Copyright © 2010 · Magnolia Press

Article



# Two new species of the genus *Niphargus* Schiödte, 1849 (Amphipoda, fam. Niphargidae) from the Frasassi cave system in Central Italy

# GORDAN S. KARAMAN<sup>1</sup>, BETTY BOROWSKY<sup>2</sup> & SHARMISHTHA DATTAGUPTA<sup>3</sup>

<sup>1</sup>Montenegrin Academy of Sciences and Arts, Podgorica, Crna Gora (Montenegro). E-mail: karaman@t-com.me <sup>2</sup>Dept. of Biology, Nassau Community College, Garden City, New York, 11530, USA. E-mail: borowsb@ncc.edu <sup>3</sup>Centre of Geobiology, Georg-August University, Gottingen, Germany. E-mail: sdattag@uni-goettingen.de

## Abstract

Two new subterranean species of the genus *Niphargus* Schiödte, 1849 (*Niphargus montanarius*, **n. sp.** and *N. frasassianus*, **n. sp.** (Crustacea, Amphipoda, Gammaridea, Niphargidae) from the Frasassi cave system in Genga, the Marches, Italy, are described and figured, and their taxonomic status within the genus *Niphargus* is discussed.

Key words: Crustacea, Amphipoda, Niphargus, taxonomy, subterranean waters

### Introduction

The genus *Niphargus* Schiödte, 1849 belonging to the family Niphargidae (Crustacea, Amphipoda, Gammaridea), contains over 300 known species and subspecies, and is widespread in subterranean waters throughout Europe. *Niphargus* species are found from England and Spain to Asia Minor and the Near East. Most of these taxa are endemic and restricted to relatively small areas, typically to karstic areas with caves and underground pools. In Italy alone there are nearly 60 *Niphargus* (sensu auct.) taxa known, with over half of these endemic to Italy [G. Karaman & Ruffo (1986); G. Karaman (1993); G. Karaman (1994), Ruffo (1995)].

Earlier collections from the Frasassi cave complex yielded one species (*Niphargus ictus*, described by G. Karaman (1986) (Grotta del Fiume, 8 Ma)(=Marche). The present paper describes two more that were collected during intensive studies of the caves and their fauna, organized by the Osservatorio Geologico di Coldigioco, near Apiro (Central Italy).

## Material and methods

The samples were collected from the subterranean waters using small hand nets, initially preserved in 98% ethanol and later transferred to 70% ethanol. Specimens were examined and dissected using a Wild M20 stereomicroscope and drawn using a camera lucida attachment. Small appendages (mouthparts, uropods, telson) were temporarily mounted in lactic acid, and later transferred to liquid of Faure. The body length of specimens examined was measured by tracing individual's mid-trunk lengths (tip of the rostrum to end of telson) using a camera lucida. All illustrations were inked manually.

Systematic section

Order Amphipoda Latreille, 1816 Suborder Gammaridea Latreille, 1802

Family Niphargidae G. Karaman, 1962

Genus Niphargus Schiödte, 1849

*Niphargus montanarius*, n. sp. (Figs 1–6)

**Material examined**. ITALY: "Lago Bugianardo", cave on left side of the Sentino River at the base of Frasassi Gorge (Genga reg., central Italy), 1 specimen, adult (male, holotype), May, 2008 (leg. Dattagupta, S.); -ibid., September, 15, 2009, 2 specimens (leg. Dattagupta, S.). The holotype and one female specimen are deposited in the Museum of Natural History in Verona, Italy.

**Diagnosis**. Large species, large unequal gnathopods bearing a row of short setae along outer margin of dactyl. Outer plate of maxilla 1 with 7 spines, each spine bearing 1 or 2 lateral teeth. Dactyl of pereopods 3–7 with several inner marginal spines (more than one on almost all dactyls); segment 2 of pereopods 5–7 narrow, unlobed. Pleopods with 2 retinacula. Epimeral plates 2–3 angular. Uropods 1–2 with rami of equal length (females) or inner ramus slightly longer than outer one (male). Uropod 3 long, second segment of outer ramus long. Telson with gaping lobes.

**Description**. Male 21.0 mm, mature (holotype):

Body slender, metasomal segments 1-3 with 3 or 4 short dorsoposterior setae each; epimeral plates 1-2 broadly angular, with slightly convex posterior margin, epimeral plate 3 angular with straight posterior margin; epimeral plate 2 with 2 subventral spines, epimeral plate 3 with 3 subventral spines (fig. 3E).

Urosomite 1 with 2 setae on each side, and with one ventroposterior spine near peduncle of uropod 1 (fig. 1I); urosomite 2 on each side with 3 slender spines and 1 seta (fig. 1I), urosomite 3 smooth.

