

Heterotrophic Flagellates from Freshwater and Soil Habitats in Subtropical China (Wuhan Area, Hubei Province)

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Summary. We studied the diversity of heterotrophic flagellates from the sandy sediments of the Yangtze River, sandy and silty sediments of Donghu Lake, soil, moss and litter from the Luojiashan and Moshan hills as well as litter from the floodplain near Donghu Lake in April 2010. Sixty-seven heterotrophic flagellate species were identified by means of phase and interference contrast light microscopy and transmission electron microscopy. The majority of the observed flagellates were bacterivorous. Local species richness of river sediment communities was significantly lower than that of lake sediments and terrestrial habitats. The communities from the terrestrial habitats were more heterogeneous than those from freshwater sediments. Common species for the aquatic habitats were *Rhynchomonas nasuta*, *Paraphysomonas* sp., *Neobodo designis*, *N. curvifilis*, *Bodo saltans* and *Spumella* spp. In the soils only *Spumella* spp. was found in the majority of samples. Most characteristic taxa for the lake sediments were *Helkesimastix faecicola*, *Petalomonas minuta*, *P. pusilla*, *Diphylleia rotans*, *Amastigomonas caudata*. Amoeboflagellates such as *Cercomonas angustus*, *C. granulifera*, *Paracercomonas crassicauda* were specific for the terrestrial habitats. There were no specific taxa in the river sediments. The majority of the heterotrophic flagellates identified in this survey have been noted in China earlier. They are common (and usually predominant) for other regions in both freshwater and soil habitats.

Key words: Heterotrophic flagellates, China, subtropics, soil, freshwater, protists.

INTRODUCTION

Heterotrophic flagellates (HF) are an obligatory component of microbial communities in all types of habitats containing fluid water (Vørs 1992, Arndt *et al.*

2000, Tikhonenkov *et al.* 2006). Thus, they play an important role in matter and energy flow in most aquatic and terrestrial ecosystems. Their potential function in classical herbivore food webs has been known for a long time, as well as the understanding of their essential role in the microbial loop (Pomeroy 1974, Azam *et al.* 1983). Since the recognition of the microbial loop, ecosystem model investigations encouraged quantitative studies of microbial loop components, HF in particular (Berninger *et al.* 1991, Gasol and Vaqué 1993).

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Most of the quantitative data from the 1980s and early 1990s lack details of taxonomic composition, especially for HF. Since then, it has become known that protozoan functional diversity, trophic relations and niches in different habitats are related to different taxonomic levels: from higher taxonomic groups to morphospecies (Arndt *et al.* 2000), and in some cases to cryptic species (Scheckenbach *et al.* 2006) and ecotype (Weisse and Montagnes 1998, Boenigk *et al.* 2005) levels. Thus, a community ecology model with a fine taxonomic resolution is sorely needed in protistan ecology to overcome problems that are untreatable by using black boxes such as ‘bacteria,’ ‘flagellates’ and ‘ciliates’ in ecosystem models.

Despite their recognized importance, the diversity and biogeography of HF in natural systems remains under-explored. In general, marine HF are better studied in comparison with freshwater. The most of publications devoted to the diversity of HF in marine sediments of Australian seashore and some tropical sites (e.g. Larsen and Patterson 1990, Patterson and Simpson 1996, Ekeboom *et al.* 1995/1996, Lee and Patterson 2000, Al-Qassab *et al.* 2002). To date, species composition of HF in some riverine (Weitere and Arndt 2003, Kopylov *et al.* 2006, Kosolapova 2007, Tikhonenkov and Mazei 2008, Kiss *et al.* 2009), lake (Mylnikov and Zhgarev 1984, Auer and Arndt 2001, Domaizon *et al.* 2003, Tikhonenkov 2007/2008, Kosolapova and Kosolapov 2009), and soil (Foissner 1991, Ekelund and Patterson 1997, Tikhonenkov *et al.* 2010) habitats of different geographical zones (from equatorial to polar) were investigated. At that, mainly planktonic habitats were investigated in the freshwaters. However, many geographical territories as well as specific habitat range in need of revealing the biodiversity structure and understanding its peculiarities.

One such case is the huge territory of China. To date, most of the publications on Chinese protistan ecology are devoted to soil, freshwater and marine ciliates (Xu and Sun 2000; Ma *et al.* 2008, 2009; Song *et al.* 2009; Zheng and Wang 2009; Zou *et al.* 2009; Li *et al.* 2010b; Xu *et al.* 2011) and testate amoebae (Wang 1977; Yang *et al.* 2004, 2006; Qin *et al.* 2007; Li *et al.* 2009; 2010a).

Recently Ning and Shen (1998a, b, 1999) reviewed faunal characteristics and distribution of species of soil protozoa in 6 typical zones of China. The distribution of species and the analyses 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.* (2010) have

discussed the diversity, distribution and biogeography of testate amoebae in China. They have underlined that the knowledge concerning rhizopods from China has improved greatly over the past 20 years. These authors have summarized the testate amoebae research in China and provided the necessary context for future research.

Data concerning the HF diversity of Central China is limited to several papers (Gong *et al.* 1990; Song 1995, 2000; Feng *et al.* 2004; Ning *et al.* 2007; Xu *et al.* 2005; Zou *et al.* 2009). At that, growth rates and production of HF in Chinese freshwater ecosystems were studied (Zhao *et al.* 2002, 2003).

Moreover there are a lot of data dealing with protozoan community changes in subtropical lakes, on the background of the eutrophication process as well as other anthropogenic impacts (Gong 1986, Jiang and Shen 2007, Yan *et al.* 2010). Most of such studies have focused on the genetic diversity of protists (Yan *et al.* 2007; Chen *et al.* 2008, 2010). However, none of the research focused on the comparison of HF morphospecies biodiversity in contrasted biotopes (i.e. soils, riverine and lake sediments) in a certain territory. The aim of our study was to reveal the species composition and HF community structure patterns in sediments of the Yangtze River and Donghu Lake as well as in the soils from the surrounding floodplains and the hills in the Wuhan Region of subtropical China.

MATERIALS AND METHODS

This investigation was carried out in April 2010 (2010.04.15–2010.04.20) in various habitats around Wuhan city, China (Fig. 1). The territory is characterized by subtropical humid monsoon climate with significant seasonal variations. Mean annual air temperature 16.7°C; mean annual precipitation 1160 mm, 75% of which falls on spring and summer (130 mm in April 2010). Samples were taken from the sandy sediments of the Yangtze River (temp. 11°C, pH = 8.0), sandy and silty sediments of Donghu Lake (temp. 15°C, pH = 8.2), soil, moss and litter from the Luojiashan and Moshan hills (*Sophora japonica* L. forests), and litter from the floodplain near Donghu lake (*Pinus massoniana* Lamb., *Acer palma* Tum., and *Cathaya argyrophylla* Chun et Kuang woods). Donghu Lake area in the eastern part of Wuchang District of Wuhan City is about 33 square kilometers. It is a natural dammed lake formed in the early Holocene attached to the right bank of the Yangtze which flows through Wuhan, and is only five kilometers away from the river. The mean depth of the lake is 2.2 m; the maximum one – 4.8 m. Moshan is a 118 m high hill, located in the southwest side of Donghu Lake. Luojiashan hill is 118.5 m high, lies southeast of Donghu Lake. Altogether 21 habitats were sampled in triplicates. The samples were taken from the surface of the sediments situated in 50 cm from water’s edge at a depth 30 cm; soil samples were taken from the 5 cm top layer.

