10.15517/RBT.V67I3.32909
Artículo

Testate Amoebae of Peru: filling the gap in the Neotropics
Amebas testadas de Perú: llenando un vacío en el Neotrópico

Anatoly Bobrov[1]
Yuri Mazei[2,3*]
Anna Buyvolova[1]
Leon Yacher[4]

[1] Department of Soil Geography, Lomonosov Moscow State University, Moscow, Russia; anatoly-bobrov@yandex.ru, buyvolova@gmail.com
[2] Department of Hydrobiology, Lomonosov Moscow State University, Moscow, Russia; yurimazei@mail.ru
[3] Department of Zoology and Ecology, Penza State University, Penza, Russia
[4] Southern Connecticut State University, New Haven, CT, USA; yacher@southernct.edu
* Correspondence

Received 23-IV-2018
Corrected 14-VII-2018
Accepted 22-IV-2019

Abstract
Testate amoebae diversity from 28 surface (0-3 cm depth) soil samples found near Cuzco (6 samples), in Machu Picchu (17 samples), in Aguas Calientes (5 samples), and one bottom sediment sample from the Lake Titicaca near the Puno collected during March of 2016 were analyzed. The 144 testate amoebae species and infra-specific taxa belonging to 27 genera were identified. Nineteen amoebae have not been identified to species level and likely represent new taxa. Species richness varied from one to 54 taxa per sample. The highest diversity was found in rainforests followed by those in meadows and agave habitats. The only bottom sample from Lake Titicaca has yielded two hydrobiont species from the genus *Difflugia*. In the course of the study, several rare species with limited geographical distribution were observed, namely *Centropyxis castaneus, C. compressa, C. deflandriana, C. latideflandriana, C.* cf. *ohridensis, C.* cf. *ovoides, C.* cf. *pannosus, C. stenodeflandriana, Cyclopyxis plana, C. profundistoma, Apodera vas, Argynnia retorta, A. spicata, Certesella certesi, Trachelcorythion pulchellum.* Our study fills a geographical gap in the distribution of some flagship species with restricted geographic distribution, e.g. *Apodera vas* and *Certesella certesi* in Peru. The results illustrate the continuity of expansion species along the Pacific coast.
Key words: biodiversity; biogeography; mountain rainforest; Peru, protista.

Resumen
La diversidad de amebas testadas de la capa superior del suelo (0-3 cm de profundidad) fue analizada en 28 de muestras recolectadas cerca de Cuzco (seis muestras), Machu Picchu (17 muestras) y Aguas Calientes (cinco muestras), así como una muestra de sedimentos del fondo recolectada en el lago Titicaca, cerca de la ciudad de Puno, en marzo 2016. Se identificaron 144 especies de amebas testadas y taxones infra-específicos pertenecientes a 27 géneros. Un total de 19 amebas no han sido identificadas a nivel de especie y probablemente sean nuevos taxones. La riqueza de especies varía de uno a 54 taxones por muestra. La mayor diversidad fue encontrada en los bosques pluviales, prados y hábitats de agave. En los sedimentos del fondo del lago Titicaca se encontraron 2 especies hidrobiónticas del género *Difflugia*. En el estudio se encontraron varias especies raras con una limitada distribución geográfica, tales como:

Centropyxis castaneus, C. compressa, C. deflandriana, C. latideflandriana, C. cf. *ohridensis, C.* cf. *ovoides, C.* cf. *pannosus, C. stenodeflandriana, Cyclopyxis plana, C. profundistoma, Apodera vas, Argynnia retorta, A. spicata, Certesella certesi* y *Trachelcorythion pulchellum.* Nuestro estudio llena un vacío en la distribución geográfica de algunas especies importantes de Perú, que tienen una distribución geográfica limitada, por ejemplo: *Apodera vas* y *Certesella certesi*. Los resultados muestran la continuidad de la expansión de las especies a lo largo de la costa del Pacífico.
Palabras clave: biodiversidad; biogeografía; pluviselva; Perú; protista.

Introduction

Peru's territory is located according to the biogeographical zoning in the world's neotropical area (Udvardy, 1975). It is included in one of the world's 25 biodiversity hotspots (Myers, Mittermeier, Mittermeier, Da Fonseca, & Kent, 2000). The uniqueness of the climatic conditions caused primarily by the presence of mountain barriers that have influenced the formation of the neotropical flora and fauna over several million years (Antonelli, Nylander, Persson, & Sanmartín, 2009). Recent biogeographic partitioning of this region rests predominately on the studies of mosses, ferns, flowering plants, insects, birds, and small mammals (Morrone 2001, 2006, 2017; Hoorn et al., 2010; Herzog, Martínez, Jørgensen, & Tiessen, 2011). Less attention is paid to unicellular organisms, which macroscale patterns may considerably differ from those of macroorganisms (Martiny et al., 2006; Azovsky & Mazei, 2013, Azovsky, Tikhonenkov, & Mazei, 2016). Among protists, testate amoebae represent a common diverse and abundant component of both aquatic and terrestrial habitats, playing a vital role in ecosystem functioning. For example, in the soils of the southern taiga, they occupy in terms of biomass second place after fungi, exceeding the biomass of bacteria (Schröter, Wolters, & De Ruiter, 2003). These microorganisms are among the larger protist groups (most taxa 5-500 μm in length) and play important roles as consumers of smaller microorganisms and in the case of some species, in primary production of endosymbiotic algae (Wilkinson & Mitchell, 2010; Jassey et al., 2015). Thus, soil-inhabiting testate amoebae as part of terrestrial ecosystems can be used as biological indicators (Payne, 2013). However, their geographical distribution remains poorly understood, although we know that most of the species are cosmopolites with several cases of limited geographic distribution (Foissner, 2006; Smith & Wilkinson, 2007; Smith, Bobrov, & Lara, 2008; Heger et al., 2011a; Azovsky & Mazei, 2013; Azovsky et al., 2016).

