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THE SUBTERRANEAN SPECIES *NIPHARGUS ZAGREBENIS* S. KAR. 1950 (FAM. NIPHARGIDAE) ON BALKAN (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 312)

SUMMARY

The subterranean species *Niphargus zagrebensis* S. Karaman, 1950 (Crustacea: Amphipoda, fam. Niphargidae) is redescribed and figured based on typical material from Zagreb, and certain variability of single taxonomical characters is given. New localities of this species are mentioned and geographic map with all known localities of *N. zagrebensis* presented. The relation of this species regarding other similar species (*N. valachicus* Dobreanu & Manolache, 1933; *N. hrabei* S. Karaman; 1932; *N. elegans* Garbini, 1894) is discussed.

Keywords: Amphipoda, *Niphargus zagrebensis*, taxonomy, distribution, subterranean, Balkan peninsula.

INTRODUCTION

The subterranean fauna of Amphipoda on Balkan peninsula (including Slovenia) is still only partially investigated, especially family Niphargidae presented by 9 genera: *Carinurella* Sket, 1971, *Chaetoniphargus* G. Karaman & Sket, 2019, *Exniphargus* G. Karaman, 2016, *Karamaniella* Sket, 1962; *Niphargobates* Sket, 1981, *Niphargobatoides* G. Karaman, 2016; *Niphargopsis* Chevreux, 1922; *Niphargus* Schiödte, 1849; *Pontoniphargus* Dancau, 1970 (see G. Karaman & Sket, 2019).

Among them, only genus *Niphargus* is presented on Balkan by numerous species and subspecies (over 100), described during last 172 years. During so long period, the methods and descriptions with figures of species have been changed drastically. As genus *Niphargus* contains so many taxa, it is necessary even more and more detailed description and figures of each taxon. Numerous taxa, described many years ago, require more detailed redescription and additional figures, to confirm taxonomical identity of species themselves.

We redescribed *Niphargus zagrebensis* S. Karaman, 1950, species considered for a long time as a subspecies of *Niphargus valachicus* Dobreanu & Manolache, 1933, to support morphologically the species status of *Niphargus*

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zagrebensis, remarkably distinct also from other two similar species, *N. hrabei* S. Kar., 1932 and *N. elegans* Garb., 1894.

MATERIAL AND METHODS

The samples of *Niphargus* were preserved in 70% ethanol. The specimens were examined and dissected in the mixture of glycerin and water, using a Wild M 20 stereomicroscope. Later, dissected specimens were transferred onto slides with Faure liquid used for final preservation of animal. The body length of examined specimens was measured by tracing individual's midtrunk lengths (tip of the rostrum to end of the telson) and drawings were made using a camera lucida attachment and manually inked.

Some morphological terminology and setae formulae follow G. Karaman's terminology (Karaman, G., 1969; 2012) for palpus article 3 of mandible [A-setae= setae on outer face of article; B-setae= setae on inner face of article; D-setae= short setae along lateral margin of article; E-setae= long setae at distal part of article], as well as for distal corner of propodus of gnathopods 1 and 2 [S-spine= corner palmar spine on outer face; L-spines= slender serrate spines sitting near S-spine on outer face; M-setae= row of long facial corner submarginal setae on outer face; R-spine= subcorner spine on inner face].

Term "setae" and "spines" are used based on its shape, not origin. This study is based on the morphological, ecological and zoogeographical data.

TAXONOMICAL PART Family NIPHARGIDAE NIPHARGUS ZAGREBENSIS S. KARAMAN, 1950 Figures 1-9

Niphargus (Supraniphargus) valachicus zagrebensis S. Karaman, 1950: 61, figs. 23-34; S. Karaman, 1954: 176; Sket, 1958: 67;

Niphargus (Phaenogammarus) valachicus zagrebensis S. Karaman, 1960: 83;

Niphargus elegans zagrebensis G. Karaman, 1972: 5; G. Karaman, 1974: 17; Sket, 1981: 89; Barnard & Barnard, 1983: 691; Kralj, 2001: 109;

Niphargus illidzensis dalmatinus (part.) S. Karaman, 1932: 200;

Niphargus valachicus zagrebensis Kralj, 2001: 182;

Niphargus zagrebensis Gottstein, 2010: 84; Gottstein et al., 2013: 41; Delić et al., 2017a: 4; Delić et al., 2017b: 6.

MATERIAL EXAMINED: CROATIA:

S-1645= Zagreb, April 1924, 1 exp. male (leg. S. Karaman); Am 628= Zagreb, Rimski jarak, torrent, August 1939, 3 exp. juv. (leg. D. Rucner);

Sp.100= Zagreb, ibid., 11.4. 1948, many exp.(leg. S. Karaman); S-6049= Zagreb, ibid., 8.5. 1949, 10 exp. (leg. S. Karaman); S-5357= Zagreb, vicinity, 11.5.1948, leg. S. Karaman, 4 females mixed with *Gammarus fossarum* Koch, 1936;

S-4222 = Opatovina near Zagreb, 4.3. 1981, 2 males juv. mixed with *Niphargus minor* Sket, 1956 (leg. M. Kerovec);

S-8405= ibid, 20.5.1981, 2 exp. (leg. M. Krvavica);

S-2416= Between Jakovlje and Luka, Hrvatsko Zagorje, subterranean waters on the field, 27.3.1974, many exp (leg. D. Rucner);

S-3930= Forest in Turopolje, 30 km S. of Zagreb, between Poščenica and Odra rivers, 9.10. 1982, many exp. mixed with *Synurella ambulans* (F. Muller, 1846) in the forest under lives with subterranean waters (leg. G. Karaman).