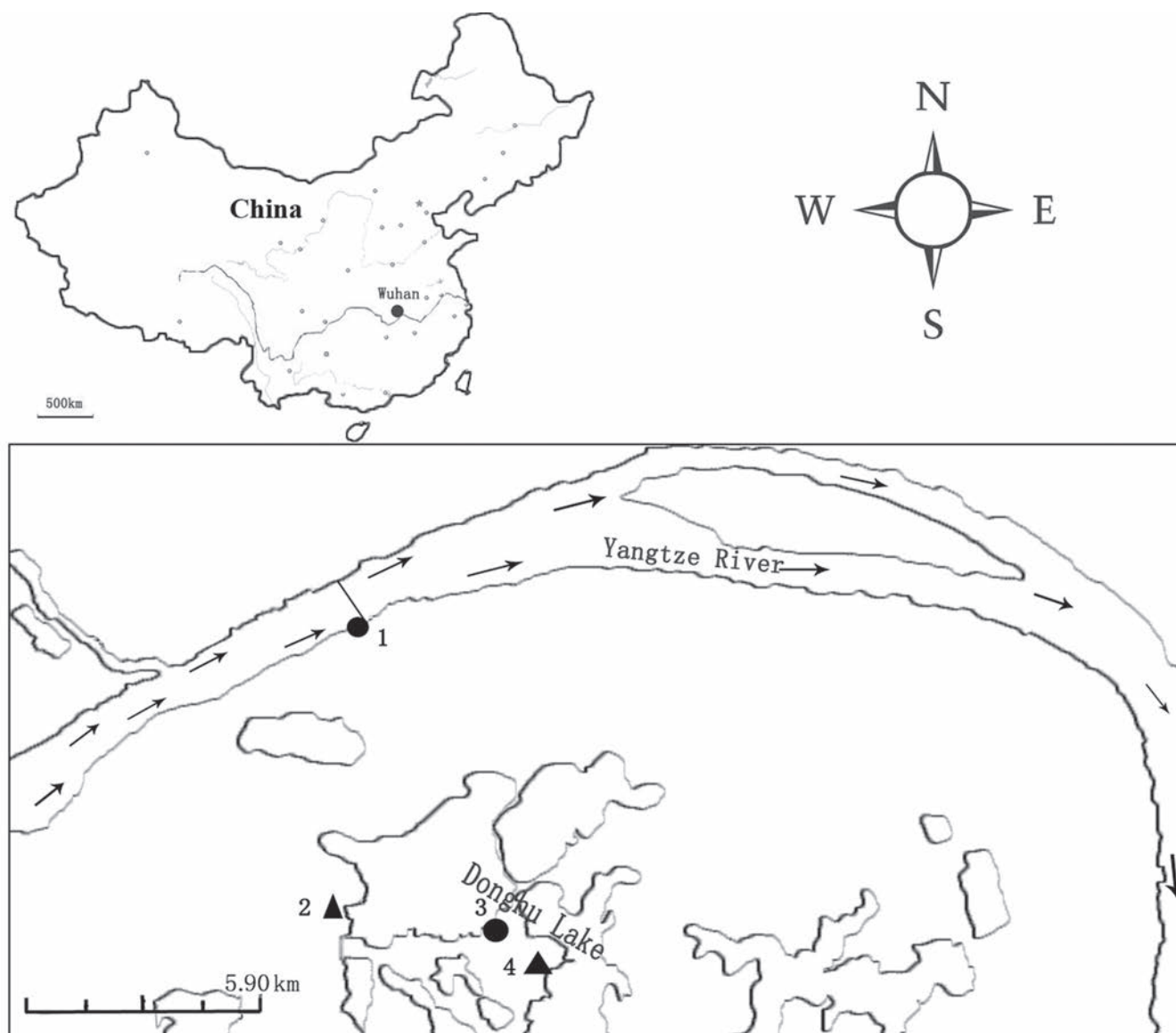


Fig. 1. Location of studied sites. 1 – Yangtze River, 2 – Moshan hill, 3 – Donghu Lake, 4 – Luojiashan hill.

The sediment samples, including water, were placed in 50-ml flasks, and maintained at approximately 3°C during transportation to the laboratory. Soil, litter and moss samples were air-dried prior to transportation. For the investigation of the species composition of the HF, heterotrophic successions were artificially initiated (Tikhonenkov *et al.* 2008). One gram of dry soil was put into Petri dishes with 5 ml Pratt's medium (or 5 ml of sediments with lake/river water) and 0.15 ml of a bacterial suspension (approximate concentration 167×10^6 ind. ml⁻¹) of *Pseudomonas fluorescens* Migula, 1895 (strain ICISC19, Institute for Cellular and Intracellular Symbiosis Collection, Russian Academy of Science) was added as a food object. The dishes were kept in a thermostat at 25°C in full darkness for 9 days. The analysis of these samples at different time intervals

allowed estimating the potential species diversity, which was higher than the active one (at the moment of the sampling) but similar to it in many aspects (Tikhonenkov 2007/2008). The samples were examined on the third, sixth (corresponding to the first most complete and heterogeneous succession stage), and ninth (the second and third stages with the uniform species structure) days of the developing heterotrophic succession in accordance with the methods of previous studies (Vørs 1992, Tikhonenkov *et al.* 2008). Two replicates of each sample were investigated.

Light microscopical observations were made with the aid of Biolam-I microscope (Russia) equipped with phase contrast and water immersion objectives giving a total magnification 770 ×, as well as a Reichert (Austria) microscope with Nomarski interference contrast

and glycerin immersion objectives (1000 ×). The microscopes were equipped with an analog video camera AVT HORN MC-1009/S, which was connected to a Panasonic NV-HS 850 video recorder. Image acquisition in VHS and S-VHS modes, followed by digitization of images and preservation of fragments of video film as AVI files, was carried out in order to facilitate more precise identification of the HF. HF were identified by observing living cells with the exception of scale-bearing species (*Thaumatomonas*). Drops of suspended scale-bearing cells were placed on copper grids coated with Formvar film and prepared as whole mounts by the method described by Moestrup and Thomsen (1980). Grids were shadowed with tungsten oxide, and were observed with a JEM-100C transmission electron microscope (TEM).

