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Some Tardigrades from Italy, with an Updated Checklist of Limno-terrestrial Species from the Country

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Abstract: In this study, 53 samples of mosses, lichens and leaf-litter collected in Italy were studied for Tardigrada. In total, 35 tardigrade taxa were identified. Three of them (*Macrobiotus sottilei*, *Milnesium dornensis* and *Ramazzottius subanomalus*) are new records for the Italian fauna. We also analysed recent reports on Italian limno-terrestrial tardigrades and prepared an updated checklist of tardigrades reported from Italy after 1995 and till the beginning of 2018.

Key words: *Macrobiotus sottilei*, *Milnesium dornensis*, new records, *Ramazzottius subanomalus*, Tardigrada, Italy

Introduction

The phylum Tardigrada currently consists of *c.* 1,300 taxa (Guidetti & Bertolani 2005, Degma & Guidetti 2007, Degma et al. 2009-2018) inhabiting terrestrial and aquatic (both freshwater and marine) environments throughout the world (Nelson et al. 2015). Italy is one of the territories where studies on tardigrades are the most advanced, which is visible from the long list of species reported from this country (see e.g. Binda et al. 1995 or Lisi et al. 2015 and literature cited therein). Nevertheless, our study shows that despite decades of sampling and description, new tardigrade species can be found in many Italian regions.

In the present paper, 53 samples collected in eight Italian provinces (Modena, Parma, Trento, Verbano-Cusio-Ossola, Verona, Olbia-Tempio, Nuoro and Oristano) were examined and 35 tardigrade taxa were found, including four new records for Italy. We also updated the list of Italian limno-terrestrial tardigrades using the recently published reports since 1995.

Materials and Methods

Sample processing

All samples were collected in northern Italy in June 2015 and in June – July 2016 by Milena Roszkowska, Łukasz Kaczmarek, Daria Grobys, Hanna Kmita and Wojciech Niedbała. Samples of mosses and lichens were collected from various types of substrata such as soil, trees, dead wood, concrete walls and rooftops. They were examined for terrestrial tardigrades using standard methods (Dastych 1980), with modifications described by Stec et al. (2015). Specimens for light microscopy were mounted on microscope slides in a small drop of Hoyer's medium, prepared according to Ramazzotti & Maucci (1983) and English translation by Beasley (1995). The slides were then placed in an incubator and dried for two days at 60°C. Dried slides were sealed with a transparent nail polish and examined under an Olympus BX41 phase contrast light microscope (PCM) with a ARTCAM–300Mi digital camera (Olympus Corporation, Shinjuku–ku, Japan).

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Comparative material

Tardigrade taxonomy followed Bertolani et al. (2014). The species were identified based on the keys in Ramazzotti & Maucci (1983), Claxton (1998), Fontoura & Pilato (2007), Michalczyk et al. (2012a, b), Morek et al. (2016), Kaczmarek & Michalczyk (2017) and Kaczmarek et al. (2011, 2017), as well as papers containing original descriptions and redescriptions, i.e. Schultze (1834), Doyère (1840), Ehrenberg (1853, 1959), Plate (1888), Richters (1900, 1902, 1903, 1904, 1911), Murray (1907, 1911), Thulin (1911, 1928), Marcus (1930, 1936), Biserov (1985), Bertolani & Rebecchi (1988, 1993), Bertolani et al. (1994, 2011), Kaczmarek & Michalczyk (2009), Pilato et al. (2012) and Ciobanu et al. (2015). Furthermore, paratypes of *Macrobiotus sottilei* Pilato, Kiosya, Lisi & Sabella, 2012, the holotype and paratypes of *Milnesium dornensis* Ciobanu, Roszkowska & Kaczmarek, 2015, and the neotype of *Milnesium tardigradum tardigradum* Doyère, 1840 were also examined and compared with specimens found during the present study.

List of samples

1. Verbano-Cusio-Ossola Province

45°55’51’’N, 08°26’20’’E, 200 m a.s.l.; next to the shopping centre in Gravellona, moss from concrete wall, 28.06.2015, coll. ŁK and MR, sample No 1.

45°57’36’’N, 07°55’02’’E, 2000 m a.s.l.; on the trail to mountain hut Zamboni Zappa, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 2.

45°57’36’’N, 07°55’02’’E, 2000 m a.s.l.; on the trail to mountain hut Zamboni Zappa, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 3.

45°57’36’’N, 07°55’02’’E, 2000 m a.s.l.; on the trail to mountain hut Zamboni Zappa, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 4.

