SOIL ORGANISMS 89 (3) · December 2017 pp. 151–155

Testate amoebae collected from moss on urban buildings with different age, height and distance to a possible source habitat – are there obvious colonization patterns?

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Received 26 June 2017 | Accepted 5 October 2017
Published online at www.soil-organisms.de 1 December 2017 | Printed version 15 December 2017

Abstract

Testate amoebae cultures were analyzed from moss samples collected at seven buildings of different age, height, and distance to a putative source habitat in Sendai City, Japan. In total, 13 amoeba taxa colonized buildings. We discussed causes as air currents, animals, or human activities. Neither amoebae from adjacent buildings, nor taxa from buildings with the same age or moss species were grouped by a cluster analysis, pointing to rather stochastic colonization patterns.

Keywords terrestrial protists | resource of urban biodiversity | urban habitat colonization | moss on the roof | Japan

1. Introduction

Recent studies demonstrate that the very first phase of primary succession or community assembly on land is dominated by small heterotrophic eukaryotes, facilitating the successive establishment of plants by e.g. improving the availability of nutrients (Hodkinson et al. 2002). In this context, immigration by air plays a pivotal role (e.g., Hodkinson et al. 2002, Wanner et al. 2015). Aquatic mosses (e.g. *Sphagnum* sp.) as well as terrestrial mosses harbour numerous taxa of testate amoebae. Even Leidy (1877) reported findings of testate amoebae in moss, which he collected from an apple tree eight feet from the ground. Recently testate amoeba communities in mosses from urban areas (mainly on walls and roofs) were studied in Hanoi [Vietnam, *Barbula indica* (Hook.)] (Nguyen-Viet et al. 2007) and Besançon [France, *Tortula ruralis* (Hedw.)] (Nguyen-Viet et al. 2004).

The aim of our study was to analyze testate amoebae in mosses from buildings of different height, age, and distance to a possible source habitat. These studies were conducted in the frame of a bachelor thesis (YO, SS) in 2007. Later on, primary data from this thesis were re-analyzed and discussed in the context of initial terrestrial colonization patterns (MW).

2. Materials and methods

The investigation sites are located in Sendai City, Japan. Seven buildings of different age, height, and location (Figs 1, 2) were selected in 2007. From these buildings, moss samples containing testate amoebae were taken (Tab. 1). To facilitate detection of rare testate amoebae, cultures of moss samples were incubated for

ISSN 1864-6417 (print) · **ISSN** 2509-9523 (digital)

50 days: sterilized 9 cm Petri dishes were provided with 1.5% agar (without any ingredients) and inoculated with a moss sample under the clean bench. Two mL of sterilized ion exchanged water was added as an overlay. After incubation, five aliquots (100 µl water suspension each) per culture were removed for microscopy. The amoebae taxa were identified directly using an inverted microscope (Nikon Eclipse TE300, 100-400x). Since prevailing winds blow from SW to NE in Japan, a forest nearby (distance 1.5 to 5.6 km) is considered as terrestrial source habitat for aerial transportation of testate amoebae (Fig. 1). Cluster analysis of testate amoeba taxa (MVSP software, Kovach Computing Services, Wales, UK) used unweighted pair group method of average linkage (UPGMA) and Sørensen's coefficient.

3. Results

In total, 13 testate amoeba taxa were found in seven buildings/locations, of which six belonged to the genus *Euglypha*. *E. rotunda* Wailes & Penard 1911 and *E.* sp. 2 occurred in all samples (Tab. 1, 2). *Euglypha* sp. 1, 2, and 4 were most probably *E. rotunda*-like morphotypes, with a shell length of approx. 44, 42, and 67 µm, respectively. *Euglypha* sp. 3 was approx. 56 µm in length. A micrograph shows an individual with five long (20 µm) apical spines. Only photos from the initial work of 2007 are available, so no further species determination is possible.

The mosses *Bryum argenteum* Hedw. / *Brachymenium exile* (Dozy & Molk.) and *Bryum argenteum* / *Funaria hygrometrica* (Hedw.) harbored the lowest amount of testate amoebae taxa (Tab. 1). Cluster analysis (Fig. 2)

