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Short communication

Testate Amoeba Diversity of a Poor Fen on Mineral Soil in the Hilly Area of Central Honshu, Japan

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Abstract. We present a short note on the species composition of testate amoebae in a poor fen on mineral soil near the Pacific Coast in the hilly area of Central Honshu, Japan. In total 45 species and subspecific taxa belonged to 21 genera and 14 families of testate amoebae were recorded. Eight species and nine subspecies are newly recorded from Japan. However, most species from the list can be considered as distributed worldwide and associated mostly to oligotrophic/acid *Sphagnum* conditions.

Keywords: testate amoebae, mineral soil, fen, Japan, *Sphagnum*, Rhizaria, Amoebozoa, Stramenopiles.

INTRODUCTION

Peatland scientists have paid relatively little attention to poor fens, especially in Asian countries. Poor fens are peatlands in which the vegetation is fed by rain as well as minerotrophic water (surface- or ground-water-fed), the water possesses a low pH and is poor in nutrients (Rydin and Jeglum 2006). Vegetation of poor fens consists of vascular plants (e.g. *Carex* spp.) and *Sphagnum* spp. In Central to Western Honshu, Japan, peat mosses (*Sphagnum* spp.) usually occur on mineral soil with little or no peat accumulation. Such fens, usually dominated by *Rhynchospora* spp., *Eriocaulon* spp. and *Utricularia* spp., are defined as wet grasslands on mineral soil (Hada 1984; Tomita 2010).

Testate amoeba assemblages on *Sphagnum* have been well studied in bogs (e.g., Jassey *et al*. 2012; Lamentowicz *et al.* 2013; Qin *et al.* 2013; Marcisz *et al*. 2014; Amesbury *et al*. 2016) and minerotrophic peatlands (e.g. Lamentowicz *et al*. 2010, 2011; Jassey *et al*. 2014), but there appear not to be any studies of the testate amoebae of *Sphagnum* fens on mineral soil, probably because this kind of habitat is rare in Europe or other parts of the world.

This is the first report of a testate amoeba assemblage on *Sphagnum* in a hillside-slope type poor fen

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developed on mineral soil. We aim to contribute to the general understanding of such ecosystems, which have been neglected but are not rare in Central to Western Honshu. Our study is also the next step towards a better understanding of the biogeography of testate amoebae.

MATERIALS AND METHODS

The sampling site is a slope type of a poor fen dominated by *Sphagnum palustre* among an open forest of *Cryptomeria japonica* (Thunb. ex L.f.) D.Don, located in Koka City, Shiga prefecture in West-Central Honshu, Japan (34.917°N, 136.083°E), at an altitude of 281 m (Fig. 1). The samples were collected on 20 February, 2012 by S.D. Shimano. Three samples of the *Sphagnum* moss of the uppermost 5 cm were sampled in several points of the fen placed 3 meters apart from each other. Testate amoebae were extracted from a 5 cm^3 quota taken from each sample irrespectively of the nature of the habitat. Samples for the analysis were prepared using a method based on wet sieving. 1 cm^3 of the sample was soaked in water for 24 hours, stirred, filtered at 0.5 mm, the suspension left to settle for a further 24 hours, and the supernatant decanted off following Mazei and Chernyshov (2011). The specimens were studied using light microscopy. The higher taxa of testate amoeba were arranged according to Meisterfeld (2000a, 2000b), Adl *et al*. (2012) and Siemensma (2016) and annotations based on Shimano and Miyoshi (2008) were

