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Gordan S. KARAMAN<sup>1</sup>

# REDESCRIPTION OF PARTIALLY KNOWN *GAMMARUS PAVLOVICI* S. KARAMAN, 1929 (FAMILY GAMMARIDAE) AND ITS VARIABILITY (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 311)

#### SUMMARY

The epigean freshwater species *Gammarus pavlovici* S. Karaman, 1929 (Amphipoda: Gammaridae) is redescribed from type-locality, Rašće springs near Skoplje (North Macedonia). This species has been only partially described and for long time considered as synonym of *Gammarus balcanicus* Schäferna, 1922 by numerous authors (G. Karaman, 1977; Barnard & Barnard, 1983, etc.).

As *Gammarus pavlovici* was one of the earliest described taxa affiliated to *G. balcanicus*, the recognition of morphological and taxonomical characters of this species is notably to show the morpho-taxonomical relation of this species regarding *Gammarus balcanicus* from type-locality (Kolašin, Montenegro). Variability of some morphological characters within the type-locality population of *G. pavlovici* is presented and some recent problems within the molecular/genetic and classic morphological approach to the recognition of single taxa are discussed.

**Keywords:** Amphipoda, Gammaridae, *Gammarus pavlovici*, *balcanicus*, taxonomy, redescription, North Macedonia

#### **INTRODUCTION**

The freshwater species *Gammarus pavlovici* (fam. Gammaridae) has been discovered and described by S. Karaman (1929b) from the large spring Rašće near Skoplje (North Macedonia). Later this species was attributed to the genus *Rivulogammarus* S. Karaman, 1931 as distinct species (S. Karaman, 1931; Schellenberg, 1937a, etc.), than as subspecies of *Gammarus (Rivulogammarus)* balcanicus Schäferna, 1922 (G. Karaman, 1966), and finally as synonym of *Gammarus balcanicus* (G. Karaman, 1977; G. Karaman & Pinkster, 1987, etc.).

The recent molecular and genetic investigations of *Gammarus balcanicus* Complex indicated that *Gammarus balcanicus* Schäferna, 1922 is limited on the *locus typicus* region in Crna Gora (Montenegro) only (Mamos et al., 2014), and that many other described taxa, affiliated to *Gammarus balcanicus* as synonyms, should be a putative distinct taxa. These opinions were later confirmed by results of various authors worked on delimitation of taxa within genus *Gammarus* in various parts of Europe (Copilaş-Ciocianu, D. & Petrusek, 2017).

<sup>&</sup>lt;sup>1</sup>Gordan S. Karaman (corresponding author: karaman@t-com.me), Montenegrin Academy of Sciences and Arts, Podgorica, MONTENEGRO

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*Gammarus pavlovici*, as one of the earliest taxa affiliated to *G. balcanicus*, is redescribed and figured from type-locality, to show the morphological characters of this species regarding these of *G. balcanicus* from type-locality (Kolašin).

### MATERIAL AND METHODS

The collected samples of *Gammarus* were preserved in 70% ethanol. The specimens were examined and dissected in the mixture of glycerin and water, using a Wild M 20 stereomicroscope. Dissected specimens were transferred onto slides with Faure liquid used for final preservation. The advantage of Faure liquid is that it is possible to dissolve the liquid on slides using water, and remove the dissected pieces for further studies. The body- length of examined specimens was measured by tracing individual's lengths from tip of the rostrum to end of the telson. 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) regarding article 3 of mandibular palpus [A= A-setae on outer face; B= B-setae on inner face; C= additional C-setae on outer face; D= lateral marginal D-setae; E= distal long E-setae]. Terms "setae" and "spines" are used based on its shape, not origin.

All studies in this work are based on the classic morphological, ecological and zoogeographical studies.

### TAXONOMICAL PART

## Order AMPHIPODA Latreille, 1816 Family GAMMARIDAE Leach, 1814

## GAMMARUS PAVLOVICI S. Karaman, 1929

Figs. 1-9

*Gammarus pavlovici pavlovici* S. Karaman, 1929b: 95, fig. 9a, d; Karaman, G., 1974: 12;

Rivulogammarus pavlovici pavlovici S. Karaman, 1931: 51, fig. 9;

*Gammarus (Rivulogammarus) pavlovici* Schellenberg, 1937a: 270; 1937b: 509;

*Gammarus (Rivulogammarus) balcanicus pavlovici* Karaman, G., 1966: 117, figs. 21, 23-26;

*Gammarus balcanicus* (part.) Karaman, G., 1977: 47; Barnard & Barnard, 1983: 464; Karaman, G. & Pinkster, 1987: 213, fig. 2S.

