species (Hou *et al.* 2011, Mamos *et al.* 2014, Wysocka *et al.* 2014). Thus, we considered it appropriate to show the morphology and distribution of the Romanian morphs of *G. balcanicus* separately, to facilitate future taxonomic and systematic studies. Aside from *G. balcanicus s.l.*, we present a putatively undescribed taxon, *G.* cf. *kischineffensis*, which is morphologically and geographically distinct although it bears some resemblance to *G. kischineffensis* (see below).

Spatial analyses. We performed spatial autocorrelation analyses using Moran's I (Moran 1950) on latitude and longitude data in order to determine the degree of geographical clustering between the localities of different species. This analysis was performed with the software SAM v4.0 (Spatial Analysis in Macroecology) (Rangel *et al.* 2010) with default settings, using a significance test with 200 permutations. This analysis requires at least 30 entries to give reliable results, thus it was performed only on taxa for which we had a sufficient number of records. These were: *G.* cf. *balcanicus*, *G. balcanicus dacicus*, *G. fossarum s.l.,* *G.* cf. *kischineffensis*, *N. valachicus* and *S. ambulans*.

Identification key. Based on available literature (Cărăuşu *et al.* 1955; G. Karaman & Pinkster 1977a; 1977b; 1987) and our own observations, an identification key was compiled to contain all morphologically distinct taxa of the respective genera (including the synonymized subspecies of *G. balcanicus s.l.* and *G.* cf. *kischineffensis*) recognized from Romanian territory. The key (provided in the Appendix) combines text with graphical depiction of all relevant identification characters based on original drawings. However, as it does not contain the taxa of Ponto-Caspian origin, the key should be used as a complement to other identification resources rather than alone.

Results and discussion

Checklist of species. The presence of 11 epigean freshwater amphipod species or species complexes previously recognized in Romania, and the two presently synonymized subspecies of *G. balcanicus*, was confirmed by our data and recent literature. The only species not encountered during our field survey was *N. hrabei*; however, a recent study reported it in south-eastern Romania (Flot *et al.* 2014), confirming its presence after more than 50 years.

Furthermore, one morphologically distinct form with a well delimited distribution pattern did not correspond to any of the morphological criteria or character combinations used to differentiate previously recognized taxa (for details, see Taxonomic remarks below) and therefore we treat it as a separate entity throughout this paper. It superficially resembles *G. kischineffensis* and we have temporarily labelled it *G.* cf. *kischineffensis*.

Distribution patterns, altitudinal ranges and habitat preferences. Below, each taxon recognized in Romania is discussed separately, in alphabetical order. Their distributions are shown in three maps: the *G. balcanicus* complex in Fig. 1, remaining *Gammarus* species except *G. fossarum s.l.* in Fig. 2, and *Niphargus, Synurella* and *G. fossarum s.l.* in Fig. 3. Altitudinal ranges of taxa are summarized in Fig. 4.

Gammarus arduus is a species with a south-eastern European distribution (G. Karaman & Pinkster 1977a). Altogether, literature records and new data from our study reveal only 6 localities scattered throughout Romania (Fig. 2). Previous reports indicated its presence in western and southern Romania. During this study it was collected from three additional sites in the south-eastern part of the country, in the Măcin Mountains. It is found in streams (Table 1) at altitudes below 300 m a.s.l. (Fig. 4).

The *Gammarus balcanicus* complex is widely distributed throughout south-eastern Europe and Asia Minor (J.L. Barnard & C.M. Barnard 1983; G. Karaman & Pinkster 1987; Özbek & Ustaoğlu 2006; Özbek *et al.* 2009). It is also the most widespread amphipod in Romania (Petrescu 1994). We present data for all three reported morphs of this taxon from the country (Fig. 1). The most common is *G.* cf. *balcanicus*, phenotypically similar to the morph from the species' type locality, which is widespread throughout the Carpathians and reaches the Dobrogea region in south-eastern Romania. Intriguingly, its occurrence decreases considerably in the central part of the country and it is absent in the south (Fig. 1). It has the widest altitudinal range among freshwater amphipods from Romania, being recorded between 16 and 1530 m, with the majority of localities situated between 300 and 600 m (Fig. 4). It mostly occurs in springs and brooks and occasionally in caves or in rivers (Table 1). It was occasionally found coexisting with *G. balcanicus dacicus*, *G. fossarum s.l.* and *G. roeselii s.l.*

TABLE 1. List of epigean freshwater amphipod taxa (excluding Ponto-Caspian species) found in Romania, their type localities, distribution ranges and habitats. Habitat abbreviations: G—groundwater, Sp—springs, St—streams, R—rivers, P—ponds, Tp—temporary ponds, L—lakes, Sw—swamps. Type locality is not listed for apparently undescribed *G.* cf. *kischineffensis*.

Taxon	Type locality	Species complex	Range	Habitats in Romania
Gamarus arduus Karaman, 1975	Malkara, Turkey	Possibly	S Europe	St
G. balcanicus Schäferna, 1922	Kolašin, Montenegro	Yes	SE Europe, Asia Minor	G, Sp, St, R
G. balcanicus dacicus Dobreanu & Manolache, 1942	Sfăcăr, Ghelinţa, Romania		Romania	Sp, St, R
G. balcanicus montanus Karaman, 1929	Skopska, Macedonia		Romania, S Europe	Sp, St
G. fossarum Koch, in Panzer, 1836	Regensburg, Germany	Yes	W and Central Europe, Asia Minor?	Sp, St, R
G. kischineffensis Schellenberg, 1937	Chişinău, Moldova	Possibly	E Europe, Asia Minor	Sp, St, R
G. cf. *kischineffensis*	-		SW Romania	Sp, St
G. komareki Schäferna, 1922	Belovo, Bulgaria	Yes	S Europe, Middle East	St
G. leopoliensis Jazdzewski & Konopacka, 1989	Brzegi Dolne, Poland		E and Central Europe	St
G. pulex Linnaeus, 1758	Öland, Sweden	Yes	NW Europe, Asia	G, Sp, St
G. roeselii Gervais, 1835	Coulanges-sur-Yonne, France	Yes	Central and SE Europe, Asia Minor	Sp, St, R, L, Sw
Niphargus hrabei Karaman, 1932	Near Budapest, Hungary		Central and E Europe	Sp, St, P, Sw
N. valachicus Dobreanu & Manolache, 1933	Bucharest, Romania		Central and SE Europe, Middle East	Sp, St, R, L, P, Tp, Sw
Synurella ambulans Müller, 1846	Greifswald, Germany	Possibly	Central, E and S Europe, Asia Minor	G, Sp, St, R, L, P, Tp, Sw