45°57’36’’N, 07°55’02’’E, 2000 m a.s.l.; on the trail to mountain hut Zamboni Zappa, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 5.

45°57’36’’N, 07°55’02’’E, 2000 m a.s.l.; on the trail to mountain hut Zamboni Zappa, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 6.

45°57’36’’N, 07°55’02’’E, 2000 m a.s.l.; on the trail to mountain hut Zamboni Zappa, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 7.

45°58’15’’N, 07°56’51’’E, 1400 m a.s.l.; 0,5 km from the parking in Macugnaga, moss from concrete wall, 27.06.2015, coll. ŁK and MR, sample No 8.

45°57’42’’N, 07°55’04’’E, 1972 m a.s.l.; Belvedere Glacier, moss from rock debris, 27.06.2015, coll. ŁK and MR, sample No 9.

45°57’42’’N, 07°55’04’’E, 1972 m a.s.l.; Belvedere Glacier, moss from rock debris, 27.06.2015, coll. ŁK and MR, sample No 10.

45°57’31’’N, 07°55’14’’E, 2018 m a.s.l.; on the trail to mountain hut Zamboni Zappa, trail No. 2, moss from rock, 28.06.2015, coll. ŁK and MR, sample No 11.

45°57’31’’N, 07°55’14’’E, 2018 m a.s.l.; on the trail to mountain hut Zamboni Zappa, trail No. 2, lichen from rock, 28.06.2015, coll. ŁK and MR, sample No 12.

45°57’10’’N, 07°55’16’’E, 2100 m a.s.l.; next to the mountain hut Zamboni Zappa, next to the outlet of melting water, moss on path covered by snow, 28.06.2015, coll. ŁK and MR, sample No 13.

45°57’05’’N, 07°55’04’’E, 2080 m a.s.l.; behind the mountain hut Zamboni Zappa on the way to the lake Lago delle Locce, next to the river, lichens from rock, 28.06.2015, coll. ŁK and MR, sample No 14.

45°57’05’’N, 07°55’04’’E, 2080 m a.s.l.; behind the mountain hut Zamboni Zappa on the way to the lake Lago delle Locce, next to the river, moss from rock, 28.06.2015, coll. ŁK and MR, sample No 15.

45°57’05’’N, 07°55’04’’E, 2080 m a.s.l.; behind the mountain hut Zamboni Zappa on the way to the lake Lago delle Locce, next to the river, moss from rock, 28.06.2015, coll. ŁK and MR, sample No 16.

45°57’05’’N, 07°55’04’’E, 2080 m a.s.l.; behind the mountain hut Zamboni Zappa on the way to the lake Lago delle Locce, next to the river, moss from rock, 28.06.2015, coll. ŁK and MR, sample No 17.

45°56’36’’N, 07°54’48’’E, 2230 m a.s.l.; next to the lake Lago delle Locce, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 18.

45°57’05’’N, 07°55’04’’E, 2080 m a.s.l.; behind the mountain hut Zamboni Zappa on the way to the lake Lago delle Locce, next to the river, moss from rock, 28.06.2015, coll. ŁK and MR, sample No 19.

45°57’01’’N, 07°54’55’’E, 2130 m a.s.l.; lateral moraine of the Belvedere Glacier, trail to the lake Lago delle Locce, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 26.

45°57’01’’N, 07°54’55’’E, 2130 m a.s.l.; lateral moraine of the Belvedere Glacier, trail to the lake Lago delle Locce, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 27.

45°56’30’’N, 07°54’51’’E, 2235 m a.s.l.; next to the lake Lago delle Locce, between melted snow and ice, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 28.

45°56’30’’N, 07°54’51’’E, 2235 m a.s.l.; next to the lake Lago delle Locce, between melted snow and ice, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 29.

45°56’34’’N, 07°54’48’’E, 2220 m a.s.l.; next to the lake Lago delle Locce, moss from rock, 27.06.2015, coll. ŁK and MR., sample No 30.

45°58’13’’N, 07°57’07’’E, 1370 m. a.s.l.; lower chair-lift station of Macugnaga, moos from roof, 27.06.2015, coll. ŁK and MR, sample No 31.

45°56’51’’N, 07°54’53’’E, 2160 m a.s.l.; on the trail to the lake Lago delle Locce, moss from rock, 27.06.2015, coll. ŁK and MR, sample No 32.

