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PENSOFT

First faunistic report of limno-terrestrial tardigrades (Tardigrada Doyère, 1840) from the Maltese Islands

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Abstract

Until now, the tardigrade fauna of the Maltese archipelago was unknown, since only the presence of unidentified tardigrades in Malta was reported in the literature. The present study includes the analysis of 11 moss and lichen samples collected in 2015 and 2017 from the islands of Malta and Gozo, and 10 historical microscopic preparations from past fieldwork by Pilato and Binda. Eleven species are reported for the Maltese archipelago, of which, three are new to science but still to be formally described. The taxonomic and faunistic data obtained are discussed in comparison with those of Sicily and North Africa, aiming to draft preliminary faunistic and biogeographic considerations.

Keywords

Biodiversity, faunistics, Mediterranean, new records, water bears

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Introduction

The study of the limno-terrestrial tardigrade fauna of the Maltese archipelago represents an important contribution, which until now never been fully reported. The presence of limno-terrestrial tardigrades had already been confirmed in the past by some research (Kotwicki et al. 2014; Schembri 2003), however, papers in which the species were to be diagnosed have never been published. In the present work 11 samples of mosses and lichens from five locations on the islands of Malta and Gozo are analyzed, in addition to 10 historical microscopic slides belonging to research work of Pilato and Binda in the 1980s. Eleven species are reported, and, of these, three are new to science, although still to be formally described.

Study Area

The Maltese archipelago, with the three main islands of Malta, Gozo, and Comino, and several small islets, is located in the Mediterranean basin, 80–90 km south of Sicily. The archipelago has an area of about 316 km^2. The Maltese islands were certainly and repeatedly connected to Sicily, North Africa, and the eastern Mediterranean regions after their definitive emergence, which

probably occurred about 4 million years ago at the end of the Lower Pliocene (Corti and Lanza 1973); the Maltese Islands show geomorphological and environmental aspects typical of the Mediterranean (Rolè 2019; Spiteri and Stevens 2019), comparable to those of Sicily (Agnesi et al. 2014). The Maltese archipelago has been relatively well studied faunistically and floristically for many groups; for example, it is possible to mention Haslam et al. (1977) (flora); Schembri et al. (1993) (animal and plant comprehensive review), and Mifsud (2013) (updates on insects). The islands support a high biodiversity, especially compared to the modest area of the archipelago (see also Deidun 2010; Deidun et al. 2019; Lanfranco et al. 2019). Thake (1985) summarized the biogeographic knowledge of both animals and plants and provided a detailed study on a group of molluscs.

Methods

Eleven samples from five locations (indicated as red dots in Fig. 1) were analyzed: Dahlet Qorrot Bay and Ramla from the island of Gozo, and Buskett, Chadwick Lakes, and Wardija from the island of Malta. The samples were collected in February 2015 and November 2017 by David Mifsud and Vera D'Urso, respectively. The samples consist of portions of moss or lichens collected on soil, beach sand, or rocks. Each sample was firstly shredded and rehydrated, then sifted with fine mesh sieves (500 µm, 100 µm, 37 µm) to extract meiofaunal animals; tardigrades were sorted under a stereomicroscope and mounted on microscopic slides with polyvinyl lactophenol; sex was undetermined for all specimens examined. All slides are deposited in the Pilato and Binda Collection (Zoological Museum of the Department of Biological, Geological, and Environmental Sciences of the University of Catania, Italy; UNICT).

All data regarding the samples and mounted animals are indicated in Tables 1 and 2, respectively.

In this study, historical materials were also analyzed: Giovanni Pilato kindly made available 10 microscopic slides (no. 5994–6003); these were never studied and contained specimens from samples collected on the island of Malta; the preparations were part of Pilato and Binda's investigations in the 1980s. Unfortunately, for preparations 5996 to 6003 the exact sampling locality is unknown; 5994 and 5995 contain animals extracted from a sample collected by G. Pilato at Il-Maqluba (indicated as green dot in Fig. 1). We have no other information on these materials. The data obtained from the study of these 10 slides were integrated with the information acquired with the study of the samples collected in 2015 and 2017 (Tables 1, 2).

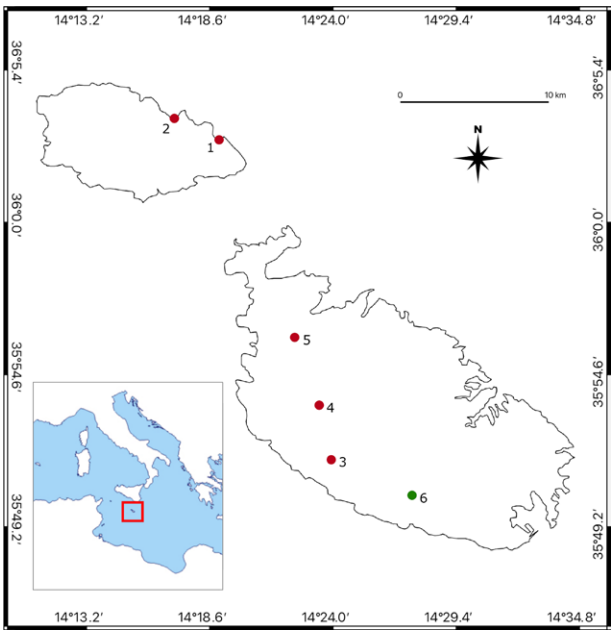

Figure 1. Map of the islands of Malta and Gozo showing the sampling sites. Localities of newly collected samples (2015, 2017) are shown as red dots, while locality of historical slides (1980s) is indicated as green dot. Gozo island: 1 = Ramla; 2 = Dahlet Qorrot Bay. Malta island: 3 = Buskett; 4 = Chadwick Lakes; 5 = Wardija; 6 = Il-Maqluba.

The map (Fig. 1) was created using Q-GIS and improved using Adobe Photoshop 2021. Observations were made under 100× objective (1000× magnification), in oil immersion, with a Leica Phase Contrast Microscope DM1000 LED equipped with micrometer for measurements. Photos were taken using a Leica Flexacam C3 digital camera installed on the above-mentioned microscope; photos were processed using Adobe Photoshop

Table 1. Data of the samples analyzed.