DIAGNOSIS

Body strong, up to 20.0 mm long, metasomal segments along dorsoposterior margin with several short setae each; urosomal segments 1 and 2 with single lateral spines mixed with single setae. Antenna 2 with slender flagellum longer than last peduncular article. Maxilla 1 inner plate with 2-4 setae, outer plate with 7 spines (6 with one lateral tooth), palpus not reaching tip of outer plate spines. Maxilliped inner plate short, with 3-4 distal spines.

Coxae relatively short, coxa 1 with subrounded ventroanterior part, coxa 4 unlobed. Gnathopods 1 and 2 with trapezoid propodus not exceeding the size of corresponding coxa and provided with row of setae along outer margin. Article 3 of both gnathopods with one distoposterior bunch of setae.

Epimeral plates with distinctly pointed ventroposterior corner in males and females, especially on epimeral plate 3. Dactylus of pereopods 3-7 along inner margin with additional spines (3-5 spines in anterior pereopods 3-4, and 2-3 spines in posterior pereopods 6 and 7; pereopod 5 often with 1-2 spines only). Article 2 of pereopods 5-7 unlobed. Pleopods 1-3 peduncles setose, with 2 retinacula.

Uropod 1 peduncle with dorsoexternal row of spines and dorsointernal row of setae (except distal spine), in males with ventrodistal finely serrate tubercle, absent in female. Inner ramus of uropod 1 remarkably elongated in male. Uropod 3 elongated in males, with long distal article of outer ramus; in female distal article short. Telson with various number of distal, marginal and facial spines.

Sexual dimorphic characters developed (uropod 1, uropod 3; tubercle on uropod 1 peduncle in males, oostegites).

DESCRIPTION: MALE 13.2 mm (Sp. 100): Body moderately strong, mesosomal segments naked, metasomal segments 1-3 along dorsoposterior margin with up to 9 short setae (fig. 4E).

Epimeral plates 1-3 with distinctly sharply pointed ventroposterior corner progressively more produced towards epimeral plate 3 (fig. 4E). Posterior margin of epimeral plates 1 and 2 sinusoid, that of epimeral plate 3 is concave, all with 6-8 short marginal setae each. Epimeral plate 2 with 2, epimeral plate 3 with 2-3 subventral spines.



Fig. 1. *Niphargus.zagrebensis* S. Kar., 1950., Zagreb, Rimski jarak, torrent, male 13.2 mm: A= head; B= antenna 1; C= accessory flagellum; D= antenna 2; E= labium; F= urosome; G= uropod 1; H= tubercle on uropod 1 peduncle; I= uropod 2; J= uropod 3.



Fig. 2. *Niphargus.zagrebensis* S. Kar., 1950., Zagreb, Rimski jarak, torrent, male 13.2 mm: A= labrum; B= right mandible, incisor and lacinia mobilis; C= left mandible, molar and lacinia mobilis; D= mandibular palpus, outer face [A= A-setae; D= D-setae, E= E-setae]; E= distal article of mandibular palpus, inner face [B= B-setae]; F= maxilla 1; G= outer plate of maxilla 1; H= maxilliped; I= peduncle of pleopod 1; J= peduncle of pleopod 2; K= peduncle of pleopod 3.



Fig. 3. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, male 13.2 mm: A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus, outer face [S= corner S-spine; L= lateral L-spines; R= subcorner R-spine; M= facial M-setae]; D-E= gnathopod 2, outer face; F= distal corner of gnathopod 2 propodus, outer face [S= corner S-spine; L= lateral L-spines; R= subcorner R-spine; M= facial M-setae].



Fig. 4. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, male 13.2 mm: A= pereopod 3; B= dactylus of pereopod 3; C= pereopod 4; D= dactylus of pereopod 4; E= epimeral plates 1-3; F= telson; G= male 15.3 mm: epimeral plates 1-3.

Urosomal segment 1 on each dorsolateral side with one spine accompanied by 2 setae; urosomal segment 2 on each dorsolateral side with one spine and one seta (fig. 1F); urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1 peduncle (fig.1G).

Head with very short rostrum, short subrounded lateral cephalic lobes and ventral excavation, eyes absent (fig. 1A).

Antenna 1 reaching nearly half of body-length; peduncular articles 1-3 progressively shorter (ratio: 57:40:19), scarcely setose (fig. 1B); main flagellum consisting of 27 articles (most o them with one short aesthetasc), scarcely setose; accessory flagellum short, 2-articulated, almost reaching half of peduncular article 3 (fig. 1C).

Antenna 2: peduncular article 3 short, at distoventral corner with bunch of setae slightly longer than article itself (fig. 1D); article 4 longer than article 5 (ratio: 69:61), along ventral margin with 3 bunches of setae (the longest setae slightly exceeding diameter of article itself), along dorsal margin with one median spine and 4 bunches of short setae; article 5 along ventral margin with 3 bunches of setae (the longest setae are rather longer than diameter of article itself), along dorsal margin with 4 bunches of short setae. Flagellum relatively slender, slightly shorter than peduncular articles 4 and 5 combined (ratio: 95:128), scarcely setose (fig. 1D). Antennal gland cone short (fig. 1D).

Mouthparts basic. Labrum much broader than long, with poorly convex distal margin (fig. 2A). Labium broader than long, with well developed inner lobes and entire convex outer lobes (fig. 1E).

Mandibles with triturative molar. Left mandible with 5-toothed incisor and 4-toothed lacinia mobilis accompanied by 6 rakers (fig. 2C). Right mandible with 4-toothed incisor and weak bifurcate serrate lacinia mobilis accompanied by 5 rakers (fig. 2B). Palpus 3-articulated: article 1 naked, article 2 with 14 setae; article 3 nearly as long as article 2, with nearly 26 marginal D-setae and 6-7 distal E-setae (fig. 2D), on outer face by one group of 7 A-setae (fig. 2D), on inner face with 5 B-setae (1-2-2) (fig. 2E).

