国立科博専報, (50), pp.21–28, 2014 年 3 月 28 日
Mem. Natl. Mus. Nat. Sci., Tokyo, (50), pp.21–28, March 28, 2014

Testate Amoebae of the Imperial Palace, Tokyo

Satoshi Shimano[1*], Anatoly Bobrov[2] and Yuri Mazei[3]

[1] Environmental Education Center, Miyagi University of Education,
Aramaki Aza-Aoba, Aoba-ku, Sendai city, Miyagi, 980–0845 Japan.
*mitesproto@gmail.com
[2] Lomonosov Moscow State University, Leninskie gory, Moscow, Russia
[3] Penza State University, Penza, Russia

Abstract. The testate amoeba fauna of the Imperial Palace, Tokyo was surveyed. Totally 54 species, 12 varieties and forms belonging to 14 genera and two taxa with unknown taxonomic position of testate amoebae were recorded there. One genus, *Planhoogenraadia*, 19 species and 11 varieties and forms are newly recorded from Japan. Testate amoebae community in Imperial Palace is characterized by low alpha-diversity and high beta-diversity. Most common taxa, found in more than half of the sampling sites, are *Cyclopyxis eurystoma* v. *parvula*, *Euglypha laevis* and *Trinema lineare*.

Key words: Amoebozoa, Imperial Palace, new record in Japan, Rizaria, soil, suspended litter, testate amoebae,

Introduction

Testate amoebae (belonged to supergroups Amoebozoa and Rhizaria: Adl *et al*., 2012) from terrestrial habitats in Japan have been inadequately studied. Soil protists of Japan have not been reviewed but Shimano & Miyoshi (2008) summarized publications on ciliates and testate amoebae as a bibliography.

The few existing data on soil testate amoebae in Japan are of special interest for biogeography and ecology. Publications dealing with specific aspects of morphology and biology of soil testate amoeba are scarce (e.g. Aoki (2003) and Aoki *et al*. (2007)). Coûteaux (1978) described several new species and one new genus found on the island of Honshu, in the valley of Shiga, 200 kilometers northwest of Tokyo. Recently, Bobrov *et al*. (2012) described two new species of testate amoebae from mountain massif Tateyama, Toyama prefecture, central Japan.

In the Imperial Palace, some prominent testate amoebae species were recorded from fresh water habitats. Tanaka & Takeda (2000) identified 21 species, *Arcella catinus* Pénard, 1890; *Arcella conica* (Playfair, 1918) Deflandre, 1926; *Arcella discoides* Ehrenberg, 1871; *Arcella polypora* Pénard, 1890; *Arcella vulgaris* Ehrenberg, 1832; *Centropyxis aculeata* (Ehrenberg, 1830) Stein, 1857; *Centropyxis hirsuta* Deflandre, 1929; *Centropyxis discoides* (Pénard, 1890) Deflandre, 1929; *Centropyxis spinosa* (Cash, 1905) Deflandre, 1929; *Lesquereusia modesta* Rhumbler, 1895; *Nebela tincta* (Leidy, 1879) Awerintzew, 1906; *Difflugia acuminata* Ehrenberg, 1838; *Difflugia tuberculata* (Wallich, 1864) Archer, 1867; *Difflugia avellana* Pénard, 1890; *Difflugia globulosa* Dujardin, 1837; *Difflugia gramen* Pénard, 1902; *Difflugia labiosa* Wailes, 1919; *Difflugia curvicaulis* Pénard, 1899; *Difflugia lanceolata* Pénard, 1890; *Difflugia corona* Wallich, 1864; *Euglypha filifera* Pénard, 1890.

Tanaka *et al*. (2006) also recorded five species, *Arcella dentata* Ehrenberg, 1832; *Difflugia oblonga* Ehrenberg, 1838; *Heleopera sphagni* (Leidy, 1874) Hopkinson, 1909; *Trinema lineare* Pénard, 1890; *Trinema enchelys* (Ehrenberg, 1838) Leidy, 1878. Thus, totally 25 species (taking into the account that *Difflugia curvicaulis* is the junior synonym of *Difflugia acuminata*: see Mazei & Warren, 2012) were recorded from the Imperial Palace. In present study, some samples were taken from several habitats, mainly from soil.