Head with short rostrum, lateral cephalic lobes short, slightly rounded, with concave ventroanterior margin (fig. 2A), eyes absent.

Antenna 1 reaching half of body length; peduncular segments 1–3 progressively shorter, peduncular segment 3 reaching nearly half of peduncular segment 2, all sparsely setose (fig. 1A); main flagellum with 25 articles, most of them with one short aesthetasc each; accessory flagellum short, 2-segmented (fig. 1A), almost reaching half the length of peduncular segment 3.

Antenna 2 peduncular segment 5 slightly longer than segment 4, each with several bunches of setae (fig. 1B); flagellum slender, nearly as long as last peduncular segment, consisting of 13 articles; antennal gland cone short (fig. 1B).

Mouthparts. Labrum broader than long, entire (fig. 3A). Labium with inner lobes (fig. 4A).

Maxilla 1: inner plate short, with 2 distal setae (fig. 3B); outer plate with 7 spines [1 or 2 spines with 2 lateral teeth each, other spines with one lateral tooth each]; palp 2-segmented, with 7 distal setae (fig. 3B). Maxilla 2 normal, both plates with marginal setae only (fig. 5A).

Maxilliped: inner plate short, with 4 or 5 distal spines intermixed with single setae (fig. 4B); outer plate with row of inner marginal spines; palp articles 3 at outer margin with 2 median setae; dactyl slender, with one median seta at outer margin; nail shorter than pedestal (fig. 4B).

Mandibles with triturative molar (fig. 1C). Left mandible with 5 incisor teeth, and 9 rakers, lacinia mobilis with 4 teeth (fig. 1D).

Right mandible with 4 incisor teeth and 8 rakers, lacinia mobilis pluritoothed (fig. 1C). Mandibular palp 3-segmented, first segment smooth; second segment with one facial and 14 marginal setae; palp segment 3 subfalciform, longer than palp segment 2, bearing on the outer face one group of A-setae (fig. 1F), on the inner face 5 groups of B-setae (fig. 1E); and along the inner margin nearly 30 short D-setae and 7 distal E-setae (fig. 1E).

Coxae 1–7 short, coxae 1–2 much smaller than corresponding gnathopods (fig. 2B, D). Coxa 1 broader than long, with slightly rounded ventroanterior corner (fig. 2B); coxae 2–3 slightly longer than broad, coxa 4 nearly as long as broad, each with very short marginal setae (fig. 3C—D). Coxae 5–7 short (fig. 4 C–E).



**FIGURE 1.** *Niphargus montanarius*, **n. sp.**, Lago Bugianardo, male 21.0 mm (holotype): A = antenna 1; B = antenna 2; C = right mandible; D = left mandible; E = mandibular palp, inner face; F= mandibular palp, outer face; G = telson; H = uropod 3; I = urosome with uropods 1–2. Scale bars: 1 = 0.25 mm (C, D); 2= 0.5 mm (E–G); 3 = 1.0 mm (A, B, I); 4 = 2.0 mm (H).

Gnathopods 1–2 relatively large, gnathopod 2 much larger than 1 (fig. 2 B,D).

Gnathopod 1: segments 3–4 along posterior margin with one posterior group of setae each (fig. 2B); segment 5 shorter than 6; segment 6 trapezoid, slightly longer than broad, with nearly 10 posterior groups of setae on the margin (fig. 2B); palm slightly convex, oblique; nearly 2/3 of propodus length, defined on outer face by one strong corner spine accompanied laterally by 3 shorter slender toothed spines (fig. 2B–C) and 2

longer setae on outer face (fig. 2B), one short spine sitting close behind a strong corner spine on the inner face (fig. 2C). Dactyl reaching posterior margin of segment 6, bearing a row of 8 short single marginal setae, nail short.



**FIGURE 2.** *Niphargus montanarius*, **n. sp.**, Lago Bugianardo, male 21.0 mm (holotype): A = head; B, C = gnathopod 1; D, E = gnathopod 2. Scale bars: 1 = 0.25 mm (C, E); 2 = 0.5 mm (A); 3 = 1.0 mm (B, D).

Gnathopod 2: segments 3–4 with one posterior group of setae each; segment 5 much shorter than 6, narrow (fig. 2D). Segment 6 large, subtriangular, but almost egg-shaped, slightly longer than broad, bearing nearly 14 posterior marginal groups of setae; palm slightly convex, but extremely oblique to the basis of segment 6 (propodus), defined on outer face by one strong corner spine accompanied by 3 short slender toothed spines sitting behind corner spine, and by 2 long setae (fig. 2D), on inner face by one short spine near the corner (fig. 2E). Dactyl not reaching posterior margin of segment 6, bearing a row of 8 short single setae along outer margin, nail short.

