

## Article

# Terrestrial Tardigrada (Water Bears) of the Słowiński National Park (Northern Poland)

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**Abstract:** In this paper, samples of mosses, lichens and cryptogams (mosses mixed with lichens) collected from Słowiński National Park (northern Poland) were studied for water bears (Tardigrada). In total, 27 tardigrade taxa were identified: 21 to the species level, one identified as „cf.” and three to the genus level, with six species (*Eremobiotus ginevrae*, *Hypsibius dujardini*, *Hypsibius scabropygus*, *Milnesium beasleyi*, *Minibiotus intermedius* and *Notahypsibius pallidooides*) being new records for Poland. Two possibly new for science species were also found, belonging to genera *Diphascion* and *Mesobiotus*. Additionally, a very rare eutardigrade *Pseudohexapodibius degenerans* has been found in the samples analyzed in the present study for the first time outside of the type locality. The effects of habitat and substrate on species richness were also investigated and showed no significant differences between mosses and lichens, as well as all substrates except for concrete walls.

**Keywords:** Europe; sand dunes; tardigrades; new records; xerophilous species; biodiversity



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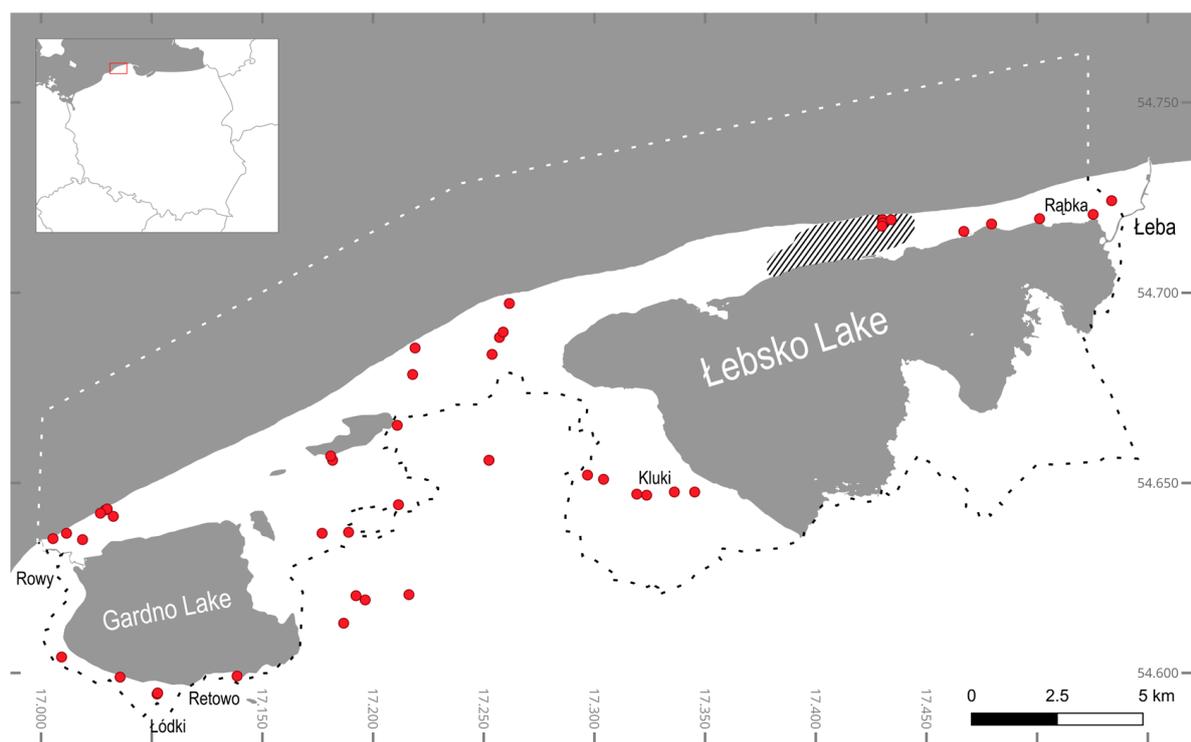
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## 1. Introduction

The Phylum Tardigrada, commonly known as water bears, is made up of small invertebrates inhabiting terrestrial, freshwater and marine environments around the world [1]. Up to now, more than 1400 species of tardigrades were described, with more being described each year [2].

Studies of tardigrades in Poland have been ongoing ever since the early XXth century [3] and to date, in total, 115 tardigrade species were reported [4–6]. However, most of the studies were realized before the advent of modern tardigrade taxonomy and can now be considered outdated. As a result, many previously reported species need to be confirmed, as they belong to species groups e.g., *Macrobiotus hufelandi* group [7], *Diphascion pingue* group [8] or to the genera where many new species were described in recent years, e.g., *Milnesium* Doyère, 1840 or *Mesobiotus* Vecchi, Cesari, Bertolani, Jönsson, Rebecchi and Guidetti, 2016 [9–12].