Materials and Methods

Samples were from mainly three area of the Imperial Palace on 1st of March in 2010, sampling sites (A–I) were shown in Fig 1. For testate amoebae sampling, several habitats were sampled with three replicants by Satoshi Shimano (the author) in each sampling point (Table 1). Especially for soil sample, both litter and soil of the uppermost 5 cm were sampled. Testate amoebae were extracted from a 5 cm^3 quot of the test substrate taken from each sample irrespectively of the nature of habitat in accordance with the method described in Mazei & Chernyshov (2011). The specimens were studied using light microscopes.

Results and Discussion

Species list

Totally 54 species, 12 varieties and forms belonged to 14 genera and two taxa with unknown taxonomic position of testate amoebae were recorded there. One genus, *Planhoogenraadia*, 19 species and 11 varieties and forms are newly recorded from Japan. The sample code in this survey (table 1) is shown for each species as numbers after dash. [(i) higher taxa follow Meisterfeld (2000a, b) and Adl *et al*. (2012). (ii) *** – genus new to Japan, ** – species new to Japan, * – varieties and forms new to Japan, † – species recorded by Tanaka & Takeda (2000) and Tanaka *et al*. (2006)].

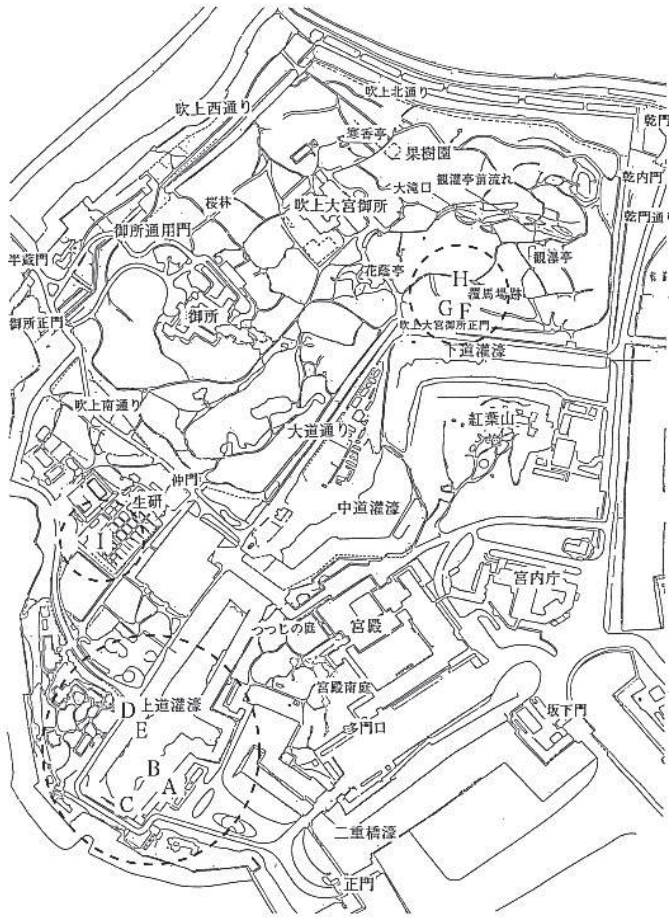

Fig. 1. The sampling points for testate amoebae in the Imperial Palace, Tokyo. A sample code (table 1) is given to each sampling point (A–G). Broken line: locations of three area for sampling points, entrance of Fuku-age-gosyo (northeast area), southwest part of Kami-doukan-bori (southwest area) and rice paddy field (west area). Map after Aoki (2000).

Table 1. The sampling points for testate amoebae in the Imperial Palace, Tokyo.