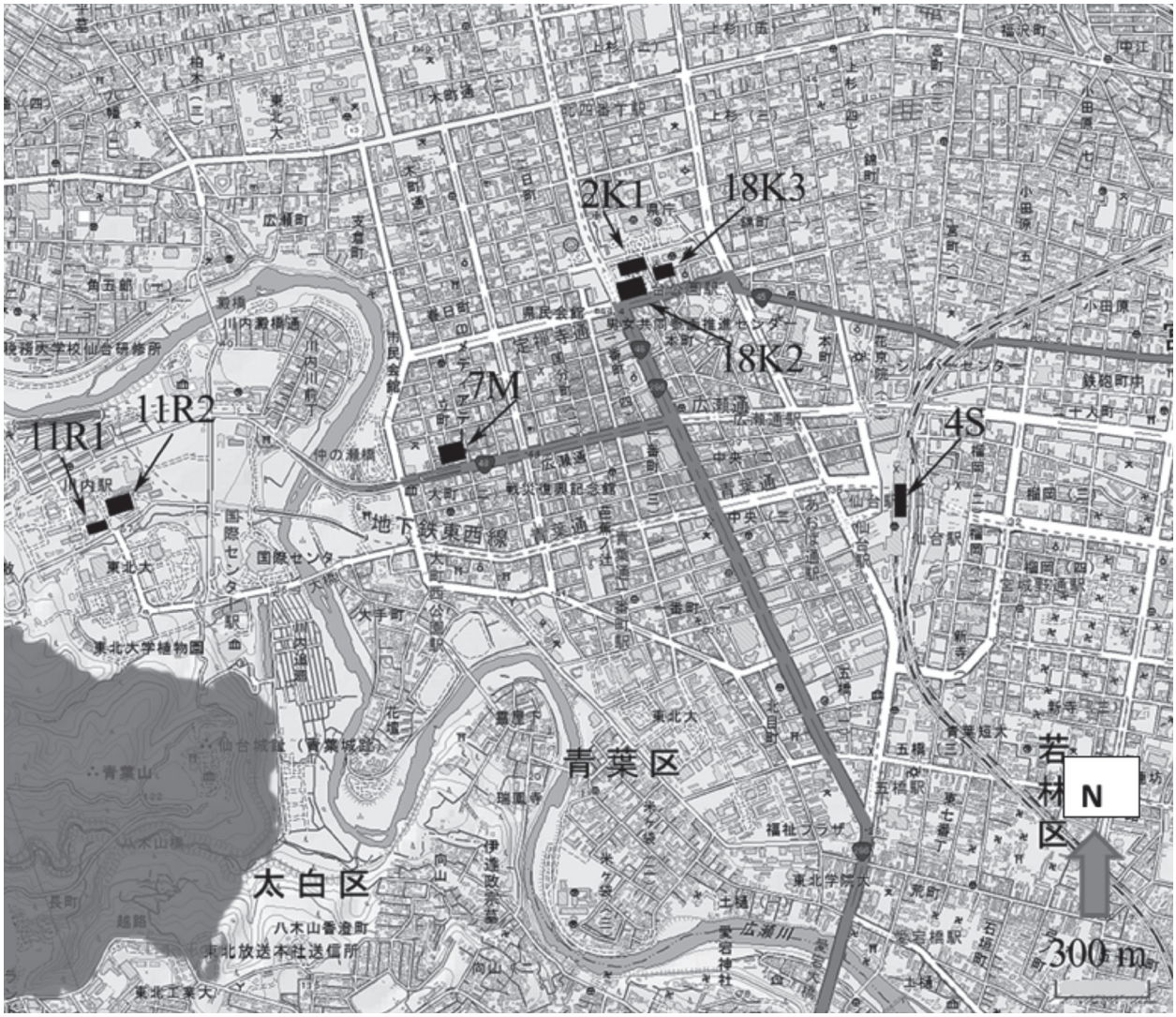
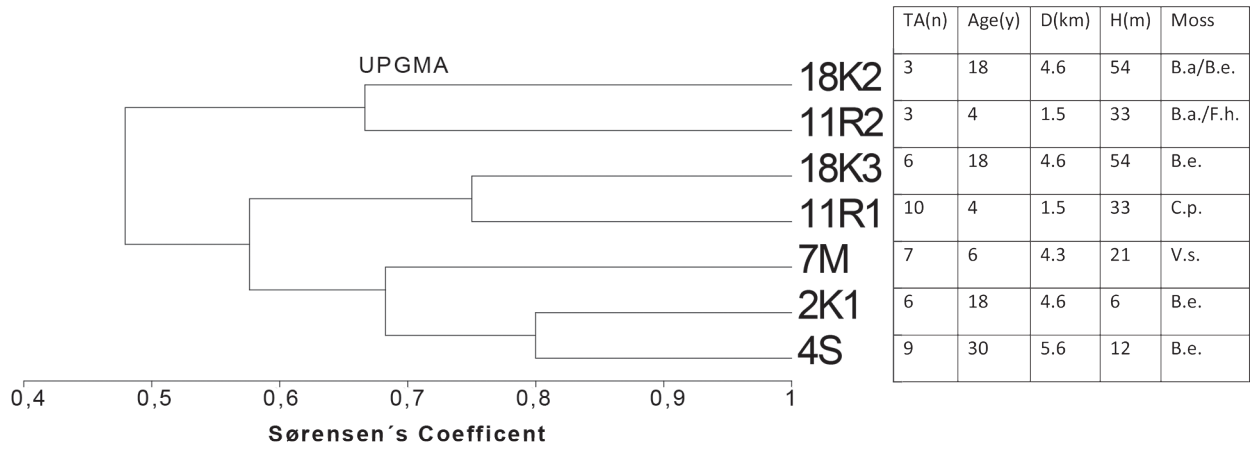


