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Tardigrada of Bory Tucholskie National Park, Zaborski Landscape Park, and their surroundings (Pomerania Province, Poland)

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Abstract: In this paper, samples of mosses and lichens collected from Bory Tucholskie National Park, Zaborski Landscape Park, and their surroundings (Pomerania Province, northern Poland) were studied for water bears (Tardigrada). In total, 38 tardigrade taxa were identified and six of them (*Hypsibius* cf. *allisoni*, *Hyp. scabropygus*, *Macrobiotus sottilei*, *Milnesium beasleyi*, *Mil. dornensis*, and *Mil. granulatum*) are new records for the Polish fauna. Eighteen taxa (only 14 identified to the species level) were found in Bory Tucholskie National Park, whereas in Zaborski Landscape Park this number was much larger, i.e. 28 taxa (including 22 identified to the species level), as well as in the areas surrounding the parks, i.e. 28 (including 23 identified to the species level). It should be also noted that the effect of substrate, habitat, and types of environmental protection on species richness was not statistical significant.

Key words: Europe, fauna, new records, tardigrades, water bears

1. Introduction

The phylum Tardigrada currently consists of about 1200 taxa (Guidetti and Bertolani, 2005; Degma and Guidetti, 2007; Degma et al., 2009–2017; Vicente and Bertolani, 2013) that inhabit terrestrial and aquatic (both freshwater and marine) environments throughout the world (Nelson et al., 2015). The first studies on Polish tardigrades were conducted over a century ago (Jakubski, 1915) and to date about 50 papers have been published reporting about 100 tardigrade taxa from the area of Poland (Nowak and Stec, 2017; see also http://tardigrada.info/E1.html and http://www.tardigrada.net/newsletter/archives.htm).

Bory Tucholskie National Park (BTNP) and Zaborski Landscape Park (ZLP) are located in the southern part of Pomerania Province in northern Poland. The BTNP was created in 1996 and covers an area of 46.13 km^2 of forests (mostly different types of pine forests like *Leucobryo-Pinetum*, *Cladonio-Pinetum*, or *Vaccinio uliginosi-Pinetum*), 21 lakes (representing all the genetic and morphologic types of lowland areas of Poland), meadows, and peatlands (http://www.pnbt.com.pl/en/). The national park is surrounded by a larger protected area, the ZLP, established in 1990. It covers an area of 340.26 km^2 to the north of the city of Chojnice. The park is a part of the Tuchola Forest Biosphere Reserve, created under a UNESCO program in 2010. The park's area is dominated by pine forests and small fragments of deciduous forests. Indispensable elements of the park are peat bogs, located in the valleys of the Brda and Zbrzyca rivers (http://zaborskipark.pl/).

To date, from the area of the ZLP and in the vicinity of the BTNP (in the neighborhood of the village of Męcikał), only six tardigrade species were reported: *Adropion scoticum* (Murray, 1905); *Astatumen trinacriae* (Arcidiacono, 1962); *Diphascon pingue* (Marcus, 1936); *Macrobiotus hufelandi* C.A.S. Schultze, 1834; *Mesocrista spitzbergensis* (Richters, 1903); and *Minibiotus intermedius* (Plate, 1888) (Dastych, 1988). Five of them (excluding *Ast. trinacriae*) are now considered as a group of morphologically similar species and correct identification is only possible based on very detailed morphometry and/or egg morphology (e.g., Claxton, 1998; Fontoura and Pilato, 2007; Kaczmarek et al., 2016; Morek et al., 2016).

In the present paper, 111 samples collected in BTNP, ZLP, and their surroundings were studied and 38 tardigrade taxa were reported, six of which are new records for Poland.

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2. Materials and methods

2.1. Sample processing

All samples were collected in BTNP, ZLP, and their surroundings in August 2015 and February 2016 by the first author (ŁK) and his father (Krzysztof Kaczmarek, KK) (see list of samples below). Samples of mosses and lichens were collected from different types of substrates like soil, trees, dead wood, concrete walls, stones, and rooftops in three types of environments, i.e. pine forest, urban, and agriculture. Samples were packed and delivered to the laboratory at the Faculty of Biology, Adam Mickiewicz University, Poznań. All samples were collected and processed following the protocol of Stec et al. (2015).

2.2. Microscopy

Specimens for light microscopy were mounted on microscope slides in a small drop of Hoyer's medium, prepared according to Ramazzotti and Maucci (1983) and the English translation by Beasley (1995), and secured with a cover slip. The slides were then placed in an incubator and dried for 2 days at 60 °C. Dried slides were sealed with transparent nail polish and examined under an Olympus BX41 phase contrast light microscope associated with an ARTCAM-300Mi digital camera (Olympus Corporation, Tokyo, Japan). Some adult specimens and eggs were prepared for SEM imaging according to Stec et al. (2015). Such prepared animals were examined under high vacuum in a Hitachi S3000N scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan).

2.3. Comparative material

Tardigrade taxonomy follows Bertolani et al. (2014). The species were identified based on the keys of Ramazzotti and Maucci (1983) (with English translation by Beasley (1995)), Dastych (1988), Fontoura and Pilato (2007), Michalczyk et al. (2012a, 2012b), and Morek et al. (2016) and more recent papers and original descriptions by Pilato (1977), Dastych (1978), Biserov (1985), Bertolani and Rebecchi (1993), Pilato et al. (2003), Miller et al. (2005), Kaczmarek and Michalczyk (2009), Bertolani et al. (2011), Pilato et al. (2012), Ciobanu et al. (2015), and Gąsiorek et al. (2016).

Furthermore, holotypes, paratypes, and neotypes of *Mac. polonicus* Pilato et al., 2003; *Mes. revelata* Gąsiorek et al., 2016; *Milnesium beasleyi* Kaczmarek et al., 2012; *Mil. dornensis* Ciobanu et al., 2015; and *Mil. tardigradum tardigradum* Doyère, 1840 were also examined and compared with material found in the present study.

2.4. Statistical analysis

To test the differences between numbers of species in particular years we used a two-tailed t-test (Zar, 1999). Furthermore, the effect of environmental factors (i.e. substrate (tree, stone, soil), habitat (urban, forest), protective types (null, landscaped park, national park)) on species richness was analyzed using a generalized linear model (GLZ) (Crawley, 2009). All statistical tests were performed with R software (R Development Core Team, 2017).

