A new species of Speleophriopsis (Copepoda: Misophrioida) from an anchialine cave in the Adriatic Sea, Mediterranean

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ORIGINAL PAPER

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# A new species of *Speleophriopsis* (Copepoda: Misophrioida) from an anchialine cave in the Adriatic Sea, Mediterranean

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Abstract A new copepod species, Speleophriopsis mljetensis, collected from an anchialine cave Bjejajka on Mljet Island (Croatia) is described. The prosome of the new species is 5-segmented; the urosome has five somites in females and six in males; the genital double-somite is symmetrical and is longer than wide; the caudal rami are symmetrical, with seta I well developed; the antennules of both sexes are 27-segmented and symmetrical; the antennal exopod is 7segmented; the maxillule possesses 15 armature elements on the praecoxal arthrite and the proximal basal endite has three slender setae and one stout claw-like seta, the fifth legs are symmetrical, 4-segmented and uniramous, and the distal segment is elongate and armed with seven and six elements in males and females, respectively. Speleophriopsis mljetensis is comparated with similar species S. balearicus and S. canariensis. The new species is mainly distinguished by the armature of the genital operculum, the fifth legs of both sexes, maxilliped distal segment armature and total length of both sexes. This is the second report of a speleophriid copepod, otherwise thought to be a Tethyan relict, from an Adriatic anchialine cave. In the Bjejajka Cave, the salinity varied between 1.5 at the surface to 39 psu at the bottom, and the temperature ranged between 14.2 and 16.6 °C. It might be assumed that it colonised Bjejajka anchialine cave from its natural deep-sea habitat. However, the distributions of

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Frano Kršinić fkrsinic@izor.hr speleophriids in the Mediterranean are difficult to explain; therefore, this finding is of particular importance, contributing to our knowledge of the global biogeography of these Tethyan relicts.

**Keywords** Adriatic Sea · Anchialine caves · Copepoda · Misophrioida · Stygofauna

#### Introduction

Misophrioid copepods primarily occur in the hyperbenthic zone in both coastal areas and deep-sea habitats (Boxshall 1989). However, many new misophrioids described in last four decades have been found in anchialine caves (Boxshall 1987; Boxshall and Iliffe 1986, 1990; Huys 1988; Jaume and Boxshall 1996a, b; Jaume et al. 1998; Jaume et al. 2001). The species of the family Speleophriidae are mainly from anchialine habitats (Boxshall and Jaume 2000). The investigation of new species of the genera Speleophriopsis Jaume & Boxshall, 1996 and Speleophria Boxshall & Iliffe, 1986 and expanding knowledge of their specific biogeography is important for understanding the dispersal and colonisation patterns of these taxa prior to the closure of the Tethys Sea (Boxshall et al. 2014). The eastern shore of the Adriatic Sea is a region of Dinaric karst characterised by porous rock and numerous related geo-morphological features, such as anchialine caves. According to a redefinition of the term 'anchialine' (Bishop et al. 2015), there are about 40 anchialine caves along the Croatian coastline. These caves extend up to 100 m from the sea and are typically small in size. From a biogeographical perspective, the fauna of the Dinaric coast and associated islands generally exhibits a paralittoral distribution (Sket 1996). Based on the sustained efforts of the Croatian Biospeleological Society in Zagreb, preliminary

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investigations of calanoid copepods in the anchialine caves have been undertaken along the Croatian coast and on nearby islands (Kršinić 2005a, b). The latest studies of anchialine caves along the eastern coast of the Adriatic have revealed two new copepod species of the family Speleophriidae. One of these, *Speleophria mestrovi* Kršinić, 2008, was found in the anchialine cave in Supurina Cave, on Vis Island in the central part of the eastern Adriatic (Kršinić 2008). The other, a species of the genus *Speleophriopsis*, was collected only in the anchialine Bjejajka Cave on the island of Mljet in the southern part of the Adriatic, and is described in the present paper.