Słowiński National Park (SPN) is located in northern Poland at the Baltic Sea coast. Originally founded in 1967, it covers an area of 32,744.03 ha, of which 21,572.89 ha is land area, situated between the towns of Łeba and Rowy (Figure 1). Its most famous feature is its moving dunes, reaching up to 50 m in height, making them the highest such structures in Europe. It encompasses a wide array of habitats, including seaside lakes, bogs, peatbogs, meadows, seaside woods and pine forests [13].



**Figure 1.** Map of sampling sites. Dashed line indicates area of the SPN. Striped field indicates area covered by sand dunes. Red dots indicate sampling sites.

To date, only two species were reported from the area of SPN, i.e., *Hypsibius pallidus* Thulin, 1911 [14] reported from the vicinity of Gardno Lake [15] and *Mesocrista revelata* Gašiorek, Stec, Morek, Zawierucha, Kaczmarek, Lachowska-Cierlik and Michalczyk, 2016 reported from old military training ground [16].

In the present study, 107 samples were collected from the entire area of the park and examined for tardigrades, all of which were then identified based on modern tardigrade taxonomy.

## 2. Materials and Methods

### 2.1. Sampling and Sample Processing

One hundred and seven moss, lichen and mixed (cryptogams) samples were collected between 27th and 30th October 2017 in SPN (Figure 1), from various types of surfaces, including tree trunks, concrete walls, dead wood and ground surface (Table 1). The collected samples were then packed in paper envelopes and dried at room temperature (ca. 18–21 °C). Sample processing was completed at the Faculty of Biology, Adam Mickiewicz University in Poznań, Poland. The tardigrades and their eggs were extracted, following the protocol of Stec et al. [17].

**Table 1.** Samples and localities containing tardigrades and/or their eggs.

Sample	Location	Habitat	Substrate	Description
1	54.629167, 17.103056	lichen	tree	Outskirts of Łódki village, willow tree
2A	54.633611, 17.086389	moss	tree	Between Retowo and Rowy villages, near bridge, birch tree
2B	54.633611, 17.086389	moss	ground surface	Between Retowo and Rowy villages, near bridge
3	54.690556, 17.182222	moss	concrete wall	Forest path to Dołgie Duże lake
4	54.691667, 17.181389	moss	tree	Forest path to Dołgie Duże lake, beech tree
23	54.647778, 17.187222	lichen	tree	Road between Smółdzin and Gardna Wielka

Table 1. Cont.

Sample	Location	Habitat	Substrate	Description
24A	54.678889, 17.211944	moss	ground surface	Pine forest
25A	54.655278, 17.216667	moss	tree	Road along Łupawa river, near graveyard
25B	54.655278, 17.216667	moss	ground surface	Road along Łupawa river, near graveyard
25C	54.655278, 17.216667	moss	concrete wall	Road along Łupawa river, near graveyard
25D	54.655278, 17.216667	moss	tree	Road along Łupawa river, near graveyard, pine tree
26	54.671667, 17.189444	moss	tree	Mixed forest, oak tree
27A	54.629444, 17.103333	moss	tree	Outskirts of Łódki village, willow tree
28B	54.655000, 17.192778	moss	tree	Pine forest
29B	54.653889, 17.196944	moss	ground surface	Pine forest
29C	54.653889, 17.196944	moss	ground surface	Pine forest
30	54.638889, 17.060000	moss	concrete wall	Water canal lock
33B	54.633889, 17.139167	lichen	tree	Camping field near Retowo village, near lake
34	54.677500, 17.079722	lichen	dead wood	White dune, near beach
35	54.677778, 17.080556	moss	ground surface	White dune, near beach
37	54.671389, 17.177500	moss	ground surface	Spruce forest
39	54.676667, 17.077500	lichen	ground surface	White dune, near beach
41B	54.670000, 17.056111	lichen	tree	Rowy, white dune, birch tree
42B	54.671389, 17.062222	lichen	tree	White dune, aspen tree
42C	54.671389, 17.062222	moss	ground surface	White dune, near beach
43	54.669722, 17.069444	cryptogams	ground surface	Gray dune
44A	54.675833, 17.083333	moss	tree	Oak forest, birch tree
45	54.682222, 17.345556	moss	tree	Kluki, path to watchtower, oak tree in a meadow
50A	54.722778, 17.257500	cryptogams	ground surface	Czołpińska dune
51A	54.713056, 17.218333	moss	concrete wall	Old military proving ground
51B	54.713056, 17.218333	moss	concrete wall	Old military proving ground
52A	54.718333, 17.254167	moss	tree	Pine forest, pine tree
53A	54.720000, 17.219444	lichen	tree	Pine branches
53B	54.720000, 17.219444	lichen	dead wood	Dead pine
53C	54.720000, 17.219444	cryptogams	tree	Pine tree
54B	54.724167, 17.259167	lichen	ground surface	Czołpińska dune
55B	54.681389, 17.323889	lichen	concrete wall	Kluki, 18th century graveyard, gravestone
55C	54.681389, 17.323889	moss	concrete wall	Kluki, 18th century graveyard, gravestone
57C	54.731667, 17.261944	moss	ground surface	Seaside path near dunes, mountain pine scrubs
59	54.699722, 17.211389	moss	tree	Near Dołgie Duże lake, oak tree
61A	54.685556, 17.304444	moss	dead wood	Outskirts of Kluki village, submerged dead birch
61B	54.685556, 17.304444	lichen	dead wood	Outskirts of Kluki village, dead birch
62A	54.690556, 17.252778	moss	tree	Smóldziński forest, oak tree
64A	54.690556, 17.252778	moss	tree	Czołpińska dune
66B	54.686667, 17.297222	moss	tree	Kluki, oak-beech forest, beech tree
67A	54.682222, 17.336389	moss	dead wood	Kluki, museum building roof
72A	54.681667, 17.319444	moss	dead wood	Outskirts of Kluki village, oak forest
72B	54.681667, 17.319444	moss	tree	Outskirts of Kluki village, birch tree
73	54.755000, 17.525278	moss	concrete wall	Outskirts of Rąbka village, shrine
76A	54.753611, 17.430278	lichen	ground surface	Łącka dune, beach
77	54.752778, 17.430278	moss	ground surface	Łącka dune, between two pine trees, dune crevice, sea facing
78	54.752500, 17.479444	moss	tree	Alder carr, alder tree
79	54.753889, 17.501111	moss	tree	Alder carr, alder tree
85	54.751944, 17.430000	lichen	tree	Łącka dune, pine forest, dune crevice, sea facing
86	54.758611, 17.533611	moss	concrete wall	Outskirts of Rąbka village, curb
87	54.750556, 17.466944	moss	dead wood	Path to Łącka dune, missile launcher site, wooden roof
88	54.753611, 17.434167	moss	ground surface	Mountain pine scrub