Sample code	Location	Sampling point	Habitat	Vegetation
1	At the top of a ridge	A	Hollow of pine tree stump	*Pinus thunbergii*
2	At the top of a ridge	A	Soil and thick litter layer of pine	*Pinus thunbergii*
3	At the top of a ridge	A	*Suspended litter on branches of a *Cinnamomum* tree	*Cinnamomum camphora, Machilus thunbergii, Pinus thunbergii*
4	Grassy field of a slope beside moat	B	Soil and litter	*Cinnamomum camphora, Machilus thunbergii, Pinus thunbergii*
5	Grassy field of a slope beside moat	B	Soil and litter pooled in the basal part of a *Machilus* tree	*Cinnamomum camphora, Machilus thunbergii, Pinus thunbergii,* only leaves of *Ginkgo biloba*
6	Wooded area beside moat	C	Soil and litter	*Camellia japonica, Fatsia japonica, Zelkova serrata, Castanopsis sieboldii*
7	Wooded area beside moat	D	Soil and litter	*Camellia japonica, Fatsia japonica, Zelkova serrata, Castanopsis sieboldii*
8	Wooded area beside moat	E	*Leaf litter in moat water	*Camellia japonica, Fatsia japonica, Zelkova serrata, Castanopsis sieboldii*
9	Evergreen wooded area, at the bottom of a hill	F	Soil and litter	*Castanopsis sieboldii, Camellia japonica, Acer palmatum*
10	Evergreen wooded area, at the top of a hill	G**	Soil and litter	*Castanopsis sieboldii, Camellia japonica, Acer palmatum*
11	Deciduous wooded area, at the bottom of a hill	H**	Soil and litter	*Acer palmatum, Cerasus jamasakura, Swida controversa, Eurya japonica, Hedera rhombea, Ophiopogon japonicus*
12	Rice field closed to the biological laboratory	I	*Soil and basal part of rice left in paddy field drained freshwater after harvesting	*Oryza sativa*

* not soil sample; ** same sampling points as Aoki (2000) (G is same as “A”, H is same as “B”).

AMOEBOZOA Luhe, 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 arenaria* f. *compressa* Chardez, 1974* – 6, 9, 11

SUBORDER DIFFLUGINA Meisterfeld, 2000

FAMILY DIFFLUGIIDAE Wallich, 1864

Genus *Difflugia* Leclerc, 1815

2. *Difflugia lucida* Penard, 1890 – 10, 12

FAMILY CENTROPYXIDAE Jung, 1942

Genus *Centropyxis* Stein, 1857

3. *Centropyxis aerophila* Deflandre, 1929 – 7, 10, 12
4. *Centropyxis aerophila* v. *minuta* Chardez, 1964* – 10
5. *Centropyxis constricta* (Ehrenberg, 1843) Deflandre, 1929 – 3
6. *Centropyxis constricta* f. *minima* Decloitre, 1953* – 12
7. *Centropyxis ecornis* (Ehrenberg, 1841) Leidy, 1879 – 9
8. *Centropyxis ecornis* f. *minima* Golemansky, 1962* – 9
9. *Centropyxis elongata* (Penard, 1890) Thomas, 1959** – 7
10. *Centropyxis* cf. *gibbosa* Rampi, 1950 (forma nova *minor*)** – 3

11. *Centropyxis latideflandriana* Bonnet, 1979** – 5, 12
12. *Centropyxis minuta* Deflandre, 1929 – 2, 9
13. *Centropyxis plagiostoma* Bonnet & Thomas, 1955 – 2
14. *Centropyxis plagiostoma* f. B (*minor*) *– see: Bobrov & Krasilnikov, 2011 – 6
15. *Centropyxis sacciformis* Hoogenraad & de Groot, 1940** – 5, 11
16. *Centropyxis sylvatica* (Deflandre, 1929) Bonnet & Thomas, 1955 – 3, 5, 9–12

FAMILY TRIGONOPYXIDAE Loeblich & Tappan, 1964
Genus *Cyclopyxis* Deflandre, 1929
17. Cyclopyxis eurystoma Deflandre, 1929 – 9
18. *Cyclopyxis eurystoma* v. *parvula* Bonnet & Thomas, 1960 – 1–10, 12
19. *Cyclopyxis kahli* Deflandre, 1929 – 6, 8

FAMILY PLAGIOPYXIDAE Bonnet & Thomas, 1960
Genus *Plagiopyxis* Pénard, 1910
20. *Plagiopyxis* cf. *barrosi* Bonnet, 1960** – 1
21. *Plagiopyxis* cf. *intermedia* Bonnet, 1959** – 8
22. *Plagiopyxis labiata* Penard, 1910** – 12
23. *Plagiopyxis minuta* Bonnet, 1959** – 3, 5–9, 11, 12
24. *Plagiopyxis minuta* v. *oblonga* Bonnet, 1959*– 6
25. *Plagiopyxis penardi* Thomas, 1958** – 6, 7, 9