Figure 1. The sampling points for testate amoebae (TA) and mosses in Sendai City, Japan. The sampling codes (location: 'R', 'M', 'K', height (in floor numbers, 3 m each) and age of buildings, and distance from forest area) are given in Fig. 2. (Source: digital country fundamental chart of the Geospatial Information Authority of Japan). In Japan, main wind direction is from SW to NE. The putative amoeba source, a forest soil, is on the lower left corner of the map (darken). The area shown covers about 100 km^2.

Table 1. Testate amoebae taxa, cultivated from mosses collected at different buildings (see Fig. 1, 2).
1 = presence of taxon in culture, 0 = taxon not found in culture. Moss species: B.a. = *Bryum argenteum* Hedw., B.e. = *Brachymenium exile* (Dozy & Molk.), C.p. = *Ceratodon purpureus* (Hedw.), F.h. = *Funaria hygrometrica* (Hedw.), V.s. = *Venturiella sinensis* (Venturi).

	B.a. / B.e.	C.p.	B.a. / F.h.	V.s.	B.e.
n =	**1**	**1**	**1**	**1**	**3**
Arcella arenaria Greef 1866	0	1	0	1	0
Centropyxis plagiostoma terricola Bonnet & Thomas 1955	0	1	0	1	1
Centropyxis sphagnicola Deflandre 1929	0	1	0	0	1
Cyclopyxis eurystoma Deflandre 1929	1	1	0	0	1
Euglypha compressa Carter 1864	0	1	0	1	1
Euglypha rotunda Wailes & Penard 1911	1	1	1	1	1
Euglypha sp. 1	0	1	0	1	1
Euglypha sp. 2	1	1	1	1	1
Euglypha sp. 3	0	0	0	1	1
Euglypha sp. 4	0	1	0	0	1
Heleopera sylvatica Penard 1890	0	1	0	0	0
Trinema enchelys (Ehrenberg 1838)	0	0	0	0	1
Trinema lineare Penard 1890	0	0	1	0	1
n	**3**	**10**	**3**	**7**	**11**



TA(n)	Age(y)	D(km)	H(m)	Moss
3	18	4.6	54	B.a/B.e.
3	4	1.5	33	B.a./F.h.
6	18	4.6	54	B.e.
10	4	1.5	33	C.p.
7	6	4.3	21	V.s.
6	18	4.6	6	B.e.
9	30	5.6	12	B.e.

Figure 2. Cluster analysis (UPGMA, Sørensen's coefficient), testate amoebae taxa. The table next to the cluster explains the sampling points (see map in Fig. 1) given in the cluster diagram, characterized by numbers of testate amoebae (TA) taxa (n), age (in years) of building (sampling point), distance (D, in km) from a possible source habitat for testate amoebae (a forest in the SW of Sendai City, see Fig.1), and height (H, in meters) of the buildings (where the moss samples with TA were taken). The moss species found in the buildings/sampling points are B.a. = *Bryum argenteum* Hedw., B.e. = *Brachymenium exile* (Dozy & Molk.), C.p. = *Ceratodon purpureus* (Hedw.), F.h. = *Funaria hygrometrica* (Hedw.), V.s. = *Venturiella sinensis* (Venturi).

combined two sites / buildings with only three testate amoebae taxa (18K2, 11R2, presence of *B. argenteum*) and three sites / buildings with the lowest sampling height (6–21 m vs. 33–54 m). Neither testate amoebae from adjacent buildings (see Fig. 1), nor testate amoebae species from buildings with the same age clustered together. However, height of the collection site seems to have an effect on the amoebal composition.

