Acta Aquatica Turcica
E-ISSN: 2651-5474 17(1), 78-87 (2021) DOI:https://doi.org/10.22392/actaquatr.756011

First Record of *Metacyclops subdolus* Kiefer, 1938 (Copepoda, Cyclopidae) from Turkey

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Research Article

Received 21 June 2020; Accepted 12 August 2020; Release date 01 March 2021.

How to Cite: Bozkurt, A. (2021). First record of *Metacyclops subdolus* Kiefer, 1938 (Copepoda, Cyclopidae) from Turkey. *Acta Aquatica Turcica*, *17*(1), 78-87. https://doi.org/10.22392/actaquatr.756011

Abstract

Copepod samples were collected from the lake in Gilindire Cave in Aydıncık District (İçel, Turkey). A total of 3 taxa including 1 rotifer, 1 copepod, and 1 amphipod were found. *Metacyclops subdolus* was reported from Turkish inland waters for the first time.

Keywords: *Metacyclops subdolus*, Zooplankton, Copepoda, Gilindire Cave, underground lake

Türkiye'den *Metacyclops subdolus* Kiefer, 1938 (Copepoda, Cyclopidae)'un İlk Kaydı

Özet

Kopepod örnekleri Aydıncık İlçesi (İçel, Türkiye) Gilindire Mağarası'ndaki gölden toplanmıştır. 1 rotifer, 1 kopepod ve 1 amfipod olmak üzere toplam 3 takson bulundu. *Metacyclops subdolus* ilk kez Türkiye iç sularından rapor edilmiştir.

Anahtar kelimeler: *Metacyclops subdolus*, Zooplankton, Kopepoda, Gilindire Mağarası, yeraltı gölü

INTRODUCTION

Gilindire Cave which is located in the southern part of Turkey (Mersin) and was discovered by a shepherd in 1999. The lake in the cave has brackish waters at the first 10 meters depth, and at the later depths it has saltwater characteristics and the average water temperature is about 25°C and absolute humidity is 80% (Nazik et al., 2001). This subterranean ecosystem, characterized by chemoautotrophic resources, was described and named as "Ophel" by Por (2007). Since the entrance of the cave is flat and narrow, there is no direct air movement from outside. Gilindire Cave, which is a multi-period and multi-origin cave in terms of geomorphological formation and development, is a very important natural formation that developed into the literature in the national sense in the science of speleology by developing in the Cambrian dolomite and limestone lithology (Nazik et al., 2001).

Some zooplanktonic organisms, such as copepods, may be acceptable markers of natural surroundings heterogeneity. Zooplankton has significant roles in groundwater food webs and ecosystems, but their natural capacities in groundwater are not sufficiently known (Gibert and Deharveng, 2002). Cyclopoid copepods compose an important part of the groundwater biodiversity in karstic habitats, and they live in planktonic or epibenthic and hyperbenthic environments in the underground source (Dussart and Defaye, 2006).

The genus *Metacyclops* Kiefer, 1927 contains 67 nominal species and subspecies (Dussart and Defaye 2006), and three of them are listed on the IUCN Red List, *M. campestris* from Brazil, *M. gasparoi* from Italy, and *M. postojnae* from Slovenia (Walter, 2018).

The genus *Metacyclops* is widespread in tropical and temperate regions, most of them have been recorded in different groundwater habitats, such as wells, caves, anchialine habitats (Pesce, 2015). However, despite its potentially rich fauna, only six species have been recorded from Turkey so far: *M. amoenus* Mann, 1940, *M. gracilis* (Lilljeborg, 1853), *M. minitus* (Claus, 1863), *M. planus* (Gurney, 1909), *M. stammeri* Kiefer, 1938 and *M. grandispinifer* (Lindberg, 1940) (Ustaoğlu, 2004; Ustaoğlu 2015).

M. subdolus has a European Mediterranean distribution (no record from North Africa); it has first been reported by Kiefer (1938) from southern Italy (La Zinzulusa, Abysso caves), then from Sardinia (Lindberg, 1956), Italy (Pesce et al., 1978; Pesce, 1985), Greece (Peloponnesos, Attica, Crete: Pesce, 1978; Pesce and Maggi, 1981, 1983), Mallorca (Can Pastilla: Lescher-Moutoué, 1981), Isreal (Dimentman and Por, 1991; Defaye and Por, 2010; Spring and Cave), and northern Negev (Defaye and Dussart, 1995).