Sample	Locality	Geographic coordinates	Altitude (m)	Date	Material
1	Gozo, Dahlet Qorrot Bay	36°02′58″N, 014°18′57″E	0	10.II.2015	Acrocarpous moss on sand
2	Gozo, Ramla	36°03′39″N, 014°17′04″E	7	10.II.2015	Acrocarpous moss on sand
3	Buskett	35°51′33″N, 014°23′55″E	192	14.II.2015	Acrocarpous moss on rock / soil
4	Buskett	35°51′33″N, 014°23′55″E	192	14.II.2015	Acrocarpous moss on rock
5	Buskett	35°51′33″N, 014°23′55″E	192	14.II.2015	Pleurocarpous moss on rock / soil
6	Buskett	35°51′33″N, 014°23′55″E	192	14.II.2015	Pleurocarpous moss on rock
7	Chadwick Lakes	35°53′31″N, 014°23′24″E	116	11.II.2015	Acrocarpous moss on soil
8	Wardija	35°55′54″N, 014°22′17″E	141	12.II.2015	Acrocarpous moss on rock / soil
9	Wardija	35°55′54″N, 014°22′17″E	141	15.XI.2017	Acrocarpous moss on rock / soil
10	Wardija	35°55′54″N, 014°22′17″E	141	15.XI.2017	Acrocarpous moss on rock / soil
11	Wardija	35°55′54″N, 014°22′17″E	141	15.XI.2017	Lichens on rock
Historical material	Il-Maqluba	35°49′51″N, 014°27′28″E	78	Not available	Not available

Table 2. List of samples, tardigrade species, and slides analyzed.

Sample	Locality	Specimens/exuviae	Eggs	Species	Slides no.
1	Gozo, Dahlet Qorrot Bay	1	0	*Ursulinius pappi*	5901
2	Gozo, Ramla	3	0	*Xerobiotus* sp.	5902–5904
		9	0	*Ursulinius pappi*	5905–5912
		5	0	*Macrobiotus* sp. nov. 2	5912–5916
		7	0	*Mesobiotus* cf. *diffusus*	5916–5923
3	Buskett	0	2	*Paramacrobiotus* cf. *sklodowskae*	5924, 5925
4	Buskett	0	1	*Paramacrobiotus* cf. *sklodowskae*	5927
5	Buskett	1	0	*Ursulinius pappi*	5928
6	Buskett	1	0	*Ursulinius pappi*	5929
7	Chadwick Lakes	30/4	16	*Ursulinius pappi*	5930–5957
		5	0	*Pilatobius* sp. nov.	5958–5962
8	Wardija	2	1	*Paramacrobiotus* cf. *sklodowskae*	5963–5965
		0	1	*Macrobiotus* sp. nov. 1	5966
		4	0	*Macrobiotus* sp. nov. 2	5967–5970
		10/3	8	*Ursulinius pappi*	5971–5981
		1	0	*Doryphoribius* sp. *doryphorus* group	5982
9	Wardija	1	0	*Bryodelphax* cf. *weglarskae*	5983
		3	0	*Hypsibius ragonesei*	5984–5986
10	Wardija	3	0	*Hypsibius ragonesei*	5987
11	Wardija	1	0	*Bryodelphax* cf. *weglarskae*	5988
		7	0	*Hypsibius ragonesei*	5989–5993
Historical material	Il-Maqluba	4	0	*Paramacrobiotus klymenki*	5994, 5995
Historical material	Unknown	16	2	*Paramacrobiotus klymenki*	5996, 5997
		0	1	*Paramacrobiotus* cf. *sklodowskae*	5996
		19	1	*Macrobiotus* sp. nov. 2	5998–6003

2021. Diagnosis at the genus level was carried out using the dichotomous keys of Pilato and Binda (2010) and updates on some genera (Michalczyk and Kaczmarek 2010; Kaczmarek and Michalczyk 2017; Kaczmarek et al. 2017, 2020; Gąsiorek et al. 2019; Guidetti et al. 2019); a provisional, general diagnosis of the species was performed with the monograph by Ramazzotti and Maucci (1983); for definitive, updated diagnosis we integrated original descriptions with updated descriptions, redescriptions, and dichotomous keys (Murray 1906; Marcus 1936; Robotti 1970; Pilato and Binda 1983; Pilato et al. 1990; Nelson 1991; Nelson and McGlothlin 1993; Abe 2004; Guidetti et al. 2013; Bingemer and Hohberg 2017; Nowak and Stec 2018; Stec et al. 2020).

Specific diagnosis also required measurements of sclerified structures, made using a micrometer, following the indications by Pilato (1981), Pilato et al. (1982), Beasley et al. (2008), and Kaczmarek and Michalczyk (2017). The pt index was also calculated, which is the percent ratio between the length of a structure and the length of the buccal tube measured from the medio-dorsal transversal ridge of the buccal armature to the base of the pharyngeal apophyses (Pilato 1981). For species with bucco-pharyngeal apparatus of the *Diphascon* type, the pbf index was also calculated, which is the percentage ratio between the length of the structures and the total length of the bucco-pharyngeal tube (Pilato et al. 2002). Morphometric data were handled using the Excel template "Parachela" (ver. 1.4) from Michalczyk and Kaczmarek (2013).

For comparison, the following material was observed in the Pilato and Binda collection. Holotype and paratypes of: *Bryodelphax weglarskae* (Pilato, 1972), slides no. 776–779; *Hypsibius ragonesei* Binda & Pilato, 1985, slides no. 3176–3177; *Paramacrobiotus gerlachae* (Pilato, Binda & Lisi, 2004), slides nos. 4864–4874; *Paramacrobiotus klymenki* Pilato, Kiosya, Lisi & Sabella, 2012, slide no. 5425. Paratypes of *Hypsibius camelopardalis* Ramazzotti & Maucci, 1983, slide no. 3582; *Macrobiotus punctillus* Pilato, Binda & Azzaro, 1990, slides no. 4430, 4432, 4433; *Paramacrobiotus sklodowskae* (Michalczyk, Kaczmarek & Węglarska, 2006), slides no. 5416. Specimens, identified by Pilato, of: *Xerobiotus pseudohufelandi* (Iharos, 1966), slides no. 3534–3539; *Ursulinius lunulatus* (Iharos, 1966), slides no. 948–953; *Mesobiotus diffusus* (Binda & Pilato, 1987) slides no. 909–912.

Results

All samples from the five localities were positive for tardigrades and/or eggs (Table 2). Reports of tardigrade species from the Maltese archipelago were completely absent from the literature; thus, all the species found are new records for the country.

Class Heterotardigrada Marcus, 1927
Order Echiniscoidea Richters, 1926
Family Echiniscidae Thulin, 1928
Genus *Bryodelphax* Thulin, 1928

***Bryodelphax* sp., *weglarskae* group**
Figure 2

New records. MALTA – **Malta island** • Chadwick Lakes; 35°53′31″N, 014°23′24″E; 116 m alt.; 11.II.2015; D. Mifsud leg.; sample 7, slide no. UNICT 5959 (1 specimen) • Wardija; 35°55′54″N, 014°22′17″E; 141 m alt.; 15.XI.2017; V. D'Urso leg.; sample 11, slide no. UNICT 5988 (1 specimen).

Identification. The two specimens found, due to the presence of ventral plates, have to be attributed to the *weglarkae* group; however, the quality of the material did not allow precise specific diagnosis.

