

Studies on Subterranean Copepods from Italy, with Descriptions of Two New Epikarstic Species from a Cave in Sicily

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(Accepted November 30, 2011)

Vezio Cottarelli, Maria Cristina Bruno, Maria Teresa Spena, and Rosario Grasso (2012) Studies on subterranean copepods from Italy, with descriptions of two new epikarstic species from a cave in Sicily. *Zoological Studies* **51**(4): 556-582. This paper presents and discusses the copepod fauna collected from several rimstone pools in Conza Cave (Palermo, Sicily). The geology, lithology, and hydrology of the cave suggest that all of the copepods collected in the pools were dislodged from the epikarst and were carried by dripping water into the pools. The copepod fauna included the first record for Sicily of the Italian endemic stygobiotic cyclopoid *Speocyclops italicus*, the 1st cave Parastenocarididae of Sicily *Parastenocaris diversitatis* sp. nov., and the stygobiotic Canthocamptuae *Bryocamptus* (*Rheocamptus*) *stillae* sp. nov. Remarks on the ecology and biology of the collected taxa are presented and discussed. http://zoolstud.sinica.edu.tw/Journals/51.4/556.pdf

Key words: Cave fauna, Speocyclops, Bryocamptus, Parastenocaris, New species.

he epikarst is the uppermost layer of karst (typically occurring in carbonate rocks such as limestone) which represents a boundary zone between superficial unconsolidated material and karstic habitats. The epikarst is "partially saturated with water and capable of delaying or storing and locally rerouting vertical infiltration to the deeper regional phreatic zone of the underlying karst aquifer" (Jones et al. 2004). Water in the epikarst zone percolates through the rock fractures and drips from the ceiling and walls of caves; the dripping often creates pools surrounded by rimstone. The epikarst is extremely heterogeneous, with numerous cracks, crevices, and semi-isolated solution pockets (Bakalowicz 2003, Williams 2008). The resulting mosaic of microhabitats hosts a variety of taxa, including a rich array of small crustaceans, especially

copepods (Brancelj and Culver 2005, Pipan 2005). The diversity of the epikarst often rivals that of the rest of the karstic aquifer (Pipan and Brancelj 2004, Brancelj and Culver 2005, Pipan and Culver 2006), and is often a source of new species (Brancelj 2009, Pipan et al. 2010). The epikarst was recently shown to host a high local richness and biodiversity in several European countries, including Italy (Pipan and Culver 2007, Dole-Olivier et al. 2009, Galassi et al. 2009, Michel et al. 2009).

In Sicily, stygobiotic microcrustaceans have been little investigated. Literature data include records for hyporheic, parafluvial, and phreatic (wells) habitats (Pesce and Galassi 1987 1988, Pesce et al. 1987 1988, Pesce 1988), whereas published data for caves, and in particular for the epikarst, do not exist. Recently, the Dipartimento di Biologia of the Univ. of Catania has been

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conducting biospeleological research in several Sicilian caves, aimed at broadening the patchy and scanty knowledge of cave microcrustaceans of this region, and increase public awareness of the fragility and vulnerability of groundwater assemblages, which are severely impacted by human activities and by the effects of climate change.

Three interesting copepods were collected from 5 rimstone pools in Conza Cave. The stygobiotic cyclopoid Speocyclops italicus Kiefer, 1938 was up to now exclusive to Italy and had never been collected in Sicily. Two harpacticoid species are new to science and are endemic to this cave: Parastenocaris diversitatis sp. nov., the 1st representative of the family Parastenocarididae from caves in Sicily, and Bryocamptus (Rheocamptus) stillae sp. nov. of the Canthocamptidae, one of the few stygobiotic cave species of this genus. Several specimens of the new species were collected, allowing us to detect the variability in some features which is useful for understanding the taxonomy of this subgenus. Parastenocaris biodiversitatis sp. nov. was described based on scanning electron microscopic (SEM) observations and showed some interesting features.

The faunistic and biogeographic value of these records and some data on the ecology and biology of the collected taxa are presented and discussed, with particular reference to the peculiar environmental conditions of the habitat in which they were collected.

Site description

Conza Cave (Palermo, Sicily, cadastral number Si Pa 60) is located in the vicinity of Palermo (38°11'13.9"N, 13°16'44.2"E). The area is characterized by a Mediterranean climate, i.e., as "Csa" in the Köppen climate classification, with hot, dry summers and mild to cool, wet winters. Meteorological data from meteorological stations near Conza Cave (Fig. 1), i.e., the total daily rainfall and cumulative monthly rainfall for the sampling period recorded by the "Osservatorio delle Acque Regione Siciliana", and the cumulative monthly rainfall and air temperature for 2011 (Meteosicilia) show a seasonal trend with a period of low or no precipitation extending from the end of May to the end of Aug., corresponding to a strong increase in air temperature. The cave opens at an elevation of 175 m, and extends for a total length of 100 m; sloping 30° upwards with a 30-m height difference (Fig. 2). The cave is developed within Upper Triassic limestone rock (a member of the "Costa della Ginestra" limestone, Sciacca Formation; see Italian Geological Map 1: 50,000 leaf 594 Partinico). This formation is composed of limestone, dolomitic limestone, stromatolitic and loferitic dolomite, megalodontic limestone, algal biolitite, and coral biolitite. The formation has a minimum thickness of 500 m; it lies on and merges laterally with white-gray dolomite with undefined stratification containing gastropod, algal, and coral fragments. The cave is known for Paleolithic and Neolithic artifacts and Pleistocenic mammal faunal remains (De Stefani 1941, Mannino et al. 1986), and for the invertebrate troglobiotic fauna (Brian 1959, Caruso 1982 1995, Caruso and Costa 1978, Spena 2007). In 1995 it was officially designated a Natural Reserve.

The cave is strongly affected by outside thermal excursions, due to its small size (Mannino et al. 1986, Spena 2007), and as a result of the karstic conditions, the surrounding area is devoid of running water. The cave is fossil; it is never flooded, and the water input to the cave is exclusively due to rainfall expressed as temporary drips. The percolation water collects in a few temporary puddles at the entrance of the cave, and in rimstone pools which are present on top of boulders and which originated from calcite dissolved in permanent or temporary drips which deposit and create a rimstone dam and an impermeable bottom layer of calcite. The rimstone pools are thus never connected to each other or to puddles on the cave floor.

Several rimstone pools are located in the first section of the cave; they disappear from 195 m in elevation (i.e., approximately -31 m from the surface level, and +23 m from the entrance level) due to the accumulation of large, collapsed material. Because the rock cover has a reduced thickness and thus a low capacity to store water, water stops dripping into the cave at least during summer months. Monitoring of the rimstone pools conducted in the last 3 yr by the Dipartimento di Biologia of Catania Univ. showed that from late spring to fall, the rimstone pools are constantly dry; some of them are also dry through the winter (Table 1). Water samples were initially collected from 5 rimstone pools characterized in table 2 and later only from pools containing copepods, i.e., all pools except pool no. 2 (Fig. 2). Before beginning the faunistic sampling, 250 mL of water was collected in Apr. 2008 from rimstone pool no. 4 and analyzed with inductively coupled plasma mass spectrometry



Fig. 1. Meteorological data. (A) Daily total rainfall for the sampling period (from the Osservatorio delle Acque Regione Siciliana, Palermo Istituto Zootecnico monitoring station), with sampling data indicated by arrows. (B) Cumulative monthly rainfall for the sampling period (from the same monitoring station) and cumulative monthly rainfall and mean monthly air temperature for 2011 (from www.meteosicilia.it, Palermo Monreale monitoring station).



Fig. 2. Conza Cave. (A) Longitudinal section along the cave main axis; (B) horizontal section, topographical relief, and positions of the rimstone pools.

Sampling date	Rimstone pool number	Water period	No. of specimens of Speocyclops italicus	No. of specimens of <i>Bryocamptus</i> (<i>R</i> .) <i>stillae</i> sp. nov.	No. of specimens of <i>Parastenocaris</i> <i>diversitatis</i> sp. nov.
30 Oct. 2009	1	water present	7		
30 Oct. 2009	3	water present		20	
30 Oct. 2009	4	water present			4
30 Oct. 2009	5	water present			
23 Nov. 2009	1	water present	25	50	
23 Nov. 2009	3	water present		9	
23 Nov. 2009	4	water present			5
23 Nov. 2009	5	water present			3
29 Dec. 2009	1	water present			
29 Dec. 2009	3	water present			
29 Dec. 2009	4	water present			6
29 Dec. 2009	5	dry			
31 Aug. 2010	1	dry			
31 Aug. 2010	3	dry			
31 Aug. 2010	4	dry			
31 Aug. 2010	5	dry			
5 Oct. 2010	1	water present	1		
5 Oct. 2010	3	water present		240	
5 Oct. 2010	4	dry			
5 Oct. 2010	5	dry			
10 Jan. 2011	1	water present			
10 Jan. 2011	3	water present		23	
10 Jan. 2011	4	dry			
10 Jan. 2011	5	dry			
9 May 2011	1	water present, no			
		samples collected			
9 May 2011	3	water present, no			
		samples collected			
9 May 2011	4	dry			
9 May 2011	5	water present			8

Table 1. Wet/dry conditions of each rimstone pool, at each sampling date, and number of specimens collected for each species

Table 2. Physical and chemical characteristics of the pools. Temperature and pH were recorded on 23 Nov. 2009 when rimstone pool no. 5 was drying out. Water samples for chemical analysis were collected on 8 Apr. 2008

	Rimstone pool no. 1	Rimstone pool no. 2	Rimstone pool no. 3	Rimstone pool no. 4	Rimstone pool no. 5
L × W × Md (cm) Temperature (°C) pH Hardness (ppm) Ca ⁺⁺ (mg/L) Mg ⁺⁺ (mg/L)	30 × 13 × 3.8	30 × 25 × 4	14 × 4 × 2.6	34 × 29 × 7 18.8 7.95 350 31.66 65.90	62 × 54 × 36 16.8 8.03
Na ⁺ (mg/L) K ⁺ (mg/L) Cl ⁻ (mg/L) Nitrates (mg/L) Sulfates (mg/L)				44.91 178.09 68.275 14.951 28.575	

L, length; W, width; Md, maximum depth.

using an Optima 2000 mass spectrometer at the Laboratorio di Igiene Ambientale e degli Alimenti of Catania Univ. (Catania, Italy).