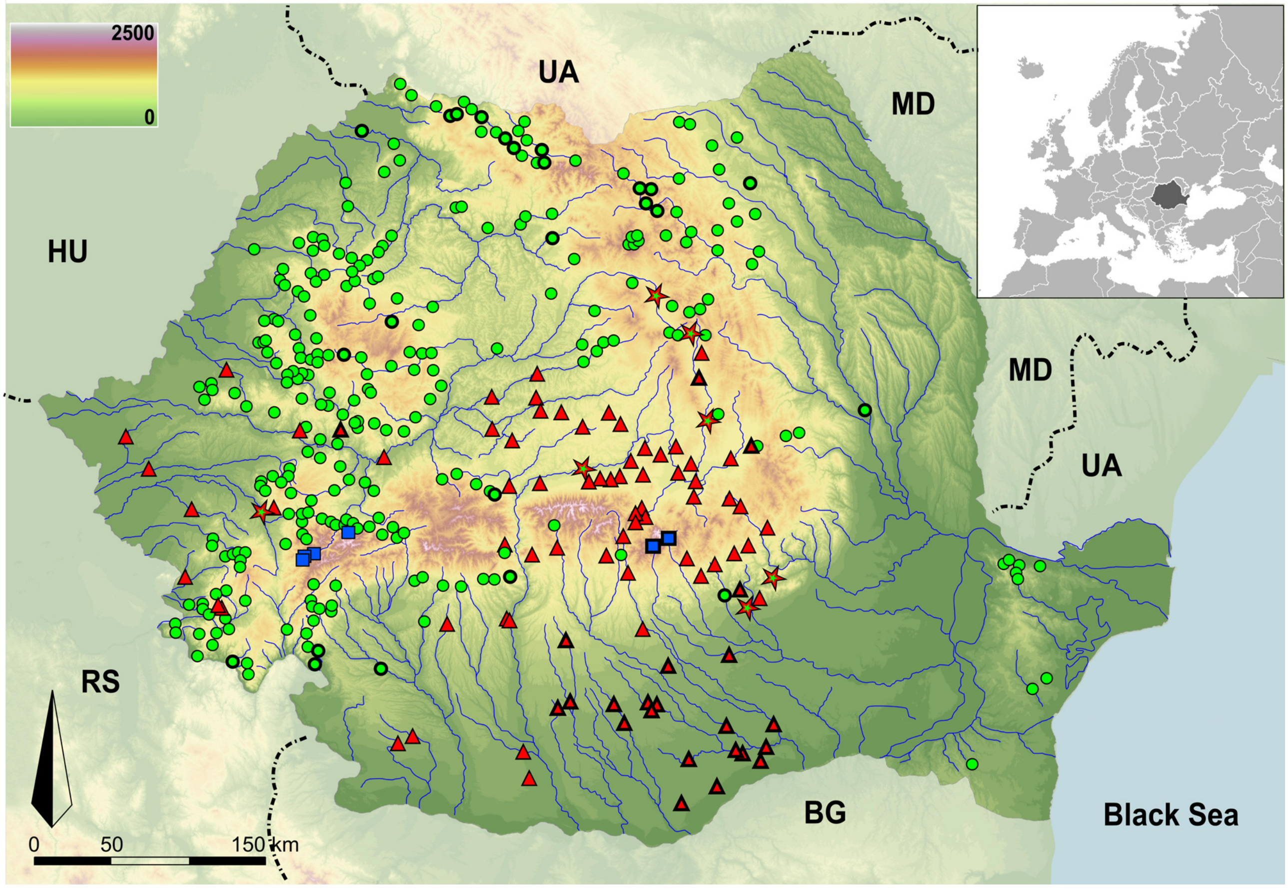


FIGURE 1. Distribution of *G.* cf. *balcanicus* (green circles), *G. b. dacicus* (red triangles) and *G. b. montanus* (blue squares) in Romania. Stars represent localities where *G.* cf. *balcanicus* and *G. b. dacicus* coexist. Shapes with thin outline—data from this study, shapes with thick outline—data from the literature. Countries are indicated by their 2-letter ISO codes: HU—Hungary, UA—Ukraine, MD—Republic of Moldova, BG—Bulgaria and RS—Serbia.

The morphologically distinct *G. balcanicus dacicus* is likewise well represented in the Romanian territory but differs in distribution from *G.* cf. *balcanicus*. It is concentrated mostly in central and southern Romania where it replaces *G.* cf. *balcanicus* and reaches the western lowlands of the country (Fig. 1). The altitudinal range of this taxon extends from 37 to 1072 m, mostly between 170 and 600 m (Fig. 4). It coexists with the same taxa as *G.* cf. *balcanicus* and inhabits small brooks, springs and lowland rivers (Table 1). *G. balcanicus montanus* has been recorded from only a few localities in the Southern Carpathians (Fig. 1). It inhabits only high altitudes that range between 880 and 1930 m (Fig. 4).

The *Gammarus fossarum* complex has a wide distribution area that spans western, central and south-eastern Europe and reaches northern Anatolia (G. Karaman & Pinkster 1977a; J.L. Barnard & C.M. Barnard 1983; Özbek & Ustaoğlu 2006). In Romania, it occurs in the western part of the Carpathians in two isolated regions, one in the north-west and the other in the south-west (Fig. 3). Its altitudinal distribution ranges from 47 to 860 m, mainly between 300 and 550 m (Fig. 4). The populations from south-western Romania also occur in rivers in the lowlands while the north-western populations are confined to springs and streams in sub-mountainous regions (Table 1, Fig. 3). In some localities *G. fossarum s.l.* coexists with *G.* cf. *balcanicus*, *G. balcanicus dacicus* and *G. roeselii s.l.*

Gammarus kischineffensis has a discontinuous distribution encompassing two distinct areas. One is restricted to north-eastern Romania, Moldova and south-western Ukraine and the other is limited to the eastern half of Turkey (G. Karaman & Pinkster 1977a, J.L. Barnard & C.M. Barnard 1983, Özbek & Ustaoğlu 2006). Thus, it is possible that the latter represents a distinct lineage. On the Romanian territory, *G. kischineffensis* occurs only in the north-eastern part of the country, being limited to the Siret and Prut River catchments and never reaches the inner Carpathian basins (Fig. 2). Nevertheless, morphologically distinct populations, which we treat separately (see below), are found throughout south-western Romania. *G. kischineffensis* is restricted to altitudes below 460 m and inhabits springs, streams and rivers (Table 1, Fig. 4).

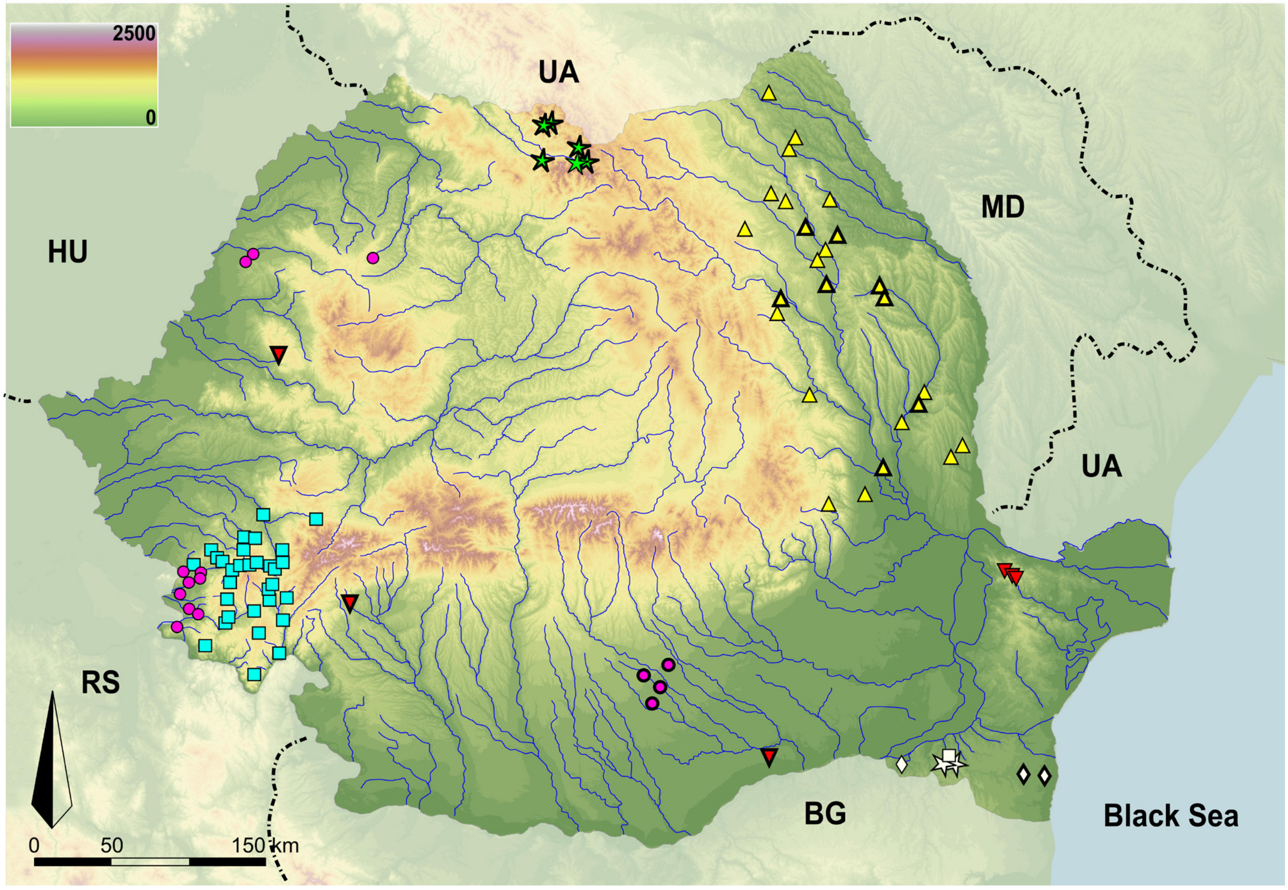


FIGURE 2. Distribution of *G. arduus* (red downward pointing triangles), *G. kischineffensis* (yellow upward pointing triangles), *G.* cf. *kischineffensis* (turquoise squares), *G. komareki s.l.* (white square), *G. leopoliensis* (green stars), *G. pulex s.l.* (white diamonds), and *G. roeselii s.l.* (pink circles) in Romania. White stars represent localities where *G. komareki s.l.* and *G. pulex s.l.* coexist. Shapes with thin outline—data from this study, shapes with thick outline—data from the literature. For country abbreviations, see Fig. 1.