## 2.2. Species Identification

Species were identified using keys, original descriptions and redescriptions of specific taxa [10,12,16,18–26]. Tardigrade taxonomy follows Bertolani et al. [27]. The abbreviations of genus names follow Perry et al. [28,29]

### 2.3. Microscopy and Imaging

In order to facilitate identification, the collected specimens were mounted on microscope slides in Hoyer's medium and secured with a coverslip. They were examined using Olympus BX41 phase contrast light microscope (PCM), equipped with an Olympus SC50 digital camera (Olympus Corporation, Shinjuku-ku, Japan) and Olympus BX53 differential interference contrast (DIC) microscope (Olympus Corporation, Shinjuku-ku, Japan).

A map was generated using QGIS 3.28.0. All figures were assembled using Inkscape 1.0.1.

### 2.4. Statistical Analysis

Two approaches, sample-size- and coverage-based, were used to generate incidence-based rarefaction and extrapolation (R/E) curves in order to assess if the tardigrade species from the various habitats and substrates were well represented and could be compared. These techniques provide a consistent sampling strategy that may be used to compare species richness across various assemblages in an equitable and insightful manner [30]. The sample-size R/E method analyzes diversity estimates in relation to sample size, whereas the coverage-based R/E curve examines the species richness of a set of groups using samples that are equally complete (equal coverage) [31]. The iNext online software (<https://chao.shinyapps.io/iNEXTOnline/> accessed on 12 March 2024) was used to create the curves and the sample coverage [32]. Its default settings were used to extrapolate the rarefaction curves which is to double each reference sample size with 100 bootstrapping replicate runs, which were performed to estimate 95% confidence intervals [33]. Furthermore, Venn diagrams were created using package VennDiagram in R (version 4.3.1).

## 3. Results

Over the course of the study, 107 samples were examined and 57 were found to be positive (53%: Table 1), containing in total 785 tardigrades (all of them belonging to Eutardigrada) and 43 eggs (Table 2). Specimens were attributed to 27 taxa, including six new for Polish fauna and two most likely new for science. In total, 21 were identified to the species level, one identified as "cf." and another three only to the genus level (see Table 2 for more details). The possibly new for science species (marked in Table 2 in bold) belong to genera *Diphascoen* Plate, 1888 [34] and *Mesobiotus*. A very rare eutardigrade *Pseudohexapodibius degenerans* (Biserov, 1990) [35], which was analyzed during the present study, was first recorded in SPN samples by Vincenzi et al. [36] (see Discussion below for more details).

Out of the habitats used in the study, moss exhibited the highest species richness as well as species diversity, while cryptogams (mixture of mosses and lichens) showed the least amount of both (Figure 2A–C). The diversity at all habitats under investigation appeared to be poor to well-represented, according to the estimated sample coverage, which had a rarefaction sample coverage percentage above 60% and extrapolation sample coverage percentage of 76% for cryptogams, rarefaction sample coverage percentage above 80% and extrapolation sample coverage percentage of 90% for lichen, and rarefaction sample coverage percentage above 85% and extrapolation sample coverage percentage of 96% for moss (Supplementary Materials S1, Figure 2B,C). In Figure 2A, the plot suggests potential sampling bias due to the different slopes and heights of curves at equivalent sample sizes. Mosses appear to have higher diversity or have been sampled more thoroughly, given the steeper curve and higher extrapolated diversity. However, in Figure 2B, the cryptogams and lichen are quite close together, suggesting similar sampling completeness despite the potentially lower total sampling effort for cryptogams. If the cryptogams curve were to level off quicker than the others, it could indicate sampling bias, but it does not seem to be the case here. Furthermore, in Figure 2C, the similar trajectories between cryptogams and lichens of up to about 75% coverage suggest comparable diversity between these two groups, while mosses may have higher true diversity or are oversampled.