Samples were collected monthly from Oct. to Dec. 2009, i.e., when the pools were permanently filled with water. By Dec., pool 5 was dry; the remaining pools dried out later on and were all dry by Aug. 2010. All pools remained dry until Oct. 2010, and after that date, we collected only once in each pool after it refilled with water to check if copepods were still present (Table 1), i.e., pools 1 and 3 in Jan. 2011 and pool 5 in May 2011; pool 4 was still dry in Apr. 2011 when the sampling campaign ended.

The non-copepod fauna was represented by stygoxene terrestrial or aquatic taxa: Tardigrada (12 specimens in pool 1), Nematoda (249 specimens in pool 3 and 1 in pool 1), Oligochaeta (72 specimens in pool 1 and 1 in pool 5), and Acari (1 specimen in pool 3 and 1 in pool 5). Very interestingly, 102 specimens of depigmented and microphtalmic Ostracoda were collected in rimstone pool 1 and are still being studied.

MATERIALS AND METHODS

Part of the water filling the selected rimstone pools (i.e., 200-380 ml for pool 1; 200-360 ml for pool 3; 400 ml for pool 4; and 400-800 ml for pool 5) was collected at each sampling date with a needle-less syringe, and preserved in a thermic bottle. Copepods were found only in rimstone pools 1, 3, 4, and 5. All specimens were sorted live under a stereomicroscope in the laboratory of the Dipartimento di Biologia, Univ. of Catania (Catania, Italy), and preserved in 70% ethanol. Selected specimens were rinsed in distilled water, dissected, and mounted in Faure's medium solution between 2 coverslips (to allow observations from 2 sides) at the Dipartimento per l'Innovazione dei Sistemi Biologici, Agroalimentari e Forestali, Tuscia Univ. (Viterbo, Italy). When mounting undissected specimens, fragments of human hair were inserted between the 2 coverslips to avoid deformation of the specimens (Karanovic 2005). Once the medium was dry, the coverslips were fixed to a microscopic slide by pieces of adhesive tape. Drawings were made at different magnifications, to a maximum of 1250x, using drawing tubes mounted on a Zeiss Axioskop® phase-contrast microscope and a Polyvar Reichert-Jung[®] interferential-contrast microscope at the Dipartimento per l'Innovazione dei Sistemi Biologici, Agroalimentari e Forestali, Tuscia Univ. (Viterbo, Italy).

The following abbreviations are used throughout the text and figures: enp, endopod; exp, exopod; A1, antennule; A2, antenna; P1-P5, 1st to 5th thoracic limbs. The nomenclature and descriptive terminology followed Huys and Boxshall (1991).

Specimens are deposited in the Natural History Museum, London (NHMUK); V. Cottarelli's collection at the Dipartimento per l'Innovazione dei Sistemi Biologici, Agroalimentari e Forestali, Tuscia Univ. (DIBAF), and R. Grasso's collection at the Dipartimento di Biologia, Catania Univ. (DB). Descriptions were the responsibility of V. Cottarelli and M.C. Bruno.

One female and 1 male paratype of *Parastenocaris diversitatis* sp. nov. were prepared for SEM. Specimens were fixed for 24 h in a 10% formaldehyde solution, washed twice in cacodylate buffer (pH 7.2), post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a graded ethanol series, critical point-dried in a Balzers Union H CPD 020 apparatus, coated with gold in a Balzers Union HMED 010 sputter coater, and observed with a 1200 JEOL JEMH EX II SEM at the Interdepartmental Center for Electron Microscopy Tuscia Univ. (Viterbo, Italy). Stubs prepared for SEM were deposited at the Interdepartmental Center for Electron Microscopy (CIME), Tuscia Univ.

TAXONOMIC ACCOUNT

Family: Cyclopidae Rafinesque, 1815 Subfamily Cyclopinae Burmeister, 1834 Genus Speocyclops Kiefer, 1937 Speocyclops italicus Kiefer, 1938

Material examined: Sicily (Italy), Grotta Conza (Si Pa 60). Rimstone pool no. 1, 30 Oct. 2009: 2 dissected $\Im \ \Im$ and 1 undissected \Im , each mounted on a slide; 4 copepodids preserved in 70% ethanol in a vial. Rimstone pool no. 1, 23 Nov. 2009: 4 dissected $\Im \ \Im$, 2 undissected $\Im \ \Im$, 1 undissected \Im , each mounted on a slide; 1 undissected \Im and 1 undissected \Im mounted on a single slide; 1 dissected \Im and 1 undissected copepodid mounted on a single slide; 14 copepodids preserved in 70% ethanol in a vial. Rimstone pool no. 1, 5 Oct. 2010: 1 partially damaged \Im preserved in 70% ethanol in a vial. All materials collected by R. Grasso, M.T. Spena, and G. Nicolosi.

Variability: All features of all specimens corresponded to those of the nominal species as redescribed by Galassi and De Laurentiis (2004a).

Remarks: S. italicus is a stygobiotic taxon endemic to Italy; it has so far been collected from wells in Umbria (Pesce and Galassi 1983, Galassi and De Laurentiis 2004a), Marche (Pesce and Maggi 1979, Pesce 1980), Molise (Pesce et al. 1987), and Basilicata (Pesce 1986, Apostolov and Pesce 1987), from caves in Campania (Kiefer 1938, Galassi and De Laurentiis 2004a, Stoch 2005), and from the hyporheic in Latium, Tuscany, and Marche (Galassi and De Laurentiis 2004a). We collected this species in locations previously not reported in the literature: i) Tuscany (Italy), Fiora River, locality "Sovana" (42°39'36.22"N, 11°37'11.25"E), 3 July 1995, hyporheic in parafluvial: 2 females, 1 male, 1 copepodid, each mounted on a slide; ii) Tuscany (Italy), Turrite Secca Stream, locality "Isola Santa'' (44°03'56.45"N, 10°18'42.13"E), 30 May 1998, hyporheic in parafluvial: 1 dissected female mounted on a slide; iii) Tuscany (Italy), Serchio River locality "Sillano" (44°13'25.85"N, 10°17'44.68"E), 31 May 1998, from hyporheic habitat in a mid-river sandbar on the left side of the river: 8 dissected females and 1 dissected male, each mounted on a slide. All materials collected by V. Cottarelli. These new data extend the distribution of this species to the hyporheic of 2 new watersheds of Tuscany (Fiora and Serchio Rivers) and represent the 1st record from caves of insular Italy (Conza Cave, Sicily).

Family Canthocamptidae Brady, 1880 Subfamily Canthocamptinae Brady, 1880 Genus *Bryocamptus* Chappuis, 1928 Subgenus *Rheocamptus* Borutsky, 1948 *Bryocamptus* (*Rheocamptus*) *stillae* Cottarelli and Bruno sp. nov.

Material examined: Sicily (Italy), Grotta Conza (Si Pa 60): dates, location, and number of specimens for each collection given in table 1. All specimens not mounted on slides preserved in vials with 70% ethanol. All materials collected by R. Grasso, M.T. Spena, and G. Nicolosi.

Type material: Holotype: ovigerous $\stackrel{\circ}{\rightarrow}$, dissected and mounted on a slide (NHMUK 2011. 8654), rimstone pool no. 3, 30 Oct. 2009. Allotype: $\stackrel{\circ}{}$, dissected and mounted on a slide (NHMUK 2011. 8655), rimstone pool no. 3, 30 Oct. 2009. Paratypes: 1 dissected and 1 undissected $\stackrel{\circ}{}$

(NHMUK 2011. 8656, 2011. 8657), 1 dissected ovigerous ♀ (NHMUK 2011. 8658) each mounted on a slide, 1 dissected β and 1 dissected $\hat{\gamma}$ on a single slide (DB), 1 undissected $\stackrel{\circ}{\rightarrow}$ and 1 dissected ♂ on a single slide (DIBAF), 1 undissected ovigerous $\stackrel{\circ}{\rightarrow}$ and 1 undissected copepodid on a single slide (DIBAF), rimstone pool no. 3, 30 Oct. 2009. Six dissected 2 2 (DB, DIBAF), 1 dissected ovigerous $\stackrel{\circ}{\rightarrow}$ (DIBAF), 1 undissected ovigerous $\stackrel{\circ}{\rightarrow}$ (DIBAF), 2 dissected ♂ ♂ (NHMUK2011. 8663, 2011. 8664), each mounted on a slide, rimstone pool no. 3, 30 Oct. 2009. Eight dissected 2(NHMUK 2011. 8659, DB, DIBAF), 7 dissected ovigerous 2 (NHMUK 2011. 8660, DIBAF), 3 undissected ovigerous 2 (DIBAF), 2 dissected ♂ ♂ (DIBAF), 3 dissected copepodids (DIBAF), each mounted on a slide; 1 undissected $\stackrel{\circ}{\rightarrow}$ and 1 copepodid mounted on a single slide (DIBAF), rimstone pool no. 1, 23 Nov. 2009. Two dissected 3 3 (NHMUK 2011. 8665, DIBAF), 3 dissected 2 2 (DB, DIBAF), 1 dissected and 1 undissected ovigerous $\stackrel{\circ}{=}$ (NHMUK 2011. 8661, 2011. 8662), 1 dissected copepodid (DIBAF) each mounted on a slide, 1 dissected δ and 1 dissected ovigerous ♀ mounted on a single slide (DIBAF), rimstone pool no. 3, 23 Nov. 2009. Two dissected and 2 undissected 2, 3 dissected and 3 undissected ovigerous 2, 2 dissected and 2 undissected $\delta \delta$, 2 dissected and 2 undissected copepodids, each mounted on a slide (DIBAF), rimstone pool no. 3, 10 Jan. 2011.