2.5. List of samples

2.5.1. Bory Tucholskie National Park (BTNP)

1. 53°51′09″N, 17°34′30″E, 124 m a.s.l.; near Drzewicz village, pine forest, lichen on tree (*Pinus sylvestris*), date: 08.2016, coll. ŁK.

2. 53°51′09″N, 17°34′30″E, 124 m a.s.l.; Drzewicz village, near bridge on Brda river, pine forest, moss on tree (*Betula pendula*), date: 08.2016, coll. ŁK.

7. 53°51′09″N, 17°34′30″E, 124 m a.s.l.; Drzewicz village, bridge on Brda river, pine forest, moss on concrete wall, date: 08.2016, coll. ŁK.

14. 53°47′19″N, 17°31′19″E, 129 m a.s.l.; Bachorze village, pine forest, moss on dead wood, date: 08.2016, coll. ŁK.

18. 53°49′04″N, 17°30′49″E, 132 m a.s.l.; Małe Swornegacie village, pine forest, lichen on soil, date: 08.2016, coll. ŁK.

46. 53°47′19″N, 17°31′19″E, 129 m a.s.l.; Bachorze village, pine forest, lichen on dead wood, date: 08.2016, coll. ŁK.

47. 53°49′04″N, 17°30′49″E, 132 m a.s.l.; Małe Swornegacie village, pine forest, lichen on tree, date: 08.2016, coll. ŁK.

66. 53°49′04″N, 17°31′55″E, 134 m a.s.l.; near Płęsno Lake, pine forest, moss on tree, date: 02.2017, coll. KK.

67. 53°50′12″N, 17°33′41″E, 131 m a.s.l.; on south of Wielkie Krzywce Lake, pine forest, lichen on tree, date: 02.2017, coll. KK.

68. 53°49′26″N, 17°33′51″E, 136 m a.s.l.; near Nierybno Lake, pine forest, moss on tree, date: 02.2017, coll. KK.

69. 53°48′37″N, 17°33′54″E, 136 m a.s.l.; south isthmus of Płęsno Lake, pine forest, moss on tree, date: 02.2017, coll. KK.

70. 53°47′29″N, 17°33′32″E, 138 m a.s.l.; near Gacno Wielkie Lake, pine forest, lichen on tree, date: 02.2017, coll. KK.

71. 53°48′37″N, 17°33′53″E, 137 m a.s.l.; near Główka Lake, pine forest, lichen on dead wood, date: 02.2017, coll. KK.

72. 53°51′09″N, 17°34′34″E, 123 m a.s.l.; near Łąckie Lake, pine forest, moss on tree, date: 02.2017, coll. KK.

73. 53°47′29″N, 17°33′32″E, 138 m a.s.l.; near Gacno Wielkie Lake, pine forest, lichen on tree, date: 02.2017, coll. KK.

74. 53°49′02″N, 17°31′56″E, 133 m a.s.l.; Płęsno Lake, near Bartuś Oak, pine forest, moss on tree, date: 02.2017, coll. KK.

75. 53°50′12″N, 17°33′41″E, 131 m a.s.l.; on south of Wielkie Krzywce Lake, pine forest, moss on tree, date: 02.2017, coll. KK.

76. 53°48′59″N, 17°33′53″E, 130 m a.s.l.; near Nierybno Lake, Pętla Lipnickiego Swamp, pine forest, lichen on tree, date: 02.2017, coll. KK.

77. 53°50′10″N, 17°33′23″E, 133 m a.s.l.; 2 km on south from Drzewicz village, near Wielkie Krzywce Lake, pine forest, lichen and moss on tree, date: 02.2017, coll. KK.

78. 53°48′59″N, 17°33′53″E, 130 m a.s.l.; near Nierybno Lake, Pętla Lipnickiego Swamp, pine forest, lichen on tree, date: 02.2017, coll. KK.

79. 53°49′04″N, 17°31′55″E, 134 m a.s.l.; near Płęsno Lake, pine forest, lichen on tree, date: 02.2017, coll. KK.

80. 53°48′52″N, 17°32′21″E, 135 m a.s.l.; near Rybie Oko Lake, pine forest, moss on soil, date: 02.2017, coll. KK.

81. 53°48′59″N, 17°32′08″E, 132 m a.s.l.; near Kacze Oko Lake, pine forest, lichen on tree, date: 02.2017, coll. KK.

82. 53°47′07″N, 17°35′20″E, 127 m a.s.l.; near Ostrowite Lake, Józefowo, forester hut, pine forest, moss on tree, date: 02.2017, coll. KK.

102. 53°49′02″N, 17°34′22″E, 138 m a.s.l.; near Bełczak Lake, pine forest, lichen on tree, date: 02.2017, coll. KK.

2.5.2. Zaborski Landscape Park (ZLP)

3. 53°49′03″N, 17°39′46″E, 129 m a.s.l.; Męcikał city, near the road to Chojnice, moss on concrete wall, date: 08.2016, coll. ŁK.

10. 53°49′29″N, 17°39′55″E, 136 m a.s.l.; Męcikał-Struga village, clearing in pine forest, moss and lichen on tree (*Betula pendula*), date: 08.2016, coll. ŁK.

12. 53°49′29″N, 17°39′55″E, 136 m a.s.l.; Męcikał-Struga village, clearing in pine forest, moss on soil, date: 08.2016, coll. ŁK.

22. 53°49′29″N, 17°39′55″E, 136 m a.s.l.; Męcikał-Struga village, pine forest, lichen on soil, date: 08.2016, coll. ŁK.

30. 53°49′12″N, 17°39′59″E, 125 m a.s.l.; Męcikał city, moss on concrete wall, date: 08.2016, coll. ŁK.

34. 53°49′29″N, 17°39′55″E, 136 m a.s.l.; Męcikał-Struga village, clearing in pine forest, moss on concrete wall, date: 08.2016, coll. ŁK.

35. 53°44′27″N, 17°30′42″E, 120 m a.s.l.; Charzkowy village, near Charzykowskie Lake, moss on rooftop, date: 08.2016, coll. ŁK.

50. 53°51′47″N, 17°30′28″E, 127 m a.s.l.; Swornegacie village, entrance from Czernica, pine forest, embankment road, moss on soil, date: 08.2016, coll. ŁK.

