PHYLOGENETIC POSITION OF THE GENUS *CHAETONIPHARGUS* KARAMAN ET SKET (CRUSTACEA: AMPHIPODA: NIPHARGIDAE) FROM DINARIC KARST. AN EXTREME CASE OF HOMOPLASY.

FILOGENETSKI POLOŽAJ RODU *CHAETONIPHARGUS* KARAMAN ET SKET (CRUSTACEA: AMPHIPODA: NIPHARGIDAE) IZ DINARSKEGA KRASA. SKRAJNI PRIMER HOMOPLAZIJE.

Boris SKET[1] & Gordan S. KARAMAN[2]

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ABSTRACT

Phylogenetic position of the genus *Chaetoniphargus* Karaman et Sket (Crustacea: Amphipoda: Niphargidae) from dinaric karst. An extreme case of homoplasy.

An alternative, phylogenetically grounded taxonomy of the recently described *Chaetoniphargus lubuskensis* Karaman & Sket (Crustacea: Amphipoda: Niphargidae) is presented. The recently described new genus and species, *Chaetoniphargus lubuskensis* from Dinaric Karst, has formerly only been taxonomically evaluated by a classical approach, based on morphology. Species of genera *Niphargobatoides*, *Niphargobates, Chaetoniphargus*, differ from *Niphargus* spp. mainly by slightly elevated number of maxilla I spines, by gradual reduction of maxillary and mandibular palps, by a strong reduction of urosomal structures. Molecularly, they appear to be nested within the *Niphargus* tree, but separately. Their collective separation from *Niphargus* would make the genus *Niphargus* paraphyletic, but for the low number of these species practically without diminishing the confusing diversity of it. A molecular phylogenetic analysis would include all here studied niphargid species as belonging to *Niphargus*. The gradual change in morphology of mentioned niphargids produces a smooth transition between Niphargidae and unrelated Metacrangonyctidae. Since other facts speak against the relatedness of both families, this is an additional case of convergence, demonstrating the need of molecular analyses when we speculate on phylogenetic relations within Amphipoda.

Key words: subterranean, taxonomy, phylogeny, Amphipoda, Niphargidae, Metacrangonyctidae, *Chaetoniphargus*.

IZVLEČEK

Filogenetski položaj rodu *Chaetoniphargus* Karaman et Sket (Crustacea: Amphipoda: Niphargidae) iz dinarskega krasa. Skrajni primer homoplazije.

Podajava alternativno, filogenetsko osnovano taksonomijo nedavno opisanega *Chaetoniphargus lubuskensis* Karaman & Sket (Crustacea: Amphipoda: Niphargidae). Pravkar opisan nov rod in vrsta iz Dinarskega krasa je bila taksonomsko ovrednotena samo s klasičnim pristopom in na osnovi morfologije. Vrste rodov *Niphargobatoides*, *Niphargobates, Chaetoniphargus*, se razlikujejo od *Niphargus* spp. predvsem po malce zvišanem številu trnov na maksili I, po postopni redukciji maksilarnih in mandibularnih palpov, po močni redukciji urosomalnih struktur. Po molekulski analizi se izkaže, da so vgnezdeni v rod *Niphargus* in to ločeno. Če jih taksonomsko ločimo iz rodu *Niphargus*, ga naredijo parafiletskega. Ker pa jih je tako malo, s tem nič ne zmanjšamo moteče raznolikosti rodu. Molekulska filogenetska analiza bi vgnezdila vse tukaj omenjene vrste kot pripadnice rodu *Niphargus,* torej rodovna imena kot sinonima imena *Niphargus*. Postopno spreminjanje v morfologiji omenjenih nifargidov nam pokaže gladek prehod med družino Niphargidae in le malo sorodno družino Metacrangonyctidae. To je dodaten primer konvergence, ki kaže nujnost molekulskega preverjanja, ko ugotavljamo filogenetske odnose znotraj skupine Amphipoda

Ključne besede: podzemeljsko, taksonomija, filogenija, Amphipoda, Niphargidae, Metacrangonyctidae, *Chaetoniphargus*.