#### Materials and methods

Material was collected during sampling expeditions carried out in March 2005 in Bjejajka Cave on Mljet Island in the Central Adriatic. Divers from the Croatian Natural History Museum (Zagreb) and the Croatian Biospeleological Society (Zagreb) made collections by hand-held hauls with a 20-cmdiameter, 125-µm mesh Nansen net, sampling between depths of 0 to 12 m. All samples were preserved in a 2.5 % formaldehyde–seawater solution neutralised with CaCO<sub>3</sub>. Specimens were dissected on slides in lactophenol. Drawings were made with the aid of a drawing tube on an Olympus BX51 differential interference contrast (DIC) microscope. Specimens were measured using an ocular micrometer. The descriptive terminology employed follows Huys and Boxshall (1991).

#### Taxonomy

Order Misophrioida Gurney, 1933 Family Speleophriidae Boxshall & Jaume, 2000 Genus Speleophriopsis Jaume & Boxshall, 1996 Speleophriopsis mljetensis sp. nov.

#### Material examined

Holotype, adult female 620  $\mu$ m in length, from Bjejajka Cave, on Mljet Island in the Central Adriatic Sea in Croatia (42°45' 55.5"N; 17°23'08.4"E), on October 04, 2004. The holotype is deposited in the Croatian Natural History Museum, Zagreb, no. HPM-BSZ,1799. Paratype series comprising eight adult females and four males from the same locality, collected during March, 2005, and are deposited in the Croatian Natural History Museum, Zagreb, no. HPM-BSZ,1800.

#### Etymology

This species name is derived from the Island of Mljet, the type locality.

#### Description

Female (Fig. 1a, b) total length (excluding caudal setae):  $600-770 \ \mu m \ (684 \pm 53.8 \ \mu m, \ n = holotype \ plus$ eight paratypes. Body cyclopiform in dorsal aspect. Nauplius eye absent. Prosome 5-segmented; cephalosome and first pedigerous somite separate. Pedigerous somite 4 with posterior margins expanded laterally in dorsal view. Rostrum well developed, rounded in dorsal aspect (Fig. 1a). Proportional lengths of prosome segments: 56:11:14:12:7 = 100. Ratio prosome:urosome including caudal rami = 1.5:1. Prosome 2.1 times long as wide. Urosome 5segmented (Fig. 1c); proportional lengths of urosomites and caudal rami 17:47:14:9:5:9 = 100. Second to fourth somites each with hyaline frill along posterior margin. Genital double-somite (Fig. 2b, c) 1.4 times longer than wide, with vestige of suture line between component somites visible ventrally; genital area symmetrical,



**Fig. 1** Speleophriopsis mljetensis sp. nov. Adult female. **a** Habitus, dorsal view; **b** habitus, lateral view; **c** genital double-somite and posterior part of urosome with caudal rami, dorsal view

located proximo-ventrally, with single small median copulatory pore, and paired genital opercula located laterally. Each operculum ornamented with naked seta, long spine and small denticle (Fig. 2b). All females with single egg, 50  $\mu$ m in diameter in medial position (Fig. 2c). Caudal rami symmetrical, about 1.3 times longer than wide. Each ramus with seven setae, four terminal setae corresponding to setae III–VI, seta I well developed, tuft of fine spinules present on inner margin of ramus (Fig. 1c).

Antennule (Fig. 2a) implanted on trapezoidal pedestal, symmetrical, 27-segmented, longer than cephalosome; segments 2 to 17 very short, segments 20 to 27 each about two times longer than wide. Aesthetascs on segments 3 and 7 hypertrophied. Armature and fusion pattern of segments as follows: segment 1 (ancestral I) two setae; segment 2 (ancestral II) one seta; segment 3 (III) two setae + aesthetasc; segment 4 (IV) one seta; segments 5 and 6 (ancestral V, VI) two setae each; segment 7 (VII) two setae + aesthetasc; segments 8–15 (VIII–XV) two setae each; segment 16 (XVI) two setae each; segment 21 (XXI) two setae + aesthetasc; segments 22 and 23 (XXII, XXIII) one seta each; segment 24 (XXIV) two setae;

segments 25 and 26 (XXV, XXVI) two setae + aesthetasc each; segment 27 (XXVII) five setae + aesthetasc.