51. 53°51′47″N, 17°30′28″E, 127 m a.s.l.; Swornegacie village, entrance from Czernica, pine forest, embankment road, lichen on soil, date: 08.2016, coll. ŁK.

52. 53°51′47″N, 17°30′28″E, 127 m a.s.l.; Swornegacie village, entrance from Czernica, pine forest, embankment road, moss on soil, date: 08.2016, coll. ŁK.

60. 53°55′43″N, 17°34′42″E, 124 m a.s.l.; Rolbik village, lichen on tree, date: 08.2016, coll. ŁK.

64. 53°45′32″N, 17°35′32″E, 152 m a.s.l.; Wyłuszczarnia nasion Klosnowo, lichen on tree (*Quercus robur*), date: 08.2016, coll. ŁK.

65. 53°45′32″N, 17°35′32″E, 152 m a.s.l.; Wyłuszczarnia nasion Klosnowo, lichen on tree (*Quercus robur*), date: 08.2016, coll. ŁK.

83. 53°54′48″N, 17°33′40″E, 147 m a.s.l.; Piecki Nature Reserve, pine forest, lichen and moss on tree, date: 02.2017, coll. KK.

84. 53°54′48″N, 17°33′40″E, 147 m a.s.l.; Piecki Nature Reserve, pine forest, lichen on tree, date: 02.2017, coll. KK.

85. 53°51′52″N, 17°38′17″E, 136 m a.s.l.; Krówna village, moss on tree, date: 02.2017, coll. KK.

86. 53°50′29″N, 17°38′33″E, 128 m a.s.l.; Czernica village, lichen on tree, date: 02.2017, coll. KK.

87. 53°55′54″N, 17°32′47″E, 129 m a.s.l.; Widno village, moss on tree, date: 02.2017, coll. KK.

88. 53°53′16″N, 17°30′28″E, 123 m a.s.l.; Zbrzyca village, lichen on rooftop, date: 02.2017, coll. KK.

89. 53°54′44″N, 17°29′50″E, 141 m a.s.l.; near Zbrzyca village, Śluza, moss on stone, date: 02.2017, coll. KK.

90. 53°53′18″N, 17°35′15″E, 135 m a.s.l.; Asmus village, forester hut, moss on tree, date: 02.2017, coll. KK.

91. 54°00′19″N, 17°37′42″E, 141 m a.s.l.; Peplin village, moss on soil, date: 02.2017, coll. KK.

92. 53°50′29″N, 17°38′33″E, 128 m a.s.l.; Czernica village, moss on concrete wall, date: 02.2017, coll. KK.

93. 53°55′54″N, 17°32′47″E, 129 m a.s.l.; Widno village, lichen and moss on tree, date: 02.2017, coll. KK.

95. 53°59′59″N, 17°39′57″E, 155 m a.s.l.; Przymuszewo village, lichen on tree, date: 02.2017, coll. KK.

96. 53°58′31″N, 17°38′21″E, 149 m a.s.l.; Parzyn village, lichen on tree, date: 02.2017, coll. KK.

97. 53°53′37″N, 17°37′10″E, 153 m a.s.l.; Antoniewo village, moss on tree, date: 02.2017, coll. KK.

98. 53°54′44″N, 17°29′50″E, 141 m a.s.l.; near Zbrzyca village, Śluza, moss on stone, date: 02.2017, coll. KK.

99. 53°58′31″N, 17°38′21″E, 149 m a.s.l.; Parzyn village, lichen on tree, date: 02.2017, coll. KK.

100. 53°58′31″N, 17°38′21″E, 149 m a.s.l.; Parzyn village, lichen and moss on tree, date: 02.2017, coll. KK.

101. 53°54′39″N, 17°30′31″E, 133 m a.s.l.; Śluza village, near Czarne Lake, moss on tree, date: 02.2017, coll. KK.

103. 53°52′37″N, 17°31′27″E, 124 m a.s.l.; Kamionka village, lichen on tree, date: 02.2017, coll. KK.

104. 53°54′48″N, 17°33′40″E, 147 m a.s.l.; Piecki Nature Reserve, pine forest, moss on tree, date: 02.2017, coll. KK.

105. 53°56′45″N, 17°38′57″E, 136 m a.s.l.; Kaszuba village, moss on tree, date: 02.2017, coll. KK.

106. 53°56′10″N, 17°32′01″E, 136 m a.s.l.; 1km from Widno village, moss on tree, date: 02.2017, coll. KK.

107. 53°56′16″N, 17°34′28″E, 127 m a.s.l.; Milachowo Lake near Rolbik village, lichen on soil, date: 02.2017, coll. ŁK.

108. 54°00′19″N, 17°37′42″E, 141 m a.s.l.; Peplin village, moss on tree, date: 02.2017, coll. KK.

109. 53°55′50″N, 17°31′30″E, 124 m a.s.l.; Laska village, moss on tree, date: 02.2017, coll. KK.

110. 53°55′50″N, 17°31′30″E, 124 m a.s.l.; Laska village, lichen on tree, date: 02.2017, coll. KK.

111. 53°56′12″N, 17°35′49″E, 139 m a.s.l.; Milachowo-Młyn village, moss on stone, date: 02.2017, coll. KK.

2.5.3. Surroundings of the parks

4. 53°50′15″N, 17°47′42″E, 137 m a.s.l.; 2 km of Czarniż village, pine forest, lichen on soil, date: 08.2016, coll. ŁK.

5. 53°49′22″N, 17°51′40″E, 143 m a.s.l.; between Bialawy and Kwieki villages, pine forest, lichen on soil, date: 08.2016, coll. ŁK.

6. 53°44′42″N, 17°47′33″E, 124 m a.s.l.; near Rytel City, Great Brda Canal, pine forest, moss on soil, date: 08.2016, coll. ŁK.

8. 53°50′15″N, 17°48′23″E, 138 m a.s.l.; 2 km of Czarniż village, pine forest, lichen on soil, date: 08.2016, coll. ŁK.

9. 53°42′43″N, 17°39′13″E, 159 m a.s.l.; near crossroad to Krojanty from the road to Czersk, 18 Pułk Ułanów Pomorskich Monument, moss on concrete wall, date: 08.2016, coll. ŁK.