[1] Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za biologijo, Ljubljana, E-mail: Boris.Sket@bf.uni-lj.si
[2] Montenegrin Academy of Sciences and Arts, Podgorica, E-mail: karaman@t-com.me

1 INTRODUCTION

Recently (Karaman & Sket 2018), the family Niphargidae (Crustacea: Amphipoda), has been enriched for an aberrant species within an independent genus. It was a negligible addition to the species number, but an important enrichment of the genera diversity. The family includes more than 400 species (Horton et al., 2018), but the big majority of them belong to the diverse genus *Niphargus*. The related 12 genera are mainly monospecific and all together contain ca 20 species. Most of them are characterized by peculiarities in mouth parts, the organ very homogeneous in the otherwise diverse *Niphargus*. The unity of the mouth apparatus, contrasting to general and even diversity of other body parts, was what probably misled taxonomists into conviction that mouth parts are a phylogenetically stable and taxonomically particularly informative group of characters. For those few niphargids with deviation from normal (*stygius* type) niphargid type, foundation of independent genera has been a norm since ever. Illustrative is the ancient *Niphargopsis* Chevreux, 1922, founded for a species with multiplied setae and spines in mandibula and maxilla I. *Chaetoniphargus* (Karaman & Sket 2018) is a member of a small group of small species; also related genera *Niphargobates* (Sket 1981), *Niphargobatoides* (Karaman 2016) and conditionally *Niphargopsis* (Schellenberg 1942) are monospecific. Morphologically, these genera, accompanied even with *Metacrangonyx* and *Longipodacrangonyx* (Boutin 1994; Boutin & Messouli 1988), form a morphologically homogeneous group of taxa, characterized by a progressive reduction of head- and urosomal appendages and telson. However, a molecular analysis and biogeography demolish this impression.

The recently described *Chaetoniphargus lubuskensis* differs from other known genera of the family Niphargidae by strongly reduced mandibular palpus consisting of only one small article, while all other known niphargids are provided with well developed 3-articulated mandibular palpus.

Considering this, we created the new genus *Chaetoniphargus*, with well defined morphological character states. It resembles the previously described genera *Niphargobates, Niphargobatoides*, as well as genera *Metacrangonyx* and *Longipodacrangonyx*. In some characters, some other small niphargids resemble this group, but they differ clearly in the 'normal' shape of the telson (*Micronipharg*us, *Niphargellus, Carinurella*; Schellenberg 1934, 1938, 1942; Sket 1971). Molecularly, they are also alien to the group. We wanted to include an alternative, phylogenetically based taxonomy with the genus description, but the editor explicitly asked to abandon such a heading. With this article we are trying to correct the insufficiency.

2 MATERIAL AND METHODS

Morphology of *Chaetoniphargus lubuskensis* was taken from the description by Karaman & Sket (2018). Some specimens were preserved in ethanol 96% and analyzed molecularly in another study (Moškrič, 2016). The Moškrič' OTU '*Niphargobates* sp. NC144' is taxonomically identical with *Chaetoniphargus lubuskensis*.

3 RESULTS

The morphology of the great majority of Niphargidae species, including *N. stygius* (Schiödte) is a continuum of states in many characters, including also very characteristic mouthparts and uropods. However, in majority of them the shape and structure of mouthparts is only slightly affected. Very seldom (in *Haploginglymus*, Mateus & Mateus 1958), the uropod III exopodite is unarticulated, but then with a long proximal article.

The following are the plesiomorphic character states of all larger (longer than 10 mm) and of the great majority of smaller (down to 2 mm) species of *Niphargus*. In only few small species, the mouth parts are apomorphic, modified, as mentioned below. Among them is the newly described niphargid *C. lubuskensis*.

Table 1: Character states in morphology of oral and urosomal body parts in Niphargidae.

	plesiomorphy	apomorphy
mandibula	normally developed, with 3-articulated palpus, with richly setiferous article 3	spines in the row between the mandibular molar and incisor multiplied and differentiated
mandibular palpus	palpus 3-articulated, with elongated articles	palpus shortened, apically reduced, to rudimentary
maxilla I outer plate	bearing very regularly 7 (exceptionally till 15) stout, simple or bifid to pectinate spines	ca 20 spines; spines multiplied and differentiated;
maxilla I palpus	overreaching the top of the outer plate; biarticulate	shortened and uniarticulate
uropod 3	longer than urosomites II+III	shorter than urosomite III
	its endopodite biarticulate	endopodite uniarticulate
telson	as long or longer than wide, deeply (more than 1/2) cleft	with broadly rounded apices; less deeply cleft
	with more than 1 spine on each lobe	spines replaced by setae

The genera *Niphargobates* and *Niphargobatoides* are morphologically well defined, so is also *Chaetoniphargus*. They apparently form a phyletic group with some changes of oral appendages in progress. In process is the multiplying of mandibular and maxillary setae and the reduction of some appendage parts (e.g. palpus), beside this also reduction of uropods III. Telson is losing its niphargid character; its clipping is getting shallower, apices less prominent and less setose.