There are a limited number of publications on Peru and contiguous areas of testate amoebae. The data represent modern and palaeo wetlands, lake sediments and terrestrial habitats (Bonnet, 1966; Haman, 1994; Krashevska, Bonkowski, & Maraun, 2007; Krashevska, Maraun, & Scheu, 2012; Swindles et al., 2014; Swindles, Lamentowicz, Reczuga, & Galloway, 2016; Patterson, Huckerby, Kelly, Swindles, & Nasser, 2015; Reczuga, Swindles, Grewling, & Lamentowicz, 2015). Two hundred and fifty four taxa of testate amoebae were found in Mexico in another study in the neotropics region (Bobrov, Krasilnikov, & García-Calderón, 2013).

The purpose of this study is to investigate the diversity and distribution of testate amoebae in Peru in different habitats as well as to discuss the geographic distribution in the neotropics of testate amoebae.

Materials and methods

The study is based upon the diversity of testate amoebae from 28 surface (0-3 cm depth) soil samples collected in Peru in March 2016 (Table 1). The collection took place nearby Cuzco (6 samples), the ancient city of Machu Picchu (17 samples) and in nearby Aguas Calientes (5 samples). Furthermore, one bottom sediment sample was extracted from Lake Titicaca, near the city of Puno (Fig. 1).

TABLE 1
Sample sites description

Nº	Place of sampling	Altitude (m)	Coordinates	Number of testate amoeba species identified
1	Cuzco. Grassland	3 607	S13° 30.254' W71° 58.864'	1
2	Cuzco. Under the tree crown	3 800	S13° 28.719' W71° 58.060'	5
3	Cuzco. Grassland	3 821	S13° 28.719' W71° 58.060'	7
4	Cuzco. Grassland	3 643	S13° 30.514' W71° 58.278'	7
5	Cuzco. Grassland	3 449	S13° 24.368' W71° 50.686'	4
6	Cuzco. Under the cactus	3 450	S13° 24.488' W71° 50.651'	5
7	Machu Picchu. Rainforest	2 468	S13° 09.917' W72° 32.621'	36
8	Machu Picchu. Rainforest	2 468	S13° 09.917' W72° 32.621'	32
9	Machu Picchu. Rainforest	2 468	S13° 09.917' W72° 32.621'	52
10	Machu Picchu. Rainforest	2 554	S13° 10.107' W72° 32.539'	23
11	Machu Picchu. Rainforest	2 554	S13° 10.107' W72° 32.539'	35
12	Machu Picchu. Rainforest	2 554	S13° 10.107' W72° 32.539'	49
13	Machu Picchu. Rainforest	2 648	S13° 10.234' W72° 32.237'	14
14	Machu Picchu. Lichen	2 694	S13° 10.177' W72° 32.082'	11
15	Machu Picchu. Grassland	2 727	S13° 10.182' W72° 32.036'	34
16	Machu Picchu. Grassland	2 706	S13° 10.185' W72° 32.117'	4
17	Machu Picchu. Rainforest	2 692	S13° 10.205' W72° 32.172'	24
18	Machu Picchu. Rainforest	2 623	S13° 10.201' W72° 32.397'	28
19	Machu Picchu. Grassland	2 529	S13° 09.988' W72° 32.636'	1
20	Machu Picchu. Rainforest	2 461	S13° 09.700' W72° 32.749'	3
21	Machu Picchu. Rainforest	2 456	S13° 09.709' W72° 32.750'	28
22	Machu Picchu. Rainforest	2 444	S13° 09.808' W72° 32.663'	1
23	Machu Picchu. Agave	2 450	S13° 09.909' W72° 32.604'	7

24	Aguas Calientes (Sacred Valley). Rainforest	2 056	S13° 09.291' W72° 31.839'	1
25	Aguas Calientes (Sacred Valley). Rainforest	2 056	S13° 09.291' W72° 31.839'	13
26	Aguas Calientes (Sacred Valley). Rainforest	2 056	S13° 09.291' W72° 31.839'	21
27	Aguas Calientes (Sacred Valley). Rainforest	2 056	S13° 09.291' W72° 31.839'	16
28	Aguas Calientes (Sacred Valley). Rainforest	2 056	S13° 09.291' W72° 31.839'	12
29	Lake Titicaca, sediments, near the town of Puno	3 825	S15° 50.120' W70° 00.935'	2

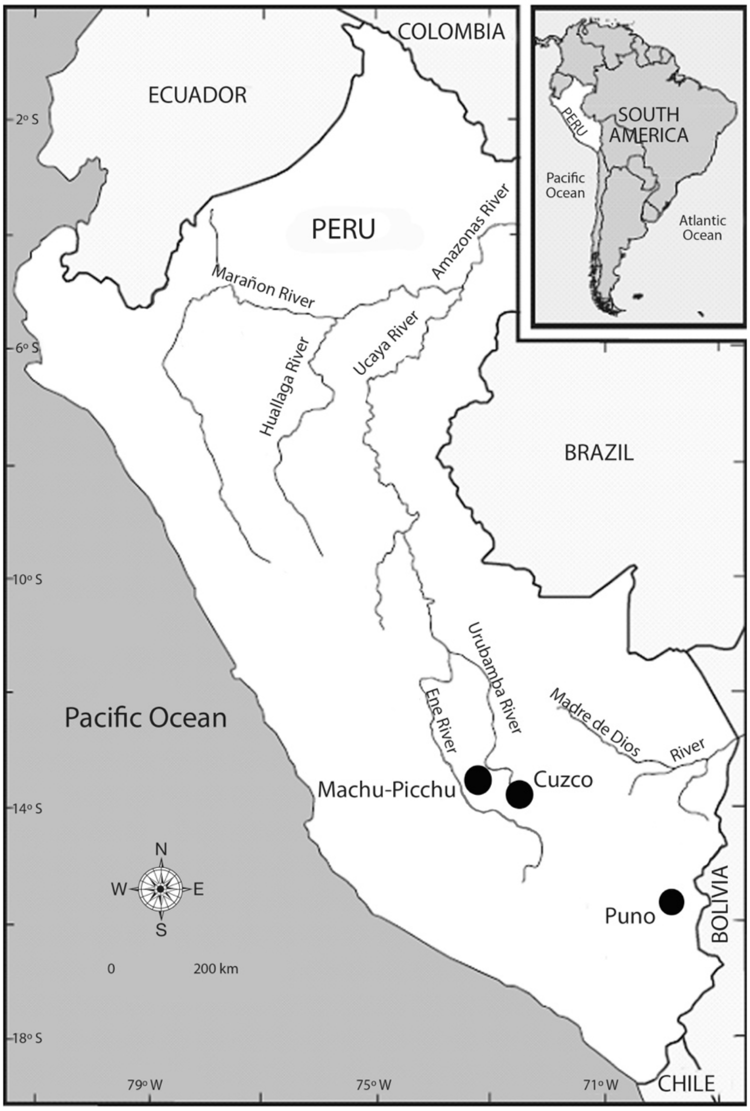

Fig. 1. Location of sampling regions (modified after Kenneth & León, 1999).