Description of female. Habitus as in figure 3. Mean length (measured from tip of rostrum to apex of caudal rami): 420 μ m (*n* = 10). Cephalothorax with smooth distal margin, several sensilla, and small oval, medially constricted, dorsal hyaline window (Figs. 3B, 4A). Position and number of sensilla on free thoracic somites as in figure 3A. Distal margins of thoracic and abdominal somites smooth (Figs. 3, 4B, C). Lateral, elliptical hyaline window on 1st free thoracic somite (Figs. 3A, 4C). Genital double-somite longer than wide, with 2 distal pores on ventral surface, small arched row of spinules on each side of genital field (Fig. 4D), paired row of lateral spinules (Fig. 4B, D), and 3 pairs of lateral sensilla (Fig. 3A). Fourth and 5th urosomites with continuous distal row of ventral spinules extending laterally (Fig. 3A), ventral part of row composed of longer spinules. Genital field (Fig. 4D) small, positioned anteriorly on midventral surface of genital double somite, consisting of 2 merged opercula derived from P6 and closing off paired genital apertures, with 1 small spine and 1 long pinnate seta; copulatory pore opening at about 2/3 of midventral surface. Anal somite (Fig. 4F) with row of strong spinules on distoventral margin extending laterally, 2 ventral pores. Anal operculum (Fig. 4E, G) convex, with 3 strong teeth, a subdistal row of small spinules, and paired sensilla.

Caudal ramus (Fig. 4E-G): Subconical, length/width ratio: 1.2, with 7 setae. Anterolateral accessory seta (seta I) reduced and inserted close to anterolateral seta (seta II). Posterolateral seta (seta III) slightly longer than seta II, and inserted near end of ramus. A group of 2 spinules near insertion of setae II and III, respectively. Dorsal seta (seta VII) composite, inserted in apical 1/4 of ramus. Terminal accessory seta (seta VI) thin, with a group of 2 spinules near its insertion and a 2nd group of 2 spinules on distomedial corner. Inner terminal seta (seta V) with breaking plane, barbed, length about 200 μ m. Outer terminal seta (seta IV) with breaking plane, pinnate, about 2.8-times length of caudal ramus.

Rostrum (Fig. 4A, H) not fused to cephalothorax, narrow, with rounded tip bearing 2 small sensilla; reaching past distal end of 1st segment of A1 (Fig. 4H).

Antennule (Fig. 4I): 8-segmented, aesthetasc



Fig. 3. *Bryocamptus* (*Rheocamptus*) *stillae* sp. nov. (A) Female, habitus, lateral view. (B) Female, habitus, dorsal view, minor ornamentation not shown. Scale bar = 100 μm.



Fig. 4. *Bryocamptus* (*Rheocamptus*) *stillae* sp. nov. (A) Female, cephalothorax. (B) Female, genital double somite, 4th and 5th urosomites, dorsal view. (C) Female, 1st and 2nd free thoracic somites, lateral view. (D) Female, genital double somite and genital field, with spermatophore attached to seminal receptacle. (E) Female, anal operculum and caudal ramus, lateral outer view. (F) Female, 5th urosomite, anal operculum and caudal ramus, ventral view. (G) Female, anal operculum and caudal ramus, dorsal view. (H) Female, rostrum and 1st antennular segment. (I) Female, antennule. (J) Female, antenna. (K) Female, anal operculum (variability), dorsal view. Scale bar = 50 μm.

on 4th segment reaching end of antennule; aesthetasc on last segment as long as segments 6 and 7. Armature formula: 1-[1], 2-[7], 3-[4], 4-[2 + ae], 5-[1], 6-[2], 7-[2], 8-[6 + ae].

Antenna (Fig. 4J): Coxa with short seta on medial margin and longitudinal spinule row on distal margin. Allobasis with transversal spinule row at 1/2 of medial margin. Endopod 1-segmented, medial margin with 3 spinules and 3 spines of approximately same length, one of which subapical. Three geniculate setae and 1 strong spine apically; hyaline frill on lateral margin. Exopod 2-segmented, 1st segment with 1 pinnate spinular seta, 2nd segment with 1 short subapical pinnate seta and 1 long and 1 short, pinnate apical setae.

Labrum as in figure 5A.

Mandible (Fig. 5B): Coxa with knob on outer surface; gnathobase with 6 teeth and short lateral pinnate seta; palp 1-segmented with 2 apical setae of different lengths.

Maxillule (Fig. 5C): Arthrite of precoxa with 6 apical spines and short lateral pinnate seta. Endite of basis with apically strong spine. Exopod with 5 lateral bare setae and 3 apical pinnate, curved setae.

Maxilla (Fig. 5D): Syncoxa with 2 endites, both with 2 apical spines and 1 seta. Basis ending in spiniform pinnate tip, with 2 setae; endopod a tubercle bearing 2 setae of different lengths.

Maxilliped (Fig. 5E): Prehensile; syncoxa with distal spinule and transverse spinule row. Basis 3-times as long as wide, with medial row of 8 spinules. Endopod 1-segmented, with apical short seta and strong, curved unipinnate claw; slightly longer than basis.

P1-P4 with 3-segmented exopods and 2-segmented endopods (Fig. 5F-I). Intercoxal sclerites of all swimming legs concave and bare (Fig. 5F-I). P1-P4 coxa with rows of spinules on lateral margin (Fig. 5F-I). Main armature formula:

P1	basis I-I	exp 0-I; 1-I; 0, 2, II
		enp 0-0; 1, 1-I, 0
P2	basis 0-l	exp 0-l; 1-l; 2, 1, ll
		enp 0-0; 1, 2, I
P3	basis 0-1	exp 0-l; 1-l; 1, 2, ll
		enp 1-0; 2, 2, I
P4	basis 0-l	exp 0-l; 1-l; 2, 2, ll
		enp 0-0; 0, 2-I, I

P1 (Fig. 5F): Basis with stout lateral seta, pore, and several spinules around its insertion; 1 medial seta with group of spinules near insertion; transversal row of hair-like spinules distally on anterior surface. Endopod slightly shorter than exopod. Exp-2 with lateral pinnate spine and long seta with pinnate tip on mediodistal corner. Exp-3 with 2 lateral pinnate spines, and 2 apical geniculate setae of different lengths. Enp-2 narrower than enp-1 and bearing very thin and short medial seta inserted proximally at about 1/2 of segment, apical geniculate seta and apical pinnate spine. Supplementary ornamentation in figure 5F.

P2 (Fig. 5G): Basis with stout lateral seta, pore, and spinules around its insertion. Exp-3 slightly shorter than exp-1 and exp-2 combined. Medial seta on exp-2 very thin and short, inserted at about 1/3 of segment. Exp-3 with 2 lateral pinnate spines, apical pinnate long seta, pinnate smaller seta on mediodistal corner, long seta with unipinnate tip at about 1/2 of medial margin. Endopod reaching distal margin of exp-2. Enp-1 bare, enp-2 narrowing in distal 1/2, with strong spine on laterodistal corner, 1 short bare seta and 1 longer pinnate seta apically, bare seta at about 1/2 of medial margin. Supplementary ornamentation in figure 5G.

P3 (Fig. 5H): Basis with long lateral seta, pore and spinules around its insertion. Exp-3 as long as exp-1 and exp-2 combined, with 2 lateral pinnate spines, long apical pinnate seta, shorter pinnate seta on mediodistal corner, long apically pinnate seta at about 1/2 of medial margin. Endopod reaching distal margin of exp-2. Enp-1 with short medial seta, enp-2 with spine on laterodistal corner, 1 short bare seta and 1 longer pinnate seta apically, 2 bare setae at about 2/3 of medial margin. Supplementary ornamentation as in figure 5H.

P4 (Fig. 5I): Basis with stout lateral seta and spinules around its insertion. Exp-3 slightly shorter than exp-1 and exp-2 combined. Exp-3 with 2 lateral pinnate spines, 2 apical setae of different lengths, very long and pinnate seta on mediodistal corner, long apically pinnate seta at about 2/3 of medial margin. Endopod reaching about 1/2 of exp-2. Enp-1 bare, enp-2 with short spine at 1/2 of lateral margin, long pinnate spine on laterodistal corner, 2 pinnate spiniform setae of different lengths apically. Supplementary ornamentation in figure 5I.