We based our recognition of different genera based on traditionally used morphological characters, supposedly important differences, which allowed to classify (divide) the family Niphargidae into 13 genera. They have been mainly applied already by Barnard & Barnard (1983).

Within the family Niphargidae, genus *Chaetoniphargus* differs from the genus *Niphargus* by strongly reduced mandibular palpus and only indistinctly triturative molar, by strongly reduced palpus of maxilla I, by uniarticulate accessory flagellum of antenna I, by short and partially reduced uropod III with uniarticulate exopodite.

Within other genera, *Chaetoniphargus* is morphologically most similar (Karaman & Sket 2018) to *Niphargobates* and *Niphargobatoides*. They resemble in the general body-shape, maxilla I, maxilliped, telson, uropod III, but *Niphargobatoides lefkodemonaki* (Sket) is still provided with a well developed 3-articulate mandibular palpus, maxilla II inner plate is larger than the outer one, its antenna I accessory flagellum is 2-articulated, uropod III with 2-articulated exopodite, telson is *Niphargus*-like, dactylus of gnathopods with several setae along outer margin. *Niphargobates orophobata* resembles *Chaetoniphargus* (in telson, maxilliped, accessory flagellum, maxilla I, uropod III), but differs remarkably by the mandible, by maxilla II with outer plate smaller than inner one.

Other genera of the family Niphargidae differ much more from the genus *Chaetoniphargus*. It is very interesting that within the family Niphargidae, most taxa (over 400) have similarly shaped mouthparts, despite the fact that they are living in very various habitats (interstitial waters, subterranean streams and lakes, springs) and that they are differently sized (from 2 to 30 mm).

4 DISCUSSION

Taxa, which are so convincingly grouped by morphological characters, cannot be grouped by molecular means.

A molecular phylogenetic analysis done recently by Moškrič (2016) shows that most OTUs mentioned in the identification key of Karaman & Sket (2018) are phylogenetically nested within the genus *Niphargus*. The taxonomic emancipation (= generic independence) of any one species renders the genus *Niphargus* paraphyletic. Although the paraphyly is necessarily acceptable in gammarid systematics (Hou & Sket 2016), it brings no practical benefits in this special case. Only

few of so erected genera include more than one species. Thus, they do not make the Niphargidae system significantly simpler.

More specifically, not only *Niphargobates* as one of the morphologically most aberrant niphargid genera, but also the recently described *Chaetoniphargus*, named by Moškrič (2016) as '*Niphargobates* sp. (NC144)', appeared to be nested within *Niphargus* (Moškrič 2016). It is also important to tell that both morphologically similar taxa are remarkably different by their phylogenetic origin. *Niphargobates orophobata* Sket is the basally split off taxon of the one group of 'giant' species (*sensu* Delić et al. 2017), containing *Niphargus steueri* Schellenberg, *N. arbiter* G. Karaman, *N. hebereri* Schellenberg. The "*Niphargobates* sp. (NC144)" (here described as *Chaetoniphargus lubuskensis*) is the closest (but still very distant) sister to another 'giant', *Niphargus orcinus* Joseph. Thus, the molecular analysis supports neither the genus *Niphargobates* nor a new genus *Chaetoniphargus.* The generic emancipation of any of them makes the rest of *Niphargus* paraphyletic, without a compensating benefit. While the fusion of both species, which are morphologically very similar, into one genus, would result even in a polyphyletic entity. The Central European species *Niphargopsis casparyi* (Pratz) is again from another molecular niphargid environment (Delić et al. 2017; Moškrič 2016); this is a species with even more spines in maxilla I, but otherwise not similar to here mentioned niphargids.

Thus, the reduction and transformation of oral appendages is a homoplasy, convergence. It is not in a functional connection with their smallness, since equally small or smaller species are not necessarily similar. The small size of all here treated species is in discrepancy with the fact, that the majority of small and the very smallest *Niphargus* spp. exhibit normally built mouth parts as well as normal appendages of the urosomite III. Among the largest niphargids are the exceedingly stout *orcinus*-type and the prominently elongated *krameri*-type species, both reaching about 30 mm length. The smallest niphargid species are below 5 mm long, some close to 2 mm, like *N. longidactylus* Ruffo (5 mm), *N. transitivus* Sket (2 mm), *N. pupetta* (Sket) (2 mm) (Sket 1971), but we know nothing about their way of feeding and functioning of the mouth parts. The functioning of this type of mouth parts is sometimes characterized as "filtratory", but it has in fact never been observed working.