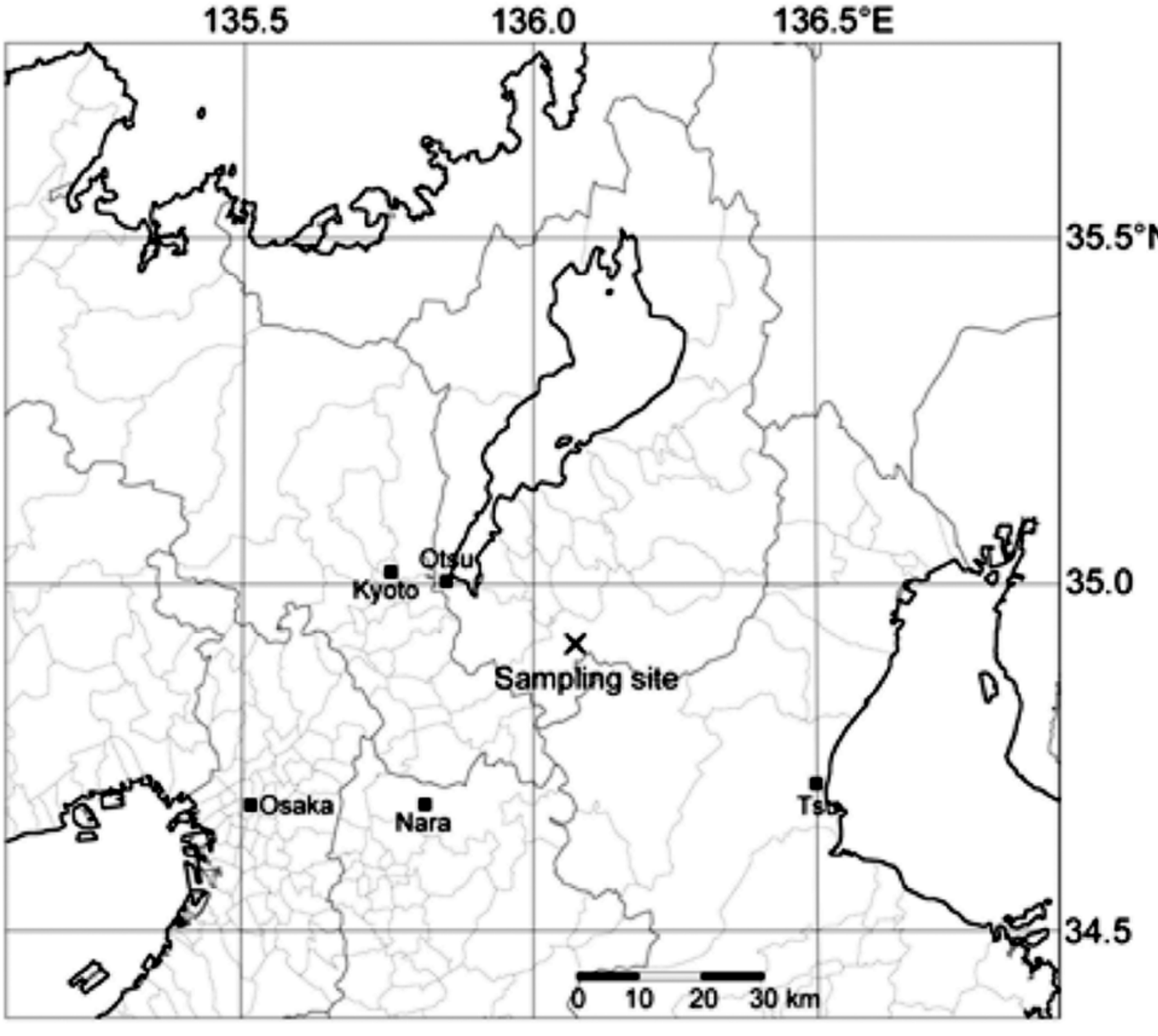

Fig. 1. Location of the poor fen on mineral soil as sampling site (×).

added to species list. A species accumulation curve was constructed based on rarefaction procedure performed in PRIMER 6.1.6 (Clarke and Gorley, 2006). The maximum expected number of species was calculated in PRIMER 6.1.6 by the nonparametric Chao2 method, which takes into consideration the theoretical number of expected rare species (Clarke and Warwick 2001).

