Front. Earth Sci. 2012, 6(1): 1–9
DOI 10.1007/s11707-012-0310-6

RESEARCH ARTICLE

Testate amoebae communities from some freshwater and soil habitats in China (Hubei and Shandong Provinces)

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Abstract Seventy-eight species and forms of testate amoebae were identified from 29 freshwater and soil habitats in three territories of China (Shandong and Hubei Provinces). Most abundant species from the genera *Plagiopyxis*, *Centropyxis* and *Trinema* represent the globally-distributed and eurybiont group of testate amoebae. The species richness was observed to be the lowest (7–12 species per biotope) in sandy sediments of the Yangtze River, but considerably higher (20–30 taxa) in soil environment. In the range of terrestrial habitats, the most remote communities from Laoshan Mountain in Shandong Province, China manifested the highest difference from others. On the other hand, communities originated in the most distant from industrial center places (Guifeng Mountain in Hubei Province, China) possess the most peculiar species composition including specific Gondwanian taxa (e.g. *Nebela bigibbosa*). In sum, the results obtained provide the evidence that the community complexity and specificity reduce in the places located within areas that are highly populated and intensively visited by humans.

Keywords testate amoebae, soil protists, freshwater protists, China

1 Introduction

Testate amoebae (testaceans) are free-living heterotrophic protists. The amoeboid cell of a testacean is protected by a shell that usually has one or two apertures through which pseudopodia extend. These protists occur worldwide and populate a variety of habitats from soil to aquatic environments (Chardez, 1965). Despite that majority of testacean species are cosmopolitans; there are taxa with limited geographical distribution (Smith and Wilkinson, 1987, Foissner, 2006, Smith et al., 2008; Bobrov et al., 2010). One of the most interesting and still poorly investigated regions is Southeast Asia. Recently some factors that could affect the biogeographic peculiarities and high species richness of testate amoebae in Asia were listed (Qin et al., 2011). They include: (i) the Asian Monsoon, which influences directly the environments of the region; (ii) the range of topographic environments, which affect a high diversity of habitats, especially at the microenvironment level. The varying extent of environmental factors during the Quaternary in Asia causes a high diversity of species in the region, including testate amoebae (Wang, 1977; Shen, 1983).

Ecology and biogeography of testate amoebae in China have been intensively studied during last two decades (Wang, 1977; Gong et al., 1990; Han and Hu, 1995; Yang et al., 2004, 2006; Qin et al., 2007, Qin and Xie, 2011; Zheng et al., 2009; Li et al., 2009, 2010). Ning and Shen (1998a, b, 1999) have reviewed faunal characteristics and distribution of species of soil protozoa in six typical zones of China. The distribution of species and the analysis of faunal similarity showed that most species of soil protozoa were neither cosmopolitan nor ubiquitous; they were restricted to certain zones and to certain types of habitat. Qin et al. (2011) have discussed diversity, distribution and biogeography of testate amoebae in China. They have summarized the testate amoebae research in China and provided the necessary context for future research. It was underlined that testate amoebae are an excellent indicator of environmental conditions, and have valuable implications for environmental changes, which is of particularly importance for China where serious problems of environmental pollution are raising during the economic growth (Qin et al., 2007, 2009). Decreasing of species

Received November 4, 2011; accepted December 15, 2011

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diversity as well as lack of endemic species (i.e., simplification of a biologic community) could be the result of such anthropogenic influence. The aim of the present study was to illustrate the cases of simple testate amoebae communities in the territories with high tourist impact in two provinces in China around large industrial centers — Wuhan City (Hubei Province) and Qingdao City (Shandong Province) cities.

2 Methods

This investigation was carried out in three territories: (i) Laoshan Mountain (Shandong Province) in April 2008; (ii) Guifeng Mountain (Hubei Province) in *Pinus massoniana* Lamb. wood in April 2010 and (iii) in different habitats around Wuhan City in April 2010, where samples were taken from sandy sediments of the Yangtze River, sandy and silty sediments of Donghu Lake, from soils in Luojia and Moshan hills (*Sophora japonica* L. forests), and litter from floodplain near Donghu Lake (*Pinus massoniana* Lamb., *Acer palma* Tum., and *Cathaya argyrophylla* Chun et Kuang. woods). Altogether 29 habitats sampled as triplicates were investigated, including 23 habitats in Hubei Province and 6 habitats in Shandong Province.