Percopods 3–4 long and slender, posterior margin of segment 6 with row of single spines accompanied by short setae (fig. 3C–D); posterior setae on segments 4–5 of percopod 3 are slightly longer than those of percopod 4. Dactyls short, not exceeding 1/3 length of segment 6, their nail slightly shorter than pedestal, bearing at outer margin one medial seta, and at inner margin 2–3 single spines (percopod 3) or 2 spines only (percopod 4) (fig. 3C–D).



**FIGURE 3.** *Niphargus montanarius*, **n. sp.**, Lago Bugianardo, male 21.0 mm (holotype): A = labrum; B = maxilla 1; C = pereopod 3; D = pereopod 4; E = epimeral plates 1–3. Scale bars: 1 = 0.25 mm (A); 2 = 0.5 mm (B); 3 = 1.0 mm (E); 4 = 1.0 mm (C, D).

Percopods 5–7 long and slender, progressively longer towards percopod 7, their segments 4–6 bearing bunches of short spines and setae along both margins (fig. 4C–E); segment 2 narrow, slightly more than twice as long as broad, with straight or slightly concave posterior margin bearing row of short setae, ventroanterior and ventroposterior lobe not developed. Dactyl of percopods 5–7 short, several times shorter than segment 6,

bearing one median plumose seta at outer margin, and one spine at inner margin (pereopods 5–6) or 2 spines (pereopod 7); nail shorter than pedestal (fig. 4C–E).

Pleopods 1–3 with 2 retinacula each. Peduncle of pleopod 1 with row of 4 short setae along anterior margin (fig. 5B); peduncle of pleopod 2 with only one anterior seta (fig. 5C); peduncle of pleopod 3 with row of posterior short setae (nearly 8 setae) (fig. 5D).

Uropod 1: peduncle with row of dorsoexternal row of spine-like setae, and with row of setae along dorsointernal margin (fig. 1I); rami slender, each with bunches of short spines and setae; outer ramus slightly shorter than inner ramus, both with short distal spines (fig. 1I).



**FIGURE 4.** *Niphargus montanarius*, **n. sp.**, Lago Bugianardo, male 21.0 mm (holotype): A = labium; B = maxilliped; C = pereopod 5; D = pereopod 6; E = pereopod 7. Scale bars: 1 = 0.5 mm(A); 2 = 0.5 mm(B); 3 = 1.0 mm(C-E).

Uropod 2: outer ramus slightly shorter than inner one, both rami with lateral and distal short spines (fig. 1I).

Uropod 3 long, peduncle short; inner ramus short, scale-like, with 2 distal and one lateral spine; outer ramus 2-segmented, long; first segment with short spines and setae along both margins; second segment slightly shorter than first one (fig. 1H), bearing short setae on both margins and at tip.

Telson short, slightly longer than broad, with gaping lobes (fig. 1G), each lobe with 4 distal short spines and one inner marginal spine; a pair of short plumose setae appears near the middle of each lobe.



**FIGURE 5.** *Niphargus montanarius*, **n. sp.**, Lago Bugianardo, male 21.0 mm (holotype): A = maxilla 2 length B, C, D = peduncle of pleopods 1–3. Female 17.0 mm: E-G = segment 2 of pereopods 5–7; H-L = dactyl of pereopods 3–7; M = uropod 1; N = uropod 2; O = uropod 3; P = telson. Scale bars: 1 = 0.5 mm (A); 2 = 0.5 mm (H–L, P); 3 = 1.0 mm (B–D); 4 = 1.0 mm (E–G); 5 = 1.0 mm (M–O).

Gills on gnathopods 1–2 and percopod 3–4 distinctly shorter than the corresponding segment 2 (figs. 2D, 3C–D). Gills on percopods 5–6 short, ovoid (fig. 4C–D).

**Female** 17.0 mm, mature: Body with setose oostegites and 22 juv. specimens in marsupium. Epimeral plate 1 broadly angular (fig. 6H), plates 2–3 with distinct ventroposterior corner and with one subventral spine (plate 2) or 3 subventral spines (plate 3) (fig. 6H).

Urosomite 1 on each dorsolateral side with 1 seta, urosomite 2 on each dorsolateral side with 2 spines, urosomite 3 smooth. Ventroposterior corner of urosomite 1 with one bunch of 2 spines (fig. 5M).

Antenna 1 slightly exceeding half of body length (ratio: 10:17), main flagellum of antenna 1 with 31 articles, that of antenna 2 with 11 articles. Maxilla 1: inner plate with 3 setae, outer plate like that in male, palp with 7 setae.