### **MATERIAL EXAMINED: North Macedonia:**

-33= Rašće spring near Skoplje, North Macedonia, May 1929, 9 exp. (leg. S. Karaman) [paralectotypes];

-138= ibid., 1930, 14 exp. (leg. S. Karaman);

-430= ibid., 1933, many exp. (leg. S. Karaman);

Sp. 496= ibid., 1934, 6 exp. (leg. S. Karaman);

S-5798= May 1960, 4 exp. (leg. G. Karaman);

S-2717= ibid., 8.5.1972, many exp. mixed with *Gammarus roeselii* f. *triacanthus* Schäferna, 1922 and *Gammarus dulensis* S. Karaman, 1929a (leg. G. Karaman);

S-5759= ibid., 17.8.1962, many exp. mixed with *Gammarus roeselii* f. *triacanthus* Schäf. 1922 and *Gammarus dulensis* S. Kar., 1929a (leg. G. Karaman).

**DESCRIPTION.Male 11.0 mm**. (S-2717): Body moderately slender, metasomal segments 1-3 with 2-4 short dorsoposterior marginal setae each (fig. 2F). Epimeral plate 1 quadrate, with poorly convex posterior margin bearing 4-5 setae, 5 setae appear at ventroanterior margin.

Epimeral plate 2 slightly pointed, with inclined posterior margin bearing 4-5 setae and with 5 facial and subventral spines. Epimeral plate 3 sharply pointed, with concave posterior margin bearing 2-4 marginal setae and with 3 subventral spines (fig. 2F).

Urosomal segments 1-3 low, poorly elevated, not compressed laterally. Urosomal segment 1 with one dorsomedian and 2 dorsolateral groups of 2 spines each, accompanied by single short setae (fig. 3D). Urosomal segment 2 on each dorsolateral side with group of 3 spines mixed with single short setae, and with one median group of 2 spines accompanied by single short setae. Urosomal segment 3 on each dorsolateral side with group of 3 spines mixed with single short setae, dorsomedian group consisting of 3 spines mixed with single short setae only. Urosomal segment 1 at ventroposterior margin with distal spine near basis of uropod 1 peduncle and with one median group of 3 setae (fig. 3D).

Head with short rostrum and short more or less subrounded (not angular) lateral cephalic lobes; eyes elliptic to poorly reniform, as long as diameter of antenna 1 peduncular article 1 (fig. 1A).

Antenna 1 reaching nearly half of body, peduncular articles 1-3 progressively shorter (ratio: 52:33:22), scarcely setose (fig. 1B); main flagellum consisting of 27 articles scarcely setose. Accessory flagellum nearly as long as last peduncular article and consisting of 4 articles (fig. 1B).

Antenna 2 relatively slender: peduncular article 3 with distal setae at ventral margin; peduncular articles 4 and 5 of equal length, scarcely setose, each with several bunches of facial and distal setae not exceeding diameter of articles themselves (fig. 1C); flagellum relatively slender, longer than last peduncular article and consisting of 12 slender articles bearing short setae up to as long as diameter of articles; many of articles with one calceola. Antennal gland cone short (fig. 1C).

Mouthparts basic. Labrum short, broader than long (ratio: 54:38), with subrounded distal margin (fig. 1D). Labium broader than long, with broad entire convex outer lobes, inner lobes absent (fig. 2A).



Fig. 1. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, male 11.0 mm: A= head; B= antenna 1; C= antenna 2; D= labrum; E= right mandible; F= right incisor; G= right lacinia mobilis; H= left incisor; I= left lacinia mobilis; J= mandibular palpus, outer face (D= D-setae; A= A-setae; E= E-setae); K= mandibular palpus, inner face (B= B-setae); L= right maxilla 1; M= left maxilla 1.



Fig. 2.*Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, male 11 mm: A= labium; B-C= gnathopod 1, outer face; D-E= gnathopod 2, outer face; F= epimeral plates 1-3.

Mandibles well developed, asymmetric to each other. **Left mandible**: molar triturative, with lateral short strong seta, incisor strong, with 5 teeth (fig. 1H), lacinia mobilis with 4 teeth (fig. 1 I), accompanied by nearly 8 strong rakers. **Right mandible**: molar triturative, with lateral long strong seta, incisor with 4 teeth (fig. 1F), lacinia mobilis bifurcate, serrate (fig. 1G), accompanied by nearly 7 strong rakers (fig. 1E). Mandibular palpus of left and right mandible symmetric to each other, consisting of 3 articles: first article short, naked, second article with nearly 18 setae (fig. 1J). Third article subfalciform, shorter than second article (ratio: 60:79), with over 20 D-setae and 6 E setae; on outer face appear one median group of 5 A-setae (fig. 1J), on inner face are attached 5 B-setae in 2 median groups (fig. 1K).