Antenna (Fig. 3a) biramous. Coxa and basis unarmed. Endopod 2-segmented, longer than exopod; proximal segment armed with two setae distally on inner margin, distal segment elongate with five setae proximally and six setae at tip; Exopod 7-segmented; armature as follows: 1,1,1,1,1,1,5.

Mandible (Fig. 3b) coxa sickle-shaped, not ornamented, gnathobase cutting edge with isolated unicuspid tooth and ten heterogeneous teeth, lateral extremity of coxa expanded. Basis longer than wide with single seta on medial margin. Endopod 2-segmented; first segment with one seta and terminal segment with seven setae. Exopod 5-segmented, setal formula 1,1,1,1,2.

Maxillule (Fig. 3c) praecoxal arthrite bearing ten stout pectinate spines, three setae on proximal part and two setae on posterior surface. Coxal epipodite armed with seven plumose setae; coxal endite with four setae. Proximal basal endite with three slender setae and one stout seta; distal basal endite with four basal exite represented by short seta. Endopod 1-segmented, bearing five slender distal setae, one stout seta, two subdistal and two lateral shorter setae. Exopod with seven distal and two lateral setae.







Fig. 3 Speleophriopsis mljetensis sp. nov. Adult female. a Antenna; b mandible; c maxillule; d maxilla; e maxilliped

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Maxilla (Fig. 3d) 6-segmented. Praecoxal endites with six and three setae, respectively. Coxal endites with three and three setae, respectively. Allobasis with proximal endite powerfully developed, ornamented with short setules, bearing five setae proximally and three setae distally. Free endopod 3-segmented, with setal formula 2,2,4.

Maxilliped (Fig. 3e) 8-segmented. Praecoxal endite with one seta; coxal endites incorporated into segment, setal formula 2,4,3. Basis triangular with three setae in inner median position, outer lateral margin ornamented with spinules. Endopod armature formula 1,2,2,2,2+1,4+1, distal segment elongate. Swimming legs 1–4 (Fig. 4a–d) armature formula as follows:

	coxa	basis	endopod	exopod		
Leg 1	0 - 1	I - I	0-1; 1, 2, 3	I - 0; I - 1; III, 1, 3		
Leg 2	0 - 1	1 - 0	$0-1; \ 0-2; \ 1, \ 2, \ 3$	I - 1; I - 1; III, I, 5		
Leg 3	0 - 1	1 - 0	0-1; 0-2; I, II, I+2	I - 1; I - 1; III, I, 5		
Leg 4	0 - 1	1 - 0	$0-1; \ 0-2; \ I, \ II, \ I+1$	I - 1; I - 1; III, I, 5		

First leg shorter than other legs. Exopod segments of legs 1–4 with spinules along outer margin, surfaces of all legs naked. On third endopod segment of legs 3 and 4, terminal setae transformed into spines.

Fifth legs (Fig. 4e) symmetrical, uniramous, 4-segmented. Coxa unarmed, basis longer than coxa, with outer plumose seta on submarginal surface, inner margin rounded. Exopod 2-segmented; proximal segment longer than basis, with naked spine on outer margin. Distal segment 2.5 times longer than proximal, ornamented distally with row of very fine setules, armed with three denticulate spines on outer margin and small naked spine on inner margin, long distal denticulate spine, with denticles along inner margin covering only distal part, and short subdistal seta.

Male (Fig. 5a, b) total length:  $620-680 \mu m$  ( $646 \pm 35.4 \mu m$ , n = 4). Prosome 2.4 times longer than wide. Cephalosome and pedigerous somites similar to those of adult female. Rostral area as in female. Prosome:urosome ratio = 1.6:1. Urosome 6-segmented (Fig. 5c); proportional lengths of urosomites and caudal rami: 7:36:23:13:5:6:10 = 100. Genital somite symmetrical, approximately as wide as long, slightly expanded dorsoventrally; paired genital apertures armed with three unequal setae representing leg 6 (Fig. 5c). Caudal rami as in female.