Besides being mutually convergent, genera *Niphargus, Niphargobates, Chaetoniphargus,* represent another case of an extreme homoplasy. The supposedly unrelated amphipods, *Metacrangonyx* spp. and *Longipodacrangonyx maroccanus* (Boutin & Messouli, 2–3 mm) are similar (Sket, Krapp-Schickel 2016) to the here treated niphargids exceedingly in some details of the head and urosomal appendages, to some degree also in the general habitus.

The animal's habitus (except that the body is laterally depressed) and size is the same as of those niphargids; mandibular palpus is uniarticulate and vestigial, maxilla I is normally developed, but with modestly elevated spines' number (in relation to typical *Niphargus* spp.), uropod III not longer than urosomite III, its exopodite uniarticulate and remarkably shorter than peduncle, endopodite vestigial; telson is semicircular, not notched at all to slightly terminally concave and without stronger spines. A difference in relation to the above treated niphargid group is the shape of gnathopod propodi and the normally 3-5 articulated accessory flagellum. In these two characters, metacrangonyctids deviate from here treated niphargids; the rest would support their belonging to Niphargidae. Considering the telson shape in *Chaetoniphargus lubuskensis* and *Metacrangonyx ilvanus* (Stoch 1997) it could be easy to imagine that the entire (non-chipped) telson of most metacrangonyctids is only the ultimate state of its transformation. The accessory flagellum of antenna I is normally 3-5 articulate in metacrangonyctids and normally biarticulate (or even uniarticulate), but never more, in niphargids.

The third, the strongest, distinguishing group of characters are in maxilla I. In both, the spines of the outer lobe are more numerous than in 'normal' *Niphargus* spp. The palpus was subjected to slight reduction, never occurring in ordinary niphargids. In metacrangonyctids, this extremity is stable in structure, and different from *Niphargus*. The biarticulate palpus in metacrangonyctids is bearing terminally some stout spines and some short setae. The inner lobe is provided with a row of pinnate marginal setae. Neither of these characters is present in any *Niphargus* sp.

This case makes us easy to imagine a generation of convergent, morphologically nearly equal, unrelated amphipod species. To support their unrelatedness, both families are geographically very distant and different. *Niphargus* inhabits the virtually whole Europe and Near East, from Ireland to Iran. Its area includes some Mediterranean islands. The species of the here treated type are limited to SE Europe (Dinarides and Greece, *Niphargopsis* the Central Europe). Metacrangonyctids occur in a wide belt along the southern shores of Mediterranean between the Israel and Islas Canarias. The only area where these groups are sympatric, are some Mediterranean islands (e.g. Elba, Stoch 1997; Ruffo & Stoch 2005). *Niphargus* spp. have never been found in Africa while metacrangonyctids never in Europe.

5 CONCLUSIONS

These facts demonstrate the high possibility of the homoplastic past of seemingly related amphipod species which means also **possibility of** mistakes in morphologically based taxonomy of Amphipoda. Although the taxonomy of Niphargidae is not yet simply solved, considering data of Moškrič (2016), *Niphargobatoides, Niphargobates, Chaetoniphargus* and *Niphargopsis* are synonymous with *Niphargus*. **These data also dispossess legitimacy to phylogenetic (and taxonomic) studies taken without any molecular verification.**

6 POVZETEK

Nedavno opisana jamska postranica *Chaetoniphargus lubuskensis* Karaman & Sket (Crustacea: Amphipoda: Niphargidae) z Velebita je bila sistematsko uvrščena na osnovi morfoloških znakov in po klasičnem postopku. Tukaj podajava filogenetsko študijo na osnovi molekulske filogenetske analize. Rod *Niphargus* vključuje približno 400 vrst, v 12 sorodnih rodov pa uvrščamo še okoli 20 vrst. Skupina rodov *Niphargobatoides, Niphargobates* in *Chaetoniphargus* so podobne živali, od *Niphargus* jih ločuje predvsem postopna redukcija obustnih okončin in zadka.