The HF were identified according with following publications: Lemmermann (1914), Skuja (1948, 1956), Larsen and Patterson (1990), Vørs (1992), Zhukov (1971, 1993), Ekeboom *et al.* (1995/1996), Patterson and Simpson (1996), Ekelund and Patterson (1997), Tong *et al.* (1997), Lee and Patterson (2000), Al-Qassab *et al.* (2002), Schroeckh *et al.* (2003), Walker *et al.* (2003), Lee *et al.* (2005), Bass *et al.* (2009), Howe *et al.* (2009), Tikhonenkov (2010). Species are distinguished in accordance with their cell shape and size, flagella movement and arrangement, and behavior and locomotion of the cell.

Using the number of species found in each habitat, a relationship between species diversity and sampling effort was calculated using a rarefaction procedure. The analysis of species-accumulation curves allowed estimates of beta-diversity comparisons of species richness in the sample sets of different size (Lawton 1999). Species accumulation curves were approximated by the power function $S = cN^z$, where N is number of samples analyzed, S – number of species identified, z – beta-diversity coefficient, and c – alpha-diversity coefficient (Scheiner 2003). The alpha-diversity coefficient (c) reflects the average number of species per sample. Beta-diversity coefficient varies from 0 (in the case of identical samples in terms of species composition) to 1 (when samples are completely different from each other by species composition).

Statistical analysis was carried out using PAST 1.89 software (Hammer *et al.* 2001).

RESULTS AND DISCUSSION

Sixty-seven HF species, including forms that have not been identified to the species level, were revealed (Table 1). The most of the species are illustrated in Figs 2–4. Morphological features of the HF species identified corresponded to those reported earlier (Skuja 1948, 1956; Larsen and Patterson 1990; Vørs 1992; Zhukov 1971, 1993; Ekeboom *et al.* 1995/1996; Patterson and Simpson 1996; Ekelund and Patterson 1997; Tong *et al.* 1997; Lee and Patterson 2000; Al-Qassab *et al.* 2002; Schroeckh *et al.* 2003; Walker *et al.* 2003; Lee *et al.* 2005; Bass *et al.* 2009; Howe *et al.* 2009; Tikhonenkov 2010).

The greatest numbers of the species in the investigated biotopes belong to euglenids, cercomonads, kinetoplastids and choanoflagellates. Euglenids are the most important component of benthic communities and compose up to 20–85% of the total community biomass following (up to 5–20%) by bodonid kinetoplastids (Dietrich and Arndt 2000, Lee 2001). These flagellates are widespread and can be observed both in freshwater and saline biotopes (Fenchel 1987, Zhukov 1993, Patterson and Simpson 1996, Arndt *et al.* 2000). Cercomonads are typical a soil and benthic groups as well, generally found in samples rich in organic matter and detritus and in sewage waters (Hänel 1979, Mylnikov and Karpov 2004). Choanoflagellates are usually represent another considerable part of the community biomass (up to 5–40%) in both marine and freshwater ecosystems (Arndt *et al.* 2000). Moreover, they are usually considered to be primarily planktonic or periphyton forms (Zhukov 1993). Together, these findings sup-



Fig. 2. 1–3 – *Agitata tremulans* (amoeboid cell with two flagella); 4 – *Allantion tachyploon* (single trailing flagellum, the posterior cell end is turned upwards at a sharp angle); 5, 6 – *Amastigomonas caudata* (small posterior pseudopodium); 7–10 – *A. aff. mutabilis* (long proboscis and big cell); 11, 12 – *Ancyromonas sigmoides* (rostrum and long posterior flagellum); 13 – *Apusomonas proboscidea* (long mastigophore); 14, 15 – *Bicosoeca lacustris* (active extruded anterior flagellum (14) or spirally constricted flagellum (15)); 16–18 – *Parabodo caudatus* (rostrum, hook-like anterior flagellum and big contractile vacuole); 19–21 – *Neobodo curvifilis* (hook-like anterior flagellum and elongated cell body); 22–25 – *N. designis* (hook-like anterior flagellum which curves back over the rostrum), (23, 24) anterior flagellum is wrapped around rostrum, (25) whole mount (TEM); 26, 27 – *Bodo minimus* (flattened egg-shaped or triangular cell body, small rostrum); 28, 29 – *Neobodo saliens* (hook-like anterior flagellum, narrow cell body, small rostrum); 30–32 – *Bodo saltans* (small hook-like anterior flagellum, cell attaches to the substratum by long posterior flagellum), (32) whole mount (TEM); 33, 34 – *Breviata anathema* (very long single anterior flagellum, small pseudopodia); 35, 36 – *Cercomonas angustus* (elongate cell body contacts to substratum by front end with short anterior and long posterior flagella); 37 – *Cercomonas longicauda* (anterior flagellum about the length of the cell, posterior one about twice the cell length); 38–40 – *Codonosiga botrytis* (choanoflagellates without lorica, colonies with 4–8 cells). Scale bars: 5 μm (1–3, 5, 6, 11, 12, 14, 15, 26, 27, 30–32), 7 μm (33, 34), 8 μm (4, 7–10, 13, 19–25, 28, 29, 35, 36), 13 μm (16–18, 37, 38, 40), 20 μm (39).

