

**What happened to the genus *Gammarus* (Crustacea: Amphipoda)  
in Central Asia**

Kaj se je zgodilo s postranicami *Gammarus* (Crustacea: Amphipoda)  
v centralni Aziji

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**Abstract:** We examined the phylogenetic relationships in the unusually structured fauna of the amphipod genus *Gammarus* in Central Asia, trying to explain its causes. Mitochondrial DNA sequence data were used in MP and ML and Bayesian analyses to generate a phylogenetic hypothesis. All of the recently collected *Gammarus* spp. specimens belong to the *G. lacustris* aggregate, except scarce marginal populations of *G. balcanicus* and *G. komareki* aggregates. Although molecularly closely related, these *G. lacustris* agg. populations are morphologically and ecologically equally diverse as are the numerous European *Gammarus* spp. Only few populations are morphologically close to the morphotype of *G. lacustris*. There is a more than 2,000 km wide gap between the eastern and western populations-species of *G. balcanicus* agg. Since *Gammarus lacustris* can be spread by birds, and its Asian relatives are molecularly significantly diverse, we suppose that it invaded Central Asia several times and from several sources. It most probably differentiated to diverse morphotypes after reaching Asia. The area of the cryophilic and less nomadic *G. balcanicus* could have been reached and conquered only by waterways. Its area was probably continuous in the past and divided by aridification during the Neogene. The strong historical aridifications probably extirpated the probably higher *Gammarus* diversity in Central Asia, including *G. balcanicus*. This opened the area and its habitats for the reiterated invasions of the particularly euryoecious *G. lacustris* s.l., followed by its morphological and ecological diversification.

**Keywords:** Amphipoda, biogeography, diversification, Palearctic

**Izvleček:** Raziskali smo filogenetske odnose v nenavadno strukturirani favni postranic *Gammarus* v Centralni Aziji in jo skušali pojasniti. Mitohondrijske sekvence DNK smo uporabili v MP in ML analizah in tako oblikovali filogenetske hipoteze. Vse postranice rodu *Gammarus*, zbrane v zadnjih letih, razen nekaterih obrobnih in poredkih populacij agregatov *G. balcanicus* in *G. komareki*, pripadajo agregatu

*G. lacustris*. Čeprav molekulsko tesno sorodne, so te populacije agregata *G. lacustris* ekološko in morfološko tako raznolike, kot so številne evropske vrste rodu *Gammarus*. Le maloštevilne populacije so morfološko blizu morfotipu *G. lacustris*. Med vzhodnim in zahodnim delom areala agregata *G. balcanicus* je tukaj 2000 km širok presledek. Ker lahko *G. lacustris* raznašajo ptiči in ker so njegovi azijski sorodniki molekulsko zelo raznoliki, predvidevamo, da so poselili Centralno Azijo večkrat in iz raznih smeri. Verjetno so se diverzificirali v različne morfotipe šele v Aziji. Bolj kriofilni in manj nomadski *G. balcanicus* s.l. je lahko osvojil svoj areal le vzdolž voda. V preteklosti je bil njegov areal gotovo zvezen in so ga razdelile šele neogenske aridifikacije. Ostre aridifikacije v preteklosti so verjetno iztrebile bogatejšo gamaridsko favno vključno z *G. balcanicus*. To je odprlo prostor in njegove habitate za ponavljane invazije posebej evriškega *G. lacustris* s.l., čemur je sledila morfološka in ekološka diverzifikacija.

**Ključne besede:** Amphipoda, biogeografija, diverzifikacija, Palearktika

## Introduction

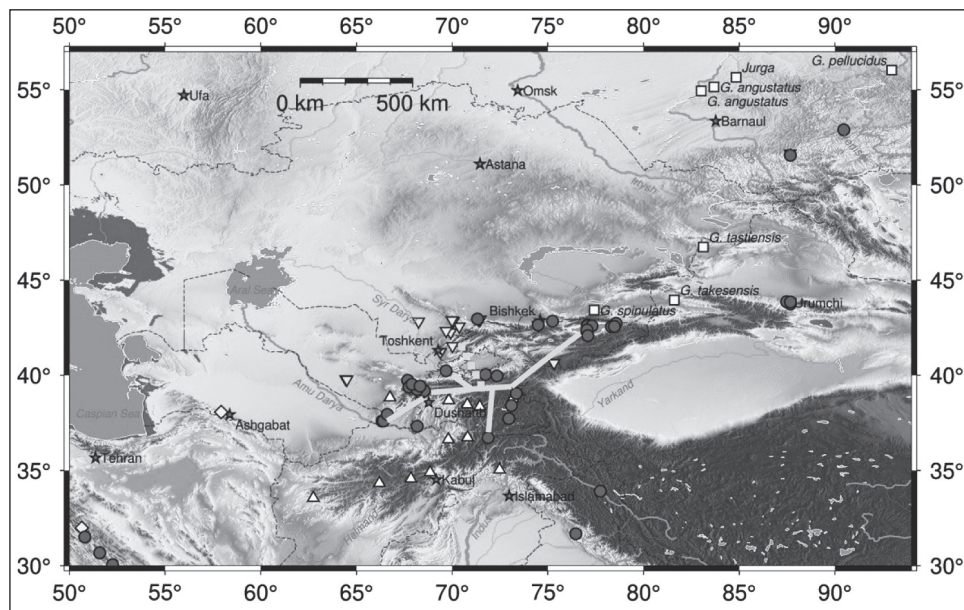
### *History of discovery and problems in taxonomy*

Amphipods of Central Asia are insufficiently studied; distributional data are fragmentary and descriptions are mostly poor, often not mutually comparable. Only a few of the previously described *Gammarus* taxa have recently been confirmed with data that are more detailed. In addition, administrative and orthography changes in the near history added to the problems of the distributional data. Just as an example: the Martynov's (1935) 'Eastern Bukhara' is not part of the recent town Buhara, Uzbekistan, it is the eastern part of the extended Bukhara Emirate; its eastern part mainly included the central and western parts of the modern Tajikistan (Chibilev 1993).

Another problem concerns the numerous findings of '*Gammarus pulex*' in Central Asia and adjacent regions. Its alleged first mention was by G.O. Sars (1901) from territories near Astana in Kazakhstan and eastern Mongolia, and then again by Sars (1903) from Lake Teleckoe, Altai. Much later, Martynov (1930b) reported four Amphipoda species for Teleckoe ozero, but *G. pulex* was not mentioned among them. However, material identified by Sars as *G. pulex* was described as new species, *G. teletzkensis* (see Martynov, 1930b). Surprisingly, *G. pulex* was listed by Martynov (1930b) from another Siberian lake and from the Biya river. Furthermore, Chevreux (1908), studying collections of prof. D.D. Pedashenko in 1906-

1908, reported *G. pulex* from a number of points located in the 'Lake Issyk-Koule, stream Gorge of Karakol, and Lake Chatyr-Koule' of Kirghizia. Afterwards, Martynov (1930a), based partly on the same collections of Pedashenko, described *Gammarus ocellatus* Martynov 1930, and *Gammarus bergi* Martynov, 1930 from Lake Ysyk Köl (Issyk Kul). Once more later, *G. ocellatus* was indicated by Martynov and Behning (1948) from the high mountain lakes of the Hissar Range in Tajikistan. According to Martynov and Behning, 1948, *G. ocellatus* is a widely distributed species in the mountainous regions of Central Asia, occurring from Tajikistan to Tien Shan in the northeast and to the Himalayas in the southeast (Ueno 1934). The question of the existence of *G. pulex* in Asia gets significantly complicated with the impossibility to accurately identify a number of previous findings due to their superficial descriptions. In Turkmenistan, *G. pulex* was reported by Birstein (1945) from rivers Šerlok and Firjuzinka (central Kopetdag); this is the only mention of *G. pulex*, where some morphological details show similarity with that European species.

The great majority of localities, treated by Martynov, are actually situated in southern Kazakhstan or very close to its border in Uzbekistan and Kyrgyzstan. The recently collected samples from Central Asia, which we could identify molecularly, are from Kyrgyzstan, E Uzbekistan, Tajikistan, and from Turkmenistan. They all virtually only contain members of the *G. lacustris* aggregate. They inhabit springs, streams, lakes, and caves, habitats,



**Figure 1:** Distribution map of *Gammarus* spp. in Central Asia and its surroundings. Asterisks with names - important cities. Reversed triangles - molecularly not defined *Gammarus* spp.; rings - molecularly defined taxa of agg. *lacustris*; pale ribbons connect localities of *G. cf. subaequalis*-Garlyk, hatched ribbon connection with Martynov's *G. subaequalis* and *G. turanus*; numerals as in the text and Supplement table S2; squares - agg. *balcanicus*; diamonds (*G. turcomanicus*) - agg. *komareki*. Upright triangles - *Sarothrogammarus* group. *Sarothrogammarus* and *G. balcanicus* groups supposedly match with entire Asian parts of the group areas. Corresponding to Supplement S3.

**Slika 1:** Karta razširjenosti vrst rodu *Gammarus* v širšem območju Centralne Azije. Zvezdica z imenom - pomembnejša mesta. Obrnjeni trikotniki - molekularno nedefinirana *Gammarus* sp. (glej tabela v dodatku S2); obročki - molekularno definirana taksa agregata *lacustris*; blede pasovi povezujejo lokalitete *G. cf. subaequalis* - Garlyk; prekinjen pas, povezava z *G. subaequalis* in *G. turanus* Martynova; številke kot v tekstu in v tabeli v dodatku S1; kvadrati - agg. *balcanicus*; rombi (*G. turcomanicus*) - agg. *komareki*. Pokončni trikotniki - rodovna skupina *Sarothrogammarus*. Skupini *Sarothrogammarus* in *G. balcanicus* sta predvidoma cela azijska dela arealov. Se ujema s sliko v dodatku S3.

which in Europe would mainly be inhabited by *G. pulex*, *G. fossarum*, and *G. balcanicus* aggregates. Based on the available data and by analyzing the geographical distribution (see the map and species list, Supplement table S2), we made an attempt to conduct a comparative morphological analysis only for two new species described in another paper (Sidorov et al. 2018). Here, we are trying to establish, what happened with the rest of the *Gammarus* phylogenetic clades/aggregates, which had evidently inhabited the region in the past and only left behind some scarce relics.

## Material and methods

### Study area

Our study area was wider Central Asia, with exclusion of the Caspian in the West, and inclusion of Krasnojarsk in the East and North and of Islamabad in the south, but excluding the Tibetan plateau.

The areas sampled were as follows. In Turkmenistan, the surroundings of Ashgabat (Ashgabat; where some localities were not accessible) and Köýtendag area in the extreme NE. In Kyrgyzstan the lake Ysyk Köl (Issyk Kul) with surroundings

and the SW of the country. In Tajikistan, the NW third of the country (sampled by C. Fišer) and the South-East (sampled by D. Palatov). In other countries, some scattered localities by different collectors. All localities and collectors are listed in the supplementary table (Supplement table S1). Geographical maps with locations of the sampling sites were constructed using an open source software Generic Mapping Tools, GMT 4.5.14. (Fig. 1).

Gammarids were sampled in springs and streams with a hand net. In lakes, small dredge and baited falls made of plastic bottles and left on deeper bottoms overnight were also used. Samples were fixed and stored in 96% ethanol with a small amount of glycerol added; such animals are appropriate for DNA analyses and still not too rigid for morphological studies. Single specimens were partly dissected and some muscle tissues or small body parts taken for DNA analysis. The rest of the bodies, including all taxonomically relevant parts, were retained as voucher specimens for subsequent morphological studies.

All samples were molecularly identified up to the level of phylogenetic clades, defined as aggregates. Note that our aggregates (abbreviations: aggs, agg) (Hou and Sket 2016) are not identical with 'groups' of Karaman and Pinkster (1977a, 1987), who explicitly define them just as morphologically (rather than phylogenetically) defined groups. Species identification was only taken as reliable if we could molecularly define morphologically appropriate samples from the toptype localities. If not, identification was marked as 'cf.'; such identification only shows a certain degree of similarity (i.e., morphotypes), allowed by the identification using available keys; it does not mean any degree of phylogenetic relatedness, let alone the taxonomical identity. In addition, a terrifying degree of homoplasy was noted in the morphological characters of Amphipoda (Hou and Sket 2011; Moškrič 2016).

#### *Molecular methods*

Total genomic DNA was extracted from specimens using the Tiangen Genomic DNA kit. Mitochondrial cytochrome oxidase subunit I (COI) and nuclear 28S rRNA genes were amplified using

primers as in Hou et al. (2007). Chromatograms were proofed and edited using Sequencher 4.2 DEMO (Gene Codes Corporation, Inc). Sequences were aligned using Clustal X (Thompson et al. 1997) and translated with the invertebrate mitochondrial genetic code to check for stop codons to detect pseudogenes in MacClade 4.06 (Maddison and Maddison 2000).

