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### An account on the non-malacostracan crustacean fauna from the inland waters of Crete, Greece, with the synonymization of *Arctodiaptomus piliger* Brehm, 1955 with *Arctodiaptomus alpinus* (Imhof, 1885) (Copepoda: Calanoida)

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#### ABSTRACT

## An account on the non-malacostracan crustacean fauna from the inland waters of Crete, Greece, with the synonymization of *Arctodiaptomus piliger* Brehm, 1955 with *Arctodiaptomus alpinus* (Imhof, 1885)

The Mediterranean bioregion is widely recognised as a biodiversity hotspot and its inland waters are among the species richest ecosystems of the northern hemisphere. However, the extent of such biodiversity has not been totally unravelled, especially in the Mediterranean islands. Here we present a first account of the crustaceans inhabiting 21 permanent and temporary ponds in Crete, the largest of the Greek islands and the fifth largest island in the Mediterranean Sea. The ponds, sampled between 2009 and 2018, cover all the island surface even though their number cannot be considered exhaustive to represent the entire non-malacostracan fauna of the island. Nevertheless, 46 taxa were identified and most of them are new records for Crete. Moreover, molecular taxonomy allowed to solve the systematic position of *Arctodiaptomus piliger* Brehm, 1955 and to synonymize this organism, previously considered endemic of the island, with *Arctodiaptomus alpinus* (Imhof, 1885). As regard branchiopods, this paper contributes a step ahead to clarify the taxonomic position of the Mediterranean *Chirocephalus* and *Ceriodaphnia* species. Finally, a review of all the non-stygobitic species present on the island is reported, including 78 taxa (21 Branchiopoda, 28 Copepoda and 29 Ostracoda). Overall, the achieved results offer new clues to solve the complex biogeographical pattern of the "entomostracan" crustaceans inhabiting the inland waters of the Mediterranean region.

Key words: Mediterranean biodiversity, temporary ponds, Calanoida, Branchiopoda, Ostracoda

#### RESUMEN

Listado de la fauna de crustáceos no malacostráceos de las aguas epicontinentales de Creta, Grecia, y sinonimización de

#### Marrone et al.

#### Arctodiaptomus piliger Brehm, 1955 con Arctodiaptomus alpinus (Imhof, 1885) (Copepoda, Calanoida)

La bioregion mediterránea es ampliamente reconocida como un hotspot de biodiversidad, y sus aguas epicontinentales se encuentran entre los ecosistemas más ricos en especies del hemisferio septentrional. Sin embargo, el alcance de dicha biodiversidad aún no ha podido ser totalmente evaluado, especialmente en las islas mediterráneas. En el presente artículo se presenta una primera relación de los crustáceos no malacostráceos que habitan en 21 lagunas permanentes y temporales de Creta, la mayor de las islas griegas y la quinta más grande del mar Mediterráneo. Las lagunas, muestreadas entre 2009 y 2018, se encuentran distribuidas por toda la superficie de la isla aunque su número no puede considerarse lo suficientemente exahustivo como para representar a la totalidad de la fauna de crustáceos no malacostráceos de la isla. No obstante, se identificaron 46 taxones, la mayor parte de ellos nuevas citas para Creta. Además, la taxonomía molecular permitió solventar la posición taxonómica de Arctodiaptomus piliger Brehm, 1955 y sinonimizar este organismo, previamente considerado endémico de la isla, con Arctodiaptomus alpinus (Imhof, 1885). En lo referente a los branquiópodos, el presente artículo constituye un avance significativo para clarificar la posición taxonómica de las especies mediterráneas de Chirocephalus y Ceriodaphnia. Finalmente, se reporta una revisión de todas las especies no hipogeas de la isla que incluye 78 taxones (21 Branquiópodos, 28 copépodos y 29 ostrácodos). En conjunto, los resultados obtenidos ofrecen nuevas pistas para resolver el complejo patrón biogeográfico de los crustáceos " entomostráceos" que viven en las aguas epicontinentales de la region mediterránea.

Palabras clave: Biodiversidad mediterránea, lagunas temporales, Calanoida, Branchiopoda, Ostracoda

#### INTRODUCTION

Biodiversity in the Mediterranean Basin is amongst the highest recorded in any biogeographic region, both in terms of species richness and endemism (Blondel *et al.*, 2010). This high diversity largely depends on the remarkable variability of the landscape and results from a complex combination of climatic, geological and paleogeographic factors which make the Mediterranean Basin a "hotspot" of biodiversity: i.e. a varied and fragmented mosaic of ecosystems and communities (Allen, 2001).

Some components of this Mediterranean environmental mosaic (e.g. terrestrial and marine flora, and vertebrate fauna) have been intensively studied in the past decades. However, natural freshwater ecosystems remain relatively unexplored habitat, especially as regard their "microscopic" biota. Although several man-made lakes are scattered all around the Mediterranean area, small water bodies and wetlands are undoubtedly the most characteristic aquatic ecosystems in the region (Alvarez Cobelas et al., 2005). Several thousands of small-sized water bodies (area < 0.001 km<sup>2</sup>) are located in Southern Europe (Meybeck, 1995) and they largely contribute to enhance regional biodiversity by hosting a number of species significantly higher than that present in large lakes (Downing, 2010), making Mediterranean small water bodies themselves "hotspots within hotspots" of biodiversity. Moreover, freshwaters are among the most threatened ecosystems in the world because of anthropic pressure, i.e. industrialization of agriculture processes and urbanization (Dudgeon et al., 2006). Actually, since the importance of small water bodies has suffered a lack of scientific recognition in the second half of the past century, several wetlands and ponds have been drained and reclaimed. These actions have contributed to the rarefaction, at a very fast rate, of these freshwater ecosystems and their biota in all the Mediterranean Region (Zacharias & Zampas, 2010; Stoch et al., 2016). The disappearing of freshwater ecosystems, coupled with the hydrological variability caused by climate change, the over-exploitation of water resources, and the changes induced in natural communities by the introduction of exotic species, highlights the urgency of biodiversity assessments in all the Mediterranean inland waters (Moreno et al., 2017). This is particularly true as regard the passively dispersed fauna inhabiting the inland waters of the Mediterranean islands and archipelagos. Actually, the success of dispersal for these species greatly depends on the number of ponds scattered across a given territory (Incagnone et al., 2015); the aquatic ecosystems on the islands acting as stepping-stones in the migration corridors of birds which transport resting stages and propagules across the Mediterranean Sea (Hindmarch & Kirby, 2002). Therefore, getting a knowledge as much as possible complete of the biodiversity



Figure 1. Location of the sampled sites. White circles: temporary waterbodies; black circles: permanent waterbodies. See table 1 for their names, codes, and coordinates. Situación de las localidades muestreadas. Círculos blancos: masas de agua temporales; círculos negros: lagos permanentes. En la tabla 1 se indican sus nombres, códigos y coordenadas.