11. 53°49′13″N, 17°57′19″E, 132 m a.s.l.; Malachin near Czersk, railroad tracks, moss on soil, date: 08.2016, coll. ŁK.

13. 53°44′08″N, 17°34′44″E, 150 m a.s.l.; Czrtołomie village, near manor house, moss on concrete wall, date: 08.2016, coll. ŁK.

15. 53°43′26″N, 17°45′34″E, 122 m a.s.l.; Suszek Lake, pine forest, moss on soil, date: 08.2016, coll. ŁK.

16. 53°50′15″N, 17°48′23″E, 138 m a.s.l.; 2 km of Czarniż village, pine forest, moss on soil, date: 08.2016, coll. ŁK.

17. 53°43′03″N, 17°34′45″E, 165 m a.s.l.; Igły village near Chojnice, moss on concrete wall, date: 08.2016, coll. ŁK.

19. 53°43′03″N, 17°34′45″E, 165 m a.s.l.; Igły village near Chojnice, moss on tree (*Populus nigra*), date: 08.2016, coll. ŁK.

20. 53°46′31″N, 17°42′56″E, 120 m a.s.l.; Mylof dam, moss on tree (*Acer platanoides*), date: 08.2016, coll. ŁK.

21. 53°43′26″N, 17°45′34″E, 122 m a.s.l.; Suszek Lake, pine forest, lichen on tree (*Betula pendula*), date: 08.2016, coll. ŁK.

23. 53°50′25″N, 17°46′04″E, 135 m a.s.l.; Czarniż village, moss on concrete wall, date: 08.2016, coll. ŁK.

24. 53°49′22″N, 17°51′40″E, 143 m a.s.l.; between Bialawy and Kwieki villages, pine forest, moss on soil, date: 08.2016, coll. ŁK.

25. 53°44′08″N, 17°34′44″E, 150 m a.s.l.; Czrtołomie village, near manor house, moss on concrete wall, date: 08.2016, coll. ŁK.

26. 53°50′25″N, 17°46′04″E, 135 m a.s.l.; Czarniż village, moss on concrete wall, date: 08.2016, coll. ŁK.

27. 53°43′32″N, 17°37′45″E, 152 m a.s.l.; Krojanty village, Krojanty Clinic, moss on rooftop, date: 08.2016, coll. ŁK.

28. 53°44′21″N, 17°32′37″E, 146 m a.s.l.; Chojniczki village, on the crossroad to Charzkowy village, moss on concrete wall, date: 08.2016, coll. ŁK.

29. 53°42′47″N, 17°56′06″E, 116 m a.s.l.; Legbąd city, fine forest, moss on concrete wall, date: 08.2016, coll. ŁK.

31. 53°44′31″N, 17°36′31″E, 149 m a.s.l.; near Powałki village, near the road to Chojniczki village, moss on tree (*Acer platanoides*), date: 08.2016, coll. ŁK.

32. 53°46′22″N, 17°42′57″E, 129 m a.s.l.; Mylof dam, pine forest, moss on soil, date: 08.2016, coll. ŁK.

33. 53°43′26″N, 17°45′34″E, 122 m a.s.l.; Suszek Lake, pine forest, moss on soil, date: 08.2016, coll. ŁK.

36. 53°44′08″N, 17°34′44″E, 150 m a.s.l.; Czrtołomie village, near manor house, moss on concrete wall, date: 08.2016, coll. ŁK.

37. 53°43′26″N, 17°45′34″E, 122 m a.s.l.; Suszek Lake, pine forest, moss on soil, date: 08.2016, coll. ŁK.

38. 53°44′21″N, 17°32′37″E, 146 m a.s.l.; Chojniczki village, on the crossroad to Charzkowy village, moss on stone, date: 08.2016, coll. ŁK.

39. 53°43′32″N, 17°37′45″E, 152 m a.s.l.; Krojanty village, Krojanty Clinic, moss on rooftop, date: 08.2016, coll. ŁK.

40. 53°46′31″N, 17°42′56″E, 120 m a.s.l.; Mylof dam, moss on concrete wall, date: 08.2016, coll. ŁK.

41. 53°44′42″N, 17°47′33″E, 124 m a.s.l.; near Rytel City, Great Brda Canal, pine forest, moss on tree (*Populus tremula*), date: 08.2016, coll. ŁK.

42. 53°42′47″N, 17°56′06″E, 116 m a.s.l.; Legbąd city, pine forest, lichen on soil, date: 08.2016, coll. ŁK.

43. 53°46′31″N, 17°42′56″E, 120 m a.s.l.; Mylof dam, moss on concrete wall, date: 08.2016, coll. ŁK.

44. 53°51′18″N, 17°42′28″E, 139 m a.s.l.; near Bursy city, Żabno village, railroad tracks, moss on concrete wall, date: 08.2016, coll. ŁK.

45. 53°46′31″N, 17°42′56″E, 120 m a.s.l.; Mylof dam, lichen on soil, date: 08.2016, coll. ŁK.

48. 53°40′07″N, 17°54′29″E, 105 m a.s.l.; near Woziwoda village, Great Brda Canal, pine forest, moss on tree (*Betula pendula*), date: 08.2016, coll. ŁK.

49. 53°40′07″N, 17°54′29″E, 105 m a.s.l.; near Woziwoda village, Great Brda Canal, pine forest, moss on concrete wall, date: 08.2016, coll. ŁK.

53. 53°43′32″N, 17°37′45″E, 152 m a.s.l.; Krojanty village, Krojanty Clinic, moss on base of tree (*Quercus robur*), date: 08.2016, coll. ŁK.

54. 53°43′30″N, 17°37′37″E, 150 m a.s.l.; Krojanty village, Krojanty Clinic, moss on rooftop of garden grill, date: 08.2016, coll. ŁK.

55. 53°43′32″N, 17°37′45″E, 152 m a.s.l.; Krojanty village, Krojanty Clinic, lichen and moss on tree (*Tilia cordata*), date: 08.2016, coll. ŁK.

56. 53°43′30″N, 17°37′37″E, 150 m a.s.l.; Krojanty village, Krojanty Clinic, moss on rooftop of garden grill, date: 08.2016, coll. ŁK.

57. 53°43′32″N, 17°37′45″E, 152 m a.s.l.; Krojanty village, Krojanty Clinic, lichen and moss on concrete wall, date: 08.2016, coll. ŁK.