The best-fit substitution model of HKY+I+ $\Gamma$  was selected for COI and GTR+I+ $\Gamma$  for 28S using jModelTest (Posada, 2008). The two fragments were analyzed separately to emphasize possible mitonuclear differences. The phylogeny was reconstructed under maximum parsimony (MP), maximum likelihood (ML), and Bayesian approach. MP analyses were performed using PAUP\* 4.0b10 (Swofford 2002). All phylogenetically uninformative characters were excluded from the analysis, and gaps were treated as missing data. Heuristic searches were conducted using tree bisection reconnection branch swapping, with a limit of one million rearrangements for each replicate. Bootstrap support indices were generated based on 1,000 bootstrap replicates with ten random-addition sequences. ML analysis was conducted using RAxML 8.2.9 (Stamatakis 2014), starting with 1,000 rapid bootstrap replications followed by a thorough tree search. Bayesian analyses were conducted using MrBayes 3.2.6 (Ronquist et al. 2012), with 8,000,000 generations sampling every 100 generations. A majority consensus tree was constructed to estimate the Bayesian posterior probabilities, after 25% burn-in.

Molecular operational taxonomic units (OTUs or MOTUs) are defined as monophyletic clades with distinguishable morphological or geographic properties.

## Results

Except for some representatives of the *G. balcanicus* agg and the only member of *G. komareki* agg, all of the recently collected *Gammarus* samples from Central Asia are members of the *G. lacustris* agg (all sensu Hou and Sket 2016; Figure 1). However, for most of the previously described taxa, samples for molecular analyses have not been obtained. Unfortunately, the same also goes for

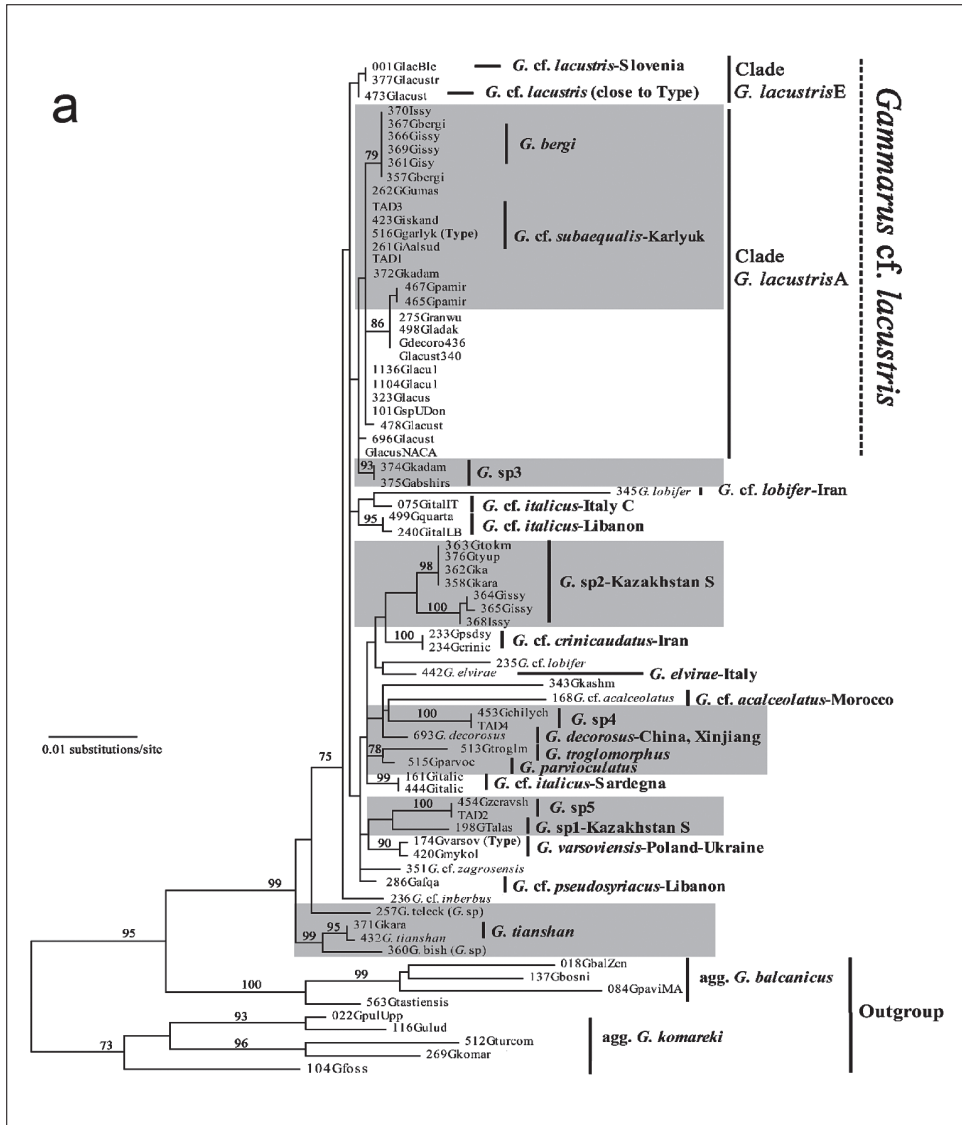
the endemic genus and species *Issykogammarus hamatus* Chevreux, 1908. None of our samples appeared to be morphologically identical to the lacustrine species *G. ocellatus* Martynov, 1930 or *G. inberbus* Karaman & Pinkster, 1977.

The phylogenetic analyses of COI and 28S markers produced very similar results. The COI dataset included a larger taxa assemblage, which allowed rough survey on the entire *G. lacustris* aggregate, while the 28S dataset was more useful for exploring the relationships between Central Asian species. The phylogenetic relationships within the *G. lacustris* agg. were largely unresolved. However, OTUs clustering for the more variable COI (Brown et al. 1979) and for 28S was similar.

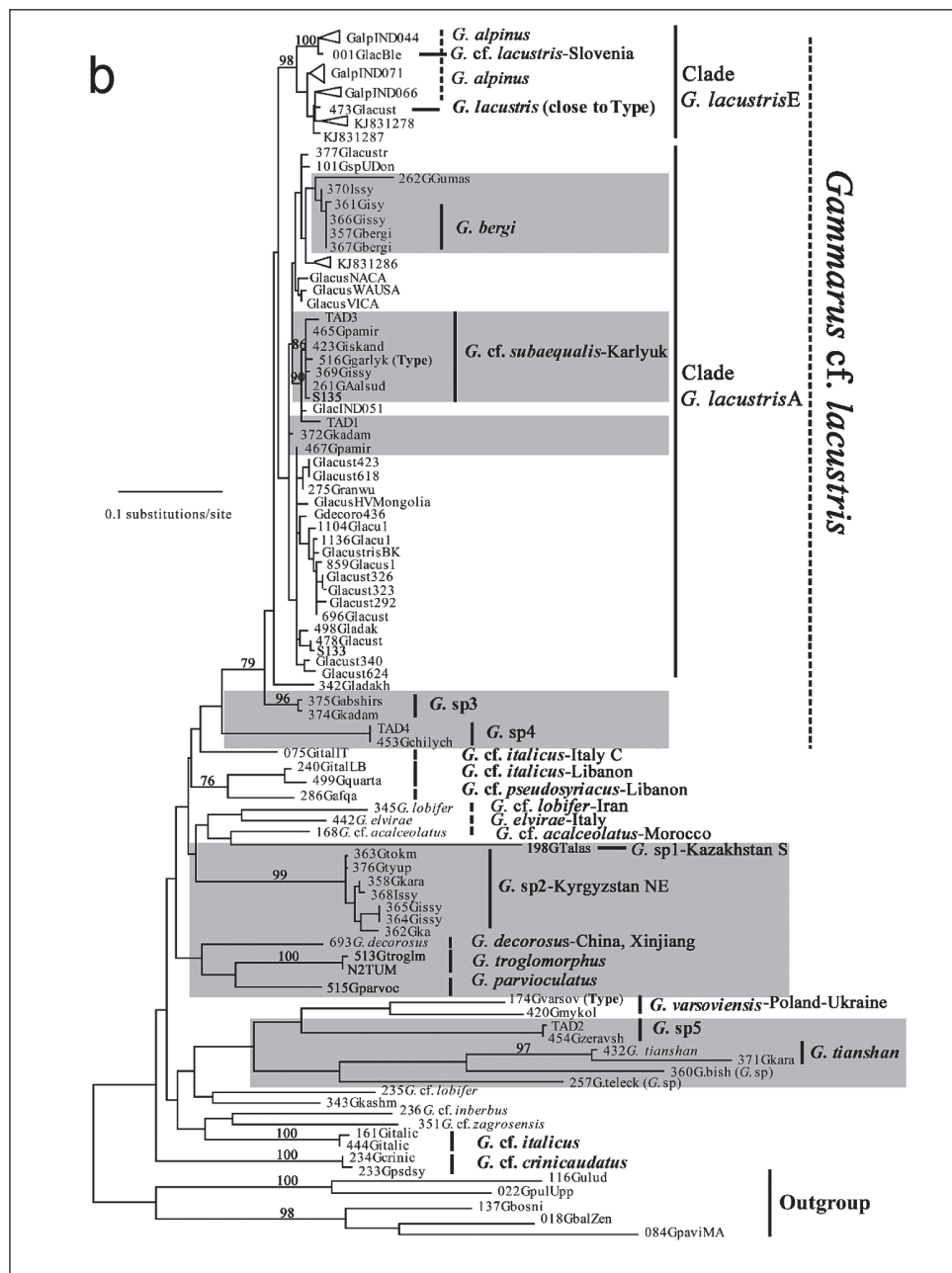
In its proximal part, the phylogram of the *G. lacustris* agg. is split into a high number of long branches. Members of these long branches are also morphologically diverse, in part already described as species living throughout western Asia and the Mediterranean (Kyrgyzstan, Turkmenistan, Iran, Lebanon, Italy, Morocco).

Terminally, the tree is split into two clades, called here *G. lacustrisA* and *G. lacustrisE*. This is a morphologically homogeneous part of the tree, most of its MOTUs have been mainly recognized by different authors and eventually published as '*G. lacustris*'. The clade *G. lacustrisE* is the European clade. The *G. lacustrisA* contains taxa from E Asia, from Himalayas (new data by ZH), some from Central Asia, but also from N America (USA, Canada), and few from Europe (Norway, Ukraine). The morphologically distinct *G. bergi* from Ysyk K l make this clade morphologically more diverse, although the majority of members have been mentioned in literature as *G. lacustris*.

The widely dispersed *G. cf. subaequalis*-Garlyk and the lacustrine *G. bergi* are hardly distinguishable in the 28S tree, while they are broadly separated in the COI tree. In addition to that, *G. bergi* is morphologically distinct, while *G. cf. subaequalis*-Garlyk belongs to the *G. lacustris* morphotype (Figs. 2a, b).







**Figure 2:** Phylogenetic tree of the aggregate *Gammarus lacustris* derived from maximum likelihood analysis of 28S (a) and COI (b) sequences. Bootstrap values (>70) are shown above branches. OTUs in both distal branches of the tree are strongly grouped and condensed. Marked subclades are from Central Asia.

**Slika 2:** Filogenetsko drevo agregata *Gammarus lacustris* po analizi maximum likelihood markerjev 28S (a) in COI (b). Vrednosti bootstrap (če >70) so nad vejami. OTUji v obeh končnih vejah so močno združeni. Označene podveje so v Centralni Aziji.

### List of taxa and their distribution

The diversity of the Central Asian *Gammarus* can be mainly illustrated by the analysis of the phylogram of the *G. lacustris* aggregate (Fig. 2a and b).

In detail, taxa, attributed to *Gammarus* or *Rivulogammarus* with revised geographical data and with some comments where necessary, are listed in the supplementary data (Supplement table S2). To avoid the accumulation of weakly supported nomenclatural combinations, we are only directly citing Martynov's taxa, since any serious revision would demand previous molecular analyses. This means that we also preserved the original nomenclature with *Rivulogammarus* S. Karaman 1931 (one of the authors - (DS) - does not agree with such solution). *Rivulogammarus* is anyway an objective junior synonym of *Gammarus* (Stock, 1969). Note that virtually all of Martynov's taxa (and subtaxa) are based on single populations. Molecularly studied samples are marked here in text with '(DNA)'. Numbers in brackets after species names are designations of OTUs in the phylogram as well as in the supplement table S1.

### *Gammarus lacustris* aggregate

The majority of Central Asian taxa and its genetic lineages are morphologically within the morphological diversity of the *G. lacustris* aggregate. Species of *G. tianshan*, *Gammarus* sp5, *G. parvioculatus*, *G. troglomorphus*, *G. decorosus*, *Gammarus* sp2, and *Gammarus* sp1 are distinctly branched from close to the base of the aggregate's phylogram. Being morphologically and molecularly so diverse, they might be the most ancient Central Asian *Gammarus* species with a long evolutionary history in the earlier biodiversity of the region. The species *G. bergi* and *G. cf. subaequalis* are nested in the clade of *G. lacustris*A with short branches, which could be explained by recent diversification of the clade *lacustris*A in Central Asia. Since *G. bergi* and *G. cf. subaequalis*-Garlyk inhabit special niches, namely a brackish lake and a streaming river, this may explain morphological differences from *G. lacustris*.