Genus *Planhoogenraadia* Bonnet, 1977
26. *Planhoogenraadia daurica* Bobrov, 2001***** – 9

FAMILY HYALOSPHENIIDAE Schultze, 1877
Genus *Hyalosphenia* Stein, 1859
27. *Hyalosphenia minuta* Cash, 1891** – 7

FAMILY HELEOPERIDAE Jung, 1942
Genus *Heleopera* Leidy, 1879
28. *Heleopera sylvatica* Penard, 1890** – 1
29. *Heleopera* sp. – 2, 11

SUBORDER PHRYGANELLINA Bovee, 1985
FAMILY PHRYGANELLIDAE Jung, 1942
Genus *Phryganella* Pénard, 1902
30. *Phryganella acropodia* (Hertwig & Lesser, 1874) Hopkinson, 1909 – 9

FAMILY CRYPTODIFFLUGIIDAE Jung, 1942
Genus *Cryptodifflugia* Pénard, 1890
31. *Cryptodifflugia oviformis* Penard, 1890 – 9
32. *Cryptodifflugia voigti* Schmidt, 1926** – 9

RHIZARIA Cavalier-Smith, 2002
CERCOZOA Cavalier-Smith, 1998
SILICOFILOSEA Adl *et al*., 2005
ORDER EUGLYPHIDA Copeland, 1956
FAMILY EUGLYPHIDAE Wallich, 1864
Genus *Euglypha* Dujardin, 1841
33. *Euglypha anodonta* Bonnet, 1960** – 5, 12
34. *Euglypha ciliata* (Ehrenberg, 1848) Wailes, 1878 – 12
35. *Euglypha cuspidata* Bonnet, 1959** – 5
36. *Euglypha filifera* v. *magna* van Oye, 1958* – 11, 12
37. *Euglypha laevis* Perty, 1849 – 1–3, 5–12
38. *Euglypha strigosa* v. *heterospina* Wailes, 1912* – 11
39. *Euglypha polylepis* (Bonnet, 1959) Bonnet & Thomas, 1960** – 11
40. *Euglypha* sp. – 10–12

Genus *Tracheleuglypha* Deflandre, 1928
41. *Tracheleuglypha acolla* Bonnet & Thomas, 1955 – 1, 3, 7, 8, 10, 11
42. *Tracheleuglypha* sp. – 6, 7, 9, 10, 12

Genus *Sphenoderia* Schlumberger, 1845
43. *Sphenoderia fissirostris* Penard, 1890** – 12

FAMILY TRINEMATIDAE Hoogenraad & de Groot, 1940
Genus *Trinema* Dujardin, 1841
44. *Trinema complanatum* Penard, 1890 – 2, 3, 11, 12
45. *Trinema complanatum* v. *elongata* Decloitre, 1973* – 6
46. *Trinema galeata* (Penard, 1890) Jung, 1942** – 3
47. *Trinema enchelys* (Ehrenberg, 1838) Leidy, 1878† – 9, 12

48. *Trinema lineare* Penard, 1890† – 1–3, 5–12
49. *Trinema lineare* v. *terricola* Decloitre, 1962* – 7
50. *Trinema penardi* Thomas & Chardez, 1958** – 3
51. *Trinema* sp. – 12

Genus *Corythion* Taránek, 1881

52. *Corythion dubium* f. *minima* Chardez, 1969* – 2, 12

incertae sedis

53. Testacea sp. 1 – 9
54. Testacea sp. 2 – 9

Biodiversity and community composition

Distribution of species richness per sampling points is shown on Fig. 2. Total number of species varied from one species in grassy field of a slope beside moat (sample code 4) to 19 in evergreen wooded area, at the bottom of a hill (sample code 9) and 24 in rice field closed to the biological laboratory (sample code 12). However, average number of species per sample in a sampling point varies insignificantly (Tukey's range test).