45°58’08’’N, 08°27’17’’E, 304 m a.s.l.; Bracchio, next the Le Oche di Bracchio hotel, lichen from dead wood, 29.06.2015, coll. ŁK and MR, sample No 33.

45°58’08’’N, 08°27’17’’E, 304 m a.s.l.; Bracchio, next the Le Oche di Bracchio hotel, moss from concrete wall, 29.06.2015, coll. ŁK and MR, sample No 34.

45°58’08’’N, 08°27’17’’E, 304 m a.s.l.; Bracchio, next the Le Oche di Bracchio hotel, moss from tree, 29.06.2015, coll. ŁK and MR., sample No 35.

45°57’41’’N, 08°27’17’’E, 202 m a.s.l.; Mergozzo, Pallanza Street along the lake, moss from concrete wall, 29.06.2015, coll. ŁK and MR, sample No 36.

45°57’41’’N, 08°27’17’’E, 202 m a.s.l.; Mergozzo, Pallanza Street along the lake, moss from concrete wall, 29.06.2015, coll. ŁK and MR, sample No 37.

45°57’42’’N, 07°55’04’’E, 1972 m a.s.l.; Belvedere Glacier, moss from rock debris mixed with soil, 27.06.2015, coll. ŁK and MR, sample No 38.

2. Modena Province

44°38’37’’N, 10°55’13’’E, 30 m a.s.l.; Modena City, moss from concrete wall, 25.06.2015, coll. ŁK and MR, sample No 20.

44°31’19’’N, 10°50’49’’E, 175 m a.s.l.; Castle of Spezzano, moss from concrete wall, 25.06.2015, coll. ŁK and MR, sample No 21.

44°31’19’’N, 10°50’49’’E, 175 m a.s.l.; Castle of Spezzano, moss from concrete wall, 25.06.2015, coll. ŁK and MR, sample No 22.

44°31’19’’N, 10°50’49’’E, 175 m a.s.l.; Castle of Spezzano, moss from concrete wall, 25.06.2015, coll. ŁK and MR, sample No 23.

44°31’19’’N, 10°50’49’’E, 175 m a.s.l.; Castle of Spezzano, moss from concrete wall, 25.06.2015, coll. ŁK and MR, sample No 24.

3. Parma Province

44°48’15’’N, 10°19’41’’E, 55 m a.s.l.; Parma city, Piazzale della Pace, moss from tree, 26.06.2015, coll. ŁK and MR, sample No 25.

4. Trento Province

45°53’10’’N, 10°50’11’’E, 140 m a.s.l.; Riva del Garda, on the way to the Bastion, moss from rocky wall, 07.07.2016, coll. DG and HK, sample No 39.

45°53’10’’N, 10°50’11’’E, 140 m a.s.l.; Riva del Garda, on the way to the Bastion, moss from dead wood, 07.07.2016, coll. DG and HK, sample No 40.

45°52’55’’N, 10°50’48’’E, 68 m a.s.l.; Riva del Garda, lawn next to the promenade, under *Quercus* sp., moss from soil, 07.07.2016, coll. DG and HK, sample No 41.

45°53’01’’N, 10°50’44’’E, 69 m a.s.l.; Riva del Garda, lawn next to the conference centre, under the palm trees, moss from soil, 07.07.2016, coll. DG and HK, sample No 42.

45°52’54’’N, 10°50’51’’E, 70 m a.s.l.; Riva del Garda, promenade next to the lake, moss from the monument, 07.07.2016, coll. DG and HK, sample No 43.

45°53’10’’N, 10°50’11’’E, 100 m a.s.l.; Riva del Garda, on the way to the Bastion, moss from concrete wall, 07.07.2016, coll. DG and HK, sample No 44.

45°53’10’’N, 10°50’11’’E, 100 m a.s.l.; Riva del Garda, on the way to the Bastion, moss from concrete wall, 07.07.2016, coll. DG and HK, sample No 45.

45°53’10’’N, 10°50’44’’E, 70 m a.s.l.; Riva del Garda, next to the road to Verona, roundabout, viale Giosuè Carducci, moss from concrete wall, 05.07.2016, coll. DG and HK, sample No 47.

45°53’08’’N, 10°50’37’’E, 69 m a.s.l.; Riva del Garda, Viale S. Francesco, 100 m from the lake, olive tree, moss from tree, 05.07.2016, coll. DG and HK, sample No 48.