A new species of *M. subdolous* was reported in a limited number of countries and regions, and for the first time the report has been in Turkey. Some supplementary drawings and descriptions from Gilindire specimens are provided as a basis for future comparison.

MATERIALS and METHODS

Gilindire cave is located in Aydıncık district of Içel province, at a longitude of 33° 24' 11.04"east, and a latitude of 36° 07' 58.08" north (Figure 1). The cave consists of three separate sections that are interconnected but occur at different periods. The cave, whose width reaches 100 m in places and the ceiling height reaches 18 m and extends between + 22 / -28 meters according to the entrance, is divided into many halls and rooms by large and thick dripstones. There is a large lake in the last part of Gilindire Cave. The length of the lake is approximately 140 m, its width is18-30 m, and the ceiling height is 35-40 m, the water level is 46 meters from the entrance of the cave and it is the same as the sea level (Nazik et al., 2001; Özşahin and Kaymaz, 2014) (Figure 2).

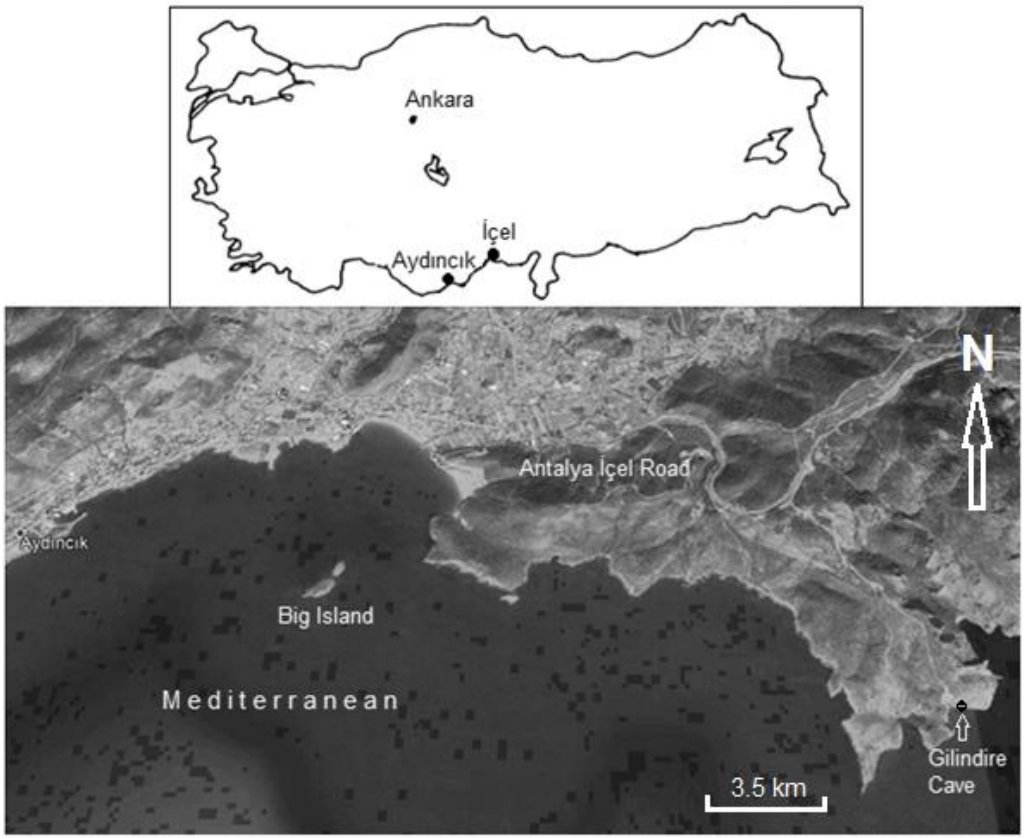

Figure 1. The location of Gilindire Cave.

It was determined that the first 10 meters of the lake water have the feature of mixing water and when it goes down from this depth, it gains seawater feature completely. It was reported that the water of the lake is largely seawater, and the groundwater leaking from the rain and reaching the lake is mostly mixed with seawater at the upper elevations of the lake (Nazik et al., 2001; Özşahin and Kaymaz, 2014).