Laoshan Mountain (N36°03′–N36°04′, E120°07′–E120°43′), 1133 m, is the major mountain in the Shandong Peninsula, rising on the coast of the Yellow Sea, the continental climate of warm temperate zone. The typical soil includes brown and saline soil. Because the terrain is complicated, there are many types of vegetation, such as forests, bushes, sand and salt plant, and agricultural cultivation.

Guifeng Mountain (N29°45′–N31°40′, E114°24′–E116°07′), 1224 m, lies on the dividing line between the south and north climate of Dabie Mountain, and the subtropical monsoon climate with humid. The vegetation is characterized by warm-temperate mixed deciduous needle-leaf and broad-leaf forest in north slope and the subtropical mixed evergreen needle-leaf and broad-leaf forest in south slope. The soil is mainly made of gneiss, slightly acid, can grow primitive ancient *Rhododendron* Forest, primitive bamboos, and wild orchids.

Wuhan (N29°58′–N31°22′, E113°41′–E115°05′), the center of hinterland of China, located at the confluence of the Yangtze River and Han River, the subtropical monsoon climate with humid. The vegetation includes evergreen broadleaf forest, deciduous broad-leaf forest, the mixed of deciduous broad-leaf and coniferous forest. There are many types of soil, mainly including rice land, brown soil, aquent, and red soil, and pH is modest.

Soil samples included ca 1 cm of litter or mosses (if present) and 1–2 cm of uppermost mineral soil. Samples of lake sediments (1–2 cm thick) were taken at the depth of 20–100 cm. For microscopic analysis soil was soaked with water for 24 h. Then samples were shaken for 10 min, filtered through the 0.5 mm mesh, centrifuged and decanted. Prepared samples were stored in the refrigerator at 4°C. For each sample, 8–15 microscopic slides were prepared and inspected. The number of shells per slide varied from 10 to 70 individuals, including both living cells and empty shells. Thus, totally we counted 150–500 (300 on the average) individuals per sample. The taxonomic scheme drawn in Meisterfield (2000a,b) was applied.

Hierarchical cluster-analysis on the basis of Raup-Crick similarity matrix was performed to classify communities by species composition. Detrended correspondence analysis (DCA) was applied to reveal patterns of community structure changes. Statistical analysis was conducted using PAST 1.89 software (Hammer et al. 2001).

3 Results

Seventy-eight species, varieties, and forms from 21 genera have been identified (Table 1, Figs. 1 and 2). Most diverse were the genera *Centropyxis* (15 taxa), *Euglypha* (10), and *Difflugia* (9). Three testate amoebae were not identified to species level. *Plagiopyxis penardi* was the most abundant species (12.7% of total abundance; up to 31.8% in individual locations) followed by *Centropyxis sylvatica* (10.3%, 37.5%, respectively) and *Trinema lineare* (6.2%, 14.0%, respectively).

The species diversity and community composition of testate amoebae varied considerably among the sites. The lowest species richness (7–12 species per biotope; 3–11 species per sample) was discovered in the Yangtze River. On the other hand, 20–30 taxa were identified from the other sites. In the sediments of the Yangtze River, the community was composed of the species of freshwater (*Cyphoderia bonneti, Pseudodifflugia gracilis, Zivkovicia spectabilis, Difflugia shurmani*) and soil (*Plagiopyxis callida, P. penardi, Centropyxis aerophila*) origin. The mixture of the pedobiont (*Centropyxis* spp., *Plagiopyxis* spp.) and eurybiont (*Trinema* spp., *Euglypha* spp., *Assulina* spp.) organisms was formed in the river's floodplain.