(1) Close to the phylogram base, a loose clade of long-branched OTUs splits, including *G. tianshan* Zhao, Meng & Hou (432; syn. *G. montanus* Hou, Li & Platvoet, 2004), which is our only taxon from the lake Teleckoe (257) and other populations from Central Asia, and also a population from Ukraine (420) and the type population of *G. varsoviensis* from Poland. Martynov (1930b, 1935) described *Gammarus teletzkensis* Martynov, 1930, *G. ocellatus* subsp. *angulatus* Martynov, 1930, *Gammarus korbuensis* forma *reducta* Martynov, 1930 from the lake Teleckoe ozero (Altyn Köl), Altaj, Russia. We could not join our *Gammarus* sp. (DNA) OTU 257 G.teleck. to any of the nominal taxa described from that lake.

(2) In the basal part of the tree, there is also a long-branched clade from Turkmenistan and Xinjiang. These are *G. decorosus* Meng, Hou & Li, 2003 (693; Xinjiang, China), *G. troglomorphus* Sidorov, Hou et Sket (513), and *G. parvioculatus* Sidorov, Hou et Sket (515), the latter ones from the extreme East of Turkmenistan. *Gammarus troglomorphus* Sidorov, Hou & Sket, 2018 (DNA) Turkmenistan E, Lebap Province, Garlyk, is one of the most extremely troglomorphic *Gammarus* species at all, eyeless and with long pereopods. It seems to be the only troglomorphic species and the only one with strongly carinate urosomites within the aggregate. It was found accompanied by a troglobiotic fish *Triplophysa starostini* (Parin, 1983) (syn. *Noemacheilus s.*, *Paracobitis s.*, *Troglcobitis s.*) in the collapse doline (karst sink) Suw Oyuk.

*Gammarus parvioculatus* Sidorov, Hou & Sket, 2018 (DNA) from Turkmenistan E, Lebap Province, Köýtendag District, Khodzhapil (= Hojeypil) was found in two very different springs near Köýtén. Although evidently an eutroglophile, it is a comparatively short-legged species with variable pigmentation and only slightly reduced eyes.

Thus, *G. troglomorphus* and *G. parvioculatus* are very morphologically different, not only at the expense of troglomorphy. On the other hand, they are molecularly sister species in both COI and 28S analyses. These molecular relations are weakly supported (with only 45%) in COI. In the 28S tree, the grouping of *G. troglomorphus* and *G. parvioculatus* is supported with a value of only 78. In the COI tree, their further sister



taxon is *G. decorosus* (OTU), an epigeal animal from Xinjiang.

(3) Phylogenetically more compact is a group of OTUs here named *Gammarus* sp. 2 (DNA), although it might be a group of independent species. They inhabit the lake Ysyk Köl (365, 368, at least up to 70 m deep) and springs (in Tokmok, Tyup, Karakol: 358, 362, 363, 376) between Karakol and Bishkek in the NE part of Kyrgyzstan. The group could not be identified either with *Gammarus ocellatus ocellatus* Martynov, 1930a, or with *Gammarus inberbus* Karaman et Pinkster, 1977b (described from the same lake at Koisara). All our gammarids from the SE part of the lake are provided with slight dorsal elevations on urosomites, while in *G. inberbus*, pleonites and urosomites are explicitly 'smooth, without dorsal elevations or excavations' (Karaman and Pinkster 1977). Moreover, our animals show less acute epimeron III and eye not as large as in *Gammarus ocellatus ocellatus*.

(4) Phylogenetically comparatively isolated group with some branches around the base of the terminal bifurcation of the COI tree, taken from Tajikistan, Kyrgyzstan, and from the Indian Ladak. In the 28S tree, these OTUs are similarly grouped, just slightly differently positioned in the tree.

*Gammarus* sp. 1 (DNA), OTU 198 G Talas, from Kazakhstan, Dzhambul oblast', Taraz, r. Talas, is phylogenetically an isolated population, molecularly most related to species from Iran, Europe (Italy), N Africa.

*Gammarus* sp. 3 (DNA), OTUs 374, 375, is from Kyrgyzstan W, Khodzhaaryk, Abshirsai, Abshir Ata.

*Gammarus* sp. 4 (DNA), OTU 453 is from Tajikistan SW, Shaartuz district, oasis Chilychor.

*Gammarus* sp. 5 (DNA) OTU (454 TAD2) and *G. tianshan* are a comparatively isolated, basally split group, including also a specimen from Teleckoe ozero; the group is similarly positioned in both phylograms.

(5) While the terminal clade *G. lacustrisE* is entirely European, *G. lacustrisA* includes few branches from N and E Europe (Norway, Ukraine), North America (USA, Canada), and eastern China, some isolated OTUs from E Asia and two important subclades from Central Asia. One of them is *G. bergi* Martynov, 1930 (DNA) (357, 361, 366, 367, 370), endemic to the brackish,

high elevation (1,607 m a.s.l.) lake Ysyk Köl. We found it up to 30 m deep on northern and southern sides of the lake (Bokonbaevo and Cholpon Ata). The species stands out of the *G. lacustris* agg. for its long and dense setosity of antennae I-II. It is roughly reminiscent to *G. komareki*, but four specimens analyzed molecularly clearly speak for its position in the *G. lacustris* agg. and away from the *G. komareki* agg. Moreover, in the tree it is surrounded by animals of the *lacustris* morphotype (i.e., morphologically identified as *G. lacustris*). An individual from deeper water (-30 m) has less setose (but still *komareki*-like) antennae. Terminal clades *G. lacustrisE* and *G. lacustrisA* loosely correspond to both allozyme races of Vainio and Väinölä (2003).

The other important subclade here has been designated as *G. cf. subaequalis*-Garlyk (Sidorov et al. 2018), morphologically related to the following Martynov's taxa. *Rivulogammarus subaequalis* Martynov, 1935, with 4 subordinate taxa (and/or synonyms; Martynov's subordinate/subspecific taxa are: subspecies, natio, morpha, forma) is from S Kazakhstan and Tajikistan; *Rivulogammarus turanus* Martynov, 1935 with 6 subordinate taxa, which was found in Uzbekistan and S Kazakhstan. As *Gammarus (Rivulogammarus) syriacus subaequalis*, the former was supposed to be (Birštejn, 1948) 'very common, widely spread in Tajikistan' (springs at fortress Hisor (= Gissar, Hissar, r. Hanakinka, kishlak near st. Hanaka, kišlak Hodžambio).

Thus, *Gammarus cf. subaequalis*-Garlyk (DNA) seems to be (a part of) the most important taxon of the Central Asia. The Martynov's *G. subaequalis* and *G. turanus* are morphologically mutually very close and may be phylogenetically identical with the molecularly studied OTU 516, described nowadays (Sidorov et al. 2018) as *G. cf. subaequalis*-Garlyk. The population from Garlyk (= Karlyuk), Köyten area in NE Turkmenistan resembles *G. turanus* particularly in the shape of pereopod V base, but it is very different in telson shape and setation. In telson and in uropod III dimensions and setation, it is closer to *G. subaequalis*. Our taxon is very different from both in urosomal spines. Molecularly very close to 516 (Karabulak, Garlyk) are OTUs TAD3 (Zeravshan Valley), 261 (lake Alaudin), 369 (Ysyk Köl, -10 m), 423 (lake Iskandar Köl), 465 (lake Karakul,

Pamir). Their phylogenetic relation (affinity) is also highly supported (90 BP). Considering the morphology and distribution, we suppose that these populations are really conspecific with one or both of the mentioned Martynov's species. Seven molecularly closely related populations are distributed within the area between the easternmost Turkmenistan, Tajikistan, and up to the eastern Kyrgyzstan with ca. 1,200 km distance between localities 516 and 369 (i.e., between Garlyk and Ysyk-Köl), while the Martynov's *G. subaequalis* and *G. turanus* are ca. 250 km away from this area to the NW. The phylogenetic relation between this taxon and *G. troglomorphus*, its very close geographical neighbor, is very weak.

If our supposition is correct, *Gammarus subaequalis* Martynov, 1935 is one of the most widespread species of the region, as indirectly already suggested by Birštejn (1948). Morphologically it is a *lacustris*-like species with a series of features occurring in phylogenetically diverse species within the genus *Gammarus*. The greatest morphological similarity outside Central Asia is with *G. crinicaudatus* Stock et al., 1998 from the southern Zagros region of Iran.

#### *Gammarus balcanicus* aggregate

Members of the *G. balcanicus* aggregate are morphologically comparatively easily recognizable. The reliability of such identification is, however, low, but in our case it does not contradict the few molecularly supported data. Characteristic of this aggregate is the poor setation of pereopods III–IV and of uropod III (Karaman and Pinkster 1987). In the latter, the spines along the exopodite outer margin are associated with only few and short finer setae.

Molecularly proven members in Central Asia are *Gammarus takesensis* Hou, Li & Platvoet, 2004 (DNA) from China, Xinjiang, Takes, and *Gammarus tastsienensis* (Hou, 2002, thesis not printed) (DNA) from China, Xinjiang, Yumin. The only morphologically recognized members of this aggregate are *Gammarus brevipodus* Hou, Li & Platvoet, 2004 from China, Xinjiang, Xinyuan, *Gammarus angustatus* Martynov, 1930a with two subordinate taxa from Russia, river Ob drainage (incl. Novosibirsk), *Gammarus pellucidus* Gur-

janova, 1930 from Russia, Krasnojarsk, *Gammarus (Rivulogammarus) balcanicus* Birštejn, 1950 from Russia, river Listvjanka, Jurginskij rajon, Kemerovska oblast' and *Rivulogammarus spinulatus* Martynov, 1935 from Kazakhstan, E of Almaty.

Thus, we can confirm the presence of the eastern assemblage of the agg. *balcanicus* along the E Kazakhstan–Xinjiang (China) border and in the adjacent areas of Russia. No member of agg. *balcanicus* could be detected in Iran. The closest western agg. *balcanicus* members are probably (not DNA-confirmed) in Asia Minor – *G. orientalis* (S. Karaman 1934) (Karaman 2017; in Central Turkey), *G. burduri* S. & G. Karaman, 1959 (SW Turkey), and in Azerbaijan – *G. alarodius* Deržavin, 1938 (in Nakhichivan, river Araks), *G. talyschensis* Deržavin, 1939 (S Azerbaijan).

#### *Gammarus komareki* aggregate

The *Gammarus komareki* aggregate is represented by *Gammarus turcomanicus* Birštejn, 1945 (DNA) (syn. *G. (Rivulogammarus) balcanicus turcomanicus* Birštejn 1945) at the edge of the area, in Turkmenistan, rivers Aşgabatka, Kişinka, Kara-su, Zolotoj ključ, Čulinka, Sakiz-jab around Aşgabat (Birštejn 1945). Molecular analysis (new data) shows the appurtenance of the spring population of Čulinka to agg. *komareki*. Although described as *G. balcanicus* ssp., its morphology is not in disagreement with such solution. This is the easternmost known population of the *G. komareki* agg. Further members of this aggregate are present in Iran (Stock et al. 1998, Zamanpoore et al. 2011), in Caucasus (Birstein 1933, Behning 1940, Behning and Popowa 1947), in E Europe, and probably in Asia Minor (Alanyali et al. 2011).

## Discussion

#### *Ruling of the G. lacustris* aggregate in Central Asia

Our gammarid samplings clearly show that - beside the *Sarothrogammarus* group in high mountains - we could only find members of *G. lacustris* in Central Asia. However, the core area is sparsely surrounded by members of the

*G. balcanicus* aggregate and by *G. komareki* in the West. We were also not able to phylogenetically classify the Martynov's (1935) taxa, originating mainly in southern Kazakhstan. Members of this aggregate even inhabit aberrant habitats, such as a brackish mountain lake and subterranean waters, the latter highly morphologically modified. The *G. lacustris* relatives here are morphologically very diverse, not as unified as the populations in Central and Northern Europe, which could be identified as a unique *G. lacustris* 'morphospecies' (Karaman and Pinkster 1977, Alther et al 2016).

Some additional taxa had been described, for which we find no close phylogenetic relations to our molecularly studied samples. These are *G. ocellatus* Martynov, 1930a with three subordinate taxa from Ysyk Köl and Teleckoe ozero, indicated later by Martynov and Behning, 1948 for a wider high mountain area in Central Asia. Farther *R. truncatus* Martynov, 1935 with 2 subordinate taxa from Uzbekistan and Tajikistan; *Gammarus gracilis* Martynov, 1935 from Kazakhstan, eastern Karatau; *Gammarus angusticoxalis* Martynov, 1935 from Kyrgyzstan; *Rivulogammarus brevicornis* Martynov, 1935 from Kazakhstan S, Karatau, springs at rivulets Kichkin, Ishfan-Mazar, Martynov (1935): 'A separate species, but close to *R. turanus*'.

Sidorov described *Gammarus montaniformis* Sidorov, 2012 from Kazakhstan, eastern Tien-Shan, and *Gammarus alius* Sidorov, 2012 from Kyrgyzstan.