In the observations made by scuba diving in the underground lake, no significant groundwater flow was detected in any direction, especially from the land direction towards the sea. Also, it was

determined that the water level did not change in the section between the lake and the sea, where the horizontal distance is about 250 m. On the other hand, in the region where the average precipitation is low, the waters leaking from the surface continue to form sediments in the Vadose section (Nazik et al., 2001; Özşahin and Kaymaz, 2014).

Copepod specimens were collected in May, June, and November 2015 by throwing the plankton net (60 µm mesh size) from the lakeshore to the interior (about 10-12 meters), pulling it from the surface to the shore, and also collected at different depth. This process was done at least 10 times. Samples were replaced into a glass jar and fixed with 4% buffered formaldehyde. Specimens were examined in a mixture of distilled water and glycerol. Drawings and measurements were made by using an Olympus microscope with a drawing-tube an ocular micrometer. Copepod body lengths were measured at 10x magnification with micrometric ocular attached to the ocular. The species were identified according to Dussart (1967), Damian-Georgescu (1970), Kiefer (1978), Pesce (1978), Defaye and Por (2010).

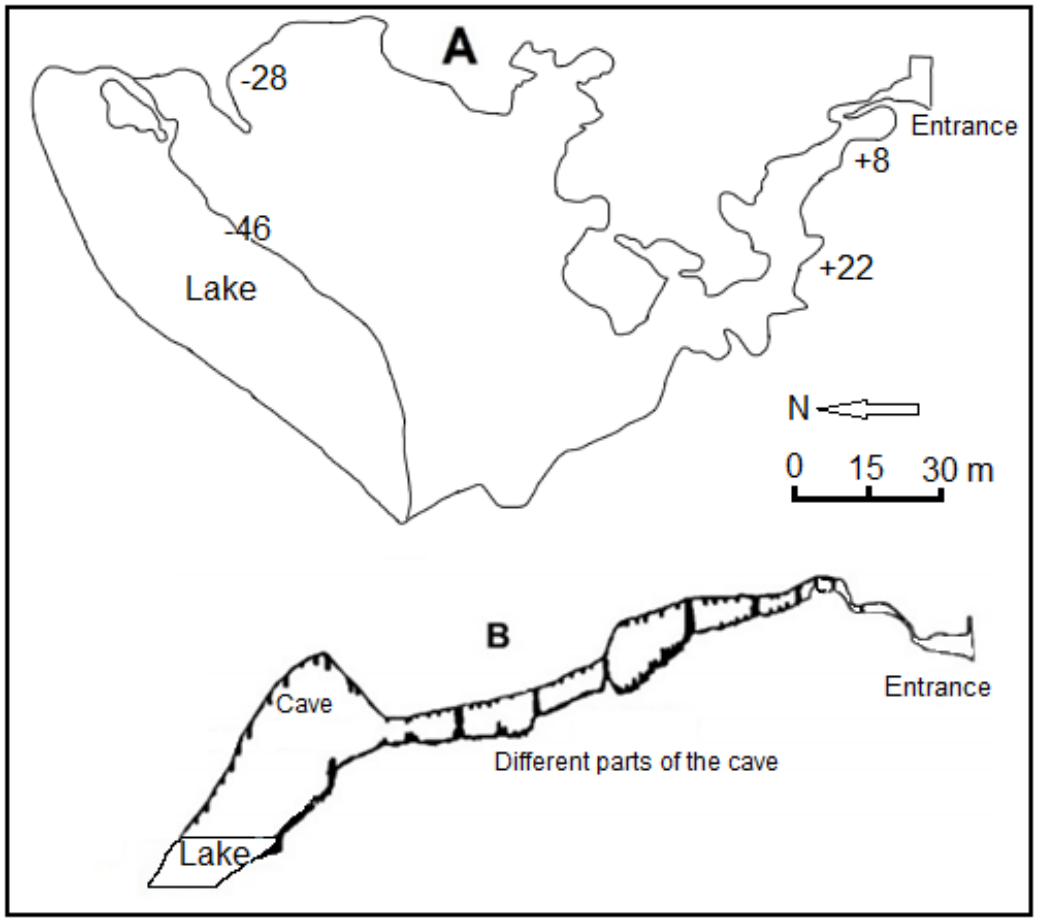

Figure 2. Plan of the Gilindire Cave (A top view, B side view) (redrawn from Nazik et al. 2001)

RESULTS

The water quality criteria (temperature, pH, electrical conductivity, salinity, and dissolved oxygen) measured by depths are given in Table. According to Nazik et al. (2001), while there was no change in temperature and pH depending on the depth, there was a significant increase in electrical conductivity and salinity, and a significant decrease in dissolved oxygen was reported.