Three types of the communities were distinguished in terms of both species composition and community structure (Figs. 3–5). A bulk of the limnophilous taxa from the genera *Cyphoderia, Cucurbitella, Difflugia, Pseudodifflugia, Zivkovicia* were characteristic for the aquatic habitats. It is interesting that originally terrestrial species *Plagiopyxis callida* tend to populate the freshwater sediments. Among the communities from the terrestrial habitats (Figs. 3 and 5) one from the Laoshan Mountain (with *P. penardi*, *Phryganella. acropodia*, *T. enchelys*, *C. lithostoma* as dominants, and *C. minuta*, *T. minuta*, *Ph.*

Table 1 Species list and relative abundance (%) of testate amoebae in different habitats in Hubei and Shandong Provinces. Abbreviations of biotopes: h – highland, f – floodplain, a – aquatic habitats; h-1 – Luojia hill, h-2 – Moshan hill, h-3 – Guifeng Mountain, h-4 – Laoshan Mountain, f-1 – Donghu Lake floodplain, f-2 – Yangtze River floodplain, a-1 – Donghu Lake, a-2 – Yangtze River

Taxon	Biotope							
	h-1	h-2	h-3	h-4	f-1	f-2	a-1	a-2
Arcella arenaria compressa Chardez, 1974	0.8	0.0	0.0	0.2	0.9	0.0	0.0	0.0
A. discoides Ehrenberg, 1843	0.0	0.0	0.0	0.0	0.0	0.0	6.8	0.0
A. rotundata Playfair, 1918	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
A. rotundata stenostoma Deflandre, 1928	0.0	0.0	0.0	0.0	0.0	0.0	2.8	0.0
Centropyxis aculeata (Ehrenberg, 1838) Stein, 1857	0.0	5.0	0.0	0.4	0.0	0.0	0.0	0.0
C. aculeata tropica Deflandre, 1929	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
C. aerophila Deflandre, 1929	4.2	10.0	2.0	5.9	7.0	0.0	2.6	13.5
C. aerophila minuta Chardez, 1964	0.8	3.3	0.0	0.0	0.9	0.0	0.0	0.0
C. aerophila sphagnicola Deflandre, 1929	0.0	0.0	0.0	3.7	1.9	0.0	11.3	0.0
C. cassis (Wallich, 1864) Deflandre, 1929	0.0	0.0	0.0	0.2	0.0	0.0	3.1	0.0
C. constricta (Ehrenberg, 1841) Deflandre, 1929	1.8	3.3	2.0	0.0	8.4	18.8	0.0	0.0
C. elongata (Penard, 1890) Thomas, 1959	0.0	0.0	2.0	0.7	0.0	0.0	0.0	0.0
C. elongata f. *minor* n.f.	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0
C. minuta Deflandre, 1929	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
C. orbicularis Deflandre, 1929	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0
C. plagiostoma Bonnet et Thomas, 1955	0.0	0.0	5.9	0.0	0.0	4.2	0.0	0.0
C. platystoma (Penard, 1890) Deflandre, 1929	0.0	0.0	0.0	0.5	0.0	0.0	4.8	0.0
C. sylvatica (Deflandre, 1929) Bonnet et Thomas, 1955	11.6	13.3	5.9	0.0	13.8	37.5	0.0	0.0
C. vandeli Bonnet, 1958	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyclopyxis eurystoma Deflandre, 1929	6.7	0.0	5.9	0.0	0.9	0.0	0.0	0.0
C. eurystoma parvula Bonnet, Thomas, 1960	9.7	0.0	5.9	0.0	5.5	2.1	0.0	0.0
C. kahli Deflandre, 1929	3.4	5.0	2.0	0.2	1.8	2.1	0.0	0.0
C. lithostoma Bonnet, 1974	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0
C. cf. *profundistoma*	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. sp.	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trigonopyxis arcula (Leidy, 1879) Penard, 1912	0.8	0.0	5.9	1.4	0.0	0.0	0.0	0.0
T. minuta Schönborn et Peschke, 1988	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Plagiopyxis barrosi Bonnet, 1960	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0
P. callida Penard, 1910	0.0	0.0	0.0	0.0	0.0	0.0	4.8	37.7
P. declivis Thomas, 1958	0.0	0.0	2.0	2.2	0.0	0.0	0.0	0.0
P. labiata Penard, 1910	0.0	0.0	0.0	0.7	0.9	0.0	0.0	0.0
P. minuta Bonnet, 1959	9.0	10.0	5.9	0.0	9.7	6.3	0.0	0.0
P. penardi Thomas, 1958	7.6	1.7	3.9	31.8	17.8	4.2	10.6	24.4
Heleopera sylvatica Penard, 1890	0.8	0.0	0.0	0.0	0.0	2.1	1.4	0.0
H. petricola amethystea Penard, 1902	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0
Nebela biggibosa Penard, 1890	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0
N. collaris (Ehrenberg, 1848) Leidy, 1879	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0
N. tincta (Leidy, 1879) Awerintzew, 1906	0.0	0.0	5.9	0.0	0.0	0.0	0.0	0.0
Schoenbornia humicola (Schönborn, 1964) Decloitre, 1964	1.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0
Cucurbitella vlasinensis Ogden et Zivkovic, 1987	0.0	0.0	0.0	0.0	0.0	0.0	5.6	0.0