Class Eutardigrada Richters, 1926
Order Parachela Schuster, Nelson, Grigarick & Christenberry, 1980
Superfamily Hypsibioidea Pilato, 1969 in Marley et al. 2011
Family Hypsibiidae Pilato, 1969
Subfamily Hypsibiinae Pilato, 1969
Genus *Hypsibius* Ehrenberg, 1848

***Hypsibius ragonesei* Binda & Pilato, 1985**
Figures 3, 4

New records. MALTA – **Malta island** • Wardija; 35°55′54″N, 014°22′17″E; 141 m alt.; 15.XI.2017; V. D'Urso leg.; sample 9, slides no. UNICT 5984–5986 (1 specimen; sample 10, slide no. 5987 (3 specimens); sample 11, slides no. UNICT 5989 (3 specimens), UNICT 5990–5993 (4 specimens).

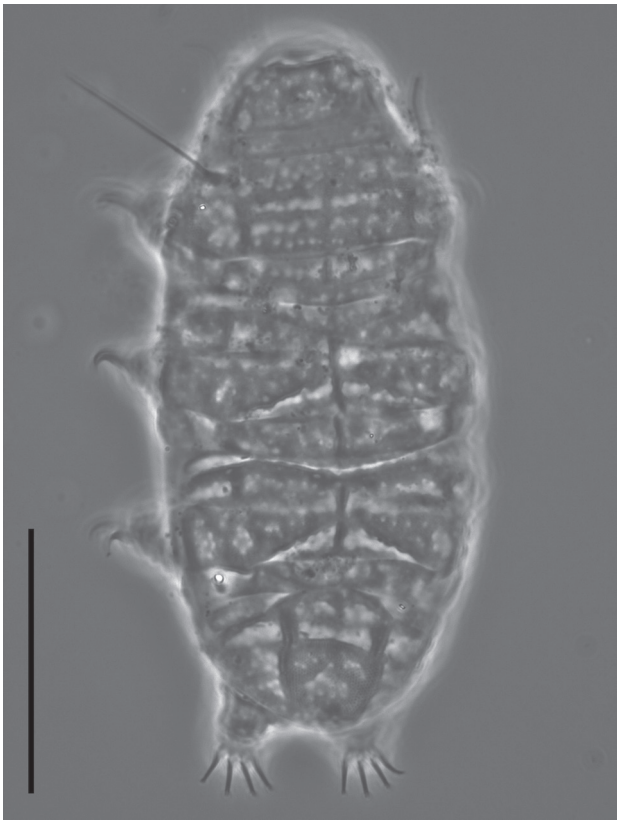

Figure 2. Habitus of *Bryodelphax* cf. *weglaskae* (slide no. UNICT 5983). Scale bar: 50 µm.

Identification. The specimens were identified by morphological and morphometric characters, using the literature and by direct comparison with the holotype and paratypes of *H. ragonesei* and one paratype of *H. camelopardalis*. Similar species are *H. camelopardalis*, *H.*

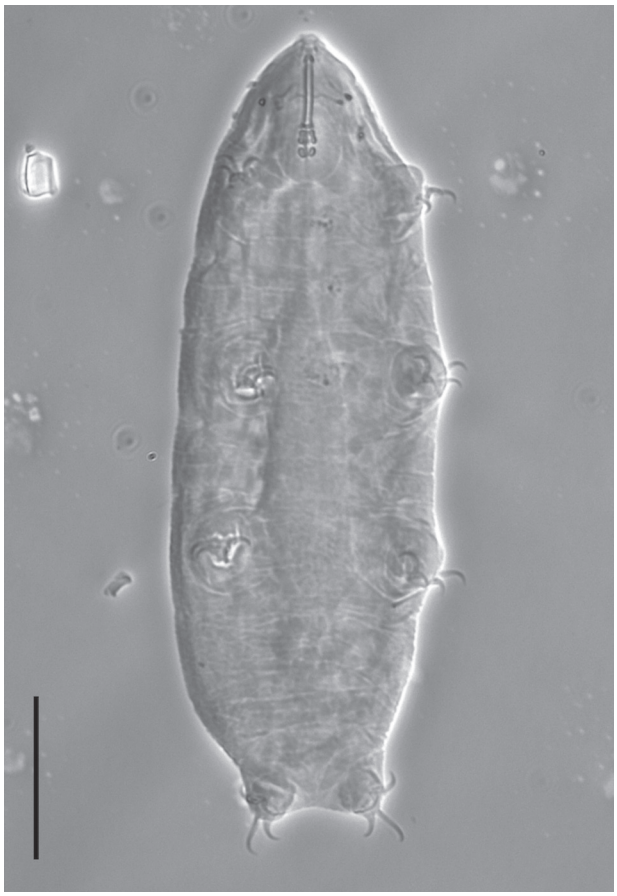

Figure 3. Habitus of *Hypsibius ragonesei* (slide no. UNICT 5992). Scale bar: 50 µm.

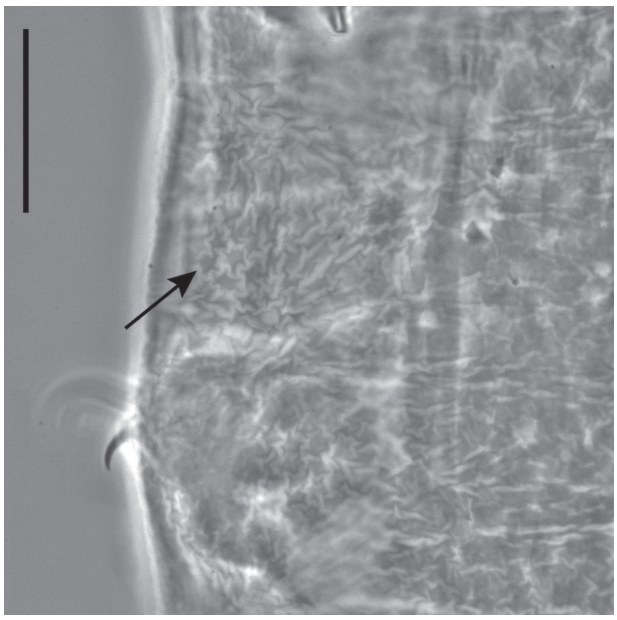

Figure 4. Detail of the cuticular roughness of *Hypsibius ragonesei* forming irregularly shaped tubercles (slide no. UNICT 5992). Scale bar: 20 µm.

roanensis Nelson & McGlothlin, 1993, *H. scabropygus* Cuénot, 1929, and *H. stiliferus* Abe, 2004, but the studied specimens show general correspondence with the morphological and morphometric characters of *H. ragonesei* (Table 3). Our Maltese specimens, attributed to the latter species, differ from *H. camelopardalis*, *H. scabropygus*, and *H. stiliferus* in some morphometric characters and in having uniformly distributed tubercles (Fig. 4); the tubercles are distributed in transverse rows in *H. camelopardalis* and *H. stiliferus*, or limited to the caudal part of the body in *H. scabropygus*. Differences from *H. roanensis* are the wider buccal tube, elongated instead of granular placoids 1 and 2, and internal and external claws more similar to each other (more different in *H. roanensis*).