*Rivulogammarus hirsutus* Martynov, 1935 (syn.: *Rivulogammarus brachyurus* Birstein, 1935, *Gammarus birsteini* Karaman et Pinkster, 1977) with 3 subordinate taxa from S Kazakhstan is morphologically very different and taxonomically intriguing. Antennae resemble *G. komareki* or *G. bergi*; according to Martynov (1935), it is close to *G. turanus*; the uropod III endopodite is shortened to 50% of exopodite or less. The uniquely long dorsal setae on urosomites, in Martynov's morph 'hirsutissimus' even on pleonites, make its belonging to agg. *lacustris* or agg. *komareki* unlikely, but not impossible.

Birštejn (1945) considered *G. hirsutus* Martynov, 1935 a junior synonym of *G. brachyurus* Birštejn, 1935; since the name *G. brachyurus* appeared to have been preoccupied (Karaman and Pinkster 1977), the name *hirsutus* may nev-

ertheless be used. The Karaman and Pinkster's (1977) *G. birsteini* has been illustrated according to specimens from Turkey; since it lacks those characteristic urosomal setae, it is evidently a species *per se*.

#### *Issykogammarus hamatus*

A specific problem is *Issykogammarus hamatus* Chevreux, 1908. It is an aberrant taxon of an uncertain position. It was obtained from different depths of the lake Ysyk Köl, Kyrgyzstan NE. It was described as a separate genus (Chevreux 1908), later attributed to principally Bajkalian acanthogammarids as a 'Baikalian Escapee' (Barnard and Barnard 1983). But, if we only overlook the recast of the pereopod coxae, the species fits easily into the diversity of the aggregate *lacustris* or even very close to the morphotype *lacustris*. The evolutionary mutability of the mentioned coxal armament in amphipods can be best documented by the marine genus *Epimeria* (Rauschert and Arntz 2015) or by the general body shape variability in Bajkalian gammarids, nested phylogenetically within *Gammarus* (Hou et al. 2016). Unfortunately, a molecular analysis was impossible and the old-fashioned presentation of the morphology, conducted even mainly on females, does not allow for a serious reevaluation of the species' taxonomy and phylogeny.

#### *Gammarus pulex* and its aggregate

*Gammarus pulex* and its aggregate in Central Asia remain a mystery. The only seemingly reliable data on *G. pulex* are those by Birštejn, 1945 from the rivers Sherlok and Firjuzinka near Aşgabat (Ashgabat) (central Kopetdag, neighbor to *G. turcomanicus*). Explicitly mentioned are curled posterior setae on pereopod III (not so in pereopod IV) and widened and densely setose flagellar articles of antenna II; we could only hardly expect these striking characters of *G. pulex pulex* (Linnaeus) in *G. lacustris* relatives. Nevertheless, both species are very similar and the Asian members of the aggregate *lacustris* are explicitly diverse.

Martynov (1930a, 1935) is persistently mentioning '*G. pulex*' for lentic(!) waters in Central Asia without giving any further data. One could suppose that he was misled by the similar *G. lacustris*, which he surprisingly did not mention at all. According to Karaman (1984), 'almost not any of these (cited here) populations belong to the *G. pulex*' - two pages of citations are given, more than 60 populations for the entire Asia between the Caspian and the Pacific shores, with exception of its Indian (Southeast Asia, Indonesia) region.

### Ecology and ethology

*Gammarus lacustris* has been characterized as an euryhaline, eurythermal, and euryoxibiotic species-ubiquist (Bazikalova 1941, Birstein and Taliev 1946, Bekman 1954, Pirozhnikov 1932, Økland and Økland 1985). In the vast territory of its rather well documented geographic distribution (see also Straškraba 1966), the morphotype '*Gammarus lacustris*' inhabits various water bodies: limnic and running waters, cold and warm, fresh and salty, rich in oxygen and hypoxic. In the Trans-Urals and in the Middle Urals, it inhabits soft fresh water with very low salt content (dry residue 60-77 mg/l) and brackish water bodies, for example in the Chelyabinsk region of the lakes Chebarkul and Cancul with dry residue 8,83 g/l (Dexbach 1952). In the Baikal region, it inhabits low-mineralized water bodies with a content of dry residue from 25 mg/l to 152 mg/l (Bekman 1954). In the lakes of the West Siberian lowland, it lives in water bodies whose salinity reaches up to 10‰ (Balabanova 1941, 1949).

A number of authors (Buschkiel 1931; Haempel 1908) pay attention to the concentration of calcium ions in the water. Therefore, Wundsch (1922) managed to show the mosaic distribution of *Gammarus* in the rivers of the right bank of the Rhine, where he stated the absence of *Gammarus* if the content of calcium ions was less than 9 mg/l. Experimentally, Bekman (1954) found out that the normal ontogenetic development of the Baikal *Gammarus* was disturbed in waters containing less than 7 mg/l of calcium.

In Central Asia, members of the *G. lacustris* agg. inhabit all types of perennial aquatic habitats.

They have mostly been found in springs, while rivers were sampled occasionally. Since temperatures of ground waters (and springs) normally approach mean yearly temperatures of the area, we can expect the temperatures of springs to range from ca. 18 °C (at Aşgabat, at 300 m a.s.l.) in the foothills to 7.5 °C in higher areas (around Ysyk Köl at 1,650 m a.s.l.). The terrain is mainly magmatic, dictating low calcium content in water; there are smaller karst i.e., carbonate areas (particularly in SE Kyrgyzstan and NE Turkmenistan), where we may expect hard waters. An even higher level of mineralization is reached in the lake Ysyk Köl itself, where water is brackish, with 6 g/l chloride/sulfate/sodium/magnesium-based salinity (Aladin and Plotnikov 1993, Baetov 2005).

Martynov (1935) mentioned stagnant waters as a common dwelling place for *G. pulex*, but we only found some normal *G. lacustris* agg. members in some smaller lakes. Unfortunately, Martynov explicitly states only ponds close to Bishkek, the area where we found *G. cf. lacustris*. Several gammarids have been described from the lake Teleckoe, as well as from Ysyk Köl. Some species may be present in the depths of some tens of meters. *Issykogammarus hamatus* and *Gammarus bergi* are definitely endemic, while we cannot confirm this for *G. inberbus* or *G. ocellatus*. *I. hamatus* exhibits characteristics of the 'lacustrine morphology' (Hou et al. 2016), while other species from this lake are morphologically trivial.

*G. troglomorphus* is an evidently highly specialized troglobiont. *G. parvioculatus* is present in both, springs and underground; its only troglomorphies are slightly smaller eyes and integumental depigmentation in some individuals. Some other troglotrophic amphipods are also *Tadzocrangonyx* spp. (*Sarothrogammarus* group of Gammaridae) and *Bogidiella* spp. (Bogidiellidae; found in Turkmenistan).

Gammarids are not provided with ontogenetic stages that were particularly suited for lateral dispersal. However, appropriate behavior may enable the adult to disperse laterally, outside the water. Segerstråle (1954) has shown that the ethology of *G. lacustris* enables it to take the advantage of transportation by birds (ornithochory). The author could experimentally establish that this is not a general ability of gammarids, since *G. pulex* was behaving differently. Therefore, we also

do not know whether this is only an ethological adaptation or is it also morphologically dependent (supported). This capability may also be either inherent in the whole aggregate or limited to the *lacustris* morphotype. Only the remotely related *Crangonyx pseudogracilis* Bousfield, 1958 was later a subject for a similar experiment (Rachalewski et al. 2013). In South Dakota, *Hyaella azteca* (Saussure) was found in duck's plumage (Rosine 1956). In Canada, the same amphipod was found in the fur of muskrat and beaver, while numerous *G. lacustris* were found in the fur of beaver (Peck 1975). We have no information about the respective behavior of other gammarids. We do not know which morphological characters contribute to the ability to hang on birds and we do not know the ethological differences and similarities between all of the numerous members of the *G. lacustris* aggregate. But, the exceedingly large distribution area of the *G. lacustris* agg. (and even of the morphotype '*G. lacustris*') shows that this group is extraordinarily capable of dispersal. It either dispersed in morphologically diverse types, or it dispersed as the morphotype *lacustris*, while its populations morphologically diversified only after reaching their recent dwelling areas.

#### *The supposed historical biogeography of Gammarus in Central Asia*

*Gammarus* in Eurasia (Palearctic). The Eurasian continent is inhabited by phylogenetically and geographically very distinct gammarid groups (Hou et al. 2011, Hou and Sket 2016). The Asian continental freshwaters east of Ural and north of 55 °N are virtually devoid of *Gammarus* and mostly inhabited by *Pallasea* and *Synurella*, which regularly significantly dominates in tundra and forest-steppe in the northern biotopes. The few known populations (Deržavin 1923, Kurenkov and Mednikov 1959, Labay 1998) are either not attributable to an aggregate, or belonging to *G. lacustris* agg., with the exception of *G. koreanus* Ueno, 1940 in Sahalin, probably belonging to the Oriental aggregate. It is also necessary to take into account the fact that *G. lacustris* was purposely introduced almost everywhere in the lakes in the Central Russian Upland, Siberia and even in Kamčatka during the Soviet times. These doings

have not always been properly documented. The eastern portion of China and areas NE from it (Japan and Korea, Russian Far East) are inhabited by the Oriental aggregate of *Gammarus*; Lake Bajkal has its own endemic fauna, nominally of diverse genera (and even families, e.g. Tahteev 2000), which are in fact phylogenetically members of *Gammarus* and probably sister to the aggregates *balcanicus* and *lacustris* (Hou and Sket 2016). In the West, the European aggregates *G. pulex*, *G. fossarum*, and *G. roeselii* do not cross the eastern borders of Europe. Two SE European aggregates are extending towards Central Asia. The *G. komareki* agg. is richly represented in Iran and with *G. turcomanicus* Birštein, 1945 (syn. *G. balcanicus turcomanicus*) in S Turkmenistan at Ašgabat (Ašgabat). The repeatedly mentioned *G. pulex* (e.g., Martynov 1935) remains a total mystery regarding Asia; molecularly, the morphologically similar aggregates *G. pulex*, *G. fossarum*, and *G. roeselii* could have only been verified for the European territory, with inclusion of Georgia (Hou et al. 2014, Supplement table S1).

The *G. balcanicus* aggregate occupies the E and SE Europe with some probable, but not molecularly proven representatives between Europe and the Caspian (see above). Molecularly proven members are only present in Ukraine and further west (Hou et al. 2014). In SE Europe, *G. balcanicus* agg. populations, cited mainly simply as '*G. balcanicus*', are evenly distributed, although populations of three other aggregates are also present there. Karaman and Pinkster (1987) mention more than 1,000 samples (of the supposed unified species!) in their area, Poland to SW USSR.

However, an additional detached area exists with a small number of reliable agg. *balcanicus* members (Hou and Sket 2016) along the border between Xinjiang and Kazakhstan, and (not molecularly proven) in the adjacent areas of southern Sibir' (Siberia, Russia). This results in more than 2,000 km wide gap between both parts of the entire agg. *balcanicus* distribution. This gap includes the heart of Central Asia. In recent years, we sampled ca. 40 springs and clear lakes in Central Asia, typical habitats of '*Gammarus balcanicus*', but not one sample contained *balcanicus*-like animals. It was molecularly proven that they all belong to the *G. lacustris* agg. Even if some undiscovered populations of the *G. balcanicus* agg. do exist



there, they must be very scarce, of an evidently relictual character.

Thus, the *G. balcanicus* agg. is only approaching Central Asia marginally in the NE. Such situation in a sedentary and highly endemic group, as agg. *balcanicus* is, can only be explained by a temporary omnipresence of the aggregate in the past. Thus, we suppose that in the past, the aggregate members used to be continuously present between Europe and NW Xinjiang, and in the South of the West Siberian Plain.

In Europe, a comparable area would be richly populated by gammarids of aggs. *G. pulex*, *G. fossarum*, and *G. balcanicus*, while the morphotype ‘*G. lacustris*’ is the only representative of the *G. lacustris* agg. There, it is mainly restricted to lakes (Alther et al. 2016; Väinölä et al. 2017). In Southern Europe and the Mediterranean, *G. lacustris* agg. is also present in springs and streams and may be represented by morphologically different species (e.g., *G. italicus*, *G. elvirae*). In Italy, only the *G. lacustris* aggregate is present along the peninsula and in the Sardegna (Sardinia) island. The others are limited (with a small exception) to the continental North of the country, while Sicily (Sicily) and the French Corse (Corsica) are devoid of a freshwater *Gammarus*. Some populations of ‘*G. italicus*’ from the central Italian lakes (later separated as *G. elvirae* Iannilli & Ruffo, 2002) are even ‘very similar to *G. lacustris*’ (Karaman 1993). However, all these areas are richly inhabited by the related *Homoegammarus* (syn. *Echinogammarus* p.p.).

Within the peninsular part of Italy (Stoch and Ruffo 2003), agg. *balcanicus* is present in two closely positioned UTM quadrates, the agg. *lacustris* in ca. 45 quadrates of 10x10 km (*G. elvirae* ca. 40 quadrates, *G. lacustris* 5 quadrates). The situation is similar in the Alpine and Scandinavian areas of central and northern parts of Europe, where agg. *pulex* is somehow limited, while the others are close to absent, and *G. lacustris* agg. (by *G. cf. lacustris*) is mainly common in lakes (Fryer 1953; Segerstråle 1954, Alther et al. 2016). On the contrary, in Slovenia, which lies at the SE edge of the Alps, we registered *G. fossarum* agg. from 120 localities (but hundreds of noted remain unregistered), *G. roeselii* agg. from 13 in the East, *G. balcanicus* agg. from 6 scattered localities in

the West, and *G. lacustris* agg. only in one lake in pre-Alps (our own data).