Proximal part of 27-segmented antennules (Fig. 6a) similar to that of female. Distal part of left antennule typically bent inwards (Fig. 6b) while, in right antennule, making loop to outside. Geniculation present between ancestral segments XX and XXI on both sides. Segment XV cup-shaped; segment XVI longer than wide and with large part incorporated in segment XV. Segments 2–15 condensed; broad articulations present between segments 16–18, with rounded margin anteriorly. Segmental setation pattern as follows: segments 1–17 as for female; segments 18 and 19, 1 seta + spine; segment 20,



**Fig. 4** Speleophriopsis mljetensis sp. nov. Adult female. **a** Leg 1; **b** leg 2; **c** leg 3; **d** leg 4; **e** leg 5

one seta; segment 21, two spines + aesthetasc (left antennule), one spine (right antennule); segments 22, one spine; segment 23, one seta, segment 24, two setae; segment 25, two setae + aesthetasc; segment 26, two setae; segment 27, five setae + aesthetasc.

Antenna, mouthparts and swimming legs 1–4 identical to those of female. Fifth legs uniramous and symmetrical (Fig. 6c). Coxa unarmed, basis longer than coxa, with outer naked seta on submarginal surface, inner margin rounded. Both exopodal segments relatively longer than in female; naked spine on outer margin of proximal segment smaller than spine in female; distal segment with three spines on outer margin, as in female, distal spine and short seta, and with two elements on inner margin, proximal pinnate seta and subdistal truncate spine ornamented with denticles on both margins.

#### Discussion

The morphological characters of the family Speleophriidae and a key to genera were provided by Boxshall and Halsey

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Fig. 5 Speleophriopsis mljetensis sp. nov. Adult male. a Habitus, dorsal view; b habitus, lateral view; c genital somite, posterior part of urosome and caudal rami, ventral view

(2004). Four described species of the genus Speleophriopsis had previously been discovered in anchialine caves. Speleophriopsis scottodicarloi (Boxshall & Iliffe, 1990) was discovered in Chalk Cave, Bermuda and S. campaneri (Boxshall & Iliffe, 1990) in Ngamduk Cave, Angaur Island, Palau, by Boxshall and Iliffe (1990). Both species are known only from females. Speleophriopsis balearicus Jaume & Boxshall, 1996 was found in Cova des Burrí at Balearic Islands, Western Mediterranean (Jaume and Boxshall 1996a), while S. canariensis Jaume & Boxshall, 1996 was discovered in Jameos de los Lagos, Lanzarote, Canary Islands (Jaume and Boxshall 1996b). The newly discovered species was found in the anchialine cave habitat on the island of Mljet, located near the deepest part of the Adriatic Sea, South Adriatic Pit, which has a maximum depth of 1250 m. The new species is similar to S. balearicus and S. canariensis; differences from S. campaneri and S. scottodicarloi are more obvious, as previously noted by Jaume and Boxshall (1996b) in their description of S. canariensis. According to Jaume and Boxshall (1996a), body segmentation and armature of all



**Fig. 6** *Speleophriopsis mljetensis* sp. nov. Adult male. **a** Right antennule; **b** distal part of left antennule; **c** leg 5

appendages are virtually identical in S. balearicus and S. canariensis, except the armature of the genital operculum and leg 5. The genital operculum of S. mljetensis sp. nov. is armed with a slender naked seta, a shorter stout spine and a small denticle. In S. balearicus, it is armed with two setae, while in S. canariensis, it carries a long plumose seta, a short stout spine and a tiny denticle. The tip of the outer spine on the first exopodal segment of leg 5 in the females does not reach the tip of the proximal spine on the second segment in S. mljetensis sp. nov. and in S. canariensis, while in S. balearicus, this outer spine is the same length as the second segment and reaches the tip of the proximal spine on the second segment. The terminal spine on the second exopodal segment is only as long as that segment in S. mljetensis sp. nov.; however, the distal seta is much shorter than in S. balearicus and is not pinnate.