Remarks. Thirteen specimens were found. The type locality is near Noto, Sicily, and no reports from Africa are known.

Subfamily Pilatobiinae Bertolani, Guidetti, Marchioro, Altiero, Rebecchi & Cesari, 2014

Table 3. Morphometric data of the measurable specimens of *Hypsibius ragonesei* found in the present study. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: *N* = number of specimens or structures measured; SD = standard deviation.

Character	*N*	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	7	160–266	663–1066	203	844	40	167
Buccopharyngeal tube							
Buccal tube length	7	22.8–25.1	—	24.1	—	0.8	—
Stylet support insertion point	7	14.4–16.5	63.1–67.4	15.6	64.9	0.7	1.5
Buccal tube external width	7	2.3–3.0	10.2–12.5	2.7	11.4	0.2	0.8
Buccal tube internal width	6	1.2–1.8	5.1–7.6	1.4	5.9	0.2	0.9
Placoid lengths							
Macroplacoid 1	7	3.7–4.4	15.6–18.0	4.1	17.1	0.3	0.9
Macroplacoid 2	7	2.3–3.1	10.3–12.3	2.8	11.5	0.3	0.9
Macroplacoid row	7	7.2–8.4	31.7–35.0	8.0	33.4	0.4	1.2
Claw I heights							
External base	1	4.0–4.0	16.5–16.5	4.0	16.5	?	?
External primary branch	1	9.7–9.7	40.2–40.2	9.7	40.2	?	?
External secondary branch	1	6.1–6.1	25.3–25.3	6.1	25.3	?	?
External base/primary branch (cct)	1	41.2–41.2	—	41.2	—	?	—
Internal base	3	4.0–4.3	16.4–17.9	4.1	17.1	0.2	0.7
Internal primary branch	3	6.2–6.8	25.7–27.5	6.5	26.9	0.3	1.0
Internal secondary branch	3	5.0–5.3	20.5–21.7	5.1	21.1	0.1	0.6
Internal base/primary branch (cct)	3	59.9–69.3	—	63.9	—	4.9	—
Claw II heights							
External base	2	4.6–4.8	19.2–19.2	4.7	19.2	0.1	0.1
External primary branch	3	8.8–11.6	38.6–46.2	10.1	41.8	1.4	3.9
External secondary branch	3	6.2–6.6	25.7–29.0	6.4	26.9	0.2	1.8
External base/primary branch (cct)	2	41.5–47.4	—	44.5	—	4.2	—
Internal base	5	3.2–4.6	14.2–18.6	4.0	16.6	0.5	1.6
Internal primary branch	4	6.5–8.6	27.5–34.1	7.3	30.4	0.9	2.9
Internal secondary branch	6	5.1–6.1	21.2–24.3	5.5	22.4	0.3	1.1
Internal base/primary branch (cct)	3	49.0–55.3	—	51.3	—	3.5	—
Claw III heights							
External base	3	4.5–5.1	18.6–21.5	4.9	20.2	0.3	1.5
External primary branch	2	8.9–9.7	36.7–41.5	9.3	39.1	0.6	3.4
External secondary branch	3	6.4–7.2	26.4–30.7	6.8	28.3	0.4	2.2
External base/primary branch (cct)	2	50.7–51.7	—	51.2	—	0.7	—
Internal base	4	3.6–4.6	14.9–18.4	4.2	17.3	0.5	1.6
Internal primary branch	4	6.8–8.3	27.9–33.3	7.4	30.7	0.8	2.9
Internal secondary branch	4	5.0–6.5	21.1–25.9	5.6	23.3	0.6	2.0
Internal base/primary branch (cct)	4	52.5–65.6	—	56.5	—	6.2	—
Claw IV heights							
Anterior base	4	4.1–5.3	18.0–21.1	4.5	18.9	0.5	1.5
Anterior primary branch	4	7.9–8.9	33.8–35.4	8.3	34.8	0.4	0.8
Anterior secondary branch	4	5.6–7.0	23.7–28.3	6.3	26.2	0.6	2.2
Anterior base/primary branch (cct)	4	51.6–59.4	—	54.2	—	3.6	—
Posterior base	2	5.0–5.3	21.5–21.8	5.2	21.7	0.2	0.2
Posterior primary branch	3	10.1–11.6	41.9–49.3	10.8	45.5	0.7	3.7
Posterior secondary branch	3	7.4–8.5	31.1–36.3	7.9	33.2	0.6	2.7
Posterior base/primary branch (cct)	2	43.6–52.1	—	47.9	—	6.0	—

Genus *Pilatobius* Bertolani, Guidetti, Marchioro, Altiero, Rebecchi & Cesari, 2014

***Pilatobius* sp. nov.**

New records. MALTA – **Malta island** • Chadwick Lakes; 35°53′31″N, 014°23′24″E; 116 m alt.; 11.II.2015; D. Mifsud leg.; sample 7, slides no. UNICT 5958–5962 (5 specimens).

Remarks. Five specimens were found. The combination of characteristics of the cuticular reticulum, placoids, claws, and some morphometric characters make this Maltese species clearly distinct from all the other congeners, but the new species is still to be formally described.

Superfamily Isohypsibioidea Sands, McInnes, Marley, Goodal-Copestake, Convey & Linse, 2008
Family Doryphoribiidae Gąsiorek, Stec, Morek & Michalczyk, 2019
Genus *Doryphoribius* Pilato, 1969

***Doryphoribius* sp., *doryphorus* group**

New records. MALTA – **Malta island** • Wardija; 35° 55′54″N, 014°22′17″E; 141 m alt.; 12.II.2015; D. Mifsud leg.; sample 8, slide no. UNICT 5982 (1 specimen).

Identification. Only one specimen was found. Due to the presence of two macroplacoids and a cuticle without gibbosities, it is attributable to the *doryphorus* group (Gąsiorek et al. 2019); however, the attribution to a described or underscribed species was not possible.