GR095

3R097

of aquatic organisms in insular inland waters is of paramount importance to have a thorough vision on the biogeography of aquatic organisms in the Mediterranean region, and to develop sound conservation plans for these characteristic ecosystems (e.g. Alfonso *et al.*, 2011; Bagella *et al.*, 2016; Diaz-Paniagua, 2015; Grillas *et al.*, 2004).

GR083

**GR084** 

GROSS

The crustacean fauna of aquatic ecosystems in the islands of the western Mediterranean Basin has been sufficiently documented, and several investigations were carried out in the last decades addressed toward the faunistic exploration of Sicily (Marrone & Mura, 2006; Marrone et al., 2005, 2006a, 2006b; Pieri et al., 2006), Maltese islands (Lanfranco, 2001), Sardinia (Ruffo & Stoch, 2005; Boix et al., 2017, and references therein), Corsica (Champeau & Thiéry, 1990; Culioli et al., 2006) and Balearic Islands (Jaume, 1989-1990; Pretus, 1990; Zamora et al., 2005). Conversely, with the exception of Corfu (Stephanides, 1948), only a few, scattered data are available for the islands located in the eastern Mediterranean Sea (e.g. Mura & Hadjstephanou, 1987; Tziortzis et al., 2014; Karagianni et al., 2018); remarkably, the availability of data for the Aegean Islands is very scarce.

In this paper, we provide a first faunal assessment of the non-malacostracan crustaceans inhabiting permanent and temporary ponds in Crete, the southernmost island of the Aegean archipelago and the fifth Mediterranean island by surface, with the aim to increase our knowledge on the biogeography of freshwater crustaceans passively dispersing across the Mediterranean Basin. To our knowledge, despite Crete has the highest amount of Mediterranean Temporary Ponds (priority habitat 3170\* according to the EU "Habitats Directive") among the administrative regions of Greece (Dimitriou et al., 2006), just a few papers dealing with Cretan inland water crustaceans are available in the scientific literature (see Kiefer, 1928; Chappuis, 1929, 1956; Klie, 1941; Brehm, 1955; Lindberg, 1956; Pesce & Maggi, 1981; Petkowski et al., 2000). Moreover, with this paper we wish to commemorate the late Prof. Maria Rosa Miracle, who recently passed away after an illness of over one year. With her work, Maria Rosa largely contributed to establish a Mediterranean vision of Limnology and promoted the importance of Mediterranean small water bodies among dozens of students and colleagues.

#### **MATERIAL AND METHODS**

Crete, the largest island of Greece and the fifth in the Mediterranean Sea, is a limestone island covering an area of 8303 km<sup>2</sup>. It is located about 100 km south of the Greek mainland, and 175 km south-west of Turkey, along a line crossing the

#### Marrone et al.

Table 1. List of the sampled sites. Geographical coordinates are expressed as decimal degrees (Map Datum: WGS84). GSD: "Greek Samples Database"; Hydr.: Hydroperiod; P: permanent; T: temporary. Relación de las localidades muestreadas. Las coordenadas geográficas se expresan en grados decimales (Datum: WGS84). GSD: "Greek Samples Database"; Hydr.: Hidroperiodo; P: permanent; T: temporal.

GSD Code	Site	Latitudine N	Longitudine E	Elevation (m a.s.l.)	Habitat type	Hydr.	Plankton sample	Sediment sample
GR082	Limni Kourna	35.329935	24.272689	16	Lake	Р	х	
GR083	Limni Agya	35.476936	23.932054	37	Reservoir	Ρ	х	
GR084	Pond 1 at Omalos (Chania)	35.324877	23.890936	1060	Pond	Т	х	х
GR085	Pond along the Exo-Apidi road	35.068422	26.124883	605	Reservoir	Ρ	х	
GR086	Marsh 1 of Aposelemis	35.334893	25.328806	0	Ground pools	Т	х	
GR087	Limni Zirou	35.072129	26.137817	566	Pond	Ρ	х	
GR088	Pond 2 at Omalos (Chania)	35.326636	23.888916	1054	Pond	Т	х	х
GR090	Marsh of Xerocampos	35.047924	26.237118	0	Marsh	Т		х
GR091	Marsh 2 of Aposelemis	35.335044	25.327582	0	Marsh	Т		х
GR092	Marsh of Vai	35.255259	26.264846	0	Marsh	Т		х
GR094	Pond 3 at Omalos (Chania)	35.325999	23.890255	1057	Pond	Т		х
GR095	Faistos rock pool	35.051189	24.814912	76	Rock pool	Т		х
GR096	Spinalonga rock pool	35.299204	25.738825	10	Rock pool	Т		х
GR097	Limni Katalyki	35.075107	24.745754	0	Pond	т		х
GR098	Athoudiaris pool	35.235600	26.224500	558	Mud puddle	т		х
GR099	Karst pool	35.051979	26.186606	539	Pond	т		х
GR100	Pond at Omalos (Dykti)	35.071527	25.454529	1330	Pond	т	х	х
GR151	Stephana pond	35.264155	24.893111	1128	Mud puddle	Т	х	
GR152	Karydi pool	35.130936	26.164500	685	Pond	т	х	
GR153	Profitis Ilias pool	35.227659	26.219754	199	Mud puddle	т		х
GR175	Cape Lithinon rock pool	34.945553	24.761992	152	Rock pool	т	x	

large islands of Karpathos and Rhodes. Cretan landscape is dominated by mountain ranges, with the highest peak reaching 2456 m a.s.l. and a mean elevation of 482 m a.s.l. The climate is typically Mediterranean at the low altitudes, but on the mountains snow coverage can easily persist until May, with bioclimatic belts ranging from the Thermomediterranean to the Supramediterranean one (Rivas-Martínez *et al.*, 2004).