**Table 2.** Tardigrade taxa recorded in present study, placed in alphabetical order. Species new for Poland marked as underlined. Species new to science marked in bold.

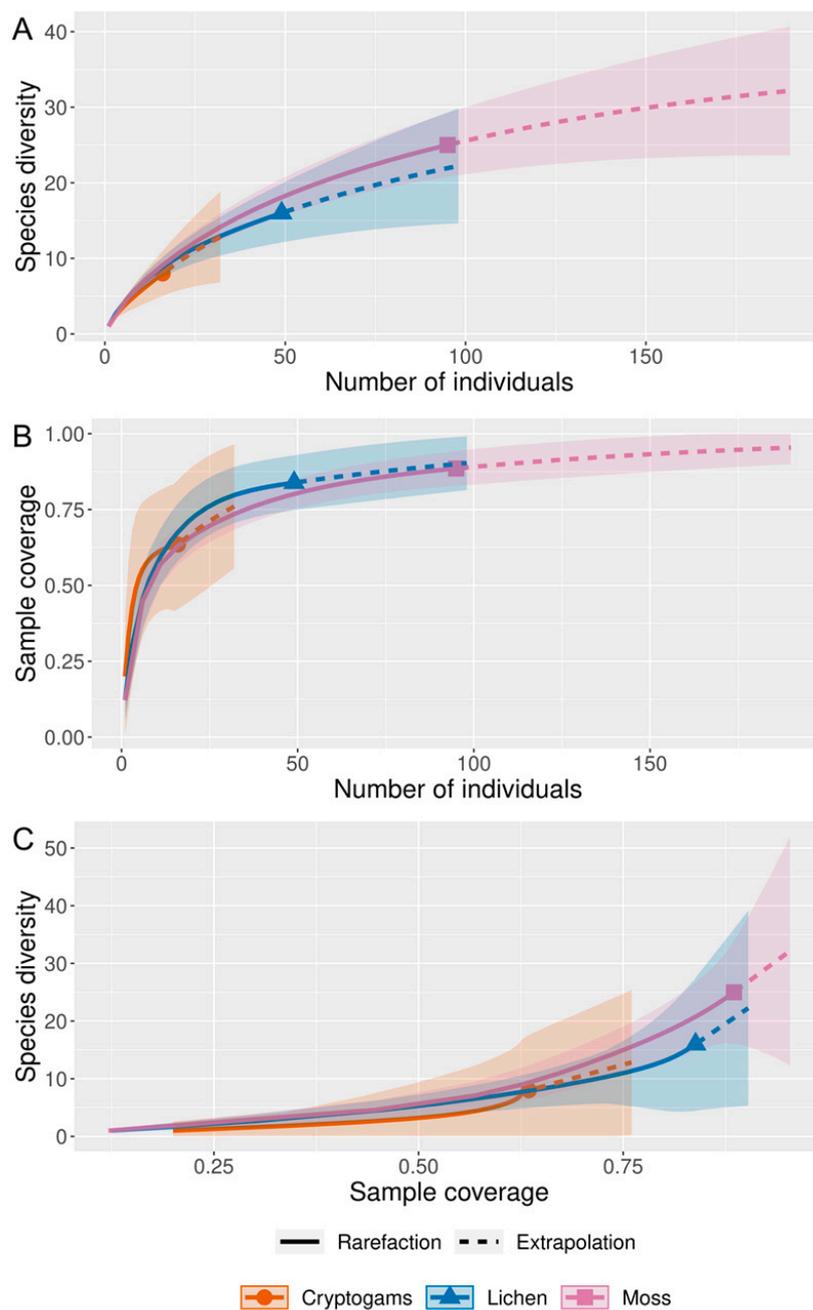
Taxon	Sample (Number of Specimens + Number of Eggs)	Remarks
<i>Adropion belgicae</i> (Richters, 1911) [37]	53C (1)	Holarctic species [38–40]. In Poland known from southern regions, mainly from Tatra Mountains [15].
<i>Adropion scoticum scoticum</i> (Murray, 1905) [41]	25B (5); 53C (1); 57C (1)	Probably a cosmopolitan complex of very similar species [38–40,42–44]. Very common in Poland with wide distribution, especially in coniferous forests [15].
<i>Dianeia sattleri</i> (Richters, 1902) [45]	39 (1)	Probably a cosmopolitan complex of very similar species [38–40,42–44]. Widely distributed in Poland [15].
<i>Diphascon pingue pingue</i> (Marcus, 1936) [46]	27A (1); 78 (5)	The <i>pingue</i> group is a complex of extremely similar species [18]. Nominal <i>Diphascon pingue</i> is probably restricted to the Holarctic [47,48]. Common in Poland. Strongly connected with forest habitats [15].
<b><u>Diphascon sp.</u></b>	39 (1)	Probably a new species from the <i>Diphascon nobilei</i> group [49]. For more details, see Discussion.
<i>Eremobiotus ginevrae</i> Lisi, Binda and Pilato, 2016 [23]	35 (4)	Species new to Poland. Up to now reported only from type locality in Italy [23].
<i>Guidettion prorsirostre</i> (Thulin, 1928) [50]	25B (1)	Species with Holarctic distribution with only few localities in other geographic regions, suggesting a species complex [38–40,43,44]. In Poland, common and widely distributed [15].
<u><i>Hypsibius dujardini</i></u> (Doyère, 1840) [7]	25D (1); 53A (9)	<i>Hypsibius dujardini</i> is a species complex [22] with a global distribution [38–40,42–44]. Widely reported in Poland [15]. However, the distribution of nominal species of the complex, redescribed in 2018, is at present unknown and up to now the only confirmed locality is Paris, France [19]. Taking this into account, this report should be considered as new for Poland.