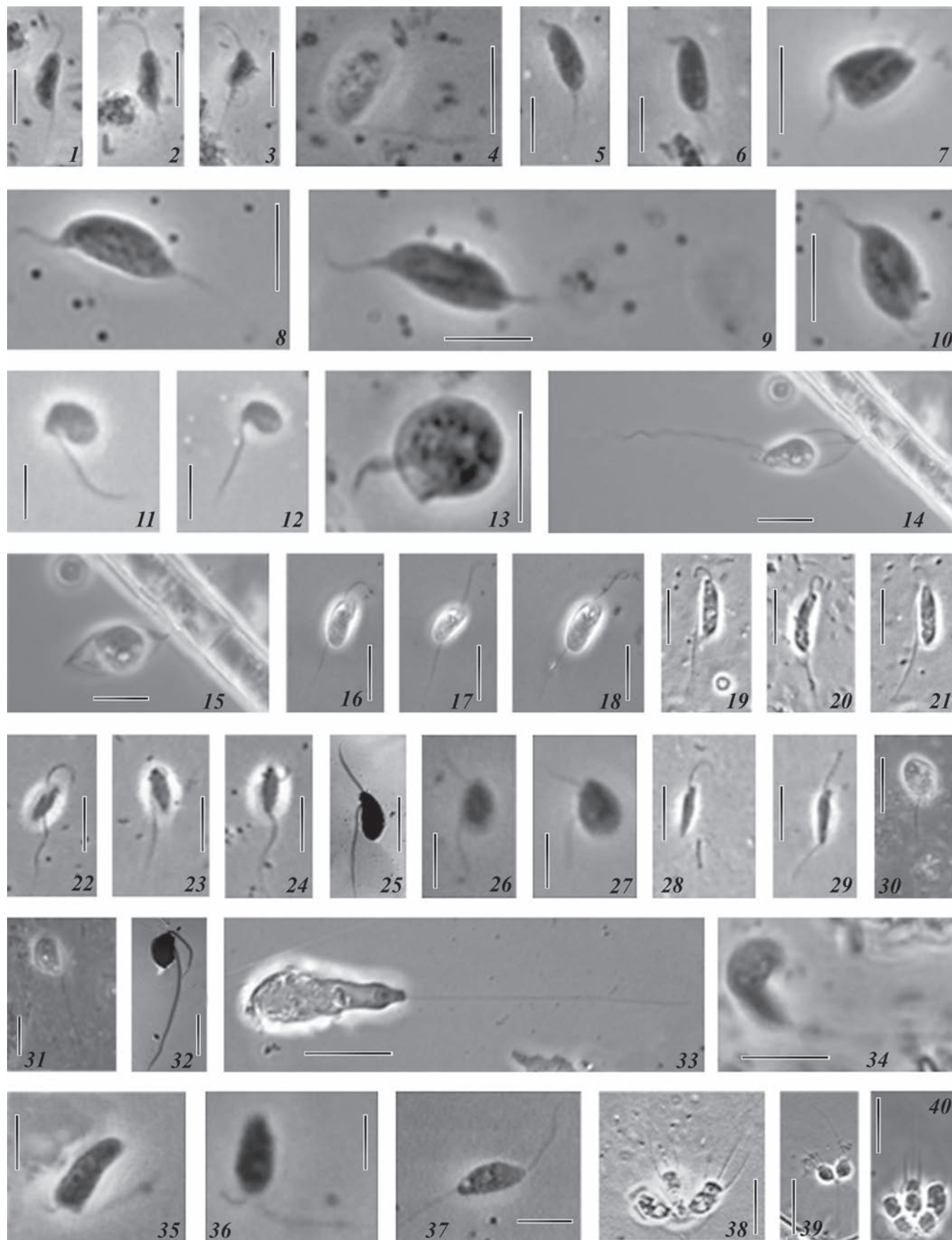
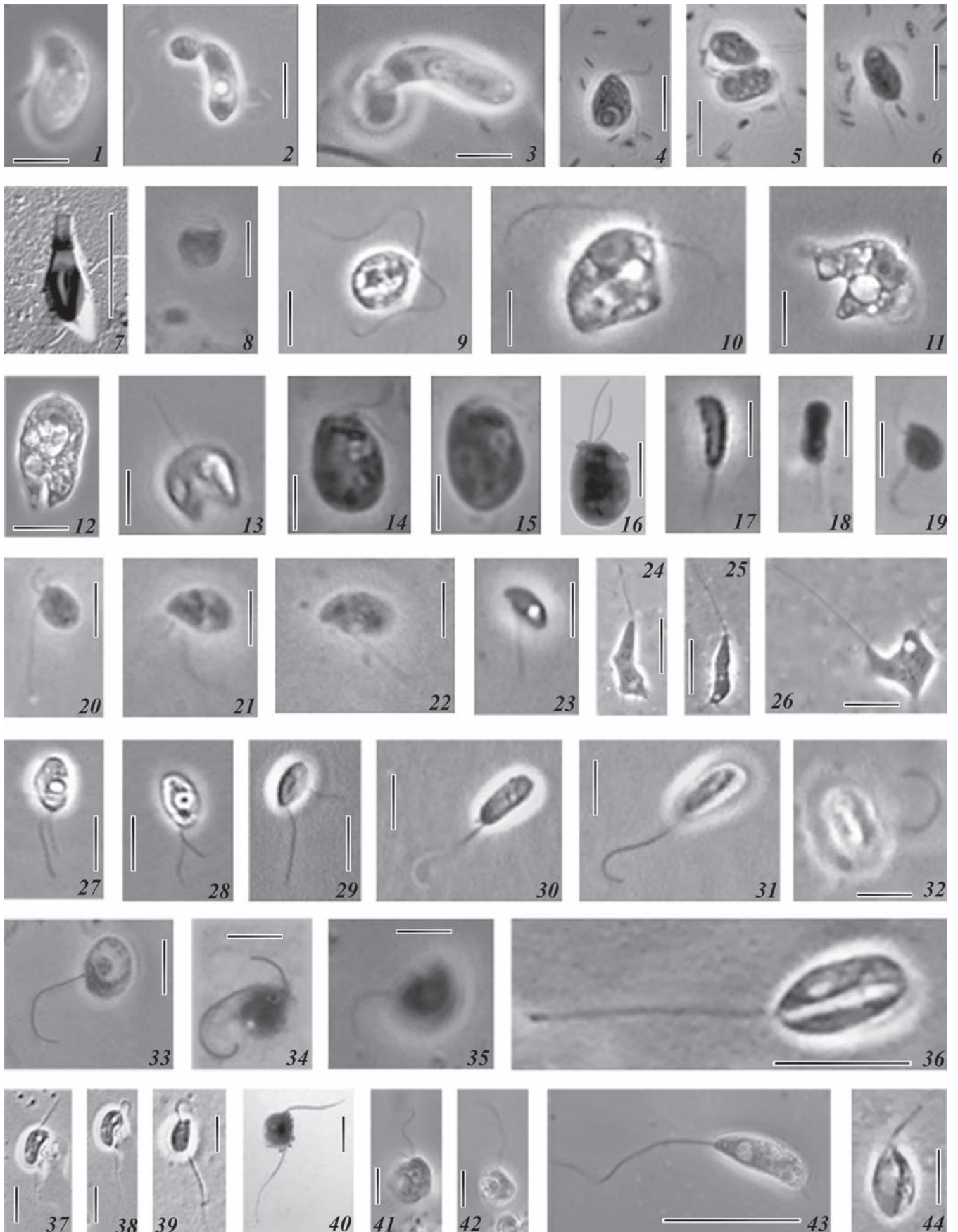


Table 1. Species list and frequency of occurrence of HF (amount of habitats, where species was found and, in brackets, total number of habitats) in different environments in Wuhan area.