RESULTS

In totally 45 species and subspecific taxa from 21 genera, 14 families of testate amoebae were recorded. 8 species and 9 subspecific taxa are newly recorded from Japan (Table 1).

The results of a rarefaction procedure show that the species-accumulation curve does not reach a plateau (Fig. 2). The curve is well fitted ($R^2 = 0.99$) by the power function $S = 22.52N^{0.67}$ (where S is number of species revealed, N is number of samples investigated). A low value of the power coefficient (0.67) reflects an unsaturated community with 22.52 as an average number of taxa per sample. Expected total number of species (Chao2) in the studied area is estimated as 72.

DISCUSSION

Among the different types of poor fens on mineral soil, a "soligenous sloping fen" is frequently observed that develops on a slope watered by a divergent flow of water seeping from the upper part of the slope (Lamentowicz *et al.* 2010; Tomita 2010). Such fens are usually situated atop granite or rhyolite (Hada 1984; Tomita 2010), and their vegetation is often dominated by *Sphagnum palustre* L., despite they are not exclusively ombrotrophic (rain-fed) and strongly acidic conditions typical of *Sphagnum* bogs under open forest of Japanese cedar *Cryptomeria japonica* (Thunb. ex L.f.) D.Don. Such fens appear not to be covered by the classification system of wetlands developed in Europe (cf. Succow and Jeschke 1986; Rydin and Jeglum 2006; Hotes 2007).

Based only on three samples from one sampling date, already 45 taxa of testate amoebae were found, of which 17 taxa are new records for Japan. This finding reveals an unexpected high diversity for this type of poor fen from Japan. A study conducted in several *Sphagnum*-dominated peatlands from north-western Poland revealed 52 species from 44 samples (Lamentowicz and Mitchell 2005).

Most of the species represent acidic conditions of a *Sphagnum* bog. The testate amoebae species composition resembles a typical ombrotrophic bog, despite they are located in the mesotrophic conditions. There are mixotrophic species present such as *Hyalosphenia papilio*, representing more open parts of the *Sphagnum* patches (Payne *et al*. 2016). There were no clear indicators of a rich fen, so we can assume that the habitat is generally poor in nutrients, although *Centropyxis* spp. might represent a higher nutrient status. As this issue is very interesting, our next study will better characterize community structure and abundance of each species.

In the present study, all determined genera and almost all species are characterized by uniquely defined tests, thus a misidentification can be excluded. However, *Valkanovia elegans* cannot be distinguished from *Assulina muscorum* (type 4), but *Valkanovia* can inhabit both upper and lower horizons, whereas *Assulina* and its forms lives exclusively in the upper horizon layer (Schönborn and Peschke 1990). All species from the list can be considered as cosmopolitan. Most likely, this fact reflects a low level of local geographic specificity for such non-zonal ecosystems like peatlands. On the opposite, in soils of zonal ecosystems in the Imperial Palace area, Tokyo, Shimano *et al*. (2014) found two species with limited geographical distribution (*Centropyxis latideflandriana* and *Planhoogenraadia daurica*), thus more species with geographical limitation can be expected for the future in Japan. Moreover, there will be a considerable amount of new or unrecorded testate amoeba taxa for Japan, the assumption is borne out by recent research papers (Aoki *et al*. 2007; Bobrov

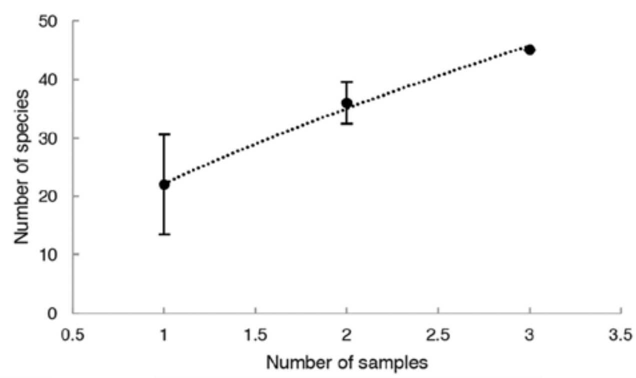

Fig. 2. Sample-based testate amoeba species accumulation curve for all three samples collected in the sampling site of the poor fen on mineral soil. The bars are standard deviations.