58. 53°43′32″N, 17°37′45″E, 152 m a.s.l.; Krojanty village, Krojanty Clinic, lichen and moss on stone wall, date: 08.2016, coll. ŁK.

59. 53°43′33″N, 17°37′50″E, 151 m a.s.l.; Krojanty village, Krojanty Clinic (Soplicowo), lichen and moss on rooftop, date: 08.2016, coll. ŁK.

61. 53°43′33″N, 17°37′50″E, 151 m a.s.l.; Krojanty village, Krojanty Clinic (Soplicowo), lichen on brick wall, date: 08.2016, coll. ŁK.

62. 53°43′33″N, 17°37′50″E, 151 m a.s.l.; Krojanty village, Krojanty Clinic (Soplicowo), moss on brick wall, date: 08.2016, coll. ŁK.

63. 53°43′33″N, 17°37′50″E, 151 m a.s.l.; Krojanty village, Krojanty Clinic (Soplicowo), moss on tree (*Cerasus* sp.), date: 08.2016, coll. ŁK.

94. 53°52′56″N, 17°38′12″E, 141 m a.s.l.; Wielkie Chełmy village, moss on concrete wall, date: 02.2017, coll. KK.

3. Results

In total, 4470 specimens (including 352 simplex/exuviae) and 282 eggs of 38 tardigrade taxa were extracted from 111 samples (Table 1).

Eighteen taxa (including 14 identified to the species level) were found in BTNP, 28 (including 22 identified to the species level) in ZLP, and 28 (including 23 identified to the species level) in the areas surrounding the parks (Tables 1 and 2).

The mean number of species per sample was 2.59 (95% CL: 2.18–2.86), varying from 3.5 in 2015 to 6.0 in 2016, but the difference among years was not significant ($t = -0.20$, $P = 0.83$). Moreover, we did not find any effect of substrates, habitat, and the types of environmental protection on species richness (GLZ: $F = 0.52$, $P = 0.59$).

4. Discussion

Tardigrade studies in Polish national parks have not been very intense and only 6 of 23 Polish national parks were studied more thoroughly. To date, 14 species were reported from Wolin National Park, 18 from Świętokrzyski National Park, 21 from Wielkopolska National Park, 24 from Ojców National Park, 27 from Bieszczady National Park, and 54 from Tatra National Park (Dastych, 1972, 1979, 1980, 1988; Hęciak, 1976; Kaczmarek and Michalczyk, 2003; Michalczyk and Kaczmarek, 2003). In other parks tardigrades were reported only occasionally (e.g., Babia Góra National Park, Białowieża National Park, and Słowiński National Park) or were not at all (see Dastych, 1988). However, all studies are outdated and the species were identified based on old descriptions and keys. Based on modern taxonomy, species such as *Mac. hufelandi*, *Mesobiotus harmsworthi* (Murray, 1907), *Paramacrobiotus richtersi* (Murray, 1911), *Hypsibius convergens* (Urbanowicz, 1925), *Hyp. dujardini* (Doyère, 1840), *Mil. t. tardigradum*, *Dip. p. pingue*, and *Ramazzottius oberhaeuseri* (Doyère, 1840) are now considered as species groups. This means that the number of species from each park may be distinctly higher than what is confirmed, e.g., by findings of a few different species from the *hufelandi* group or from the genus *Milnesium* in present studies (see Table 1). In conclusion, although the country of Poland was sampled for tardigrades quite extensively (Dastych, 1988), the Polish tardigrade fauna is still very poorly known, especially in Polish national parks.

The number of 18 taxa (only 14 identified to the species level) found in the BTNP is rather low in comparison to most studied national parks in Poland. This is not very surprising taking into consideration that terrestrial ecosystems are very homogeneous. The park is covered in 83% by forests, 98% of which are different types of pine forests, i.e. *Leucobryo-Pinetum*, *Cladonio-Pinetum*, or *Vaccinio uliginosi-Pinetum* (http://www.pnbt.com.pl/en/forest_eco_systems-375). In the present study only terrestrial tardigrades were collected and examined and it is highly probable that a few additional freshwater species could inhabit the park's numerous lakes and rivers.

Interestingly, a much larger number of species was found in the regions not included in the BTNP, i.e. 28 taxa (including 22 identified to the species level) in the ZLP and 28 taxa (including 23 identified to the species level) in the areas surrounding the parks (Table 2). This is most likely due to the much larger mosaic of microhabitats available for tardigrades, such as urban and agriculture areas, and a higher variety of forest ecosystems (including deciduous forests).

Many species found in the present study were widely reported from Poland in the past; however, most of them are now considered as species groups based on modern taxonomy and their real distribution is unknown (Table 1). Among the found species, many are xerophilous and forest species, which is obviously connected with the environmental conditions of the studied area. Six taxa (four identified to the species level) are new records for the Polish tardigrade fauna: *Hyp.* cf. *allisoni*; *Hyp. scabropygus*

Table 1. Tardigrades found in the present study (in alphabetical order).