Left and right maxilla 1 asymmetrical to each other. **Left maxilla 1**: inner plate triangular, with row of mesial marginal setae (fig. 1M); outer plate with numerous serrate distal spines; palpus 2-articulated, second article slightly curved, narrow, bearing nearly 8 distal short spine-like setae. **Right mandible**: inner and outer plates like these in left mandible; palpus article 2 dilated, slightly curved, bearing 5-6 distal short strong spines (fig. 1L).

Maxilla 2 longer than broad, with inner plate slightly smaller than outer one bearing distolateral and faciolateral row of setae (fig. 3A); outer plate with distal setae only.

Maxilliped: inner plate longer than broad, with row of distal short spines and lateral row of setae (fig. 4A); outer plate not reaching distal tip of palpus article 2, with distal setae and lateral row of short spines. Palpus 4-articulated, article 2 along outer margin with one median and one distal bunch of setae; article 3 at outer margin with 2 median and one distal bunch of setae; article 4 (dactylus) with short nail and 3-4 short setae at inner margin near nail (fig. 4A), at outer margin with one median seta.

Coxa 1 longer than broad (ratio: 63:51), slightly dilated distally, subrounded ventral margin scarcely setose (fig. 2B).

Coxa 2 remarkably longer than broad (ratio: 68:43), ventral (distal) part subrounded and poorly more narrowed than proximal one, bearing nearly 4 short marginal setae; 3-4 short setae are attached at ventroposterior corner of the plate (fig. 2D).

Coxa 3 remarkably longer than broad (ratio: 77:42), with almost parallel lateral margins and subrounded ventral (distal) margin bearing 4 short setae only (fig. 3B).

Coxa 4 dilated, poorly longer than broad (ratio: 85:82), with strong ventroposterior lobe and poorly convex ventral margin bearing 1-2 short setae only (fig. 3D).

Coxae 5-7 much shorter than coxae 1-4. Coxa 5 much broader than long (ratio: 64:38), with short anterior lobe and 2 posterior marginal setae (fig. 4B). Coxa 6 smaller than coxa 5, broader than long (ratio: 49:28), anterior lobe small, posterior margin scarcely setose (fig. 4C). Coxa 7 entire, much broader than long (ratio: 49:25), ventral margin convex, with 3 posterior setae (fig. 4D).



Fig. 3. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, male 11 mm: A= maxilla 2; B= pereopod 3; C= pereopod 4; D= urosome with uropods 1-2; E= urosome, dorsal projection; F= uropod 3.



Fig. 4. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, male 11 mm: A= maxilliped; B= pereopod 5; C= pereopod 6; D-E= pereopod 7.

Gnathopod 1: article 2 along anterior and posterior margin with numerous long setae, setae in distal part are shorter than these in proximal part of article (fig. 2B); article 3 at posterior margin with one distal bunch of setae. Article 5 triangular, shorter than propodus (ratio: 35:50), along posterior margin with 3-4 transverse rows of short setae, along anterior margin with one median and one distal bunch of setae. Propodus longer than broad (ratio: 105: 62), pyriform, along posterior margin with 4 transverse rows of straight setae and several marginal spines; palm long, strongly inclined, with one corner and several facial spines; in the middle of palm is attached one strong spine accompanied by one bunch of setae much longer than spine itself. Dactylus along outer margin with one median seta, inner margin naked; nail short (fig. 2C).

Gnathopod 2: article 2 along anterior and posterior margin with long straight setae, especially in proximal part (fig. 2D); article 3 at posterior margin with one distal bunch of setae; article 5 triangular, shorter than propodus (ratio: 40:48), along posterior margin with 5-6 transverse rows of straight setae, along anterior margin with one median and one distal bunch of setae. Propodus quadrate, longer than broad (ratio: 95:60), along posterior margin with nearly 8 transverse rows of straight setae (fig. 2E), palm concave, inclined, with strong corner spine and one median palmar spine accompanied by bunch of long setae; dactylus along outer margin with one median seta, inner margin naked, nail short.

Pereopods 3 and 4 moderately strong. Pereopod 3: article 2 along both margins with long setae in proximal part and shorter setae in distal part. Articles 4-6 of unequal length (ratio: 55:38:39). Article 4 along posterior margin with 5 bunches of short straight setae not exceeding diameter of article itself, along anterior margin with 3 bunches of single setae mixed with spines (fig. 3B). Article 5 along anterior margin with distal bunch of short spines and single short setae, along posterior margin with 4 groups of short spines mixed with short straight setae; article 6 along posterior margin with 6 pairs of short spines, along anterior margin with distal bunch of setae. Dactylus short and strong, at inner margin with one seta near basis of the nail.