45°53’06’’N, 10°50’36’’E, 74 m a.s.l.; Riva del Garda, Viale Liberazione, 100 m from the lake and from the conference centre, cypress, moss from tree, 05.07.2016, coll. DG and HK, sample No 49.

45°52’51’’N, 10°50’59’’E, 75m a.s.l.; Riva del Garda, 30 m from the lake, Via Filzi and Via Rainer Rilke junction, moss from concrete chest, 04.07.2016, coll. DG and HK, sample No 50.

5. Verona Province

45°45’59’’N, 10°48’54’’E, 130 m a.s.l.; Malcesine City, Via Novene Vecia, close to the street leading to the chair-lift to Monte Baldo, moss from concrete wall, 05.07.2016, coll. DG, sample No 46.

6. Olbia-Tempio Province

40°54’42’’N, 09°05’45’’E, 482 m a.s.l.; Sardinia, near Tempio Pausania, Pausania Inn. Hotel, leaf-litter from *Quercus sobur* forest, 19.06.2016, coll. WN, sample No 51.

7. Nuoro Province

40°12’18’’N, 09°20’54’’E, 625 m a.s.l.; Sardinia. Gennargentu Mtns., Orgosolo, leaf-litter from *Quercus ilex* forest, 17.06.2016, coll. WN, sample No 52.

8. Oristano Province

40°05’20’’N, 08°52’04’’E, 247 m a.s.l.; Sardinia, 30 km NE from Oristano, well of St. Catherine, leaf-litter from olive grow, 17.06.2016, coll. WN, sample No 53.

Results

In total, 1308 specimens (including 127 simplex/exuviae) and 178 eggs of 35 tardigrade taxa were found in 37 positive samples (Table 1). Five species were found in Modena Province, 10 in Trento, 26 in Verbano-Cusio-Ossola Province, two in Verona Province, three in Olbia-Tempio Province and one in Oristano Province. Tardigrades did not occur in the samples from two provinces (Parma and Nuoro). Three species, *Macrobiotus sottilei, Milnesium dornensis* and *Ramazzottius subanomalus* (Biserov, 1985), are new records for Italy (see Table 1). We also found

Table 1. Alphabetical list of species found in the present study (provinces: 1 – Verbano-Cusio-Ossola; 2 – Modena; 4 – Trento; 5 – Verona; 6 – Olbia-Tempio; 8 – Oristano). Parma and Nuoro provinces were not included because no tardigrades were found in the samples from these provinces. *Species new for the Italian fauna.

Species	Samples No	1	2	4	5	6	8
1. *Bryodelphax parvulus* Thulin, 1928	45			X			
2. *Calohypsibius ornatus* (Richters, 1900)	12, 15	X					
3. *Dianea sattleri* (Richters, 1902)	36	X					
4. *Diphascon p. pingue* (Marcus, 1936)	17, 51	X				X	
5. *Echiniscus b. blumi* Richters, 1903	5, 35	X					
6. *E. mediantus* Marcus, 1930	31	X					
7. *E. m. merokensis* Richters, 1904	3	X					
8. *E. testudo* (Doyère, 1840)	24, 37, 41, 47, 49	X	X	X			
9. *Hypsibius dujardini* (Doyère, 1840)	37, 51	X				X	
10. *H. microps* Thulin, 1928	9	X					
11. *H. pallidus* Thulin, 1911	31	X					
12. *Isohypsibius p. prosostomus* Thulin, 1928	21, 22		X				
13. *Macrobiotus crenulatus* Richters, 1904	19	X					
14. *M. furcatus* Ehrenberg, 1859	12	X					
15. *M. hufelandi* C. A. S. Schultze, 1834	3, 14, 15, 16, 20, 21, 31, 48, 49	X	X	X			
16. *M. hufelandi* group	5, 6, 8, 9, 11, 12, 21, 22, 24, 30, 32, 33, 50	X	X	X			
17. *M. sandrae* Bertolani & Rebecchi, 1993	40			X			
18. *M. sottilei* Pilato, Kiosya, Lisi & Sabella, 2012*	46, 47, 51			X	X	X	
19. *M. vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011	36, 37	X					
20. *Mesobiotus* sp.	3	X					
21. *Milnesium alpigenum* Ehrenberg, 1853	5, 7, 8, 14, 16, 31	X					
22. *M. dornensis* Ciobanu, Roszkowska & Kaczmarek, 2015*	47, 49			X			
23. *M. t. tardigradum* Doyère, 1840	31	X					
24. *Minibiotus intermedius* (Plate, 1888)	31	X					
25. *Murrayon hibernicus* (Murray, 1911)	11, 12	X					
26. *Paramacrobiotus areolatus* (Murray, 1907)	35, 36	X					
27. *P. richtersi* (Murray, 1911)	20, 23		X				
28. *P. richtersi* group	43, 53			X			X
29. *Pilatobius recamieri* (Richters, 1911)	9, 12, 29	X					
30. *Pseudechiniscus suillus* (Ehrenberg, 1853)	40			X			
31. *Ramazzottius affinis* Bertolani, Guidetti & Rebecchi, 1994	46				X		
32. *R. oberhaeuseri* (Doyère, 1840)	14	X					
33. *R. subanomalus* (Biserov, 1985)*	31	X					
34. *R. tribulosus* Bertolani & Rebecchi, 1988	31	X					
35. *Ramazzottius* sp.	5, 45	X		X			