Poprejšnja molekulska analiza kaže, da morfološka podobnost teh rodov ne odseva sorodstvenih, filogenetskih vezi. Vsi rodovi so sicer vgnezdeni v rodu *Niphargus*, vendar ločeno, so torej plod konvergenčne evolucije. Medtem, ko ima vseh 400 vrst nifargov sklop obustnih okončin oblikovan po tipu tipske vste *Niphargus stygius*, pa imajo omenjeni trije rodovi konvergenčno preoblikovanega, različno reduciranega. Podobno je s telzonom in uropodi III. A homoplazija je šla še dlje. Ti trije nifargobatski rodovi so se morfološko močno približali sistematsko-filogenetsko dokaj oddaljeni družini Metacrangonyctidae. Mandibula je postala pri obeh družinah zaradi pokrnitve palpa skoraj enaka, uropod III se je razvil podobno konvergentno, pa je maxila I pri vseh vrstah metakrangoniktidov skoraj enaka, družinsko značilna. Telzon predstavlja navidezno gladko filetsko vrsto *Niphargus+Niphargobatoides – Niphargobates – Chaetoniphargus – Metacrangonyx ilvanus* – druge *Metcarangonyx* spp.

Tukaj imamo opravka še z enim primerom konvergence, ki kaže nujnost molekulskega preverjanja pri sistematskih študijah na postranicah (Crustacea: Amphipoda).

REFERENCES - LITERATURA

Barnard, J.L & Barnard, C.M., 1983: *Freshwater amphipods of the World. I. Evolutionary patterns. II.* Handbook and bibliography.- Hayfield Associates: Mt. Vernon, Virginia, 1983, pp. XIX +849 pages, 50 figs., 7 graphs, 98 maps, 12 tables.

Boutin, C., 1994: *Phylogeny and biogeography of metacrangonyctid amphipods in North Africa* Hydrobiologia 287 (1): 49–64.

Boutin, C. & Messouli, M., 1988: *Longipodacrangonyx maroccanus, n. gen., n. sp., Nouveau Representant du groupe Metacrangonyx dans les eaux souterraines du Maroc Crustaceana*. Supplement, No. 13, Studies on Amphipoda (Proceedings of the VIth International Colloquium on Amphipod Crustaceans, Ambleteuse, France, 28 June-3 July 1985) pp. 256-271.

Chevreux, E., 1922: *Sur un nouveau genre d`amphipodes de la faune francaise.* Bulletin du Muséum National d`Histoire Naturelle, Paris, 7: 487-488.

Delić, T., Švara, V., Coleman, C. O., Trontelj, P., Fišer, C., 2017: *The giant cryptic amphipod species of the subterranean genus Niphargus (Crustacea, Amphipoda).* Zoologica scripta, 2017, pp. 1-13.

Horton, T., Lowry, J., De Broyer, C., Bellan-Santini, D., Coleman, O., Daneliya. M., Dauvin, J.-C., Fišer, C., Gasca, R., Grabowski, M., Fuerra-Garcia, J. M., Hendrycks, E., Holsinger, J., Hughes, L., Jauma, D., Jazdzewski, K., Just, J., Kamaltynov, R. M., Kim, Y.-H., King, R., Krapp-Schickel, T., Lecroy, S., Lörz, A.-N., Senna, A.R., Serejo, C., Sket, B., Tanberg, A. H., Thomas, J., Thurston, M., Vader, W., Väonölö, R. R, Vonk, R., White, K., Zeidler, W., 2018: World *Amphipoda Database; Niphargus* Schiödte, 1849: World Register of Marine Species.

Hou, Z. & Sket, B., 2016: *A review of Gammaridae (Crustacea: Amphipoda): the family extent, its evolutionary history, and taxonomic redefinition of genera*. *Zoological Journal of the Linnean Society,* 176 (2): 323–348, https://doi.org/10.1111/zoj.12318

Karaman, G., 2016: *Two new genera of the family Niphargidae from Greece (Contribution to the Knowledge of the Amphipoda 287).* Agriculture & Forestry, Podgorica, 62 (1): 7-27.

Karaman, G. & Sket, B., 2018: *A new genus and species of Niphargidae from Velebit, Croatia*. Zootaxa (in print)

Mateus, A. & Mateus, E., 1958: *Un nouveau genre et une nouvelle espece d`Amphipode troglobie du Portugal*. Publicaçoes do Instituto de Zoologia "Dr. Augusto Nobre", Faculdade de Ciencias do Porto, Coimbra, 59: 1-15.

Moškrič, A., 2016: *Postavitev filogenetskega ogrodja rodu slepih postranic Niphargus (Crustacea: Amphipoda) na podlagi molekulskih metod: doktorska disertacija (The formation of a phylogenetic framework for the genus Niphargus (Crustacea: Amphipoda) using molecular methods).* Univerza v Ljubljani, Biotehniška fakulteta, Disertacija.