Gammarus cf. *kischineffensis* is a newly recognized form. Due to its morphological distinctness and allopatric distribution, it seems likely that it is a species separate from *G. kischineffensis* in a strict sense (see Taxonomic remarks). It is encountered in the south-western part of the country, in the Almăjului, Aninei and Semenic Mountains (Fig. 2). Altitudinally, it was found between 140 and 860 m in springs and streams (Table 1, Fig. 4). It occasionally coexists with *G. fossarum s.l.* and *G.* cf. *balcanicus*.

The *Gammarus komareki* complex has a distribution range that extends from Bulgaria and northern Greece, throughout the northern half of Turkey into the north-western part of Iran (G. Karaman & Pinkster 1977a; Grabowski & Pešič 2007; Özbek & Ustaoğlu 2006; Zamanpoore *et al.* 2011). It is known only from three localities in south-eastern Romania from the Dobrogea Region (Fig. 2). It occurs at altitudes below 100 m and inhabits slow flowing rivers with rich vegetation, coexisting with *G. pulex s.l.* (Copilaş-Ciocianu 2013) (Table 1, Fig. 4).

Gammarus leopoliensis occurs only in the northern half of the Carpathian region in Poland, Ukraine, Slovakia, Hungary and Romania (Grabowski & Mamos 2011; Papp & Kontschán 2011). Its distribution in Romania is confined to the northern part of the country (Fig. 2), to streams at high altitudes, from 600 to 1150 m (Table 1, Fig. 4) (Papp *et al.* 2008).

The *Gammarus pulex* complex has a western, central and northern European distribution with patchy populations being encountered in south-east Europe, Asia Minor and throughout Asia (G. Karaman & Pinkster 1977a; J.L. Barnard & C.M. Barnard 1983; Özbek & Ustaoğlu 2006). It is encountered only in south-eastern Romania, in the Dobrogea Region (Fig. 2), in groundwater, springs, and streams, at altitudes that do not exceed 100 m (Table 1, Fig. 2), cohabiting with *G. komareki s.l.* in some sites (Fig. 2).

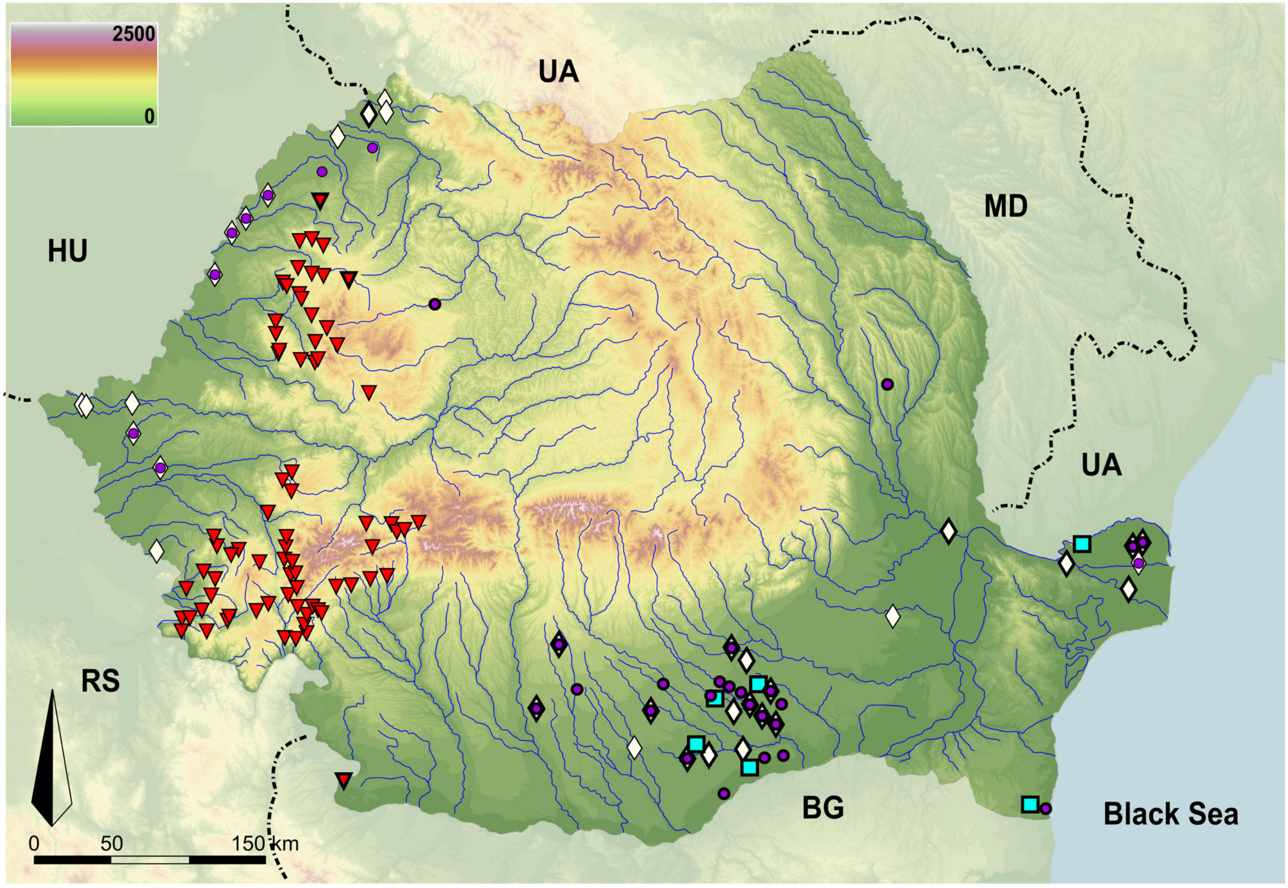


FIGURE 3. Distribution of *G. fossarum s.l.* (red triangles), *N. hrabei* (turquoise squares), *N. valachicus* (white diamonds), and *S. ambulans* (violet circles) in Romania. Diamonds with violet circle inside represent localities where *N. valachicus* and *S. ambulans* coexist. Shapes with thin outline—data from this study, shapes with thick outline—data from the literature. For country abbreviations, see Fig. 1.

The *Gammarus roeselii* complex is distributed across western, central and south-eastern Europe as well as the western part of Turkey (G. Karaman & Pinkster 1977a; J.L. Barnard & C.M. Barnard 1983; Jazdzewski & Roux 1988; Özbek & Ustaoğlu 2006). It is present in the western and southern parts of Romania in a few distinct patches (Motaş *et al.* 1962; Pârvulescu 2009) (Fig. 2). It is a typical lowland taxon, occurring mostly at altitudes below 200 m (Fig. 4). *G. roeselii s.l.* is ecologically the most plastic gammarid in Romania, being found in springs, streams, rivers, and occasionally in lakes and swamps (Motaş *et al.* 1962, Table 1). It can co-occur with *G.* cf. *balcanicus*, *G. balcanicus dacicus* and *G. fossarum s.l.*

Niphargus hrabei occurs in central and south-eastern Europe from the Small Hungarian Plain to the Danube Delta (Cărăuşu *et al.* 1955; J.L. Barnard & C.M. Barnard 1983; Meijering *et al.* 1995; Nesemann *et al.* 1995). It is found only in the south-eastern lowlands of Romania and the Danube Delta in springs, streams, ponds and swamps at altitudes below 350 m (Table 1, Figs 3–4).