The region of study (Cuzco and Machu Picchu) belongs to the system of cultivated land, forest and scrubland and is located on the western border of biodiversity priority areas with a high degree of endemity (Neugarten et al., 2015). The region has been influenced by human activity for many centuries, leading to a combination of manmade habitats, paramo grassland, *Polylepis* thickets, partially degraded virgin forest and former cultivated lands that has reverted back to forests or scrubs (Parker, Parker, & Plenge, 1982). Soils in Machu-Picchu represent Dystric Regosols (Arenic) type, and soils in Cuzco represent Eutric Regosols type (Gardi et al., 2015).
Samples for testate amoebae were prepared using a method based on wet sieving. 1 cm^3 of sample was soaked in water for 24 h, stirred, filtered at 0.5 mm, the suspension left to settle for a further 24 h, and supernatant decanted off following the methods applied by Mazei, Blinokhvatova, and Embulaeva (2011). No back-filtering step was used as this leads to the loss of small taxa; relatively large mesh size (500 µm) was used to retain the largest shells of testate amoebae (Payne, 2009; Avel & Pensa, 2013). The specimens were revealed under a light using a biological microscope (Zeiss Axioplan 2) at x200 and x400 magnifications.
Species accumulation curve was constructed based on rarefaction procedure performed in PRIMER 6.1.6 (Clarke & Gorley, 2006). The maximum expected number of species was calculated in PRIMER 6.1.6 by the nonparametric Chao2 method, which takes into consideration the theoretical number of expected rare species (Clarke & Warwick, 2001).

Results

A total of 144 taxa were found in 29 samples:

Arcella *arenaria compressa*
Centropyxis *aculeata*
C. acuminata
C. aerophila
C. aerophila cornata
C. aerophila deflandrei
C. aerophila minuta
C. aerophila sphagnicola
C. cassis
C. constricta
C. constricta gigas
C. constricta minuta
C. deflandriana
C. delicatula
C. discoides
C. elongata
C. elongata minor
C. gibba
C. latideflandriana
C. minuta
C. orbicularis
C. ovalis
C. cf. *pannosus*
C. plagiostoma
C. plagiostoma lata
C. plagiostoma longa

C. plagiostoma minor
C. plagiostoma terricola
C. pyriformis
C. stenodeflandriana
C. sylvatica
C. sylvatica minor
C. sp. (1-9)
Cyclopyxis cf. *arcelloides*
C. arcelloides gibbosa
C. eurystoma
C. eurystoma parvula
C. intermedia
C. kahli
C. kahli cyclostoma
C. lithostoma
C. machadoi
C. penardi
C. plana
C. puteus
C. sp.
Trigonopyxis *arcula*
T. arcula major
T. minuta
Plagiopyxis *barrosi*
P. bathystoma
P. callida
P. callida grandis
P. declivis
P. declivis oblonga
P. labiata
P. minuta
P. minuta oblonga
P. penardi
P. penardi oblonga
Geamphorella *lucida*
Schoenbornia *humicola, Sch. smithi*
Difflugia *decloitrei*
D. lucida
D. oblonga
D. pristis
Awerintzewia *cyclostoma*
A. sp.
Heleopera *foissneri*
H. petricola
H. petricola amethystea
H. petricola humicola
H. petricola major
H. sphagni
H. sylvatica
Hyalopshenia *insecta*
H. minuta
Nebela *bohemica*

N. collaris
N. griseola
N. lageniformis
N. minor
N. parvula
N. tubulata
N. wailesi, N. sp. (1-2)
Certesella *certesi*
Argynnia *caudata*
A. dentistoma
A. retorta
A. spicata
A. vitraea
Apodera *vas*
Porosia *bigibbosa*
Quadrulella *quadrigera*
Q. symmetrica
Phryganella *acropodia*
Valkanovia *elegans*
V. delicatula
Assulina *muscorum*
Euglypha cf. *acantophora*
E. ciliata glabra
E. cristata
E. cristata decora
E. compressa
E. cuspidata
E. dolioliformis
E. filifera pyriformis
E. laevis
E. polylepis
Tracheleuglypha *acolla*
T. acolla minor
Sphenoderia *fissirostris*
S. macrolepis
S. sp.
Corythion *dubium*
C. dubium minima
C. orbicularis
C. pulchellum
Trinema *complanatum*
T. grandis
T. enchelys
T. lineare
T. lineare minuscula
T. lineare terricola
T. penardi
T. cf. *caudatum*
Cryptodifflugia *minuta*
Testate amoebae unidentified (spec. 1-6)

Nineteen taxa (13.2 % of the total number of taxa recorded) have not been identified to the species level. Twenty three taxa (16.0 %) have a limited geographical distribution: *Centropyxis acuminata, C.* cf. *compressa*, *C. deflandriana, C. delicatula, C. latideflandriana, C.* cf. *ovalis, C.* cf. *pannosus, C. pyriformis, C. stenodeflandriana, Cyclopyxis lithostoma, C. machadoi, C. plana, Geamphorella lucida, Awerintzewia cyclostoma, Plagiopyxis barrosi, Heleopera foissneri, Certesella certesi, Argynnia retorta, A. spicata, Apodera vas, Quadrulella quadrigera, Sphenoderia macrolepis, Trinema* cf. *caudatum* (Fig. 2). Over a half of the species found in the samples belong to five genera: *Centropyxis, Cyclopyxis, Plagiopyxis, Euglypha,* and *Nebela*.