Crustacean samples were collected throughout the island from November 2009 to January 2018; the geographical coordinates and elevation of each site were recorded using a GPS, and an alphanumeric code referring to a "Greek Samples Database" (GSD) set up by FM and FS, was attributed to each of them (Fig. 1; Table 1). When water bodies were experiencing their inundated phase, a 200 µm mesh-sized hand net was used to sample along shorelines, through submerged vegetation and in open waters, paying attention to collect crustaceans in all the microhabitats present in each sampled site. Collected crustacean samples were fixed in situ using 90 % ethanol. Due to the different phenology of the species and to the seasonal succession of the crustacean assemblages of temporary waters, the presence of several taxa might be overlooked in sampling surveys with no or few temporal replicates; in such cases, the ex-situ re-hydration of dry sediment, known as "Sars' method" (van Damme & Dumont, 2010), is a useful complement to the study of the actual crustacean diversity occurring in a given area. Accordingly, sediment samples were collected from most of the sampled water bodies and cultured in laboratory. About 300 gr of sediment were collected from each water body, integrating sediment samples from the deepest parts and the shores. When collected wet, the sediment was first dried at room temperature in open plastic bags. Sediment subsamples from each site were then rehydrated in laboratory using 4-litres plastic aquaria where a thin layer of sediment (about 5 mm) was introduced with 3 litres of distilled water. Cultures were run at the University of Palermo during winter months and kept at temperature between 12 and 16 °C with a 12-hours photoperiod and a feeble aeration. Cultures were maintained for about two months and monitored each second day. The hatched crustaceans were collected monthly by filtering the water over a 125  $\mu$ m plankton net. Some specimens belonging to poorly-represented species in the cultures were individually collected by pipetting. The procedure described above was repeated four times and designated as "Hydration Experiments" 1-4 (Table S1, available at http://www.limnetica.net/en/limnetica).

Crustacean samples collected in the wild or coming from laboratory cultures were sorted in laboratory under a dissecting stereomicroscope. Branchiopods were identified according to Alonso (1996), Kotov & Alonso (2010) and Sinev *et al.* (2012); copepods according to Dussart (1967, 1969), Kiefer (1971, 1978), Borutzky *et al.* (1991), Einsle (1993) and Ranga-Reddy (1994); ostracods according to Meisch (2000) and Mazzini *et al.* (2014).

Undissected crustacean specimens were stored in 95 % ethanol at -20 °C, dissected soft parts were stored in glycerol in sealed microscope slides, and ostracod valves were dried and stored in micropaleontological slides.

Voucher specimens of *Arctodiaptomus alpinus* (Imhof, 1885) from GR088 (MZUF634) and GR100 (MZUF635), and *Chirocephalus diaphanus* from GR094 (MZUF637) and GR099 (MZUF636), were deposited in the Museo di Storia Naturale, Sezione di Zoologia "La Specola", Università di Firenze, Italy (MZUF); other specimens are currently stored in FM's collection at the Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche of the University of Palermo, Italy, and are available for loan on request.

Because of the unsettled systematics of the large branchiopod genus *Chirocephalus* and, in particular, of the species attributed so far to the *C. diaphanus*-group sensu Brtek (1995), two specimens of *Chirocephalus diaphanus* s.l. from sites GR084 and GR099 (Crete, Greece) were studied by amplifying and sequencing a fragment of the mitochondrial gene encoding for the cytochrome oxidase subunit I (COI), a molecular marker widely used in the molecular systematics and phylogeography of *Chirocephalus* (e.g.: Ketmaier *et al.*, 2012; Reniers *et al.*, 2013; Zarattini *et al.*, 2013; Cottarelli *et al.*, 2017). Comparative

#### Marrone et al.



**Figure 2.** Bayesian consensus phylogram based on a 408-bp-long fragment of the mitochondrial gene encoding for the cytochrome oxidase subunit I (mtDNA COI). Node support is reported as nodal posterior probability/ML bootstrap; nodal posterior probability lower than 0.5 is represented as an unresolved polytomy; "\*" indicates bootstrap support lower than 50. Accession numbers of sequences derived from GenBank are shown in brackets. Cretan samples are reported in bold. *Árbol filogenético de consenso Bayesiano basado en el fragmento largo 408-bp del gene mitocondrial codificado por la subunidad I citocromo oxidasa (mtDNA COI). El soporte de los nodos se muestra como la probabilidad posterior nodal /ML bootstrap; la probabilidad posterior nodal inferior a 0.5 se representa como una politomía no resuelta. "\*" indica un soporte boostrap inferior a 50. Entre paréntesis los números de acceso de las secuencias derivadas del GenBank. En negrita las muestras de Creta.* 

sequences belonging to other *C. diaphanus* s.l. populations and other *Chirocephalus* species were downloaded from GenBank and included in the analyses (Fig. 2).

Calanoid copepods were prepared according to Dussart and Defaye (2001), and line drawings of specimens of the genus *Arctodiaptomus* were made using a compound microscope equipped with a camera lucida. Drawings were based on specimens collected in GR084, an ancient temporary pond located on the Omalos plateau (Chania, Crete). Two specimens of *Arctodiaptomus* cf. *piliger* from sites GR088 and GR099 (Crete, Greece), one specimens of *A. alpinus* from Greek mainland (Central Greece), one specimen of *Arctodiaptomus* cf. *alpinus* from Mongolia (Tov province), one specimen of *Arctodiaptomus belgrati* Mann, 1940 from Romania (Ciuc basin), and one specimen of *Arctodiaptomus kerkyrensis* Pesta, 1935 from Italy (Basilicata) were studied by amplifying and sequencing a fragment of the mitochondrial gene encoding for the cytochrome b (Cyt-b). Cyt-b sequences proved to be informative about the molecular systematics of the genus