Pereopod 4 like pereopod 3 but with rather shorter setae along posterior margin. Articles 4-6 of unequal length (ratio: 50:35:36), article 4 along posterior margin with 5 groups of short setae, along anterior margin with one median and one distal spine mixed with single short setae (fig. 3C). Article 5 along posterior margin with 4 groups of short spines mixed with single short setae, along anterior margin with distal bunch of spine and short setae; article 6 along posterior margin with 6 pairs of short spines; dactylus short and strong.

Pereopods 5-7 moderately strong. Pereopod 5 slightly shorter than pereopods 6 and 7, article 2 longer than broad (ratio: 72:52), along anterior margin with row of short spines and proximal group of setae, along posterior margin with nearly 8 short setae, ventroposterior dilatation obtuse (fig. 4B). Articles 4-6 of poorly unequal length (ratio: 53:54:53), article 4 along anterior margin with 5 bunches of short setae, along posterior margin with 2 bunches of 1-3 short spines and setae; article 5 along both margins with 3 bunches of short spines and single short setae. Article 6 along anterior margin with 5 groups of 2-3 short spines, along posterior margin with 3 groups of short spines; dactylus short and strong, with one strong seta at inner margin near basis of the nail, nail shorter than pedestal.

Pereopod 6: article 2 longer than broad (ratio: 80:48), along anterior margin with row of short single spines and proximal bunch of setae, along posterior margin with 7-8 short setae, ventroposterior dilatation obtuse, on inner face with proximal anterior group of submarginal setae (fig. 4C). Articles 4-6 of unequal length (ratio: 61:66:64); articles 4 and 5 along both margins with groups of short spines accompanied by single short setae; article 6 along anterior margin with 5 groups of short spines, along posterior margin with 3 lateral groups of short setae and distal bunch of short spines and setae. Dactylus short and strong, like that in pereopod 5.

Pereopod 7: article 2 longer than broad (ratio: 83:52), along anterior margin with row of nearly 5 spines, distal bunch of spine and short setae, and proximal group of setae, along posterior margin with nearly 9 short setae; on inner face appear distal subventral short spine and seta (fig. 4D), ventroposterior dilatation short, obtuse. Articles 4-6 of unequal length (ratio: 53:63:60); article 4 at anterior margin with 3 bunches of short spines, posterior margin with 2 groups of spines; article 5 along anterior margin with 4 bunches of short spines, along posterior margin with 2 groups of spines. Article 6 along anterior margin with 6 groups of short spines, along posterior margin with 3 median groups of short spines and setae. Dactylus like that of pereopods 5 and 6, at inner margin with strong seta near basis of the nail, along outer margin with one median plumose seta (fig. 4E); nail shorter than pedestal (ratio: 17:44).

Pleopods 1-3 with 2 retinacula. Peduncle of all pleopods with several bunches of short setae (fig. 5B, C, D).

Uropod 1: peduncle with dorsoexternal row of spines, dorsointernal margin with one median and 1-2 distal spines (fig. 3D); one spine is attached at outer face of peduncle; outer ramus is poorly longer than inner one, both rami with 2-3 lateral and 4 distal short spines.

Uropod 2: rami nearly equal or inner ramus is poorly longer than outer one, both rami with 2 lateral and 4-5 distal spines (fig. 3D).

Uropod 3: peduncle with one lateral and several distal spines; inner ramus reaching half of outer ramus, provided with plumose setae along both margins and with 3 lateral and one distal spine. Outer ramus 2-articulated, first article along outer margin with 4 bunches of spines mixed with single smooth setae, along inner (mesial) margin with numerous plumose setae (fig. 3F); second article short, not exceeding diameter of first article and provided with 2-3 lateral and 3-4 distal simple setae.

Telson slightly longer than broad (ratio: 80:75), each lobe with 2 distal spines accompanied by 2 simple setae (the longest setae exceeding length of spines), a pair of short simple setae attached on dorsal surface of each lobe, as well as a pair of very short plumose setae sitting in upper half of lobes (fig. 5A).



Fig. 5. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, male 11 mm: A= telson; B= peduncle of pleopod 1; C= peduncle of pleopod 2; D= peduncle of pleopod 3;

**Female ovig. 8.2 mm**: E= antenna 1; F= antenna 2; G= epimeral plates 1-3; H= telson.



Fig. 6. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, female ovig. 8.2 mm: A-B= gnathopod 1, outer face; C-D= gnathopod 2, outer face; E= peduncle of pleopod 1; F= peduncle of pleopod 2; G= peduncle of pleopod 3.