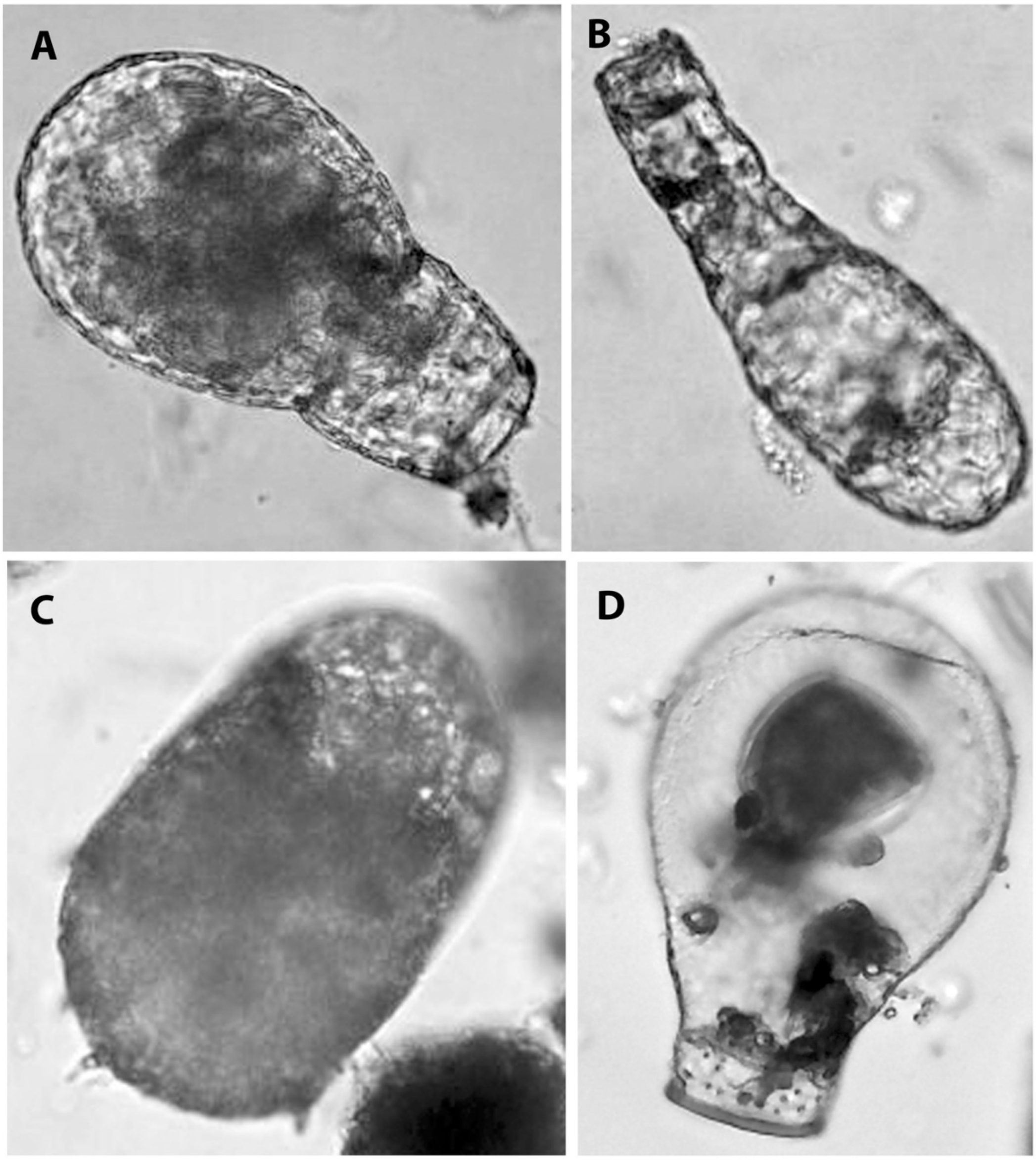

Fig. 2. Micrographs of some rare species of testate amoebae in studied regions (magnification 400x): A. - *Apodera vas;* B. - *Argynnia retorta;* C. - *Centropyxis* cf. *compressa;* D. - *Certesella certesi.*

Different habitats yielded various species richness (Table 1). Results of rarefaction procedure (Fig. 3) show that species-accumulation curve does not reach a plateau. The curve is well fitted ($R^2 = 0.98$) by the power function $S = 20.56N^{0.57}$ (where S is the number of species revealed, N is the number of samples investigated). Low value of power coefficient (0.57) reflects an unsaturated community with 20.56 as an average number of taxa per sample. Expected total number of species (Chao2) in the studied area is estimated at 189.

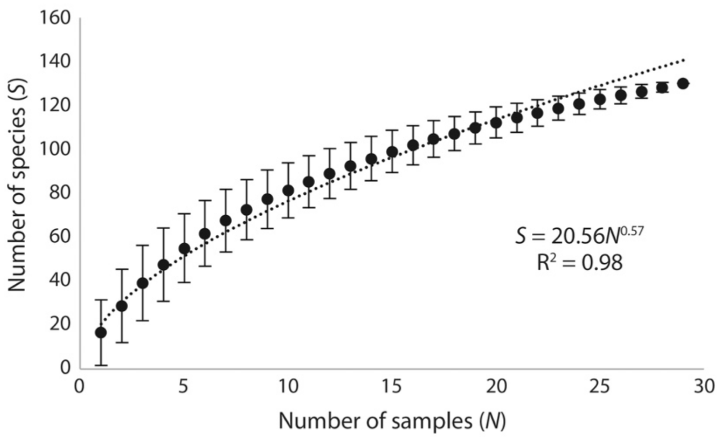

Fig. 3. Relations between number of samples investigated (*N*) and number of species identified (*S*). Whiskers - standard deviation.