Niphargus valachicus has a large and fragmented range, spanning from the Pannonian Plain along the Lower Danube, and reaching the Danube Delta (Cărăuşu *et al.* 1955; J.L. Barnard & C.M. Barnard 1983). It is also present in Turkey along the southern shore of the Black Sea and reaches the south of the Caspian Sea in Iran (Fišer *et al.* 2009; Hekmatara *et al.* 2013). In Romania, it is a common species in the lowlands, being found in swamps, canals, temporary ponds, and large rivers in sympatry with *S. ambulans* (Cărăuşu *et al.* 1955; Copilaş-Ciocianu & Pârvulescu 2012; Table 1). It inhabits the western and southern plains of Romania (Fig. 3), being encountered between 0 and 360 m with most localities ranging around 100 m (Fig. 4). It often coexists with *S. ambulans* and *G. balcanicus dacicus*, and occasionally with *G. roeselii s.l.* and *N. hrabei* (Motaş *et al.* 1962).

Synurella ambulans is widespread in central, eastern and southern parts of Europe (G. Karaman 1974; Sidorov & Palatov 2012). In Romania it has a distribution similar to *N. valachicus*, co-occurring in the same habitats and at the same altitudes (Table 1, Figs 3–4).

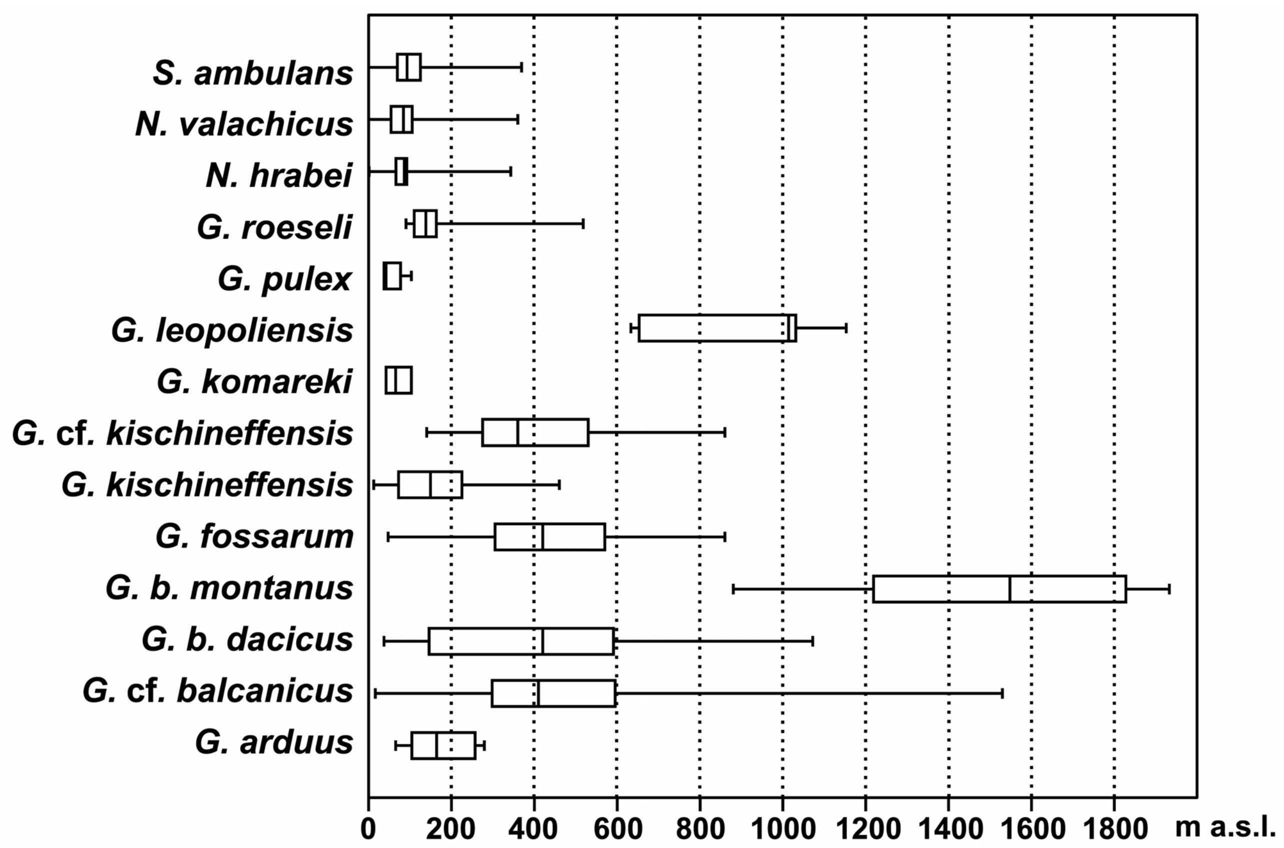


FIGURE 4. Boxplots representing the altitudinal ranges of studied epigean amphipod taxa in Romania.

Biogeographical patterns. Freshwater amphipods in Romania have patchy and often non-overlapping distribution patterns. A distinction can be made between the distributions of *Gammarus* and *Niphargus* /*Synurella*. *Gammarus* species exhibit high altitudinal variation and allopatric distributions, contrasting with *Niphargus* and *Synurella* which are sympatric and restricted to the lowlands.

The distribution patterns of the analysed species are significantly non-random ($I > 0$, $p \leq 0.05$). However, the positive autocorrelation distance values varied between genera. Maximum significant positive autocorrelation distances ranged between 19 and 180 km for different *Gammarus* taxa ($p \leq 0.01$) and reached 327 km and 296 km for *N. valachicus* ($p = 0.03$) and *S. ambulans* ($p = 0.005$), respectively (Fig. 5). Thus, the spatial autocorrelation analyses further emphasize this patchy distribution pattern by revealing that populations of *Gammarus* are significantly autocorrelated for shorter distances than *N. valachicus* and *S. ambulans*. This means that *Gammarus* species have more geographically clustered distributions than the latter two.

Four *Gammarus* taxa have well-delimited, wide distributions in Romania; these are *G.* cf. *balcanicus*, *G. balcanicus dacicus*, *G. fossarum s.l.* and *G. kischineffensis*. The former two have an intertwined, complementary distribution pattern that is mostly non-overlapping, while the latter two are restricted to the western and eastern parts of the country, respectively (Figs 1–3). The remaining taxa, *G. arduus*, *G. leopoliensis*, *G. komareki s.l.*, *G. pulex s.l.* and *G. roeselii s.l.* are not so widespread, most likely due to the fact that the territory of Romania only marginally overlaps with their distribution areas. The full distribution of *G.* cf. *kischineffensis* is at present unknown, it is possible that it extends into neighbouring Serbia.

The allopatric distributions displayed by *Gammarus* species in Romania are typical for the genus (G. Karaman & Pinkster 1977a; 1977b; 1987; Väinölä *et al.* 2008). Molecular phylogenetic analyses indicate that the frequent allopatry observed in many *Gammarus* species is the result of geological vicariant events of Tertiary age and that the majority of extant freshwater species originated in the Late Tertiary (Hou *et al.* 2007; 2011; 2013; Wysocka *et al.* 2014). During this period, the Carpathian Mountain range was a geomorphologically highly dynamic archipelago surrounded by the shallow Central Paratethys Sea (Popov *et al.* 2004). This constantly changing topography was characterized by different timings of landmass uplift and drastic variations in sea level (Harzhauser

& Piller 2007, Kováč *et al.* 2007). Sea level fluctuations and the vicariance they create are considered to be of significant importance in the evolution of many, especially subterranean, freshwater amphipod taxa (Notenboom 1991; Holsinger 1994). We hypothesize that the dynamic geomorphology of the Carpathian region during the Late Tertiary has left its footprint on the contemporary ranges of *Gammarus* species in Romania.

