Testate amoeba diversity of the Tokai Hilly Land Spring-fed Mires, a group of poor fens on the Pacific Coast of Central Honshu, Japan

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Abstract We present a short note on the species composition of testate amoebae in Tokai Hilly Land Spring-fed Mires, a group of poor fens. Totally 39 species and 6 subspecies 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 fens, new record to Japan, *Sphagnum*, Rhizaria, Amoebozoa, Stramenopiles

Running title: Testate amoeba diversity of poor fens, Japan

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 minerotrophic water (surface- or ground-water-fed), the water possesses a low pH and is poor in nutrients. 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 by Tomita (2010). They have been neglected by researchers despite their occurrence near human dwellings, but recently their ecological importance have been increasingly recognized. The Tokai Hilly Land Spring-fed Mires, a group of poor fens on the Pacific coast of Central Honshu, were inscribed as a registered wetland under the Ramsar Convention in July, 2012. This was the first registration of a poor fen on mineral soil as a Ramsar wetland in Japan. In addition, poor fens on mineral soil are potentially interesting as geological and archaeological study sites, because most of them seem to have been strongly affected by human activities (Tomita 2010). Basic ecological knowledge about poor fens in Japan is, however, still scant except concerning the vegetation itself (e.g., Hada 1984; Tomita 2010).

Among the different sorts of poor fens on mineral soil, a "hillside-slope type" is frequently observed that develops on a slope watered by a divergent flow of spring water seeping from the upper part of the slope (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 the lack of the ombrotrophic (rain-fed) and strongly acidic conditions typical of *Sphagnum* bogs under openforest of Japanese cider *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; Hotes 2007).

Testate amoebae (Rhizaria, Amoebozoa and Stramenopiles; Adl et al. 2012) commonly inhabit mosses including *Sphagnum*. They are known as good environmental indicators of hydrology (Mitchell et al. 2008), acidity and calcium concentration (Opravilová and Hajek 2006), Pb-loading (Nguyen-Viet et al. 2007, 2008), and other environmental parameters (e.g., Wanner 1999). In addition, they are also useful as bioindicators of past environmental changes because their thecae often remain in the sediment as fossils and allow species-level identification (Tolonen 1986; Payne et al. 2012; Lamentowicz et al. 2015). Testate amoeba assemblages on *Sphagnum* have been well studied in bogs (e.g., Jassey et al. 2011; 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.

This is the first report of testate amoeba assemblage on *Sphagnum* in a hillside-slope type poor fen developed on mineral soil. We aim to contribute to the ecological understanding of such fens, which have been neglected but are not rare in Central to Western Honshu. The present study also provides a basis for paleoenvironmental studies of such fens as sites of archaeological interest.

Materials and methods

The sampling site was a hillside-slope type poor fen dominated by *S. palustre* among an open forest of *Cryptomeria japonica* (Thunb. ex L.f.) D.Don, located in Koka City, Shiga prefecture in west-central Honsyu, Japan (34.917°N, 136.083°E), at an altitude of 281 m (Fig. 1). The samples were collected in 20 February, 2012 by Satoshi Shimano (the author). The *Sphagnum* moss of the uppermost 5 cm were sampled in several points of the fen. Testate amoebae were extracted from a 5 cm^3 quota taken from each sample irrespectively of the nature of the habitat in accordance with the method described in Mazei and Chernyshov (2011). The specimens were studied using light microscopy. The higher taxa of testate amoeba were arranged according to Meisterfeld (2000a, b), Adl *et al*. (2012) and Siemensma (2016) and annotations based on Shimano and Miyoshi (2008) were added to species list.

Results

Species list

Totally 40 species and 6 subspecific taxa from 21 genera, 14 families of testate amoebae were recorded. 8 species and 9 subspecific taxa are newly recorded from Japan.

List of taxa (* – new to Japan)

AMOEBOZOA Lühe, 1913 emend. Cavalier-Smith, 1998
TUBULINEA Smirnov, Nassonova, Berney, Fahrni, Bolivar & Pawlowski, 2005
TESTACEALOBOSIA de Saedeleer, 1934
ORDER ARCELLINIDA Kent, 1880
SUBORDER ARCELLINA Haeckel, 1894

FAMILY ARCELLIDAE Ehrenberg, 1843

Genus *Arcella* Ehrenberg, 1832

1. *Arcella discoides* Ehrenberg, 1871
2. *Arcella discoides foveosa* Playfair, 1918 *
3. *Arcella* sp.

