Uralocrangonyx gen.n. (Amphipoda: Crangonyctidae) from the Southern Ural, Russia

Uralocrangonyx gen.n. (Amphipoda: Crangonyctidae) с Южного Урала, Россия

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ABSTRACT. Integrative analysis revealed that the stygobiotic Crangonyx chlebnikovi Borutzky, 1928 (Amphipoda: Crangonyctidae) from the Southern Ural, Russia, should be placed into a separate new genus, Uralocrangonyx gen.n. Together with the European Crangonyx subterraneus Bate, 1859 and Crangonyx paxi Schellenberg, 1935 as well as North American Bactrurus spp., the new genus forms the “Bactrurus” Clade according to the following morphological features: free urosomites; the dense armature of palm of gnathopods I–II; the absence of sternal gill/processes; the presence of 8–9 hooks in clade-related genera in the structure and armature of sinus. At the same time, the new genus differs from the anterolateral lobe bearing moderate inferior antennal on pereonal segments II–III; and head with a concave gnathopods I–II; the absence of sternal gill/processes

KEY WORDS: Diversity, Crustacea, Amphipoda, Crangonyctidae, new genus, subterranean, Southern Ural, Russia.

Интегративный анализ показал, что южно-уральский стигбононт Crangonyx chlebnikovi Borutzky, 1928 (Amphipoda: Crangonyctidae) должен быть выделен в новый род, Uralocrangonyx gen.n. Совместно с европейскими Crangonyx subterraneus Bate, 1859 и Crangonyx paxi Schellenberg, 1935, а также североамериканскими Bactrurus spp., новый род формирует единую кладу “Bactrurus” на основании следующих морфологических признаков: свободные уросомиты; мощное вооружение ладони хелипед I–II; отсутствие грудных жабр/отростков на переональных сегментах II–III; и вогнутая переднебоковая доля головы с хорошо выраженным антенальным синусом. В то же время, от родственных родов клады новых видов хорошо отличается строением (вооружением) обоих хелипед и наличием 8–9 крючков в ретинакулах на уроподах. Выделение нового рода меняет всю таксономическую систему бывшего рода Crangonyx sensu lato, и мы предлагаем восстановить род Eucrangonyx Stebbing, 1899 для североамериканских видов Crangonyx sensu lato (с типовым видом Eucrangonyx gracilis (S.I. Smith, 1871)). Crangonyx islandicus Svavarsson et Kristjannson, 2006, эндемичный для Исландии, следует также выделить в отдельный род, поскольку он филогенетически не связан ни с европейским родом Crangonyx Spence Bate, 1859 s.str., ни с одним из видов неарктических Eucrangonyx Stebbing, 1899, а относится к кладе “Stygobromus” семейства Crangonyctidae.

Introduction

The Holarctic family Crangonyctidae (Crustacea: Amphipoda) is represented by a very ancient group of crustaceans that appeared at the end of the Cretaceous in Gondwana [Copilaş-Ciocianu et al., 2019]. Modern representatives of the main clades are scattered across the divergent continents [Kornobis et al., 2011; Copilaş-Ciocianu et al., 2019; Palatov, Marin, 2020; Marin, ...
Palatov, 2021a). There are still many unresolved phylogenetic issues concerning the family, which require special attention. Representatives of the genus *Crangonyx* Spence Bate, 1859 are presently known from in North America [Zhang, Holsinger, 2003], Iceland [Svarsvasson, Kristjansson, 2006], Western Europe [Holsinger, Skalski, 1980; Holsinger, 1986; Sidorov et al., 2018], in the Southern Ural [Sidorov et al., 2012], and even Northern Africa (Morocco) [Messouli, 2006]. Central Asian species referring to the genus *Crangonyx* [Holsinger, 1986] were recently moved to the family Gammaridae, mainly to the genus *Tadzocrangonyx* Karaman et Bernard, 1979 [Sidorov et al., 2018]. The type species of the genus *Crangonyx* Spence Bate, 1859, *C. subterraneus* Spence Bate, 1859, was described from a well at Ringwood, Hampshire, South East England [Bate, 1859], while currently it is known from many locations in the south of England [Holsinger, 1986; Johns et al., 2015; Durkota et al., 2019] and northwestern Europe (Northern France, Netherlands, Germany, Switzerland and Luxembourg) [Stock, 1961, 1962; Hoffmann, 1962; Holsinger, Skalski, 1980; Holsinger, 1986; Citoleux, 2007; Alther et al., 2017; Sidorov et al., 2018]. The occurrence of the species in Czech Republic and Poland [Berezina, Iuris, 2008; Sidorov et al., 2018] are suspicious as no descriptions or genetic data have been published. *Eu crankonyx vej dovskyi* Stebbing, 1899 and *Niphargus moniei* Wrzesniowski, 1890 are junior synonyms of this species [Horton et al., 2021].