P5 (Fig. 5J): Baseoendopods not fused; medial expansion of baseoendopod slightly longer



Fig. 5. *Bryocamptus* (*Rheocamptus*) *stillae* sp. nov. (A) Female, labrum. (B) Female, mandible. (C) Female, maxillule (precoxal arthrite disarticulated). (D) Female, maxilla. (E) Female, maxilliped. (F) Female, P1. (G) Female, P2. (H) Female, P3 (intraspecimen variability). (I) Female, P4. (J) Female, P5. (K) Male, 2nd urosomite and P6, lateral view. (L) Male, anal operculum and caudal ramus, lateral outer view. (M) Male, 5th urosomite, anal operculum, and caudal ramus, ventral view. (N) Male, antennule, disarticulated (segments numbered with Arabic numerals). (O) Female, P5 (variability). Scale bar = 50 μm.

than exopod, with 2 pores, bearing from lateral to medial margin: short subapical serrate seta, 3 apical pinnate setae of increasing length, and pinnate subapical seta; distal margin incised between each pair of 3 medialmost setae. Small knob between baseoendopodal lobe and exopod insertion. Exopod about 1.4-times longer than broad, with 2 lateral and 1 subapical pinnate setae, middle 1 longest; very long apical pinnate seta, medial pinnate seta as long as lateral subapical 1.

P6 (Fig. 4D): With 2 setae, 1 very short.

Description of male. Habitus similar to that of female but proportionally thinner. Mean length (measured from tip of rostrum to apex of caudal rami): 400 μ m (n = 6). Second urosomite (Fig. 5K) without ventral spinules or lateral spinule row at 2/3 of somite; 3rd-5th urosomites with posterior ventral row of spinules extending laterally. Hyaline windows as in female. Anal somite and anal operculum as in female (Fig. 5L, M). Caudal ramus (Fig. 5L, M) similar to that of female in shape, without group of 2 spinules on distomedial corner; seta VII proportionally longer and seta VI shorter than in female. Rostrum, A2, mouthparts, exopods, intercoxal sclerites, coxae, and bases of P1-P4 as in female. Spermatophore as in figure 4D.

Antennule (Fig. 5N): 8-segmented, geniculate. Fifth and 6th segments with sclerotized plates. Aesthetascs on 4th segment shorter than in female. Armature formula: 1-[1], 2-[8], 3-[4], 4-[4 + ae], 5-[0], 6-[0], 7-[0], 8-[8 + ae].

P1 (Fig. 6A): Endopod similar to that of female but medial seta on P1-enp2 inserted subapically.

P2: Endopod (Fig. 6B) 2-segmented, extending beyond end of exp-2. Enp-1 bare; enp-2 with 2 lateral groups of 2 spinules, medial seta inserted at 1/2 of margin, 2 apical setae of different lengths, longer 1 pinnate, and subdistal knob-like apophysis on medial corner.

P3 (Fig. 6C, D): Endopod 3-segmented, modified, extending beyond end of exp-2.; enp-1 with medial seta; spiniform process on enp-2 extending beyond end of corresponding exopod, ending in barbed tip. Enp-3 with 2 apical setae of different lengths, longer 1 unipinnate with subapical unipinnate tuft.

P4 (Fig. 6E): Endopod 2-segmented, proportionally smaller than in female; enp-1 bare; enp-2 similar to that of female but apical spiniform setae proportionally longer.



Fig. 6. Bryocamptus (Rheocamptus) stillae sp. nov. (A) Male, P1. (B) Male, P2. (C) Male, P3. (D) Male, P3 endopod. (E) Male, P4. (F) Male, P5. (G) Male, P6. Scale bar = 50 μm.

P5 (Fig. 6F): Baseoendopods fused, medial portion of each baseoendopod slightly expanded, with 2 pinnate spines subequal in length. Exopods small, asymmetric, 1 exopod bearing 2 setae of subequal length on lateral margin, slightly longer seta on distolateral corner, long pinnate apical seta, shorter pinnate seta on distomedial corner; 2nd exopod with 1 supplementary lateral pinnate seta.

P6 (Figs. 5K, 6G): A narrow plate bearing 2 pinnate and 1 normal setae of different lengths on each side, middle 1 longest and medialmost 1 bare.

Variability: Four females and 3 males the anal operculum of which with 4 or 5 (1 female, Fig. 4K) teeth instead of 3. One female 1 P3enp-2 of which with only 1 seta on medial margin. In females, setation of last segment of P2 and P3 endopods and of P5 baseoendopod varied among specimens and in some specimens between right and left legs of same specimen (for instance, 1 specimen bearing 3 and 5 setae on right and left P5 baseoendopods, respectively) (Fig. 5H, O, Table 3), and setation of last segment of P4 endopod almost constant (3 of 33 females bearing 2 instead of 3 setae on one of 2 endopods). Right P5 exopod always with 5 setae, whereas left 1 with 4 setae in 11 specimens and 5 in remaining 18 specimens. In males, setation of last segment of P2 and P3 endopods not varying, and setation of P4 endopod slightly variable (1 specimen of 8 with 2 setae instead of 3), whereas P5 exopod highly variable with one of 2 exopods often (in 6 of 8 specimens right 1) with 6 setae and other exopod with 5 setae (Table 3). In females, 6 setae never recorded on P5 exopod (possibly a dimorphic character).

Etymology: The species epitheton is the feminine singular genitive of the Latin noun *stilla* = drop, meaning "of the drop", and refers to the drips and drop-filled rimstone pools where this species was collected.

Remarks: Bryocamptus (Rheocamptus) stillae sp. nov. belongs to the pygmaeus-typhlops group sensu Borutsky (1952), which includes taxa with only 2 lateral spines on the last segment of P2-P4 exopods and a 1- (typhlops group) or 2-segmented (pygmaeus group) P4 endopod. Both sexes of *B.* (*R.*) stillae sp. nov. have a 2-segmented P4

	Number of setae	Number of specimens
Female right and left P2-enp-2	R 3, L 3	1
	R 3, L 4	2
	R 4, L 3	5
	R 4, L 4	18
Female right and left P3-enp-2	R 4, L 4	3
	R 4, L 5	8
	R 5, L 4	4
	R 5, L 5	15
Female right and left P4-enp-2	R 2, L 3	1
	R 3, L 2	2
	R 3, L 3	30
Female right and left exp-P5	R 5, L 4	18
	R 5, L 5	11
Female right and left baseoenp P5	R 4, L 5	1
	R 5, L 3	2
	R 5, L 4	12
	R 5, L 5	14
Male right and left P2-enp-2	R 3, L 3	8
Male right and left P3-enp-3	R 2, L 2	8
Male right and left P4-enp-2	R 3, L 3	7
	R 3, L 2	1
Male right and left exp P5	R 6, L 5	6
	R 5, L 6	2
Male right and left baseoenp P5	R 2, L 2	8

Table 3. Combination of the number of setae on the right (R) and left (L) legs in the examined specimens of *B*. (*R*.) *stillae* sp. nov.

endopod, indicating a stronger affinity of the new species with the pygmaeus group. Several morphological features of B. (R.) stillae sp. nov. (e.g., depigmentation, lack of a naupliar eye, an elongated body, long aesthetasc on A1, reduced body ornamentation, and the armature of the appendices) are also common in other stygobiotic taxa of Bryocamptus (Rheocamptus) (Karanovic and Bobic 1998). Among species of the pygmaeus group, B. (R.) stillae sp. nov. mostly resembles the rare cave species B. (R.) dentatus Chappuis, 1937. The 1st specimen of this species was 1st collected from a cave near S. Sebastian in Spain (Chappuis 1937), later 1 specimen from the anchihalyne Zinzulusa Cave in southern Italy (Apulia) (Chappuis 1938), and several specimens from a cave in the French Pyrenees (Chappuis and Rouch 1959). In 1998, it was collected from a spring in a karstic region of northeastern Italy (Stoch 2000-2006). Unfortunately, Chappuis (1938) did not describe or draw the Italian specimen of B. (R.) dentatus, and we were unable to examine Stoch's Italian specimens. The available descriptions and illustrations of the Spanish and French specimens are incomplete according to recent taxonomic standards, e.g., the mouth parts are not described (with the exception of the mandibular palp). From the analysis of the remaining features, B. (R.) stillae sp. nov. and B. (R.) dentatus share the shape and ornamentation of the anal operculum, and the same size and armature of exopods P1, P2, P4, and P5, although the same armature is present in other species of the subgenus Rheocamptus which are not related to those discussed here, such as B. (R.) pygmaeus (G. O. Sars, 1863). Endopods P1-P3 of the female of the two species look similar, but in B. (R.) stillae sp. nov., the armature is highly variable: many specimens have a reduced number of setae/spines. Such a reduction is not present in B. (R.) dentatus, for which specimens collected at locations quite distant from each other share the same armature of endopods P2 and P4. In this same species, however, French and Spanish specimens differ in the armature of P3enp-2 and P3exp-3: enp-2 has 5 and 4 setae, and exp-3 has 5 and 6 setae/spines, respectively for the two populations (Chappuis and Rouch 1959). Significant differences between B. (R.) stillae sp. nov. and the Spanish and French populations of B. (R.) dentatus include: 1) the length of the A1 aesthetasc in both sexes, which in the latter does not reach the end of the last segment of A1 and is longer in the former; 2) the mandibular palp with 3

or 4 setae in B. (R.) dentatus and with 2 setae, one of which is very small, in B. (R.) stillae sp. nov.; 3) the ornamentation of the last two urosomites, represented by a continuous distal spinule row extending from lateral to ventral in both somites for both sexes of B. (R.) stillae sp. nov., with the ventral part composed of longer spines; the same spinule row extends only laterally and ventrally and is interrupted ventrally in the corresponding somites of B. (R.) dentatus from Spain (Chappuis 1937); the same spinule row is joined ventrally by a range of shorter spines in specimens from the Pyrenees (Chappuis and Rouch 1959); 4) P2enp-2 of males bears 1 long distal seta in B. (R.) dentatus, whereas B. (R.) stillae sp. nov. bears 2 apical setae of different lengths, but both are shorter than the only 1 present in B. (R.) dentatus; 5) P4enp-2 of the new species has 3 distal setae in almost all of the examined specimens of both sexes, whereas in B. (R.) dentatus, this segment bears 1 more seta at midlength of the medial margin; 6) the P5 baseoendopod of females differs: it bears 6 setae in B. (R.) dentatus, and 4 or 5 setae in B. (R.) stillae sp. nov., and the relative lengths of the setae differ between the 2 species; 7) the caudal rami are cone-shaped in B. (R.) stillae sp. nov, and quadrangular and more elongated in B (R.) dentatus; seta I is longer, seta IV is thinner and shorter, and there are additional spines in B. (R.) stillae sp. nov.; and 8) a hyaline window on the 1st free thoracic somite is only present in B. (R.) stillae sp. nov.