Ruffo, S., Stoch, F. (eds.), 2005: *Checklist e distribuzione della fauna italiana*. Ministero dell' ambiente e della tutella del' territorio.

Schellenberg, A., 1934: *Eine neue Amphipoden-Gattung aus einer belgischen Höhle nebst Bemerkungen über die Gattung Crangonyx*. Zoologischer Anzeiger, 106 (9): 215-218.

Schellenberg, A., 1938:. *Niphargellus, eine neue subterrane Amphipodengattung an der Ost- und Westgrenze des Reiches*. Zoologischer Anzeiger, 122 (9-10): 245-248.

Schellenberg, A., 1942: *Flohkrebse oder Amphipoda*. In: Die Tierwelt Deutschlands und der angrenzenden Meeresteile. Jena: Fischer

Sket, B., 1971: *Vier neue aberrante Niphargus-Arten (Amphipoda, Gammaridae) und einige Bemerkungen zur Taxonomie der Niphargus-ähnlichen Gruppen*. Razprave, Slovenska Akademija Znanosti in Umetnosti, Ljubljana, 14 (1): 1-25.

Sket, B., 1981: *Niphargobates orophobata n. g., n. sp. (Amphipoda, Gammaridae, s. l.) from cave waters in Slovenia (NW Yugoslavia)*. Biološki vestnik, Ljubljana, 29 (1): 105-118.

Sket, B., 1990: *Is Niphargobates lefkodemonaki sp. n. (Crustacea: Amphipoda) from Kriti (Greece) a Zoogeographical Enigma?* Zoologische Jahrbücher, Abteilung für Systematik, 117: 1-10.

Sket, B. (Krapp-Schickel, T.), 2016: *Interview with Boris Sket*. Amphipod newsletter, 40: 2-10.

Stoch, F., 1997: *Metacrangonyx ilvanus n. sp., the first Italian representative of the family Metacrangonyctidae (Crustacea: Amphipoda).* Annales limnologie, 33 (4) 1997: 255-262.

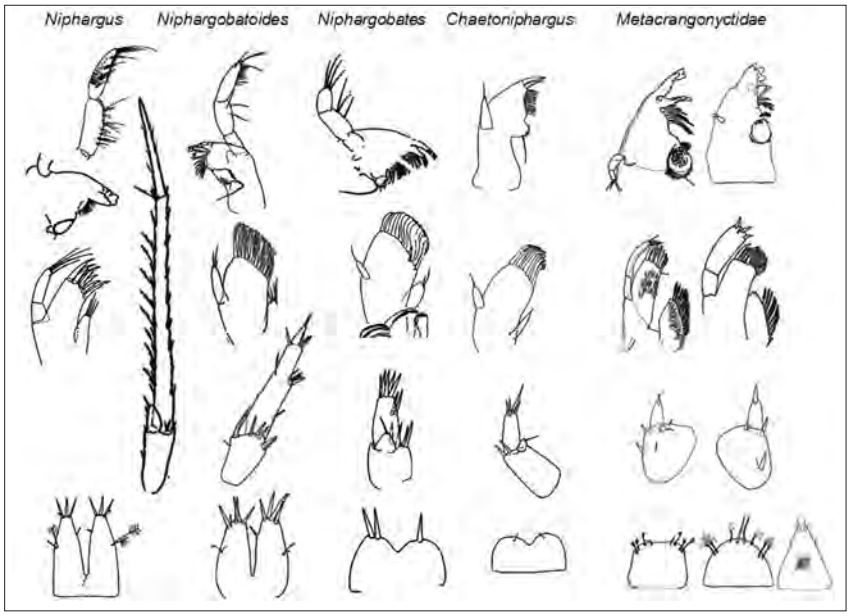

Figure 1, Some taxonomically relevant body parts of Niphargidae and Metacrangonyctidae. In columns follow: genus (or family) name // mandibula // maxilla I // uropod III // telson. Drawings are not in scale. Taken from C. Boutin & M. Messouli,G. Karaman, B. Sket, F. Stoch.
Slika 1, Nekateri taksonomsko pomembni deli telesa pri družinah Niphargidae in Metacrangonyctidae. V stolpcih si sledijo: ime rodu (ali družine) // mandibula // maxilla I // uropod III // telson. Risbe niso v merilu. Povzeto po C. Boutin & M. Messouli, G. Karaman, B. Sket, F. Stoch