Maxilla 1: inner plate with 3 setae; outer plate with 7 spines (6 spines with one lateral tooth; one spine with finely serrate margin and one tooth) (fig. 2G); palpus 2-articulated, not reaching distal tip of outer plate spines (fig. 2F), provided with 6 distal setae.

Maxilla 2: outer plate slightly larger than inner on, both plates with distomarginal setae only (fig. 5A).

Maxilliped: inner plate short, with 3 distal pointed spines mixed with single setae (fig. 2H); outer plate not exceeding 2/3 of palpus article 2, along inner (mesial) margin with nearly 12 short spines; palpus 4-articulated; article 3 along outer margin with one median and one distal bunch of long setae, along inner margin with numerous setae in distal part and row of short facial spine-like setae; article 4 with short nail and bunch of 1-2 ventral setae near basis of the nail.

Coxae 1-7 relatively short. Coxa 1 as long as broad, with subrounded ventroanterior corner, provided with nearly 7 short setae (fig. 3A). Coxa 2 slightly broader than long (ratio: 50:47), along ventral margin with 5-6 short setae (fig. 2D). Coxa 3 almost as broad as long (ratio: 54:53), along ventral margin with nearly 6 setae (fig. 4A). Coxa 4 slightly broader than long (ratio: 53:50), without ventroposterior lobe, and with nearly 5-6 short marginal setae (fig. 4C).

Coxa 5 nearly as long as coxa 4, broader than long (ratio: 67:42), anterior lobe short, subrounded (fig. 5B). Coxa 6 shorter than coxa 5, broader than long (ratio: 60:37), anterior lobe relatively shallow (fig. 5D). Coxa 7 entire, broader than long (ratio: 50:26), with convex ventral margin (fig. 5F).

Gnathopods 1 and 2 relatively small, with propodus as large as corresponding coxa (fig. 3A, D). Gnathopod 1: article 2 along anterior and posterior margin with numerous long setae; article 3 along posterior margin with one distal bunch of setae; article 5 shorter than propodus (ratio: 30:40), along anterior margin with distal bunch of setae (fig. 3A). Propodus almost quadrate, broader than long (ratio: 74:70), along posterior margin with 7 transverse rows of setae; palm almost straight, inclined almost to the half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and with row of 6 facial M-setae (fig. 3B, C), on inner face by one submarginal R-spine (fig. 3C). Dactylus reaching posterior margin of propodus, along outer margin with row of 8 single setae, along inner (mesial) margin with several short setae only (fig. 3B).

Gnathopod 2: article 2 along anterior margin with row of single long setae, along posterior margin with several bunches of long setae (fig. 3D); article 3 at posterior margin with one distal bunch of setae. Article 5 poorly shorter than propodus (ratio: 38:42), along anterior margin with distal bunch of setae. Propodus poorly rhomboid, broader than long (ratio: 88:78), along posterior margin with 8 transverse rows of setae (fig. 3E); palm almost straight, inclined less than half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and by 5 facial M-setae, on inner face by one subcorner R-spine (fig. 3F). Dactylus reaching posterior margin with several short setae (fig. 3F).

Percopods 3 and 4 moderately stout. Percopod 3 poorly longer than percopod 4, article 2 along anterior margin with several proximal long setae and several distal short setae (fig. 4A), along posterior margin with bunches of long setae in proximal part. Articles 4-6 of different length (ratio: 50:32:35): article 4 at posterior margin with several bunches of relatively short setae (the longest setae not exceeding diameter of article itself), at anterior margin with single facial and distal setae; article 5 along posterior margin with 4 groups of setae (the longest setae exceeding diameter of article itself); article 6 along posterior margin with 5 pairs of short spines. Dactylus strong, shorter than article 6 (ratio: 19:35), along inner margin with 3-4 strong spines, along outer margin with one median plumose seta (fig. 4B); nail shorter than pedestal (ratio: 22:30).

Pereopod 4: article 2 at anterior margin with proximal bunch of long setae and row of short distal setae, along posterior margin with numerous bunches of long setae (fig. 4C). Articles 4-6 of unequal length (ratio: 42:28:33); article 4 along both margins with setae not exceeding diameter of article itself; article 5 along posterior margin with 2 single spines accompanied by several short setae; article 6 along posterior margin with 5 groups of single or pairs of short spines. Dactylus strong, remarkably shorter than article 6 (ratio: 19:33), along ventral margin with 3-4 strong spines, along outer margin with one median plumose seta (fig. 4D); nail shorter than pedestal (ratio: 22:32).

Pereopods 5-7 relatively stout. Pereopod 5 remarkably shorter than pereopods 6 and 7 (fig. 5B, D, F), article 2 dilated, longer than broad (ratio: 72:48), along anterior margin with nearly 8 groups of spine-like setae or short setae, posterior margin slightly concave in the middle, bearing nearly 11 short marginal setae, ventroposterior lobe not developed (fig. 5B). Articles 4-6 of unequal length (ratio: 30:43:44); article 4 along anterior margin with 3 groups of short setae and distal 2 spines, along posterior margin with one median and 2 distal spines. Articles 5 and 6 along both margins with several bunches of short spines and some distal long setae. Article 6 remarkably shorter than article 2 (ratio: 44:72). Dactylus short and strong, much shorter than article 6 (ratio: 19:44), along inner margin with 1-2 strong spines, along outer margin with one median seta (fig. 5C); nail shorter than pedestal (ratio: 19:30).