Table 1. List of taxa from the Tokai Hilly Land Spring-fed Mires (* – new to Japan).

AMOEBOZOA Lühe, 1913 emend. Cavalier-Smith, 1998
ORDER ARCELLINIDA Kent, 1880

FAMILY ARCELLIDAE Ehrenberg, 1843
Genus *Arcella* Ehrenberg, 1832
1. *Arcella discoides* Ehrenberg, 1871
2. *Arcella discoides foveosa* Playfair, 1918 *
3. *Arcella* sp.

FAMILY DIFFLUGIIDAE Wallich, 1864
Genus *Difflugia* Leclerc, 1815
4. *Difflugia bacillifera* Pénard, 1890
5. *Difflugia globulosa* Dujardin, 1837
6. *Difflugia globulus* (Ehrenberg, 1848)
7. *Difflugia oblonga* Ehrenberg, 1838

Genus *Wailesella* Deflandre, 1928
8. *Wailesella eboracensis* (Wailes and Pénard, 1911) Deflandre, 1928

FAMILY CENTROPYXIDAE Jung, 1942
Genus *Centropyxis* Stein, 1857
9. *Centropyxis aculeata* (Ehrenberg, 1838) Stein, 1857
10. *Centropyxis aculeata dentistoma* Decloître, 1949 *
11. *Centropyxis aculeata minima* van Oye, 1938 *
12. *Centropyxis constricta* (Ehrenberg, 1843) Deflandre, 1929
13. *Centropyxis sylvatica* (Deflandre, 1929) Bonnet & Thomas, 1955

FAMILY PLAGIOPYXIDAE Bonnet and Thomas, 1960
Genus *Bullinularia* Deflandre, 1953
14. *Bullinularia indica* (Pénard, 1907) Deflandre, 1953 *

FAMILY HYALOSPHENIIDAE Schultze, 1877
Genus *Hyalosphenia* Stein, 1859
15. *Hyalosphenia insecta* Harnisch, 1938 *
16. *Hyalosphenia papilio* (Leidy, 1874) Leidy, 1879

FAMILY HELEOPERIDAE Jung, 1942
Genus *Heleopera* Leidy, 1879
17. *Heleopera petricola amethystea* Pénard, 1899 *
18. *Heleopera rectangularis* Bonnet, 1966 *

FAMILY NEBELIDAE Taránek, 1882
Genus *Nebela* Leidy, 1874
19. *Nebela barbata* (Leidy, 1874)
20. *Nebela marginata* Pénard, 1902 *
21. *Nebela parvula* Cash, 1909
22. *Nebela* sp. 1

Genus *Porosia* Jung, 1942
23. *Porosia biggibosa* (Pénard, 1890) Jung, 1942

Genus *Argynnia* Vucetich, 1974
24. *Argynnia* sp.

Genus *Physochila* Jung, 1942
25. *Physochila griseola* (Pénard, 1911) Jung, 1942

FAMILY CRYPTODIFFLUGIIDAE Jung, 1942
Genus *Cryptodifflugia* Pénard, 1890
26. *Cryptodifflugia oviformis* Pénard, 1890
27. *Cryptodifflugia oviformis fusca* Bonnet & Thomas, 1955 *

FAMILY PHRYGANELLIDAE Jung, 1942
Genus *Phryganella* Pénard, 1902
28. *Phryganella acropodia australica* Playfair, 1917 *