Inner plate of maxilliped with 4 distal spines, palp segment 3 at outer margin with 1 or 2 median setae. Mandibular palp segment 2 with 17 setae, palp segment 3 with 26 D-setae, one group of A-setae and 6 groups of B-setae.

Coxa 1 broader than long, coxae 2–3 slightly broader than long, coxa 4 nearly as long as broad (fig. 6 length A–D).



**FIGURE 6.** *Niphargus montanarius*, **n. sp.**, Lago Bugianardo, female 17.0 mm: A - D = coxae 1-4; E = gnathopod 1; F = right gnathopod 2; G= left gnathopod 2; H = epimeral plates 1–3. Scale bars: 1 = 1.0 mm (A-F, H).

Gnathopods 1–2 large. Gnathopod 1: much smaller than 2, segment 6 slightly longer than broad, with 9 groups of setae along posterior margin; palm, convex, inclined 2/3 of propodus length, bearing one strong corner spine accompanied laterally on outer face by 4 slender toothed spines, and on inner face by one short subcorner spine (fig. 6E); dactyl reaching posterior margin of segment 6, with row of 8 setae along outer margin (fig. 6E).

Gnathopod 2: segment 6 almost egg-shaped, slightly longer than broad, with 12–14 groups of setae along posterior margin; palm defined on outer face by 1 or 2 strong corner spines, accompanied by 3 or 4 slender toothed spines lateral to the corner spine and on the inner face by one short subcorner spine (fig. 6F–G); dactyl not reaching posterior margin of segment 6, with 7 short setae along outer margin (fig. 6G).

Percopods 3–4 as in male, but dactyls of percopod 3 along inner margin with 1–3 spines each (fig. 5H); dactyl of percopod 4 along inner margin with 3 or 4 spines (fig. 5 I).

Segment 2 of percopods 5–7 with narrow segment 2 more than twice as long as broad, without distinct ventroposterior and ventroanterior lobe (fig. 5 E–G). Dactyl of percopods 5 and 7 with 2 spines at inner margin, that of percopod 6 with one spine along inner margin (fig. 5 J–L).

Pleopods 1–3 with 2 retinacula each. Peduncle of pleopod 1 with 4 posterior and one anterior seta; that of pleopod 2 with 2 anterior setae only; peduncle of pleopod 3 with 4 anterior setae.

Uropods 1–2 slender. Uropod 1: peduncle with row of dorsointernal strong setae and dorsoexternal row of spines (fig. 5M), rami of equal length, bearing lateral and distal short spines and lateral bunches of simple setae.

Uropod 2: rami of equal length, each with short lateral and distal spines (fig. 5N).

Uropod 3 relatively long, reaching half of body-length. Inner ramus short, scale-like, with 2 distal spines; outer ramus 2-segmented: first segment with spines along both margins and with single plumose setae along inner margin; second segment reaching nearly 1/3 of first segment, with simple setae along both margins and tip (fig. 5O).

Telson nearly as long as broad, with gaping lobes bearing 3 distal spines each and up to 1 inner marginal spine; a pair of short plumose setae appears near the middle of each lobe (fig. 5P).

Coxal gills relatively short, not reaching tip of corresponding segment 2 of percopods. Oostegites broad, with marginal setae.

**Variability**. The presence of 2 strong corner spines on segment 6 of one gnathopod 2 in the female is atypical (usually only one spine is present), but this is common within the *Niphargus* taxa.

The number of spines along the inner margin of the dactyl of pereopods 3–7 is variable, but there are usually at least several spines on each dactyl.

**Etymology**. This species is named for Prof. Dr. Alessandro Montanari, Director of the Osservatorio Geologico di Coldigioco located near Apiro, the Marches, Italy in honor of his comprehensive studies of the Frasassi cave system and the geological history of this part of Italy in general.

**Remarks.** *Niphargus montanarius*, **n. sp.** is distinctly different from all other known *Niphargus* taxa in Italy. While large unequal gnathopods are present in some other taxa in Italy (especially in the subgenus *Orniphargus*, as *N. patrizii* Ruffo & Vigna-Tagl., 1968, *N. parenzani* Ruffo & Vigna-Tagl., 1968, as well as *N. sodalis* G. Kar., 1984 from Teramo, various species of the *N. stygius* group, etc.), *N. montanarius* is unique in its combination of characters: long uropod 3 in males and females, more numerous spines on dactyls of pereopods 3–7, poorly developed armature on urosomites, and spines of outer plate in maxilla 1 with 1 or 2 lateral teeth each.

A shorter dactylus of gnathopod 2 not reaching the posterior margin of segment 6 is also present in large specimens of *Niphargus cornicolanus* Iannilli & Vigna-Tagl., 2005, known from Pozzo del Merro, 32 La (Lazio, central Italy), but this species differs significantly from *N. montanarus* by the short uropod 3 in males and females, the narrow long telson, broader segment 2 of pereopods 5–7, etc.