**Figure 3.** Bayesian consensus phylogram based on a 313-bp-long fragment of the mitochondrial gene encoding for the cytochrome b (mtDNA Cyt-b). Node support is reported as nodal posterior probability/ML bootstrap; nodal posterior probability lower than 0.5 is represented as an unresolved polytomy, "\*" indicates bootstrap support lower than 50. Accession numbers of sequences derived from GenBank are shown in brackets. Cretan samples are reported in bold. *Árbol filogenético de consenso Bayesiano basado en el fragmento largo 313-bp del gene mitocondrial codificado por la subunidad I citocromo oxidasa (mtDNA Cyt-b). El soporte de los nodos se muestra como la probabilidad posterior nodal /ML bootstrap; la probabilidad posterior nodal inferior a 0.5 se representa como una politomía no resuelta. "\*" indica un soporte boostrap inferior a 50. Entre paréntesis los números de acceso de las secuencias derivadas del GenBank. En negrita las muestras de Creta.* 

Arctodiaptomus (Marrone et al., 2014); accordingly, this molecular marker was chosen with the aim of comparing the allegedly endemic Cretan species Arctodiaptomus piliger Brehm, 1955 and different populations of the closely related A. alpinus (Imhof, 1885). Moreover, comparative sequences belonging to an A. alpinus population from the Italian Alps and other species of the genera Arctodiaptomus and Diaptomus were downloaded from GenBank and used in the analyses (Fig. 3).

DNA extraction and amplification were performed following the protocols and procedures described in Marrone *et al.* (2010, 2013) for copepods, and Cottarelli *et al.* (2017) for anostracans. Chromatograms were visualised and edited using Chromas Lite 2.01 (Technelysium Pty Ltd., South Brisbane, Australia), and aligned in ClustalX (Thompson *et al.*, 1997). MEGA7 (Kumar *et al.*, 2016) was used to translate the Cyt-b and COI sequences to amino acids in order to check for the possible presence of frameshifts or stop codons, which would indicate the presence of sequencing errors or pseudogenes. The sequences were deposited in GenBank under Accession Numbers MG924484-MG924489 (*Arctodiaptomus* spp.) and MG924490-MG924491 (*Chirocephalus diaphanus* s.1.).

Bayesian inference (BI) of phylogeny and maximum likelihood (ML) analyses were performed on the Cyt-b dataset as implemented by MrBayes 3.2 (Ronquist *et al.*, 2012) and PhyMl v.3 (Guindon & Gascuel, 2003), respectively. The choice of the best evolutionary model was made using MrModeltest 2.2 (Nylander 2004) on the basis of Akaike Information Criterion (AIC) for both analyses and datasets; accordingly, a Hasegawa-Kishino-Yano nucleotide substitution model with gamma distributed rate variation among sites (HKY+G; nst=2) was used

173

for the "Chirocephalus dataset", and a General Time Reversible model with gamma distributed rate variation among sites and a proportion of invariant sites (GTR+I+G; nst=6) was used for the "Arctodiaptomus dataset". Node supports were evaluated by their posterior probabilities in the BI and by 1000 bootstrap replicates in the ML analyses. The BI analyses were performed with two independent runs of 1 x 106 generations and four Markov chains using default heating values. Trees and parameter values were sampled every 100 generations resulting in  $10 \times 10^3$  saved trees per analysis. An initial fraction of 2000 trees (20 %) was conservatively discarded as burn-in. For all analyses and both datasets, standard deviation of split frequencies reached values lower than 0.018288, and values of the potential scale reduction factor (PSRF) were comprised between 0.999 and 1.002 for all the partitions with frequency  $\geq 0.10$  in at least one run, indicating convergence of the runs.

#### RESULTS

Four permanent and 17 temporary water bodies spread throughout Crete, from sea level up to 1330 m a.s.l., were sampled (Fig. 1 and Table 1; Table S2 and Fig. S1 available at http://www.limnetica. net/en/limnetica). Zooplankton samples were available from 8 sites only; sediment samples were collected in further 10 sites, since these were dry when sampling was performed. The crustacean fauna occurring in 3 additional sites was studied based both on direct crustacean samplings and sediment culturing (Table 1; Table S1 available at http://www.limnetica.net/en/limnetica).

Overall, 46 non-malacostracan crustacean taxa (17 Branchiopoda, 10 Copepoda, and 19 Ostracoda) were collected in the present study, and 27 of these are new for the Cretan fauna. These results therefore bring to 78 the number of non-malacostracans species up to now known for the island (Table 2). Most of the collected taxa were identified at species level, although this proved not to be possible for the cladocerans *Ilyocryptus* sp. from GR083 and GR094 and *Leydigia* sp. from GR084, and the ostracods *Cypridopsis* sp. (from GR152), *Candona* sp. (from GR094), *Ilyocypris* sp. (from GR089,

GR094, GR099), and *Eucypris* sp. occurring in GR092 and GR151.

A single anostracan species morphologically ascribed to Chirocephalus diaphanus Prévost, 1803 was found in five of the studied sites (Table 2; Fig. S2 available at http://www.limnetica.net/ en/limnetica). Upon aligning our Cretan Chirocephalus sequences and those downloaded from GenBank, and having trimmed the tails of the sequences which were not present in all the individuals, we obtained a properly aligned 408 bp-long fragment of the mtDNA COI. BI and ML trees, rooted on Chirocephalus kerkyrensis Pesta, 1936 (a species belonging to the *bairdi*-species group of the genus Chirocephalus, see discussion in Cottarelli et al., 2017), showed a consistent topology at the major nodes (Fig. 2). The sequences of the Cretan Chirocephalus specimens form the sister clade of C. diaphanus s.l. sequences from Bulgaria and Romania, i.e. were included in the "eastern clade" of C. diaphanus s.l., which should be ascribed to the binomen Chirocephalus romanicus Stoicescu, 1992 according to Reniers et al. (2013).

The widespread *Ceriodaphnia* sp., observed in 6 out of the 21 studied sites, corresponds to the taxon ascribed to *Ceriodaphnia quadrangula* Müller, 1785 by Alonso (1996). However, this is, in fact, a different widespread west-Palearctic species pending a formal description (M. Alonso, unpublished data).

Three species of calanoid copepods were collected in the frame of this survey: *Calanipeda aquaedulcis* Kritschagin, 1873, *Neolovenula alluaudi* (Guerne & Richard, 1890), and a diaptomid belonging to the genus *Arctodiaptomus*. The morphology of both males and females of the latter species are presented in figures 4 and 5, respectively.