Coxal gills ovoid, not exceeding the ventral tip of corresponding article 2 of legs; the smaller coxal gills appear on pereopod 7 (figs. 2D; 3B, C; 4B, D, E).

**FEMALE with 14 eggs in marsupium, 8.2 mm**: Body stout, metasomal segments with 2 dorsoposterior marginal short setae. Urosomal segments like these in males, low; first and second urosomal segment with one median and 2 dorsolateral groups of 2-3 short spines and short setae (fig. 9B). Urosomal segment 3 with 2 dorsolateral groups of 3 spines mixed with single short setae, median group is consisting of 2 short setae only. Urosomal segment 1 at ventroposterior corner near basis of uropod 1 peduncle with one spine and one lateral bunch of long setae (fig. 7C).

Epimeral plate 1 subangular, convex posterior margin bearing 4 short setae, at ventroanterior margin appear a bunch of setae (fig. 5G). Epimeral plate 2 pointed, with 2-3 posterior marginal setae, 3 facial spines appear in ventrodistal part. Epimeral plate 3 sharply pointed, with 2-3 posterior marginal setae and with row of 5 subventral spines.

Head like that in male, eyes elliptic to poorly reniform, not exceeding diameter of antenna 1 peduncular article 1.

Antenna 1 mostly like that in male but slightly shorter. Peduncular articles progressively shorter (ratio: 50:30:19), scarcely setose, but some setae can be longer than diameter of articles themselves (fig. 5E); main flagellum consisting of 19 slender articles scarcely setose. Accessory flagellum consisting of 4 articles (fig. 5E), longer than last peduncular article.

Antenna 2 relatively slender; peduncular articles 4 and 5 nearly of equal length or article 5 poorly longer, both articles along ventral margin with 4 bunches of setae (the longest setae exceeding diameter of articles themselves); flagellum relatively slender, consisting of 8 articles bearing setae as long as or longer than diameter of articles themselves, calceola absent (fig. 5F). Antennal gland cone poorly exceeding distal tip of peduncular article 3 (fig. 5F).

Mouthparts like these in male.

Coxa 1 longer than broad (ratio: 70:50), slightly dilated ventrally, bearing 5 short setae at convex ventral margin (fig. 6A). Coxa 2 longer than broad (ratio: 80:43), with 5 short setae at ventral convex margin (fig. 6C). Coxa 3 longer than broad (ratio: 82:50), at ventral convex margin with 5 short setae (fig. 7A). Coxa 4 slightly longer than broad (ratio: 83:65), with large ventroposterior lobe and scarce number of ventral marginal setae, several short setae are attached along posterior margin (fig. 7B).

Coxa 5 much shorter than 4, remarkably broader than long (ratio: 67:43) with small anterior lobe (fig. 8A). Coxa 6 is remarkably smaller than coxa 5, broader than long (ratio: 50:30) (fig. 8B). Coxa 7 only slightly smaller than coxa 6, entire, much broader than long (ratio: 50:25), with 2 setae at posterior margin (fig. 8C).



Fig. 7. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, female ovig. 8.2 mm: A= pereopod 3; B= pereopod 4; C= ventroposterior corner of urosomal segment 1.

Female ovig. 7.0 mm: D= pereopod 3; E= pereopod 4.



Fig. 8. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, female ovig. 8.2 mm: A= pereopod 5; B= pereopod 6; C= pereopod 7; D= uropod 1; E= uropod 2.



Fig. 9. *Gammarus pavlovici* S. Karaman, 1929, Rašće spring, Skoplje, female ovig. 8.2 mm: A= uropod 3; B= urosome , dorsal projection. **Female ovig. 7.0 mm**: C= pereopod 5; D= pereopod 6; E= pereopod 7.

Gnathopods 1-2 smaller than these in male. Gnathopod 1: article 2 along anterior and posterior margin with numerous long setae in proximal and median part, setae in distal part of article are shorter; articles 3-4 like these in male. Article 5 triangular, almost as long as propodus, along posterior margin with several transverse rows of setae, along anterior margin with distal bunch of setae (fig. 6A). Propodus subpyriform, longer than broad (ratio: 83:54), along posterior margin with 3 transverse rows of setae and several marginal spines; palm inclined, slightly convex and defined by 2 strong corner spines; median palmar spine absent, but replaced by group of 4-5 long setae (fig. 6B). Dactylus along outer margin with one median seta, inner margin naked.