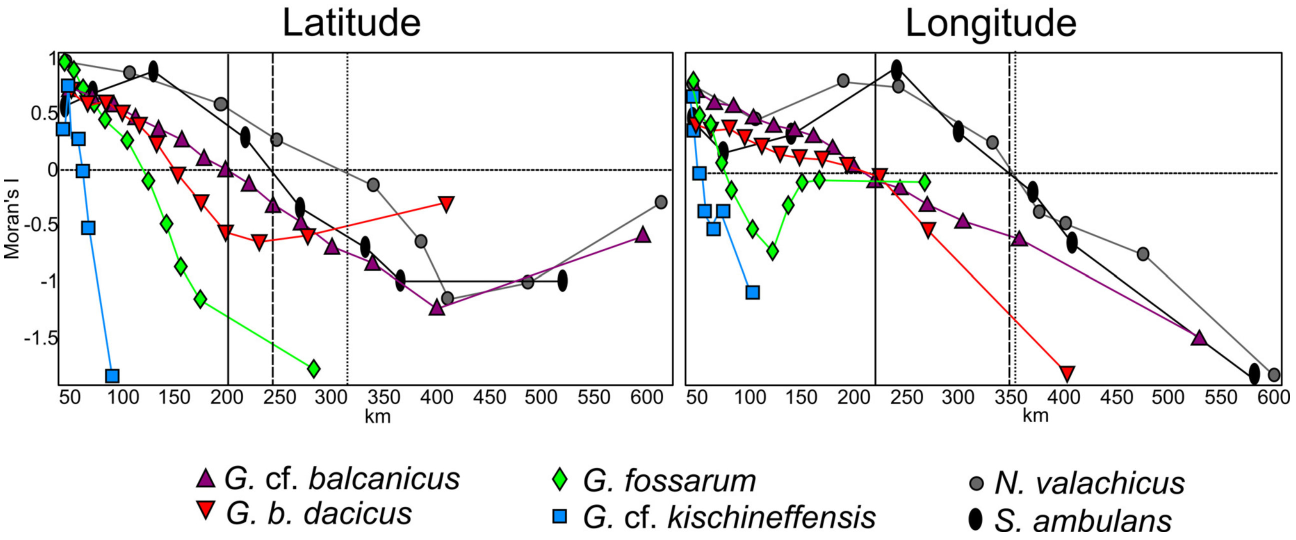


FIGURE 5. Spatial correlograms on latitude and longitude data for the analyzed taxa. Maximum positive autocorrelation distance threshold is represented by a vertical line for *Gammarus*, dashed vertical line for *S. ambulans*, and dotted line for *N. valachicus*.

Niphargus valachicus, *N. hrabei* and *S. ambulans* are unusual amongst their congeners because of their predominantly epigean lifestyle, wide distributions and ecological plasticity (Straškraba 1972; Meijering *et al.* 1995; Nesemann *et al.* 1995; Sidorov & Palatov 2012). Both *Niphargus* species are sympatric with *S. ambulans* throughout their distribution range, sharing the same ecological requirements (Motaş *et al.* 1962; Meijering *et al.* 1995; Nesemann *et al.* 1995), and in many cases coexisting in the same habitat (Motaş *et al.* 1962; Straškraba 1972; Akbulut *et al.* 2001; Juhász *et al.* 2006; Copilaş-Ciocianu & Pârvulescu 2012). The co-existence of *Niphargus* and *Synurella* seems to be quite old, since both genera are known from Baltic amber that dates back to the Eocene, i.e., is at least 35 million years old (Jażdżewski & Kupryjanowicz 2010; Jażdżewski *et al.* 2014).

Molecular data indicate that *Niphargus* colonized south-eastern Europe at the beginning of the Oligocene (~25 million years ago) (McInerney *et al.* 2014). Although *N. valachicus* was not included in that study, its distribution reflects the extent of the Paratethys Sea during the Early Pliocene (~5 million years ago), when—as it has been hypothesized—it may have colonized available freshwater habitats through coastal lagoons (Sket 1981). Straškraba (1972) suggested that the present-day distribution of *N. valachicus* is linked with the more recent Pleistocene extent of the Paratethys. However, during the Pliocene and Early Pleistocene, the extent of the Paratethys was more or less the same, without major fluctuations (Popov *et al.* 2004). Thus, it is possible that the hypothesized freshwater colonization may have taken place anytime during this time frame. The lowland regions of Romania, where *N. valachicus* is nowadays present, fit to this general pattern since they were continuously submerged under the waters of the Paratethys during the Pliocene/Pleistocene (Popov *et al.* 2004). Due to its large and fragmented range, it is possible that this taxon might harbour independently evolving lineages (Fišer *et al.* 2009).

Straškraba (1972) also suggested a connection between the distribution of *N. hrabei* and the extent of the Paratethys during the Pleistocene. Since this species is morphologically, ecologically and biogeographically similar to *N. valachicus* (Straškraba 1972; Nesemann *et al.* 1995), it is possible that it follows the same pattern. It is considered that *N. hrabei* is expanding its range at present (Ketelaars 2004). Both species are sympatric in the Lower Danube basin in Romania and coexist in some instances (Motaş *et al.* 1962). *S. ambulans*, although having a wide distribution in Europe, has a problematic taxonomy and probably represents a species complex, as suggested by its morphological variability and ecological plasticity (Meijering *et al.* 1995; Konopacka & Blazewicz-Paszkowycz 2000; Sidorov & Palatov 2012). It belongs to the crangonyctoid group which is considered one of the most ancient groups of freshwater amphipods (J.L. Barnard & C.M. Barnard 1983). Its unclear taxonomy and lack of molecular data restrain biogeographical interpretations for this taxon.

Aside from historical factors, we presume that the contrasting patterns (allopatric vs. sympatric) observed between *Gammarus* and *Niphargus*/*Synurella* are due to the fact that these taxa have different ecological preferences. Romanian *Gammarus* species, except for *G. roeselii s.l.*, are seemingly more restricted in their habitat preferences, favouring springs and streams along an extensive altitudinal gradient (Table 1, Fig. 4). Therefore, their spread might be limited by the availability of these suitable habitats. Competition and interspecific predation, common between *Gammarus* species (e.g. MacNeil & Dick 2012), might also be important factors contributing to their distribution patterns. On the other hand, *Niphargus* and *Synurella* are more euryoecious, preferring a wide spectrum of habitats ranging from groundwater and springs to stagnant waters or temporary ponds, but along a much narrower altitudinal gradient (Table 1, Fig. 4). This is probably due to the fact that niphargids and crangonyctids seem to be more tolerant to low oxygen levels than gammarids (Dick *et al.* 1997; Simčič & Brancelj 2006). Thus, they can occupy a wider variety of habitats and prevail at lower altitudes, in which they probably face lower competition pressure from gammarids. The mechanisms that permit these two genera to coexist are unclear, given the fact that freshwater amphipods often exhibit high levels of intra-guild predation (MacNeil *et al.* 1997; 1999; Luštrik *et al.* 2011).

Taxonomic remarks. We present the morphological differences between the three morphs of *G. balcanicus* reported from Romania, as pointed out by Cărăuşu *et al.* (1955). *G. balcanicus dacicus* morphologically differs from *G.* cf. *balcanicus* by the longer endopod of uropod 3 (up to 90% the length of the exopod), the pointed infero-posterior corners of epimere 2 and the presence of long setae (as long as the urosome spines) on the dorso-posterior side of the metasome segments (Fig. 6). It also has a very distinct distribution that is complementary to that of *G.* cf. *balcanicus* and in some localities they are known to coexist and still maintain their morphological distinctness (Fig. 1). Based on this evidence, we consider that this taxon is a distinct entity and should be 'resurrected' if not even elevated to a specific status, an issue that remains to be resolved by further molecular research.

In the case of *G. balcanicus montanus*, the situation is less clear. Its main morphological differences from *G.* cf. *balcanicus* are its smaller size and a short endopod of uropod 3 (about 50% the length of the exopod) (Fig. 6). Its distribution is not geographically separated but it is supposedly ecologically distinct, inhabiting only high altitude springs or brooks. It might represent an ecomorph of *G.* cf. *balcanicus*, although both substantially overlap altitudinally. Molecular analyses are needed to resolve the taxonomic status of *G. balcanicus montanus*.