Among species of the *typhlops* group, *B*. (*R*.) stillae sp. nov. is closest to those characterized by a small mandibular palp bearing only 2 setae, such as B. (R.) typhlops (Mrázek, 1893) and B. (R.) borus Karanovic and Bobic, 1998. The latter and B. (R.) stillae sp. nov. share a similar armature of P2 and P3 endopods in females, of the P2 endopod in males, and of P5 and exp-3 of P2-P4 in both sexes. The shape and armature of the caudal rami are also similar, but the hyaline window on the 1st free thoracic somite is missing in both B. (R.) typhlops and B. (R.) borus. The main difference between B. (R.) stillae sp. nov. and species of the typhlops group is the segmentation of endopod P4 which is 1-segmented in the latter, suggesting that the species of the typhlops group are more evolved towards stygobization than those of the pygmaeus group. On the other hand, the reduced armature of most of the swimming legs and the reduced size of P4enp-1 in both sexes of B. (R.) stillae sp. nov. indicate that this species belongs to the *pygmaeus* group; but among species of this group, it is closest to the typhlops group.

The variability of B. (R.) stillae sp. nov. does not overlap with the one of the closest taxa. Several other authors recorded high variability in several morphological characters in species of Bryocamptus, particularly in the subgenus Rheocamptus, such as B. (R.) dentatus and B. (R.) borus (Chappuis 1938, Chappuis and Rouch 1959, Karanovic and Bobic 1998). Such variability hinders the taxonomic attribution of specimens, and in our opinion, renders descriptions based on 1 specimen (i.e., B. (R.) madarensis Apostolov, 1969, B. (R.) pirgos Apostolov, 1969, B. (R.) alosensis Apostolov, 1998, and B. (R.) aberrans Apostolov and Pesce, 1991) questionable.

A taxonomic key, summarizing the features useful in distinguishing *B. stillae* sp. nov. from its closest species, and a list of species within the larger species-groups which share characters with *B.* (*R.*) *stillae* sp. nov. is given as follows:

- 1. Two-segmented P4 endopod (i.e., pygmaeus group) 2

Family Parastenocarididae Chappuis 1940 Subfamily Parastenocaridinae Chappuis 1940 Genus Parastenocaris Kessler 1913 Parastenocaris diversitatis Cottarelli and Bruno, sp. nov.

Material examined: Sicily (Italy), Grotta Conza (Si Pa 60), dates, location, and number of specimens for each collection given in table 1. All material collected by R. Grasso, M.T. Spena, and G. Nicolosi.

Type material: Holotype: male (NHMUK 2011. 8666), dissected and mounted on 2 different slides, rimstone pool no. 4, 23 Nov. 2009. Allotype: ♀ (NHMUK 2011. 8667), dissected and

mounted on slide, rimstone pool no. 4, 29 Dec. 2009. Paratypes: 1 3 (NHMUK 2011. 8668) dissected and mounted on 2 different slides, 2 dissected (NHMUK 2011. 8669, DIBAF) and 1 undissected 3 (DIBAF) each mounted on slide, rimstone pool no. 4, 30 Oct. 2009; 1 dissected 3 (DB), 1 undissected (DIBAF), and 1 dissected $\stackrel{\circ}{\downarrow}$ (NHMUK 2011. 8671), each mounted on slide, rimstone pool no. 5, 23 Nov. 2009; 1 dissected ♀ (DB) and 2 dissected & (DIBAF) each mounted on slide, rimstone pool no. 4, 29 Dec. 2009; 2 undissected 3 3 (NHMUK 2011. 8670, DIBAF) and 1 undissected ♀ (NHMUK 2011. 8672) each mounted on slide, rimstone pool no. 5, 9 Apr. 2011. One male and $1 \stackrel{\circ}{\downarrow}$, rimstone pool no. 4, 30 Oct. 2009, prepared for SEM, on 1 stub (CIME).

Description of male: Body vermiform, slender, unpigmented, eyeless; habitus as in figure 7A. Mean length (measured from tip of rostrum to apex of caudal rami): 0.380 μ m (*n* = 5). Hyaline frills of cephalothorax, free thoracic somites, and urosomites smooth (Fig. 7A). Cephalothorax with elliptical dorsal integumental window (Fig. 7A), pore, and 14 sensilla. In lateral view, tergites of 3 free thoracic somites bearing 9, 9, and 9 sensilla, respectively; urosomites 1 to 5 respectively with 5, 0, 7, 7, and 0, sensilla. Urosomites 2-5 with dorsal elliptical integumental window of shapes and sizes as in figure 7A. Anal somite (Fig. 8A, B) with paired sensilla on dorsal side. Anal operculum (Fig. 8A, B) convex, with smooth distal margin. Caudal rami (Figs. 8A, B, 11A) shorter than last abdominal somite; length/width ratio 3.0, with strong dorsal pointed apophysis. Anterolateral accessory seta (I) and posterolateral seta (III) short and subequal, anterolateral seta (II) twice as long as setae I and III, all 3 setae inserted together at 3/4 length of caudal ramus. Outer terminal seta (IV) long (length seta/length caudal ramus: 1.67), unipinnate. Inner terminal seta (V) without fracture plane. Terminal accessory seta (VI) short (length seta/length caudal ramus: 0.45) and smooth, with row of spinules near its insertion. Dorsal seta (VII) articulate, short (length seta/length caudal ramus: 0.91). Spermatophore as in figure 8C.

Rostrum (Figs. 7A, 8D): Small, reaching end of 1st segment of antennule, with 2 apical sensilla.

Antennule (Figs. 8D, 11C, D): Geniculate, 8-segmented. First segment bare. Second segment with 5 normal and 1 uniserially plumose setae. Third segment with 4 distal setae. Fourth segment represented by U-shaped sclerite with 2 short setae. Fifth segment bearing a seta on its ventral side and distal tubercle with 2 equal setae and aesthetasc, slightly constricted at midlength, and not reaching end of antennule; dorsal side of segment with triangular and pointed apophysis matching one on 7th segment. Sixth segment bare. Seventh segment bare, prolonged into a dorsal apophysis. Eighth segment with 8 setae and short, thin, apical aesthetasc. Armature formula: 1-[0], 2-[1 uniplumose + 5 bare], 3-[4 bare],4-[2 bare], 5-[2 + ae], 6-[0], 7-[0], 8-[8 bare + ae].

Antenna (Fig. 8E): Coxa unarmed; allobasis with transverse row of 3 spinules on medial margin.

Exopod 1-segmented, with short, pinnate apical seta. Endopod bearing 2 unipinnate geniculate setae and 2 shorter spiniform unipinnate setae at apex. One long subapical transformed unipinnate seta with spinule near its insertion. Six spinules along mediodistal margin of endopod.

Labrum as in figure 11B.

Mandible (Figs. 8F, 11B): Coxal gnathobase bare, cutting edge with 4 strong teeth. One-segmented palp, with 2 distal setae of subequal length.

Maxillule (Figs. 8G, 11B): Precoxal arthrite

(A) (B)

Fig. 7. Parastenocaris diversitatis sp. nov. (A) Male, habitus, lateral view. (B) Female, habitus, lateral view. Scale bar = 100 μm.



Fig. 8. *Parastenocaris diversitatis* sp. nov. (A) Male, anal somite, anal operculum, and caudal ramus, lateral view. (B) Male, anal somite, anal operculum, and caudal rami, dorsal view. (C) Male, spermatophore. (D) Male, antennule, disarticulated (segments numbered with Arabic numerals). (E) Male, antenna. (F) Male, mandible. (G) Male, maxillule. (H) Male, maxilla. (I) Male, maxilliped. (J) Male, P1 basis and endopod. (K) Male, P1 basis and exopod. Scale bar = 50 μm.

with 3 modified spines. Coxa with 2 apical setae of subequal length, basis with 1 apical bare seta.

Maxilla (Figs. 8H, 11B): Syncoxa with 2 endites; proximal endite with short bare seta; distal endite with 1 bare and 1 enlarged pinnate setae. Allobasis prolonged into apical pinnate claw, distally spinulose; endopod represented by a small tubercle arising proximally along medial margin of allobasis with 2 setae of equal length.

Maxilliped (Figs. 8I, 11B): Prehensile. Syncoxa small and unarmed; basis slim and elongate, unarmed; endopod represented by distally unipinnate claw.

P1 (Fig. 8J, K): Basis with thin medial seta and hook with sinuous margin (arrowed in Fig. 8J), lateral seta and spinule row. Exopod (Fig. 8K) 3-segmented, as long as endopod. Third segment with 2 geniculate and 1 normal pinnate apical setae, and 1 subapical pinnate seta. Endopod (Fig. 8J) 2-segmented; enp-1 with proximal longitudinal row of 3 spinules and distal longitudinal row of 2 spinules along medial margin; enp-2 with long, geniculate pinnate seta, and short pinnate seta on apex.