#### *Phylogeography of Gammarus taxa in Köýtendag*

Very instructive, predicting the next surprises in the *Gammarus* fauna of Central Asia, are the relations among the eastern Lebab (Köyten-Garlyk (=Karlyuk)), extreme East of Turkmenistan) species. Two cave-related species (*G. troglomorphus*, *G. parvioculatus*) are morphologically the most dissimilar species of Central Asia, and among the most divergent species pairs within *Gammarus* at all, which is only to some extent the result of troglomorphy. As exposed above, both cave-related species are extremely morphologically different, and molecularly loosely, but firmly related. Their localities are ca. 40 km apart, while their probably (with low support again) closest relative is *G. decorosus* Meng, Hou & Li, 2003 from ca. 2,000 km away in Urumqi, Xinjiang, which is not inclined to inhabit subterranean waters. All of the mentioned species represent relatively long branches in the proximal part of the aggregate phylogram, with contrast to the basal *G. tianshan* and others.

The third species from the Köyten-Garlyk area is clearly epigeal, geographically the closest neighbor of *G. troglomorphus* at Garlyk, but evidently broadly dispersed. We tentatively named it *G. cf. subaequalis*-Garlyk (Sidorov et al. 2018). See above for some more details. It is a member of a small clade of 7 samples (our OTUs 261, 423, 465, 516, 369, 359, S135) in the tree from the 1,000 km long stripe between Garlyk (= Karlyuk) in Turkmenistan, Tajikistan, and Ysyk Köl area in Kyrgyzstan. Only future field sampling can enable us to tell whether these series of populations is conspecific with the named Martynov’s taxa. The samples of *G. cf. subaequalis*-Garlyk are molecularly close or nearly identical, and as a group very close to the branchlets of the terminal part of the tree, its clade *lacustrisA*, including numerous samples from Eurasia and North America. The endemic *G. bergi* Martynov from the lake Ysyk-Köl is also included. Only ca. 300 km to the NW from the central part of this area are Martynov’s (1935) localities of *G. subaequalis* and *G. turanus*, which are morphologically similar and might be



both, mutually conspecific and conspecific with the mentioned group.

#### *The climatic past possibly influencing the distribution of gammarids in Central Asia*

Central Asia has been the area of the highest aridity because of the rain shadow caused by Tibetan Plateau and some other mountain ranges (Caves et al. 2014). This aridity was increasing during the Cenozoic. The precipitation should have 'more than halved' over the Neogene. It is likely that this aridity extirpated the majority of the previously evidently existing agg. *balcanicus* populations; this opened the territory for the invasion of the vagile and euryoecious *G. lacustris* agg., which may have happened several times during short climatically less adverse periods.

The Central Asian taxa are very diversely positioned in the phylogram; some have branched off close to the tree root, while some are members or sisters of the most distal branches. One has to bear in mind that the distribution area of the entire *G. lacustris* agg. covers the entire area of the genus *Gammarus* and even crosses the other gammarid species (Hou et al. 2011, Hou and Sket 2016, Väinölä et al. 2017). To a great degree, it occurs even in the shape, morphotype of *G. lacustris*, and is therefore often morphologically identified and published as '*Gammarus lacustris*'.

Such distribution can probably be explained by the ability of *G. lacustris* to be transported by birds, as shown by Segerstråle 1954, hand in hand with the above-mentioned ecological universality of '*G. lacustris*'. Waterfowl from the North (here Jamal and Tajmyr,) migrate in a wide front, but birds often fly along the coast of the seas and along large rivers (here Irtyš) and Central Asian lakes (Thompson et al. 2001, Šnitnikov 1980, Kydyraliev 1990). Therefore, the populations of amphipods can be steadily replenished in their basins. The erratic spreading of this group is also supported by the fact that molecularly very close populations occur in very distant localities, including the sisterhood between the populations from North America and Ysyk Köl.

The morphological diversity of the *G. lacustris* agg. members shows its evolutionary plasticity and

a possibility that its populations (and future species) could have distributed at different times, but every one probably still in the 'original' *lacustris* morphotype, i.e. conquistador's appearance. Thus, numerous subsequent colonizing groups formed *lacustris*-morphotype populations and diversified only after colonization of new territories, in this case in Central Asia. Such diversification might be a consequence of the enforcement between at least some lake (e.g., in Ysyk Köl) populations. No case of species sympatry within the agg. *lacustris* has been recognised outside Central Asia.

Precipitation quantity had been fluctuating in the near past (Wolff et al. 2017), and it was probably so during the whole Cenozoic period as well. Therefore, the *G. balcanicus* agg. could have spread its area from the West into Central Asia and beyond either before the onset of aridification, or also during a longer or shorter wetter period within its course.

The only additional gammarids (i.e., non-*Gammarus*) in Central Asia are members of the eastern (Asian) clade of the *Sarothrogammarus* group of genera (Hou and Sket 2016, Barnard and Barnard 1983), including *Tadzocrangonyx* spp. They are ecologically specific, living mainly in springs and to some extent in the subterranean, between 2,000 and 3,000 m a.s.l., and hardly ever below 1,000 m a.s.l. They are limited to the SE part of Central Asia: Uzbekistan - Tajikistan - N Afghanistan - N Pakistan. It is possible that different ecological regime in high elevations prevented extirpation of the *Sarothrogammarus* gammarids.

## Conclusions

(1) Recent samplings of gammarids in the core area of Central Asia, approximately between E Turkmenistan - N to E Kirgystan - Tajikistan, which we were able to study molecularly, delivered only members of the *G. lacustris* aggregate. The area is sparsely surrounded by members of the *G. balcanicus* aggregate. Members of the related *Sarothrogammarus* group of genera are only present in springs and mainly in higher elevations.

(2) The *G. lacustris* aggregate appeared to consist of species with high phenotypic and evolutionary plasticity, resulting in a morphologically diverse and geographically widely distributed

taxon. Its distribution area is broader than that of the rest of the genus *Gammarus*. In addition, within the genus' area, it is present where other species are absent – including in Central Asia.

(3) While the *G. balcanicus* relatives and other Palearctic groups are evidently only able to disperse along streams, the *G. lacustris* relatives are able to disperse laterally outside water, by ornitochory. We do not know how general this ability is within the aggregate.

The *Gammarus* assortment in C Asia consists of species from all parts of the phylogram. They evidently immigrated in part in different times from far abroad, but some speciation must have happened within the area, to a smaller extent probably even within the lake Ysyk Köl (Issyk Kul) alone.

No members of the generally European clade *G. lacustris*E were found in Central Asia, but Central Asian species are in close relationship with other species from Europe, Asia, and North Africa – and even N America.

(4) The absence of other *Gammarus* groups in this wide arid area and its paleoclimatic history indicates that the – probably originally richer - amphipod fauna in the lower regions could have been extirpated in particularly arid periods. The *G. lacustris* relatives could survive to some extent for their euryoecity and be restored by a steady import and immigration. The *G. balcanicus* populations could only survive sheltered in springs in marginal positions of the area.

So, a great gap in the Gammaridae diversity appeared in Central Asia

(5) The morphological diversity of the *G. lacustris* agg. in Central Asia and also in general nearly equals the diversity of the European *Gammarus* spp.

The generally very poor subterranean fauna in Central Asia is represented by the troglomorphic and highly troglomorphic *G. troglomorphus*, and by the very stocky and still occulated eu-troglophile *G. parvioculatus*.

## Povzetek

Ob raziskovanju podzemeljske biotske pestrosti v Köytendag, Turkmenistan, v okviru misije britanskega Royal Society for the Protection of

Birds, smo opazili nenavadno sestavo favne postranic rodu *Gammarus*. To nas je napeljalo na podrobnejšo raziskavo postranic v Centralni Aziji.

Postranice (Amphipoda) v Centralni Aziji so pomankljivo raziskane. Opisi so večinoma skromni. Velike težave so z geografskimi podatki. Zaradi administrativnih in pravnopisnih sprememb so stara krajevna imena spremenjena, nova pa neustaljena. Tako npr. staro ime 'Eastern Bukhara' ni del današnjega mesta Buhara, Uzbekistan, temveč vzhodni del obsežnega Emirata Buhara, ki je vključeval predvsem osrednje in zahodne dele sodobnega Tadžikistana. Druga skupina problemov zadeva številne najdbe domnevnega *Gammarus pulex* v Centralni Aziji, ki tam, kot kaže, sploh ni prisoten. Po drugi strani pa Martynov (1935) sploh ne omenja *G. lacustris*, ki je s sorodniki prevladujoča skupina vrst. Martynov je okoli leta 1935 napisal nekaj člankov in obsežno raziskavo z dokaj površnimi in skromno ilustriranimi opisi, večinoma iz (danes) južnega Kazahstana. Izgleda, da je njegov *Gammarus subaequalis* Martynov, 1935 zelo podoben ali identičen nekaterim našim vzorcem in zastopa tam najbolj razširjeno vrsto.

Zdaj zbrani vzorci so večinoma iz Kirgizije, vzhodnega Uzbekistana, Tadžikistana in iz Turkmenistana. Ti omogočajo tudi molekulska analizo, težko pa jih je povezati s taksoni, ki so jih postavili Martynov, Birštejn in Schellenberg. V dodani tabeli je pregled vseh že prej objavljenih podatkov.

Naša raziskava zajema v Turkmenistanu Aşgabat z okolico in Köýtendag na skrajnem severovzhodu; v Kirgiziji jezero Ysyk Köl (Issyk Kul) z okolico ter jugozahod dežele; v Tadžikistanu kraje na severozahodu in jugovzhodu. Vse lokalitete in zbiralci so naštet v tabeli. V največji meri smo vzorčili izvire in jezero, le malo pa reke. Živali smo molekulska identificirali do agregatov, 'skupin vrst', ki se sploh ne ujemajo z izključno morfološko določenimi skupinami Karamana in Pinksterja. Za določitev vrst se zanesemo le na vzorce iz topotipskih lokalitet, v kolikor so tudi morfološko primerni. Pri vseh drugih veljajo imena le pogojno in so označena s 'cf.'.

Za molekulska analizo smo vzeli majhne dele živali, preostanek takšnih osebkov smo spravili za poznejšo natančnejšo morfološko in taksonomsko obdelavo. DNK smo izolirali s pomočjo Qiagen kita in pomnožili mitohondrijski COI ter jedrni 28S gen s primeri, ki so opisani v Hou et al. (2007). S

programom jModelTest (Posada, 2008) sta bila izbrana modela substitucij HKY + I + G za COI in GTR + I + G za 28S. Filogenijo smo rekonstruirali z metodama največje varčnosti (MP) in največjega verjetja (ML). Podporo razvejitev smo preverjali z bootstrap analizo (metodo vezanja).

Razen maloštevilnih pripadnikov agregata *balcanicus* in vzorca iz agregata *komareki* smo vse vzorce molekulsko razkrili kot pripadnike agregata *lacustris*. Filogenetske analize s COI in 28S so dale zelo podobne rezultate. Žal nismo imeli na voljo aberantnega endema *Issykogammarus hamatus* Chevreux.

Od proksimalnega dela filograma za agregat *lacustris* se cepijo številne dolge veje. Tudi taksoni s teh vej so morfološko raznoliki, deloma opisani kot samostojne vrste, ki živijo v zahodni Aziji in v evropskem Mediteranu. Na vrhu je drevo razcepljeno v dve veji. Taksoni na njih so si podobni, v literaturi so največkrat označeni kot *G. lacustris*; zelo drugačen je jezerski *G. bergi*. Ena teh vej je evropska; druga iz severovzhodne Evrope sega po eni strani v Severno Ameriko in po drugi v obsežne dele Azije. *Gammarus* cf. *subaequalis*-Garlyk je zelo razširjen po Centralni Aziji in je po morfotipu *lacustris*.

Pestrost centralnoazijskih gamaridov torej lahko ponazorimo z analizo filograma za agregat *lacustris*. Velja upoštevati, da imajo skoraj vsa taksa in subtaksa Martynova za osnovo le posamezne populacije. V drevesu agregata *lacustris* je še mešanica vrst, ki so, in takih, ki niso prisotne v Centralni Aziji. Izgleda, da je najbolj razširjen *G. cf. subaequalis*-Garlyk. Med dolgimi vejami pri bazi drevesa omenimo nedavno odkriti podzemeljski vrsti iz Turkmenije. *G. parvioculatus* je očitno evtroglifna žival z malce zmanjšanimi očmi, sicer pa bolj kratkonoga v primerjavi z večino povsem površinskih vrst. Nasprotno pa je *G. troglomorphus* menda najbolj troglomorfna vrsta svojega rodu. Ti vrsti iz severovzhodne Turkmenije sta si filogenetsko sestrski, obenem pa med seboj morfološko najbolj različni vrsti v Aziji; različni si nista le na račun troglomorfnosti. Njima najbližji sorodnik je povsem površinska vrsta *G. decorosus* iz kitajskega Xinjianga. Od že znanih vrst v jezeru Ysyk Köl smo nekatere našli in molekulsko določili, nekaterih pa nismo našli. Endemna jezerska vrsta *G. bergi* nekoliko spominja na *G. komareki*, a jo mole-

kulski podatki zanesljivo postavljajo v agregat *lacustris*.