The distinct form *G.* cf. *kischineffensis* is morphologically different from its presumed relative *G. kischineffensis* (Fig. 7). Geographically, their ranges are separated by ca. 300 km (Fig. 2). The main distinctive features of *G.* cf. *kischineffensis* are 1) pointed infero-posterior corners of the 2nd and 3rd epimeres that are straight in *G. kischineffensis* (Fig. 7a); 2) the inner side of the telson lobes that has one spine; this spine is absent in *G. kischineffensis* (Fig. 7b); and 3) the presence of long setae (longer than or as long as the width of the underlying segment) in the upper quarter of the external margin of the 3rd uropod exopodite (these are present along the upper half in *G. kischineffensis*) (Fig. 7c). Based on its morphological and geographical distinctness, we propose that this morph might be an undescribed species, a hypothesis that will be tested by further morphological and molecular studies.

Conclusions

Romania has a diverse fauna of epigean freshwater amphipods and further taxonomic studies are needed to truly recognize this diversity. Morphological variation in the *G. balcanicus* complex and *G.* cf. *kischineffensis* suggest the presence of undescribed taxa, and it is likely that molecular studies will reveal additional cryptic diversity (as it has been the case with *G.* cf. *balcanicus*). The distributions of the epigean freshwater amphipods in Romania are characterized by their patchiness and altitudinal variability. Coupled with their low dispersal abilities and the heterogeneous topography/geology of this region, these animals constitute a suitable model system for studying biogeography and phylogeography at a fine scale, with implications for further research in ecology, adaptation and speciation of freshwater amphipods.

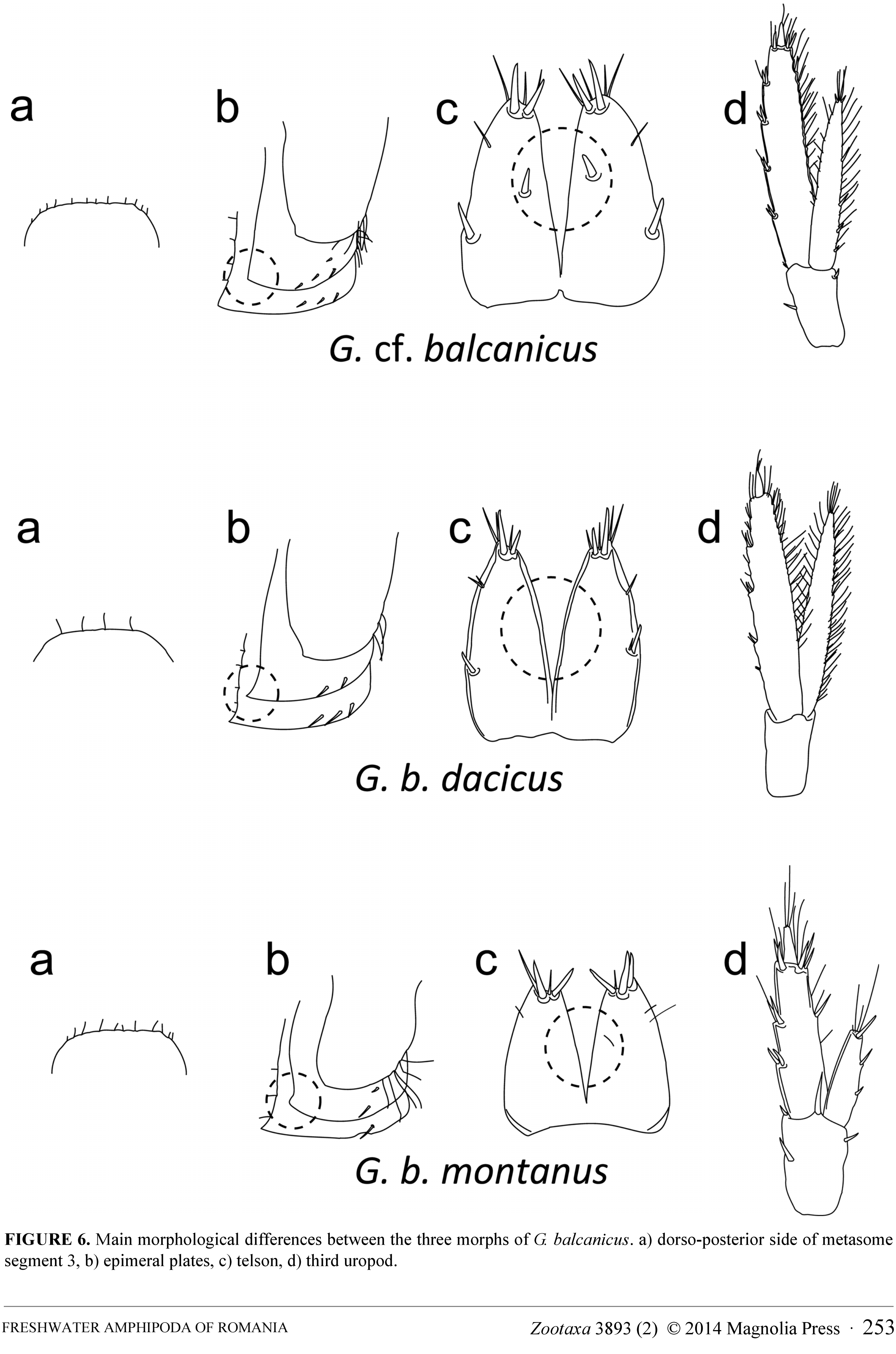
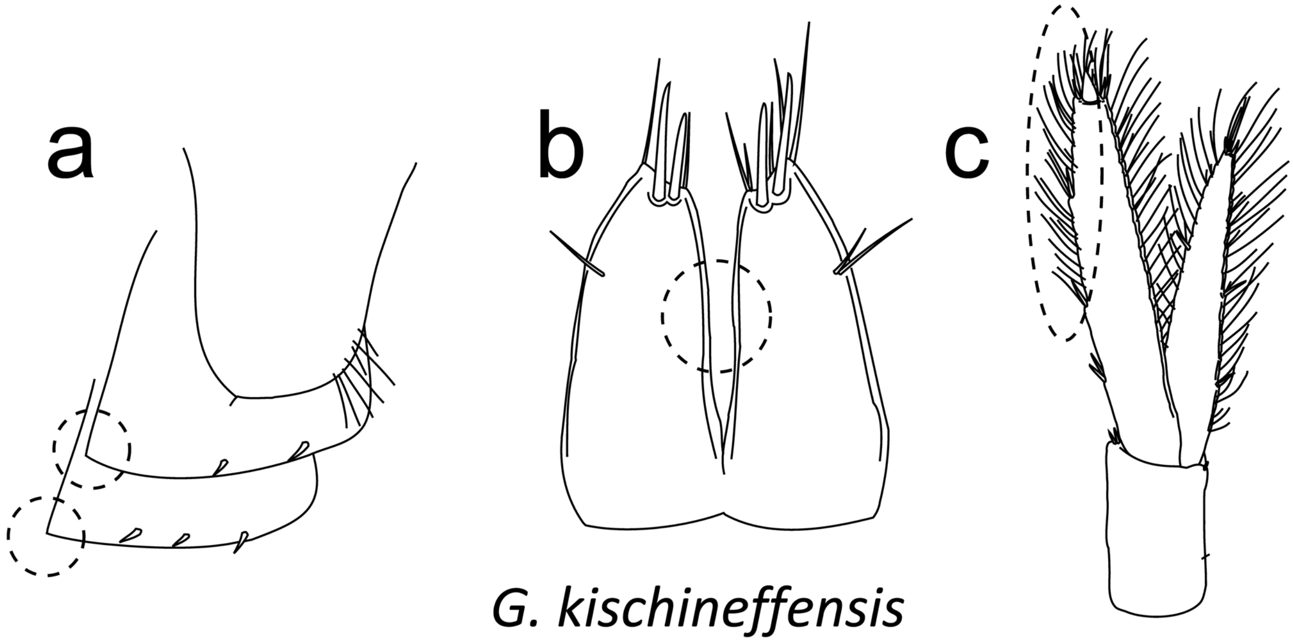
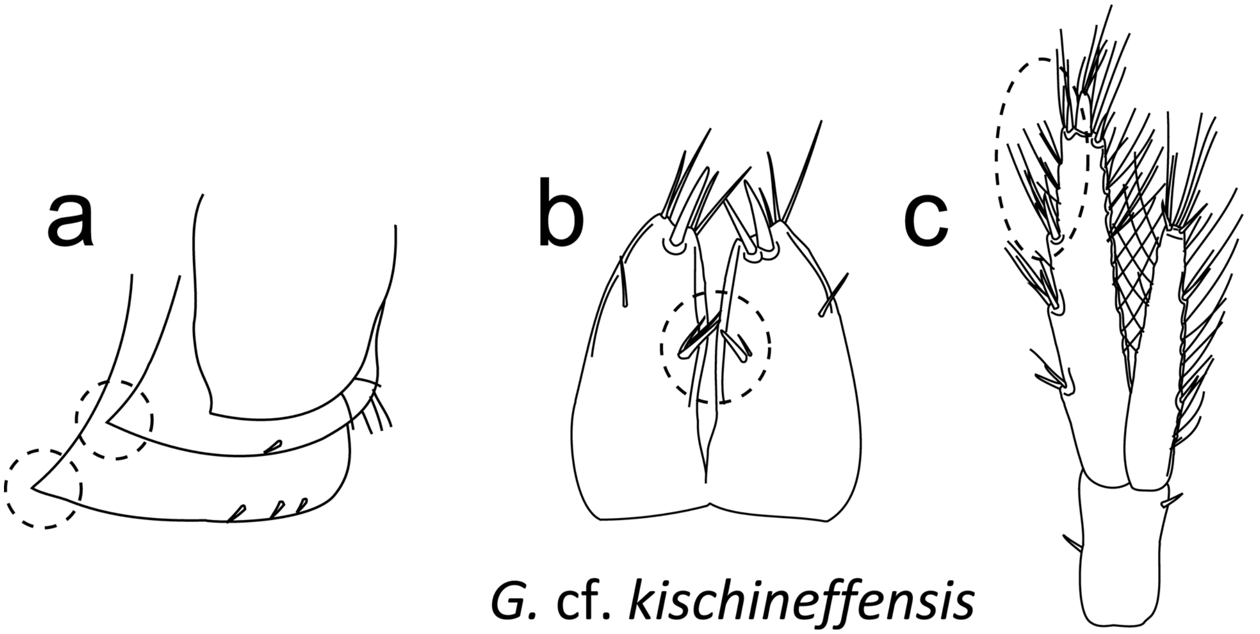