P2 (Fig. 9A): Basis with pore and row of spinules around exopod insertion, without lateral seta. Exopod 3-segmented, exp-1 with strong lateral spine inserted distally at 3/4 of margin; remaining armature shown in figure 9A. Endopod reaching midlength of exp-1, represented by cylindrical segment, slightly enlarged apically, with apical seta surrounded by 3 short spinules.

P3 (Figs. 9B, 11E): Slender and elongate, basis with long lateral seta, lateral pore (Fig. 9C), and longitudinal row of spinules (Figs. 9C, 11E). Endopod reduced to short seta. Exp-1 distally slender, proximal longitudinal row of 2 spines and distal longitudinal row of 4 spines on lateral margin. Distal thumb represented by short, leaflike, pointed segment; exp-2 fused with exp-1 and prolonged into long, inwardly curved apophysis with blunt tip.

P4 (Fig. 9E): Basis with lateral seta, pore, and row of spinules near exopod insertion; exopod 3-segmented, slender; all segments approximately same length, exp-1 slightly enlarged; armature shown in figure 9E. Endopod slightly shorter than 1st 2 segments of exopod, bearing 1 lateral pointed tip and 1 medial inwardly curved expansion ending in a bilobate tip on its proximal part; distal part ending in thin, long, pinnate tip. Three curved spines of increasing length near insertion of endopod, medialmost 1 longest, all with blunt tip.

P5 (Figs. 9F, 11F): Without intercoxal sclerite,

composed of 2 rectangular plates fused at their bases, with 1 proximal pore. On free distal margin, from medial to lateral: short seta, 2 longer setae the medial 1 longest, long basipodal lateral seta.

Description of female: Mean length (measured from tip of rostrum to apex of caudal rami) 0.382 um (n = 4). Habitus as in figure 7B. Genital somite and following 2 urosomites with oval dorsal integumental windows similar to those of male (Fig. 7B). Genital somite and 1st abdominal somite fused, forming genital double-somite (Figs. 7B, 9G). Genital field located in proximal 1/3 of genital double-somite (Fig. 9G). Body ornamentation, anal operculum, rostrum, antenna (Fig. 11H), oral appendages (Fig. 11H), maxilliped, P1 exopod, P2 basis and exopod, and P4 exopod as in male. Anal somite with lateral pore (Fig. 9H). Caudal rami (Figs. 9H, 11G) similar to those of male but proportionally slightly shorter, length/width ratio 2.7; proximal part of seta V transformed, enlarged, and laterally flattened in proximal part.

Antennule (Figs. 9I, 11H): 7-segmented, aesthetasc on 4th segment longer and thicker than that of male, reaching end of 7th segment. Armature formula: 1-[0], 2-[1 pinnate +4 bare], 3-[4 bare], 4-[2 + ae], 5-[0], 6-[1], 7-[8 bare + (1 + ae)]. Apical acrothek represented by 1 seta and slender aesthetasc longer than male's.

P1 (Fig. 10A): Basis (Fig. 11I) with thin lateral seta, seta on medial margin shorter than in male. Enp-1 with proximal and distal longitudinal row of 2 spinules along medial margin and row of 3 spinules along lateral margin.

P2 endopod (Fig. 10B): Slightly thinner than in male, apical seta surrounded by 4 short spinules.

P3 (Fig. 10C): Basis with lateral seta; exopod 2-segmented, as normal for Parastenocarididae. Exp-1 with proximal and distal transverse row of 2 long spinules along lateral margin. Endopod (Figs. 10D, 11J) a small, cylindrical, pointed segment, slightly pinnate, reaching 2/3 of exp-1.

P4: Basis (Fig. 10E) as in male with additional row of 5 small spinules below endopod insertion; endopod (Figs. 10E, 11K) a small, cylindrical, pointed segment, pinnate in apical 1/2, almost as long as exp-1.

P5 (Figs. 10F, 11L): Without intercoxal sclerite, but right and left legs coalescent; legs trapezoidal, more elongate than in male, with larger pore. On free distal margin, from medial to lateral: strong, outwardly curved spiniform process, 2 subequal setae, long basipodal lateral seta.

Variability: The features appear to be con-

stant in the type series except for the number of spines in the outer rows of P3-exp1 in males, which is 3 or 4 in the distal row (Fig. 9D).

Etymology: The species epitheton is the feminine singular genitive of the Latin noun *diversitas* = diversity, meaning "of the diversity",

and refers to the high diversity of microcrustaceans in groundwater, which nowadays is particularly endangered by human impacts and climate change.

Remarks: The family Parastenocarididae is a taxon with a very specialized ecology and biology



Fig. 9. *Parastenocaris diversitatis* sp. nov. (A) Male, P2. (B) Male, P3. (C) Male, P3 basis, lateral view. (D) Male, P3, lateral view (variability). (E) Male, P4. (F) Male, P5. (G) Female, genital double-somite and genital field, ventral view. (H) Female, anal somite, anal operculum, and caudal ramus, lateral view. (I) Female, antennule. Scale bar = $50 \mu m$

for life in groundwater, where these harpacticoids are often one of the dominant families. However, P. diversitatis sp. nov. does not fit into the genus Parastenocaris Kessler as defined based on revisions (Reid 1995, Galassi and De Laurentiis 2004b), which attribute to the genus only those species which are morphologically close to P. brevipes Kessler, 1913, and which correspond to the definition of Parastenocaris sensu stricto given by Reid (1995). Therefore, P. diversitatis sp. nov. must be temporarily included in Parastenocaris sensu lato according to Galassi and De Laurentis (2004b), or in Parastenocaris incertae sedis according to Schminke (2010), taking into account that neither group represents species-group with a phylogenetic implication, but they are "taxonomic repositories" to be used until the revision of the family is completed and the genus Parastenocaris is officially restricted to those species that are morphologically similar to P. brevipes.

Three species of *Parastenocaris* sensu lato were so far collected in Sicily: *P. trinacriae* Pesce, Galassi and Cottarelli, 1988 and *P. kalypso* Pesce, Galassi and Cottarelli, 1988, from freshwater wells in Trapani Province (northeastern Sicily), and *P. luciae* Cottarelli Bruno and Berera, 2008, in the interstitial of the banks of a small river mouth in Palermo Province (northeastern part of the island, west of Trapani). However, notwithstanding the spatial proximity of these species, *P. diversitatis* sp. nov. does not share relevant affinities with *P. trinacriae*, *P. kalypso*, or *P. luciae*, which belong to different phyletic groups.

Parastenocaris diversitatis sp. nov. is characterized by a strong, dorsal, pointed apophysis on each caudal ramus in both sexes. Only a group of species endemic to Italy shares this particular feature of the caudal rami: P. federici Stoch, 2000; P. lorenzae Pesce, Galassi and Cottarelli, 1995; and P. pasquinii Cottarelli, 1972, as redescribed by Bruno and Cottarelli (1998). Also a species from Italy and Slovenia, P. gertrudae Kiefer, 1968, as redescribed by Stoch (2000), has "a distal sclerotized tooth, wide and blunt" on the dorsal side of the caudal ramus; but according to us, this species belongs to a different phyletic lineage and is not further discussed. The remaining 3 species were previously attributed to the *minuta*-group Lang, 1948, which is today considered polyphyletic (Galassi and De Laurentiis 2004b) and thus are not valid. Among these 3 species, P. diversitatis sp. nov. has the closest affinities with P. pasquinii,



Fig. 10. *Parastenocaris diversitatis* sp. nov. (A) Female, P1 basis and endopod. (B) Female, P2. (C) Female, P3 exopod. (D) Female P3 basis and endopod. (E) Female P4 basis and endopod. (F) Female, P5. Scale bar = 50 µm.

an endemic species from the psammolittoral zone of 2 volcanic lakes of Latium, central Italy (Bruno and Cottarelli 1998), and with *P. lorenzae* from the krenal of a spring in the Sangro River watershed, in Abruzzo (east-central Italy). *Parastenocaris diversitatis* sp. nov. shares the presence of a dorsal apophysis on the caudal rami with those 2 species. Similarities and differences in the main features of males of *P. diversitatis* sp. nov. and *P. pasquinii* are as follows: a) the presence of 1 seta and 1 hook on the P1 basis, near the endopod insertion; b) similar shape and ornamentation of P2; c) similar shape of P3 (although the number of spines on the P3 exopod and the lengths of the thumb and distal apophysis differ); d) enp-P4 is leaf-like, with projections, in both species, but the endopod is proportionally shorter in *P. diversitatis* sp. nov., and the number and size of spinules near its insertion differ between the 2 species; e) P5 is rectangular, with a thin spine and 3 setae in *P. diversitatis* sp. nov.; P5 is larger, triangular with a strong tip, 3 setae, and 1 spine in *P. pasquinii*; and f) the anal operculum and caudal rami are similar, but the caudal rami of *P. diversitatis* sp. nov. are proportionally shorter and larger, without the pore at the basis of the main apical seta. Differences between the females of *P. diversitatis* sp. nov. and *P. pasquinii* are few: a) a much longer medial