*Issykogammarus hamatus* je endemna jezerska vrsta. Jezero je visokogorsko (1,650 m n.m.) in somorno. Žival ima bočne trnaste izrastke, kar je značilno le za vrste iz starih jezer. Zato so ga uvrstili med živali tega tipa. Vendar pa se po vseh drugih morfoloških značilnostih dokaj ujema z *G. lacustris*.

*G. turcomanicus* je bil kot *G. balcanicus turcomanicus* napačno uvrščen. Pri Aşgabat u je najbolj vzhodno nahajališče (te vrste in) agregata *komareki*. Populacije agregata *G. balcanicus* se zadirajo v naše območje iz južne Rusije (Sibirije) proti jugozahodu, v osrednjem delu Centralne Azije jih ni, pojavijo pa se spet na jugovzhodu Evrope. Med obema deloma areala je pas brez njih, širši kot 2,000 km. To so živali, vezane na stalne, hladnejše vode. Agregat *pulex* za Azijo je in ostaja misterij. Starejši avtorji ga omenjajo pogosto, večinoma brez opisov, zdaj pa ga nismo našli in te vrste v Aziji verjetno ni. Od drugih rodov v Srednji Aziji živi rodovna skupina *Sarothrogammarus*; tukajšnja podskupina je vezana na višje lege in jo široka vrzel (ca. 2,500 km) ločuje od mediteransko-atlantske podskupine rodov in vrst.

V Centralni Aziji smo našli gamaride v vseh tipih voda. Tako v mehkih, kot v trdih in v somornici, v potokih, bistrim mlakah, jezerih in izvirih, od ca. 300 do nad 2,000 m n.m. V geološki preteklosti je Srednja Azija utrpela aridifikacije, ki so nedvomno povzročile siromašenje voda ter izumiranje manj evriekih gamaridov. Zlasti v vlažnejših obdobjih je bilo tako olajšano poseljevanje zelo evriekemu *G. lacustris*.

Za razliko od srednje in severne Evrope, kjer je agregat *lacustris* zelo vezan na jezera, se tukaj pojavlja v vseh tipih voda. Gamaridi nimajo stadijev, posebej prirejenih za razširjanje, pač pa so za *G. lacustris* ugotovili zmožnost razseljevanja s ptiči. Domnevamo, da so se na ta način razširjali v Srednjo Azijo in po njej. Na ta račun domnevna vrsta *G. lacustris* (torej morfotip tega imena) zaseda območje, ki presega siceršnji areal rodu *Gammarus* in celo družine Gammaridae.

Rod *Gammarus* v Srednji Aziji zastopa skoraj izključno agregat *G. lacustris*, le po robu semkaj segata še agregata *balcanicus* in *komareki*. Domnevamo, da so takšni poselitvi prispevala izumiranja gamaridov v izjemno sušnih obdo-

bjih. Tem je sledilo poseljevanje z evrieko vrsto *G. lacustris*, ki se morda edina razširja tudi s ptiči, in se je diverzificirala po naselitvi v Srednji Aziji. Zdaj tako na območju najdemo med drugim tudi ekstremno troglomorfno vrsto tega agregata in zelo aberantno jezersko vrsto.

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### List of supplements (web edition)

**Supplement S1:** List of molecularly analyzed samples of the *Gammarus lacustris* aggregate, adequate to the tree in Fig. 2. Top samples (from the tree top to first Tajikistan samples) are only few representatives of the morphotype *lacustris* from Europe, the rest are all available samples of the aggregate. Highlighted samples are from Central Asia s.l..

**Supplement S2:** List of *Gammarus* spp., described from Central Asia, but not studied molecularly. Nomenclature and taxonomic subdivision are taken from original papers; the recent geographic, administrative, and orthographic changes have been considered as much as possible. For practical reasons, the original naming of taxa has been retained.

**Supplement S3:** Distribution map of *Gammarus* spp. in Central Asia. Red asterisks with names - important cities. Reversed triangles - molecularly not defined *Gammarus* spp., letters indicate abbreviated taxonomic names (see Suppl. S2). Rings - molecularly defined taxa of agg. *lacustris*, numerals are OTU designations (as in the second column in Suppl. S1); pale ribbons connect localities of *G. cf. subaequalis*-Garlyk, hatched ribbon connection with Martynov's *G. subaequalis* and *G. turanus*. Squares - agg. *balcanicus*; diamond (*G. turcomanicus*) - agg. *komareki*. Adequate to Figure 1 in the paper.

**Supplement S4:** Additional taxonomical clarification. (1) Type population of *Gammarus lacustris* Sars, 1863. (2) Identity of '*G. balcanicus*' from Alma-Ata. (3) Corrected classification of '*Anisogammarus madyensis* Chaudri, Ghauri & Mahoon, 1987.

## Supplement / Dodatek

**Supplement S1:** List of molecularly analyzed samples of the *Gammarrus lacustris* aggregate, corresponding to the tree in Fig. 2. Top samples (from the tree top to first Tajikistan samples) are only few representatives of the morphotype *lacustris* from Europe, the rest are all available samples of the aggregate. Highlighted samples are from Central Asia s.l..

**Dodatek S1:** Popis molekularno analiziranih vzorcev agregata *Gammarrus lacustris*, ki se ujema s sliko 2. Vrhnji vzorci (od vrha drevesa do prvih tadžikistanskih vzorcev) so le redki predstavnik morfolojskega tipa *lacustris* iz Evrope, preostalo pa so vsi dosegljivi vzorci agregata. Zasenčeni (obarvani) vzorci so iz Centralne Azije s.l..

Taxon - working names; (Type - from type locality)/ Takson - delovna imena (Type - iz tipске lokalitete)	Designation of OTUs in the tree/ Oznaka OUT v drevesu	Locality (r = stream; c = cave; N, S, W, E = north, south, west, east)/ Lokaliteta (r = potok; c = jama; N, S, W, E = sever, jug, zahod, vzhod.)	Collector(s) or Reference (with year)/ Zbiralec ali vir podatka (z letnico)	Habitat/ Habitat	Country/ Država	Voucher number/ Številka vaučerja	COI	28S
G. alpinus Alther, Fiser & Altermatt 2016	GalpIND (7 OTUs)	Alps	Alther et al. 2016	lakes	Austria S (Europe)		KX283210	
G. cf. lacustris	001GiacBle	lake Blejsko jezero, Bled	B. Sket	lake	Slovenia NW (Europe)		JF965915	JF965728
G. alpinus	GalpIND (50 OTUs)	Alps	Alther et al. 2016	lakes	Austria N (Europe)		KX283184	
G. alpinus	GalpIND (6 OTUs)	Alps	Alther et al. 2016	lakes	Austria N (Europe)		KX283188	
G. alpinus	GalpIND (9 OTUs)	Alps	Alther et al. 2016	lakes	France (Europe)		KX283195	
G. lacustris G.O. Sars 1864 (close to Type)	473Giacust	lake Birisjoen, Vågå, Oppland	J. Vainio	lake	Norway S (Europe)	SLOCHN473	MK066420	MK066380
G. lacustris	KJ831288	lake Ulvenvann (?Oslo?)	K.Ostbye et al.	lake	Norway S (Europe)		KJ831288	
G. lacustris	KJ831277	lake Tinho, Eidfjord, Hordaland (Bergen)	K.Ostbye	lake	Norway SW (Europe)		KJ831277	
G. lacustris	KJ831281	Sandagrotta Cave, Skrim, Kongsberg S	K.Ostbye	cave	Norway S (Europe)		KJ831281	
G. lacustris	KJ831279	Sandagrotta Cave, Skrim, Kongsberg S	K.Ostbye	cave	Norway S (Europe)		KJ831279	
G. alpinus	GalpIND064	GalpIND064	Alther et al. 2016	lake	Alps (Europe)		KX283190	
G. lacustris	KJ831278	Sandagrotta	K.Ostbye	cave	Norway S (Europe)		KJ831278	
G. lacustris	KJ831280	Sandagrotta	K.Ostbye	cave	Norway S (Europe)		KJ831280	
G. lacustris	KJ831287	lake Ulvenvann	K.Ostbye	lake	Norway S (Europe)		KJ831287	
G. lacustris	377Giacustr	lake Tinho, Eidfjord, Hordaland	K.Ostbye	lake	Norway S (Europe)	SLOCHN377	MK066421	MK066381
G. cf. kischineffensis	101GspUDon	r. Severski Donec, Biškin, Veliki Burluk, Kharkiv	P. Trontelj	river	Ukraine (Europe)		JF965910	JF965722
G. sp	262 GGumas	Gorno Badakhshan, SE of Gumas	C.Fiser	spring	Tajikistan		JF965966	JF965796
G. cf. bergi	370Issy	lake Ysyk Köl, littoral, Cholpon Ata	B.Sket	lake	Kyrgyzstan	SLOCHN370	MK066422	MK066382
G. bergi Martynov 1930 (close to Type)	361Glsy IK	lake Ysyk Köl, ittoral, Barskoon, NW Kichi Jargylchak	B.Sket	lake	Kyrgyzstan	SLOCHN361	MK066423	MK066383
G. bergi	366Gissy	Lake Ysyk Köl, -30m, 12 km E Kadzhi Say, Bokonbaevo	B.Sket	lake	Kyrgyzstan	SLOCHN366	MK066424	MK066384
G. bergi	357Gbergi	Lake Ysyk Köl littoral, Bokonbaevo, 12 km E Kadzhi Say	B.Sket	lake, -30m	Kyrgyzstan	SLOCHN357	MK066425	MK066385
G. bergi	367Gbergi	Lake Ysyk Köl, -10m, Cholpon Ata KG,	B.Sket	lake, -10m	Kyrgyzstan	SLOCHN367	MK066426	MK066386
G. cf. lacustris	KJ831286	lake Lille Lauerann, Kongsberg	K.Ostbye	lake	Norway S (Europe)		KJ831286	
G. cf. lacustris	KJ831282	lake Lille Lauerann, Kongsberg	K.Ostbye	lake	Norway S (Europe)		KJ831282	
G. cf. lacustris	KJ831285	lake Lille Lauerann, Kongsberg	K.Ostbye	lake	Norway S (Europe)		KJ831285	
G. cf. lacustris	KJ831283	lake Lille Lauerann, Kongsberg	K.Ostbye	lake	Norway S (Europe)		KJ831283	
G. cf. lacustris	KJ831284	lake Lille Lauerann, Kongsberg	K.Ostbye	lake	Norway S (Europe)		KJ831284	
G. cf. lacustris	GiacusNACA		K.Ostbye		Canada	AY529052	AY529052	AY529073
G. cf. lacustris	GiacusWAUSA				USA	AY926673	AY926673	
G. cf. lacustris	GiacusVICA				Canada	AY926672	AY926672	