(*Continued*)

Taxon	Biotope							
	h-1	h-2	h-3	h-4	f-1	f-2	a-1	a-2
Difflugia cf. *bifurcata*	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
D. dujardini Chardez, 1957	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
D. globulosa Dujardin, 1837	0.0	0.0	0.0	0.0	0.0	0.0	7.9	0.0
D. globulus (Ehrenberg, 1848) Medioli et Scott, 1983	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
D. cf. *lemani*	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0
D. minuta Rampi, 1950	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
D. nana Coûteaux, 1976	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
D. penardi Hopkinson, 1909	0.0	5.0	0.0	0.0	2.6	0.0	4.5	0.0
D. shurmanni van Oye, 1932	0.0	0.0	0.0	0.0	0.0	0.0	10.0	5.4
Lagenodifflugia montana (Ogden et Zivkovic, 1983) Ogden, 1987	0.0	1.7	0.0	0.0	1.3	0.0	0.0	0.0
L. vas (Leidy, 1874) Medioli et Scott, 1983	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
L. sp.	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0
Pontigulasia incisa Rhumbler, 1896	0.0	1.7	0.0	0.0	0.0	0.0	0.0	0.0
Zivcovicia spectabilis (Penard, 1902) Ogden, 1987	0.0	0.0	0.0	0.0	0.0	0.0	3.3	2.8
Phryganella acropodia (Hertwig et Lesser, 1874) Hopkinson, 1909	0.8	0.0	0.0	12.7	0.0	0.0	7.1	0.0
Ph. hemisphaerica Penard, 1902	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0
Pseudodifflugia gracillis Schlumberger, 1845	0.0	0.0	0.0	0.0	0.0	0.0	4.1	8.0
P. gracilis terricola Bonnet et Thomas, 1960	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0
Assulina muscorum Greef, 1888	0.8	0.0	3.9	3.4	3.3	16.7	0.0	0.0
Tracheleuglypha dentata Deflandre, 1938	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Euglypha ciliata (Ehrenberg, 1848) Leidy, 1878	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
E. ciliata glabra Wailes, 1915	0.0	3.3	0.0	1.0	0.0	0.0	0.0	0.0
E. compressa Carter, 1864	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0
E. compressa glabra Wailes, 1915	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0
E. cristata decora Jung, 1942	0.0	3.3	2.0	0.0	0.0	0.0	0.0	0.0
E. cuspidate Bonnet, 1959	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
E. laevis (Ehrenberg, 1832) Perty, 1849	7.3	3.3	5.9	0.0	1.8	2.1	0.0	0.0
E. strigosa (Ehrenberg, 1871) Leidy, 1878	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
E. strigosa glabra Wailes, 1898	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0
E. tuberculata Dujardin, 1841	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0
Cyphoderia bonneti Štěpánek, 1967	0.0	0.0	0.0	0.0	0.0	0.0	0.9	8.2
Corythion dubium Tarának, 1881	0.0	0.0	5.9	0.4	0.0	0.0	0.0	0.0
C. dubium minima Chardez, 1969	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C. orbicularis (Penard, 1910) Iudina, 1996	0.0	0.0	0.0	0.2	0.0	0.0	0.9	0.0
Trinema complanatum Penard, 1890	8.9	8.3	5.9	3.7	3.8	2.1	0.0	0.0
T. enchelys (Ehrenberg, 1838) Leidy, 1878	0.5	0.0	5.9	8.8	0.8	0.0	0.0	0.0
T. lineare Penard, 1890	14.0	5.0	5.9	5.8	10.2	2.1	6.4	0.0
T. sp.	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Number of species	28	23	24	30	24	12	20	7