Gnathopod 2: article 2 along both margins with numerous long setae in proximal part, setae rather shorter in distal part. Article 5 triangular, narrow, nearly as long as propodus, along posterior margin with several transverse rows of setae, along anterior margin with 2 bunches of setae (fig. 6C). Propodus quadrate, longer than broad (ratio: 81:47), along posterior margin with 6 transverse rows of setae, along anterior margin with 3 bunches of long straight setae. Palm slightly inclined, with 2 corner spines, median palmar spine absent and replaced by group of 5-6 long setae; dactylus like that in gnathopod 1 but shorter, along outer margin with one median seta (fig. 6D).

Percopods 3 and 4 more setose and with longer setae than these in male. Percopod 3: article 2 along anterior and posterior margin with numerous long setae (fig. 7A). Articles 4-6 of unequal length (ratio: 48:32:34). Articles 3 and 4 along posterior margin with distal bunch of longer setae. Article 5 along posterior margin with numerous bunches of simple setae longer than diameter of articles themselves (fig. 7A), along anterior margin with 4 bunches of long setae. Article 5 along posterior margin with nearly 4 bunches of long straight setae remarkably longer than diameter of article itself, along anterior margin with distal bunch of setae only. Article 6 along posterior margin with 5 groups of short spines mixed with short single setae, along anterior margin with distal bunch of setae. Dactylus short and strong, like that in percopod 7.

Pereopod 4: pilosity of articles 2-6 like that in pereopod 3, with numerous straight long setae (fig. 7B). Articles 4-6 are of unequal length (ratio: 43:31:32), dactylus short and strong, like that in pereopod 3.

Percopods 5-7 more setose and with longer setae than these in male. Percopod 5: article 2 longer than broad (ratio: 70:51), along anterior margin with row of short spines and proximal pair of short setae, along posterior margin with nearly 8 setae (fig. 8A); articles 4-6 of unequal length (ratio: 52:45:48); article 4 along anterior margin with 5 bunches of setae (the longest setae exceeding diameter of article itself), along posterior margin 3 single spines and several long setae are attached; article 5 along anterior margin with 3 bunches of setae mixed with single spines, along posterior margin with 2 bunches of setae mixed with spines; article 6 along anterior margin with 5 pairs of short spines, along posterior margin with 2 bunches of setae and single spines; dactylus short and strong. Pereopod 6: article 2 longer than broad (ratio: 80:51), with subangular ventroposterior corner, along anterior margin with row of short spines and with 2 proximal groups of setae, along posterior margin with nearly 7 longer setae; on inner face is attached one subventral seta. Articles 4-6 of poorly unequal length (ratio: 57:55:58); article 4 along anterior margin with 3-4 bunches of longer setae and distal bunch of spines, along posterior margin with 3 bunches of spines mixed with single setae (fig. 8B); article 5 at anterior and posterior margin with 2 bunches of spines mixed with single setae longer than spines (fig. 8B); article 6 along anterior margin with 4 groups of short spines, along posterior margin with 3 groups of short setae and one distal spine. Dactylus short and strong.

Percopod 7: article 2 longer than broad (ratio: 84:55), along anterior margin with row of short spines and 3 groups of proximal setae, along posterior margin with nearly 13 setae, on inner face appear one subventral spine and 2 setae. Articles 4-6 of poorly unequal length (ratio: 47:49:50), bearing less number of setae than these on percopods 5 and 6 (fig. 8C). Article 4 along anterior margin with 3 bunches of spines mixed with setae (setae not exceeding diameter of article), along posterior margin with 2 bunches of spines mixed with single setae. Article 5 along anterior margin with 3 bunches of spines mixed with single setae, along posterior margin with 2 bunches of spines mixed with single setae, along anterior margin with 2 bunches of spines mixed with single setae, along anterior margin with 5 groups of short spines; dactylus short and strong.

Pleopods 1-3 with 2 retinacula. Peduncle of all pleopods covered with several bunches of long setae (fig. 6E, F, G).

Uropod 1: peduncle with dorsoexternal and dorsointernal row of spines (fig. 8D); outer ramus poorly longer than inner one, both rami with lateral and with 4 distal short spines.

Uropod 2: inner ramus poorly longer than outer one, both rami with lateral and with distal 5 spines.(fig. 8E)

Uropod 3 slightly shorter than that in male; peduncle with single lateral and distal spines; inner ramus reaching nearly half of outer ramus, along outer margin with 2-3 lateral and distal short spines accompanied usually with single plumose setae (fig. 9B), inner (mesial) margin is naked. Outer ramus consisting of 2 articles: first article along outer margin with 4 bunches of spines mixed with simple setae, along inner (mesial) margin with row of plumose and single setae; second article short, not exceeding diameter of first article and bearing 4 distal simple setae.