Six samples from various types of habitats near the city of Cuzco (altitudes from 3607 m to 3450 m) yielded twenty taxa of testate amoebae of the cosmopolitan group. Most of these also belong to eurybiontic species, with the exception of several taxa, such as *Plagiopyxis labiata, Heleopera petricola, Valkanovia delicatula, Cryptodifflugia minuta*, each which usually inhabit permanently or temporarily in wet habitats enriched with organic substances.

Twelve samples from the rainforest near Machu Picchu (heights from 2 444 m to 2 692 m) revealed the most diverse testate amoebae community (108 taxa). In addition, 17 testate amoebae from these samples were not determined to the species level. Besides, the location has yielded almost all species with a limited geographical distribution. Representatives from all ecological groups of testate amoebae were noted here with the prevalence of hygro- and hydrophilic species from the genera *Arcella, Difflugia, Trigonopyxis, Centropyxis, Cyclopyxis, Heleopera, Hyalosphenia, Nebela, Porosia, Argynnia, Quadrulella, and Sphenoderia.* The environmental condition is due to high humidity resulting from significant seasonal amount of precipitation, fog and cloudiness. The highest diversity (52 taxa) was observed in the sample # 9 (Table 1), a population which is represented by all ecological groups of testate amoebae - that is eurybiont, pedobiont, sphagnobiont, hydrobiont, acidophilic, and calcific.

In a single sample from lichen habitat near Machu Picchu, 11 taxa were found, represented by xerophilic species from the genera *Assulina, Corythion*, as well as eurybionts from the genera *Centropyxis* (including rare species *C. deflandriana* and *C. latideflandriana), Cyclopyxis,* and *Trinema*.

In three samples from grassland habitats near Machu Picchu, 37 testate amoebae taxa were identified. Communities include high number of hygrophilous species *Arcella arenaria compressa, Trigonopyxis arcula, T. minuta, Centropyxis cassis, C. constricta, C. elongata, C. orbicularis, Heleopera petricola amethystea, H. petricola humicola, H. sylvatica, Nebela tubulata, N. wailesi, Argynnia caudata, A. spicata, Quadrulella quadrigera,* and *Difflugia lucida*, reflecting a wet hydrological regime of the grasslands due to specific climatic features of this mountainous region of Peru. The calciphilic testate amoebae species dwelling the region, are for example *Centropyxis plagiostoma, C. plagiostoma lata, C. plagiostoma minor,* and *Cyclopyxis kahli* indicate near-neutral pH of the soil fluids. Among the rare species, only one was found, namely *Centropyxis deflandriana*.

Eleven testate amoebae taxa were found in one sample taken from the soils near agave, among which one can distinguish a group of calciphilic species: *Centropyxis plagiostoma, C. plagiostoma minor*, and exclusively pedobiont species *Plagiopyxis minuta* and *P. penardi*. The findings of a moderately hygrophilous species *H. petricola humicola* indicate relatively favorable soil moisture conditions. Besides, the location provided a rare species of *Centropyxis acuminata*.

Forty-two testate amoebae taxa were found in five samples at Aguas Calientes at an altitude of 2 056 m.a.s.l. When comparing with the higher elevated forests of Machu-Picchu (above 2 444 m.a.s.l.), the number of hydrophilic taxa decreased. There is no species of the genus *Trigonopyxis*. Species from the genera *Heleopera, Nebela, Argynnia* are rare. All of this most likely reflects decreasing humidity levels below the slope. Topography is a predictor of species composition (Basnet, 1992).
The only bottom sample from Lake Titicaca yielded only two hydrobiont species from the genus *Difflugia*, i.e. *D. oblonga* and *D. pristis*.

Discussion

The broadleaved forest biome, also known as Amazonia or Amazon Jungle, covers most of the Amazon River basin and forms part of the Neotropic bioregion. A significant part of the Amazon basin falls on the territory of Peru. The age of the rainforest is estimated to be about several tens of millions of years (Mark, Yadvinder, Oliver, & Sharon, 2005). In fact, the age of the Amazonia region has been determined to be approximately 55 million years (Burnham & Johnson, 2004; Morley, 2000). The largest in South America, the Amazon rainforest is a place of an unprecedented biodiversity (Turner, 2001). The diversity of plant species is the highest in the world: as has been reported, 0.25 km^2 (62 acres) in the Ecuadorian rainforest supports more than 1 100 species of trees (Wright, 2001). Considering that Ecuador holds about 2 % of the area that comprises the Amazon, the exceptional biodiversity of the entire forest need be noted.
In this study, we have attempted to understand to what extent the region of the Peruvian rainforests is unique in respect to testate amoebae as a common eukaryotic microbial component of terrestrial ecosystems (Geisen et al., 2017). The findings of 144 testate amoebae taxa in 29 samples, including 19 taxa not identified to the species level, is indicative of a high potential level of terricolous testate amoebae diversity, which can be caused by a diversity of microhabitat types produced by higher vascular plants and the antiquity of the Amazonian landscapes, and accentuate the importance of this part of the neotropics as a hotspot of biodiversity (Myers et al., 2000).
Previously, even a greater percentage of testate amoeba species of an uncertain taxonomic position was noted for Mesoamerica, in particular Mexico, where this figure exceeded 30% of the total biodiversity described (Bobrov et al., 2013). In the more northern part of the neotropics, 254 taxa of testate amoebae were found (Bobrov et al., 2013). The highest species diversity of testate amoebae was attributed to the soils of the tropical rainforests (126 taxa) and wetlands (144 taxa), including organisms with a limited geographical distribution (from the genera *Cornuapyxis, Ellipsopyxis, Hoogenraadia, Planhoogenraadia, Apolimia, Certesella, Apodera* and *Alocodera*).
In the course of the study of terricolous testate amoebae of the neotropical area of Ecuador's mountain habitats (Krashevska et al., 2007, 2012) 135 testate amoebae taxa were identified in 36 samples. The vast majority of the identified testate amoebae were rather common and can be found also in temperate areas. Nine species (6.7 % of the total list) were tropical, thus were reckoned by authors as relicts of Gondwana (Krashevska et al., 2007, 2012).
Earlier studies of testate amoebae in Amazonian *Sphagnum* ombrotrophic bogs revealed 47 taxa (Swindles et al., 2014, 2016) and provided the description of a new species, namely *Arcella peruviana* (Reczuga et al., 2015). Another study revealed the possibility to use testate amoebae as a bioindicator of the aquatic environment in the Amazon basin (Arrieira, Schwind, Alves, & Lansac-Tôha, 2017).
This study revealed several rare species. Most located in limited geographical areas (genera *Certesia, Apodera, Argynnia,* and some representatives of the genera *Centropyxis* and *Cyclopyxis*) inhabiting the rainforests. Most likely, due to the high humidity of the upper soil

layer and the microhabitat diversity of environmental conditions contributed to the limited distribution. Likely, high spatial heterogeneity appears in the region's diversity of soil types because of the features of its history and its complex relief (Quesada et al., 2011). Both factors, namely high spatial heterogeneity and high humidity, created the proper conditions for the high biodiversity, maintenance, including that of the soil-dwelling testate amoebae.