<i>Hypsibius convergens</i> (Urbanowicz, 1925) [51]	26 (1)	<i>Hypsibius convergens</i> is a species complex [22] with a global distribution [38–40,42–44] and nominal species-type locality in Lithuania. The nominal species still needs a modern redescription. This is a species complex. <i>Hypsibius pallidus</i> is a common European species, but it has also been reported from non-European localities [38–40,42,43]. Very common in Poland and widely distributed [15].
<i>Hypsibius pallidus</i>	24A (2); 29C (1)	Species with wide Holarctic distribution [38–40]. The original description is uncertain and the species needs a modern redescription [53]. Species new for the Polish fauna.
<u><i>Hypsibius scabropygus</i></u> Cuénot, 1929 [52]	2B (1); 61B (1)	
<i>Macrobotus cf. hufelandi</i>	1 (1); 2A (3); 2B (12); 3 (1); 23 (17); 25C (3); 25D (1); 29B (6); 29C (1); 30 (2); 37 (2); 33B (9); 43 (4); 50A (2); 52A (2); 53A (6); 53B (3); 53C (16); 55C (10); 57C (5); 66B (18); 67A (75); 72A (1); 72B (1); 73 (8); 77 (19); 79 (10); 86 (66); 87 (30)	Accurate identification of these specimens was impossible due to lack of eggs.
<i>Macrobotus hufelandi</i> C.A.S. Schultze, 1834 [54]	4 (5+1); 25B (75+1); 51A (3+1); 59 (32+1); 62A (3+1)	This species belongs to a cosmopolitan and species-rich complex [10]. In the past, <i>Mac. hufelandi sensu stricto</i> was reported almost everywhere [39], but its range is probably much more restricted. However, at present, its true distribution is unknown. In Poland, it was reported from many localities [15], but most of the reports are old and it is obvious that some of them belong to other members of the <i>hufelandi</i> group.
<i>Macrobotus vladimiri</i> Bertolani, Biserov, Rebecchi and Cesari, 2011 [55]	55B (0+1)	To date, species known only from Italy (type locality) and Poland [4,5,55].