Nearctic species of the genus *Crangonyx* are represented by about 50 species, distributed in North America [Zhang, Holsinger 2003; Horton et al., 2021]. Stebbing [1899] proposed to allocate a separate genus *Eu crankonyx* Stebbing, 1899 for *Crangonyx gracilis* S.I. Smith, 1871, known from Ontario, Canada. To date, this generic name is reduced to a junior synonym to the genus *Crangonyx*. *Crangonyx islandicus* Svarsvasson et Kristjansson, 2006 is endemic to Iceland [Svarsvasson, Kristjansson 2006; Kornobis et al., 2011, 2012], while *Crangonyx africans* Messouli, 2006 is only known from Morocco, Northern Africa [Messouli, 2006]. There is some doubt that the latter species belongs to the family *Crangonyctidae*. *Crangonyx paxi* Schellenberg, 1935 is known only from a small pond of the Solini mine in the Klesnica Valley in Sudetenland (western Poland) [Schellenberg, 1935, 1942]. Its taxonomic position was debated, but finally Holsinger & Skalski [1980] came to conclusion that *C. paxi* is a separate species, a deviant representative of the genus *Crangonyx*. Finally, *Crangonyx chlebnikovi* Borutzky, 1928 was described from the Great Mechka Cave [Borutzky, 1928], while its subspecies, *Crangonyx chlebnikovi maximovitshi* Pankov et Pankova, 2004 was found in the neighboring Kungur (Ice) Cave, Kungur District, the Southern Ural, Russia [Pankov, Pankova, 2004]. Sidorov et al. [2010] indicated that this species is related to North-American genus *Bactrurus* Hay, 1902.

In this article, we represent a complete morphological description and molecular genetic data of the representatives of the genus *Crangonyx* s.l. from the Southern Ural, as well as with a discussion on the known distribution and taxonomic position within the family.

**Material and methods**

Amphipods were collected with a hand net in various caves (subterranean) water resources in the Kungur District, Perm Krai, the Southern Ural, Russia (see Fig. 1). Totally, amphipods were found in three different but neighboring caves, namely the Great Mechka Cave (57°36′36.0″N 56°37′13.0″E) (the type locality of *Crangonyx chlebnikovi* Borutzky, 1928), the Kungur Ice Cave (57°26′28.1″N 57°00′29.9″E) (the type locality of *C. chlebnikovi maximovitshi* Pankov et Pankova, 2004) and the Babinogorskaya Cave (57°27′02.4″N 56°51′32.9″E). After sampling, the specimens were fixed in 90% solution of ethanol.

Photographs were made with a digital camera attached to a Olympus ZX10 and Olympus CX21. The scanning electron microscopic (SEM) images were made using the Vega3 Tescan in the Yu.A. Orlov Paleontological Museum of the Paleontological Institute of the Russian Academy of Sciences, Moscow.

Cytochrome c oxidase subunit I (COI) mtDNA has been proving as extremely informative gene marker in previous studies at both species [Avise, 1993] and higher taxonomic levels [Palatov, Marin, 2020, 2021; Marin, Palatov, 2021a, b]. The COI mtDNA gene marker was amplified with the using of the universal primers LCO1490 (5′–GGTCAATTCAACAAATATATATGC–3′) and HC02198 (5′–TAAACTTCAGGGTGACCAAAAAATCA–3′) [Folmer et al., 1994]. Polymerase chain reaction (PCR) were performed under the standard conditions. Dataset of aligned sequences of COI mtDNA gene markers, about 617 base pairs in length used in the study were taken from the GenBank (NCBI) and author’s database.