Table. Physical-chemical properties of Aynalı Lake in Gilindire Cave (quotation from Nazik et al., 2001).

Depth (m)	Temp (°C)	pH	EC (µS/cm)	Salinity (ppt)	DO (% sat)	DO (mg/L)
1.2	21.65	7.29	4334	2.4	86.6	6.81
2.6	21.65	7.25	5106	2.8	84.1	6.60
3.9	21.65	7.27	6397	3.5	79.7	6.22
5.6	21.64	7.26	9002	5.1	75.9	5.87
6.8	21.64	7.27	11131	6.3	74.4	5.71
8.3	21.64	7.29	13426	7.7	71.5	5.44
9.9	21.64	7.30	17012	10.0	69.6	5.22
11.3	21.64	7.31	20691	12.3	61.5	4.55
13.1	21.65	7.30	25844	15.7	53.7	3.89
14.2	21.65	7.31	30923	19.2	46.2	3.28
16.0	21.65	7.30	36708	23.2	43.6	3.02
17.7	21.65	7.32	41700	26.8	46.2	3.13
20.0	21.65	7.33	44831	29.0	48.9	3.27
22.1	21.65	7.34	46796	30.5	47.5	3.15
23.8	21.66	7.35	47899	31.3	44.2	2.92
25.3	21.66	7.35	48294	31.6	42.1	2.77
27.0	21.67	7.33	48419	31.7	43.5	2.87

In this study, 1 rotifer (*Philodina* sp.), 1 copepod (*Metacyclops subdolus*), and 1 amphipod (under review) were identified in the cave.

Taxonomic account:
Order Cyclopoida Burmeister, 1835
Family Cyclopidae Rafinesque, 1815
Subfamily Cyclopinae Rafinesque, 1815
Genus *Metacyclops* Kiefer, 1927
Metacyclops subdolus Kiefer, 1938 (Figures 3-5)

Redescription of the female (Figures 3-4). Nineteen specimens were measured and body lengths ranged between 0.586 mm and 0.778 mm, except for caudal seta, and the mean was 0.682 mm. Body about slender, widest at the posterior part of the cephalothorax in dorsal view (Figure 3A). Cephalothorax nearly 1.8 times as long as the following pedigerous somites. Prosome/urosome ratio 1.7; body length/width ratio 2.5; cephalothorax/genital double-somite width ratio is about 2.9. Somite bearing the P5 slightly wider than genital double-somite and a serial endowed with lateral hairs.

Figure 3. *Metacyclops subdolus* Female. A) Habitus, dorsal; B) Caudal rami and anal somite, ventral; C) Antennule; D) Antenna; E) P5 and P6, lateral; F) Genital field, ventral. Scale bars: A 250 μm; B, C, D 100 μm; E, F 50 μm.