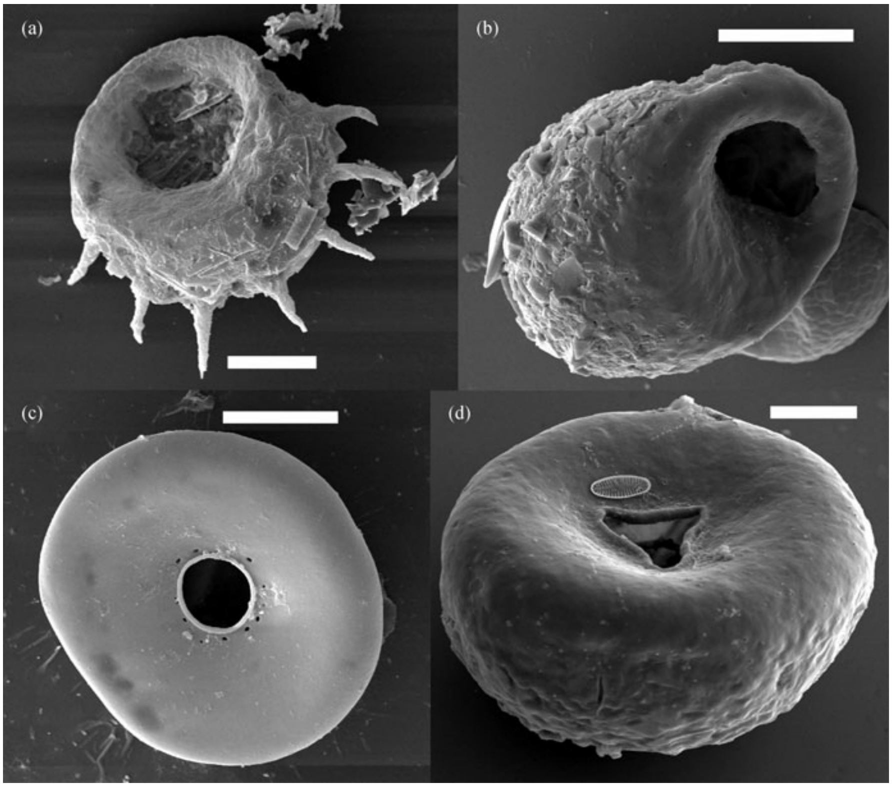

Fig. 1 Scanning electron micrographs of selected testate amoebae from the investigated sites. (a) *Centropyxis aculeata*, (b) *Centropyxis aerophila*, (c) *Arcella arenaria compressa*, (d) *Trigonopyxis arcula*. Scale bar: 20 µm