A consensus of complementary sequences was obtained with MEGA 7.0. The best evolutionary substitution model was determined using MEGA 7.0 and jModeltest2.1.41 as GTR+G+I. A phylogenetic analysis was conducted using PhyML 3.0 (http://www.atgc–montpellier.fr/phyml/) [Guindon et al., 2010] using several models based on BIC (Bayesian Information Criterion) and AIC (Akaike Information Criterion). The phylogenetic trees with a higher bootstrap probability were used for graphically display relationships within the family. Bootstrap support is presented for ML analysis. Pairwise genetic divergences (p-distances) was calculated using MEGA 7.0 with the Kimura 2-Parameter (K2P) model of evolution [Kimura, 1980].

The body length (bl., mm), the dorsal length from the distal margin of head to the posterior margin of telson, without uropod III and both antennas, is used as a standard measurement. The type material is deposited in the collection of Zoological Museum of Moscow State University, Moscow, Russia (ZMMU).

**Results**

**PHYLOGENETIC PART.** According to the obtained phylogenetic reconstruction (tree) (Fig. 2), *Crangonyx sensu lato* (s.l.) is a polyphyletic group.
Fig. 1. The map of distribution and general lateral view of *Uralocrangonyx chlebnikovi* (Borutzky, 1928) comb.n. (fixed in ethanol). The red asterisk and blue triangle on the map also show the known (unpublished) records of blind underground crustaceans in the Southern Urals, which presumably belong to the genus *Uralocrangonyx* gen.n.

Рис. 1. Карта распространения и общий вид *Uralocrangonyx chlebnikovi* (Borutzky, 1928) comb.n. (фиксированные в спирте). Красная звездочка и синий треугольник на карте показывают также известные (не опубликовано) находки слепых подземных ракообразных на южном Урале, которые предположительно относятся к роду *Uralocrangonyx* gen.n.
Fig. 2. The phylogenetic reconstruction (tree) of molecular phylogenetic (COI mtDNA gene marker) scenario (GTR+G+I model (AIC)) of the family Crangonyctidae (additional sequences from GenBank (NCBI). Outgroup include species of the genus Pseudocrangonyx (Pseudocrangonyctidae) and Crymostygus thingvallensis (Crymostygidae).

Photo of C. subterraneus by Jules Carter (from https://hcrs.brc.ac.uk/species/crangonyx-subterraneus); E. pseudogracilis by Malcolm Storey (from https://www.discoverlife.org/mp/20p?see=I_MWS48333&res=640).
The lineage of *C. subterraneus* [Spence Bate, 1859], and possibly *C. paxi*, which is apparently related to *C. subterraneus* [Holsinger, Skalski, 1980], represent the genus *Cragononx* sensu stricto (s.). The Nearctic *Bactrurus* spp., and two Palearctic species, the western European *Crangonyx subterraneus* Spence Bate, 1859 (*Crangonyx* s.str.) and *Crangonyx chlebnikovi* Borutzky, 1928 from the Southern Ural, form a well-supported separate clade — the “*Bactrurus*” Clade (see Fig. 2). At the same time, *C. chlebnikovi* represents a well-supported monophyletic lineage separated from *C. subterraneus* (see below), and its designation into a separate genus is well justified.

The North American *Cragononx* s. l. is phylogenetically separated from the “*Bactrurus*” Clade (see Fig. 2), and we propose to resurrect for it the generic name *Eucragononx* Stebbing, 1899, with the type species *Cragononx gracilis* S.I. Smith, 1871 [Stebbing, 1899]. The Nearctic *Crangonyx* spp., *Sicfera* spp. and the Palearctic *Amurocrangonyx arsenjevi* (Derzhavin, 1927) form a well-supported separate clade — the “*Eucragononx*” Clade.