The distribution of *Centropyxis latideflandriana* and *C. stenodeflandriana* includes the Australian biogeographical regions (Bonnet, 1979; Meisterfeld & Tan, 1998), oriental (Bonnet, 1979; Balik, 1995; Bobrov, Mazei, & Tiunov, 2010), and the Southern part of Palearctic zone (Bonnet, 1979; Bobrov, 2001; Bobrov et al., 2013). Nine species of the genus *Centropyxis* are now found exclusively outside the Holarctic: *Centropyxis acuminata, C. deflandriana, C. delicatula, C. latideflandriana, C.* cf. *ovalis, C. cf. pannosus, C. pyriformis, C. serrahni, C. stenodeflandriana* (Bobrov et al., 2010).

Two "flagship" (sensu Foissner, 2006) species, namely *Apodera vas* and *Certesella certesi* were found in the same locations during our study. The former species seemed to be more common for Mesoamerica (Heger, Lara, & Mitchell, 2011b) and tends to inhabit in wetter habitats (freshwater sediments, wetlands) in comparison to the later taxon (Lansac-Tôha, Velho, Takahashi, Aoyagui, & Bonecker, 2001; Miranda & Mazzoni, 2015). Geographic distribution of *Apodera vas* is well documented beyond the limits of Holarctic (Mitchell & Meisterfeld, 2005; Smith & Wilkinson, 2007; Smith et al., 2008). The hypothesis on gondwanian origin of *Apodera vas* and later distribution through Pacific Ocean islands has previously been advanced (Smith & Wilkinson, 2007). In South America, the main findings of *Apodera vas* is limited to the narrow band of the Pacific coast along the Andes mountains from Tierra del Fuego, Cape Horn and the Patagonia in the South to Mexico in the North: Argentina (Vucetich, 1975), Chile (Zapata & Fernandez, 2008; Fernandez & Zapata, 2010-2011; Fernandez, Zapata, Meisterfeld, & Baessolo, 2012; Fernandez, Lara, & Mitchell, 2015), Southern Ecuador (Krashevska et al., 2007), Costa Rica, Guatemala (Laminger, 1973), Mexico (Golemansky, 1967; Laminger, 1973; Heger et al., 2011b; Bobrov et al., 2013). Almost all of the above-mentioned publications indicate joint occurrences of *Apodera vas* and *Certesella certesi,* latter sometimes replaced by the morphologically close *Certesella martiali* (Vucetich, 1978).

Heger et al. (2011b) investigated the northern limits of *Apodera vas* and *Certesella certesi* distribution within Mesoamerica based on the Great American Interchange idea (Simpson, 1940). The analysis of more than 200 samples has revealed a continuous geographic distribution of both the *Apodera vas* and the *Certesella certesi* within the intermediate zone ranging from the Neotropical realm toward the Nearctic realm, i.e. Panama, Costa Rica, Nicaragua, Salvador, Honduras, Guatemala, and Mexico. Also, joint occurrences were noted in four countries: Panama, Costa Rica, Guatemala, and Mexico.

In the eastern plains of South America outside the Andes, *Apodera vas* was found in water ponds (Lansac-Tôha et al., 2001; Miranda & Mazzoni, 2015). Neither there, nor in the swamps of the Peruvian Amazon, the joint findings of *Apodera vas* and *Certesella certesi* has ever been detected (Swindles et al., 2014).

Our study fills a geographical gap that consisted in an insufficiency of collected data on the findings of *Apodera vas* and *Certesella certesi* in Peru, and illustrates the continuity of these species expansion along the Pacific coast.

Acknowledgments

The work was supported by the grants of Russian Foundation for Basic Research (16-04-00451 and 15-29-02518 to AB - sampling and microscopic analysis) and Russian Science Foundation (14-50-00029 to YuM - general synthesis). We are grateful to Natalia Kirillina for editing first variant of English text.

References

Antonelli, A., Nylander, J. A. A., Persson, C., & Sanmartín, I. (2009). Tracing the impact of the Andean uplift on Neotropical plant evolution. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 9749-9754.

Arrieira, R. L., Schwind, L. T. F., Alves, G. M., & Lansac-Tôha, F. A. (2017). Estudos da biodiversidade de amebas testáceas para estratégias voltadas à preservação: uma revisão. *Revista em Agronegócio e Meio Ambiente, Maringá (PR), 10*, 567-586.

Avel, E., & Pensa, M. (2013). Preparation of testate amoebae samples affects water table depth reconstructions in peatland palaeoecological studies. *Estonian Journal of Earth Sciences, 62*, 113-119.

Azovsky, A., & Mazei, Y. (2013). Do microbes have macroecology? Large-scale patterns in diversity and distribution of marine benthic ciliates. *Global Ecology and Biogeography, 22*, 163-172.

Azovsky, A., Tikhonenkov, D., & Mazei, Y. (2016). Global diversity and distribution of the smallest eukaryotes: biogeography of marine benthic heterotrophic flagellates. *Protist, 167*, 411-424.

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

Basnet, K. (1992). Effect of topography on the pattern of trees in Tabanuco (*Dacryodes excels*) dominated rain forest or Puerto-Rico. *Biotropica*, *24*, 31-42.