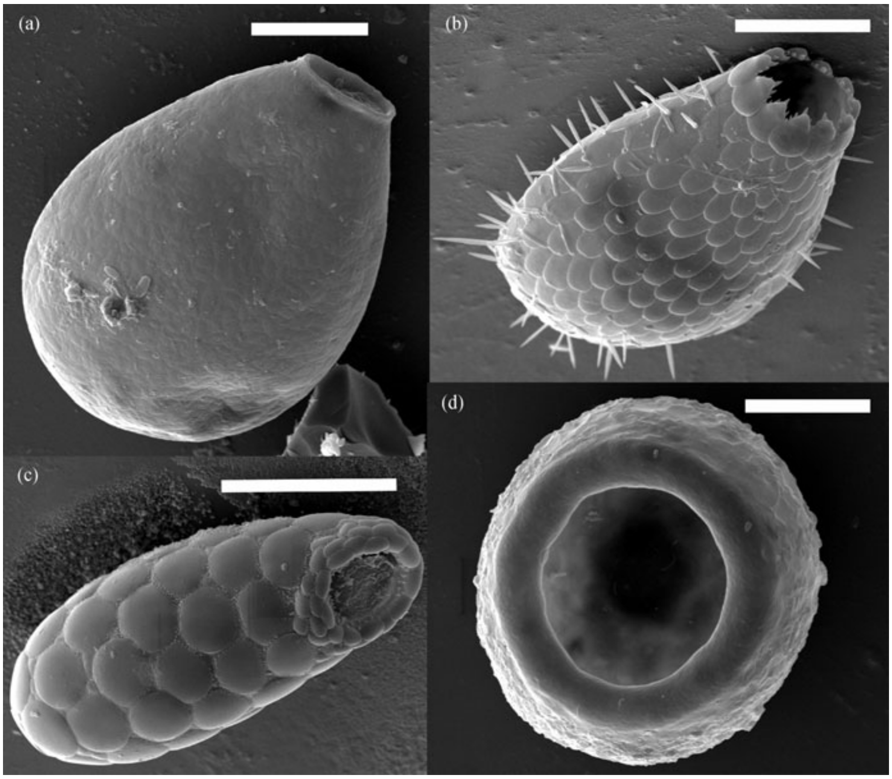

Fig. 2 Scanning electron micrographs of selected testate amoebae from the investigated sites. (a) *Nebela tincta*, (b) *Euglypha strigosa*, (c) *Trinema enchelys*, (d) *Phryganella hemisphaerica*. Scale bar: 20 µm

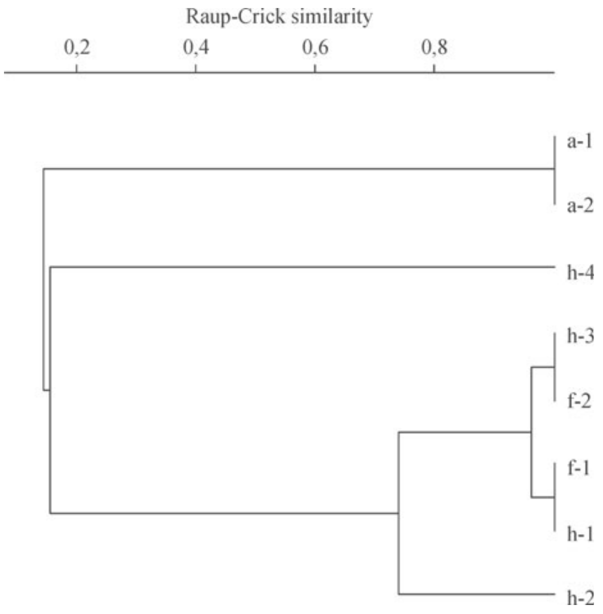

Fig. 3 Results of testate amoebae communities' classification (cluster-analysis) by species composition. See Table 1 for abbreviation

hemisphaerica, *E. ciliata*, *E. compressa*, *E. strigosa*, *E. s. glabra*, *E. tuberculata*, *C. orbicularis* as characteristic species) was distinguished from the others located in the Hubei Province.

Most peculiar composition of the community was formed in the soils of the pine wood of Guifeng Mountain. This place is located far from the large economic centers. However, it has considerable tourist impact. Testate amoebae community in this place was characterized by high diversity of the bryophilic species including those of Gondwanian origin (*Heleopera petricola amethystea*, *Nebela bigibbosa*, *N. tincta*, *Pseudodifflugia gracilis terricola*).

4 Discussion

The Sino-Japanese Floristic Region of East Asia harbors the most diverse of the world's temperate flora, and was the most important glacial refuge for its Tertiary representatives ("relics") throughout Quaternary ice-age cycles. Historical biogeography, using methods of phylogeographic studies provides several scenarios of the modern flora of China (Qiu et al., 2011). Protozoan fauna can be very strong argument toward the reasoned selecting of one of the proposed scenarios as it was shown for the testate amoebae of the Far East of Russia (Bobrov, 2001). In the tertiary forests of this region, testate amoebae of Gondwana-tropical group was found. Thus, with a high degree of probability, such findings await more work in the territory of China.