Morphospecies	River sediments	Lake sediments	Soil, litter and mosses
AMOEOBOZA (Lühe, 1913) emend. Cavalier-Smith, 1998			
Eumycetozoa Zopf, 1884, emend. Olive, 1975 [not Mycetozoa de Bary, 1873]			
<i>Hyperamoeba flagellata</i> Aléxiéeff, 1923	0(6)	1(6)	0(9)
Mastigamoebidae Goldschmidt, 1907			
<i>Incertae sedis</i> AMOEOBOZA: Spongomonadida (Hibberd, 1983) emend. Karpov, 1990			
<i>Phalansterium</i> sp.	0(6)	0(6)	1(9)
<i>Spongomonas uvella</i> Stein, 1878	0(6)	0(6)	2(9)
OPISTHOKONTA Cavalier-Smith, 1987, emend. Cavalier-Smith and Chao, 1995, emend. Adl <i>et al.</i> , 2005			
Choanomonada Kent, 1880			
<i>Codonosiga botrytis</i> Kent, 1880	0(6)	0(6)	2(9)
<i>Salpingoeca amphoridium</i> Clark, 1868	0(6)	0(6)	1(9)
<i>S. gracilis</i> Clark, 1868	0(6)	1(6)	0(9)
<i>S. massarti</i> de Saedeleer, 1927	0(6)	0(6)	1(9)
<i>S. megachelia</i> Ellis, 1929	0(6)	0(6)	1(9)
<i>S. minor</i> Dangeard, 1910	0(6)	0(6)	1(9)
RHIZARIA Cavalier-Smith, 2002			
Cercozoa Cavalier-Smith, 1998, emend. Adl <i>et al.</i> , 2005			
<i>Agitata tremulans</i> Bass and Cavalier-Smith, 2009	1(6)	0(6)	3(9)
<i>Cercomonas angustus</i> (Skuja, 1948) Mylnikov and Karpov, 2004	0(6)	0(6)	3(9)
<i>C. granulifera</i> (Hollande, 1942) Mylnikov and Karpov, 2004	0(6)	0(6)	2(9)
<i>C. longicauda</i> Dujardin, 1841	1(6)	0(6)	0(9)
<i>C. ovatus</i> (Klebs, 1892) Tikhonenkov, 2007	0(6)	0(6)	1(9)
<i>C. radiatus</i> (Klebs, 1892) Mylnikov and Karpov, 2004	0(6)	1(6)	0(9)
<i>Cercomonas</i> sp. 1	0(6)	0(6)	2(9)
<i>Cercomonas</i> sp.2	0(6)	0(6)	2(9)
<i>Paracercomonas crassicauda</i> (Dujardin, 1836) Bass and Cavalier-Smith, 2009	0(6)	0(6)	3(9)
<i>Helkesimastix faecicola</i> Woodcock et Lapage, 1914	0(6)	4(6)	0(9)
<i>Bodomorpha reniformis</i> Zhukov, 1975	0(6)	0(6)	1(9)
<i>Bodomorpha minima</i> Hollande, 1942	2(6)	0(6)	4(9)
<i>Allantion tachyploon</i> Sandon, 1924	1(6)	1(6)	2(9)
<i>Metromonas simplex</i> (Griessmann, 1913) Larsen and Patterson, 1990	0(6)	0(6)	1(9)
<i>Metromonas</i> sp. sensu Tikhonenkov, 2007/2008	0(6)	0(6)	1(9)
<i>Protaspa</i> aff. <i>gemmifera</i> Larsen and Patterson, 1990	0(6)	0(6)	1(9)
<i>P. simplex</i> Vørs, 1992	1(6)	1(6)	3(9)
<i>Protaspa</i> sp.	0(6)	0(6)	1(9)
<i>Thaumatomonas seravini</i> Mylnikov and Karpov, 1993	1(6)	0(6)	1(9)
CHROMALVEOLATA Adl <i>et al.</i> , 2005			
Cryptophyceae Pascher, 1913, emend. Schoenichen, 1925			
<i>Goniomonas truncata</i> (Fresenius, 1858) Stein, 1887	1(6)	1(6)	1(9)
Kathablepharida Okamoto et Inouye, 2005			
<i>Kathablepharis ovalis</i> Skuja, 1948	0(6)	1(6)	0(9)

<i>Kathablepharis</i> sp.	0(6)	1(6)	0(9)
Stramenopiles Patterson, 1989, emend. Adl <i>et al.</i> , 2005			
<i>Bicosoeca lacustris</i> Skuja, 1948	0(6)	1(6)	0(9)
<i>Cyathobodo</i> sp.	0(6)	0(6)	1(9)
<i>Paraphysomonas</i> sp.	6(6)	6(6)	3(9)
<i>Spumella</i> spp.	5(6)	5(6)	9(9)
<i>Pteridomonas pulex</i> Penard, 1890	0(6)	0(6)	1(9)
Alveolata Cavalier-Smith, 1991			
<i>Colpodella angusta</i> (Dujardin, 1841) Simpson and Patterson, 1996	0(6)	1(6)	0(9)
<i>Colponema edaphicum</i> Mylnikov and Tikhonenkov, 2007	0(6)	0(6)	1(9)
EXCAVATA Cavalier-Smith, 2002, emend. Simpson, 2003			
Fornicata Simpson, 2003			
<i>Hexamita crassa</i> Klebs, 1893	0(6)	1(6)	0(9)
<i>H. mutabilis</i> Zhukov <i>et al.</i> , 1978	0(6)	2(6)	0(9)
<i>Trepomonas agilis</i> Dujardin, 1841	0(6)	1(6)	0(9)
Euglenozoa Cavalier-Smith, 1981, emend. Simpson, 1997			
<i>Entosiphon sulcatum</i> (Dujardin, 1841) Stein, 1878	0(6)	2(6)	1(9)
<i>Peranema dolichonema</i> Larsen and Patterson, 1990	0(6)	0(6)	1(9)
<i>Petalomonas minor</i> Larsen and Patterson, 1990	0(6)	1(6)	1(9)
<i>P. minuta</i> Hollande, 1942	0(6)	5(6)	0(9)
<i>P. ornata</i> Skvortzow, 1957	0(6)	2(6)	0(9)
<i>P. pusilla</i> Skuja, 1948	0(6)	3(6)	0(9)
<i>Petalomonas</i> sp. 1	0(6)	0(6)	1(9)
<i>Ploeotia plana</i> (Christen, 1959) Schroeckh, Lee and Patterson, 2003	0(6)	0(6)	1(9)
<i>Notosolenus apocamptus</i> (Stokes, 1884) Stokes, 1884	0(6)	0(6)	1(9)
<i>N. mediocanellatus</i> (Stein, 1878) Schroeckh, Lee and Patterson, 2003	0(6)	1(6)	0(9)
<i>Rhynchomonas nasuta</i> (Stokes, 1888) Klebs, 1892	6(6)	6(6)	0(9)
<i>Bodo angustatus</i> (Dujardin, 1841) Bütschli, 1883	0(6)	0(6)	1(9)
<i>B. minimus</i> Klebs, 1893	0(6)	0(6)	2(9)
<i>B. saltans</i> Ehrenberg, 1832	3(6)	4(6)	0(9)
<i>Neobodo curvifilis</i> (Griessmann, 1913) Moreira, López-García et Vickerman, 2004	3(6)	5(6)	0(9)
<i>N. designis</i> (Skuja, 1948) Moreira, López-García et Vickerman, 2004	5(6)	5(6)	3(9)
<i>N. saliens</i> (Larsen and Patterson, 1990) Moreira, López-García et Vickerman, 2004	2(6)	1(6)	0(9)
<i>Parabodo caudatus</i> (Dujardin, 1841) Moreira, López-García et Vickerman, 2004	0(6)	1(6)	2(9)
<i>P. nitrophilus</i> Skuja, 1948	1(6)	6(6)	2(9)
<i>Phyllomitus apiculatus</i> Skuja, 1948 sensu Mylnikov, 1986	1(6)	2(6)	0(9)
APUSOZOA Cavalier-Smith, 1997			
<i>Ancyromonas sigmoides</i> Kent, 1880	2(6)	3(6)	1(9)
<i>Amastigomonas caudata</i> Zhukov, 1975	0(6)	3(6)	0(9)
<i>A. aff. mutabilis</i> (Griessmann, 1913) Molina and Nerad, 1991	0(6)	0(6)	1(9)
<i>Apusomonas proboscidea</i> Aléxéieff, 1924	0(6)	0(6)	1(9)
<i>Diphylleia rotans</i> Massart, 1920	0(6)	4(6)	1(9)
Eukaryotes <i>incertae sedis</i>			
<i>Breviata anathema</i> Walker, Dacks and Embley, 2006	0(6)	0(6)	1(9)



port the opinion that a great number of water column HF are ubiquitous species (Vørs 1992, Ekeboom *et al.* 1995/1996, Lee and Patterson 1998).