Five cyclopoid and two harpacticoid species were collected during this survey (Table 2). Among these, the cyclopoids *Acanthocyclops einslei* Mirabdullayev & Defaye, 2004, the widespread *Metacyclops minutus* (Claus, 1863), and the brackish water harpacticoid *Cletocamptus retrogressus* Schmankevitsch, 1875 are new records from Crete.

#### Crustaceans from inland waters of Crete

**Table 2.** Checklist of the non-stygobitic taxa cited for Crete island; codes of the sites of occurrences according to table 1. References: 1: Kiefer (1928); 2: Chappuis (1929); 3: Klie (1941); 4: Brehm (1955); 5: Lindberg (1956); 6: Chappuis (1956); 7: Pesce & Maggi (1981); 8: Petkowski *et al.* (2000); 9: Karaouzas *et al.* (2000); 10: Present work.; <sup>1</sup>: "*C. quadrangula*" sensu Alonso (1996) and probably sensu Brehm (1955); <sup>2</sup>: described as *A. piliger* by Brehm (1955); <sup>3</sup>: uncertain identification; cited as *Cyclops strenuus* (Chappuis, 1929) and *Cyclops rubens rubens* (Lindberg, 1956); <sup>4</sup>: reported as *Cyclops bicuspidatus odessanus* (Chappuis, 1929) and *Acanthocyclops bicuspidatus f. odessana* (Lindberg, 1956). *Relación de los taxones no estigobíticos citados en la isla de Creta; códigos de las localida-des donde aparecen de acuerdo con la tabla 1. Referencias: 1: Kiefer (1928); 2: Chappuis (1929); 3: Klie (1941); 4: Brehm (1955); 5: Lindberg (1956); 6: Chappuis (1956); 7: Pesce & Maggi (1981); 8: Petkowski et al. (2000); 9: Karaouzas et al. (2000); 10: Present work.; <sup>1</sup>: "C. quadrangula" sensu Alonso (1996) y probablemente sensu Brehm (1955); <sup>2</sup>: descrito como A. piliger por Brehm (1955); <sup>3</sup>: identificación dudosa; citado como Cyclops strenuus (Chappuis, 1929) y Cyclops rubens rubens (Lindberg, 1956); <sup>4</sup>: reportado como Cyclops bicuspidatus odessana (Lindberg, 1956); <sup>4</sup>: reportado como Cyclops bicuspidatus of dessana (Lindberg, 1956); <sup>4</sup>: reportado como Cyclops bicuspidatus of sensu a (Lindberg, 1956); <sup>4</sup>: reportado como Cyclops bicuspidatus of dessana (Lindberg, 1956); <sup>4</sup>: reportado como Cyclops bicuspidatus f. odessana (Lindberg, 1956); <sup>4</sup>: reportado como Cyclops bicuspidatus of dessana (Lindberg, 1956).* 

Таха	References	Sites of occurrence (Present work)
BRANCHIOPODA		
Anostraca		
Chirocephalidae		
Chirocephalus diaphanus s.l. Prévost, 1803	9, 10	GR084, GR088, GR094, GR099, GR175
Anomopoda		
Daphniidae		
Daphnia (Ctenodaphnia) atkinsoni Baird, 1859	10	GR151
Daphnia (Ctenodaphnia) chevreuxi Richard, 1896	10	GR088, GR094, GR099
Simocephalus vetulus (Müller, 1776)	4, 10	GR084, GR087, GR100
Ceriodaphnia sp. <sup>1</sup>	4, 10	GR084, GR087, GR088, GR094, GR099, GR152
Ceriodaphnia reticulata (Jurine, 1820)	4, 10	GR085
Moinidae		
<i>Moina brachiata</i> (Jurine, 1820)	10	GR099
Moina micrura Kurz, 1875	10	GR084, GR088
Ilyocryptidae		
<i>Ilyocryptus</i> sp.	10	GR083, GR094
Macrothricidae		
Macrothrix hirsuticornis Norman & Brady, 1867	10	GR084, GR088, GR094, GR099, GR100, GR151
Chydoridae		
Chydorus sphaericus (Müller, 1776)	4, 5, 10	GR084, GR100
Ovalona anastasia (Sinev, Alonso, Miracle & Sahuquillo, 2012)	10	GR084
Ovalona nuragica (Margaritora, 1971)	10	GR084
Alona affinis (Levdig, 1860)	4.5	
Alona salina Alonso, 1996	10	GR091, GR096
Alonella excisa (Fischer, 1854)	4.5	
Alonella exigua (Lillieborg, 1853)	5	
Alonella nana (Baird, 1843)	5	
Coronatella rectangula (G.O. Sars. 1862)	10	GR085, GR087, GR097, GR099, GR100
Levdiaia iberica Kotov & Alonso, 2010	10	GR088
Levdiqia sp.	10	GR084
COREPODA		
Calanoida		
Beeudodiantomidae		
Calanineda aquaedulois Kritschagin 1873	4 10	CP082
Diaptomidae	4, 10	GRU02
Arctodiaptomus (Rhabdodiaptomus) alpinus (Imhof, 1855) <sup>2</sup>	4, 10	GR084, GR088, GR099, GR100, GR152
Neolovenula alluaudi (Guerne & Richard, 1890)	7, 10	GR084, GR085
Cyclopoida Cyclopidae		
Macrocyclons albidus (Jurine 1820)	5	
Fucyclons serrulatus (Eischer 1851)	257	
Ectocyclops schulatus (Lischer, 1031)	2, 3, 7	
Tropocyclops practicus (Roon, 1860)	2 5 10	GR085 GR087
Paracyclops fimbriatus (Fischer, 1953)	2, 3, 10	
1 aracyclops IIIIbrialus (FISCIEI, 1003)	0, 1	