Family Isohypsibiidae Sands, McInnes, Marley, Goodall-Copestake, Convey & Linse, 2008
Genus *Ursulinius* Gąsiorek, Stec, Morek & Michalczyk, 2019

***Ursulinius pappi* (Iharos, 1966)**
Figure 5

New records. MALTA – **Gozo island** • Dahlet Qorrot Bay; 36°02′58″N, 014°18′57″E; 0 m alt.; 10.II.2015; D. Mifsud leg.; sample 1, slide no. UNICT 5901 (1 specimen) • Ramla; 36°03′39″N, 014°17’04″E; 7 m alt.; 10.II.2015; D. Mifsud leg.; sample 2, slides no. UNICT 5905–5912 (9 specimens) – **Malta island** • Buskett Woodlands; 35°51′33″N, 014°23′55″E; 192 m alt.; 14.II.2015; D. Mifsud leg.; sample 5, slide no. UNICT 5928 (1 specimen); sample 6, slide no. UNICT 5929 (1 specimen) • Chadwick Lakes; 35°53′31″N, 014°23′24″E; 116 m alt.; 11.II.2015; D. Mifsud leg.; sample 7, slides no. UNICT 5930 (1 specimen; 1 exuvia with 4 eggs), UNICT 5931–5942 (15 specimens), UNICT 5943 (1 specimen; 1 exuvia with 4 eggs), UNICT 5944 (1 specimen; 1 exuvia with 5 eggs), UNICT 5945–5953 (9 specimens), UNICT 5954 (1 exuvia with 4 eggs), UNICT 5955–5957 (3 specimens) • Wardija; 35°55′54″N, 014°22′17″E; 141 m alt.; 12.II.2015; D. Mifsud leg.; sample 8, slides no. UNICT 5972 (1 exuvia with 3 eggs), UNICT 5971 (1 specimen), UNICT 5973–5978 (8 specimens), UNICT 5980 (1 specimen), UNICT 5979 (1 exuvia with 2 eggs), UNICT 5981 (1 exuvia with 3 eggs).

Identification. The specimens were identified by morphological and morphometric characters (Table 4) using the literature and by direct comparison with specimens of *U. lunulatus.* The species differs from other congeners for morphometric characters and in having 10 rows of large reticulated gibbosities, arranged as follows: X: 2-4-6-4-6-4-6-4-4-2 (Michalczyk and Kaczmarek 2010).

Remarks. Fifty-two specimens and seven exuviae, containing 2–5 eggs each, were found. The type locality is near Csot, in Hungary; this species is known from Sicily (Lisi 2015) and Morocco (McInnes et al. 2017).

Superfamily Macrobiotoidea Thulin, 1928
Family Macrobiotidae Thulin, 1928
Genus *Macrobiotus* C.A.S. Schultze, 1834

***Macrobiotus* sp. nov. 1**

New records. MALTA – **Malta island** • Wardija; 35°55′54″N, 014°22′17″E; 141 m alt.; 12.II.2015; D. Mifsud leg.; sample 8, slide no. UNICT 5966 (1 egg).

Identification. A single egg was found, with conical processes, ending with the filament or truncated with a modest terminal enlargement, and showing a delicate *hufelandi*-like reticulum on the chorion. This falls within

Figure 5. Habitus of *Ursulinius pappi* (slide no. UNICT 5956). Scale bar: 50 µm.

Table 4. Morphometric data of the measurable specimens of *Ursulinius pappi* found in the present study. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: *N* = number of specimens or structures measured; SD = standard deviation.

Character	*N*	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	11	136–294	668–1073	204	853	50	130
Buccopharyngeal tube							
Buccal tube length	11	19.6–29.7	—	23.7	—	3.5	—
Stylet support insertion point	11	12.5–20.5	60.1–70.4	15.7	65.8	2.9	3.0
Buccal tube external width	10	2.5–4.1	10.8–16.5	3.3	13.9	0.5	1.7
Buccal tube internal width	10	1.9–3.1	7.2–12.3	2.4	10.1	0.5	1.4
Placoid lengths							
Macroplacoid 1	9	3.4–4.7	14.1–18.8	4.0	16.3	0.4	1.4
Macroplacoid 2	10	1.9–3.7	9.1–12.5	2.6	11.0	0.5	1.1
Macroplacoid row	9	6.5–9.5	29.5–33.8	7.8	31.8	0.9	1.5
Claw I heights							
External base	5	3.8–5.2	17.6–20.3	4,4	18.9	0.6	1.1
External primary branch	5	6.9–10.8	32.9–38.4	8.6	36.4	1.6	2.1
External secondary branch	6	5.1–7.5	24.0–27.9	6.3	26.0	0.9	1.7
External base/primary branch (cct)	5	48.3–55.7	—	52.0	—	2.7	—
Internal base	5	3.0–4.4	14.8–17.9	3.9	16.4	0.6	1.3
Internal primary branch	4	5.1–7.2	23.8–30.6	6.6	27.0	1.0	2.9
Internal secondary branch	5	4.3–6.8	21.5–24.8	5.4	22.7	1.0	1.4
Internal base/primary branch (cct)	4	55.1–62.2	—	59.5	—	3.2	—
Claw II heights							
External base	9	3.3–5.2	16.6–21.6	4.4	19.1	0.7	1.4
External primary branch	8	6.7–9.2	32.1–39.0	8.0	35.1	0.8	2.6
External secondary branch	9	4.9–7.0	23.0–29.3	5.9	25.9	0.8	2.1
External base/primary branch (cct)	8	48.7–59.6	—	54.1	—	4.1	—
Internal base	6	3.1–4.0	14.5–18.4	3.7	16.2	0.4	1.4
Internal primary branch	5	5.3–7.5	26.9–34.8	6.7	30.8	0.9	3.5
Internal secondary branch	5	4.2–6.5	21.3–24.8	5.1	23.0	1.0	1.6
Internal base/primary branch (cct)	4	51.0–57.8	—	54.5	—	3.2	—
Claw III heights							
External base	5	4.0–5.4	15.3–22.5	4.8	19.3	0.6	2.9
External primary branch	5	7.5–9.9	32.5–39.3	8.9	35.5	0.9	3.0
External secondary branch	6	5.4–7.9	23.6–31.4	6.8	26.5	0.9	2.8
External base/primary branch (cct)	5	47.1–59.1	—	54.0	—	4.3	—
Internal base	4	3.4–4.7	15.2–18.6	4.1	17.0	0.5	1.4
Internal primary branch	4	6.3–7.6	25.3–32.1	7.0	29.9	0.7	3.2
Internal secondary branch	6	4.7–7.5	21.9–26.4	6.0	23.9	0.9	1.7
Internal base/primary branch (cct)	3	52.5–61.3	—	56.1	—	4.7	—
Claw IV heights							
Anterior base	2	3.9–4.7	16.9–20.1	4.3	18.5	0.5	2.3
Anterior primary branch	3	7.2–9.2	33.1–36.6	8.1	34.4	1.0	1.9
Anterior secondary branch	2	4.3–5.6	20.0–21.7	4.9	20.9	0.9	1.2
Anterior base/primary branch (cct)	2	51.1–55.0	—	53.1	—	2.8	—
Posterior base	3	4.2–5.0	17.5–21.4	4.7	19.4	0.4	2.0
Posterior primary branch	5	7.9–11.4	38.2–41.0	9.0	40.0	1.4	1.2
Posterior secondary branch	5	4.9–8.9	24.6–30.1	6.6	26.3	1.6	2.2
Posterior base/primary branch (cct)	2	42.7–53.1	—	47.9	—	7.3	—

the possible morphotypes of the *hufelandi* group according to Kaczmarek and Michalczyk (2017); in particular, it has clear similarities with the eggs of three species: *M. kristenseni* Guidetti, Peluffo, Rocha, Cesari & Moly De Peluffo, 2013, *M. nebrodensis* Pilato, Sabella, D'Urso & Lisi, 2017, and *M. recens* Cuénot, 1932. However, the detailed characteristics of the egg found make the Maltese species clearly distinct from all the other congeners, but the new species is still to be formally described.