Most common species are *Cyclopyxis eurystoma* v. *parvula* (found in 72% of sampling points), *Euglypha laevis* (50%), *Trinema lineare* (50%). 29 species were recorded only in one sample (from the total 36 samples investigated). It reflects in high values of beta-diversity estimated as a power function in the species-accumulation curve (Fig. 3). In contrast, alpha-diversity (measured as an intercept in the species-accumulation curve on Fig. 3) is low and can be treated as 5.4 testate amoebae taxa per sample. Thus testate amoebae community in Imperial Palace is characterized by low alpha-diversity and high beta-diversity and seems to be beta-dominant.

Most of the species refers to be eurybionts. There are calcephilic (*Centropyxis plagiostoma, Cyclopyxis kahli*) and acidophilic species (*Centropyxis ecornis, C. ecornis* v. *minima, C. elongata, C.* cf. *gibbosa* f. *minor*, *Heleopera sylvatica, Hyalosphenia minuta, Difflugia lucida, Sphenoderia fissirostris.*

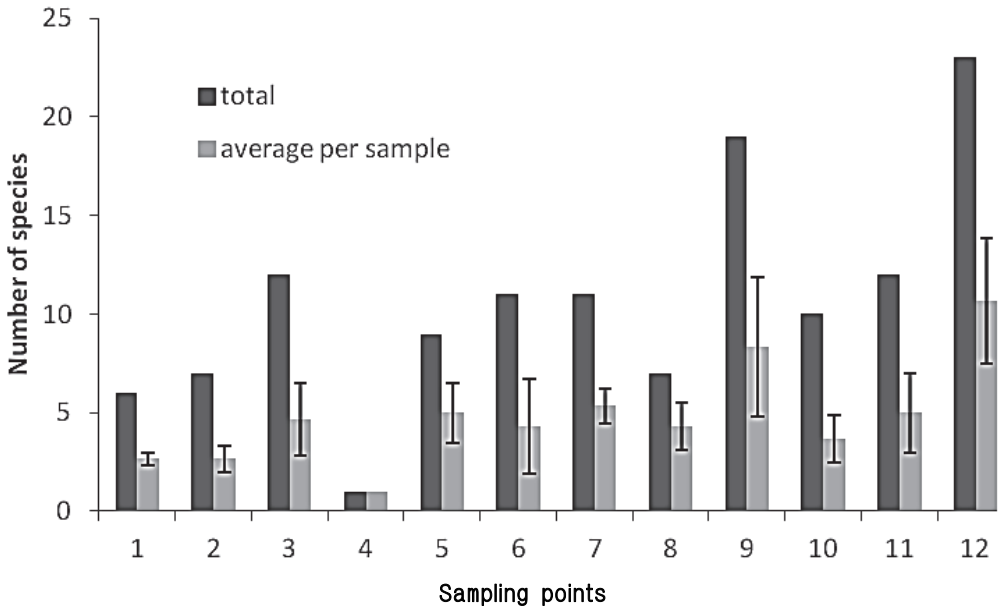

Fig. 2. Number of species at different sampling points (total for three replicates at each sampling point and average per sample). Whiskers: standard error of the mean. Average number of species per sample in a sampling point varies insignificantly (Tukey's range test).