few specimens, which we were unable to identify at the species level due to the lack of eggs. These were identified as: *Macrobiotus hufelandi* group, *Mesobiotus* sp., *Paramacrobiotus richtersi* group and *Ramazzottius* sp. These specimens may belong to the species reported in this paper or to other taxa.

We also examined known data on terrestrial and freshwater tardigrades published in recent years (1995 to 2018), i.e. after the last Italian tardigrade checklist (Binda et al. 1995), and prepared the updated list of Italian limno-terrestrial tardigrades (Table 2).

Table 2. Alphabetical list of new records of terrestrial and freshwater tardigrade species reported from Italy after the publication of Binda et al. (1995)

Species	Source
1. *Adropion marcusi* (Rudescu, 1964)*	Guidetti et al. (1999)
2. *Adropion vexatum* Pilato, Sabella, D'Urso & Lisi, 2017	Pilato et al. (2017)
3. *Bindius triquetrus* Pilato, 2009	Pilato (2009)
4. *Borealibius zetlandicus* (Murray, 1907)	Pilato et al. (2006)
5. *Bryodelphax iohannis* Bertolani, Guidi & Rebecchi, 1996	Bertolani et al. (1996)
6. *Claxtonia pardalis* (Degma & Schill, 2015)	Degma & Schill (2015)
7. *Diphascon dolomiticum* Pilato & Bertolani, 2005	Pilato & Bertolani (2005)
8. *Diphascon humicus* Bertolani, Guidetti & Rebecchi, 1994	Bertolani et al. (1994)
9. *Diphascon hydrophilum* Pilato, Binda, Bertolani & Lisi, 2005	Pilato et al. (2005)
10. *Diphascon nelsonae* Pilato, Binda, Bertolani & Lisi, 2005	Pilato et al. (2005)
11. *Diphascon platyungue* Pilato, Binda, Bertolani & Lisi, 2005	Pilato et al. (2005)
12. *Diphascon procerum* Pilato, Sabella & Lisi, 2014	Pilato et al. (2014)
13. *Diphascon serratum* Pilato, Binda, Bertolani & Lisi, 2005	Pilato et al. (2005)
14. *Diphascon ziliense* Lisi, Sabella & Pilato, 2014	Lisi et al. (2014)
15. *Eohypsibius nadjae* Kristensen, 1982	Bertolani & Rebecchi (1996)
16. *Eremobiotus ginevrae* Lisi, Binda & Pilato, 2016	Lisi et al. (2016)
17. *Grevenius rusticus* (Pilato, Sabella & Lisi, 2015)	Pilato et al. (2015)
18. *Grevenius zappalai* (Pilato, Sabella & Lisi, 2015)	Pilato et al. (2015)
19. *Hypsibius pallidoides* Pilato, Kiosya, Lisi, Inshina & Biserov, 2011	Lisi (2015)
20. *Hypsibius pradellii* Bertolani & Rebecchi, 1996	Bertolani & Rebecchi (1996)
21. *Macrobiotus biserovi* Bertolani, Guidi & Rebecchi, 1996	Bertolani et al. (1996)
22. *Macrobiotus insuetus* Pilato, Sabella & Lisi, 2014	Pilato et al. (2014)
23. *Macrobiotus joannae* Pilato & Binda, 1983	Rebecchi et al. (2000)
24. *Macrobiotus nebrodensis* Pilato, Sabella, D'Urso & Lisi, 2017	Pilato et al. (2017)
25. *Macrobiotus polonicus* Pilato, Kaczmarek, Michalczyk & Lisi, 2003	Lisi (2015)
26. *Macrobiotus serratus* Bertolani, Guidi & Rebecchi, 1996	Bertolani et al. (1996)
27. *Macrobiotus vladimiri* Bertolani, Biserov, Rebecchi & Cesari, 2011	Bertolani et al. (2011)
28. *Mesobiotus patiens* (Pilato, Binda, Napolitano & Moncada, 2000)	Pilato et al. (2000)
29. *Mesobiotus simulans* (Pilato, Binda, Napolitano & Moncada, 2000)	Pilato et al. (2000)
30. *Microhypsibius bertolanii* Kristensen, 1982	Guidetti & Bertolani (2001)
31. *Milnesium almatyense* Tumanov, 2006	Lisi (2015)
32. *Milnesium minutum* Pilato & Lisi, 2016	Pilato & Lisi (2016)
33. *Milnesium vorax* Pilato, Sabella & Lisi, 2016	Pilato et al. (2016)
34. *Murrayon dianeae* (Kristensen, 1982)	Bertolani & Rebecchi (1996)
35. *Mixibius parvus* Lisi, Sabella & Pilato, 2014	Lisi et al. (2014)
36. *Paramacrobiotus pius* Lisi, Binda & Pilato, 2016	Lisi et al. (2016)
37. *Pilatobius secchii* (Bertolani & Rebecchi, 1996)	Bertolani & Rebecchi (1996)
38. *Pseudobiotus kathmanae* Nelson, Marley & Bertolani, 1999	Nelson et al. (1999)
39. *Ramazzottius affinis* Bertolani, Guidetti & Rebecchi, 1993	Bertolani et al. (1993)