Macrobiotus sp. nov. 2

New records. MALTA – **Gozo island** • Ramla; 36° 03′39″N, 014°17′04″E; 7 m alt.; 10.II.2015; D. Mifsud leg.; sample 2, slides no. UNICT 5914–5916 (3 specimens) – **Malta island** • Wardija; 35°55′54″N, 014°22′17″E; 141 m alt.; 12.II.2015; D. Mifsud leg.; sample 8, slides no. UNICT 5967–5970 (4 specimens) • [unknown locality]; [unknown date]; G. Pilato leg.; slides no. UNICT 5998,

5999 (6 specimens), UNICT 6000 (8 specimens), UNICT 6001 (1 specimen, 1 egg), UNICT 6002 (1 specimen), UNICT 6003 (3 specimens).

Remarks. Twenty-six specimens and one egg were found. The combination of cuticle and egg characters, and some morphometric traits, make the Maltese species clearly distinct from all the other congeners, but the new species is still to be formally described.

Genus *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi & Guidetti, 2016

Mesobiotus* cf. *diffusus

Figure 6

New records. MALTA – **Gozo island** • Ramla; 36°03′ 39″N, 014°17′04″E; 7 m alt.; 10.II.2015; D. Mifsud leg.; sample 2, slides no. UNICT 5917–5923 (7 specimens).

Identification. The specimens were identified by morphological and morphometric characters using the literature and by direct comparison with the holotype and paratypes of *M. diffusus*. Our specimens show general correspondence with that species, which differs from the other congeners for its unique combination of characters: smooth cuticle (also on legs), oral cavity armature with three rows of teeth (I–III) and without additional teeth, eyes absent, lunules I–III smooth and IV crenate, and morphometric characters. However, because we did not find eggs that are attributable to the specimens, we prefer not to report the species as belonging to *M. diffusus* sensu stricto.

Remarks. Seven specimens were found. The type locality of *Mesobiotus diffusus* (Binda & Pilato, 1987) is in North Africa, the species has been reported from Sicily (Lisi 2015), so its presence in Malta is very plausible.

Genus *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009

Paramacrobiotus* cf. *sklodowskae

Figures 7, 8

New records. MALTA – **Malta island** • Buskett Woodlands; 35°51′33″N, 014°23′55″E; 192 m alt); 14.II.2015; D. Mifsud leg.; sample 3, slides no. UNICT 5924, 5925 (4 eggs); sample 4, slide no. UNICT 5927 (1 egg) • Wardija; 35°55′54″N, 014°22′17″E; 141 m alt.; 12.II.2015; D. Mifsud leg.; sample 8, slides no. UNICT 5963, 5964 (4 specimens), UNICT 5965 (1 egg).

Identification. The specimens were identified by morphological and morphometric characters using the literature and by direct comparison with the holotype and paratypes of *P. gerlachae* and paratypes of *P. sklodowskae.* The species belongs to the *richtersi* group because of the presence of microplacoid and reticulated areoles around the egg processes (egg in Fig. 8). It differs from

Figure 6. Habitus of *Mesobiotus* cf. *diffusus* (slide no. UNICT 5918). Scale bar: 50 µm.

Figure 7. Habitus of *Paramacrobiotus* cf. *sklodowskae* (slide no. UNICT 5964). Scale bar: 50 µm.

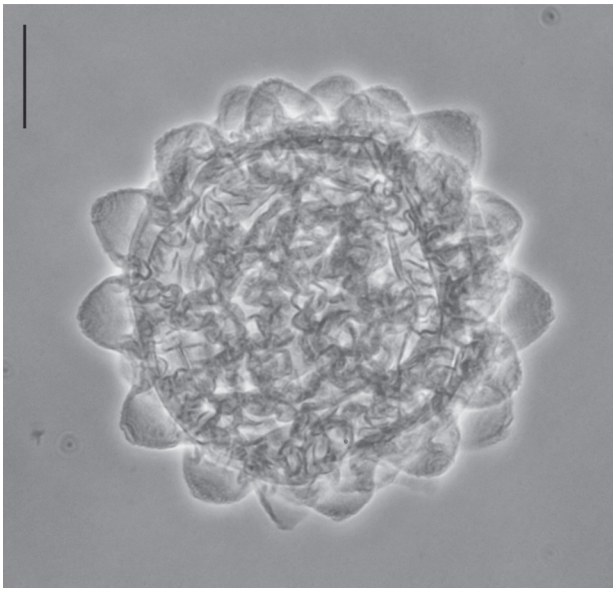

Figure 8. Egg of *Paramacrobiotus* cf. *sklodowskae* in toto (slide no. UNICT 5996). Scale bar: 20 µm.

the other species of the same group by the following combination of characters: presence of granulation on legs I–III, eggs with more than six areoles around each process, processes without elongated apices, and other morphological and morphometric details. However, due to the scarcity of specimens and the lack of eyespots in our specimens (eyespots are present in *P. sklowdoskae* sensu stricto), we cannot attribute our specimens to the species in the strict sense.

Remarks. Two specimens and four eggs were found. The type locality of *P. sklodowskae* Michalczyk, Kaczmarek & Węglarska, 2006 is Cyprus, and this species has been reported from Tunisia (Gąsiorek et al. 2017) but not from Sicily. Despite that, this species' occurrence in the Maltese archipelago is still biogeographically plausible.

Paramacrobiotus klymenki Pilato, Kiosya, Lisi & Sabella, 2012

Figures 9, 10

New records. MALTA – **Malta island** • Il-Maqluba; 35°49′51″N, 014°27′28″ E; 78 m alt.; G. Pilato leg. [unknown date], slides no. UNICT 5994, 5995 (4 specimens) • [unknown locality]; G. Pilato leg. [unknown date], slides no. UNICT 5996 (1 specimen), UNICT 5997 (15 specimens, 2 eggs).