In terms of biogeography of testate amoebae, the territory of China is of undoubted interest, since it is

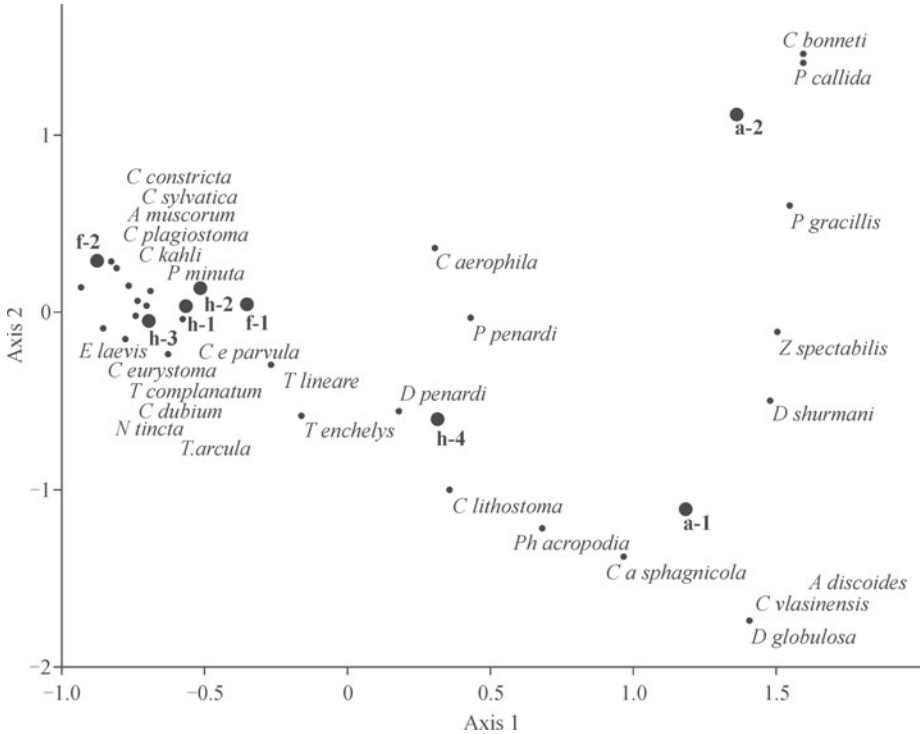

Fig. 4 Results of the ordination of testate amoebae communities. Axis 1 explains 35.9% and Axis 2 explains 20.2% of the total community variance