* The species has been reported by Guidetti at al. (1999) as *Diphascon* cf. *marcusi*. According to Gąsiorek et al. (2016), this is a taxon with unclear taxonomic position, which needs a redescription.

Discussion

Studies on limno-terrestrial tardigrades in Italy have a long history. In the latest published checklist, Binda et al. (1995) reported 192 taxa from this country but few of them were suggested as doubtful and in fact can represent different taxa. For example, *Echiniscus ramazzottii* Binda & Pilato, 1969 was presented by Binda et al. (1995) as possible synonym of *Echiniscus blumi blumi* Richters, 1903. Moreover, Binda et al. (1995) suggested that also *Echiniscus bisetosus* Heinis, 1908, *Echiniscus mediantus* Marcus, 1930 and *Echiniscus trisetosus* Cuénot, 1932 could be possible synonyms of *Echiniscus blumi blumi*, although the existence of all these species was partially justified and they were characterised as separate morphospecies by Guil (2008). On the other hand, the same author pointed out that these taxa can be only a variance of the nominotypical subspecies *Echiniscus blumi blumi* and molecular analyses are needed before final conclusion about their validity. Specimens of another species reported from Italy, *Mesobiotus furciger* (Murray, 1907), were probably mistakenly identified (due to old-fashioned taxonomy) and should be now considered *Mesobiotus pilatoi* (Binda & Rebecchi, 1992) (Binda & Rebecchi 1992). Furthermore, Binda et al. (1995) considered also that *Ramazzottius thulini* Pilato, 1971 could be a possible synonym of *Ramazzottius oberhaeuseri* (Doyère, 1840); however, these have recently been confirmed as distinct valid species (Pilato et al. 2013). Additionally, *Diphascon humicus* Bertolani, Guidetti & Rebecchi, 1994 and *Ramazzottius affinis* Bertolani, Guidetti & Rebecchi, 1993 were omitted by Binda et al. (1995) due to delays in print.

In the present study, we analysed all data concerning Italian limno-terrestrial tardigrades, published after 1995 and counted 39 species (32 of them described as new to science; Table 2). It should be pointed out that most of new species were described from a relatively small area, i.e. Sicily. The next three new records are presented in this paper (Table 1). Taking into consideration all species reported by Binda et al. (1995) (excluding *Echiniscus ramazzottii* and *Mesobiotus furciger*), later data and new records represented in this article, the total number of limno-terrestrial tardigrades in Italy is increased to 233. This is a very high number, especially in comparison to other much larger areas, like the entire continents of Africa with only 169 known species, Central America with 100 species, South America with 237 species, or North America with 274 species (Kaczmarek et al. 2014, 2015, 2016, McInnes et al. 2017, Roszkowska et al. 2018). As it is noted in Tables 1 and 2, new species and new records are still reported from Italy quite frequently.

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