Other *Niphargus* species have large gnathopods as well, but these feed by filtering mud and detritus, and the shapes of the gnathopods are modified for this purpose (for example *Niphargus trullipes* Sket, 1958 from Vjetrenica cave in Herzegovina). The large size and shape of its extreme gnathopods suggests that it may be a predator, which is very unusual in gammarid amphipods.

(Figs 7–12)

**Material examined**. ITALY: "Pozzo dei Cristalli", in Frasassi cave system (Genga region, central Italy), May 27, 2008, 2 specimens (leg. Dattagupta, S.) — holotype and paratype.

-Tunnel of the sulfide spring ("Sorgente sulfurea del tunnel"), Frasassi cave system [Genga region, central Italy), 14 September, 2009, many specimens [leg. Karaman, G. & Karaman, B.].

- ibid., 15. September, 2009, many specimens [leg. Borowsky, B. & Borowsky, R.].

- "Grotto sulfureo, ramo sulfureo" (Frasassi cave system), 2008 and 2009, 5 specimens (leg. Dattagupta, S.).

Holotype and paratype are deposited in the Museum of Natural History in Verona, Italy.

**Diagnosis**. Body stout. Epimeral plates 1–3 with slightly rounded ventroposterior corners in males. Gnathopods 1–2 relatively small, only slightly unequal in size and shape, with dactyl reaching posterior margin of segment 6 and provided with a row of long setae along outer margin. Pereopods 3–7 short and stout, their dactyl short, with one spine along inner margin. Segment 2 of pereopods 5–7 less than twice as long as broad, Pleopods with 2 retinacula each. Uropods 1–2 with inner ramus much longer than the outer one. Second segment of uropod 3 in males exceeding half of first segment, in females is much shorter. Telson short, bearing short distal, marginal and facial spines. Outer plate of maxilla 1 with 7 spines; some of these spines with 2 or 3 lateral teeth sitting in the opposite sides of the spine itself.

**Description. Male** 15 mm, mature (holotype):

Body strong and stout, metasomal segments 1–3 with 3 or 4 dorsoposterior short marginal setae each. Epimeral plates 1–3 with slightly rounded ventroposterior corner; plates 2–3 with 3 or 4 short subventral spines each (fig. 9H). Urosomite 1 on each side with one spine-like seta, urosomite 2 with 2 dorsolateral spines on each side, urosomite 3 smooth (fig. 7G). Urosomite 1 with ventroposterior short spine near the basis of peduncle of uropod 1 (fig. 7G).

Head with short rostrum and short slightly rounded lateral cephalic lobes (fig. 7A), eyes absent.

Antenna 1 reaching one third of the body-length; peduncular segments 1–3 progressively shorter; peduncular segment 3 slightly exceeding half of peduncular segment 2 (fig. 7B), all sparsely setose; main flagellum consisting of 19 articles (most of them with one short aesthetasc each); accessory flagellum short, 2-segmented (fig. 7D).

Antenna 2: peduncular segment 5 shorter than 4, each with bunches of longer setae (fig. 7C); flagellum longer than last peduncular segment, consisting of 11 articles; antennal gland cone short (fig. 7C).

Coxae 1–4 short, coxae 1–3 nearly as long as broad (figs 8B–D, 9A), coxa 4 slightly broader than long (high), all with short marginal setae (fig. 9C). Coxa 1 with slightly rounded ventroanterior corner. (fig. 8B). Coxae 5–7 short (fig. 10B, D, F).

Labrum entire, similar to that of *N. montanarius*. Labium without inner lobes, similar to that of *N. montanarius*. Maxilla 1: inner plate with 2 setae, outer plate with 7 spines: inner spine with 3–5 lateral strong setae, 6 spines with 2 lateral strong teeth sitting at opposite sides of spines (fig. 7E, F); palp 2-segmented, short, with 6 long distal setae slightly shorter than last segment (fig. 7E).

Maxilla 2: both plates with numerous marginal setae only (fig. 8A).

Maxilliped: inner plate short, with 3 distal spines intermixed with single setae (fig. 10A); outer plate not exceeding half of posterior margin of palp segment 2, with row of marginal spines; palp segment 3 at outer margin with one medial and one distal bunch of setae (fig. 10A); palp segment 4 with nail shorter than pedestal.

Left mandible: incisor with 5 teeth and 9 rakers, lacinia mobilis with 4 teeth (fig. 11B). Right mandible: incisor with 4 teeth and 8 rakers, lacinia mobilis pluridentate (fig. 11A).