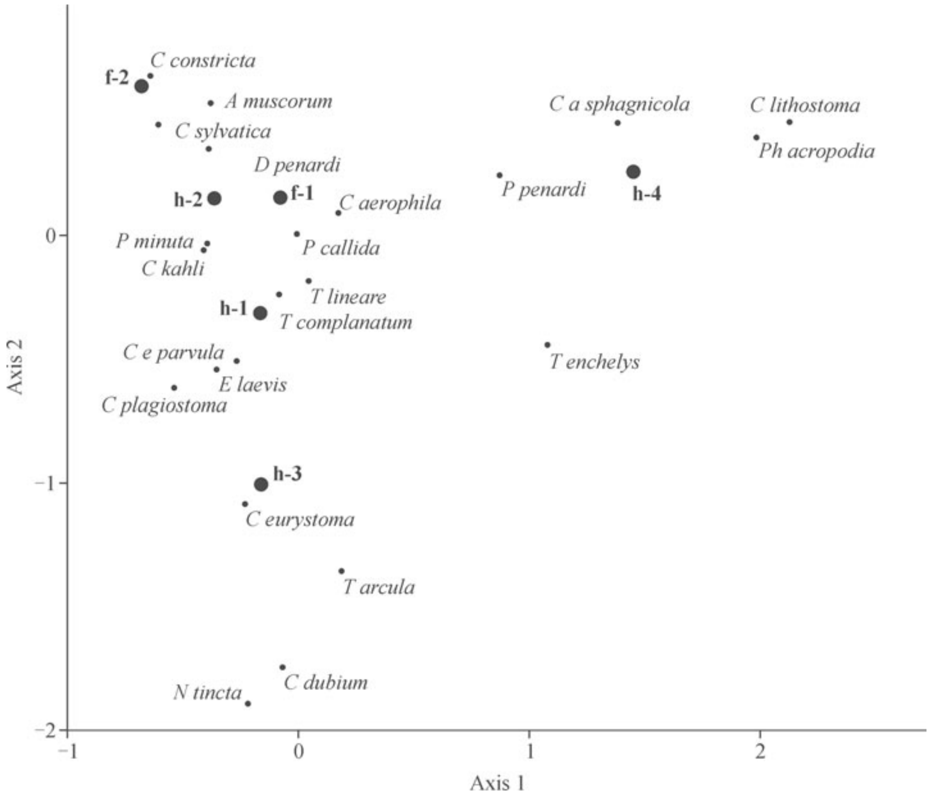

Fig. 5 Results of the ordination of testate amoebae communities (without aquatic habitats). Axis 1 explains 42.9% and Axis 2 explains 26.3% of the total community variance

included in the two biogeographical Realms (Palaearctic and Indomalayan). The boundary between them passes through a vast territory in the latitudinal direction and is due to climatic and topographic features. The investigated area belongs to the Palaearctic Realm, Oriental Deciduous Forest (Udvardy, 1975). This fact as well as the increasing recreational load determines the relative poverty of the testate amoebae and the absence of rare species. Almost all species revealed during this study belong to cosmopolitan taxa that form the basis of the population of the Palearctic. The exceptions are *Centropyxis litophila* and *C.* cf. *profundistoma*. First species is rather common for soils of the neotropics. In particular it was identified in the mountain soils of Mexico (Bobrov and Krasilnikov, 2011). The second one was found in the southern border of the Palaearctic (Foissner and Korganova, 1995).

Soil testate amoebae fauna in China is poorly studied. The majority of publications are devoted to the aquatic organisms (Yang et al., 2004; Qin et al., 2009; Qin and Xie, 2011). To some extent, this article partially fills this gap. As we see, future studies of testate amoebae in China should be directed to the inventory of testate amoebae fauna in nature reserves and recreational areas with low human load, and located in the border between Palaearctic and Indomalayan. There is a high probability to find rare and new species in those regions.

Aquatic studies of testate amoebae in China usually yield extremely important findings. For example, in Mulan Lake in the Hubei Province, a part of the Palaearctic Realm, Oriental Deciduous Forest, two new prominent species *Difflugia mulanensis* (Yang et al., 2005) and *Pentagonia zhangduensis* (Qin et al., 2008) were described. Another case is an observation in China of the rare species *Difflugia biwae* (Yang and Shen, 2005), previously described in an ancient tectonic Biwa Lake (Honshu, Japan). These facts allow supposing, that in the lakes of China the number of endemics is higher than that in terrestrial habitats. These habitats as possible refugees for endemic taxa are very promising targets of research in terms of discovering rare and peculiar forms and thus broadening our understanding of macroecological patterns of microbes. However, during our study we have not found any peculiar taxa in the large Donghu Lake, most probably because of high anthropogenic impact (Jiang and Shen, 2007) and limited number of samples.

Acknowledgements This work was supported by Joint Cost-share Grant of Russian Foundation for Basic Research (No. 10-04-91155-GFEN-a) to Yuri Mazei, Initiative Grant of Russian Foundation for Basic Research (No. 11-04-01171-a) to Anatoly Bobrov and the National Natural Science Foundation of China (Grant No. 31011120092) to Yingchun Gong.

Author contributions: AB conducted laboratory and microscopic analysis including species identification, and worte the paper, YuM designed the study, conducted all statistical analysis and worte the paper, VCh conducted microscopic analysis including species indentification and counting, YG and WF organised the field work and wrote the paper.

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