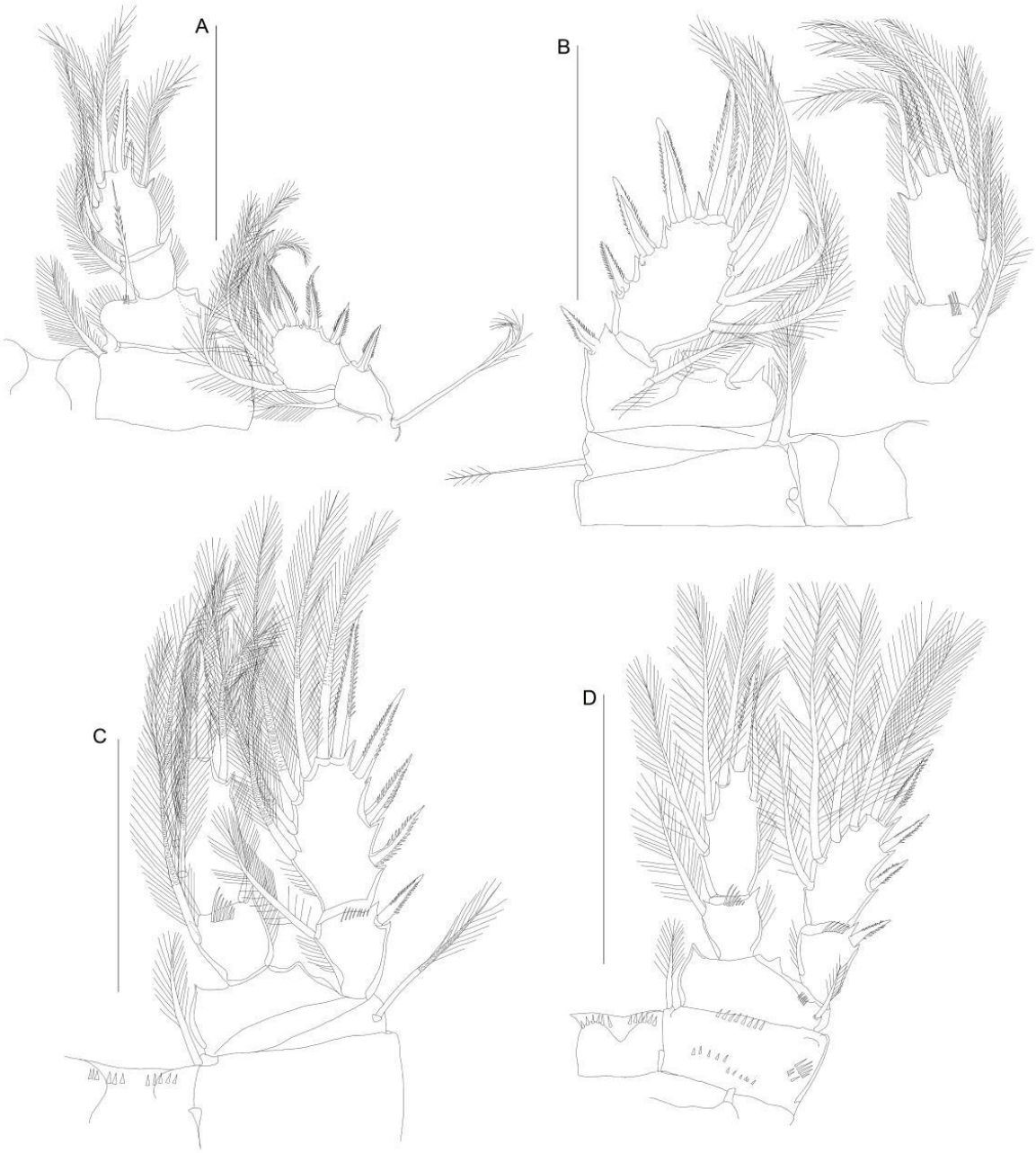

Figure 4. *Metacyclops subdolus* female swimming legs, A) P1, anterior; B) P2, anterior; C) P3, anterior; D) P4, posterior. Scale bars: 100 μm (A-D).

Genital double-somite (Figures 3A, F), slightly longer than wide (about 1 to 1.2 times as long as wide), other abdominal segments wider than long. In ventral view, the seminal receptacle consisting of two parts, with the anterior part compressed, and a small, bulbous posterior part (Figure 3F), and a common structure for *Metacyclops*. While copulatory pore is located at about 0.40 of the somite length from the anterior margin, gonopores are located in lateral position, protected by the reduced P6. The following two urosomites (Figure 3A) are almost the same length, 0.4 and 0.3 times as long as the genital somite, and slightly narrowing towards the anal somite, respectively (Figure 3B). Anal somite is slightly shorter than the preceding somite, with a smooth margin, convex, and bearing a short anal operculum, located in the anterior half of the somite. Without ornamentation on the distal margin of any somite, only 10-13 small ventral spinules on the distal margin of the anal somite, at the base of each of the furcal rami. Furcal rami (Figures 3A, B) 2.2 times wider. Furcal setae: the median external (lateral) seta inserted about 70% from the base of the furcal ramus; the outermost terminal seta shorter than the innermost and the dorsal seta; external median terminal seta is 2.31 as long as the dorsal seta,