Identification. The specimens were identified by morphological and morphometric characters (Table 5) using the literature and by direct comparison with paratypes of *P. klymenki*. The studied specimens show a general correspondence with the morphological and morphometric characters of *P. klymenki*; this species falls within the *areolatus* group due to the absence of a microplacoid in the pharynx, but differs from all the other species of the group in the following unique combination of characters: absence of eyes, crenate and well-developed lunules on legs IV, egg processes with elongated apices (Fig. 10),

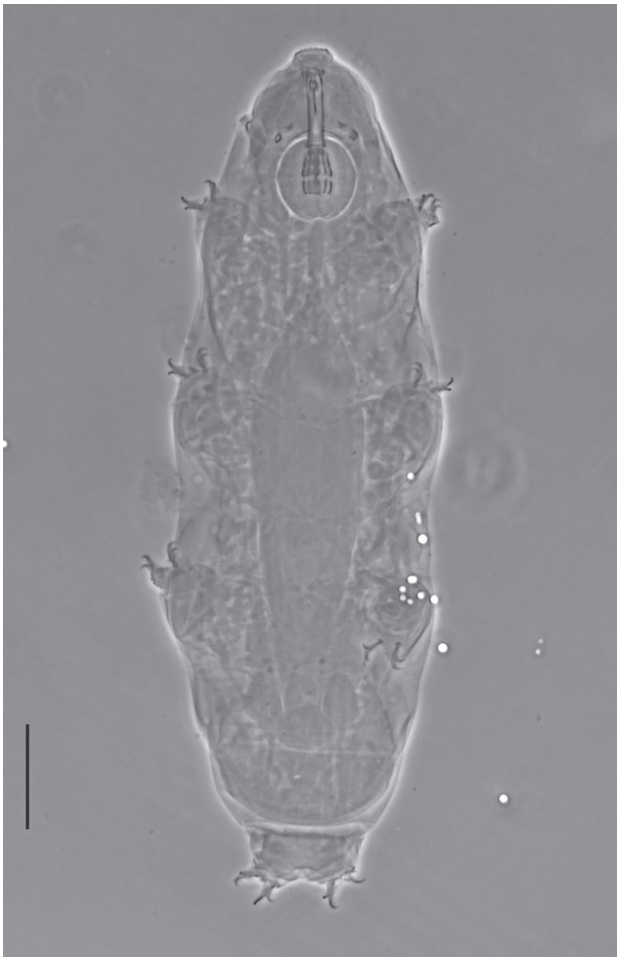

Figure 9. Habitus of *Paramacrobiotus klymenki* (slide no. UNICT 5997). Scale bar: 50 µm.

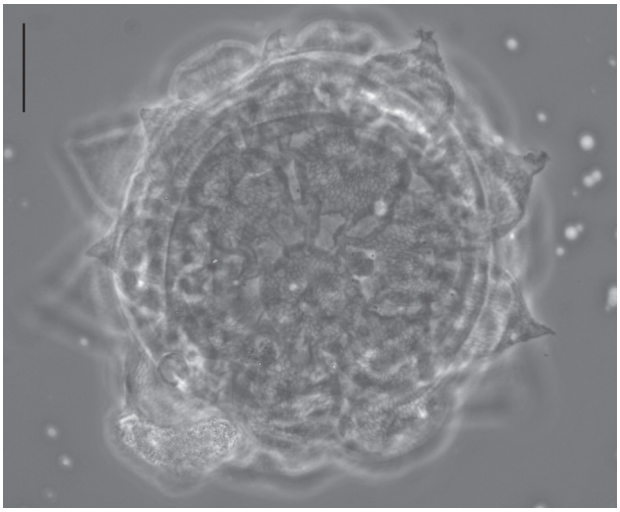

Figure 10. Egg of *Paramacrobiotus klymenki* in toto (slide no. UNICT 5997). Scale bar: 20 µm.

unsculptured apices of the egg processes, and other more detailed morphological and morphometric characters (Table 5). The only egg in good condition and embryonated has a diameter excluding processes of 65.2 µm, diameter including processes of 91.4 µm, process height of 13.4–14.9 µm, process base width of 16.7–19.3 µm, process base/height ratio 125–141%, 21 processes on the

Table 5. Morphometric data of the measurable specimens of *Paramacrobiotus klymenki* found in the present study. Range refers to the lowest and the highest values among all measured specimens. *Pt* values are provided in italics. Abbreviations: *N* = number of specimens or structures measured; SD = standard deviation.

Character	*N*	Range		Mean		SD	
		µm	*pt*	µm	*pt*	µm	*pt*
Body length	10	232–456	763–1172	339	963	64	132
Buccopharyngeal tube							
Buccal tube length	11	21.9–44.3	—	34.4	—	7.0	—
Stylet support insertion point	10	17.0–35.5	77.6–80.1	28.2	79.2	5.2	0.8
Buccal tube external width	11	3.9–10.0	16.7–22.6	6.9	19.9	1.9	1.9
Buccal tube internal width	11	3.0–8.1	12.7–18.2	5.7	16.4	1.6	1.8
Ventral lamina length	10	11.9–26.9	54.1–62.8	21.0	58.9	4.2	2.5
Placoid lengths							
Macroplacoid 1	8	3.4–8.9	15.3–20.1	6.2	17.1	1.7	1.9
Macroplacoid 2	8	2.2–5.4	10.1–13.6	4.2	11.6	1.0	1.1
Macroplacoid 3	7	5.6–9.4	15.6–21.1	6.9	18.1	1.3	1.8
Macroplacoid row	6	10.3–25.3	46.8–57.0	17.0	49.8	5.0	3.9
Claw I heights							
External primary branch	9	6.8–12.2	26.2–30.8	10.0	28.5	1.7	1.7
External secondary branch	8	5.3–9.4	20.0–24.2	7.9	22.1	1.3	1.6
Internal primary branch	7	6.7–11.8	25.9–30.6	9.7	27.7	1.9	1.7
Internal secondary branch	7	4.8–9.3	20.3–22.2	7.5	21.2	1.6	0.7
Claw II heights							
External primary branch	7	7.1–12.9	27.3–33.5	10.6	30.6	2.0	2.2
External secondary branch	7	5.0–9.9	22.4–25.8	8.2	23.6	1.7	1.3
Internal primary branch	6	6.7–12.2	26.7–30.4	9.7	28.5	1.9	1.5
Internal secondary branch	6	4.9–9.7	21.5–24.0	7.7	22.3	1.7	0.9
Claw III heights							
External primary branch	6	7.0–13.4	26.4–33.7	10.4	29.7	2.1	2.7
External secondary branch	6	5.2–10.0	21.1–26.1	8.1	22.9	1.6	1.8
Internal primary branch	4	6.8–10.6	26.6–32.3	9.4	29.2	1.8	3.0
Internal secondary branch	4	4.9–8.4	20.6–24.9	7.2	22.3	1.6	1.9
Claw IV heights							
Anterior primary branch	6	7.9–14.5	32.7–37.9	12.0	35.8	2.3	2.3
Anterior secondary branch	6	5.6–9.9	22.3–26.2	8.3	24.8	1.6	1.4
Posterior primary branch	6	8.5–15.9	34.3–38.8	12.7	36.8	2.5	1.9
Posterior secondary branch	6	5.6–10.0	25.0–28.4	8.6	26.2	1.7	1.2

hemisphere, and 10 processes on the egg circumference.