#### Table 2. (cont.)

Cyclops sp. <sup>3</sup>	2, 5	
Megacyclops viridis (Jurine, 1820)	5, 7, 10	GR085
Acanthocyclops einslei Mirabdullayev & Defaye, 2004	10	GR083
Diacyclops bisetosus (Rehberg, 1880)	7	
Diacyclops lubbocki (Brady, 1869) <sup>4</sup>	2, 5, 7, 10	GR086, GR091
Diacyclops crassicaudis (G.O. Sars, 1863)	1, 2, 7	
Thermocyclops oblongatus (G.O. Sars, 1927)	5, 7	
Metacyclops minutus (Claus, 1863)	10	GR098, GR151
Harpacticoida		
Canthocamptidae		
Mesochra aestuarii Gurney, 1921	6	
Canthocamptus staphylinus (Jurine, 1820)	2, 6, 10	GR084
Attheyella (Attheyella) crassa (Sars, 1863)	2	
Bryocamptus (Bryocamptus) minutus (Claus, 1863)	2, 6	
Bryocamptus (Rheocamptus) pygmaeus (Sars, 1863)	2, 6	
Bryocamptus (Rheocamptus) zschokkei (Schmeil, 1893)	2	
Elaphoidella denticulata Chappuis, 1929	2	
Maraenobiotus vejdovskyi Mrazek, 1893	6	
Maraenobotus brucei carpathicus Chappuis, 1928	6	
Cletocamptus retrogressus Schmankevitsch, 1875	10	GR086, GR091, GR097
Ameiridae		
Nitokra lacustris (Shmankevich, 1875)	6	
Laophontidae		
Onychocamptus mohammed (Blanchard & Richard, 1891)	6	
ΟΣΤΡΑCODA		
Bodoconida		
Candonidao		
Candona lindhari Datkovaki 1060	10	CR099
Candona Indiren Pelkovski, 1909	10	GR000
Candona neglecia Sais, 1887	10	 CR004
Candona sp.	10	GR094
Condenencia kingelaii (Brady & Debatteen, 1970)	3	
Candonopsis kingsien (Brady & Robertson, 1670)	3	
	10	CR088 CR004 CR000
llyocypris sp.	10	GR000, GR094, GR099
liyocypris divice Klie, 1090	3	
llyocypris divisa Kile, 1920	2 10	 CR001
liyocypris gibba (Ramuoni, 1006)	3, 10	GR091
Cuprididee	10	GR100
Cyprididae	10	
Cypris bispiriosa Lucas, 1849	10	GR083, GR087
Eucypris kerkyrensis Stephanides 1937	8	 CR001 CR000 CR100
Eucypris virens (Junne, 1820)	3, 10	GR091, GR099, GR100
Eucypris sp.	10	GR092, GR151
	8, 10	GR098, GR100, GR151
Herpetocypris chevreuxi (Sars, 1896)	3, 8, 10	GR084
Herpetocypris reptans Cole, 1965	3	
Stenocypria fischeri (Lilijeborg, 1883)	10	GR100
Heterocypris barbara (Gauthier & Brehm, 1928)	10	GR086, GR094, GR097, GR099, GR100, GR151
Heterocypris incongruens (Ramdohr, 1808)	3, 8, 10	GR086, GR091, GR096, GR098, GR100, GR151
Heterocypris salina (Brady, 1868)	3, 8	
<i>Cypria ophtalmica</i> (Jurine, 1820)	3	
Cypridopsis vidua (O.F. Müller, 1776)	10	GR083
Cypridopsis hartwigi G.W. Müller, 1900	10	GR100
Cypridopsis elongata (Kaufmann, 1900)	10	GR100
Plesiocypridopsis newtoni (Brady & Robertson, 1870)	3	
Sarscypridopsis aculeata (Costa, 1847)	10	GR085, GR091, GR097, GR152
Potamocypris arcuata (Sars, 1903)	10	GR088, GR094, GR098, GR099, GR100
Lymnocytheridae		
Paralimnocythere psammophila (Flössner, 1965)	10	GR100

#### Morphological description of the *Arctodiaptomus* specimens from Crete

Male: Body length 1.410 mm, shorter than in female (Fig. 4a). Urosome asymmetrical (Fig. 4b) with a strong spine on the right side of the first genital somite and scattered small setae (in variable number) on the other somites. Geniculate right antennula (Fig. 4e) with spines at the segment 8 and 10-13; length proportions as follows: 13>11>10>8>12. Antepenultimate segment of right antennula with a spinous process shorter than the penultimate segment and with a rounded-hyaline terminal end (Fig. 4f), variable in different specimens (Fig. 4g). Rostral spines as in Fig. 4h. Posterior surface of the second endopodite segment of leg 2 with a weakly sharpened "Schmeil's organ" (Fig. 4d). Right fifth leg (P5, Fig. 4c) with chitinous processes on the inner side of basis and on the second exopodite segment; distal claw with a proximal 'curve' as in A. steindachneri (Richard, 1897); lateral spine as long as one half of distal claw.

Female: Size 1.615 mm. Habitus as in Fig. 5a. Urosome slightly asymmetrical (Figs. 5b, 5c) with spines on both sides of the genital somite in variable position, but with the left spine always pointed down with respect to the right one. Antennula (Fig. 51) with a long seta on the first segment ("dentifer") reaching the 8th segment. Two setae on segments 11 and 13, one seta on segments 15 and 17. P5 (Fig. 5d) with robust coxal spines and small sensilla variable both in number and position (Figs. 5e, 5g). Endopodite of fifth pair of legs indistinctly 2-segmented with setulae on the inner side (Figs. 5f, 5h, 5i); a setula can be present only on a single segment or in both the indistinct segments; variability was observed even in the same specimen in left and right legs (Figs. 5h, 5i).

By analysing the morphological features of the collected specimens, it was noticed that the morphological characters (and the drawings) originally used by Brehm (1955) to describe the species *Arctodiaptomus piliger* do not allow to distinguish it from the congeneric *A. alpinus* (Imhof, 1885). These results were confirmed by the molecular analyses based on a 313 bp-long fragment of the Cyt-b gene. In both the BI and ML trees (Fig. 3), which showed a concordant topology at the major nodes, Cretan Arctodiaptomus cf. piliger sequences clustered with Arctodiaptomus alpinus s.s. sequences from Italy and Greece. Conversely, Arctodiaptomus cf. alpinus reported for Mongolia by Marrone et al. (2015) forms the sister group of the studied European A. alpinus populations. The 26 % of uncorrected p-distance between the Mongolian sample and the European A. alpinus s.s. populations is even higher than that observed between the sister species A. similis (Baird, 1859) and A. irregularis Dimentman & Por, 1985 (Marrone et al., 2014), thus suggesting that the Mongolian taxon might actually represent a cryptic species related to A. alpinus s.s.