Taxon	Samples	Remarks
1. *Adropion prorsirostre* (Thulin, 1928)	2 (2), 66 (2), 80 (1), 91 (2), 96 (4)	Species with Holarctic distribution with only single localities in other geographic regions suggests a species complex (Kaczmarek et al., 2016). In Poland common and widely distributed (Dastych, 1988).
2. *Adr. scoticum* (Murray, 1905)	69 (2)	*Adropion scoticum* is probably a cosmopolitan complex of very similar species (Kaczmarek et al., 2016). In Poland common and widely distributed, especially in coniferous forests (Dastych, 1988).
3. *Astatumen trinacriae* (Arcidiacono, 1962)	3 (1), 6 (2), 31 (1)	Species with mainly Holarctic distribution (Kaczmarek et al., 2016). In Poland common and widely distributed, especially in forests, but not numerous (Dastych, 1988).
4. *Diphascon pingue pingue* (Marcus, 1936)	2 (1), 48 (4), 66 (4), 68 (3), 75 (3), 77 (2), 80 (1), 82 (11), 109 (2)	The *pingue* group is a complex of extremely similar species (see, e.g., Fontoura and Pilato, 2007 for the diagnostic key to the group). Nominal *Dip. pingue* is probably restricted to the Holarctic (Pilato and Binda, 1998, 1999). In Poland common and widely distributed and strongly connected with forests (Dastych, 1988).
5. *Echiniscus blumi* Richters, 1903	35 (31)	This species probably has a northern Holarctic distribution and other records (e.g., in tropical southern American states) should be reexamined (Kaczmarek et al., 2015, 2016). Xerophilous species. To date reported mainly from southern, upland, and mountain regions of Poland, but known also from the northeastern corner of Poland (Dastych, 1988).
6. *Ech. testudo* (Doyère, 1840)	28 (1), 29 (2), 30 (44), 35 (56), 40 (25), 49 (8), 59 (5)	Species widely distributed in the Arctic and Europe, with a high dispersal potential (Jørgensen et al., 2007, 2013). The reports from the other localities, e.g., from South America, need confirmation (Kaczmarek et al., 2015). Rather common and numerous xerophilous species, distributed mainly in southern and western Poland (Dastych, 1988).
7. *Hypsibius* cf. *allisoni*	92 (48)	Specimens found in present study correspond well with original description of *Hyp. allisoni* (Horning et al., 1978). However, this description is inaccurate and it is highly possible that our specimens represent another taxon. **It is new taxon for the Polish fauna.**
8. *Hyp. convergens* (Urbanowicz, 1925)	66 (3), 69 (2), 107 (1), 109 (8)	*Hypsibius convergens* is a species complex (Kaczmarek and Michalczyk, 2009) with global distribution (McInnes, 1994) and the nominal species still needs a modern redescription. In Poland very common and widely distributed (Dastych, 1988).
9. *Hyp. dujardini* (Doyère, 1840)	3 (17), 13 (4), 27 (1), 36 (11), 39 (72), 55 (2), 56 (2), 57 (27)	*Hypsibius dujardini* is a species complex (Kaczmarek and Michalczyk, 2009) with global distribution (McInnes, 1994) and the nominal species still needs a modern redescription. Hygrophilous species, in Poland common and widely distributed (Dastych, 1988).
10. *Hyp. microps* Thulin, 1928	2 (2), 5 (1), 48 (1), 66 (1), 68 (5), 82 (1), 97 (8), 105 (18)	This is probably a species complex, similar to the *pallidus* and co*nvergens-dujardini* complexes (Kaczmarek and Michalczyk, 2009). Nominal *Hyp. microps* has Holarctic distribution (McInnes, 1994). To date in Poland reported only from Pieniny Mts. (Dastych, 1988).
11. *Hyp. pallidus* Thulin, 1911	2 (5), 4 (1), 10 (1), 12 (1), 14 (5), 16 (1), 18 (1), 22 (1), 23 (1), 24 (1), 29 (1), 51 (4), 54 (2), 55 (16), 56 (11), 72 (21), 75 (2), 77 (1), 79 (1), 89 (1), 90 (1), 91 (11), 98 (1), 103 (2), 106 (14)	This is probably a species complex, similar to the *microps* and *convergens-dujardini* complexes (Kaczmarek and Michalczyk, 2009). *Hypsibius pallidus* is a common European species but it has also been reported from non-European localities (see Kaczmarek et al., 2016). In Poland very common and widely distributed (Dastych, 1988).

Table 1. (Continued).

Taxon	Samples	Remarks
12. *Hyp. scabropygus* Cuénot, 1929	89 (1)	Species with wide Holarctic distribution (McInnes, 1994); however, the original description is uncertain and the species need a modern redescription (see Zawierucha et al., 2014). **Species new for the Polish fauna**.
13. *Isohypsibius latiunguis* (Iharos, 1964)	33 (4)	Known only from Hungary (type locality), Italy, and Poland (McInnes, 1994). In Poland (up to now fourth locality), very rare lowland species.
14. *Iso. lunulatus* (Iharos, 1966)	3 (6)	Species with wide Holarctic distribution with only a single record from South America, which should be considered erroneous (Kaczmarek et al., 2015, 2016). In Poland, a rare species reported mainly from uplands. This is the northernmost locality of this species in Poland.
15. *Iso. prosostomus* Thulin, 1928	7 (1), 9 (2), 13 (10), 17 (1), 31 (1), 48 (2), 62 (3), 87 (1)	Largely Palearctic or Holarctic distribution with very few reports from other regions (McInnes, 1994). In Poland very common and widely distributed (Dastych, 1988).
16. *Iso. sattleri* (Richters, 1902)	13 (5), 29 (1), 42 (4)	Many taxonomic confusions caused because this species has questionable cosmopolitan distribution (McInnes, 1994), and the true geographic range is now unknown. In Poland common and widely distributed but not numerous (Dastych, 1988).
17. *Macrobiotus furcatus* Ehrenberg, 1859	58 (4), 63 (19)	Binda and Pilato (1992) redescribed this species and transferred it to the genus *Minibiotus* R.O. Schuster, 1980. However, Bertolani et al. (2014), based on molecular and morphological data, stated that it does not belongs to the genus *Minibiotus* and retransferred it temporarily to genus *Macrobiotus* C.A.S. Schultze, 1834. Based on its rather wide distribution (McInnes, 1994), it is highly probable that it is a species complex. In Poland reported up to now only from Pieniny and Tatra Mts. (Dastych, 1988). This is the northernmost locality of this species in Poland.
18. *Mac. h. hufelandi* C.A.S. Schultze, 1834	4 (19+4), 18 (27+4), 32 (11+4), 48 (18+1), 55 (25+1), 66 (33+3), 68 (126+15), 69 (55+1), 75 (107+2), 80 (35+1), 82 (117+6), 106 (14+2), 111 (42+1)	This species belongs to a cosmopolitan and species-rich complex (e.g., Kaczmarek et al., 2016). In the past, *Mac. hufelandi* sensu stricto was reported almost everywhere (McInnes, 1994), but its range is probably much more restricted. However, at present, its true distribution is unknown. In Poland it was also reported from many localities (Dastych, 1988), but most of the reports are old and it is obvious that some of them belong to other members of the *hufelandi* group. Even in the present study a few different species from this group were found, i.e. *Mac. macrocalix* Bertolani and Rebecchi, 1993; *Mac. polonicus*; *Mac. sottilei*; and *Mac. vladimiri*.
19. *Mac. macrocalix* Bertolani and Rebecchi, 1993	74 (64+8), 89 (5+1)	Species known from Italy (type locality), Poland (Małopolska Province), and Sweden, probably with European or Palearctic distribution (Kaczmarek and Michalczyk, 2002; Cesari et al., 2009). This is the second record of this species in Poland.
20. *Mac. polonicus* Pilato et al., 2003	23 (26+7), 26 (82+11), 59 (33+6)	This is the first report of this species beyond the type locality (Wielkopolska Province, W Poland) (Pilato et al., 2003).
23. *Mac. sottilei* Pilato et al., 2012	3 (68+5), 29 (55+20), 49 (110+3)	To date, species known only from the type locality in Belarus (Pilato et al., 2012). **Species new for the Polish fauna**.
21. *Mac. vladimiri* Bertolani et al., 2011	20 (87+2), 62 (1), 74 (65+37), 87 (13+2), 98 (9+5), 100 (96+22)	To date, species known only from Italy (type locality) and Poland (Małopolska Province) (Bertolani et al., 2011; Nowak and Stec, 2017).