SUBORDER DIFFLUGINA Meisterfeld, 2000

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. *Physochilla griseola* (Pénard, 1911) Jung, 1942

SUBORDER PHRYGANELLINA Bovee, 1985

FAMILY CRYPTODIFFLUGIIDAE Jung, 1942

Genus *Cryptodifflugia* Pénard, 1890

26. *Cryptodifflugia oviformis* Pénard, 1890
27. *Cryptodifflugia oviformis fusca* Bonnet & Thomas, 1955 *

SUBORDER PHRYGANELLINA Bovee, 1985

FAMILY PHRYGANELLIDAE Jung, 1942

Genus *Phryganella* Pénard, 1902

28. *Phryganella acropodia australica* Playfair, 1917 *

RHIZARIA Cavalier-Smith, 2002

CERCOZOA Cavalier-Smith, 1998

SILICOFILOSEA Adl *et al.*, 2005

ORDER EUGLYPHIDA Copeland, 1956

SUBORDER EUGLYPHINA Kosakyan et al., 2016

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
LABYRINTHULOMYCETES Dick, 2001
ORDER AMPHITREMIDA Poche1913
FAMILY AMPHITREMIDAE Poche, 1913
Genus *Amphitrema* Archer, 1869
45. *Amphitrema wrigthianum* Archer, 1869

Discussion

Based on only a few samples from one sampling date, already 45 taxa of testate amoebae were found, of which 17 species or subspecies 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 *Sphagnum* bog. Testate amoebae species composition resembles typical ombrotrophic bog, despite they are located in the mesotrophic conditions. There are mixotrophic species present such as: *Archerella flavum* and *Hyalosphenia papilio* that represent 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 of nutrients, however taxa *Centropyxis* spp. - might representing a higher nutrient status. Next study will better characterize communities 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). Most species from the list can be considered as cosmopolitan. However, 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 et al. 2012; Shimano et al. 2014; Bobrov and Kosakyan 2015) and bibliographies

(Shimano and Miyoshi 2008, Shimano et al., in prep.). 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 suppl. Tab. 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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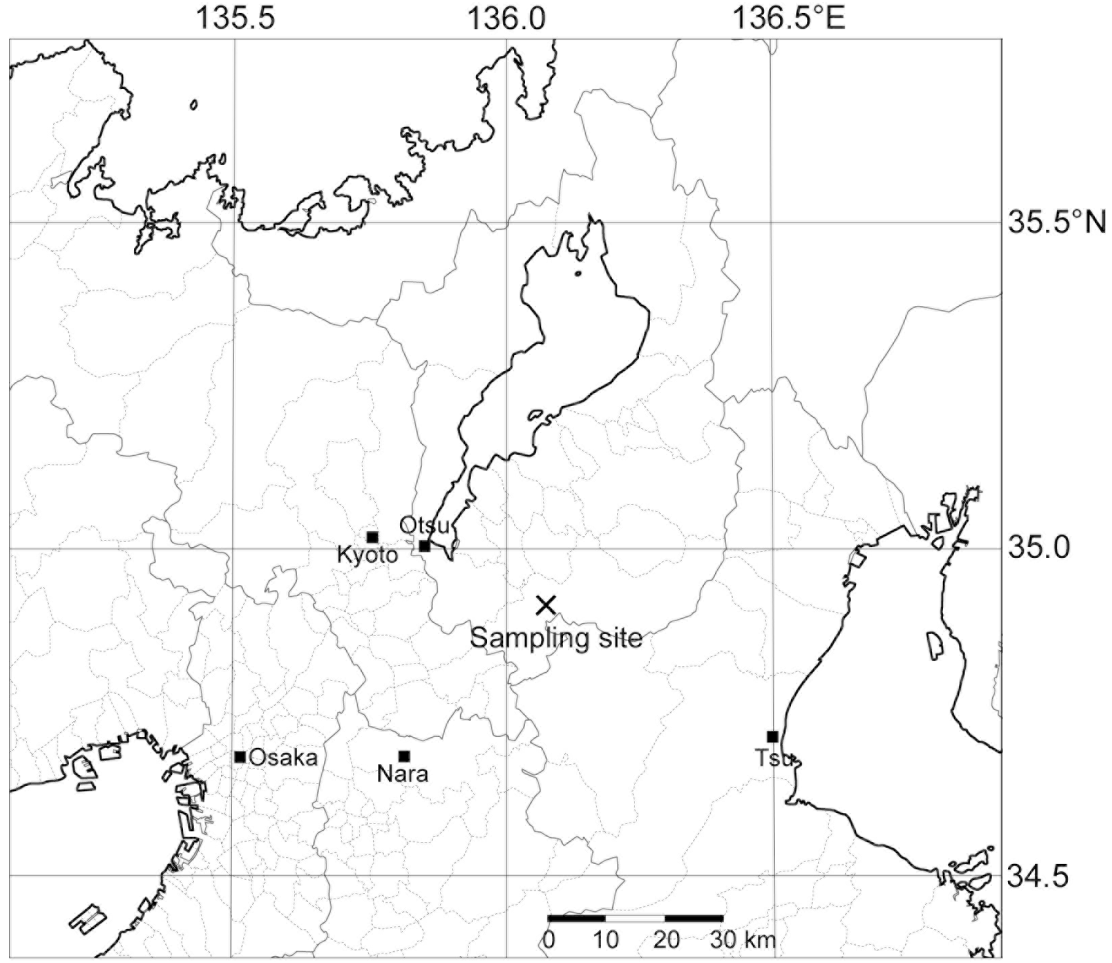

Fig. 1 Location of the Tokai Hilly Land Spring-fed Mires as sampling site (×)

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.
SO4 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, NH4-N, NO2-N, and NO3-N were colorimetrically determined using an autoanalyzer (AACS-II: Bran + Luebbe, Tokyo, Japan).