FIGURE 6. Main morphological differences between the three morphs of *G. balcanicus*. a) dorso-posterior side of metasome segment 3, b) epimeral plates, c) telson, d) third uropod.



G. kischineffensis



G. cf. *kischineffensis*

FIGURE 7. Main morphological differences between *G. kischineffensis* and *G.* cf. *kischineffensis*. a) epimeral plates, b) telson, c) third uropod.

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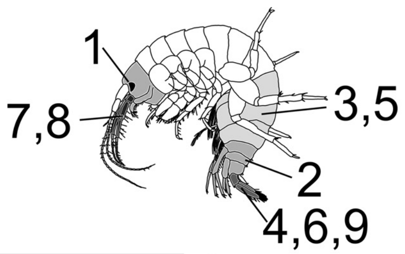
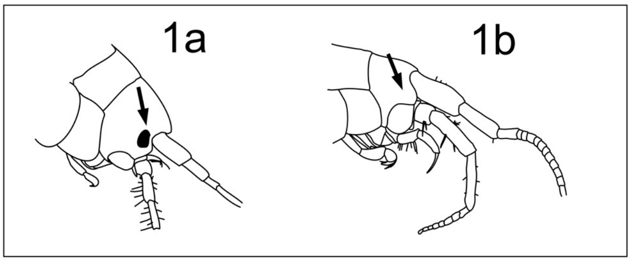
Pictorial identification key to the epigean freshwater *Gammarus*, *Niphargus* and *Synurella* species of Romania

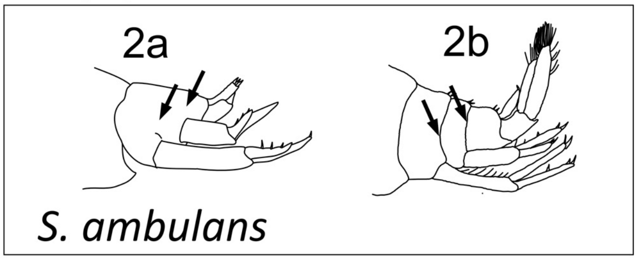
This key should be used as a complement to other identification resources rather than alone.

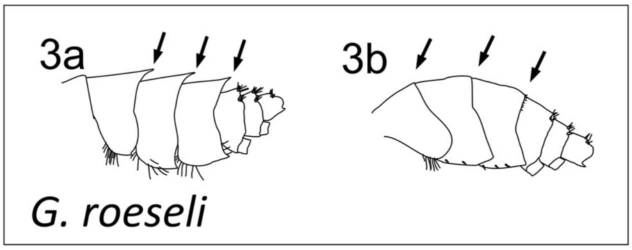
Meaning of abbreviations: A1—first antenna, antennules; A2—second antenna; E2—second epimeral plate; E3—third epimeral plate; P6—sixth pereiopod; P7—seventh pereiopod; U3— third uropod.

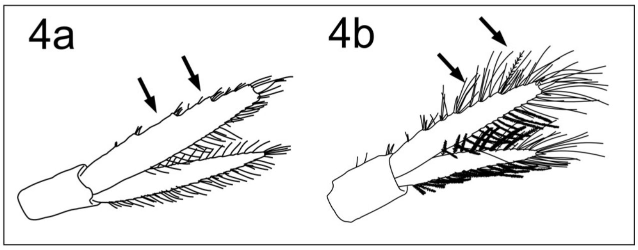
1a.	Eyes present	2
1b.	Eyes absent	13
2a.	Urosome segments fused	*S. ambulans*
2b.	Urosome segments free	3
3a.	Metasome segments with large dorsal spines	*G. roeseli*
3b.	Metasome segments without dorsal spines	4
4a.	Setation of external margin of U3 outer ramus is scarce and as long as or shorter than the width of underlying segment	5
4b.	Setation of external margin of U3 outer ramus is dense and longer than the width of the underlying segment	7
5a.	Postero-inferior corner of E2 pointed, 4 setae on the dorso-posterior side of metasome segments	*G. balcanicus dacicus*
5b.	Postero-inferior corner of E2 straight/slightly pointed, many setae on the dorso-posterior side of metasome segments	6
6a.	Body length up to 8 mm, inner ramus of U3 is half length of the outer ramus	*G. balcanicus montanus*
6b.	Body length longer than 8 mm, inner ramus of U3 is longer than half length of the outer ramus	*G. balcanicus*
7a.	Setation of A2 peduncle dense and ca. 2x longer than the width of the underlying segment	*G. komareki*
7b.	Setation of A2 peduncle scarce and shorter than or slightly exceeding the width of the underlying segment	8
8a.	Flagellum of A2 swollen and bearing flag-like brush of setae as long as or exceeding the width of the underlying segment	*G. pulex*
8b.	Flagellum of A2 slender	9
9a.	Inner ramus of U3 reaches at most half length of the outer ramus	*G. fossarum*
9b.	Inner ramus of U3 is longer than half length of the outer ramus	10
10a.	Posteroinferior surface of P7 basis with setae, distal margin of E2 setiferous	*G. arduus*
10b.	Posteroinferior surface of P7 basis without setae, distal margin of E2 not setiferous	11
11a.	Postero-inferior corners of E2 & E3 pointed, upper quarter of external margin of U3 outer ramus setiferous	*G.* cf. *kischineffensis*
11b.	Postero-inferior corner of E2 straight, upper half of external margin of U3 outer ramus setiferous	12
12a.	First and 2nd peduncle segments of A1 have equal lengths, flagellum of A2 without calceoli	*G. leopoliensis*
12b.	First peduncle segment of A1 longer than the 2nd one, flagellum of A2 with calceoli	*G. kischineffensis*
13a.	Dactylus of P6 and P7 bears 1 spine	*N. hrabei*
13b.	Dactylus of P6 and P7 bears at least 5 spines	*N. valachicus*