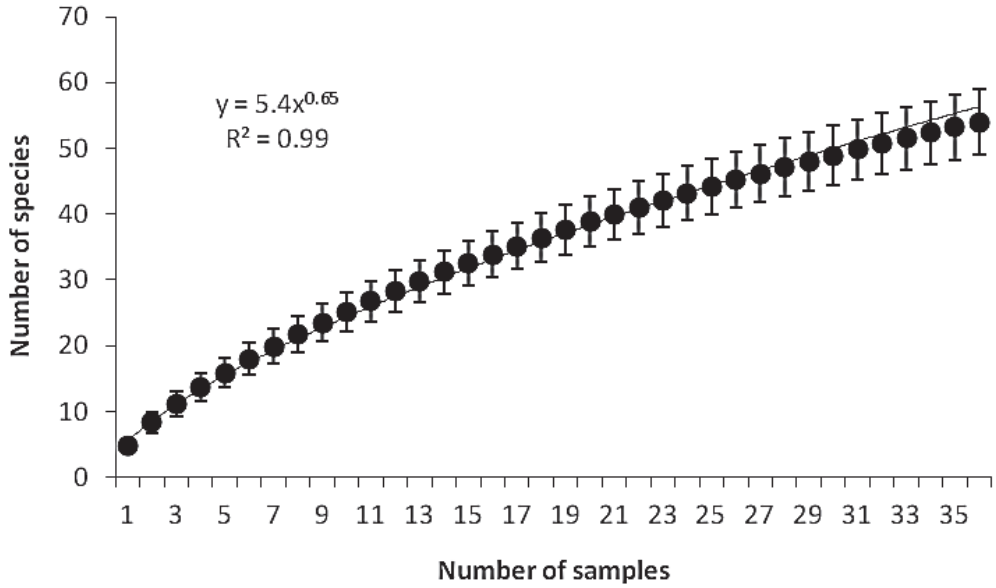

Fig. 3. Rarefaction curve of the species richness per number of species.

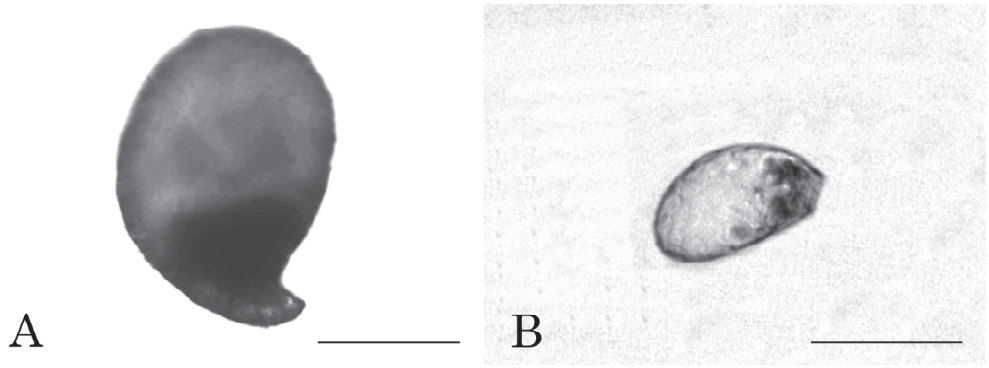

Fig. 4. Rare species found in samples of the Imperial Palace. A: *Planhoogenraadia daurica*, B: *Tracheleuglypha* sp. Scale bars=50µm.

Two species from the list have limited geographical distribution, namely *Centropyxis latideflandriana* and *Planhoogenraadia daurica* (fig. 4A). Former species is known as belonging to Gondwana-tropical group. Our data represent the northernmost finding of the species (Balik, 1995; Bobrov *et al*., 2010). Later species was described (Bobrov, 2000) from the forest soil in the territory of the Sikhote-Alin Reserve (Russian Far East). This is second record of the species found in the Imperial Palace.

Four species were unidentified in this study. For instance, rare species *Tracheleuglypha* sp. (fig. 4B) was found in five samples (sample code 6, 7, 9,10 and 12). It differs from the close related species *Tracheleuglypha selachiistoma* Bartos, 1963 by having small test and, subterminal aperture. It will be described as a new species in future study.

Acknowledgements

We wish to express my sincere gratitude to the Gardens Division, Maintenance and Works Department, Internal Subdivisions, Imperial Household Agency for their kind support on field research.

References

Adl, S. M., A. G. B. Simpson, C. E. Lane, J. Lukeš, D. Bass, S. S. Bowser, M. W. Brown, F. Burki, M. Dunthorn, V. Hampl, A. Heiss, M. Hoppenrath1, E. Lara, L. le Gall, D. H. Lynn, H. McManus, E. A. D. Mitchell, S. E. Mozley-Stanridge, L. W. Parfrey, J. Pawlowski, S. Rueckert, L. Shadwick,C. L. Schoch, A. Smirnov & F. W. Spiegel, 2012. The revised classification of eukaryotes. *Journal of Eukaryotic Microbiology*, 59: 429–493.

Aoki, J., 2000. Oribatid mites from the Imperial Palace, Tokyo. *Memoirs of the National Science Museum*, *Tokyo*, (35): 151–164 (in Japanese).