Telson slightly broader than long (ratio: 82:74), each lobe with 1-2 spines accompanied by 4 setae (the longest setae exceeding length of spines); dorsal face of each lobe is covered by one proximal spine and 1-2 single setae, as well as by pair of short plumose setae in distal part of each lobe (fig. 5H).

Coxal gills large, ovoid, reaching or exceeding ventral margin of corresponding article 2 of pereopods (gnathopod 2 and pereopods 3-5) (figs. 6C; 7A, B; 8A), or gills are shorter (pereopods 6 and 7) (fig. 8B, C).

Oostegites moderately narrow, appear on gnathopod 2 and pereopods 3-5, with long marginal setae (figs. 6C; 7B; 8A).

# VARIABILITY OF MORPHOLOGICAL CHARACTERS BY SPECIMENS FROM TYPE-LOCALITY

Body of females usually slightly more stout than that in male, with slightly longer extremities. The shape of eyes, usually semireniform, sometimes more elliptic, always reaching diameter of antenna 1 peduncular article 1. Lateral cephalic lobes subangular to obtusely subrounded. Metasomal segments 1-3 at dorsoposterior margin with 2-4 short setae only; urosomal segments 1-3 low, never compressed laterally, with one dorsomedian and 2 dorsolateral groups of elements (spines and setae), consisting of 2-3 spines accompanied by 1-4 short setae; only dorsomedian group on urosomal segment 3 always without spines, but with 2-4 short setae.

Antenna 1 reaching 2/5- 3/5 of body length, scarcely setose, rather longer, with higher number of articles in males than in females; accessory flagellum consisting of 3-4 articles, as long as or longer than last peduncular article.

Regarding the variability of morphological characters in females, to avoid possibility to compare abnormal or intersex specimens, we compare the females with eggs in marsupium. In the same sample two types of females were observed: females with strong pilosity of body, especially pereopods (figs. 7A, B; 8A, B, C), and females with relatively scarce pilosity of pereopods (figs. 7D, E; 9C, D. E).

In males in hands the pilosity of body and pereopods is more or less constant.

We suppose that this variability of pilosity is not just simple variability of single specimens, but probably exist the seasonal variability of the pilosity of body. Rather similar seasonal differences in the pilosity of body is observed in *Echinogammarus tibaldii* Pinkster & Stock, 1970 in Italy, where the species *E. bolo* G. Karaman & Tibaldi, 1973 and *E. roco* G. Karaman, 1973, despite the remarkable morphological differences, were later considered by some authors as seasonal different forms of *E. tibaldii* (**Pinkster, 1988**). There are no molecular/genetic investigations of this problem to support this conclusion.

This kind of possible seasonal variability has been mentioned already by G. Karaman (1977) in some populations of *Gammarus balcanicus* (sensu auct.) from Bosnia and Herzegovina (Buna River; Bosna River), North Macedonia (Bjelica River near Kičevo; Skopska Crna Gora Mts.), Croatia (Jadro River near Split; Krka River near Knin, etc.] where various types of morphological differences have been observed. The seasonal variability of *Gammarus* population is still poorly known, including molecular/genetic data of this phenomenon, and needs more detailed investigations.

*Gammarus pavlovici* (from type-locality) is very similar to *Gammarus balcanicus* Schäf. (from type-locality) by various general characters (scarce pilosity of body, scarce pilosity of article 2 of pereopods 5-7, presence of

calceola in males, slender antenna 2), but more detailed observations show differences [lacking dorsolateral groups of spines and setae on urosomal segment 1 in *balcanicus*, present in *pavlovici*; rather longer inner ramus of uropod 3 in *balcanicus*, shorter in *pavlovici*, etc.] (for details, see **Karaman, G., 1977**). For this reason, for delimitation of *G. balcanicus* and *G. pavlovici*, as well as for other taxa of *Gammarus balcanicus* superspecies is necessary use also many other characters (molecular/genetic, zoogeography, ecology, etc.]. As populations of *G. balcanicus* Complex are still in process of differentiation, the category subspecies must be not ignored, as well as presence of various intermediate populations.

**LECTOTYPE**: Male 8.0 mm (No. 33) and paralectotypes are deposited in KARAMAN's Collction in Podgorica, Montenegro.

LOCUS TYPICUS: Rašće spring near Skoplje, North Macedonia.