Bobrov, A. (2001). Findings of tropical group testate amoebae (Protozoa, Testacea) at the far east (Sikhote Alin' Reserve). *Biology Bulletin, 28*, 401-407.

Bobrov, A., Krasilnikov, P., & García-Calderón, N. E. (2013). Biogeography of testate amoebae in the soils of Mexico. *Biodiversity and Conservation, 22*, 2837-2855.

Bobrov, A., Mazei, Y., & Tiunov, A. (2010). Testate amoebae of a monsoon tropical forest of South Vietnam. *Acta Protozoologica*, *49*, 311-325.

Bonnet, L. (1966). Le peuplement thécamoebien de quelques sols du Chili. *Protistologica, 2*, 113-140.

Bonnet, L. (1979). Nouveaux Thécamoebiens du sol (X). *Bulletin de la Société d'histoire naturelle de Toulouse, 115*, 106-118.

Burnham, R. J., & Johnson, K. R. (2004). South American palaeobotany and the origin of Neotropical Forests. *Philosophical Transactions of the Royal Society, 359*, 1595-1610.

Clarke, K., & Gorley, R. (2006). PRIMER v. 6: User Manual/Tutorial. Plymouth, UK: PRIMER-E Ltd.

Clarke, K., & Warwick, R. (2001). *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation* (2nd ed.). Plymouth, UK: PRIMER-E Ltd.

Fernandez, L. D., Lara, E., & Mitchell, E. A. D. (2015). Check list, diversity and distribution of testate amoebae in Chile. *European Journal of Protistology, 51*, 409-424.

Fernandez, L. D., & Zapata, J. M. (2010-2011). Seasonal variation in the testate amoebae community of a temperate peatland from Southern Chile. *Boletin de la Sociedad de Biología de Concepción, Chile, 80*, 27-39.

Fernandez, L. D., Zapata, J. M., Meisterfeld, R., & Baessolo, L. (2012). First records and community pattern of Arcellinida Inhabiting a pristine and remote island from Southeastern Pacific, Chile. *Acta Protozoologica, 51*, 139-154.

Foissner, W. (2006). Biogeography and dispersal of micro-organisms: a review emphasising protists. *Acta Protozoologica, 45*, 111-136.

Gardi, C., Angelini, M., Barcelo, S., Comerma, J., Cruz, G. C., Encina, R. A., … Ravina Da Silva, M. (2015). *Soil Atlas of Latin America and the Caribbean.* Luxembourg: European Commission – Publications Office of the European Union.

Geisen, S., Mitchell, E. A. D., Wilkinson, D., Adl, S., Bonkowski, M., Brown, M., … Lara, E. (2017). Soil protistology rebooted: 30 fundamental questions to start with. *Soil Biology and Biochemistry, 111*, 94-103.

Golemansky, V. (1967). Tecamebianos muscicolas (Rhizopoda, Testacea) de Mexico. *Revista de la Sociedad Mexicana de Historia Natural, 18*, 73-77.

Haman, D. (1994). A thecamoebinid assemblage from lake Cocococha, Tambopata Reserve, Madre-De-Dios Province, Southeastern Peru. *Journal of Foraminiferal Research, 24*, 226-232.

Heger, T., Booth, R., Sullivan, M., Wilkinson, D., Warner, B., Asada, T., … Mitchell, E. A. D. (2011a). The re-discovery of *Nebela ansata*: a forgotten microbe illustrates the knowledge gap between microbial and visible biodiversity. *Journal of Biogeography, 38*, 1897-1906.

Heger, T., Lara, E., & Mitchell, E. A. D. (2011b). Arcellinida testate amoebae (Amoebozoa: Arcellinida): model of organisms for assessing microbial biogeography. In D. Fontaneto (Ed.), *Biogeography of Microscopic Organisms: Everything Small Everywhere?* (pp. 111-129). Cambridge: Systematics Association and Cambridge University Press.

Herzog, S. K., Martínez, R., Jørgensen, P. M., & Tiessen, H. (Eds.). (2011). *Climate Change and Biodiversity in the Tropical Andes*. Inter-American Institute for Global Change Research and Scientific Committee on Problems of the Environment: SCOPE.

Hoorn, C., Wesselingh, F. P., Ter Steege, H., Bermudez, M. A., Mora, A., Sevink, J., ... Antonelli, A. (2010). Amazonia through Time: Andean Uplift, Climate Change, Landscape Evolution, and Biodiversity. *Science, 330*, 927-931.

Jassey, V. E. J., Signarbieux, C., Hättenschwiler, S., Bragazza, L., Buttler, A., Delarue, F., … Lara, E. (2015). An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. *Scientific Reports, 5*, 16931.

Kenneth, R. Y., & León, B. (1999). *Peru's humid eastern montane forests: Centre for Research on the Cultural and Biological Diversity of Andean Rainforests (DIVA). An overview of their physical settings, biological diversity human use and settlement, and conservation needs.* (Report No. 5). Rønde: DIVA.

Krashevska, V., Bonkowski, M., & Maraun, M. (2007). Testate amoebae (Protista) of an elevational gradient in the tropical mountain rain forest of Ecuador. *Pedobiologia, 51*, 319-331.

Krashevska, V., Maraun, M., & Scheu, S. (2012). How does litter quality affect the community of soil protists (testate amoebae) of tropical montane rainforests? *Microbial Ecology, 80*, 603-607.

Laminger, H. (1973). Die Testaceen (Protozoa, Rhizopoda) einiger Hochgebirgsgewa¨sser von Mexiko, Costa Rica und Guatemala. *Internationale Revue der gesamten Hydrobiologie und Hydrographie, 58*, 273-305.

Lansac-Tôha, F. A., Velho, L. F. M., Takahashi, E. M., Aoyagui, A. S. M., & Bonecker, C. (2001). On the occurrence of testate amoebae (Protozoa, Rhizopoda) in Brazilian inland waters. V. Families Hyalospheniidae, Plagiopyxidae, Microcoryciidae, Cryptodifflugiidae, Phryganelidae, Euglyphidae, Trinematiidae and Cyphoderiidae. *Acta Scientiarum Biological Sciences, Maringá, 23*, 333-347.