*Crangonyx islandicus* Svavarsson et Kristjánsson, 2006, endemic to Iceland, is phylogenetically unrelated neither to the European *Crangonyx* s.str. nor to the North American *Eucragononx*, but belongs to a phylogenetic distinct “*Stygobromus*” Clade (see Fig. 2). Moreover, this species represent a distinct lineage, which should be separated into a new genus.

**TAXONOMIC PART**

**Order Amphipoda Latreille, 1816**

**Infraorder Gammarida Latreille, 1802**

**Family Crangonyctidae Bousfield, 1973**

*Uralocragononx gen.n.*

**DIAGNOSIS.** Relatively large-sized species amphipod, with males smaller than females, without secondary sexual dimorphism. *Body* unpigmented, smooth. *Eyes* completely reduced. *Head* with moderate inferior antenal sinus. *Lateralia* with 16 strong pectinate setae. *Antenna I* with slender aesthetascs and 2-segmented accessory flagellum. *Pereonal segments II–III* without sternal gill/processes. *Gnathopod I* smaller than GnII, both with teardrop-shaped propodus (palm); ventral palmar margin with deep ventroproximal cavity and armed with two deep rows of notched robust setae (teeth) along the entire length. *Pleon* with free urosomites. *Pleopods* with 8 hooks in retinacules in both sexes. *Uropod III* with outer ramus about 3 times as long as wide, rather wide, with several clusters of marginal and a tuft of distal setae. *Telson* entire, rectangular, wider than long, with marginal clusters of spines.

**DIFFERENTIAL DIAGNOSIS.** The new genus clearly separates from all Palearctic crangonyctid genera by 1) head with a well-marked anterolateral sinus (vs. distally rounded); 2) lateralia with 16 strong pectinate setae (vs. 10 setae in *Pontonyx* Palatov et Marin, 2021, 10–13 — in *Amurocrangonyx* Sidorov et Holsinger, 2007 and 8 — in all genera from the “*Syncrella*” Clade (see Fig. 2)); 3) teardrop-shaped palm of gnathopods I–II with deep ventroproximal cavity and ventral palmar margin armed with 2 deep rows of notched robust setae (teeth) along the entire length; 4) the absence of sternal gill/processes on pereonal segments II–III; 5) 8–9 hooks in the retinacules of pleopods (vs. less than 4 hooks in other Palearctic crangonyctid genera with the exception of *Volgonyx* Marin et Palatov, 2021 bearing 8–9 hooks in the retinacules); 6) relatively slender basis of ambulatory pereiopods, especially PVII; and 7) large outer rami of uropod III, which is about 3 times as long as wide.

**ETYMOLOGY.** The genus is named after the region where it is distributed, the Southern Ural; the meaning is “Crangonyx from Ural”.

*Uralocragononx chlebnikovi* (Borutzky, 1928) **comb.n.**

Figs 3–8.

*Cragononx chlebnikovi* Borutzky, 1928

*Cragononx chlebnikovi* maximovitshi Pankov et Pankova, 2004

**MATERIAL EXAMINED.** Russian Federation, Southern Ural, Perm Krai, Kungur District: 1♀ (bl. 16.5 mm) (ZMMU Mb-1220), 7♂♂ (bl. 16–19 mm) (LEMMI), Kungur Ice Cave, 57°26′N 56°37′E, in subterranean lake, 12.07.2020; 3♂♂ (bl. 16.0–16.5 mm), Great Mechina, 57°36′36.0″N 56°37′13.0″E, in subterranean lake, coll. O.I. Kadebskaya, 20.09.2017; 4♀♀ (bl. 16–19 mm), Kungur Ice Cave, in subterranean lake, coll. O.I. Kadeb-
Fig. 3. *Uralocrangonyx chlebnikovi* (Borutzky, 1928) comb.n., ♀: a — antenna I; b — accessory flagellum of antenna I; c — antenna II; d — gnathopod I; e — distoventral corner of chela of GnI; f — gnathopod II; g — distoventral corner of chela of GnII.