Percopod 6: article 2 dilated, longer than broad (ratio: 83:53), along anterior margin with row of 6 groups of short spine-like setae, along posterior, almost straight margin, with nearly 13 short setae, ventroposterior lobe not developed (fig. 5D). Articles 4-6 of unequal length (ratio: 55:66:72): article 4 at anterior margin with several single or bunches of short setae and single short spines, along posterior margin with 2 median and 2-3 distal spines. Articles 5 and 6 along both margins with several bunches of short spines (spines not exceeding diameter of articles). Article 6 shorter than article 2 (ratio: 72:83). Dactylus much shorter than article 6 (ratio: 22:72), at inner margin with 2 strong spines, at outer margin with one median plumose seta (fig. 5E); nail shorter than pedestal (ratio: 23:72).

Pereopod 7: article 2 longer than broad (ratio: 85:54), along anterior margin with 6 groups of spine-like setae, along posterior poorly convex margin with nearly 17 short setae, ventroposterior lobe not developed (fig. 5F). Articles 4-6 of unequal length (ratio: 49:65:87), article 2 at anterior margin with 3 groups of setae and one spine, along posterior margin with 3 groups of spines. Articles 5 and 6 along both margins with several bunches of short spines and short setae. Article 2 almost as long as article 6 (ratio: 85:87). Dactylus much shorter than article 6 (ratio: 27:87), along inner margin with 2-3 strong spines, at outer margin with one median plumose seta (fig. 5G), nail shorter than pedestal (ratio: 24:50).



Fig. 5. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, male 13.2 mm: A= maxilla 2; B-C= pereopod 5; D-E= pereopod 6; F-G= pereopod 7.



Fig. 6. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, female 13.1 mm: A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus, outer face [S= corner S-spine; L= lateral L-spines; R= subcorner R-spine; M= facial M-setae]; D-E= gnathopod 2; F= urosome with uropods 1-2.

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 along anterior external margin with 1-2 distal setae (fig. 2 I); peduncle of pleopod 2 at anterior external margin with one distal seta (fig. 2J); peduncle of pleopod 3 at anterior external margin with 9 median setae, along posterior margin with 2 setae (fig. 2K).

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae (except distal spine); one finely serrate tubercle is attached ventrally at top of peduncle (fig. 1H) (see detailed description in: S, Karaman 1950: 63, figs. 36, 37); Inner ramus with 5 distal and single lateral spines including 2 short setae; outer ramus much shorter than outer one, with 5 distal and several lateral spines (fig. 1G).

Uropod 2: rami of nearly subequal length, bearing single lateral and 5 distal short spines each (fig. 1 I).

Uropod 3 long, narrow: peduncle slightly longer than broad, with distal short spines; inner ramus short, scale-like, with group of lateral and distal spines. Outer ramus 2-articulated: first article along outer margin with 7 groups of short spines, along inner margin with 6 groups of short spines; single plumose setae are attached near spines at inner (mesial) side of the article (fig. 1J); second article rather shorter than first one (ratio: 73:126), with bunches of short simple setae along both margins and tip.

Telson incised less than 2/3 of telson-length, poorly longer than broad (ratio: 89:84); each lobe with 3 distal spines; single spines are attached along inner (mesial) and outer margin as well as on dorsal surface (fig. 4F); a pair of short plumose setae are attached near the median part of outer margin on each lobe.

Coxal gills on gnathopod 2 and pereopods 3-4 large, ovoid (figs. 3D; 4A, C), these of pereopods 5 and 6 rather smaller (fig. 5B, D).

FEMALE, ovigerous, with 20 eggs in marsupium, 13.1 mm:

Body strong, metasomal segments 1-3 along dorsoposterior margin with 6-8 short setae (fig. 8C). Urosomal segment 1 on each dorsolateral side with one spine and 1-3 setae (fig. 6F); urosomal segment 2 on each dorsolateral side with 2 spines and 2-3 setae; urosomal segment 3 naked. Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1 peduncle (fig. 6F).

Epimeral plates 1-3 similar to these in male, with ventroposterior corner distinctly pointed, progressively more developed towards epimeral plate 3 (fig. 8C). Epimeral plates 1 and 2 with poorly sinusoid posterior margin provided with several short setae each; posterior margin of epimeral plate 3 concave, provided with 7 short setae. Epimeral plate 2 with 4 and epimeral plate 3 with 4-6 subventral spines (fig. 8C).

Head like that in male. Antenna 1 almost reaching half of body-length, scarcely setose like that in male; peduncular article 3 relatively short; main flagellum consisting of 22 articles, scarcely setose; accessory flagellum reaching half of peduncular article 3 (fig. 8A).



Fig. 7. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, female 13.1 mm: A-B= pereopod 3; C= pereopod 4; D= pereopod 5; E= pereopod 6; F-G= pereopod 7.



Fig. 8. *Niphargus.zagrebensis* S. Kar., 1950, Zagreb, Rimski jarak, torrent, female 13.1 mm: A= accessory flagellum; B= antenna 2; C= epimeral plates 1-3; D= telson; E= uropod 3.

Antenna 2: peduncular article 3 with distoventral bunch of long setae (fig. 8B); peduncular article 4 slightly longer than article 5 (ratio: 58:54), both articles along ventral margin with 3 bunches of ventral setae longer than diameter of articles themselves, along dorsal margin with 4 groups of short setae each. Antennal gland cone short (fig. 8B).

Mouthparts like these in male. Mandibles with 6 rakers. Mandibular palpus article 3 with nearly 30 D-setae, 5-6 E-setae, on outer face by one bunch of 7-8 A-setae, on inner face with 3 groups of B-setae (2-3-2).