#### DISCUSSION

To date, the microcrustaceans and large branchiopods of the inland waters of Crete were poorly known. While several papers dealing with the stygobitic copepods of Crete were published in the XX century (e.g. Chappuis, 1929, 1956; Lindberg, 1956; Pesce & Maggi, 1981, 1983 and references therein), the Cretan surface non-malacostracan crustaceans, to our knowledge, were the object of a few studies only (Chappuis, 1929; Klie, 1941; Brehm, 1955; Lindberg, 1956; Karaouzas et al., 2015). Although the present work admittedly does not provide an exhaustive list of Cretan inland water non-malacostracan crustaceans, it paves the way to further studies, and allows to carry out a first characterization of Cretan fauna. The vast majority of observed taxa are first records for Crete and/or for the eastern Mediterranean basin, further stressing the need of better exploring these areas.

A single anostracan species was observed; this result, although unexpected when the anostracan fauna of other large Mediterranean islands is considered (Brtek & Thiéry, 1995), is in line with the paucity of large branchiopod records to date available for Greece (Abatzopoulos *et al.*, 1999). Its morphology is compatible with those described for the subspecific taxa *C. diaphanus carinatus* Daday, 1910 (*locus typicus*: valley of river Vardar\Axios, Greece), *C. diaphanus pentheri* Pesta, 1921 (*locus typicus*: Kukës, Alba-



**Figure 4.** Arctodiaptomus alpinus from Crete (GR084); Male. a: habitus; b: urosome; c: fifth pair of legs; d: second leg; e: right antennula (segments 7-13); f: antepenultimate segment of the right antennula; g: as in f (different specimen). Scale bar: 0.1 mm. Drawings by GA. Arctodiaptomus alpinus de Creta (GR084); Macho. a: habitus; b: urosoma; c: quinto par de patas; d: segunda pata; e: anténula derecha (segmentos 7-13); f: antepenúltimo segmento de la anténula derecha; g: como en f (ejemplar diferente). Barra de escala: 0.1 mm. Ilustraciones de GA.

Limnetica, 38(1): 167-187 (2019)



**Figure 5.** Arctodiaptomus alpinus from Crete (GR084); Female. a: habitus; b, c: urosome; e, d, g: fifth pair of legs; f, h, i: endopodite of fifth leg; l: A1. (Specimen 1: a, b, d, l; Specimen 2: c, g, h, I; Specimen 3: e, f). Scale bar: 0.1 mm. Drawings by GA. Arctodiaptomus alpinus de Creta (GR084); Hembra. a: habitus; b, c: urosoma; e, d, g: quinto par de patas; f, h, i: endopodito de la quinta pata; l: A1. (Ejemplare 1: a, b, d, l; Ejemplare 2: c, g, h, I; Ejemplare 3: e, f). Barra de escala: 0.1 mm. Ilustraciones de GA.

nia) and C. diaphanus romanicus Stoicescu, 1962 (locus typicus: Oltenita, Romania), although, as stressed by several authors (e.g. Belk & Brtek, 1995; Marincek & Petrov, 1995; Mura et al., 2002; Reniers et al., 2013) a proper distinction of C. diaphanus subspecies is not always straightforward, and a revision of this species-group is needed. The molecular analyses of the Cretan Chirocephalus from GR084 and GR099 clustered the two analysed specimens within the "eastern clade" of C. diaphanus, which included samples from Romania and Bulgaria. According to Reniers et al. (2013) this clade should be worth of species status under the binomen Chirocephalus romanicus. However, in the light of the wide morphological plasticity of C. diaphanus s.l. and of the morphological similarity among its eastern subspecies, "C. romanicus" might in fact prove to be just a junior synonym of C. pentheri or C. carinatus. Accordingly, pending a revision of the systematics and taxonomy of the diaphanus-group of the genus Chirocephalus, which includes samples from the type localities of all the described taxa, we prefer to report the Cretan Chirocephalus as C. diaphanus s.l., refraining from assigning it to any other taxon of specific or subspecific rank. In Greece, C. diaphanus s.l. was already reported for Zante (Stephanides, 1948), Boeotia (Pesta, 1921) and Macedonia (Daday, 1910). Moreover, Karaouzas et al. (2015) report the presence of Chirocephalus sp. from Omalos temporary pond (Crete), i.e the site coded as GR084 in the present study.

In the last decades, careful morphological studies confirmed that, as anticipated by Frey (1982), the paradigm of cosmopolitanism of freshwater taxa was inapplicable to anomopods, and that their actual diversity was largely underestimated (Forró et al., 2008). Accordingly, several new species were recently described, even in well-studied regions, especially among the radopods. These taxa, originally considered endemic to their *terra typica*, are now being observed even in relatively-distant regions, thus better defining their distribution. This is the case for some alleged west-Mediterranean endemic taxa as Ovalona anastasia (Sinev, Alonso, Miracle & Sahuquillo, 2012), Ovalona nuragica (Margaritora, 1971), Alona salina Alonso, 1996 and Levdigia iberica

Kotov & Alonso, 2010, whose presence on an island of the eastern Mediterranean Sea was rather unexpected and further stresses the need for a careful re-examination of the anomopod fauna of the eastern Mediterranean countries.

The observed Cretan copepod fauna is rather poor when compared to other large Mediterranean islands (e.g. Sicily, Corsica, or Sardinia). These are usually inhabited by 6-11 diaptomid species, and even the relatively-small Balearic Islands and Corfu respectively host five and six diaptomid species (Marrone et al., 2009). These numbers are significantly higher than the two diaptomid species observed on Crete. This paucity is likely to be ascribed to the inadequacy of sampling and/or of the used culturing techniques for the hatching or raising of calanoid copepods. Actually, Crete has a climate, geographic location and physiography which should allow the presence of a much richer diaptomid fauna (Marrone et al., 2017). The same applies to cyclopoid and harpacticoid copepods, whose observed species richness is almost completely due to the few samples collected during the wet phase of the water bodies, while only three species, i.e. Diacyclops lubbocki (Brady, 1869), Metacyclops minutus (Claus, 1863) and Cletocamptus retrogressus Schmankevitsch, 1875 hatched in the cultures (see Table S1, available at http://www.limnetica. net/en/limnetica). It is then obvious that different culturing methods are to be developed and implemented to efficiently use the Sars' method for censing the copepods occurring in a given area. Until culturing methodologies are optimised, the study of copepods should be primarily based on collections in the field. It is likely that an increased sampling effort will lead to the finding of several other copepod taxa, including representatives of the diaptomid genera Mixodiaptomus and Hemidiaptomus which are among the most typical copepod inhabitants of Mediterranean temporary ponds (Miracle, 1982; Sahuquillo & Miracle, 2013).