G. cf. subaequalis-Garlyk	TAD3	Zeravshan valley	C. Fišer	spring	Tajikistan	JF965968	JF965798
G. cf. subaequalis-Garlyk	465Gpamir	lake Karakul, Pamir	D. Kryvokhyzha	lake	Tajikistan NE	MK066427	MK066387
G. cf. subaequalis-Garlyk	423GIskand	lake Iskandarkul	C. Fišer	lake	Tajikistan	MK066428	MK066388
G. cf. subaequalis-Garlyk ('Type')	516Garlyk	Kara Bulak, Karyluk, Koytendag	B. Sket	stream	Turkmenistan E	MG655630	MK066389
G. cf. subaequalis-Garlyk	369Gissy	lake Ysyk Köl, -10m, Cholpon Ata,	B. Sket	lake -10m	Kyrgyzstan	MK066429	MK066390
G. cf. subaequalis-Garlyk	261GAalsud	lake Alaudin	C. Fišer	lake	Tajikistan	JF965965	JF965795
G. cf. subaequalis-Garlyk	S135	Pamir, GBAO, Ishkoshim distr. vil. Zumudg	D. Palatov	stream	S135	MK160078	
G. cf. subaequalis-Garlyk	TAD1	Khujaand, Sogdijskaja obl.	C. Fišer	spring	Tajikistan	JF965967	JF965797
G. cf. alpinus	GlacIND048(-050), 8 OTUs	Alps	Alther et al. 2016	lake	Alps (Europe)	KX283247	
G. sp.	372 Gkadam	Kyzyl Bulak, Kadamzhai	B. Sket	stream	Kyrgyzstan -SW	MK066430	MK066391
G. sp.	467 Gpamir	lake Bulunkul, Pamir	S. Litvinchuk	lake	Tajikistan SE	MK066431	MK066392
G. cf. lacustris	Glacus423	Altun, Xinjiang	Hou et al 2007	lake	China	EF570321	
G. cf. lacustris	Glacus 618	618 Xiaianza, Tibet (?)	Hou et al 2011		China Tibet	EF570322	
G. cf. lacustris	275Gramvu	lake Ranwu, Yale	A. Brancej		China Tibet	MK066432	MK066393
G. cf. lacustris	GlacusHV Mongolia	? lake Hovsgol	Macdonald et al 2005		Mongolia	AY926670	
G. decorosus Meng, Hou & Li 2003	Gdecoro436	Urumqi	Hou et al 2011		China Xinjiang	EF570303	EF582949
G. cf. lacustris	1104Glacul	Aixa Zuoqi, Inner Mongolia	Hou et al 2014		China Inner Mongolia	JF965918	JF965733
G. cf. lacustris	1136Glacul	Selenga	Hou et al 2011	river	Mongolia	JF965917	JF965732
G. cf. lacustris	Glacustris BK	Olhon Island, lake Bajkal	??? V. Tahteev	lake	Russia Bajkal	AY926671	
G. cf. lacustris	859 GlacusI	Haidong, Xunhua, Qinghai	D. Qi		China Qinghai	MK066433	
G. cf. lacustris	Glacust 326	Fangshan	Hou et al 2007		China Beijing	EF570318	
G. cf. lacustris	Glacust 323	Fangshan	Hou et al 2007		China Beijing	EF570317	EF582964
G. cf. lacustris	Glacust292	Beijing, Yanqing	Hou et al 2007		China	EF570315	
G. cf. lacustris	696Glacust	Changji, Urumqi,	CHN	CHN 696 (with 436)	China Xinjiang	JF965916	JF965730
G. cf. lacustris	498Gladak	Leh, Ladakh	G. Mazepa		India J&K	MK066434	MK066395
G. cf. lacustris	478Glacust	lake Shira	K.P. Vereshchagina, E.S. Zadereev, A.G. Degermebdzhy	lake	Russia Khaassia	MK066435	MK066394
G. cf. lacustris	S133	Khovd aimag, Chono-Kharaikhin-gol river	D. Palatov	stream	Mongolia	MK160077	
G. cf. lacustris	Glacus340	Hoh Xil	CHN		China Qinghai	EF570320	EF582965
G. cf. lacustris	Glacus624	Qinghai	Hou et al 2007		China	EF570323	
G. cf. lacustris	342Gladak	Ladakh	G. Mazepa		India J&K	MK066436	
G. sp3	374Gkadam	Abshir Ata, Abshirsai, Khodzhaaryk	B. Sket,	spring	Kyrgyzstan SW	MK066437	MK066396
G. sp3	375Gabhshirs	Abshir Ata-waterfall, Abshirsai, Khodzhaaryk	B. Sket,	spring-waterfall	Kyrgyzstan SW	MK066438	MK066397
G. sp4	TAD4	oasis Chilychor, Shaartuz distr.	Hou et al 2011	spring	Tajikistan SW	JF965970	JF965800
G. sp4	453Chilych	oasis Chilychor, Shaartuz d., Khatlonskaya oblast	G. Mazepa		Tajikistan SW	MK066439	MK066398
G. cf. italicus	075GitalIT	Lazio, Rieti, Terme di Cotilia	C. Fišer	spring	Italia (Europe)	JF965904	JF965716
G. cf. pseudosyrtaicus	286Grafqa	El Mnaitra, Qartaba	B. Sket,	spring	Libanon	MK066440	MK066415
G. cf. italicus	240GitalLB	spring Labour	L. Simon	spring	Libanon	JF965907	JF965719
G. cf. pseudosyrtaicus	499Gquarta	El Aaqoura, Qartaba	B. Sket,	spring	Libanon	MK066441	MK066399
G. cf. lobifer	345Globifer	Yasooj, Chaharmah	V. Akmalii (det. Akmalii & BS)		Iran	MK066442	MK066416

G. elvirae Iannilli & Ruffo 2002 (Type)	442Gelvira	Fontana Tiburto, Ciciliano, Lazio	I&d F.Stoch	spring	Italy (Europe)	SLOCHN442	MK066443	MK066400
G. cf. aculeolatus	168GGoigou	Timahoitte, Goigou	R.Verovnik	stream	Morocco (Africa)		JF965831	JF965636
G. sp1	198 GTalas	river Talas, Dzhambul oblast', Taraz	G.Mazepa	stream	Kazakhstan		JF965963	JF965791
G. sp2 (short antennae)	363Gtokm	Tokmok, Biškek	B.Sket	spring	Kyrgyzstan	SLOCHN363	MK066448	MK066402
G. sp2	376Gtyup	4km NW Tyup, Karakol	B.Sket	spring	Kyrgyzstan	SLOCHN376	MK066449	MK066403
G. sp2	358Gkara	5km NW Karakol	B.Sket	spring	Kyrgyzstan	SLOCHN358	MK066450	MK066404
G. sp2	368Gissy	lake Ysyk Köl, -10m, Cholpon Ata	B.Sket	lake	Kyrgyzstan	SLOCHN368	MK066451	MK066405
G. sp2	365Gissy	lake Ysyk Köl, -70m, Cholpon Ata	B.Sket	lake, -70m	Kyrgyzstan	SLOCHN365	MK066452	MK066406
G. sp2	364Gissy	lake Ysyk Köl, -70m, Cholpon Ata	B.Sket	lake, -70m	Kyrgyzstan	SLOCHN364	MK066453	MK066407
G. sp2	362Gka	10km NE Karakol	B.Sket	spring	Kyrgyzstan	SLOCHN362	MK066454	MK066401
G. decorosus Meng, Hou & Li 2003	693GGdeoro	Urumqi, Xinjiang	CHN		China Xinjiang		JF965875	JF965684
G. troglomorpus Sidorov, Hou & Sket 2018 (Type)	513Gtroglm	Suw Oyuk, Karlyuk	B.Sket	groundwater	Turkmenistan	SLOCHN513	MG655628	MK066408
G. troglomorpus	N2TUM	Suw Oyuk, Karlyuk	B.Sket	groundwater	Turkmenistan	SLOCHN513a	MK066444	
G. parvioculatus Sidorov, Hou & Sket 2018 (Type)	515Gparvoc	Hojeipil, Koytendag	B.Sket	spring	Turkmenistan	SLOCHN515	MG655629	MK066409
G. varsoviensis Jazdzewski, 1975 (Type)	174-varsov	Secymin village	M. Grabowski		Poland (Europe)		JF965987	JF965818
G. cf. varsoviensis	420Gmykol	Povd. Bug, Pervomaysk d., Mykolayiv Reg.	S.Sidorovsky	stream	Ukraine (Europe)	SLOCHN420	MK066445	MK066418
G. sp.	TAD2	Zeravshan, Sogdiyskaja oblast	L.G. Mazepa	spring	Tajikistan		JF965969	JF965799
G. cf. komareki	454Gzeravsh	kishlak Veshkand, Sugd	L.G.Mazepa		Tajikistan	SLOCHN454	MK066455	MK066410
G. tianshan Zhao, Meng & Hou 2017 (?Type)	432 G. tianshan	Zhaosu, Xinjiang CHN 432	CHN		China		EF570327	EF582971
G. tianshan	371 Gkara	371 Karakol	CHN		Kyrgyzstan	SLOCHN371	MK066456	MK066412
G. sp.	360 bish	Ala Archa, Biškek	B.Sket	spring	Kyrgyzstan	SLOCHN360	MK066446	MK066411
G. sp.	257Gteleck	lake Teleckoe ozero	M.Schletterer	lake	Russia		JF965964	JF965792
G. cf. lobifer	235Globif	Khuzestan Prov., Choram	A.Sari & B.Sket	spring	Iran		JF965920	JF965735
G. sp.	343Gkashm	Kashmir ?	G.Mazepa		India J&K	SLOCHN343	MK066457	MK066413
G. cf. inberbus (?)	236Gimberb	Kohgiluyeh prov., S Yasuj	A.Sari & B.Sket	spring	Iran		JF965902	JF965714
G. cf. zagrosensis	351Gizagros	Sepidan, Fars	V.Akmali (det. Akmali & BS)		Iran	SLOCHN351	MK066458	MK066417
G. italicus Goedmakers & Pinkster 1977 (Type)	161Gitalic	Sassari (Sardegna),	F.Stoch		Italia (Europe)		JF965905	JF965717
G. italicus	444Gitalic	Badde Selo, Sardegna	F.Stoch		Italia (Europe)	SLOCHN444	MK066447	MK066414
G. cf. crinicaudatus Stock,	234Gernic	Bakhtiari Prov., Lordegan	A.Sari & B.Sket	spring	Iran		JF965874	JF965683
G. cf. pseudosyniacus	233Gpsdsy	Bakhtiari Prov., Lordegan	A.Sari & B.Sket	spring	Iran		JF965937	JF965759
<b>OUTGROUP / ZUNANJIKI</b>								
G. cf. uludagi	116Gulud	island Evia	G.Urbanič		Greece (Europe)		JF965986	
G. pulex Linne 1758	022GpulUpp	r. Håga, Kvambo, Uppsala	D.Andersson		Sweden (Europe)		JF965939	
G. bosniacus Schäferma 1922 (Type)	137Gbosni	Vrela Bosne, Ilidža, Sarajevo	B.Sket	spring-lake	Bosnia & Herzegovina (Europe)		JF965872	
G. cf. balcanicus	018GbalZen	Zenica	T.Rada	spring	Bosnia & Herzegovina (Europe)		JF965837	
G. pavlovici S.Karaman 1931 (Type)	084GpavMA	Rašće, Skopje	B.Sket	spring-waterwork	Macedonia (Europe)		JF965930	
Gammars ?turcomanicus	563Giasstiensis						EF570347	EF582993
	512Gturcom		B.Sket	springs	Turkmenistan	SLOCHN512	MK066459	MK066419
	269Gkomar						JF965913	JF965725
	104Gfoss						JF965886	JF965696



**Supplement S2:** List of *Gammarus* spp., described from Central Asia, but not studied molecularly. Nomenclature and taxonomic subdivision are taken from original papers; the recent geographic, administrative, and orthographic changes have been considered as much as possible. For practical reasons, the original naming of taxa has been retained.

**Dodatek S2:** Popis vrst rodu *Gammarus*, opisanih iz Centralne Azije, vendar ne molekulsko analiziranih. Nomenklatura in taksonomska delitev so vzeti iz izvornih del; sodobne geografske, administrativne in pravopisne spremembe smo upoštevali, kolikor je bilo mogoče. Iz praktičnih razlogov smo ohranili izvorno poimenovanje.

### Aggregate *lacustris*

The majority of following taxa or populations are morphologically within the diversity of the aggregate *lacustris*.

- (1) *Rivulogammarus turanus* Martynov, 1935 with subordinate taxa:  
*R. t. turanus* Martynov, 1935, Uzbekistan, Tashkent province, Chimgan, river Chimganka, near the sanatorium;
- R. t. morpha subnivalis* Martynov, 1935, Uzbekistan, Tashkent province, source of the river Chimganka;
- R. t. natio kulikenensis* Martynov, 1935, Uzbekistan, 9 km from Tashkent, spring at town Kuylyuk;
- R. t. subsp. coxalis* Martynov, 1935, Kazakhstan SE, range Alexandrovskij, Taraz (= Aulie-Ata), gorge Kulan, near Dmitrievka;
- R. t. forma karabasicus* Martynov, 1935, Kazakhstan S, E Karatau mountains, torrent Karabas-tau;
- R. t. karabasicus* morpha *excisus* Martynov, 1935, Kazakhstan S, E Karatau, at the mount Bahai-bas-tau near Galkino.

- (2) *Rivulogammarus subaequalis* Martynov, 1935, with subordinate taxa (and/or synonyma):  
*R. s. subaequalis* Martynov, 1935, Kazakhstan S, eastern Karatau, limnocene at Galkino (= Zertas);  
*R. s. forma zarudnyi* Martynov, 1935, Kazakhstan S, in Bulaki, Karatau, (position insecure);  
*R. s. forma bianchii* Martynov, 1935, Kazakhstan S, Taraz (= Aulie-ata), close to Talas;  
*R. s. bianchii* morpha *compressus* Kazakhstan S, Jambyl Province, spring at Taraz (= Aulie-ata) and surroundings;

*Gammarus (Rivulogammarus) syriacus subaequalis* (Martynov 1935), Tajikistan, springs at fortress Hisor (= Gissar, Hissar), r. Hanakinka, kishlak near st. Hanaka, kišlak Hodžambio; 'very common, widely spread in Tajikistan' (Birštein, 1948)

- (3) *Gammarus korbuensis* Martynov 1930.  
Distribution: Russia, Altaj, river Korbu, affluent of Teleckoe ozero (Altyn Köl), Altaj.  
With *Gammarus korbuensis* forma *reducta* Martynov 1930, Russia, Teleckoe ozero (Altyn Köl), Altaj.

- (4) *Gammarus teletzkensis* Martynov 1930 (syn. *Gammarus pulex* G. O. Sars 1903)  
Distribution: Russia, Altaj, Teleckoe ozero (Altyn Köl),

- (5) *Gammarus ocellatus* Martynov, 1930 with subordinate taxa  
*G. o. ocellatus* Martynov, 1930  
Distribution: Kyrgyzstan, Ysyk Köl (Issyk-Kul Lake; Martynov, 1930a); Tajikistan, Iskander-kul Lake, mouth of the Serima river (Iskander-kul Lake basin) and small Verin-kul lake (unknown locality) (Martynov, Behning, 1948).