Table 2. Cont.

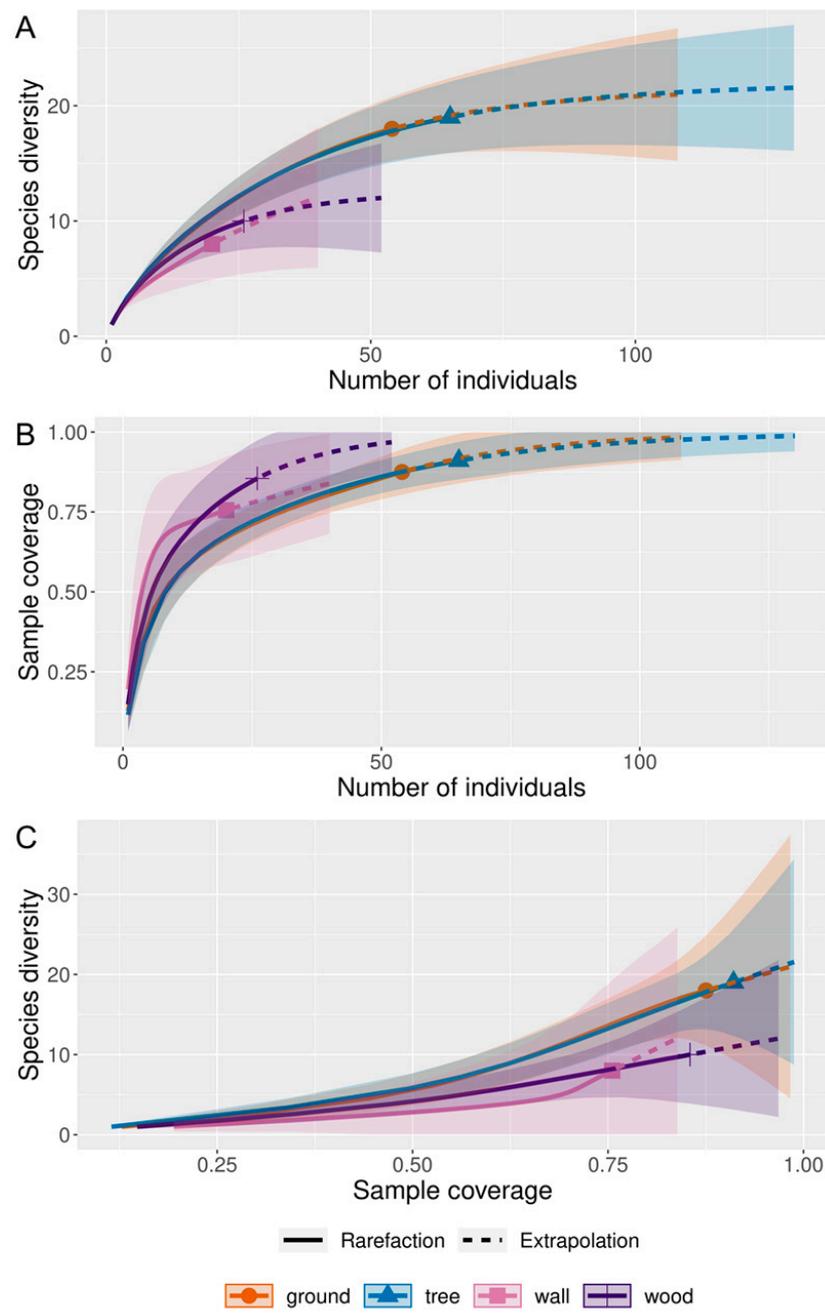
Taxon	Sample (Number of Specimens + Number of Eggs)	Remarks
<i>Mesobiotus</i> sp.	24A (1); 25A (1); 27A (1); 29C (1); 59 (2)	Accurate identification of these specimens was impossible due to lack of eggs.
<u><i>Mesobiotus</i> sp.</u>	61A (11+1)	Probably a new species, but more material is necessary for a formal description of it. See Discussion for more details.
<i>Mesocrista revelata</i>	1 (1); 37 (4); 25B (4); 53B (1); 53C (10); 72A (3)	It is known from few localities in Poland. However, it is highly probable that most if not all reports of <i>Mec. spitzbergensis</i> [56] in Poland should be considered as <i>Mec. revelata</i> [4,16]. If this is the case, this species is probably widely distributed in Poland.
<i>Milnesium beasleyi</i> Kaczmarek, Jakubowska and Michalczyk, 2012 [57]	30 (1); 34 (8); 41B (5); 42B (4)	This species was reported from Turkey (type locality) and Poland [4,57].
<i>Milnesium tardigradum tardigradum</i> Doyère, 1840 [7]	51B (13)	In the past, species considered as cosmopolitan and reported around the world. However, following redescrptions [58,59] and genetic analyses, confirmed localities are in Africa (Republic of South Africa), Asia (Japan) and Europe (Denmark, France, Germany, Great Britain, Hungary, Poland, Russia and Switzerland) [11,60,61].
<i>Milnesium dornensis</i> Ciobanu, Roszkowska and Kaczmarek, 2015 [62]	1 (1); 25B (2); 27A (1); 33B (1); 42B (2); 42C (2); 43 (1); 51B (2); 53A (4); 61A (1); 72A (1); 79 (1); 85 (2); 86 (2)	The species is known only from Poland and Romania (type locality) [4,62] and recently probably found in Tunisia (reported as <i>Mil. Cf. dornensis</i> ) [63].
<u><i>Minibiotus intermedius</i></u> (Plate, 1888) [34]	61A (1); 53B (1); 78 (1)	<i>Minibiotus intermedius</i> used to be considered cosmopolitan for many years [39]. However, modern taxonomy has shown that it is a species complex [64]. It was recently redescrbed based on material from type locality in Germany [21]. In Poland, it was reported from many localities [15]. Its distribution needs to be confirmed based on a recent redescription of this species. In this situation, the only confirmed locality should be considered Germany. Taking it into account, this record should be considered as the first confirmed locality of this species from Poland.
<i>Notahypsibius pallidoides</i> (Pilato, Kiosya, Lisi, Inshina and Biserov, 2011) [65]	33B (1); 34 (2); 39 (2); 50A (1); 54B (4); 64A (2); 67A (2); 85 (1); 87 (1); 88 (2)	Up to now, species known from Austria, Croatia, Russia and Ukraine [26,65], however some reports of <i>Hys. pallidus</i> may in fact belong to this species. Species new for Poland.
<i>Paramacrobiotus fairbanksi</i> Schill, Förster, Dandekar and Wolf, 2010 [66]	28B (5+3); 45 (14+1); 53C (26)	A true cosmopolitan species, confirmed based on morphological and genetic studies [67–69].
<i>Paramacrobiotus</i> sp.	44A (1)	Accurate identification of this specimen was impossible due to lack of eggs and overall low number of specimens.
<i>Pseudohexapodibius degenerans</i>	35 (2); 39 (2+1); 76A (33+15); 88 (6+1)	Species up to recently only known from type locality in Ukraine [35]. Recorded by [36] using data collected in this study.
<i>Ramazzottius</i> sp.	23 (21); 25B (1); 33B (4); 34 (5); 41B (4); 42B (15); 51B (6); 88 (3)	Accurate identification of the species was impossible due to lack of eggs.
<i>Xerobiotus pseudohufelandi</i> (Iharos, 1966) [70]	35 (2); 39 (32+16)	Known from few localities in Europe, Russia and Tunisia [39,71]. In Poland, known only from few localities [15,36]