Table 1. (Continued).

Taxon	Samples	Remarks
22. *Mac.* sp. (*hufelandi* group)	2 (18), 6 (27), 7 (1), 10 (4), 11 (3), 13 (1), 14 (3), 21 (2), 22 (2), 24 (1), 30 (34), 36 (1), 43 (34), 45 (3), 46 (24), 60 (2), 64 (11), 72 (10), 73 (1), 76 (1), 77 (26), 79 (23), 88 (19), 90 (25), 93 (20), 95 (1), 96 (1), 102 (32), 104 (10), 105 (11), 107 (39), 109 (15)	The accurate identification of the species was not possible due to lack of eggs, especially as a few species of the *hufelandi* group were found in the present study (*Mac. h. hufelandi*, *Mac. macrocalix*, *Mac. polonicus*, *Mac. sottilei*, and *Mac. vladimiri*).
24. *Mesobiotus h. harmsworthi* (Murray, 1907)?	53 (34+6), 101 (29+2)	Originally described from Franz-Josef Land in Arctic Russia, *Mes. harmsworthi* is now considered a group of species, all requiring careful taxonomic analysis of individuals and eggs (see Kaczmarek et al., 2011 for a diagnostic key). A partial redescription of the nominal species was published by Pilato et al. (2000), but it was made based on specimens collected in completely different climatic conditions (Italy, Mediterranean climate) and it is doubtful. Specimens found in the present study correspond well to the Italian redescription. In Poland *Mes. harmsworthi*-like specimens are common and widely distributed (Dastych 1988), but based on figures presented by Dastych (1988) it is obvious that they belong to at least two different species from the *harmsworthi* group.
25. *Mes.* sp.	2 (3), 6 (2), 72 (2), 77 (17), 96 (1), 102 (10), 104 (11), 108 (4)	The accurate identification of the species was not possible due to lack of eggs.
26. *Mesocrista revelata* Gąsiorek et al., 2016	48(2), 66(1), 69(5), 71(5), 76(1), 79(2), 80(1)	This is the fourth report of this recently described species in Poland; however, it is highly probable that most or even all reports of *Mec. spitzbergensis* (Richters, 1903) in Poland should be considered as *Mec. revelata* (Gąsiorek et al., 2016). In this situation, in Poland the species is probably widely distributed, strongly connected with coniferous forests
27. *Milnesium beasleyi* Kaczmarek et al., 2012	36 (5), 63 (2)	This is the first report of this species beyond the type locality in Turkey (Kaczmarek et al., 2012). **Species new for the Polish fauna.**
28. *Mil. dornensis* Ciobanu et al., 2015	4 (5), 21 (4), 34 (1), 46 (5), 48 (3), 68 (6), 69 (1), 80 (1), 91 (2), 98 (1), 100 (2), 101 (60), 106 (1), 110 (2)	The species was known only from Romania (type locality) and recently reported as *Mil.* cf. *dornensis* from Tunisia (Gąsiorek et al. 2017). **Species new for the Polish fauna**.
29. *Mil. granulatum* Ramazzotti, 1962	48 (1)	Species described from Chile, but also reported from Argentina, Colombia, Italy, Romania, and USA (Bartels et al., 2014; Ciobanu et al., 2014; Roszkowska et al., 2016). **Species new for the Polish fauna**.
30. *Mil. t. tardigradum* Doyère, 1840	10 (20), 11 (11), 26 (8), 27 (4), 29 (5), 34 (8), 35 (4), 36 (10), 39 (14), 42 (1), 43 (20), 44 (21), 49 (10), 51 (11), 54 (2), 56 (14), 61 (135), 62 (3), 92 (14), 97 (1)	This species belongs to a cosmopolitan genus (e.g., Michalczyk et al., 2012a, 2012b). In the past, *Mil. t. tardigradum* sensu stricto was reported almost everywhere (McInnes, 1994), but its range is probably much more restricted. However, at present, its true distribution is unknown. In Poland it was also reported from many localities (Dastych, 1988), but most of the reports are old and it is obvious that some of them belong to other members of the genus. Even in the present study a few different species from this genus were found, i.e. *Mil.*, *Mil. dornensis*, and *Mil. granulatum*.

Table 1. (Continued).