The highest amount of species were from the genera *Cercomonas* (7 species), *Salpingoeca* (5), *Petalomonas* (5), *Protaspa* (3), *Bodo* (3), *Neobodo* (3). The most common species are *Spumella* spp. (observed in 90% locations), *Paraphysomonas* sp. (71.4), *Neobodo designis* (66.6), *Rhynchomonas nasuta* (57.1), *Parabodo nitrophilus* (42.8), *Neobodo curvifilus* (38.1), and *Bodo saltans* (33.3). However, thirty-three species (49% of the total species richness) were detected in one sample only, which serves as evidence of great microhabitat heterogeneity and diversity of rarer species in the community.

The majority of flagellates observed were bacterivorous (according to Larsen and Patterson (1990), Sanders (1991)). Among encountered flagellates, 9 species of possible predators were identified: *Allantion tachyploon*, *Colpodella angusta*, *Colponema edaphicum*, *Diphylleia rotans*, *Kathablepharis ovalis*, *Kathablepharis* sp., *Metromonas simplex*, *Metromonas* sp., *Phyllomitus apiculatus* (Larsen and Patterson 1990, Zhukov 1993, Mylnikov and Mylnikova 2008, Mylnikov and Tikhonenkov 2007, Mylnikov 2009). Feeding process and huge food vacuoles with prey of some species are shown on Figs 3–1–3–5; 3–27, 3–33. The greatest number of predatory organisms was observed in the lake sediments. These species consume smaller flagellates and other protists, and therefore, like some ciliates and Metazoa, constitute ‘top-down control’ on assemblages of bacterivorous, cyanobacterivorous, omnivorous, and mixotrophic microorganisms. Some flagellates (such

as *Paraphysomonas*, *Goniomonas*, and *Ploetia*) have been concerned to omnivorous forms. Bacterio-detritophage collector feeders and filter feeders, which play important roles in abundance control, production and structure of bacterial communities (Berninger *et al.* 1991, 1993; Weisse 1991; Sanders *et al.* 1992), were prevalent at all sites.

Three species of diplomonads (genera *Hexamita* and *Trepomonas*) were observed in the lake sediments. These organisms with reduced mitochondria are generally considered to be the most common flagellates in anoxic habitats (Mylnikov 1991, Brugerolle and Müller 2000).

As in any study of this type, the total number of species recorded increased with an increase in sampling effort. To take into account the effect of unequal sampling within the types of habitat, we considered the relation between the number of morphospecies found, S , and number of habitats, N . To do this, the mean species numbers were calculated for repeated draws of N habitats chosen randomly from the total dataset of 21 habitats using the rarefaction procedure. The species accumulation curve (Fig. 5) fit well ($R^2 = 0.99$) with power function $S = 10.84N^{0.62}$. The curve does not flatten out (power coefficient is more than 0.5), so the species list obtained for these sites is far from being complete, and each new sample should yield new species. The local species richness in lake and terrestrial habitats (shown as diamond and triangle, respectively, in Fig. 5) fit the rarefaction curve well, and individual deviations do not exceed the confidence limits. Thus, the between-site differences in local species richness are mainly determined by unequal sampling efforts. However, the local

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Fig. 3. 1–3 – *Colpodella angusta*, (1) general view (flagella out of focus) and (2, 3) sucking of a prey; 4–7 – *Colponema edaphicum*, (4) big food vacuole, rostrum, (4, 6) general view, (5) division, (6) two heterodynamic flagella and (7) extrusive organelle (toxicyst), TEM; 8 – *Cyathobodo* sp. (truncated cell, two equal flagella, stalk out of focus); 9–13 – *Diphylleia rotans* (two isokont flagella, big longitudinal groove, big anterior nucleus); 14–16 – *Goniomonas truncata* (flattened cell with two slightly unequal flagella, band of ejectisomes near the anterior end, big contractile vacuole, big peristome), (16) whole mount (TEM); 17, 18 – *Helkesimastix faecicola* (small rostrum, gliding amoeboid cell, usually only posterior flagellum is visible); 19, 20 – *Bodomorpha minima* (rostrum, triangular or oval cell, small size); 21–23 – *B. reniformis* (big rostrum, small anterior flagellum, front nucleus, lateral contractile vacuole); 24–26 – *Hyperamoeba flagellata* (very long single anterior flagellum, posterior contractile vacuole, spicular pseudopodia); 27–29 – *Kathablepharis ovalis* (oval cell, flagella usually directed backward, big food vacuole); 30–33 – *Metromonas simplex* (30, 31) attached flattened cells, (32) nodding movement of cell, and (33) cell with captured prey; 34, 35 – *Metromonas* sp. sensu Tikhonenkov, 2007/2008, (34) – attached flattened cell and (35) nodding movement; 36 – *Notosolenus apocampius* (single anterior flagellum and visible longitudinal groove); 37–40 – *Parabodo nitrophilus* (visible front contractile vacuole, hook-like anterior flagellum, rostrum, flattened cell body), (40) whole mount (TEM); 41, 42 – *Paraphysomonas* sp. (stalk, spherical cell body, two heterocont (one long and one very short) flagella on cell top); 43 – *Peranema dolichonema* (big gliding cell, long anterior flagellum and big front nucleus); 44 – *Petalomonas minor* (small cell with single anterior flagellum and longitudinal ridge). Scale bars: 1 μm (7), 3 μm (17, 18), 4 μm (14–16), 5 μm (1–3, 8, 19–23, 30–32, 34, 35, 41, 42, 44), 10 μm (4–6, 10–12, 27–29, 33), 13 μm (37–40), 15 μm (9, 13, 24–26, 36), 30 μm (43).