Similarly, for substrates from which samples were extracted, the ground surface and trees had the highest tardigrade species richness and diversity while concrete walls had the lowest (Figure 3A–C). The diversity in the substrates under investigation appeared to be average to well-represented, according to the estimated sample coverage, which had a rarefaction sample coverage percentage above 75% and extrapolation sample coverage percentage of 84% for concrete walls, rarefaction sample coverage percentage above 80% and extrapolation sample coverage percentage of 97% for dead wood, rarefaction sample coverage percentage above 85% and extrapolation sample coverage percentage of 98% for the ground surface, and rarefaction sample coverage percentage above 90% and

extrapolation sample coverage percentage of 98% for trees (Supplementary Materials S2, Figure 3B,C). In Figure 3B, the curves plateau towards 1, particularly for “wood”, indicating that further sampling might only add a few additional species. The “wall” has a lower coverage, which may imply either less comprehensive sampling or inherently lower species richness. Also, in Figure 3C, given that all habitats seem to reach similar levels of coverage, the diversity differences are likely genuine rather than artifacts of sampling bias.



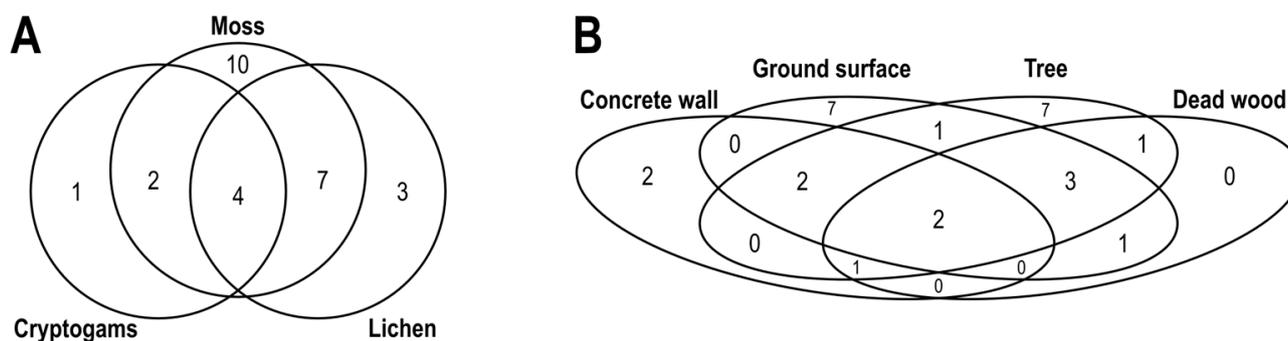
**Figure 2.** Rarefaction and extrapolation for habitats. (A) Sample-size-based rarefaction and extrapolation sampling curve; (B) sample completeness curve; (C) coverage-based rarefaction and extrapolation sampling curve. The solid lines of the curves represent rarefaction, the dashed lines represent extrapolation and the shaded areas represent the 95% confidence intervals (based on a bootstrap method with 100 replications).



**Figure 3.** Rarefaction and extrapolation for substrates. (A) Sample-size-based rarefaction and extrapolation sampling curve; (B) sample completeness curve; (C) coverage-based rarefaction and extrapolation sampling curve. The solid lines of the curves represent rarefaction, the dashed lines represent extrapolation and the shaded areas represent the 95% confidence intervals (based on a bootstrap method with 100 replications).

Upon comparing the taxa composition across all habitats, only four taxa were found in all habitats (*Macrobiotus cf. hufelandi*, *Mesocrista revelata*, *Milnesium cf. alpigenum* and *Notahypsibius pallidoide*), ten taxa were only found in mosses, three in lichens and one in cryptogams, mosses and lichens shared eight and moss and cryptogams only two, but no common taxa were between lichens and cryptogams (Figure 4A). Furthermore, when comparing taxa composition across all substrates, there was only one common taxon (*Macrobiotus cf. hufelandi*) present between all the substrates, two taxa were exclusive

to concrete walls, none to dead wood, seven to the ground surface and seven to trees (Figure 4B).