4. Discussion

In the initial work conducted in 2007, moss samples collected from different buildings were used to cultivate testate amoebae to find out if there are testate amoebae at all. Therefore no mosses from the putative terrestrial source habitat were analyzed for testate amoebae communities. Later we re-analyzed this basic data set for the detection of immigrating testate amoebae. Culturing of the moss samples containing testate amoebae facilitated the detection of even very rarely occurring taxa, but amoebal densities were not interpretable any more. Moreover, some amoeba taxa (e.g., *Centropyxis* spp., *Cyclopyxis* spp.) need to collect mineral particles from the environment for shell building. In our cultures, these particles were only available from substrate attached to the inoculated moss plants. Thus, amoebae with self-synthesized shell platelets (e.g, *Euglypha* spp.) were probably more selected.

It remains unclear if the identity of moss species is an important driver of colonization patterns for testate amoebae. Different mosses may provide different micro-environmental conditions, e.g., with respect to moisture content. In our study, the lowest amoeba taxa numbers appeared where the moss *Bryum argenteum* was present. This moss occurs worldwide and is characteristic for N-rich urban habitats, tolerating high pollution levels and preferring high light intensity (Nebel & Philippi 2000). However, this moss was collected always together with an

Table 2. Testate amoebae taxa, cultivated from mosses collected at different buildings (see Fig. 1, 2).
1 = presence of taxon in culture, 0 = taxon not found in culture.

	4S	11R1	11R2	7M	2K1	18K2	18K3
Arcella arenaria Greef 1866	0	1	0	1	0	0	0
Centropyxis plagiostoma terricola Bonnet & Thomas 1955	1	1	0	1	1	0	0
Centropyxis sphagnicola Deflandre 1929	0	1	0	0	0	0	1
Cyclopyxis eurystoma Deflandre 1929	1	1	0	0	1	1	1
Euglypha compressa Carter 1864	1	1	0	1	1	0	0
Euglypha rotunda Wailes & Penard 1911	1	1	1	1	1	1	1
Euglypha sp. 1	1	1	0	1	0	0	1
Euglypha sp. 2	1	1	1	1	1	1	1
Euglypha sp. 3	1	0	0	1	0	0	0
Euglypha sp. 4	0	1	0	0	0	0	1
Heleopera sylvatica Penard 1890	0	1	0	0	0	0	0
Trinema enchelys (Ehrenberg 1838)	1	0	0	0	1	0	0
Trinema lineare Penard 1890	1	0	1	0	0	0	0
n	**9**	**10**	**3**	**7**	**6**	**3**	**6**

other species. Moreover, *Ceratodon purpureus* (Hedw.) prefers also disturbed urban habitats (Nebel & Philippi 2000), and here we found numerous amoebae taxa.

Animals (e.g., birds, mammals, insects, mites) and human activities are identified as distribution vectors for testate amoebae (Chardez 1965, Lara et al. 2011, Wilkinson 2010, Wilkinson et al. 2012). Numerous studies point out that aerial immigration is one of the most important dispersal strategies for colonization of new land surfaces by unicellular eukaryotes. Rivera et al. (1994) used four sampling points (two and five meters above ground level) in the metropolitan area of Mexico City to collect cysts of naked amoebae suspended in the air. The main source habitat was the soil, and the authors could clearly demonstrate that there are a lot of viable cysts of naked amoebae in the air of an urban environment. Wanner et al. (2015) caught 12 species of testate amoebae of airborne origin by using 70 adhesive traps over a 91-day exposure. *Phryganella acropodia* (Hertwig & Lesser, 1874) and *Centropyxis sphagnicola* (Deflandre, 1929) occurred most frequently in the adhesive traps. The analysis of the 'target substrate' of aerial immigration pointed to a shift from a stochastic (variable) beginning of community assembly to a more deterministic (stable) course. In our study, a big urban area is more or less permanently touched by strong winds blowing from a big natural resource habitat (forest soil) in the target direction (the buildings), indicating that aerial immigration may be the most important driver. This finding points in the same direction as our cluster analysis, which sorted by sampling height. However, in our study, age and location of the sampling sites had no effect on amoebal taxa, pointing, as in Wanner et al. (2015), to a stochastic beginning of community assembly.

To conclude, this study confirms that testate amoebae are able to colonize new terrestrial habitats, even urban buildings at an heigth of 54 meters. In this study, it was not possible to pinpoint the exact cause for immigration. Air currents are suggested to be a main cause. Early phases of colonization may be predominantly driven by stochastic events.

5. Acknowledgments

We would like to thank Ms. Miho Tanaka (Mushi Bunko, Kurashiki City) for her identification of mosses. This study was supported by The Japan Society for the Promotion of Science (JSPS, grant number S16738, MW) and by the JSPS KAKENHI (15H02858, SS). Klaus Birkhofer (Dept. Ecology, BTU Cottbus-Senftenberg, Cottbus, Germany) and anonymous referees contributed with helpful discussions.

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