Maxilla 1: inner plate with 3-4 setae, outer plate with 7 spines (6 spines with one lateral tooth, one spine with one lateral tooth and finely serrate margin, palpus 2-articulated, not reaching tip of outer plate spines and provided with 6 setae.

Maxilliped inner plate with 4 distal spines; palpus article 4 at inner margin with 2 setae near basis of nail.

Coxae 1-4 slightly longer than these in male. Coxa 1 longer than broad (ratio: 42:30), with subrounded ventroanterior corner and bearing nearly 7 marginal setae (fig. 6A). Coxa 2 slightly longer than broad (ratio: 50:40), along ventral convex margin with nearly 12 short setae (fig. 6D). Coxa 3 longer that broad (ratio: 53:45), at ventral margin with nearly 12 setae (fig. 7A). Coxa 4 as long as broad, along ventral margin with 6-8 setae (fig. 7C).

Coxa 5 broader than long (ratio: 60:41), with anterior subrounded lobe as long as coxa 4 (fig. 7D); coxa 6 distinctly smaller than coxa 5, bilobed, broader than long (ratio: 46:32) (fig. 7E); coxa 7 entire, broader than long (ratio: 43:22) (fig. 7F).

Gnathopods 1 and 2 relatively small, almost as large as corresponding coxa (fig. 6A, D). Gnathopod 1: article 2 along both margins with numerous long setae; article 3 at posterior margin with one distal bunch of setae (fig. 6A). Article 5 nearly as long as propodus, along anterior margin with distal bunch of setae. Propodus trapezoid, slightly larger than long (ratio: 69:63), along posterior margin with 7 transverse rows of setae (fig. 6B). Palm convex, inclined slightly less than half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3-4 serrate L-spines and 8 facial M-setae (fig. 6C), on inner face by one subcorner R-spine. Dactylus reaching posterior margin with several short setae (fig. 6B).

Gnathopod 2 rather larger than gnathopod 1: article 2 along both margins with numerous long setae; article 3 at posterior margin with one distal bunch of setae (fig. 6D). Article 5 almost as long as propodus, along anterior margin with 3 groups of setae. Propodus trapezoid, rather inclined, broader than long (ratio: 68:80), along posterior margin with 9 transverse rows of setae (fig. 6E). Palm convex, inclined nearly 1/3 of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 serrate L-spines and 6 facial M-setae, on inner face by one subcorner R-spine. Dactylus reaching posterior margin of

propodus, along outer margin with 13 single or paired setae, along inner margin with several short setae (fig. 6E).

Percopods 3 and 4 moderately stout. Percopod 3 only slightly larger and with slightly longer setae than percopod 4, article 2 with longer proximal setae along anterior and posterior margin and with shorter setae in distal part (fig. 7A). Articles 4-6 of unequal length (ratio: 40:25:30); article 4 along both margins with several bunches of setae (the longest setae slightly exceeding diameter of article); article 5 along posterior margin with 3 bunches of setae (the longest setae exceeding diameter of article itself), along anterior margin with 2 groups of short setae; article 6 along posterior margin with 6 paired short spines, along anterior margin with 3 groups of short setae. Dactylus much shorter than article 6 (ratio: 16:30), along inner margin with 5 strong spines, nail shorter than pedestal (fig. 7B).

Percopod 4: pilosity of articles 2-4 like that in male. Article 5 along posterior margin with 3 groups of single spines mixed with short setae, along anterior margin with distal group of short setae (fig. 7C). Dactylus like that of percopod 3, along inner margin with 5 spines.

Pereopod 5 remarkably shorter than pereopods 6 and 7 (fig. 7D, E, F), article 2 dilated, unlobed, longer than broad (ratio: 58:38), along anterior margin with row of spine-like setae, along posterior margin with nearly 13 short setae. Articles 4-6 of equal length; article 4 at anterior margin with 5-6 groups of short setae and distal spine, along posterior margin with 2 median and 2 distal spines (fig. 7D). Article 5 along anterior margin with 2 groups of short spines and distal anterior setae, at posterior margin with 2 groups of spines and 2 short setae; article 6 along anterior margin with 3 bunches of spines, along posterior margin with distal bunch of spines and setae. Article 2 longer than article 6 (ratio: 58:36). Dactylus much shorter than pedestal (ratio: 14:36), at inner margin with 2 spines, nail is shorter than pedestal.

Pereopod 6: article 2 longer than broad (ratio: 67:46), along anterior margin with 6 groups of spine-like setae and distal group of setae, along posterior almost straight margin with nearly 12 short setae, ventroposterior lobe not developed (fig. 7E). Articles 4-6 of unequal length (ratio: 48:54:60); article 4 along anterior margin with 5 bunches of short setae and single spines, along posterior margin with 4 groups of spines; articles 5 and 6 along both margins with several bunches of short spines often mixed with single short setae. Article 2 longer than article 6 (ratio: 67:60). Dactylus much shorter than article 6 (ratio: 19:60), at inner margin with 3 spines, nail is shorter than pedestal.

Pereopod 7: article 2 longer than broad (ratio: 70:48), along anterior margin with row of spine-like setae and distal long setae, along posterior slightly convex margin with nearly 16 short setae, ventroposterior lobe not developed (fig. 7F). Articles 4-6 of unequal length (ratio: 42:55:68); article 4 along anterior margin with several groups of short setae, along posterior margin with 3 bunches of spines; articles 5 and 6 along both margins with several bunches of short setae. Article 2 slightly longer than

article 6 (ratio: 70:68). Dactylus much shorter than pedestal (ratio: 20:68), along inner margin with 3 strong spines, along outer margin with one median plumose seta (fig. 7G); nail shorter than pedestal (ratio: 20:46).