Рис. 3. *Uralocrangonyx chlebnikovi* (Borutzky, 1928) комб.н., ♂: a — антенна I; b — дополнительный жгутик антенны I; c — антенна II; d — гнатопод I; e — дистовентральный угол клаши GnI; f — гнатопод II; g — дистовентральный угол клаши GnII.
Fig. 4. *Uralocrangonyx chlebnikovi* (Borutzky, 1928) comb.n., $\varphi$: $a$ — labrum (upper lip); $b$ — labium (lower lip); $c$ — lateralia; $d, f$ — mandible; $e, g$ — incisor process and pars incisiva of mandible; $h$ — maxilla I; $i$ — same, distal margin of outer lobe; $j$ — maxilla II; $k$ — maxillipede.

Рис. 4. *Uralocrangonyx chlebnikovi* (Borutzky, 1928) comb.n., $\varphi$: $a$ — верхняя губа; $b$ — нижняя губа; $c$ — латералия; $d, f$ — мандибула; $e, g$ — режущий отросток и pars incisiva (режет) мандибулы; $h$ — максилла I; $i$ — то же, дистальный край наружной доли; $j$ — максилла II; $k$ — максиллипedef.
Fig. 5. Uralocrangonyx chlebnikovi (Borutzky, 1928) comb.n., &a; a — pereopod III; b — dactylus of PIII; c — pereopod IV; d — dactylus of PIV; e — pereopod V; f — dactylus of PV; g — pereopod VI; h — dactylus of PVI; i — pereopod VII; j — dactylus of PVII.

Рис. 5. Uralocrangonyx chlebnikovi (Borutzky, 1928) comb.n., &a; a — переопод III; b — дактилус PIII; c — переопод IV; d — дактилус PIV; e — переопод V; f — дактилус PV; g — переопод VI; h — дактилус PVI; i — переопод VII; j — дактилус VII.
Fig. 6. Uralocrangonyx chlebnikovi (Borutzky, 1928) comb.n., a–c — epimeral plates I–III; d — telson; e — pleopod III; f — retinacula of pleopod III; g — uropod I; h — uropod II; i — uropod III.

Рис. 6. Uralocrangonyx chlebnikovi (Borutzky, 1928) comb.n., a–c — эпимеральные пластинки I–III; d — тельсон; e — плеопод III; f — ретинакула плеоподы III; g — уропода I; h — уропода II; i — уропода III.
Fig. 7. Uralocrangonyx chlebnikovi (Borutzky, 1928) comb.n., a — head; b, c — aesthetases of antenna I; c — antenna II; d — distal segment of mandibular palp; e — retinacula of pleopod I; f — hooks of retinacula; g — urosomal segments.

Рис. 7. Uralocrangonyx chlebnikovi (Borutzky, 1928) comb.n., a — голова; b, c — эсцетаски антенны I; c — антенна II; d — дистальный сегмент щупика мандибулы; e — ретинакула плеоподы I; f — крючки ретинакулы; g — уросомальные сегменты.
Fig. 8. *Uralocrangonyx chlebnikovi* (Borutzky, 1928) comb.n., $\exists$: $a$ — palm (chela) of gnathopod I; $b$ — proximoventral margin of palm of GnI; $c$ — palm (chela) of gnathopod II; $d$ — distoventral margin of palm (chela) and cutting margin of dactylus of GnII; $e, f$ — proximoventral margin of palm (chela) of GnII.

Рис. 8. *Uralocrangonyx chlebnikovi* (Borutzky, 1928) comb.n., $\exists$: $a$ — ладонь (клешня) гнатоподы I; $b$ — проксимовентральный край ладони GnI; $c$ — ладонь (клешня) гнатоподы II; $d$ — дистовентральный край ладони и режущий край дактилюса GnII; $e, f$ — проксимовентральный край ладони GnII.
Three of four main clades of the family (Fig. 2) includes representatives in both Neartic and Palaeartic, while the “Synurella” Clade is represented only by Palaeartic species, and their diversity has not yet been fully studied [Palatov, Marin, 2020, 2021; Marin, Palatov, 2021a, b]. We hope that a more detailed study of all species of the family using integrative approach will allow in the future to provide a more detailed revision of the family and revising the issues of its origin.

Conflicts of Interest
The authors declare no potential conflict of interest.

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