internal median terminal seta 3.8 times as long as furcal rami. All furcal setae plumose. Antennule 11-segmented (Figure 3C), short, barely reaching posterior margin of cephalothorax, setation from proximal to distalmost segments (number of setae in parentheses; a, aesthetasc; s, spine): 1(8), 2(4), 3(6), 4(2), 5(1 + s), 6(2), 7(3), 8(2 + a), 9(2), 10(2 + a), and 11(7 + 1a). Segment 1 is ornamented with spinules (Figure 3C). Segment 3 shows suture of residues of two original segments, so probably consisting of two fused segments. In the 5th segment, one of the two setae spiniform and short. The antenna (Figure 3D) consists of 4-part basipodite and 3-endopod. Basipodite bearing 2 internal setae inserted antero-distally (Figure 3D), and externally the long exopodal seta inserted distally. Ornamentation is visible on the basipodite as two lateral groups of 5 spinules on the external frontal edge. Endopodite bearing is divided into three segments 1, 9, and 7 setae, respectively. All three ornamented with a row of hairs or spines on the outer margin, discontinuous on the distal segment.

Swimming legs P1-P4 (Figures 4A-D), both rami 2-segmented. Setal formula (setal arrangement of exp-2 of P1-P4) 5,5,5,5. Spine formula (arrangement of spines on exp-2 of P1-P4) 3,4,4,3. The spine and setal formula are as follows (spines in Roman numerals, setae in Arabic numerals; legend: outer/inner spine or seta; outer/terminal/inner).

	Coxopodite	**Basipodite**	**Exopodite**	**Endopodite**
P1	0-1	1-I	I-1; III, 2, 3	I-1; 1, 1-I, 3
P2	0-1	1-0	I-1; III, I-1, 4	0-1; 1, I-1, 4
P3	0-1	1-0	I-1; III, I-1, 4	0-1; 1, I-1, 4
P4	0-1	1-0	I-0; II, I-1, 4	0-1; 1, I, 3

Ornamentation of setae and spinules are shown in the figures. According to this P1 (Figure 4A), intercoxal sclerite with paired lateral lobes produced posteriorly, both the frontal and caudal surfaces smooth without transverse spinule or setule row. No spinules on Coxa smooth; 1 plumose seta located in the inner distal. Basis armed with 1 pinnate seta distomedially, its tip almost reaches the top of enp-2; lateral seta well developed, plumose. Inner seta present on both enp-1 and exp-1; enp-2 bearing 4 plumose setae along its inner margin, not modified to pinnate or spinous form.

P2-P3 (Figures 4B, C), paired lateral lobes of intercoxal sclerite; lateral seta on basis short, plumose distally; inner seta on both enp-1 and exp-1; enp-2 bearing 5 plumose setae along its inner margin, not modified to pinnate or spinous form. The distal margin of intercoxal sclerite of P3 with transverse spinule.

P4 (Figure 4D), intercoxal sclerite with paired lateral lobes poorly produced posteriorly with 5-6 spinules on inner distal margin. Coxa with transverse row of 8-9 sharp spinules along the posterior margin; 1 inner distal seta well developed, plumose. Basis, lateral seta too short, plumose. Enp-1 with 1 inner distal seta; exp-1 lacking inner seta. P4 enp-2 extends 2.05 times as long as wide; slightly shorter than enp-2 (about 0.84 times as long as enp-2) apically armed with single spine; the outer distal seta crosses tip of apical spine.

P5 (Figure 3E), protopodal segment completely incorporated into fifth pedigerous somite, with outer basal seta, situated rather dorsolaterally and flanking 5-6 spinules at its base; free exopodal segment very small, subapically bearing 1 spine inner sand 1 outer apical plumose seta; spine slightly shorter than exopod; outer apical seta about 5.6 times longer than the inner spine. No colour observed. Egg-sacs paired, each bearing 6 medium size-eggs. P6 (Figure 3E), bearing two slender and short (slightly longer in the males) setae subequal in size.

Male (Figures 5A-C). Fourteen specimens were examined and body lengths excluding caudal seta ranged between 0.556 mm and 0.626 mm, with an average of 0.592 mm. Anterior part of the body has the same shape as that of a female. Urosome of 5 somites, a row hair laterally, as in female; the genital somite the largest, twice as long as wide (Figure 5A); the three following urosomites, it slightly narrows towards the anal somite. Anal somite in the same width as the preceding somite (Figure 5B). Anal operculum (Figure 5A) as in female, short, with distal margin convex and no ornamentation on sinus. A few spinules at the base of the furcal rami go laterally from the middle of the ramus. Furcal rami 2.15 times as long as broad. A spine is present at the insertion of the outermost terminal seta. No ornamentation at the posterior margin of any somite.