Remarks. Twenty specimens and two eggs were found. The species was previously only reported from Belarus.

Genus *Xerobiotus* Bertolani & Biserov, 1996
(considered valid by Massa et al. 2021)

***Xerobiotus* sp.**
Figure 11

New records. MALTA – **Gozo island** • Ramla; 36°03′ 39″N, 014°17′04″E; 7 m alt.; 10.II.2015; D. Mifsud leg, sample 2, slides no. 5902–5904 (3 specimens).

Identification. The specimens were identified by morphological characters using the literature and by direct comparison with specimens of *X. pseudohufelandi*. The studied specimens show general correspondence with the morphological traits of the genus (claws of the *Xerobiotus* type, bucco-pharyngeal apparatus of the *Macrobiotus* type) according to Bertolani and Biserov (1996).

Remarks. Three specimens were found. The scarcity of the available material prevents a certain identification to species.

Discussion

In the absence of previous studies, all 11 species found in this work are newly recorded from the Maltese archipelago. It is certain that this number is strongly underestimated with respect to the number of species of limno-terrestrial tardigrades that may be present, especially in considering that only 12 or 13 sites were sampled, which are not fully representative of the variety of microhabitats present on the islands. However, we believe that some preliminary faunistic and biogeographic considerations are worthwhile.

In Sicily and small circum-Sicilian islands, 120 limno-terrestrial species are known (Arcidiacono 1962, 1964; Binda 1969, 1978; Binda and Pilato 1969a, 1969b, 1971, 1972, 1984, 1985, 1987; Binda et al. 1980; Pilato 1969, 1971a, 1971b, 1971c, 1973a, 1973b, 1973c, 1974, 2009; Pilato and Catanzaro 1988, 1989; Pilato et al. 1982, 1989, 2000, 2014, 2015, 2016; Lisi et al. 2014, 2016; Lisi 2015; Pilato and Lisi 2016; Pilato et al. 2017, 2019). As for North Africa, we refer to the African countries bordering the Mediterranean, in the region commonly called "Mediterranean Africa": Algeria, Tunisia, Morocco, Libya.

Figure 11. Habitus of *Xerobiotus* sp. (slide no. UNICT 5916). Scale bar: 50 µm.

and Egypt. Only 82 species are known from these countries (Guidetti et al. 2009; McInnes et al. 2017; Schill et al. 2007), of which 36 species reported from Algeria, three from Egypt, 22 from Libya, 29 from Tunisia, and 35 from Morocco. These numbers are very low considering the vastness of these countries and the variety of habitats they host.

The tardigrade fauna known to date of the Maltese archipelago is compared with those of North Africa and Sicily in Table 6. For the purposes of comparison, we consider the species with "cf." as attributable to the actual species. Thus, of the 11 species found in Malta, two species (18.2%; *Mesobiotus* cf. *diffusus*, *Ursulinius pappi*) are shared with Sicily and North Africa, three species (27.3%; *Ursulinius pappi, Mesobiotus* cf. *diffusus, Paramacrobiotus* cf. *sklodowskae*) are shared with North Africa, three species (27.3%; *Hypsibius ragonesei*, *Ursulinius pappi, Mesobiotus* cf. *diffusus*) are shared with Sicily, and three species (27.3%; *Pilatobius* sp. nov., *Macrobiotus* sp. nov. 1, *Macrobiotus* sp. nov. 2) are endemic to the Maltese archipelago. Three more species (27.3%; *Bryodelphax* sp. *weglarskae* group, *Doryphoribius* sp. *doryphorus* group and *Xerobiotus* sp.) require identification and cannot be included in this comparison. Although percentages have been provided, these are statistically unreliable evaluations of faunal comparisons, but only draw preliminary considerations awaiting confirmation. The Maltese archipelago seems to show equal faunal affinity with Sicily than North Africa; this could be explained by the key position of the Maltese archipelago in the Mediterranean between Sicily and North Africa reflecting in this intermediate faunal affinity. However, so far, only Sicily with its 120 species is relatively well-studied, while just 11 species are known from the Maltese archipelago, and only 82 species from the North African region, despite the vastness of the region and its rich variety of micro-environments. Therefore, given the incompleteness of studies in the Maltese archipelago and North Africa, reflecting in poor, statistically non-significant, data, it is possible that additional study may reveal a different scenario. For example, it could be expected a higher faunal affinity with Sicily due to the longer lasting geological connection with that archipelago during the Pliocene. This is supported by the fossil record of the metazoan faunas of both territories (Hunt and Schembri 1999), by the presence of numerous current Sicilian–Maltese endemics, and by the similarities of the modern faunas of the Maltese archipelago and Sicily. It is possible to consider a large part of the Maltese biota as a subset of the Sicilian biota (Schembri 1993).

The faunistic and biogeographic commonalities of the Maltese archipelago with Sicily and North Africa are not surprising, however, it is interesting that three species (those new to science) are currently exclusive to the Maltese archipelago. This suggests that this small archipelago has great potential for taxonomic and biogeographical study of limno-terrestrial tardigrades, and our results reported here clearly indicate that the study

Table 6. Correspondence between Maltese species and the tardigrade faunas of Sicily and North Africa. ? = species of unknown identity.

Species	Present in North Africa	Present in Sicily	Absent in Sicily and North Africa	New to science
Bryodelphax sp. *weglarskae* group	?	?	?	?
Hypsibius ragonesei	–	+	–	–
Pilatobius sp. nov.	–	–	+	+
Doryphoribius sp. *doryphorus* group	?	?	?	?
Ursulinius pappi	+	+	–	–
Macrobiotus sp. nov. 1	–	–	+	+
Macrobiotus sp. nov. 2	–	–	+	+
Mesobiotus cf. *diffusus*	+	+	–	–
Paramacrobiotus klymenki	–	–	+	–
Paramacrobiotus cf. *sklodowskae*	+	–	–	–
Xerobiotus sp.	?	?	?	?

of the tardigrade fauna of the Maltese archipelago is still incomplete. Soil, litter, and freshwater samples have not been collected, which would be further enrich our knowledge of Maltese tardigrades.

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Authors' Contributions

Conceptualization: OL, DC, DM. Data curation: OL, DC, DM. Funding acquisition: DM, OL. Investigation: OL, DC, DM. Methodology: OL, DC. Writing – original draft: OL, DC, DM. Writing – review and editing: OL, DC, DM.

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