The fifth legs of the males also differ. In *S. canariensis*, the first and second exopodal segments are thicker than in the other two species, whose segments are more elongate, especially in the new species. In *S. balearicus* only, the second spine on the inner margin of the second exopodal segment extends beyond the tip of the distal spine, while the distal pinnate seta is also long. Finally, the second seta on the inner margin of the second segment is replaced by a spine ornamented with denticles along both sides, only in *S. mljetensis* sp. nov.

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Species	Cephalosome posterior margin	Basis leg 5 inner margin		Swimming legs surfaces	Antenna	Antenna endopod distal segment
S. balearicus	Expanded posterolaterally	Not produced distally Produced distally into a rounded outgrowth		With small denticles	6-segmented	Ornamented
S. canariensis	Expanded posterolaterally Not expanded posterolaterally			to a With small denticles	6-segmented 7-segmented	Ornamented Not ornamented
S. mljetensis sp. nov.		Not produced dista	lly Naked			
	Maxillule exopod setation	Maxillule exopod last seta	Maxilli segmer	iped first endopodal nt armature	Maxilliped distal segment armature	Adult female length up to
S. balearicus	3,2,6	Not well developed	Two setae		Five long setae on tip	1090 µm
S. canariensis	3,2,6	Not well developed	ot well developed Two se		Five long setae on tip	680 µm
S. mljetensis sp. nov.	2,2,6	Very well developed	One seta		Four long setae on tip + one shorter on latera margin	770 μm al

Table 1 Additional comparative characteristics for species Speleophriopsis balearicus, S. canariensis and S. mljetensis sp. nov.

In *S. mljetensis* sp. nov., the caudal rami are more divergent, and the inner surface is ornamented with scattered denticles. The articulations between segments 16–18 in the male antennule are broad and with anteriorly rounded margin. Segment 21 of the left antennule bears two spines and an aesthetasc, while same segment of the right antennule bears only one spine. In addition, the new species is distinguishable from *S. balearicus* and *S. canariensis* by the features noted in Table 1.

In the Bjejajka Cave, the salinity varied between 1.5 at the surface and 39 psu at the bottom, and the temperature ranged between 14.2 and 16.6 °C. The oxygen concentration was at its lowest at 4 m depth a value of 0.14 mg  $O_2L^{-1}$ , while significantly higher values were found near the bottom, during January 2009 (Žic et al. 2011). The highest population density of the new species was found in the deeper parts of the cave, where there were significantly higher values of oxygen and salinity. Increased concentrations of dissolved organic carbon (DOC) and trace metals like Zn and Cu are found in the water and sediment (Cuculić et al. 2011) of Bjejajka Cave, and bacterial abundance was higher in the cave than in the surrounding seawater (Krstulović et al. 2013). The new species was found only in the cave on the island of Mljet, which is located 25 nautical miles from the deepest part of the Adriatic Sea. Therefore, it might be assumed that it colonised Bjejajka anchialine cave from its natural deep-sea habitat, as it was hypothesised earlier for misophrioids by Boxshall (1989). However, distributions of speleophriids in the Mediterranean are difficult to explain, given the Messinian Salinity Crisis (MSC), according to which the Mediterranean Sea was dry well after the closure of the Tethys Sea, as noted by Jaume and Boxshall (1996a) and Boxshall and Jaume (2000). Previously described species of the genus Speleophria, discovered in the Southern Adriatic, the Eastern Mediterranean Sea (Kršinić 2008), as well as a new species of the genus *Speleophriopsis* are of particular importance, contributing to our knowledge of the biogeography of these Tethyan relicts. As mentioned by Jaume and Boxshall (1996b), it is necessary to find the possible Quarternary glaciation habitat refuges in the area of the South Adriatic and in the deepest part of the Mediterranean, the Ionian Sea.

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