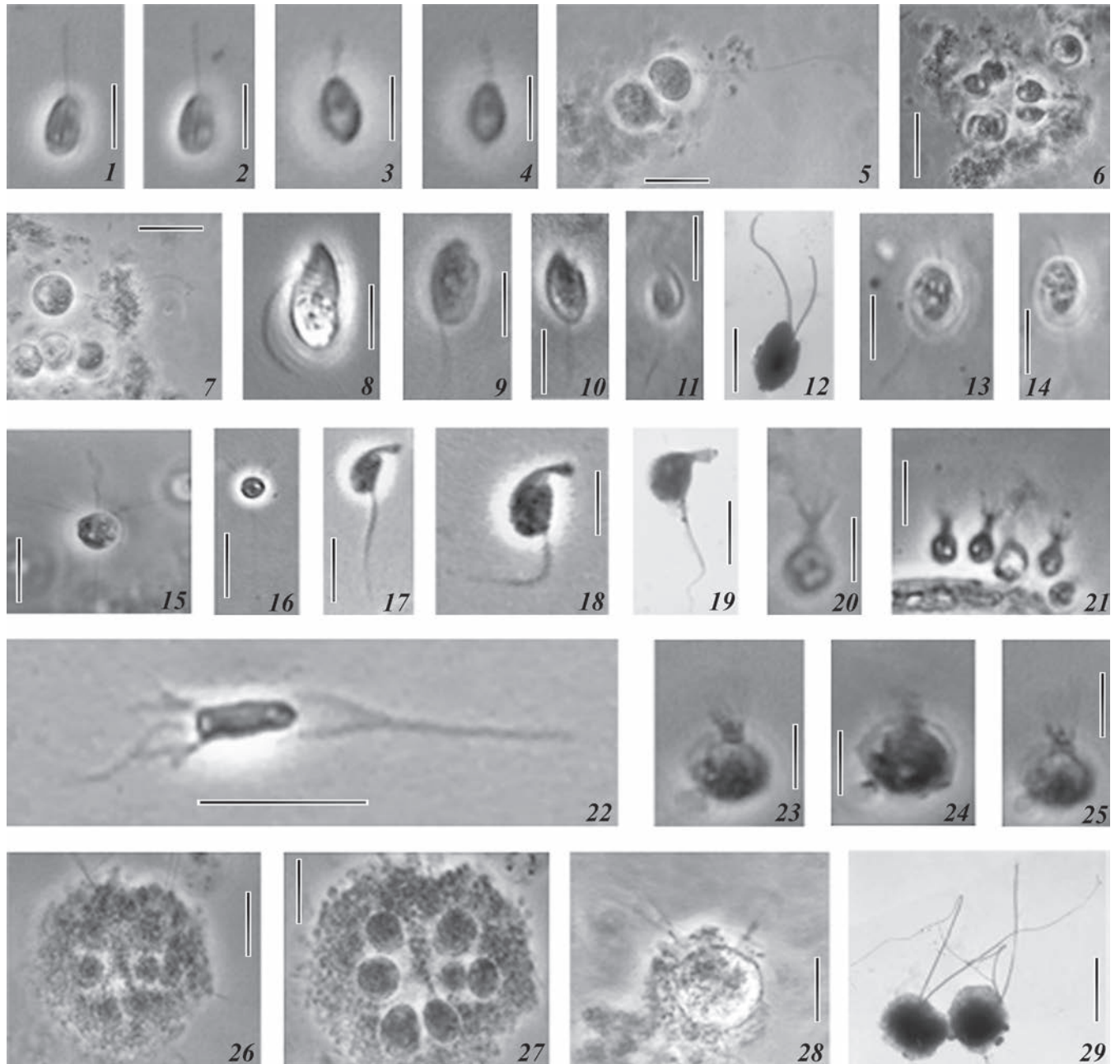


Fig. 4. 1, 2 – *Petalomonas minuta* (small cell with single anterior flagellum and longitudinal groove); 3, 4 – *P. pusilla* (small cell with single anterior flagellum and truncate anterior end); 5–7 – *Phalansterium* sp. (colonies of unikont spherical cell surrounded by mucilage); 8–12 – *Phyllomitus apiculatus* (cells with visible flagellar pocket, two heterodynamic flagella, anterior flagellum curves back over the rostrum (8), big food vacuoles, (12) whole mount (TEM); 13, 14 – *Protaspa simplex* (gliding spherical cells, cell body wags in unison with the flagellar beat); 15, 16 – *Pteridomonas pulex* (single apical flagellum emerging from a slight depression at the top of the cell, flagellum is surrounded by 12 stiffarms, body attaches to substrate by thin stalk); 17–19 – *Rhynchomonas nasuta* (oval cell bears undulating proboscis and long posterior flagellum), (19) whole mount (TEM); 20, 21 – *Salpingoeca amphoridium* (cell with narrow neck inside amphora-like lorica); 22 – *S. gracilis* (cylindrical cell inside cyathiform lorica); 23–25 – *S. minor* (spherical cell with narrow neck inside round lorica); 26–29 – *Spongomonas uvella*, (spherical cells with two isokont long acronematic flagella form colonies surrounded minute granules) (29) whole mount (TEM). Scale bars: 4 μm (3, 4), 5 μm (18, 23–25, 28, 29), 6 μm (1, 2), 8 μm (5, 7, 17, 19), 10 μm (6, 8–10, 12–14, 20, 27), 15 μm (11, 15, 21, 22, 26), 25 μm (16).

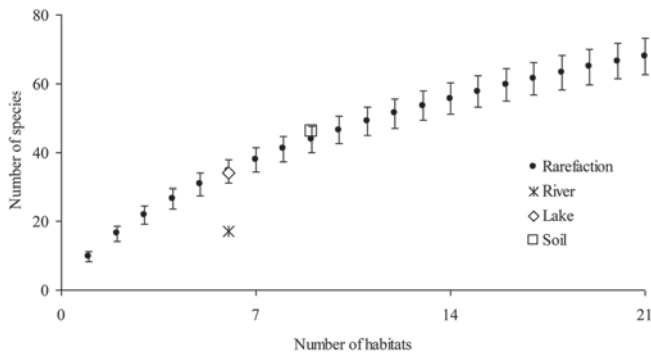


Fig. 5. Species accumulation curve. Whiskers – standard deviation.

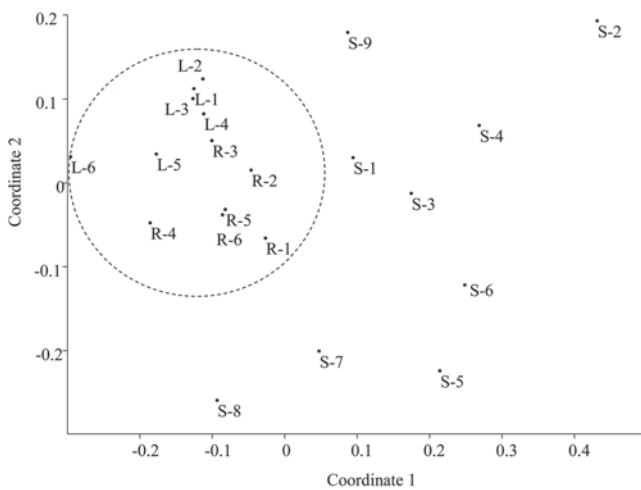


Fig. 6. Results of HF communities ordination (MDS-analysis) by species composition. S1–S9 – terrestrial (mainly soil) habitats, L1–L6 – lake sediments, R1–R6 – river sediments.

species richness of communities from the river habitats (star in Fig. 5) is characterized by a significantly lower level than predicted by the rarefaction curve. This value can be a result of too low sampling effort in the river or, most probably, by the extremely nonstable sandy biotope on the bank of the river.