**Figure 4.** Venn diagrams of species richness (A) for habitats and (B) for substrates.

#### 4. Discussion

At the time of this publication, seven out of 23 national parks in Poland have been studied for tardigrades. So far, 54 tardigrade species have been reported in Tatra National Park, 27 in Bieszczady National Park, 24 in Ojców National Park, 21 in Wielkopolski National Park, 18 in Świętokrzyski National Park, 14 in Wolin National Park and 14 in Bory Tucholskie National Park [4,15,72–77]. However, it should be stressed that the vast majority of those reports have been made using now outdated taxonomic descriptions and keys. Many of the recorded taxa have been since identified as species groups/complexes, such as the *Diphascion pingue* group, *Hypsibius dujardini-convergens* group, *Mesobiotus harmsworthi* group, *Milnesium tardigradum* complex, *Minibiotus intermedius* group, *Paramacrobotus richtersi* group and different morphogroups in genus *Macrobotus* [10,11,18–20,78,79], suggesting that the actual diversity within those areas is higher than those studies suggest.

Little to no tardigrade species have been reported from SPN, rendering almost all species recorded here as new for this region. The only exceptions are *Mec. revelata* and *Psh. degenerans* which were first recorded in SPN by Gąsiorek et al. and Vincenzi et al. [16,36]. In the present study, we reported 27 taxa (21 identified to the species level) from SPN (see also Table 2). Two species have been found that are probably new for science. However, due to an insufficient number of specimens and eggs, a formal description of these taxa is at this moment impossible. A new species of *Diphascion* belongs to the *Diphascion nobilei* group, which is characterized by the presence of wide bases of claws on the fourth pair often equipped with smaller or larger teeth. The second species, from the genus *Mesobiotus*, belongs to the *Mesobiotus coronatus* group which is characterized by eggs with conical processes with a crown of thickenings situated near processes bases. Six of the recorded species are considered new for Poland, including two relatively recently described from Italy and Turkey, i.e., *Eremobiotus ginevrae*, *Mil. beasleyi*. Another newly recorded species is *Hys. scabropygus*, which is widely distributed in Europe. The last three species considered as new for Poland are *Hys. dujardini*, *Minibiotus intermedius* and *Notahypsibius pallidoides*, with the former two of them having been reported in Poland in the past, i.e., [4,15,73]. The reports in question were published before the publishing of recent redescriptions of these two taxa. Therefore, in this study, we can confirm the presence of these species in Poland according to up-to-date taxonomy. The last species, *Not. pallidoides*, has possibly been reported from Poland (including SPN) as *Hys. pallidus* [15], but this hypothesis can only be confirmed by a reexamining of the specimens reported in previous studies.

However, the total species diversity in SPN can be higher because only terrestrial samples have been collected during the course of this study. It is important to remember that a significant area of SPN is covered by lakes and sea (Figure 1), both of which can be home to aquatic and marine tardigrade species, respectively. It should be also noted that our study did not collect any samples of soil (as opposed to mosses and lichens growing on

the ground surface) or leaf litter, which are known to be a habitat for tardigrades [80,81]. These factors suggest that the actual species richness of the SPN can be even higher than hereby presented.

We found that there was no statistically significant difference between species diversity in mosses and lichens in SPN (Supplementary Materials S1). Similar to our result, studies in Costa Rica [82] and in Great Smoky Mountains National Park (USA) [83] also showed no significant differences in estimates of tardigrade diversity between mosses and lichens, including species richness and the Shannon index. Moreover, in a study by Guidetti et al. [84], similar to our study, most species were recorded in mosses and lichens, with only a comparatively small number found in cryptogams (mosses mixed with lichens). However, they also concluded that there was a considerable heterogeneity in species richness for some of the habitats, as well as noted the poor effects of habitat categories on species richness in the multiple regression, where other environmental variables were controlled [84]. When comparing species diversity across different sampling substrates (i.e., concrete walls, ground surface, trees and dead wood), we have found there to be in general no significant differences between different substrates. The only exception are the samples collected from concrete walls, which showed significantly lower diversity when compared to all other sampling substrates (Supplementary Materials S2). This decrease in diversity could be attributed to the man-made nature of such structures. They are more likely to be periodically cleaned off mosses and lichens housing tardigrades, leading to reduced diversity. Despite this, two species, *Mil. tardigradum* and *Mac. vladimiri*, were only found in the samples collected from concrete walls. While the former is considered a cosmopolitan species, the latter has only been reported from few localities and appears to show a mild preference for habitats associated with rocks and concrete and brick constructions, i.e., xerothermic habitats [4,5,55]. When compared to our findings, some studies reported no differences between any substrates of the collected samples [82], while others showed that the samples collected from rock surfaces had higher species richness than those taken from tree trunks [85], and further more papers presented the opposite results, displaying higher species richness for the samples' substrates from tree trunks when compared to rocks [83,86].

In order to better understand the role of substrates and habitats in tardigrade diversity, it would be beneficial to conduct a broader range assessment of various conditions, including precipitation, temperature, as well as characteristics of the habitat sampling substrates. Furthermore, as shown in our study, to confidently compare tardigrade species richness across substrates and habitat categories, a greater than expected number of specimens is required, as per our rarefaction curves (Figures 2 and 3). Insufficient numbers of specimens may not reach a rarefaction plateau, showing that trends of species diversity across habitats or substrate types should be viewed with caution when presented without sufficient context.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/d16040239/s1>, Supplementary Material S1: Coverage-based R/E data for habitats; Supplementary Material S2: Coverage-based R/E data for substrates.

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