Fig. 11. *Parastenocaris diversitatis* sp. nov. (A) Male, anal somite, anal operculum, and caudal rami, dorsal view. (B) Male, labrum, mouth parts, and P1 basis. (C) Male, antennule, dorsal view. (D) Male, antennule, lateral inner view. (E) Male P3. (F) Male, P5. (G) Female, anal somite, anal operculum, and caudal ramus, lateral view. (H) Female, antennule, antenna, and mouth parts. (I) Female, P1 basis. (J) Female, P3 basis and endopod. (K) Female, P4 basis and endopod. (L) Female, P5. Scale bars: A-E, G, H, L = 10 μm; F, I, J, K = 5 μm.

seta near the enp-P1 insertion in *P. pasquinii*; b) enp-P4 with a similar shape and ornamentation but it is proportionally longer and thicker in P. pasquinii; both species have a spinular row near the insertion of enp-P4 which is composed of 3 spinules in *P. pasquinii* (in the original description, Cottarelli 1972) and 5 in P. diversitatis sp. nov.; c) different shape and ornamentation of P5, bearing 1 less seta in P. diversitatis sp. nov.; and d) differences in the caudal rami are the same as those described for males; moreover, in several females of both species, the main apical seta (seta V) is transformed in the same way, although this might be due to convergence. In fact, the same transformation is present in females of species belonging to other phyletic lineages such as in several (but not all) populations of P. italica Chappuis, 1953 (Noodt 1955, Chappuis 1957 1958, Kiefer 1968, Cottarelli 1972, Schminke 1991), and P. mateusi (Noodt and Galhano, 1969). It was interesting to note that P5 of both sexes of P. pasquinii and P. diversitatis sp. nov. has a large excretory pore (Figs. 9F, 10F, 11F, L), but we observed a similar pore on P5 of some Parastenocaris sensu lato. We think that, in fact, the pores on P5 are widespread in the Parastenocarididae: for instance, they are present in P. proserpina Chappuis, 1938, P. amalasuntae Bruno and Cottarelli, 1998, and Parastenocaris sp. from Thailand (Cottarelli, unpubl. data). Because all of those species are phylogenetically very distant from *P. pasquinii* and *P. diversitatis* sp. nov., we do not think this feature should be discussed within the pool of affinities.

In terms of the affinities of P. diversitatis sp. nov. and P. lorenzae, males of the 2 species differ in the shape of the caudal rami (which are proportionally shorter and larger in *P. lorenzae*), in the ornamentation of the P1 basis (in P. lorenzae the "hook" is missing), and in the shape, size, and armature of P5. The armature and ornamentation of P2 and exp-P3 are very similar in the 2 species (enp-P3 is shorter and smaller in P. lorenzae), and enp-P4 is also similar, but 4 spines of decreasing length are inserted on the basis near the endopod insertion in P. lorenzae and 3 in P. diversitatis sp. nov. Females of the 2 species share a similar shape and ornamentation of the P2, P3, and P4 endopods (but enp-P4 in P. lorenzae lacks the basal spinule row); P5 is similar, but the new species has 1 less seta. Differences in the caudal rami are the same as those described for males.

In conclusion, *P. diversitatis* sp. nov., *P. pasquinii*, and *P. lorenzae* represent a homo-

geneous and distinct group, characterized by the strong morphological affinities listed above; the most important 1 being the dorsal apophysis of the caudal rami. Such an apophysis is also present in P. federici: this species according to us is not phylogenetically related to the other species discussed here, and differs from them in several informative characters such as the structure of the P4 endopod and the shape, armature and ornamentation of P3 in males, the presence of a thin seta on the P1 basis, and the shape and armature of P5 in both sexes. To sum up, the dorsal apophysis of the caudal rami seems to have developed independently in different phyletic lineages, being; for instance, present in males of P. gertrudae, and might be related to the stygobization process. The functional meaning of this structure is discussed in a paragraph on the ecology of P. diversitatis sp. nov.

A taxonomic key, summarizing the features useful in distinguishing *P. diversitatis* sp. nov. from its most affine species is given as follows:

- Caudal rami more elongated (L/W > 2.5) and large; male, P1 basis with hook: male, P4 basis with 3 spines of decreasing length inserted near endopod insertion; female, P4 basis with basal spinule row near endopod insertion .. 3
- P5 rectangular with a thin spine and 3 setae in males and trapezoidal with outwardly curved spiniform process and 3 setae in females; male, caudal rami without pore at basis of seta V; female, row of 5 spinules near insertion of enp-P4..
- *P. diversitatis* sp. nov.
 P5 large, triangular, with a strong tip, 3 setae, and 1 spine in males and trapezoidal with outwardly curved spiniform process and 4 setae in females; male, caudal rami with pore at basis of seta V; female, row of 3 spinules near insertion of enp-P4 *P. pasquinii*

DISCUSSION

The description of the ecology of *Bryocamptus* (*Rheocamptus*) stillae sp. nov. and *Parastenocaris diversitatis* sp. nov. must be considered preliminary, being based on the result of sampling surveys conducted only to describe the aquatic fauna of Conza Cave. In fact, samples were qualitative, and sampling techniques not exhaustive: for instance, drift filtration units (sensu Pipan 2005) to collect animals directly from the drips were installed in the cave only recently, and the resulting data will be discussed in a future paper. Nonetheless, these preliminary collections provide the first data on epikarstic fauna from Sicily; the species list presumably will increase in the future, considering that "the fauna of epikarst is often very rich in species rivalling or exceeding that of other aquatic cave habitats" (Culver and Pipan 2009).

The scarce rainfall characteristic of the Conza Cave area, its short distance from the sea (about 1 km linear distance), the small size of the cave, and the reduced ceiling thickness (abut 14 m) imply that, at least during the dry season, the volume of epikarstic water is very reduced. In Conza Cave, all of the water filling the rimstone pools is meteoric and percolates through the epikarst during rainfall periods. The rimstone pools are isolated: the cave is fossil, and the structure and position of the rimstone pools prevent them from even accidentally being filled from water collecting in the cave. Some of the rimstone pools are dry for most of the year (Grasso, pers. comm.); during our sampling campaign, rimstone pools 1 and 3 were dry from (presumably) spring 2010 to Dec. 2010; rimstone pool 5 was dry from Nov. 2009 to Apr. 2011, and rimstone pool 4 presumably from late winter 2009 to early spring 2010, and was still dry in Apr. 2011. In spite of the stressful environmental conditions, the epikarstic system of Conza Cave represents an important refuge for stygobiotic taxa: the water present in the network of microfractures ensures the survival of local populations of aquatic taxa during dry periods, and copepods collected in trickles and pools are the result of downward drift, as reported by Brancelj and Culver (2005) for fossil galleries. The persistence of copepod populations is supported by the notion that every year in the period of percolation, the drip pools are recolonized by stygobiotic copepods. It appears that a few meters of unconsolidated soil and fractured carbonatic rock can sustain stable populations of specialized and euriecious taxa, as already reported for other karstic systems (Brancelj 2002).

Brancelj (2002) showed that in caves where interconnections between water bodies filled by dripping are weak or nonexistent, colonization is passive, with a 'persist or perish' option, and numbers of specimens and taxa depend on the intensity of water flow, and the size and interconnections within the drainage basin of each single dripping point. Considering that the rimstone pools of Conza Cave are only filled by percolating water and they are dry for long periods, the scope of our repeated sampling, conducted before and after a prolonged dry phase, was to prove that: 1) the pools were colonized by organisms dripping from the epikarst; and 2) the epikarstic habitat of Conza Cave, albeit not very extended vertically and with scarce water input, can represent the main permanent habitat for stygobiotic copepods. Because there are no data on the existence of resting stages in species of Speocyclops, stygobiotic Bryocamptus, and Parastenocaris, which excludes their survival in dry pools, the presence of these copepods in pools immediately after they refill with dripping water would prove these hypotheses to be true. As shown in table 1, when rimstone pools were refilled with water after a prolonged dry period, copepods were in fact present, and the species composition in each pool did not change. More specifically, after rimstone pool 3 was rewetted, 240 specimens (mainly adults, some of which were coupling, and some females with egg sacs) of B. (R.) stillae sp. nov. were collected there. Pool 4 was still dry at the time of the last sampling (9 Apr. 2011), but the small amount of water (980 cm³) present in pool 5 yielded 8 specimens of P. diversitatis sp. nov. (4 males and 4 females).

The results of 3 yr of sampling (although with qualitative and temporally uneven methods) showed that the copepod fauna composition in the rimstone pools remained relatively constant, and even in a limited space such as the epikarst of Conza Cave, different taxa had very different and restricted distributions. In fact, we detected constant differences in stygobiotic species compositions among rimstone pools which represent an approximation of the composition of the fauna in the drips and thus in the epikarst above, indicating that the epikarstic copepod fauna probably varies on a very small spatial scale. Many epikarst copepods are known to have a linear extent of only a few hundred meters (Pipan et al. 2006, Moldovan et al. 2011), and the epikarst fauna is not uniformly distributed but rather divided into "blocks" probably characterized by different environmental condition and as a consequence, by different faunal assemblages (Brancelj 2002, Meleg et al. 2011). In fact, even if all the pools of Conza Cave are very close to each other (in a range of about 30 m), their copepod fauna divides them into 2 groups. One group was represented by rimstone pools 4 and 5 (the ones with P. *diversitatis* sp. nov. only) which are about 7 m

apart, and a 2nd group was represented by pools 1 and 2 (where *B.* (*R.*) *stillae* sp. nov. and *S. italicus* were collected) which are about 10 m apart. The distance between the 2 groups ranges about 10-16 m (Fig. 1). Faunistic differences in groups of pools which revealed 2 spatially distinct groups of water bodies within a cave over a spatial distance of about 70 m were reported by Brancelj (2002) in Velika Pasjica cave, Slovenia. It is very likely that the epikarst of Conza Cave is represented by 2 separate percolation units which feed the 2 groups of rimstone pools, and that these 2 "islands" could even be characterized by microfractures of different sizes (smaller fractures could be present in the area where *P. diversitatis* sp. nov. lives).