*G. o.* subsp. *angulatus* Martynov 1930, Teleckoe ozero, Altaj, Russia.

- (6) *Gammarus inberbus* Karaman & Pinkster, 1977

Distribution: Kyrgyzstan, Koysara (Koysara; and some undefined places in) Ysyk Köl (Issyk-Kul Lake; Karaman & Pinkster, 1977).

All our gammarids from SE part of the lake are provided with slight dorsal elevations on urosomites, while in *G. inberbus* pleonites and urosomites are explicitly 'smooth, without dorsal elevations or excavations' (Karaman & Pinkster, 1977).

- (7) *Rivulogammarus truncatus* Martynov, 1935

with subordinate taxa:

*R. t. truncatus* Martynov, 1935, Uzbekistan S (eastern Buchara, localization not reliable), village Sangirdak, r. Surkhan (Surkhanderya?) (? S) (Synonym *Gammarus sangirdaki* G. Karaman, 1984).

*R. t.* (Mart.) subsp. *montanus* Martynov 1935 (homonym, nec *G. pavlovici montanus* S. Karaman 1929, nec *Gammarus montanus* Hou, Li & Platvoet, 2004; synonym *Gammarus sangirdaki hissari* G. Karaman, 1984.), Tajikistan, locality Han-tahta, northern Hissar range.

- (8) *Gammarus gracilis* Martynov, 1935, Kazakhstan, eastern Karatau, near Shymkent (= Tshimkent).  
Syn. *Gammarus chimkentii* G. Karaman, 1984.

According to the author, this is a separate species, but morphologically and geographically very close to *G. turanus*.

- (9) *Gammarus angusticoxalis* Martynov, 1935

Kyrgyzstan, near Bishkek (= Pishpek), rivers Dzhungan and Ala-archa (Chu river basin?) (Martynov, 1935). Uropod III endopodite less than 50% exopodite length.

- (10) *Gammarus montaniformis* Sidorov, 2012

Kazakhstan, eastern Tien-Shan, Dzungarian Alatau, ca 2200 m a.s.l. (Sidorov, 2012).

Characterised by comparatively short uropod III endopodite.

- (11) *Rivulogammarus brevicornis* Martynov, 1935,

Kazakhstan S, Karatau, springs at rivulets Kichkin, Ishfan-Mazar.

Martynov: 'A separate species, but close to *R. turanus*'.

- (12) *Gammarus altius* Sidorov, 2012

Distribution: Kyrgyzstan, lake Chatyr-kul, 3555 m a.s.l.

Its unique character is a pair of hooks on the epistome of the upper lip.

### Aberrant taxa of uncertain position

- (13) *Rivulogammarus hirsutus* Martynov, 1935 (syn.: *Rivulogammarus brachyurus* Birštein, 1935, *Gammarus biršteini* Karaman et Pinkster, 1977) with subordinate taxa:

*R. h. hirsutus* Martynov, 1935, S Kazakhstan, NE Karatau; Syr Darya Mountains, Talas-Alatau (Birštein 1935);



*R. h.* var. *oligochaetus* Birštejn, 1935, S Kazakhstan, Syr-Darya Mountains; Karatau Mountains; near Buchujtau;

*R. h.* morpho *hirsutissimus* Martynov 1935, Kazakhstan, Kel'ty-Mashat, eastern Karatau.

(14) *Issykogammarus hamatus* Chevreux 1908

Distribution: Kyrgyzstan NE, only in the lake Ysyk Köl, from different depths.

It was described as a separate genus. But if we only overlook the recast of the pereopod coxae, the species fits easily into the diversity of the aggregate *lacustris*. Unfortunately, a molecular analysis was impossible and the old-fashioned presentation of the morphology, even done mainly on the female, does not allow a serious reevaluation of the species' taxonomy and phylogeny.

#### **Aggregate *balcanicus***

Members of the agg. *balcanicus* are comparatively easily recognizable morphologically. The reliability of such an identification is however low, but in our case it does not contradict the few molecularly supported data. Characteristic is the poor setation of pereopods III-IV and of uropod III. In the latter, the spines along the exopodite outer margin are joined by only few and short finer setae.

(15) *Rivulogammarus spinulatus* Martynov, 1935

Distribution: Kazakhstan, mountain river (torrent) Issyk in Semireche (= Zhetisu) district (E of Almaty).

(16) *Gammarus pellucidus* Gurjanova, 1930.

Distribution: Russia, Krasnojarsk, river Enisej, Ladejskij perekat.

(17) *Gammarus angustatus* Martynov 1930 with subordinate taxa

*G. a. angustatus* Martynov 1930, Russia, upper Ob, torrent Injuška, affluent of Ini; Kyrgyzstan NE, river Alamedinka near Biškek.

*G. a.* forma *obensis* Martynov 1930, Russia, Novosibirsk, river Ob.

(17a) *Gammarus brevipodus* Hou, Li & Platvoet, 2004 from China, Xinjiang, Xinyuan, W of Urumqi.

(17b) *Gammarus 'balcanicus'* (*Gammarus (Rivulogammarus) balcanicus* Birštejn, 1950) from Russia, Oblast' Kemerovo, Jurginskij rajon)

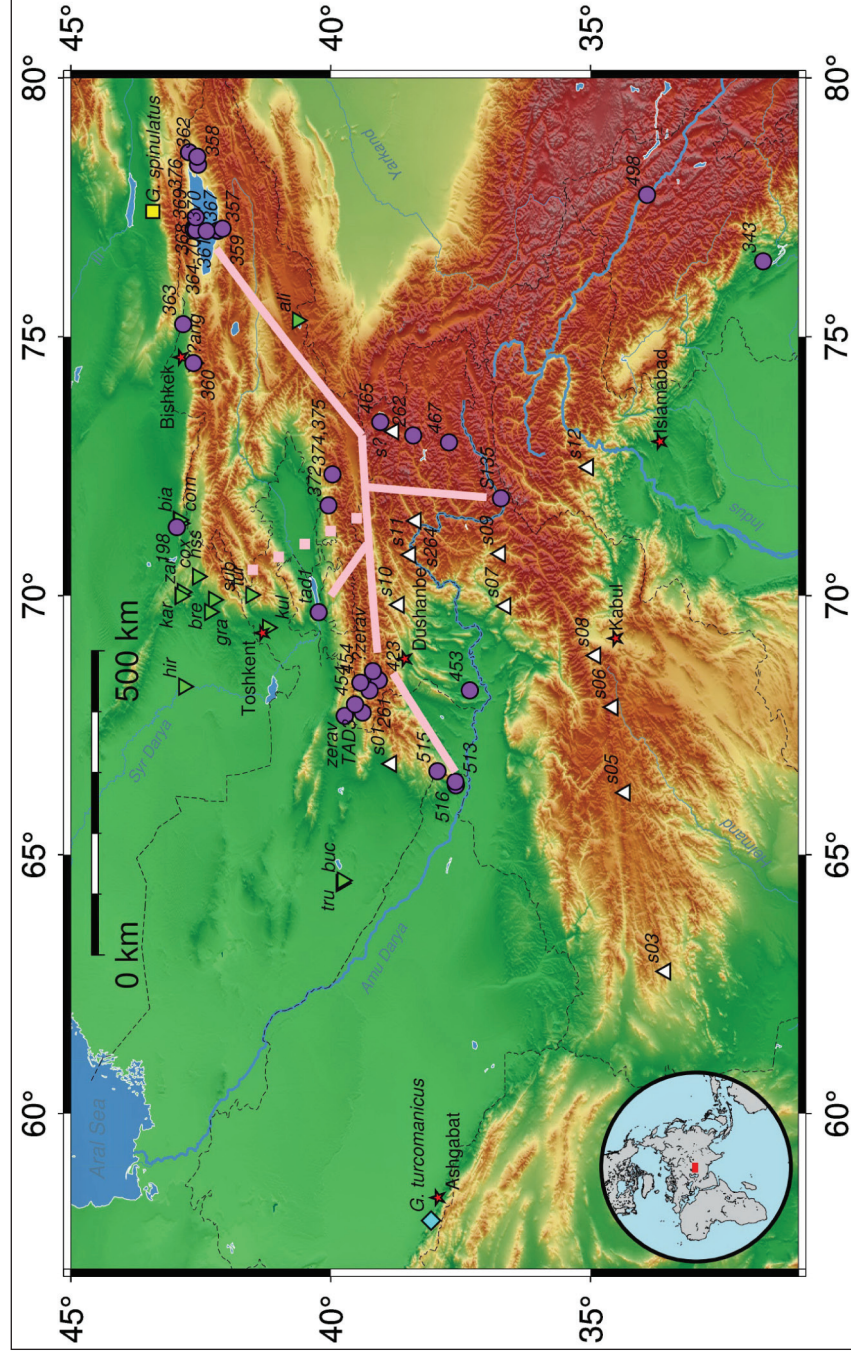
#### **Aggregate *pulex***

(18) The only seemingly reliable indications on *G. pulex* are by Birštejn 1945 from the rivers Sherlok and Firjuzinka near Aşgabat (Ashgabat) (central Kopetdag, neighbour to *G. turcomanicus*).

(19) *Rivulogammarus pulex* forma *mesasiaticus* Martynov 1936 from Kyrgyzstan NE. Two 'sub-forms', one in springs and in river, another in stagnant waters (ponds) near Tokmok and Biškek (= Frunze) do not exhibit any characters specific to *G. pulex*. Our samples from surroundings of Tokmok and Biškek contain only members of the agg. *lacustris*.

**Supplement S3:** Distribution map of *Gammarus* spp. in Central Asia. Red asterisks with names - important cities. Reversed triangles - molecularly not defined *Gammarus* spp., letters indicate abbreviated taxonomic names (see Suppl. S2). Rings - molecularly defined taxa of agg. *lacustris*, numerals are OTU designations (as in the second column in Suppl. S1); pale ribbons connect localities of *G. cf. subaequalis*-Gartlyk, hatched ribbon connection with Martynov's *G. subaequalis* and *G. turanus*. Squares - agg. *balcanicus*; diamond (*G. turcomanicus*); agg. *komareki*; upright triangles - *Sarothrogammarus* group. Corresponding to Figure 1 in the paper.

**Dodatek S3:** Karta razširjenosti *Gammarus* spp. v Centralni Aziji. Rdeče zvezdice z imeni - pomembni kraji. Obrnjeni trikotniki - molekularsko nedefinirane *Gammarus* spp., črke so okrajšana taksonomska imena (glej Dodatek S2). Krožci - molekularsko definirana taksa agregata *lacustris*, številke so oznake OTUjev (kot v drugem stolpcu v Dodatku S1); svetle proge povezujejo lokalitete tipa *G. cf. subaequalis*-Gartlyk, prekinjena proga povezavo z Martynov's *G. subaequalis* in *G. turanus* Martynova. Kvadratniki - agregat *balcanicus*; romb (*G. turcomanicus*) - agregat *komareki*; pokončni trikotniki - skupina *Sarothrogammarus*. Se ujema s Sliko 1 v članku.



**Supplement S4:** Additional taxonomical clarification

**Dodatek S4:** Dodatna taksonomska pojašnila.

**(1) Type population of *Gammarus lacustris* Sars, 1863.**

*Gammarus lacustris* the name-bearing species of the aggregate lacustris and topotype population of it, was described by Sars without defining the type locality. Karaman & Pinkster (1977) selected 'Norway, Selsvand, Våge' as 'restricted type-locality'. Since we could not detect such a locality, W. Väder (pers. com.) helped us with identification of Selsvand as Selsvatn(et) in the community of Vågå, province of Oppland in mid Norway. This is the correct spelling of the selected type locality. Our sample 473 is from the lake Birisjøen, Vågå Municipality, ca. 200 km NNW Oslo and only ca 35. km SW from Selsvatnet, hydrographically in the same system; we consider the sample 473 as belonging to the topotype (meta)population of *Gammarus lacustris* Sars, 1863.

**(2) Identity of 'G.balcanicus' from Alma-Ata.**

The origin of *G. balcanicus* of MacDonald et al., 2005, reportedly from Alma-Ata, after a joint reconsideration with some authors, could not be localized with certainty. Molecularly, this OTU is related to populations from Krym (Ukraine). On the other hand, it is very likely that *R. spinulatus* from the same area (E of Almaty) really belongs to the agg. balcanicus, as much as morphological characters are reliable.

**(3) Corrected classification of '*Anisogammarus madyensis* Chaudri, Ghauri & Mahoon, 1987**

The morphology of '*Anisogammarus madyensis*' clearly positions the species into the *Sarothrogammarus* group of genera, more precisely the genus *Sarothrogammarus*. We will provisionally classify it as *Sarothrogammarus madyensis* (Chaudri, Ghauri & Mahoon, 1987). Its provenience in the Swat Valley is not in contrast with the group biogeography, it only slightly extends its area and adds Pakistan. On the other hand, *Anisogammaridae* are alien to the area of W Asia.