RHIZARIA Cavalier-Smith, 2002
ORDER EUGLYPHIDA Copeland, 1956

FAMILY EUGLYPHIDAE Wallich, 1864
Genus *Euglypha* Dujardin, 1841
29. *Euglypha compressa glabra* Cash, Wailes & Hopkinson, 1915 *
30. *Euglypha cuspidata* Bonnet, 1959 *
31. *Euglypha laevis* Perty, 1849
32. *Euglypha tuberculata* Durjardin, 1841

FAMILY ASSULINIDAE Lara et al., 2007
Genus *Assulina* Leidy, 1879
33. *Assulina muscorum* Greeff, 1889
34. *Assulina seminulum* (Ehrenberg, 1848) Leidy, 1879
35. *Assulina scandinavica* Pénard, 1890 *

Genus *Placocista* Leidy, 1879
36. *Placocista spinosa* (Carter, 1865) Leidy, 1879

Genus *Valkanovia* Tappan, 1966
37. *Valkanovia elegans* (Schönborn, 1964) Tappan, 1966

FAMILY SPHENODERIIDAE Chatelain et al., 2013
Genus *Sphenoderia* Schlumberger, 1845
38. *Sphenoderia splendida* (Playfair, 1918)

FAMILY TRINEMATIDAE Hoogenraad & de Groot, 1940
Genus *Trinema* Dujardin, 1841
39. *Trinema complanatum* Pénard, 1890
40. *Trinema lineare* Pénard, 1890
41. *Trinema lineare minuscula* Chardez, 1971 *

Genus *Corythion* Taránek, 1881
42. *Corythion dubium* Taránek, 1882
43. *Corythion dubium orbicularis* Pénard, 1911 *
44. *Trachelocorythion pulchellum* (Pénard, 1890) Bonnet, 1979 *

STRAMENOPILES Patterson 1989, emend. Adl *et al.* 2005
ORDER AMPHITREMIDA Poche1913

FAMILY AMPHITREMIDAE Poche, 1913
Genus *Amphitrema* Archer, 1869
45. *Amphitrema wrigthianum* Archer, 1869

et al. 2012; Shimano *et al*. 2014; Bobrov and Kosakyan 2015) and bibliographies (Shimano and Miyoshi 2008). These few studies already resulted in more than 350 species, including three species new for science (Bobrov *et al*. 2012; Bobrov and Kosakyan 2015).

In Supplemental Table A, some environmental data are given. pH as a major environmental factor is still in the range as discussed by Lamentowicz and Mitchell (2005).

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Supplemental Table. A list of data of the water sample and environments, collected in the sampling site of the Tokai Hilly Land Spring-fed Mires

Environmental factors	value
Water temperature ℃	8.0
pH	5.7
EC mS/m	2.5
NH_4-N μM	0.54
NO_2-N μM	0.20
NO_3-N μM	6.07
PO_4-P μM	0.02
TDN μM	13.09
TDP μM	0.10
Si mg/L	3.693
F mg/L	N.D.
Cl mg/L	1.576
Br mg/L	N.D.
SO_4 mg/L	1.498
Li mg/L	0.008
Na mg/L	1.087
K mg/L	0.380
Mg mg/L	0.038
Mn mg/L	N.D.
Ca mg/L	0.043

Electric conductivity (EC) and pH were checked at site with a B-173 conductivity meter (Horiba, Kyoto, Japan) and a B-212 pH meter (Horiba, Kyoto, Japan), respectively. Water temperature was measured with an alcohol thermometer. Water samples for water analyses in the laboratory were filtered by syringe-driven filter units with 0.22 μm pore size hydrophilic Polyethersulfone (PES) membrane. Each water sample was partly kept in a refrigerator (for SRSi determination) and the rest in a freezer (for the other analyses). In the laboratory, major anions and cations were analyzed by ion column chromatography (DX-AQ: Nippon Dionex, Osaka, Japan). SRSi, SRP, NH_4-N, NO_2-N, and NO_3-N were colorimetrically determined using an autoanalyzer (AACS-II: Bran + Luebbe, Tokyo, Japan).