Aoki, Y., M. Hoshino & T. Matsubara, 2007. Silica and testate amoebae in a soil under pine–oak forest. *Geoderma*, 142: 29–35.

Aoki, Y., 2003. Accurate enumeration and identification of Testacea (Protozoa, Rhizopoda) in forest soil using scanning electron microscopy. *Journal of Microbiological Methods*, 55: 791–795.

Balik, V., 1995. Testate amoebae (Protozoa: Rhizopoda) from a primary mountain rain forest in the Tam-Dao region (Vietnam). *Acta Societatis Zoologicae Bohemoslovenicae*, 59: 1–16.

Bobrov, A., 2000. *Planhoogenraadia daurica* Bobrov is a new species of testate amoebae (Protozoa, Testacea) from Primor'e (The Far East). *Zoologicheskiy Zhurnal*, 79: 108–110 (in Russian).

Bobrov, A. & P. Krasilnikov, 2011. Testate amoebas of pine forests in Mexico. *Biology Bulletin,* 38: 400–405.

Bobrov, A., Yu. Mazei & A. Tiunov A., 2010. Testate amoebae of a monsoon tropical forest of South Vietnam. *Acta Protozoologica*, 49: 311–325.

Bobrov, A., S. Shimano & Yu. Mazei, 2012. Two new species of testate amoebae from the mountain forests soil of Japan and redescription of the genus *Deharvengia*. *Acta Protozoologica*, 51: 55–63.

Coûteaux, M.-M., 1978. Quelques thécamoebiens du sol du Japon. *Revue d'Écologie et de Biologie du Sol*, 15: 119–128.

Mazei, Yu. & V. A. Chernyshov, 2011. Testate amoebae communities in the southern tundra and forest-tundra of Western Siberia. *Biology Bulletin* 38: 789–796.

Mazei, Yu. & A. Warren, 2012. A survey of the testate amoeba genus *Difflugia* Leclerc, 1815 based on specimens in the E. Penard and C.G. Ogden collections of the Natural History Museum, London. Part 1: Species with shells that are pointed aborally and/or have aboral protuberances. *Protistology*, 7: 121–171.

Meisterfeld, R., 2000a. Order Arcellinida Kent, 1880. In: The Illustrated Guide to the Protozoa, vol. 2, 2nd ed. Lee, J.J., Leedale, G.F., Bradbury, P. (eds), Allen press Inc., Lawrence, Kansas, USA, pp. 827–860.

Meisterfeld, R., 2000b. Testate amoebae with filopodia. In: The Illustrated Guide to the Protozoa, vol. 2, 2nd ed. Lee, J.J., Leedale, G.F., Bradbury, P. (eds) Allen press Inc., Lawrence, Kansas, USA, pp. 1054–1084.

Shimano, S. & N. Miyoshi, 2008. A bibliography of publications on free-living protists (ciliates and testate amoebae) recorded from Japan – preliminary version, April 2008. *The Japanese Journal of Protozoology*, 41: 133–152 (in Japanese). Up-

dated version May, 2010: https://sites.google.com/site/bibliographyjapan

Tanaka, M. & M. Takeda, 2000. Protozoans, rotifers, cladocerans and copepods from the Inside Moats of Imperial Palace, Tokyo [Plates 1–16]. *Memoirs of the National Science Museum*, *Tokyo* (35): 233–244 + plat. 17.

Tanaka, M., M. Takeda & M. Nagano, 2006. Protozoans, Rotifers, Cladocerans and Copepods from the Inside Moats of the Imperial Palace, Tokyo. II. *Memoirs of the National Science Museum*, *Tokyo*, (43): 419–439.

皇居の有殻アメーバ類

島野智之・Anatoly Bobrov・Yuri Mazei

皇居の有殻アメーバ類を調査した．14属と2つの分類学的位置が未確定の種の合計54種，12の variety あるいは forma が得られた．1属（*Planhoogenraadia*），19種と，11の variety あるいは forma が日本新記録であった．皇居では，採集地点からは低いアルファ生物多様性，高いベータ生物多様性がみられた．もっとも普通に出現した種は，採集地点（sampling sites）の半分以上の試料から出現した *Cyclopyxis eurystoma* v. *parvula*, *Euglypha laevis* そして，*Trinema lineare* の3種であった．