Antennule (Figure 5C), 16-segment, relatively longer than in female; geniculate between segments 7 and 9, and between segments 14 and 15; segments 14, 15 and 16 are elongated; Setal formula: 1(8),

2(4), 3(2), 4(2), 5(1), 6(2), 7(1), 8(2), 9(2), 10(2), 11(2), 12(1), 13(0), 14(0), 15(1), 16(10), most setae smooth. The aesthetascs present on segments 1(3), 4(1), and 9(1) are all long. First segment with a proximal, oblique row of 6 spinules. Antenna without significant differences from that of the female. Segmentation of swimming legs P1-P4 identical to that in the female, without sexual dimorphism. P5 similar to the female, with apical external seta half as long as genital somite. P6 (Figure 5B) is composed of two short elements located close to each other at the outer corner, on a small cuticular plate.

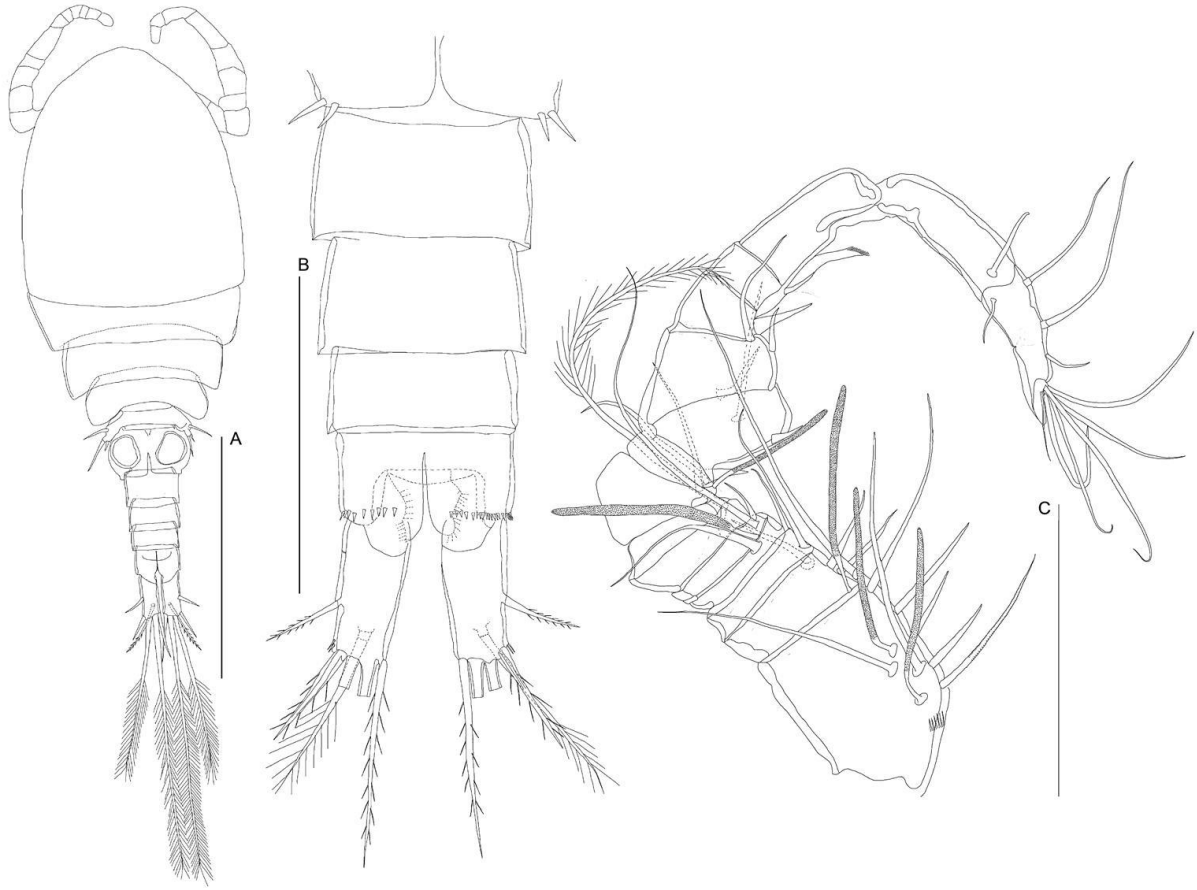

Figure 5. *Metacyclops subdolus* Male. A) Habitus, dorsal; B) P6, caudal rami and anal somite, ventral; C) Antennule. Scale bars: A 250 μm, B, C 100 μm.