We think that these data confirm our hypotheses and, in conclusion, Conza Cave hosts a specialized fauna of epikarst specialists, which are adapted to living in a selective habitat. The number of stygobiotic taxa collected was lower than those recorded for much-larger caves with more-favorable hydrology and climate (Pipan and Brancelj 2004, Moldovan et al. 2007, Galassi et al. 2009), and also of caves of comparable extent, hydrology, and structure (e.g., in the small karstic cave Velika Pasjica in Slovenia, Brancelj (2002) collected 1989 specimens of 1 cyclopoid and 11 harpacticoid taxa from 19 puddles and basins on 8 sampling dates, of which three of these taxa were new to science). Nonetheless, Conza Cave hosts an interesting fauna worthy of appropriate protection measures.

Bryocamptus (R.) stillae sp. nov. was never collected in phreatic or hyporheic habitats or in springs in Sicily (Pesce and Galassi 1987 1988, Pesce et al. 1987 1988, Pesce 1988, Cottarelli unpubl. data), which suggests this is not a widespread species but rather has a restricted distribution. The morphological features discussed above indicate that this is a stygobiotic species, and although its biology is presently unknown, some features related to reproduction also indicate that it is a true stygobiont. In fact, B. (R.) stillae sp. nov. has an extended reproductive period, as already observed for several stygobiotic harpacticoids (Rouch 1968): coupling adults, ovigerous females, copepodids, and some nauplii were collected throughout the entire sampling period. The number and size of eggs also suggest stygobiotic specialization, because extended reproductive periods and the production of a few, large eggs are the results of strong adaptations to trophic conditions of the habitat and are typical of K- or A-selection (adversity), which is

common in troglobiotic and stygobiotic taxa. For harpacticoids, the reported number of eqgs is > 10, and the egg diameter is around 40 μ m for epigean species (with the exception of cold-stenothermic species such as Hypocamptus Chappuis and Bryocamptus (Arcticocamptus) Chappuis, which produce 2 eggs of large diameter), whereas all hypogean ones produce fewer than 10 eggs. which have diameters typically around 50 μ m (Rouch 1968). Ovigerous females of B. (R.) stillae sp. nov. were always carrying a few eggs (6 and in a few cases, 5) of large size (with a mean diameter of 60 µm, measured from 30 eggs from 5 ovigerous females collected randomly in different samples). This is the greatest diameter so far recorded for stygobiotic species of Bryocamptus (Rheocamptus).

Although B. (R.) stillae sp. nov. displays several morphological adaptations typical of stygobiotic harpacticoids and is probably a true "epikarst specialist" sensu Pipan et al. (2010), i.e., found only in epikarst habitats, the species does not show all of the peculiar adaptations discussed by Brancelj (2006 2009) for all species of 2 canthocamptid genera exclusive to the epikarst (i.e., Morariopsis Kiefer and Paramorariopsis Brancelj) and to a lesser extent, of some epikarstic species of Elaphoidella (E. tarmani Brancelj, 2009 and E. millennii Brancelj, 2009), and also partly present in Lessinocamptus Stoch (i.e., "short and robust setae on legs; body elongated with short antennules; short, robust and very divergent caudal rami with the seta V relatively short, strong and wide apart; setae I, III, IV short, robust and arcuated, seta VI short, spiniform and arcuated" (Brancelj 2009). These morphological adaptations help these harpacticoids avoid or reduce vertical drifting (Brancelj 2009), a useful survival strategy considering that "in the unsaturated zone (including the epikarst) there is a characteristic predominance of vertical water movement along small cracks and fissures" (Brancelj 2006). Because periods of intense percolation are not necessarily correlated with high abundance or biodiversity of stygobiotic copepods (Moldovan et al. 2007), stygobiotic organisms can avoid drifting, especially if cave water is not the breeding ground for stygobiotic copepods, as reported by Brancelj (2002) for Speocyclops infernus (Kiefer, 1930). In Conza Cave, the rimstone pools dry out for long periods of time, which would not allow survival of stygobiotic species that do not have resting stages, and are recolonized after rewetting by copepods drifting in with the drips. Our data suggest that specimens

of B. (R.) stillae sp. nov. can probably avoid drifting downstream during the rare but very strong seasonal rainfall events by holding to the rock substrate thanks to A1 and A2, which are relatively short and robust, the elongated body, the strong spinules of the ventral body surface and caudal rami, and possibly the few strong teeth on the anal operculum and the ventral row of strong spinules on the anal somite. Teeth instead of spinules are in fact present on the anal operculum in most stygobiotic Bryocamptus, and these features are probably related to stygobization. In fact, all of the adaptations listed above for B. (R.) stillae sp. nov. are also present in other stygobiotic Bryocamptus which are not epikarst specialists; it would be more parsimonious if they were present in the lineage of B. (R.) stillae sp. nov. before its ancestor taxon became specialized to life in the epikarst, and were maintained because they provided advantages in this habitat. On the other hand, the lack of exclusive adaptations to the epikarst by B. (R.) stillae sp. nov. may indicate that this species is a recent colonizer. Among copepods collected in Conza Cave, B. (R.) stillae sp. nov. seems to be the species with the widest ecological valence, the best adaptations to life in rimstone pools where this species is present with ovigerous females and larval stages, and the greatest sensitivity to drifting, given its high abundance in the pools. However, dripping of specimens from the epikarst to the pools causes a decline in the original epikarstic populations, because all of the specimens ending up in the pools are destined to perish when the pools dry out. In fact, given the hydrological conditions of Conza Cave, the abundant drifting of B. (R.) stillae sp. nov. does not imply a higher chance of dispersal and colonization, and populations of copepods in drips and pools are considered sink populations exposed to severe competition and predation, the community structure of which changes over time (Brancelj 2002 2006).

The reduced size and cylindrical and elongated body typical of all *Parastenocaris* allows the members of this taxon to live in habitats ranging from small interstitial spaces to deep phreatic water and several aquatic habitats of caves. If according to Brancelj et al. (2010), "reduction is not a compelling factor for living in a narrow space" for *Parastenocaris*, the epikarst was probably not among the primary subterranean habitats which this lineage colonized. Therefore, even if selective pressures of the epikarstic habitat did not result in peculiar adaptations in P. diversitatis sp. nov., the general morphology of Parastenocaris represents a preadaptation for colonizing the epikarst: some morphological peculiarities which appeared along the phyletic lineage leading to P. diversitatis sp. nov. would turn out to be particularly useful for life in the epikarst. For instance, the apophysis on the caudal rami of P. diversitatis probably helps anchor the animal to the substratum, preventing vertical drift. Such an apophysis is not exclusive to Parastenocaris collected in the epikarst; it was recorded in species (P. pasquinii and P. lorenzae) respectively known from lacustrine psammon and krenal, but also in both sexes of a species known only from rimstone pools in caves (P. federici) and in males of a 2nd species (P. gertrudae) collected from rimstone pools in caves and also in the hyporheic habitat (Stoch 2000). Parastenocaris diversitatis sp. nov. was rare in rimstone pools compared to B. (R.) stillae sp. nov. and even to the already rare S. italicus. This might not have been due to the low densities of populations of *P. diversitatis* sp. nov. in the epikarst, but to its reduced size and peculiar body shape which probably allow these animals to penetrate into very small fractures of the rock, which are inaccessible to other copepods (which are larger, longer, stouter, and therefore less flexible). The capillary action of water percolating in small-diameter fractures probably helps specimens resist the traction force of water and avoid drifting, thus ensuring its survival in the only suitable habitat, i.e., the epikarst.

Speocyclops italicus does not seem to possess morphological features directly related to colonization of epikarstic habitats, although this and all other species of the genus share typical characters of stygobiotic cyclopoids (e.g., reduced size, legs, and antennular segmentation), similar to what was discussed for Parastenocaris, which would explain the presence of this taxon in the epikarst of Conza Cave. Speocyclops italicus was rarely collected together with *B. stillae* sp. nov. possibly due to competition between the two taxa, a phenomenon common in populations which collect in pools (Brancelj 2006). This species was first described by Kiefer (1938) from Castelcivita Cave (Salerno Province, southern Italy), and it colonizes several groundwater habitats, although in caves, according to Stoch (2005), S. italicus is mainly present in percolating water. This species is widely distributed in central and southern Italy where it shows a wide ecological tolerance (Galassi and De Laurentiis 2004a); as a consequence, S. *italicus* is probably a highly competitive species,

as already reported by Brancelj (2006) for *S. infernus*, which is such a competitive species that it determines the secondary structure of copepod populations in drip pools. The same author in fact reported how members of the genus *Parastenocaris* in the epikarst are very vulnerable and have little resistant to interspecific competition.

Acknowledgments: R. Grasso wishes to thank M. Vaccarella, President of the Club Alpino Italiano, Sicilian section, and G. Mento, Director of the Conza Cave Natural Reserve, for supporting and encouraging biospeological research in Conza Cave, and to Prof. S. Sciacca, director of the Laboratorio di Igiene Ambientale e degli Alimenti of Catania University, for analysing the water samples. Rainfall data were provided by the Osservatorio delle Acque Regione Siciliana. Funding was provided by the "Assessorato Regionale Territorio e Ambiente della Regione Siciliana" to R. Grasso. Two anonymous reviewers are greatly acknowledged for their challenging reviews, which considerably improved the manuscript.

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