A multidimensional scaling (MDS) analysis (Fig. 6) were performed to ordinate the local communities from the different habitats by species composition. The riverine and the lake communities are more similar to each other than soil ones. This fact indicates that HF communities from terrestrial habitats are more heterogeneous than those from freshwater sediments first of

all because we sampled more heterogeneous soil habitats (moss, litter, soil) compared to freshwater (sandy/silty sediments). Common species (occurring in more than half of the samples) for the aquatic habitats were *Rhynchomonas nasuta*, *Paraphysomonas* sp., *Neobodo designis*, *N. curvifilis*, *Bodo saltans* and *Spumella* spp. The last taxon is the only common HF in the soils (Table 1). Otherwise, most characteristic taxa (Table 1) for lake sediments are *Helkesimastix faecicola*, *Petalomonas minuta*, *P. pusilla*, *Diphylleia rotans*, *Amastigomonas caudata*. Amoeboflagellates (*Cercomonas angustus*, *C. granulifera*, *Paracercomonas crassicauda*) are specific for terrestrial habitats. There are no unique taxa in river sediments.

To date, information on community structure patterns of HF in various biotopes is absent for the Wuhan Region. Data concerning the HF biodiversity of Central China are limited to several papers. During investigations of the protozoan fauna of the Three-Gorges area in the Yangtze River (at the section from Fengjie to Yichang) 40 HF species were identified (Gong *et al.* 1990). They noted that in comparison with the protozoan fauna of Tibet, some lakes in middle and lower reaches of Yangtze River showed that most protozoan species can be found in all of these areas, implying that their distributions tend to be cosmopolitan.

Species composition and seasonal changes of total protozoan abundance were examined in the course of protozoan investigation at the Dongting Lake Outlet of the Yangtze River (Song 2000). Investigation of soil protozoa in the wetland treatment system of a Pb-Zn mine in Fankou showed that the abundance of flagellates was higher than that of ciliates and amoebae (Feng *et al.* 2004). It was suggested that the dominance of flagellates in the wetland system was contributed by the type-specific porous structures of soils, and its food source. Studies of flagellates in the National Nature Reserve of Baishuijiang, Gansu found unique species composition of HF (Ning *et al.* 2007). Xu *et al.* (2005) have listed 31 HF species collected from polyurethane foam units in Chaohu Lake. During studies of community characteristics of soil flagellates and amoebae in Mayan Forest Region, Zou *et al.* (2009) have showed that Amoebida was the dominant group, Kinetoplastida and Euglenida were the subdominant groups, Dinoflagellida, Choanoflagellida, Pelobiontida, and Diplomonada were the incidental groups, and *Bodo minimus*, *Bodo ovatus*, and *Oikomonas termo* were the dominant species. The result of the study showed that there were abundant species as well as endemic and rare species

in the community of soil HF and amoebas, the species composition of soil flagellates and sarcodes in the Maayan Forest Region of the National Nature Reserve of Xiaolong Mountains was unique.

Recently Ning and Shen (1998a, b, 1999) have reviewed faunal characteristics and distribution of species of soil protozoa in 6 typical zones of China. They revealed that diversity and standing crop of soil protozoa in various typical zones did not show an increasing or decreasing trend from south to north of China. On the other hand, the species distribution and analyses 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. Our data support this position. Local communities in different terrestrial habitats seem to be heterogeneous.

Among the morphospecies of heterotrophic euglenids identified from the various investigated freshwater biotopes, some forms (*Notosolenus apocamptus*, *Peranema dolichonema*, *Petalomonas minor*, *P. minuta*, and *P. pusilla*) had been previously observed in marine waters (Larsen and Patterson 1990, Tikhonenkov *et al.* 2006). Morphological features of these species corresponded with those reported elsewhere (Larsen and Patterson 1990, Patterson and Simpson 1996, Lee and Patterson 2000, Al-Qassab *et al.* 2002), with the exception of contractile vacuoles, which are absent in marine organisms. It is possible that in this case we observe different 'physiological species,' for which morphological criteria cannot be applied to distinguish them taxonomically. According to the recent report by Koch and Ekelund (2005), individuals of the same 'morphospecies' *Bodo designis*, isolated from different marine, freshwater and soil habitats, showed a different degree of tolerance to salinity changes and were characterized by high genetic heterogeneity. Thus they proposed that the clones investigated belong to several different species.

The majority of identified heterotrophic flagellates were noted in China earlier (Skvortzov 1957, Xu *et al.* 2005, Chen *et al.* 2008, Zou *et al.* 2009). New and unique HF were not revealed for the studied area during the present investigation. However, 10 taxa could not be identified to the species level. The HF morphospecies from examined biotopes are common (and often predominant) for other regions and geographical zones in riverine (Weitere and Arndt 2003, Kopylov *et al.* 2006, Kosolapova 2007, Tikhonenkov and Mazei 2008, Kiss *et al.* 2009) and lake (Mylnikov and Zhgarev 1984,

Auer and Arndt 2001, Domaizon *et al.* 2003, Tikhonenkov 2007/2008, Kosolapova and Kosolapov 2009) habitats, as well as in soils (Foissner 1991, Ekelund and Patterson 1997, Tikhonenkov *et al.* 2010).

To date, mainly planktonic habitats were investigated in the freshwaters and the data on benthic HF remains fragmentary. In the planktonic communities of rivers and lakes choanoflagellates, chrysomonads, bicosoecids are usually prevailing in terms of species richness (Zhukov *et al.* 1998, Kosolapova 2007, Kiss *et al.* 2009, Kopylov *et al.* 2009, Kosolapova and Kosolapov 2009). On the other hand, cercomonads, euglenids, and kinetoplastids are more common in soils (Foissner 1991, Tikhonenkov *et al.* 2010).

Peculiarity of the soil as a habitat for soil protists is defined by the necessity of movement in narrow interstitial spaces (pores, capillaries), "discontinuity" of water distribution and a diversity of its physical forms, as well as the volatility of moisture content (Fedorov 1972). Cercomonads have amoeboid cells; the overwhelming majority of heterotrophic euglenids are dorso-ventrally flattened. Those not flattened (some kinetoplastids) tend to be flexible, whereas larger species tend to be long and narrow (Alongi 1991). The ability to produce resting cysts, which help surviving unfavorable (dry) conditions, is well-known for many cercomonads and glissomonads (Mylnikov and Karpov 2004, Howe *et al.* 2009). These characters are thought to be adaptations to life in small cavities and thin water films surrounding the soil particles (Ekelund and Patterson 1997).

During such short and moderate research in poor investigated central subtropical China we have found species rich and heterogeneous communities of HF; the species list obtained for studied freshwater and soil sites is far from being complete, and each new sample should yield new species. The majority of the heterotrophic flagellates identified in this survey have been noted in China earlier. They are common (and usually predominant) for other regions in both freshwater and soil habitats.

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