DISCUSSION

The genus *Metacyclops* is widespread in tropical and temperate regions, the most productive in the regions of European, African and South American, poorly known for North America and Australia. At present the genus includes 67 valid species and subspecies, most of which are recorded in different groundwater habitats, such as wells, caves, anchialine habitats (Defaye and Por, 2010).

Current taxonomic studies (Herbst 1988, Reid 1987, 1991, Fiers 2001, Karanovic 2004a) recognize four groups based on the spine formula of the terminal exopodite segment of the 1-4 legs (designated as spine formula of legs 1-4). The first group with 3443 spine formula contains 52 of the 62 species of the genus (The group including *M. subdolus*). The second (3442) and third (3433) groups each contain one species, *M. mortoni* Pesce, De Laurentiis and Humphreys, 1996 and *M. cushae* Reid, 1991, respectively. The fourth group among the *Metacyclops* species is the trispinosus-group, which has 3333 spine formula (Karanovic, 2004b; Mercado-Salas et al, 2013).

The genus *Metacyclops* is widely distributed around the world and has been found in diverse (various) biotopes, both epigean and hypogean. In epigean biotopes, many widespread (common) species are frequently encountered, such as the type-species, *M. gracilis* (Lilljeborg, 1853), as well as *M. minutus* (Claus, 1863), *M. planus* (Gurney, 1909), and *M. mendocinus* (Wierzejski, 1892). In

hypogean biotopes, we can mention, for example, *M. subdolus* Kiefer, 1938, recorded from groundwaters of peri-Mediterranean countries (Defaye and Por, 2010).

Metacyclops is a very ancient genus and has probably colonized fresh waters very early, before the break-up of Pangaea (Boxshall and Jaume, 2000), many species of the genus are endemic, as the species recently described from Western Australian groundwaters (Karanovic, 2004a, 2004b). After the colonization of hypogean fresh waters occurred, it led to the diversification of more or less closely related taxa on different plates and continents. Further examinations in underground waters and a total correction of the *Metacyclops* genus will be important to comprehend the connections between the species of these commonly conveyed variety (Defaye and Por, 2010).

The occurrence of the species is certainly related to the characteristics of the biotope. The lake of Gilindire Cave has dual source of water: fresh groundwater mixed saltwater. The *M. subdolus* population in the lake consisted of a large number of adults, copepodite and nauplii. The reason for the abundance of *M. subdolus* is thought to be the absence of another creature feeding on it.

M. subdolus prefers sulfidic and slightly brackish groundwater (Defaye and Por, 2010), and has a European Mediterranean distribution (no record from North Africa); it has first been reported by Kiefer (1938) from southern Italy (La Zinzulusa, Abysso caves), then from Sardinia (Lindberg, 1956), Italy (Pesce et al., 1978; Pesce, 1985), Greece (Peloponnesos, Attica, Crete: Pesce, 1978; Pesce and Maggi, 1981, 1983), Mallorca (Can Pastilla: Lescher-Moutoue, 1981). *M. subdolus* has already been identified from Israel by Dimentman and Por (1991) from slightly brackish springs near the Dead Sea, the northern Negev by Defaye and Dussart (1995) and finally by Ayyalon Cave (Defaye and Por, 2010).

The waters where *M. subdolus* has been found with light brackish water until now are spring water, caves and wells. Therefore it confirms that *M. subdolus* is a type of groundwater species that prefers slight brackish waters.

Minor differences were detected in some characters of *M. subdolus* in Gilindire cave. İn the previous definitions, intercoxal scleritis of P1-P4 ornamented with 2 rows of spinules on ventral margin but in the present, intercoxal scleritis of third and fourth legs ornamented with a row of spinules on ventral margin, first and second smooth. Two-row spinule present ventral margin of P4 coxa but in previous, coxa smooth. P5: the base of the seta inserted on the somite has a row of spinules.

Acknowledgments: The author would like to thank Dr. Yavuz Mazlum from Iskenderun Technical University for critical review of the manuscript, and also thank Dr. Janet W. Reid (Virginia Museum of Natural History, Martinsville, Virginia, USA) for sending some of the useful literatures and for confirming the identification of *Metacyclops subdolus*.

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