Palp of both mandibles 3-segmented: first segment smooth; second segment with 13–15 setae; palp segment 3 short, almost a long as palp segment 2, bearing at posterior margin 26 D-setae and 7 long distal E-setae (fig. 11C); there are 5 single B-setae on the inner face (fig. 11C), and one group of 7 A-setae on the outer face (fig. 11D).



**FIGURE 7.** *Niphargus frasassianus*, **n. sp.**, Pozzo dei Cristalli, male 15 mm (holotype): A = head; B = antenna 1; C = antenna 2; D = accessory flagellum; E, F = maxilla 1; G = urosome with uropods 1–2; H = telson. Scale bars: <math>1 = 0.17 mm (D); 2 = 0.25 mm (E); 3 = 0.25 mm (H); 4 = 0.25 mm (A, C); 5 = 1.0 mm (B); 6 = 1.0 mm (G).

Gnathopods 1–2 relatively small, stout, their segment 6 is not larger than corresponding coxae (fig. 8B, D). Gnathopod 1 slightly smaller than gnathopod 2, segments 3–4 of both gnathopods along posterior margin with one group of setae (fig. 8B, D), segment 5 is slightly shorter than segment 6.

Gnathopod 1: segment 6 slightly longer than broad, with almost parallel lateral margins, and along posterior margin with 6 groups of setae (fig. 8C). Palm convex, oblique almost to half of segment 6 length, defined on outer face by one strong corner spine accompanied laterally by 3 slender toothed spines and 5 long setae on the face (fig. 8C), one short subcorner spine on the inner face (fig. 8C); dactyl reaching posterior margin of segment 6, strong, bearing row of 6 long marginal setae along outer margin (fig. 8C).

Gnathopod 2: segment 6 slightly longer than broad, trapezoidal shape, with 8 groups of setae along posterior margin (fig. 8E), palm slightly convex, oblique to nearly 2/5 of segment 6 length, defined on outer

face by one strong corner spine accompanied laterally by 3 slender toothed spines and 5 long setae (fig. 8E), on inner face by one short subcorner spine (fig. 8F); dactyl reaching posterior margin of segment 6, with 8 strong setae along outer margin (fig. 8E).



**FIGURE 8.** *Niphargus frasassianus*, **n. sp.**, Pozzo dei Cristalli, male 15 mm (holotype): A = maxilla 2; B, C = gnathopod 1; D–F = gnathopod 2. Scale bars: 1 = 0.25 mm (A); 2 = 0.5 mm (C, E); 3 = 1.0 mm (B, D).

Pereopods 3–4 nearly equal, stout, with strong articles (fig. 9A, C); dactyls short and stout, with one spine at inner margin, nail nearly as long as pedestal (fig. 9B, D).

Percopods 5–7 short and stout, progressively longer towards percopod 7, segment 2 less than twice as long as broad, with straight or slightly concave posterior margin bearing very short marginal setae (fig. 10B, D, F), ventroanterior lobe not developed, ventroposterior area enlarged, but without distinct lobe; segments 3–6 short and broad, segments 4–6 with bunches of strong spines and single setae on both margins; dactyl short and stout, with one spine on inner margin and one median seta on outer margin. Nail of dactyl on percopod 5 nearly as long as pedestal (fig. 10C), those of percopods 6–7 shorter than pedestal (fig. 10E, G).

Pleopods 1–3 with 2 retinacula each. Peduncle of pleopod 1 with 3 anterior marginal setae (fig. 9E); peduncle of pleopod 2 with one anterior marginal seta (fig. 9F); peduncle of pleopod 3 with 2 short posterior marginal setae (fig. 9G).



**FIGURE 9.** *Niphargus frasassianus*, **n. sp.**, Pozzo dei Cristalli, male 15 mm (holotype): A, B = pereopod 3; C, D = pereopod 4; E–G = peduncle of pleopods 1–3; H = epimeral plates 1–3. Scale bars: 1=0.5 mm (E–G); 2 = 1.0 mm (H); 3=1.0 mm (A, C).

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae (except distal spine) (fig. 7G). Outer ramus reaching nearly 3/5 of inner ramus, both with lateral and distal spines, inner ramus with 2 bunches of long setae also (fig. 7G).

Uropod 2: outer ramus slightly shorter than inner one, both rami with lateral and distal spines (fig. 7G).

Uropod 3 elongated, inner ramus short, scale-like, with 2 lateral and distal short spines; outer ramus long, 2-segmented, first segment with bunches of short spines and single setae along both margins; second segment exceeding half of first segment, bearing short setae at both margins (fig. 11E).

Telson slightly broader than long, with gaping lobes, each lobe with 4 distal spines (fig. 7H); several single spines appear at both margins and face of each lobe; a pair of short plumose setae appears near the middle of each lobe.