Mark, M., Yadvinder, M., Oliver, P., & Sharon, C. (2005). New views on an old forest: assessing the longevity, resilience and future of the Amazonian rainforest. *Transactions of the Institute of British Geographers, 30*, 477-499.

Martiny, J. B. H., Bohannan, B. J. M., Brown, J. H., Colwell, R. K., Fuhrman, J. A., Green, J. L., … Staley, J. T. (2006). Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology, 4*, 102.

Mazei, Y., Blinokhvatova, Y., & Embulaeva, E. (2011). Specific features of the microspatial distribution of soil testate amoebae in the forests of the Middle Volga region. *Arid Ecosystems, 1*, 46-52.

Meisterfeld, R., & Tan, L.W. (1998). First records of testate amoebae (Protozoa: Rhizopoda) from Mount Buffalo National Park, Victoria. *The Victorian Naturalist (Melbourne), 115*, 231-238.

Miranda, V., & Mazzoni, R. (2015). Testate amoebae (Protozoa Rhizopoda) in two biotopes of Ubatiba stream, Maricá, Rio de Janeiro State. *Acta Scientiarum Biological Sciences, Maringá, 37*, 291-299.

Mitchell, E. A. D., & Meisterfeld, R. (2005). Taxonomic confusion blurs the debate on cosmopolitanism versus local endemism of free-living protists. *Protist, 156*, 263-267.

Morley, R. (2000). *Origin and Evolution of Tropical Rain Forests.* Chichester: Wiley Press.

Morrone, J. J. (2001). *Biogeografía de América Latina y el Caribe* (Vol. 3.). Zaragoza: Manuales & Tesis SEA.

Morrone, J. J. (2006). Biogeographic areas and transition zones of Latin America and the Caribbean Islands based on panbiogeographic and cladistics analyses of the entomofauna. *Annual Review of Entomology*, *51*, 467-494.

Morrone, J. J. (2017). *Neotropical Biogeography: Regionalization and Evolution*. Boca Ratón: CRC Press.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature, 403*, 853-858.

Neugarten, R., Ceotto, P., Acero, N., Coutinho, B., Flores-Gutiérrez, R., Hierholzer, M., … Wright, M. (2015). *Mapping Essential Natural Capital in Amazonia*. Arlington, VA: Conservation International.

Parker, T. A., Parker, S. A., & Plenge, M. A. (1982). *An Annotated Checklist of Peruvian Birds.* Vermillion, South Dakota: Buteo Books.

Patterson, R. T., Huckerby, G., Kelly, Th. J., Swindles, G. T., & Nasser, N. A. (2015). Hydroecology of Amazonian lacustrine Arcellinida (testate amoebae): A case study from Lake Quistococha, Peru. *European Journal of Protistology, 51*, 460-469.

Payne, R. J. (2009). The standard preparation method for testate amoebae leads to selective loss of the smallest taxa. *Quaternary Newsletter, 119*, 16-20.

Payne, R. J. (2013). Seven reasons why protists make useful bioindicators. *Acta Protozoologica, 52*, 105-113.

Quesada, C. A., Lloyd, J., Anderson, L. O., Fyllas, N. M., Schwarz, M., & Czimczik, C. I. (2011). Soils of Amazonia with particular reference to the RAINFOR sites. *Biogeosciences, 8*, 1415-1440.

Reczuga, M., Swindles, G. T., Grewling, Ł., & Lamentowicz, M. (2015). *Arcella peruviana* sp. nov. (Amoebozoa: Arcellinida, Arcellidae), a new species from a tropical peatland in Amazonia. *European Journal of Protistology, 51*, 437-449.

Schröter, D., Wolters, V., & De Ruiter, P. C. (2003). C and N mineralization in the decomposer food webs of a European forest transect. *Oikos, 102*, 294-308.

Simpson, G. G. (1940). Mammals and land bridges. *Journal of the Washington Academy of Sciences, 30*, 137-163.

Smith, H. G., Bobrov, A., & Lara, E. (2008). Diversity and biogeography of testate amoebae. *Biodiversity and Conservation, 17*, 345-363.

Smith, H. G., & Wilkinson, D. M. (2007). Not all free-living microorganisms have cosmopolitan distributions - the case of *Nebela* (*Apodera*) *vas* Certes (Protozoa: Amoebozoa: Arcellinida). *Journal of Biogeography, 34*, 1822-1831.

Swindles, G. T., Lamentowicz, M., Reczuga, M., & Galloway, J. M. (2016). Palaeoecology of testate amoebae in a tropical peatland. *European Journal of Protistology, 55*, 181-189.

Swindles, G. T., Reczuga, M., Lamentowicz, M., Raby, C. L., Turner, T. E., Charman, D. J., … Mullan, D. J. (2014). Ecology of testate amoebae in an Amazonian peatland and development of a transfer function for palaeohydrological reconstruction. *Microbial Ecology, 68*, 284-298.

Turner, I. M. (2001). *The ecology of trees in the tropical rain forest.* Cambridge: Cambridge University Press.

Udvardy, M. D. F. (1975). *A classification of the biogeographical provinces of world.* Morges, Switzerland: IUCN.

Vucetich, M. C. (1975). Tecamebianos de mallines y otros ambientes lenticos de la Patagonia Andina (Rhizopoda Testacea). *Neotropica, 21*, 104-112.

Vucetich, M. C. (1978). Commentarios sobre le genero *Certesella* Loeblich & Tappan, 1961 y estudio de la estereo ultraestructura tecal de tres especies austroamericanas (Rhizopoda: Testaceolobosa). *Obra del Centenario del Museo de La Plata, 6*, 305-313.

Wilkinson, D. M., & Mitchell, E. A. D. (2010). Testate amoebae and nutrient cycling with particular reference to soils. *Geomicrobiology Journal, 27*, 520-533.

Wright, S. J. (2001). Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia, 130*, 1-14.

Zapata, J., & Fernandez, L. (2008). Morphology and morphometry of *Apodera vas* (Certes, 1889) (Protozoa: Testacea) from two peatlands in Southern Chile. *Acta Protozoologica, 47*, 389-395.