Taxon	Samples	Remarks
31. *Minibiotus intermedius* (Plate, 1888)	18 (1), 21 (8), 66 (9), 69 (1), 72 (5), 75 (1), 77 (48+3), 88 (1), 90 (20), 102 (3), 104 (14), 105 (1), 106 (2), 110 (1), 111 (3)	Described from Chile (Plate, 1888), *M. intermedius* was considered cosmopolitan for many years (McInnes, 1994); however, modern taxonomy has shown that it is a species complex (Claxton, 1998). In Poland common and widely distributed (Dastych, 1988).
32. *Paramacrobiotus* cf. *richtersi*	3 (10+13), 6 (1), 7 (10+4), 11 (48+25), 29 (1+6), 40 (0+1), 42 (10+8), 44 (25+1), 48 (1), 57 (5+3), 58 (9+1), 62 (1), 83 (1), 111 (1)	*Paramacrobiotus richtersi* (Murray, 1911) has uncertain diagnosis and distribution, and it belongs to a widely distributed species complex (unpublished data). Due to the uncertain diagnosis of this species it is not possible to identify collected specimens to the species level. In Poland the taxon is found in many localities (Dastych, 1988), but most of the reports are old and it is obvious that some of them belong to other members of the group.
33. *Ramazzottius oberhaeuseri* (Doyère, 1840)	17 (35+7), 19 (70+1), 36 (43+1), 39 (25+11), 56 (23+9), 59 (12+2), 61 (11+5), 100 (39+2)	*Ramazzottius oberhaeuseri* is a species complex with a cosmopolitan distribution (McInnes, 1994; Pilato et al., 2013), but most of the records of this species need confirmation. Very common and numerous xerophilous species, widely distributed in Poland (Dastych, 1988).
34. *Ram. subanomalus* (Biserov, 1985)	61 (11+1)	Species known from Poland (Wielkopolska Province), Russia, and Turkmenistan (McInnes, 1994; Biserov, 1999; Stec et al., 2016). This is the second report of this species in Poland.
35. *Ram.* sp. 1	9 (33), 10 (8), 13 (2), 23 (4), 29 (2), 30 (1), 31 (30), 35 (17), 43 (8), 44 (2), 49 (5), 54 (2), 55(1), 62 (1), 63 (69), 64 (2), 72 (1), 87 (3), 101 (17)	The accurate identification of this species with sculptured cuticle was not possible due to lack of eggs. It is possible that most of the specimens belong to *Ram. oberhaeuseri* or *Ram. subanomalus*.
36. *Ram.* sp. 2	25 (1), 28 (3), 104 (1)	The accurate identification of this species with smooth cuticle was not possible due to lack of eggs.
37. *Tenuibiotus willardi* (Pilato, 1977)	7 (20+4)	Species known from several Arctic, sub-Arctic, and mountainous Holarctic localities (McInnes, 1994, but see also comments in Zawierucha et al., 2016). Xerophilous species, in Poland rare and not numerous but widely distributed (Dastych, 1988).
38. *Xerobiotus xerophilus* (Dastych, 1978)	5 (2), 11 (1), 16 (1), 51 (1)	Species known from Germany and Poland (type locality) (McInnes, 1994). Xerophilous species; in Poland extremely rare and connected with soil mosses (Dastych, 1988).

Cuénot, 1929; *Mac. sottilei* Pilato et al., 2012; *Mil. beasleyi*; *Mil. dornensis*; and *Mil. granulatum* Ramazzotti, 1962. Species that were found most often in the samples were *Hyp. pallidus* Thulin, 1911; *Mac. h. hufelandi*; *Mil. dornensis*; *Mil. t. tardigradum*; *Min. intermedius*; and *Par.* cf. *richtersi*. Almost all of them have rather wide distributions in Poland and Europe, but they also belong to the species complexes and their true geographic range is unknown. In the present study a few species very rare in Poland were also found: *Hyp. microps* Thulin, 1928; *Isohypsibius latiunguis* (Iharos, 1964); *Iso. lunulatus* (Iharos, 1966); *Mac. furcatus* Ehrenberg, 1859; *Mac. macrocalix* Bertolani and Rebecchi, 1993; *Mac. polonicus*; *Mac. vladimiri* (Bertolani et al., 2011); *Ram. subanomalus* (Biserov, 1985); *Tenuibiotus willardi* (Pilato, 1977); and *Xerobiotus xerophilus* (Dastych, 1978). However, some of them (especially from the *hufelandi* group) probably have wider distribution and their present limited distribution in Poland is an artifact caused by incorrect identification in the past (in older publications, all of them were considered as *Mac. hufelandi* sensu stricto). We have not found differences in the number of species between years; thus,

Table 2. Taxa (in alphabetic order) from different areas (BTNP – Bory Tucholskie National Park, ZLP – Zaborski Landscape Park, SP – surroundings of the parks).

Area	Taxa
BTNP	*Adr. prorsirostre*, *Adr. scoticum*, *Dip. p. pingue*, *Hyp. convergens*, *Hyp. microps*, *Hyp. pallidus*, *Iso. prosostomus*, *Mac. h. hufelandi*, *Mac. hufelandi* gr., *Mac. macrocalix*, *Mac. vladimiri*, *Mes.* sp., *Mec. revelata*, *Mil. dornensis*, *Min. intermedius*, *Par.* cf. *richtersi*, *Ram.* sp. 1, *Ten. willardi*
ZLP	*Adr. prorsirostre*, *Ast. trinacriae*, *Dip. p. pingue*, *Ech. blumi*, *Ech. testudo*, *Hyp.* cf. *allisoni*, *Hyp. convergens*, *Hyp. dujardini*, *Hyp. microps*, *Hyp. pallidus*, *Hyp. scabropygus*, *Iso. lunulatus*, *Iso. prosostomus*, *Mac. hufelandi*, *Mac. h. hufelandi* gr., *Mac. macrocalix*, *Mac. sottilei*, *Mac. vladimiri*, *Mes. h. harmsworthi*, *Mes.* sp., *Mil. dornensis*, *Mil. tardigradum*, *Mil. intermedius*, *Par.* cf. *richtersi*, *Ram. oberhaeuseri*, *Ram.* sp. 1, *Ram.* sp. 2, *Xer. xerophilous*
SP	*Ast. trinacriae*, *Dip. p. pingue*, *Ech. testudo*, *Hyp. dujardini*, *Hyp. microps*, *Hyp. pallidus*, *Iso. latiunguis*, *Iso. prosostomus*, *Iso. sattleri*, *Mac. furcatus*, *Mac. h. hufelandi*, *Mac. hufelandi* gr., *Mac. polonicus*, *Mac. sottilei*, *Mac. vladimiri*, *Mes. h. harmsworthi*, *Mes. harmsworthi* gr., *Mec. revelata*, *Mil. beasleyi*, *Mil. dornensis*, *Mil. granulatum*, *Mil. tardigradum*, *Par.* cf. *richtersi*, *Ram. oberhaeuseri*, *Ram. subanomalus*, *Ram.* sp. 1, *Ram.* sp. 2, *Xer. xerophilous*

the results suggest that the tardigrade communities in all habitat types are stable. On the other hand, no influence of substrate, habitat, or protective type on species richness indicated that the community of tardigrades probably depends on more detailed environmental components, which are not detected by standard methods.

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