Coxal gills not reaching distal tip of corresponding segment 2 (fig. 9A).



**FIGURE 10.** *Niphargus frasassianus*, **n. sp.**, Pozzo dei Cristalli, male 15 mm (holotype): A = maxilliped; B, C = pereopod 5; D, E = pereopod 6; F,G = pereopod 7. Scale bars: 1 = 0.5 mm (A); 2 = 1.0 mm (B, D, F).

**Female** 10.0 mm, mature (paratype, with setose oostegites): Antenna 1 reaching one third of the body-length; main flagellum consisting of 18 articles; flagellum of antenna 2 is with 7 articles.

Urosomal segment 1 with one dorsolateral seta on each side (fig. 12 I); urosomite 2 with 2 spines on each dorsolateral side (fig. 12I). Coxa 1 broader than long, coxae 2–3 slightly longer than broad, coxa 4 nearly as long as broad (fig. 12A–D).

Mouthparts as in male. Outer plate of maxilla 1 as in male, 6 spines provided with 2 opposite lateral strong teeth (fig. 11F). Inner plate of maxilliped with 3 distal spines.

Mandibular palp segment 2 with 11 setae; palp segment 3 with 23 D-setae and 6 long distal E-setae; on outer face on group of 5 A-setae, on inner face 5 single B-setae.



**FIGURE 11.** *Niphargus frasassianus*, **n. sp.**, Pozzo dei Cristalli, male 15 mm (holotype): A = right mandible; B = left mandible; C = mandibular palp, inner face; D = tip of mandibular palp, outer face; E = uropod 3. Female 10 mm (paratype): F = maxilla 1, tip of outer plate; G = propodus and dactylus of gnathopod 1; H = propodus and dactylus of gnathopod 2; I = uropod 3.

Gnathopods 1–2 have slightly different size, their segment 6 trapezoid, slightly longer than broad, with palm oblique slightly less than half of posterior margin of segment 6, almost straight. Gnathopod 1: posterior margin of segment 6 with 5 groups of setae (fig. 11G), 5 facial setae are present near corner spine.

Gnathopod 2: posterior margin of segment 6 with 6 groups of setae, 4 setae present near corner spine (fig. 11H).

Percopods as in males, but segment 2 of percopods 5–7 slightly shorter, with poorly developed ventroposterior lobe (fig. 12E–G).

Epimeral plates 1–2 broadly angular, with convex posterior margin (fig. 12H) and subventral spines, epimeral plate 3 with produced almost subrounded ventroposterior corner (fig. 12H). Oostegites broad, setose.

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae (except distal spine), inner ramus only slightly longer than outer one, both with lateral and distal strong spines (fig. 12I).



**FIGURE 12.** *Niphargus frasassianus*, **n. sp.**, Pozzo dei Cristalli, female 10 mm (paratype): A-D = coxae 1-4; E-G= segment 2 of pereopods 5–7; H = epimeral plates 1–3; I = urosome with uropods 1–2; J = telson. Scale bars: 1 = 0.25 mm (J); 2 = 0.5 mm (I); 3 = 0.5 mm (A–D); 4 = 0.5 mm (H); 5 = 1.0 mm (E–G).

Uropod 2: inner ramus slightly longer than outer one, both rami with lateral and distal spines (fig. 12I).

Uropod 3 short, first segment of outer ramus with bunches of spines along both margins, and with single plumose setae along inner margin (fig. 11I); second segment of outer ramus short, with marginal setae.

Telson slightly broader than long, lobes slightly gaping, each with 3 distal single marginal and facial spines (fig. 12J).

**Variability.** The stable characters are the specific shape of the spines on the outer plate of maxilla 1, the presence of one spine along the inner margin of the dactyl of pereopods 3–7, and a short segment 2 of pereopods 5–7 with a marked enlarged ventroposterior corner (not distinct lobe).

**Etymology.** This species is based on the name of the Frasassi cave system in central Italy, where it is found.

**Remarks.** *Niphargus frasassianus* differs from all other known *Niphargus* species of Italy by the shape of the spines on the outer plate of maxilla 1: some spines have 2 or 3 lateral teeth on the opposite side of the spine, a modification that may permit specialized feeding, especially sediment straining.

A robust body with short strong gnathopods and percopods is present in some other species in the Adriatic region, including *Niphargus spinulifemur* S. Kar., 1954 known from Zazid (= Sassetto) in Istria, Croatia. But this species differs significantly from *N. frasassianus* in its strongly pointed epimeral plates, its substantially greater number of setae on the gnathopods, the different shape of spines on maxilla 1, etc.

*N. frasassianus* may be close to the *Niphargus puteanus* complex of species which also have strong bodies and pereopods (some with and some without more numerous spines on pereopod dactyls and pointed to subangular epimeral plates), but it differs from all of them in the shape of maxilla 1.