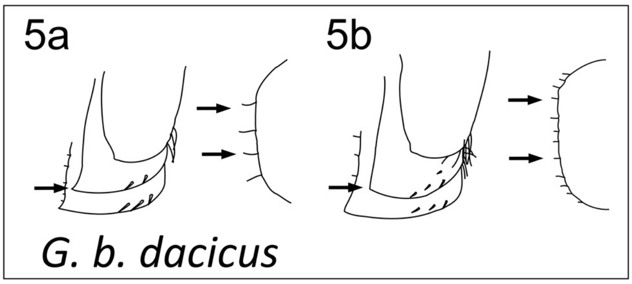

1
7,8
3,5
2
4,6,9

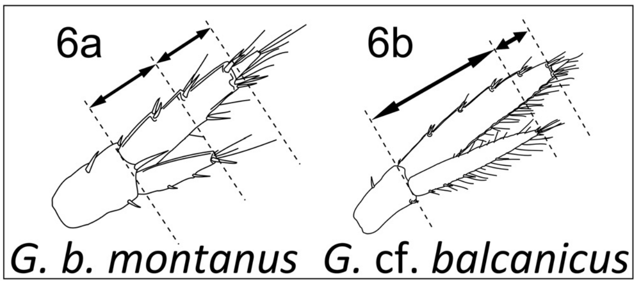

1a
1b

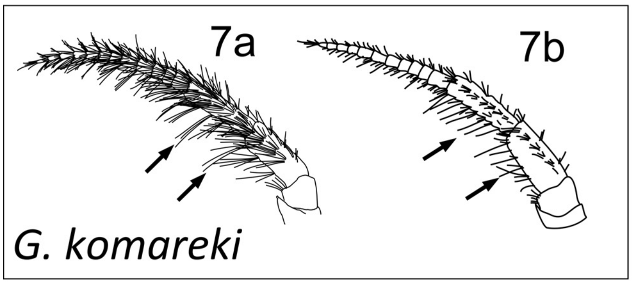

2a
2b
S. ambulans

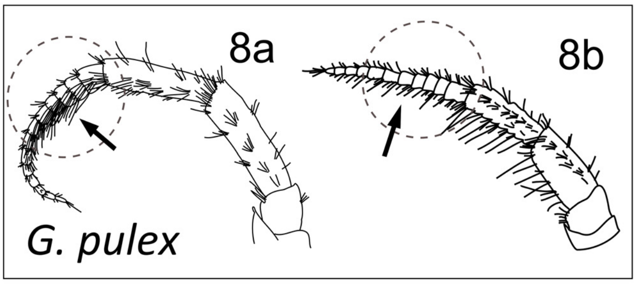

3a
3b
G. roeseli

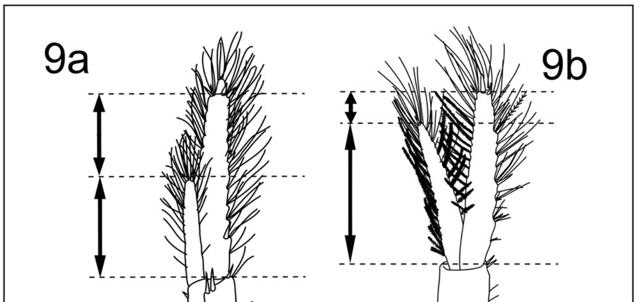

4a
4b

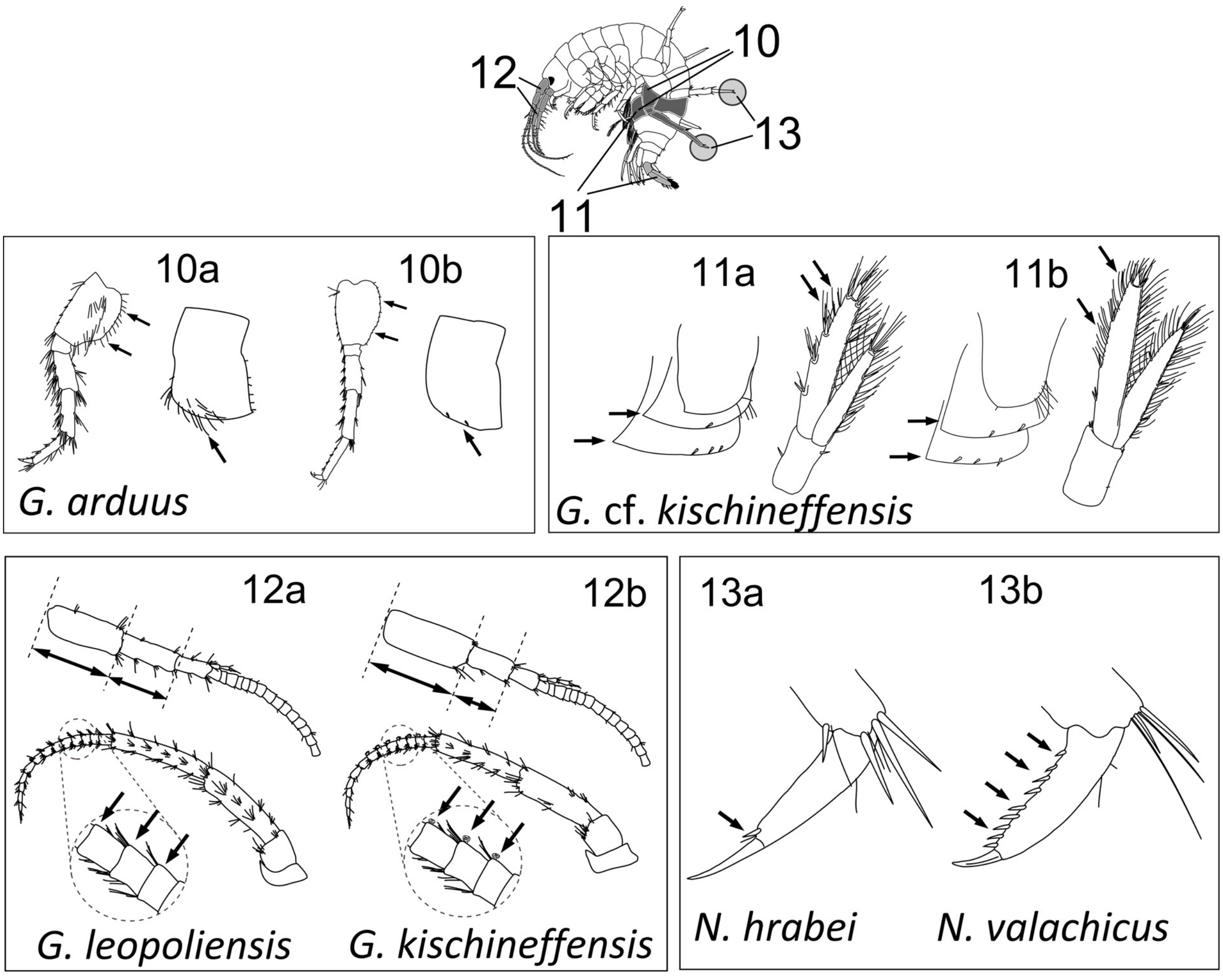

5a
5b
G. b. dacicus


6a
6b
G. b. montanus
G. cf. balcanicus


7a
7b
G. komareki


8a
8b
G. pulex


9a
9b
G. fossarum


12
10
13
11
10a
10b
G. arduus
11a
11b
G. cf. kischineffensis
12a
12b
G. leopoliensis
G. kischineffensis
13a
13b
N. hrabei
N. valachicus