Pleopods 1-3 with 2 retinacula. Peduncle of pleopod 1 with 5 setae along anterior external margin; peduncle of pleopod 2 with 1 seta at anterior external margin; peduncle of pleopod 3 with 9 setae along anterior external margin and 2 setae along posterior margin.

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae except distal spine (fig. 6F). Inner ramus is not produced, nearly as long as peduncle, provided with several lateral groups of strong spines; 4 spines are attached at the tip of article. Peduncle without distal ventral serrate tubercle. Outer ramus is only moderately shorter than inner one, with several strong lateral spines and with 2 simple long median setae; at the tip are attached 4 spines (fig. 6F).

Uropod 2 with inner ramus poorly longer than outer one, both rami with several lateral spines, on top of each ramus appear 4-5 short unequal spines (fig. 6F).

Uropod 3 narrow, elongated: peduncle nearly twice as long as broad (fig. 8E); inner ramus scale-like, with one lateral and 2 distal spines. Outer ramus 2-articulated: first article with 7 groups of strong spines along outer margin (fig. 8E), along inner (mesial) margin are attached several groups of single or paired spines mixed with single long plumose setae. Distal article of outer ramus much shorter than first one (ratio: 32:133), along both margins and tip with short simple setae.

Telson broader than long (ratio: 80:66), incised almost 2/3 of telsonlength; each lobe with 3 long distal spines and single outer and inner marginal (mesial) slender spines, as well as with one bunch of 1-3 facial spines mixed with 0-1 short seta (fig. 8D); a pair of short plumose setae is attached near the middle of outer margin of each lobe.

Coxal gills on gnathopod 2 and pereopod 5 are ovoid, large (figs. 6D, 7A, C), these of pereopods 5 and 6 are rather smaller (fig. 7D, E).

Oostegites very large, occur on pereopods 2-5, setose along margin (fig. 7C).

VARIABILITY

Sexual dimorphic characters are visible through different uropod 3, size of inner ramus of uropod 1, slightly different length of coxae, oostegites in female, presence of sexual papillae on ventral side of metasomal segment 3 in male.

In males, inner ramus of uropod 1 is remarkably elongated, up to twice longer than outer one, in large specimens rami are paddle-shaped.

In females coxae are rather longer, telson often rather broader, inner ramus of uropod 1 only slightly longer than outer one, both rami are not paddle-shaped; oostegites present.

Size of the species is up to 20.0 mm, with antenna 1 reaching 2/5 to half of body-length.

Maxilla 1 inner plate with 2-4 setae, outer plate with 6 spines bearing one lateral tooth each, palpus not reaching tip of outer plate spines.

Maxilliped: inner plate with 3-4 distal pointed spines mixed with single setae.

Propodus of gnathopods 1-2 with 5-8 facial M-setae, 3 L-spines are attached always laterally from S-spine; dactylus of both gnathopods along outer margin with row of several single or paired setae.

Dactylus of pereopods 3-7 is with elevated number of spines along inner margin: 3-5 spines on pereopods 3 and 4; dactylus of pereopod 5 with 2, occasionally only one spine; dactylus of pereopods 6 and 7 with 2-3 spines along inner margin. We have not observed significant difference in number of spines on dactylus of pereopods in males and females.

Peduncles of pleopods 1-3 is with rather variable number of setae along anterior margin. In male 15.0 mm anterior external margin in peduncle of pleopod 1 is provided with 7 setae and posterior margin with 2 setae; anterior external margin of pleopod 2 peduncle is provided with 2 setae; anterior external margin of pleopod 3-peduncle is provided with 9 setae.

Ventroposterior corner of epimeral plates 1-3 are more or less distinctly pointed in males and females, Stanko Karaman mentioned and figured (1950) that in some large specimens (male 19.0 mm) the ventroposterior pointed corner of epimeral plates is less produced, but distinctly pointed, what agree with our observations also. We observed similar variability by various other *Niphargus* species also, that very large specimens ("senile specimens") have epimeral plates less pointed or more subrounded than adult specimens of median size within the same population. Epimeral plate 2 in both sexes is provided usually with 2-4 subventral spines, epimeral plate 3 with 2-6 subventral spines.

Urosomal segments 1 in males usually with 0-1 spine and 1-3 setae, urosomal segment 2 with 1-2 spines accompanied by single setae; urosomal segment 3 is always naked; Urosomal segment 1 in females usually with 1-2 spine often accompanied by 1-3 setae.

Uropod 1 in males with outer ramus reaching 2/3 of inner ramus (in smaller specimens) to almost as long as inner ramus (in large specimens), both rami with several lateral and 4-5 distal short spines; ventrodistal tubercle on uropod 1 in males is always present.

In females rami of uropod 1 are almost of equal length or inner ramus is poorly longer, both rami with several lateral and 4-5 distal short spines.

Uropod 2 in males and females with nearly equal length or inner ramus poorly longer than outer one, both rami with several lateral and 4-5 distal short spines.

Telson with rather variable shape, scarcely longer than broad to slightly broader than long, with 3-4 distal spines and bearing 1-2 outer marginal and mesial spines and setae; single facial spines and setae are always present.



Fig. 9. Distribution of Niphargus.zagrebensis S. Kar., 1950 on Balkan

LOCUS TYPICUS: Zagreb, Croatia

Holotype: and paratypes are deposited in Karaman's Collection in Podgorica, Montenegro under the numbers:Sp.100/1-7.

LOCALITIES CITED:

CROATIA:

Zagreb and vicinity (S. Karaman, 1950); Sket, 1958; G. Karaman, 1972; G. Karaman, 1974; Sket, 1981; Kralj, 2001; Delić, 2017a; present data);

Čučerje, Dubrava near Zagreb (Delić et al., 2017a);

Between Jakovlje and Luka; Turopolje (present data);

SLOVENIA:

Brežice; Cerklje; Kostanjevica in Krakovski forest; limnocrene spring near Krka River in village Malnice (Sket, 1958; G. Karaman, 1972; G. Karaman, 1974; Sket, 1981);

Kočevske poljane, Dolenjske toplice; spring in Jurišah, Juriše, Ozalj; Božakovska jama Cave, Božakovo, Metlika (Delić et al., 2017a);

Gadina, Loka, Črnomelj (Delić et al., 2017a; Delić et al., 2017b).

REMARKS AND AFFINITY

Niphargus zagrebensis belongs to the group of species with elevated number of spines on dactylus of pereopods and presence of distal tubercle on uropod 1 in male. strongly pointed ventroposterior corner of epimeral plates, by presence of ventrodistal tubercle on uropod 1 in males and by poorly unequal uropod 3 in males and females, with typus subgeneris: *Niphargus thermalis* Dudich, 1941a, including in it also *N. mediodanubialis* Dudich, 1941b, *N. hrabei* S. Karaman, 1932 and *N. valachicus* Dobreanu & Manolache, 1933.

N. thermalis Dudich, 1941 is considered as a valid species, and *N. mediodanubialis* Dudich 1941b [loc. typ.:swamps near Szeged, Southeast Hungary] and its form *aschizotelson* Dudich 1941b [loc. typ.: wells in Révülöp, Hungary] have uncertain taxonomical position (Balázs et al., 2015).

S. Karaman (1950) removed all *Niphargus* taxa with elevated number of spines on dactylus of pereopods into a new subgenus *Niphargus* (*Supraniphargus*) n. sbg. with typus subgeneris: *Niphargus illidzensis* Schäferna, 1922.

S. Karaman (1960) removed from subgenus *Supraniphargus* all taxa living in epigean waters with tubercle on uropod 1 in male and strongly pointed epimeral plates into subgenus *Niphargus (Phaenogammarus) (N. elegans* Garbini, 1894, *N. valachicus* Dobreanu & Manolache, 1933, *N. zagrebensis*, S. Karaman, 1950, *N. hrabei* S. Karaman, 1932, *N. thermalis* Dudich, 1941, *N. potamophilus* Birstein, 1954, *N. cubanicus* Birstein, 1954).

At the present time, the taxonomic categories on generic, subgeneric and species levels are in the process of redefinition and valorization by various authors, based on numerous different methods, often contradictory, and we don't discuss this problem here.

We consider *Niphargus zagrebensis* a good distinct species, rather similar to *Niphargus hrabei* S. Karaman, 1932 described from Nana Parkan in one swamp near Danube river in Slovakia between Komorn (=Komarno) and Budapest (Hungary), later mentioned by some authors in some other localities also.

N. hrabei has also strongly acute epimeral plates, presence of distoventral tubercle on uropod 1 in males, but differs from *zagrebensis* by shorter inner ramus of uropod 1 in males, lower number of spines on some dactyls of pereopods, by shorter distal article on uropod 3 outer ramus in male, by maxilla 1, armature of telson, etc. It is necessary to redescribe *Niphargus hrabei* to understand better the taxonomical relations between these two species.

Niphargus valachicus [loc. typ.: Bucarest, Romania] differs remarkably from *N. zagrebensis* by different armature of urosomal segments 1 and 2, by very elevated number of spines on dactylus of all pereopods, etc,

Niphargus elegans Garbini, 1894 [loc. typ.: S. Pancrazio, Verona, Italy] differs from *N. zagrebensis* by higher number of spines on dactylus on pereopods 3-7, by gnathopods 1-2, by different maxilla 1, etc.

ECOLOGY:

Niphargus zagrebensis was found in the springs, wells and other subterranean waters as well as in the epigean waters in ditches with waters, but always connected with temporary or stable water sources in the vicinity.

Stanko Karaman described this species from vicinity of Zagreb (Rimski potok-torrent) and some other torrents near Zagreb), and it was collected later from the same region (Čućerje, Dubrava; Opatovina), sometimes mixed with the epigean species *Gammarus fossarum* Koch, 1836. In the Turopolje region we collected *N. zagrebensis* in small limnocrene waters under leaves in the forest, mixed with semisubterranean species *Synurella ambulans* (F. Muller, 1846). In the subterranean waters in Opatovina near Zagreb this species was collected mixed with *Niphargus minor* Sket, 1956.

Niphargus zagrebensis is semisubterranean species, blind, with strong body's cuticula, but in the process of penetration actively again into epigean waters. Similar tendency to conquest the epigean waters it was observed by some other *Niphargus* species also: *Niphargus valachicus* Dobreanu & Manolache, 1933, species very common in Danube river basin from Slovenia till Romania, Asia Minor till Iran (G. Karaman, 1998), *N. elegans* Garbini, 1894, species also very common in superficial (epigean) waters collected sometimes with *Synurella ambulans* and various *Echinogammarus* species (G. Karaman, 1993).

REFERENCES

- Balázs, G., Angyal, D & Kondorosy, E. 2015. *Niphargus* (Crustacea: Amphipoda) species in Hungary: literature review, current taxonomy and the updated distribution of valid taxa .- Zootaxa 3974 (3): 361–376. 6 figs.
- 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.
- Birstein, J.A. 1954. Nakhozhdenie podzemnogo bokoplava *Niphargus* (Crustacea, Amphipoda) v nizov`iakh Dona i v basseine Kubani. Zoolog. Zhurnal, 33 (5): 1025-1031
- Delić T., Švara, V., Coleman, C.O., Trontelj, P. & Fišer, C. 2017a. The giant cryptic amphipod species of the subterranean genus *Niphargus* (Crustacea, Amphipoda). Zool. Scr. 2017;00:1–13. <u>https://doi.org/10.1111/zsc.12252</u>, supplement.
- Delić, T., Trontelj, P., Rendoš, M. & Fišer, C. 2017b. The importance of naming cryptic species and the conservation of endemic subterranean amphipods. - Scientific Reports | 7: 3391 | DOI:10.1038/s41598-017-02938-z; Supplementary information.
- Dobreanu, E. & Manolache, C. 1933. Beitrag zur Kenntnis der Amphipodenfauna Rumäniens. -Notationes Biologicae, Bucarest, 1 (3): 103-108.
- Dudich, H. 1941a. Niphargus aus einer Therme von Budapest. Annales Musei natur. hungar. Budapest, 34 (Zool.):165-175.
- Dudich, E. 1941b. *Niphargus mediodanubialis* sp. nov., die am weitestem verbreitete *Niphargus*-Art des mittleren Donaubeckens.- Fragmenta Faunistica Hungarica, 4.(3): 61-73.
- Garbini, A. 1894. *Gammarus* ciechi in acque superficialis basse. Academia d'Agricoltura Arti e Commercio di Verona, 70 (3): 107-112.
- Gottstein, S. 2010. Priručnik za određivanje podzemnih staništa u Hrvatskoj prema Direktivi o staništima EU.- Državni zavod za zaštitu prirode, Zagreb, 99 pp., 79 figs.

- Gottstein, S., Hudina, S., Lucid, A., Maguire, I., Ternjej, I. &.Žganec, K. 2013. Crveni popis rakova (Crustacea) slatkih i bočatnih voda Hrvatske.- Hrvatsko biološko drustvo 1885, Zagreb, pp. 51.
- Karaman, G. 1969g. XXVII. Beitrag zur Kenntnis der Amphipoden. Arten der Genera *Echinogammarus* Stebb. und *Chaetogammarus* Mart. an der jugoslawischer Adriaküste. - Glasnik Republičkog zavoda za zaštitu prirode i Prirodnjačke zbirke u Titogradu, 2: 59-84, 51 figs.
- Karaman, G. 1972. Le probleme du Genre Niphargus en Yougoslavie. Actes du Ier Colloque International sur le genre Niphargus-Verona, 15-19 Aprile 1969, Museo Civico di Storia Naturale, Verona, Memorie fuori serie, 5: 1-10.
- Karaman, G. 1974. Catalogus Faunae Jugoslaviae, Crustacea Amphipoda (Contribution to the Knowledge of the Amphipoda 60). - Consilium Academiarum Scientiarum Rei Publicae Socialisticae Foederativae Jugoslaviae, Academia Scientiarum et Artium Slovenica, Ljubljana, 3 (3): 1-44.
- Karaman, G. 1993. Crustacea Amphipoda di acqua dolce. Fauna d'Italia, vol. XXXI: 1-337, Edizione Calderini Bologna, Italia.
- Karaman, G. 1998. First discovery of the Family Niphargidae (Gammaridea) in Iran. (Contribution to the Knowledge of the Amphipoda 234).- Glasnik Odjeljenja prirodnih nauka, Crnogorska akademija nauka i umjetnosti, Podgorica, 12: 9-22, 4 figs.
- Karaman, G. 2012. Further investigations of the subterranean genus *Niphargus* Schiödte, 1849 (fam. Niphargidae) in Serbia. (Contribution to the Knowledge of the Amphipoda 264). Agriculture and Forestry, Podgorica, 58 (2): 45-64, 7 figs.
- Karaman, G. & Sket, B. 2019. New genus and species of the family Niphargidae (Crustacea: Amphipoda: Senticaudata), *Chaetoniphargus.lubuskensis* gen. nov., sp. nov. from Croatia.- Zootaxa 4545 (2): 249–263, figs. 1-8.
- Karaman, S. 1932. 5. Beitrag zur Kenntnis der Süsswasser-Amphipoden (Amphipoden unterirdischer Gewässer).- Prirodoslovne razprave, Ljubljana, 2: 179-232, 25 figs.
- Karaman, S. 1950. Niphargus.ilidzensis Schaeferna i njegovi srodnici u Jugoslaviji. [Supraniphargus.ilidzensis Schaeferna und seine Nächstverwandten in Jugoslavien].-Srpska Akademija Nauka, Posebna Izdanja knj.158, Odelenje Prirodnomatematičkih nauka, Beograd, 2: 51-85, figs. 1-40.
- Karaman, S. 1954. Die Niphargiden des slovenischen Karstes, Istriens sowie des benachb. Italiens.- Acta, Musei Macedonici Sceintiarum Naturalium, Skopje, 2 (8): 159-180, figs. 1-48.
- Karaman, S.1960. Weitere Beiträge zur Kenntnis der Jugoslavischen Niphargiden.- Glasnik prirodnjačkog muzeja Beograd, Serie B, 15: 75-90, figs. 1-19.
- Kralj, K. 2001. Raznolikost faune rakušaca (Amphipoda) slatkih i bočatih voda Hrvatske.-Diplomski rad, Sveučilište u Zagrebu, Prirodoslovno-matematički fakultet, Biološki odsjek, 224 pp.
- Sket, B. 1958a. Prispevek k poznavanju naših amfipodov. Biološki Vestnik, Ljubljana, 6: 66-75, figs. 1-13.
- Sket, B. 1981. Distribution, ecological character and phylogenetic importance of *Niphargus valachicus* (Amphipoda, Gammaridae s. l.).- Biološki Vestnik, Ljubljana, 29 (1): 87-103.