### Conclusions

The subterranean waters of the Frasassi cave system are inhabited by at least three species of the genus *Niphargus: N. ictus*, G. Kar., 1986, *N. montanarius*, **n. sp.** and *N. frasassianus*, **n. sp.** It is highly likely that more will be discovered, as the caves continue to be explored, both in the Frasassi system and in other subterranean waters in Italy.

The existence of so many species within one genus (nearly 60 known taxa in Italy alone) warrants indepth genetic and ecological studies to determine their zoogeographic and phylogenetic relationships. On the other hand, extremely large variety of the body shape within genus *Niphargus* (sensu auct.) indicate the probability that this genus is not monophyletic and can be probably split in several genera.

#### Acknowledgements

We thank Prof. Dr. Alessandro Montanari for his guidance and logistical support as well as his assistance in collecting animals. We also wish to thank Prof. Dr. Diana Galassi for providing us with amphipod samples.

#### References

- Iannilli, V. & Vigna-Taglianti, A. (2005) New data on the genus *Niphargus* (Amphipoda, Niphargidae) in Italy, with description of a new species of the orcinus group. *Crustaceana*, 77 (10), 1253–1261.
- Karaman, G. (1962) Beitrag zur Kenntnis der Niphargiden (Amphipoda) Jugoslawiens. Annales Zoologici, Warszawa, 20 (6), 39–45.
- Karaman, G. (1984) Description of several new *Niphargus* Species (Gammaridea, Niphargidae) from southern Europe (Contribution to the Knowledge of the Amphipoda 136). *Poljoprivreda i šumarstvo, Titograd*, 30 (2–3), 39–64.
- Karaman, G. (1986) New data on the genus *Niphargus* Schiodte (Fam. Niphargidae) in Italy and adjacent regions (Contribution to the Knowledge of the Amphipoda 138). *Bollettino del Museo Civico di Storia Naturale, Verona*, 12, 209–228. [Dated 1985, published 1986].
- Karaman, G. (1993) Crustacea Amphipoda di acqua dolce. Fauna d'Italia, vol. XXXI, 1–337, *Edizione Calderini* Bologna, Italia.
- Karaman, G. (1994) New and interesting species of the genus *Niphargus* Schiödte 1849 (Fam. Niphargidae) from Italy (Contribution to the Knowledge of the Amphipoda 212). *Glasnik Odjeljenja prirodnih nauka, Crnogorska akademija nauka i umjetnosti, Podgorica*, 10, 91–111.
- Karaman, G. & Ruffo, S. (1986) Amphipoda: *Niphargus*-Group (Niphargidae sensu Bousfield, 1982). *In:* Botosaneanu, L. (Ed.), *Stygofauna Mundi, A Faunistic, Distributional, and Ecological Synthesis of the World Fauna inhabiting*

Subterranean Waters (including the Marine Interstitial). E.J. Brill/ Dr. W. Backhuys, Leiden. Pp. 514–534.

- Karaman, S. (1954) Die Niphargiden des slovenischen Karstes, Istriens sowie des benachb. Italiens. Acta, Musei Macedonici Sceintiarum Naturalium, Skopje, 2 (8), 159–180.
- Latreille, P.A. (1802) Histoire naturelle, Générale et Particulière des Crustacés et des insectes 3. F. Dufart, Paris, xii + 468 pp.
- Latreille, P.A. (1816) Nouveau dictionnaire d'histoire naturelle, appliquée aux arts, à l'agriculture, à l'economie rurale et domestique, à la médicine, etc. 2nd edition. Vol. 1. Paris.
- Ruffo, S. (1995) Amphipoda, in: Crustacea Malacostraca II (Tanadacea, Isopoda, Amphipoda, Euphausiacea). *Checklist delle specie della Fauna Italiana*, 30, 28–52.
- Ruffo, S. & Vigna-Taglianti, A. (1968) Alcuni *Niphargus* delle acque soterranee dell'Italia centro-meridionale e considerazioni sulla sistematica del gruppo *orcinus* (Amphipoda, Gammaridae ). *Memorie del Museo Civico di Storia Naturale, Verona*, 16, 1–29.
- Schiödte, J.C. (1849) Bidrag til den underjordiske Fauna. Det kongelige danske Videnskabernes Selskabs Skrifter. Femte Raekke. Naturvidenskabelig og mathematisk Afdeling. Andet Bind. Kjobenhavn, 2 (5),1–39.
- Sket, B. (1958) Einige interessante Funde der Malacostraca (Crust.) aus der Herzegowina und Crna Gora. *Bulletin Scientifique*, 4(2), 53.