**ECOLOGY**. In type-locality of *G. pavlovici*, spring Rašće near Skoplje (North Macedonia), three *Gammarus* species were observed in mixed populations: *Gammarus roeselii* f. *triacanthus* Schäferna, 1922 (sensu auct.), *Gammarus dulensis* S. Karaman, 1929b, *Gammarus pavlovici* S. Karaman, 1929b and *Niphargus macedonicus* S. Karaman, 1929a. All specimens are with distinct morphological characters of corresponding species, and no transitive specimens among *Gammarus* specimens have been observed.

## DISCUSSION

*Gammarus balcanicus* Complex [or *Gammarus balcanicus* multispecies] show large plasticity and variability of numerous morphological characters between various populations as well as between specimens of one population as respond on different ecological and other conditions and events.

One species (multispecies) is consisting of numerous populations with rather different morphological characters each. If these populations with stable different morphological characters, settled well limited areal, these populations were considered, according classical taxonomy, as possible subspecies (as recognized category). If some of them acquire reproductive isolation, these populations were considered a distinct species.

Recently numerous scientists are providing various molecular/genetic studies of *Gammarus* taxa, with remarkably different approach to delimitation of various taxonomical categories.

**Hebert et al.** (2003) have opinion than the taxonomic expertise is collapsing, and they suggested: "We are convinced that the sole prospect for a sustainable identification capability lies in the construction of systems that employ DNA sequences as taxon 'barcodes'. We establish that the mitochondrial gene cytochrome c oxidase I (COI) can serve as the core of a global bioidentification system for animals."

Sukumaran & Knowles, L. (2017) discussing about multispecies, and delimitation of taxa, considered "speciation as an extended process rather than an instantaneous event and carry out species delimitation inference on these data

under the multispecies coalescent." They suggested "that the multispecies coalescent diagnoses genetic structure, not species, and that it does not statistically distinguish structure associated with population isolation vs. species boundaries. Because of the misidentification of population structure as putative species, our work raises questions about the practice of genome-based species discovery."

About importance of recognition and delimitation of species and other taxonomical categories **Sukumaran & Knowles (2017)**, pleading that: "Not all populations become species. Instead, speciation theory points to a continuum for the probability that a population lineage will evolve into a new species. Depending on the extent and duration of isolation and the form and strength of selection, speciation becomes more or less a protracted process, with new lineages only gradually and stochastically evolving from the initially isolated lineages into true species over time". They well underlines that "Misidentification of population structure as putative species is therefore emerging as a key issue that has received insufficient attention, especially with respect to methodologies for delimiting taxa based on genetic data alone".

The introduction of cryptic species and the problem of recognition of cryptic species regarding rather morphologically different populations of already known taxa, remains very serious problem in taxonomy today, and many authors have different approach and suggestions. **Fišer et al. (2018)** by this way, cited **Ryberg (2015)** that "cryptic species are *de facto* taken into consideration by an increasing number of biodiversity studies that use sequence clusters rather than nominal species as units for taxonomic diversity".

Taxonomical investigations are today in transitional period when the "classical taxonomy", based mainly on external morphology is considered by new genetic-molecular researchers as invalid or scarce valid, suggesting that only genetic-molecular approach [despite using limited methods of research at the moment] is crucial and valid. Combination of all kinds of research (which include morphology, molecular and genetic studies, ecology, histology, anatomy, etc.) will resolve the problems of delimitation of various taxa and understanding taxonomical position evolutive and of single populations, without underestimation of any of them. Similar opinion wrote also Copilas-Ciolpan et al (2018) mentioning that "The multidisciplinarity of integrative taxonomy is particularly useful for clarifying the systematics of speciose groups that are poorly differentiated morphologically, and this approach can also illuminate their evolutionary history and biogeography".

In this light, **Thomson et al.** (2018) mentioned: "Discovery of new organisms together with advances in methodology continue unabated, leading to a constant reevaluation of the boundaries between taxonomic entities. Species (and higher taxa) comprise related organisms that may be clustered together differently depending on which sets of criteria are emphasized". They remember that "Through taxonomic research, our understanding of biodiversity and classifications of living organisms will continue to progress. Any system that

restricts such progress runs counter to basic scientific principles, which rely on peer review and subsequent acceptance or rejection by the community, rather than third-party regulation".

Further studies of these problems by numerous scientists will help us to understand the domains and limitations of various methods for the classification and phylogeny of various *Gammarus* populations, but always with "open mind" approach.

The increasing flow of described new species of Amphipoda in the next future, **Arfianti et al. (2018)** predicted, using a nonhomogeneous renewal process model, discovery 5600 to 6.600 new Amphipoda by the year 2100], what will show the richness of Amphipoda taxa, but increasing number of delimitation problems of various taxa also.

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