The three calanoid copepods recorded in the frame of this survey were already known to occur on the island (Brehm, 1955; Pesce & Maggi, 1981; Alfonso & Belmonte, 2013). Interestingly, we found abundant populations of the alleged Cretan endemic diaptomid *Arctodiaptomus* 

*piliger* in five temporary ponds spread throughout the island. The species was only sketchy described by Brehm (1955) and never collected again since its original description so that providing more details on its morphology and ecology was considered pivotal according to Kiefer (1978), Ranga-Reddy (1994) and Błędzki & Rybak (2016). Arctodiaptomus piliger was ascribed to the bacillifer-group sensu Kiefer (1971) based on the occurrence of two setae on the 13th antennulary segment. According to the original description, the only morphological character which would be discriminative of A. piliger and which would have differentiated this taxon from the closely-related A. alpinus was the presence of "long hairs" on the endopodites of female fifth pair of legs. This character, however, has no taxonomic value since it is routinely reported for most Arctodiaptomus species, including Arctodiaptomus alpinus (cf. Dussart, 1967; Petkovski, 1983; Stella, 1984; Kiefer, 1971, 1978; and personal observations). Moreover, the molecular identification of Cretan Arctodiaptomus specimens from two sites located more than 210 km apart on the island, consistently showed their conspecificity with Arctodiaptomus alpinus populations from the Italian Alps and from Central Greece. Morphological re-examination and molecular analyses of alleged Arctodiaptomus piliger are thus concordant in supporting the synonymization of this species with the widespread Arctodiaptomus alpinus, a species also occurring on mainland Greece (present data) and spread throughout the Palearctic (Dussart & Defaye, 2002; Flössner et al., 2005; but see also: Marrone et al., 2015), which is here formally indicated as the senior synonym of A. piliger. The possible occurrence of Arctodiaptomus alpinus on Crete suggested by Kiefer (1971, pag. 148) and reported (but not discussed) by Ranga-Reddy (1994), Jersabek et al. (2011, Fig.7) and Błędzki & Rybak (2016, figure at pag. 491) is thus here fully confirmed. Our findings provide some unprecedented information on the ecology of the species, which was usually considered a "cold stenothermal species, occurring preponderantly in the lakes of high places in the Alps (altitude 2000-2700 m)" (Ranga-Reddy, 1994) and a "real high-altitude species with a strong preference for ultraoligotrophic alpine lakes above the timberline" (Błędzki & Rybak, 2016). Conversely, in Crete the species was collected in eutrophic temporary ponds and pools frequented by livestock, and located in the range 539-1330 m a.s.l. In some cases, it co-occurred with *Neolovenula alluaudi*, a Paradiaptominae copepod considered typical of steppic regions (Jaume, 1989-1990; Alfonso & Belmonte, 2013).

All the cyclopoid and harpacticoid species collected so far from surface waters in Crete are widespread in the Mediterranean bioregion as well as across the Palearctic, confirming their high dispersal and colonization ability. *Metacy-clops minutus* was found so far only in temporary ponds and pools, showing a preference for small and ephemeral water bodies (Dussart, 1969; Einsle, 1993); it was the species most commonly obtained by the "Hydration Experiments".

To our knowledge, Klie (1941) and Petkowski et al. (2000) were the only authors who provided data on the ostracod fauna of Crete, altogether listing 15 species; among these, 10 were not found in the present study. Accordingly, considering both the data of Klie (1941) and the present data, the ostracod fauna of Crete at present includes 29 taxa. Among ostracods, the findings of Ilyocypris sp., a taxon already recorded from Greece (Mazzini et al., 2014), peninsular Italy (Alfonso et al., 2016) and Tunisia (unpublished data), and Eucypris sp. are noteworthy. These taxa are currently under study and might be species new to Science pending a formal description. Moreover, the specific allocation of both Candona sp. and Cypridopsis sp. remained uncertain, due to either absence of males or presence of exclusively juvenile specimens.

Based on the currently available data, the non-malacostracan crustacean fauna of the inland waters of Crete is a typically Mediterranean one, including both steppic and more temperate taxa. However, the well-developed orography of the island also allows the presence of species, as the calanoid copepod *Arctodiaptomus alpinus*, which are typical of colder climates. These organisms might represent in Crete relic taxa which colonised the island during Pleistocene glaciations and found at higher altitudes microthermal refuges where to survive the Holocene climate warming. Interestingly, in accordance to what observed in other large Mediterranean islands, no endemic taxa were found among the species inhabiting surface water bodies, while the microcrustacean stygofauna of Crete and other Mediterranean islands is well-diversified and characterised by the presence of strict endemics like the cyclopoid copepods of the genus Speocyclops (Lindberg, 1955, 1956; Pesce & Maggi, 1983) or the harpacticoid copepods of the genus Elaphoidella (Chappuis, 1929, 1956). This pattern is likely to be ascribed to the high potential for passive dispersal shown by organisms producing resting stages, and to their consequent ability of both rapidly colonising newly suitable habitats and likely maintaining an effective gene-flow among populations (see Kappas et al., 2017), although some contrasting evidences on this topic are also available (Incagnone et al., 2015).

The occurrence in Crete of several anomopod species currently considered endemic of the western Mediterranean area highlights the importance of the so-called Wallacean shortfall (Whittaker *et al.*, 2005) for certain "less charismatic" crustacean groups as the non-malacostracan crustaceans, and its bearing on our current understanding of their diversity patterns and biogeography.

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Con el apoyo de:



