



## **Diversity of Zygomycetes associated with the rhizosphere of woody plants from Ile-Alatau national park (Kazakhstan)**

**Rakhimova YV, Kyzmetova LA, Assylbek AM and Yermekova BD**

*Institute of Botany and Phytointroduction, Almaty, Kazakhstan*

Rakhimova YV, Kyzmetova LA, Assylbek AM, Yermekova BD 2019 – Diversity of Zygomycetes associated with the rhizosphere of woody plants from Ile-Alatau national park (Kazakhstan). Current Research in Environmental & Applied Mycology (Journal of Fungal Biology) 9(1), 53–65, Doi 10.5943/cream/9/1/6

### **Abstract**

Total 9 species of Zygomycetes belonging to six different genera, were identified from the rhizosphere of various woody plants in Ile-Alatau state national Natural Park. *Mucor mucedo* was observed as the most prevalent Zygomycete species. This species was isolated from the rhizosphere of apple, apricot, aspen, birch, oak, pine, poplar, spruce, rowan and willow. In addition to *Mucor mucedo* three additional species of the genus *Mucor* were noted: *M. fuscus* isolated from the rowan rhizosphere, *M. silvaticus* found in the apple rhizosphere, *M. strictus* was detected in the birch rhizosphere. *Absidia spinosa*, isolated from the rhizosphere of apple, apricot, hawthorn, inn, poplar, rowan, spruce and willow, was the next prevalent fungus in the studied rhizosphere samples. Only one species of *Cunninghamella*, *C. echinulata*, from hawthorn rhizosphere was detected. *Actinomucor elegans* was isolated also from the hawthorn rhizosphere. *Piptocephalis arrhiza* isolated from the apricot rhizosphere, grown together with species of the genus *Mucor*, as their parasite. *Rhizopus stolonifer* was isolated from the spruce rhizosphere.

Eight species of Zygomycetes are found in the rhizosphere of mixed forest trees, three species are found in the soil of spruce forests. Two species are common to mixed and spruce forests. The greatest number of soil fungi species is typical for mixed and small-leaved forest at the altitude range 1400–2000 m above sea level, much less of them in higher ranges. *Absidia spinosa* and *Mucor mucedo* are dominant for these altitudes. The number of soil Zygomycetes varies in the rhizosphere of different forest species. The greatest number of soil fungal species (3 species) is observed in the rhizosphere of apple, apricot, birch, hawthorn, rowan and spruce.

**Key words** – columella – coniferous forest – fungal colony – merosporangium – mixed forest – occurrence frequency – small-leaved forest – sporangiospore – sporangium – zygosporangium

### **Introduction**

Ile-Alatau state national Natural Park was established by the decree of the Government of the Republic of Kazakhstan on February 22, 1996 to preserve the unique landscapes, flora and fauna. The Park's area is 198,669 hectares. National park is located on the Northern macroslope of Zailisky Alatau, the most Northern range of the Tien Shan. Range is almost 380 km long, 30–40 km wide. The highest peak Talgar reaches 5017 m above sea level. The climate is diverse and differentiated by high-altitude climatic zones. Vegetation is characterized with specific spectrum of

altitudinal belts. There is no continuous belt of coniferous forests and alpine tundra belt. A distinctive feature of Zailisky Alatau ridge is the wide spread of all subtypes of mountain steppes.

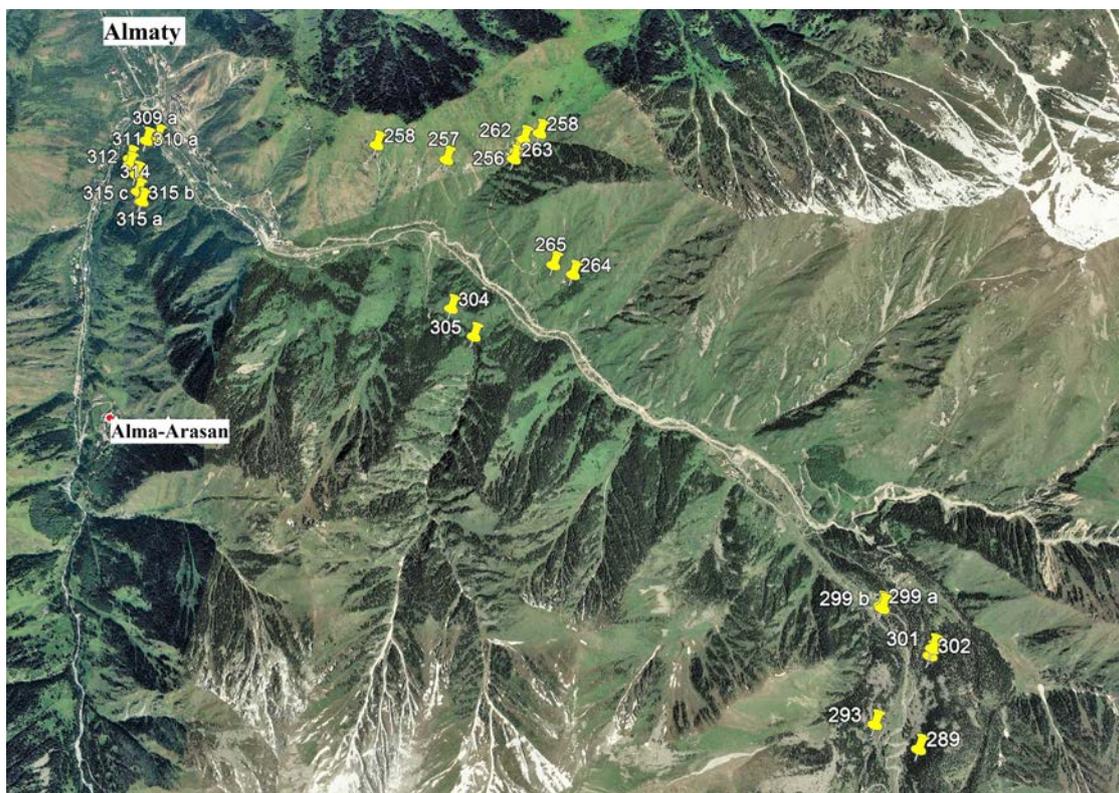
In mycological respect, Zailisky Alatau ridge is sufficiently studied. A variety of ecological conditions of the ridge, as well as the peculiar composition of host plants, could not affect the number and ratio of different groups of fungi, which are taken into account here more than 2000 species (Schwarzman 1962). However, the soil mycobiota of has been poorly studied. The list of soil fungi species in the mixed forests of Zailisky Alatau consisted of only 4 species of micromycetes, 20 species were found in coniferous forests (Schwarzman 1962). To our research Zygomycetes is not marked in the soils of the national park.

The purpose of this study was to add species of Zygomycetes to the list of soil fungi that have been detected in the forests of Zailisky Alatau, to provide short descriptions, remarks on the species, descriptions of the fungal colonies, and microphotographs.

## Materials & Methods

### Sampling sites

Soil samples of the forests of the Ile-Alatau national Park (Fig. 1) were collected during the growing season of 2018 (April, July, and August). At the sampling sites the rhizosphere of the trees of dark coniferous, small-leaved and mixed forests on mountain-forest soils in Zailisky Alatau were sampled. Dark coniferous forests are composed of the main forest-forming species of the Shrenk spruce (*Picea schrenkiana* Fisch. & C. A. Mey.). Underwood consists of rowan, aspen, various species of rose hips, honeysuckle, juniper, and willow. Lower zone in Zailiysky Alatau and valleys of the rivers are occupied by the mixed deciduous-fir woods. In addition to spruce, the dominant species were apple, apricot, willow, birch, aspen.



**Fig. 1** – Map showing soil collection sites in central part of Ile-Alatau National Park

### Soil sampling

Samples were collected randomly in the soil horizon of 5-20 cm, after removal of the top litter layer, in the rhizosphere of various woody plants. The geographic location of each sample

collection site was recorded using digital GPS (Germin). In total, 44 samples were collected in three replicates, from which 35 species of soil fungi were isolated, among them 9 species of Zygomycetes (from 25 samples) (Table 1).

**Table 1** Data on soil samples from which Zygomycetes were isolated

Samples	Altitude above sea level	Latitude, N	Longitude, E	Rhizosphere of
256	1924	43°06'24.1"	76°57'12.8"	spruce
257	1927	43°06'23.8"	76°56'46.4"	poplar
258	1930	43°06'28.7"	76°56'21.2"	poplar
262	1926	43°06'27.1"	76°56'15.9"	apricot
263	1937	43°06'22.0"	76°57'11.0"	spruce
264	1923	43°05'50.1"	76°57'30.0"	birch
265	1923	43°05'53.0"	76°57'23.1"	apple
289	2509	43°03'44.6"	76°59'19.3"	spruce
293	2480	43°03'50.6"	76°59'05.1"	spruce
299 a	2312	43°04'19.8"	76°59'11.1"	willow
299 b	2312	43°04'19.8"	76°59'11.1"	aspen
301	2377	43°04'08.5"	76°59'27.2"	spruce
302	2395	43°04'06.4"	76°59'26.9"	rowan
304	1831	43°05'43.4"	76°56'44.9"	spruce
305	1864	43°05'35.0"	76°56'52.9"	spruce
309 a	1477	43°06'37.0"	76°54'53.1"	hawthorn
310 a	1504	43°06'36.2"	76°54'49.1"	oak
310 b	1504	43°06'36.2"	76°54'49.1"	apricot
311	1556	43°06'31.4"	76°54'43.8"	birch
312	1582	43°06'29.5"	76°54'43.8"	spruce
313	1628	43°06'26.0"	76°54'46.6"	apple
314	1667	43°06'21.9"	76°54'47.9"	pine
315 a	1703	43°06'18.3"	76°54'49.6"	rowan
315 b	1703	43°06'18.3"	76°54'49.6"	apple
315 c	1703	43°06'18.3"	76°54'49.6"	pine

### Isolation of fungi and preparation of medium

Soil fungi were isolated by the serial dilution method (Polyksenova et al. 2004). Suspensions with standard level of dilution (1:10,000) were inoculated on agar. After the appearance of sporulation or signs of development of the mycelium, the isolates were transferred to a nutrient medium (potato sucrose agar). Petri dishes were labeled, sealed with paraffilm in order to avoid contamination, and incubated at 25°C.

Potato sucrose agar was made from 1800 g of potatoes per 4500 ml of water, 40 g of sucrose, 40 g of agar. The medium was autoclaved at 120°C for 30 minutes. A pinch of streptomycin (30 mg/ml) was added to the autoclaved medium to avoid the bacterial growth (Polyksenova et al. 2004).

### Growth of colonies

Radial growth of fungal colonies on solid media was measured and calculated based on the average of perpendicular diameter measurements. Fungal growth patterns and their sizes were registered by a digital camera Canon 600E.

### Macroscopic and microscopic characterization of colonies and identification

Fungal cultures were examined when they were 5–10 days old. Appearance on medium, color, texture, and growth rate of fungal colonies were analyzed.

For light microscopy, small pieces of fungal colonies (growing up to 10 days on potato sucrose agar) were cut, placed in a drop of distilled water on a microscope slide without any staining, examined and photographed using a photomicroscope Polyvar with Nomarski interference contrast optics. The diamensions of sporangiophores, sporangia, columellae, dimensions of sporangiospores and other fungal structures were recorded. Mean sizes were calculated and the differences between the isolates were tested. The obtained data were compared with the available literature.

Specimens were identified based on the taxonomic keys on soil Zygomycetes (Pidoplichko & Mil'ko 1971, Watanabe 2002). The systematics of the taxa were in accordance with Kirk et al. (2008) and they were listed in alphabetical order. Names of fungi are given according to Index Fungorum.

Occurrence frequency of fungal species was established according to the formula:  $A=B/C \times 100\%$ , where A – occurrence frequency of fungal species; B – number of samples, in which the species has been detected; C – total number of investigated samples (Mirchink 1988).

## Results

The Zygomycetes detected in the rhizosphere of various woody plants were identified as members of 9 species which fall into the genera *Cunninghamella*, *Absidia*, *Actinomucor*, *Mucor*, *Rhizopus*, *Piptocephalis*.

*Zygomycota* Moreau

*Mucoromycotina* Benny

*Mucorales* Dumort.

*Cunninghamellaceae* Naumov ex R.K. Benj.

*Cunninghamella echinulata* (Thaxt.) Thaxt. ex Blakeslee

In the hawthorn rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1477 m a.s.l., 43°06'37.0"N, 76°54'53.1"E, 29.08.2018, YV Rakhimova.

*Mucoraceae* Dumort.

*Absidia spinosa* Lendn.

In the apple rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1628 m a.s.l., 43°06'26.0"N, 76°54'46.6"E, 29.08.2018, YV Rakhimova.

In the apricot rhizosphere

Big Almaty gorge, North-West exposure slope, mixed forest, 1926 m a.s.l., 43°06'27.1"N, 76°56'15.9"E, 27.04.2018 YV Rakhimova; in the same gorge, slope of the Big Almaty peak, mixed forest, 1504 m a.s.l., 43°06'36.2"N, 76°54'49.1"E, 29.08.2018, AM Assylbek.

In the hawthorn rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1477 m a.s.l., 43°06'37.0"N, 76°54'53.1"E, 29.08.2018, AM Assylbek.

In the pine rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, the edge of the pine forest, 1667 m a.s.l., 43°06'21.9"N, 76°54'47.9"E, 30.08.2018, YV Rakhimova.

In the poplar rhizosphere

Big Almaty gorge, North-West exposure slope, mixed forest, 1930 m a.s.l., 43°06'28.7"N, 76°56'21.2"E, 27.04.2018, LA Kyzmetova.

In the rowan rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1703 m a.s.l., 43°06'18.3"N, 76°54'49.6"E, 30.08.2018, YV Rakhimova.

In the spruce rhizosphere

Big Almaty gorge, North exposure slope, coniferous forest,, 2377 m a.s.l., 43°04'08.5"N, 76°59'27.2"E, 09.08.2018, AM Assylbek; Ayusai gorge, South-Eastern exposure slope, coniferous forest, 1831 m a.s.l., 43°05'43.4"N, 76°56'44.9"E, 27.08.2018, LA Kyzmetova.

In the willow rhizosphere

Big Almaty gorge, North-West exposure slope, mixed forest, 2312 m a.s.l., 43°04'19.8"N, 76°59'11.1"E, 06.08.2018, UK Jetigenova.

***Actinomucor elegans*** (Eidam) C.R. Benj. & Hesselt.

In the hawthorn rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1477 m a.s.l., 43°06'37.0"N, 76°54'53.1"E, 29.08.2018, AM Assylbek.

***Mucor fuscus*** Bainier (*M. petrinsularis* Naumov)

In the rowan rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1703 m a.s.l., 43°06'18.3"N, 76°54'49.6"E, 30.08.2018, YV Rakhimova.

***Mucor mucedo*** Fresen.

In the apple rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, coniferous forest, 1628 m a.s.l., 43°06'26.0"N, 76°54'46.6"E, 29.08.2018, YV Rakhimova; in the same gorge, mixed forest, 1703 m a.s.l., 43°06'18.3"N, 76°54'49.6"E, 30.08.2018, YV Rakhimova.

In the apricot rhizosphere

Big Almaty gorge, North-West exposure slope, mixed forest, 1926 m a.s.l., 43°06'27.1"N, 76°56'15.9"E, 27.04.2018 YV Rakhimova; in the same gorge, slope of the Big Almaty peak, mixed forest, 1504 m a.s.l., 43°06'36.2"N, 76°54'49.1"E, 29.08.2018, AM Assylbek.

In the aspen rhizosphere

Big Almaty gorge, North-West exposure slope, mixed forest, 2312 m a.s.l., 43°04'19.8"N, 76°59'11.1"E, 06.08.2018, UK Jetigenova.

In the birch rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, birch forest, 1556 m a.s.l., N43°06'31.4", E76°54'43.8", 29.08.2018, YV Rakhimova.

In the oak rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1504 m a.s.l., 43°06'36.2"N, 76°54'49.1"E, 29.08.2018, UK Jetigenova.

In the pine rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, the edge of the pine forest, 1703 m a.s.l., 43°06'18.3"N, 76°54'49.6"E, 30.08.2018, YV Rakhimova.

In the poplar rhizosphere

Big Almaty gorge, North-West exposure slope, mixed forest, 1930 m a.s.l., 43°06'28.7"N, 76°56'21.2"E, 27.04.2018, LA Kyzmetova; in the same gorge, mixed forest, 1957 m a.s.l., 43°06'23.8"N, 76°56'46.4"E, 27.04.2018, LA Kyzmetova .

In the spruce rhizosphere

Big Almaty gorge, South West exposure slope, coniferous forest,, 2480 m a.s.l., 43°03'50.6"N, 76°59'05.1"E, 03.08.2018, YV Rakhimova; Ayusai gorge, South-Eastern exposure slope, coniferous forest, 1864 m a.s.l., 43°05'35.0"N, 76°56'52.9"E, 27.08.2018, LA Kyzmetova; Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1582 m a.s.l., 43°06'29.5"N, 76°54'43.8"E, 29.08.2018, AM Assylbek.

In the rowan rhizosphere

Big Almaty gorge, North exposure slope, coniferous forest, 2395 m a.s.l., 43°04'06.4"N, 76°59'26.9"E, 10.09.2018, YV Rakhimova.

In the willow rhizosphere

Big Almaty gorge, North-West exposure slope, mixed forest, 2312 m a.s.l., 43°04'19.8"N, 76°59'11.1"E, 06.08.2018, UK Jetigenova.

***Mucor silvaticus*** Hagem (*M. hiemalis* f. *silvaticus* (Hagem) Schipper)

In the apple rhizosphere

Big Almaty gorge, mixed forest, 1923 m a.s.l., 43°05'53.0"N, 76°57'23.1"E, 27.04.2018, YV Rakhimova.

***Mucor strictus*** Hagem

In the birch rhizosphere

Big Almaty gorge, birch forest, 1923 m a.s.l., 43°05'53.0"N, 76°57'23.1"E, 27.04.2018, YV Rakhimova.

***Rhizopus stolonifer*** (Ehrenb.) Vuill. (*Rh. nigricans* Ehrenb.)

In the spruce rhizosphere

Big Almaty gorge, coniferous forest, 1937 m a.s.l., 43°06'22.0"N, 76°57'11.0"E, 27.04.2018, AM Assylbek.

***Zoopagomycotina*** Benny

***Zoopagales*** Bessey ex R.K. Benj.

***Piptocephalidaceae*** J. Schröt.

***Piptocephalis arrhiza*** Tiegh. & G. Le Monn.

In the apricot rhizosphere

Big Almaty gorge, slope of the Big Almaty peak, mixed forest, 1504 m a.s.l., 43°06'36.2"N, 76°54'49.1"E, 29.08.2018, AM Assylbek.

*Mucor mucedo* was observed as the most prevalent species in rhizosphere of apple, apricot, aspen, birch, oak, pine, poplar, spruce, rowan and willow. The colonies of isolates are white, dirty-white, beige, or gray, fluffy, very fast-growing (Table 2). Hyphae broad, nonseptate or sparsely septate. Sporangioophores erect; sporangia spherical and multispored, columellae of various shapes: cylindrical, rarely ovoid or pear-shaped, smooth (Fig. 2), (30.0–100.0 × 20.0–90.0) μm. The largest columellae are observed in fungi isolated from the rhizosphere of poplar and spruce (Table 3). Sporangiospores hyaline, cylindrical (Fig. 3), rounded at the ends, (10.0–14.0 × 6.5–8.0) μm. Rhizoid and zygospores were absent.

**Table 2** Macroscopic characters of different isolated Zygomycetes

Fungal species	Characters of colony			Growth rate
	Color	Texture	Reverse	
<i>Absidia spinosa</i>	Grayish	Fluffy	Slightly yellowish	Fast
<i>Actinomucor elegans</i>	Unpainted, then grayish	Fluffy	Slightly beige	Fast
<i>Cunninghamella echinulata</i>	Unpainted, later smoky	Arachnoid, fluffy	Beige	Moderate
<i>Mucor mucedo</i>	Dirty white, beige or grey	Fluffy	Slightly greenish-yellowish	Very fast
<i>Mucor fuscus</i>	Ash-colored	Fluffy	Unpainted or slightly beige	Fast
<i>Mucor silvaticus</i>	Pale ash	Fluffy	Slightly beige	Fast
<i>Mucor strictus</i>	Gray	Fluffy	Slightly yellowish	Fast
<i>Piptocephalis arrhiza</i>	Unpainted	-	-	Low
<i>Rhizopus stolonifer</i>	Brownish-gray	Felt	Brownish	Fast

**Table 3** Dimensions of columellae of different isolates of *Mucor mucedo*,  $\mu\text{m}$ 

Samples	Length of columella	Width of columella
257	50.0–100.0	40.0–90.0
258	80.0–90.0	70.0–80.0
289	50.0–100.0	30.0–90.0
293	50.0–90.0	40.0–50.0
302	60.0	30.0–40.0
313	30.0–80.0	20.0–50.0

In addition to *Mucor mucedo* three species of this genus were noted. *Mucor fuscus* was isolated from the rowan rhizosphere. Colonies are ash-colored, fast-growing (Table 2). Sporangiohores hyaline, erect, branched, with short lateral branches bearing grayish brown spherical sporangia (Fig. 4). Columella cylindrical or ovoid, smooth. After the dehiscence of sporangia, hyaline, spherical (sometimes angular) sporangiospores release. Diameter of sporangiospores 8.0–10.5  $\mu\text{m}$ .

*Mucor silvaticus* was found in the apple rhizosphere. Colonies are fast-growing (Table 2), fluffy, pale ash. Sporangiohores hyaline, erect, long; sporangia spherical. Sporangia first colorless, then brownish gray, spherical. Columella pale brown or colorless, spherical or elipsoid. Sporangiospores hyaline, cylindrical. Dimensions of sporangiospores (5.5–6.5  $\times$  2.5–4.0)  $\mu\text{m}$ .

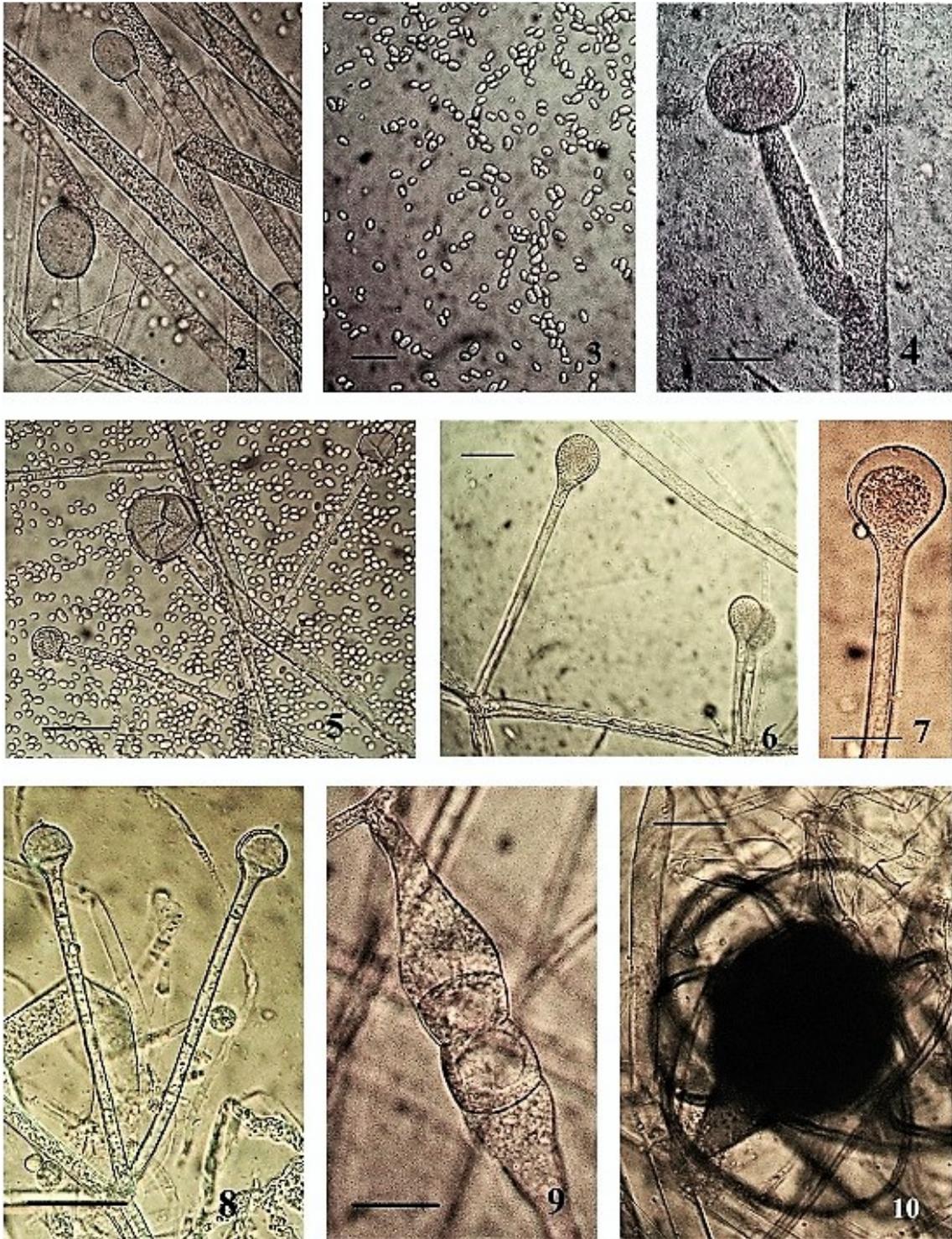
*Mucor strictus*, isolated from the birch rhizosphere, was characterized by fast-growing, fluffy, gray colonies (Table 2). Sporangiohores hyaline, erect, long, sometimes curved or branched. Sporangia spherical or slightly flattened; columella pale brown, ovoid (Fig. 5). Diameter of spherical sporangiospores 5.0  $\mu\text{m}$ .

*Absidia spinosa* was isolated from the rhizosphere of apple, apricot, hawthorn, pine, poplar, rowan, spruce and willow, and was observed less frequently than *Mucor*. Most often, the species is noted in the rhizosphere of spruce and apricot. Colonies are fast-growing, fluffy beige, grayish (Table 2). Rhizoids branched. Straight, brownish sporangiohores are situated in whorls on stolons (Figs 6, 7). Diameter of sporangiohores varies from 7.0 to 10.5  $\mu\text{m}$ , while the diameter of sporangia – from 24.0 to 40.0  $\mu\text{m}$  (Table 4). Dehiscence of sporangium takes place after maturation of globose sporangiospores. Columella hemispherical with a thin appendage (Fig. 8). Gametangia of the same size (Fig. 9). Zygosporangium, which develops after conjugation of gametangia, has simple, sickle-shaped curved appendages (Fig. 10).

**Table 4** Dimensions of different isolates of *Absidia spinosa*,  $\mu\text{m}$  (a dash in a column indicates no measurements)

Samples	Diameter of			
	sporangiohores	sporangium	columella	zygosporangium
256	7.0–10.5	24.0–31.5	7.0–24.0	–
301	7.5	30.8	21.5–26.0	90.0
304	–	–	–	50.0–80.0
309 a	–	–	20.0–21.5	–
313	7.0–8.5	32.0	–	–
314	8.5–9.5	36.5–40.0	–	–
315 B	7.5–8.5	32.0–35.0	–	–

Only one species of *Cunninghamella* (*C. echinulata*) from hawthorn rhizosphere was identified. Colonies are fluffy, unpainted, white at first, later smoky (Table 2). Conidiophores straight, with verticillate or solitary branches. Conidia yellowish brown, spherical (Fig. 11), 10–15  $\mu\text{m}$  in diameter, echinulate, with oil globules. Vesicles (Fig. 12) hyaline, pale brown, globose, subglobose or back pear-shaped, of different sizes. Apical vesicles (25.5–35.5  $\times$  20.5–30.5)  $\mu\text{m}$ ; vesicles on branches – (15.5–20.5  $\times$  10.5–15.5)  $\mu\text{m}$ .



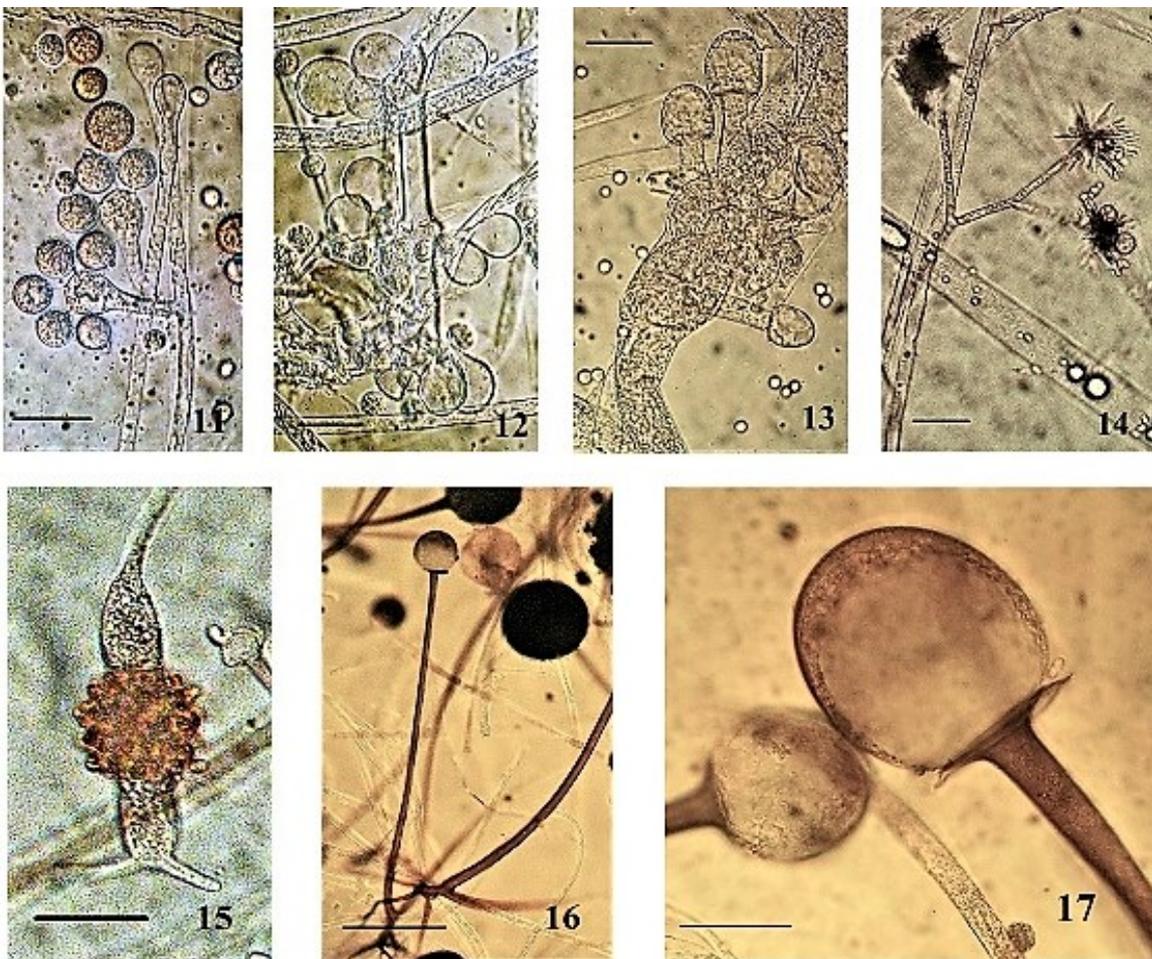
**Figs 2–10** – Species of the genera *Mucor* and *Absidia*. 2 columella of *Mucor mucedo*. Scale Bar: 50  $\mu\text{m}$ . 3 sporangiospores of *Mucor mucedo*. Scale Bar: 30  $\mu\text{m}$ . 4 sporangium of *Mucor fuscus*. Scale Bar: 35  $\mu\text{m}$ . 5 columella of *Mucor strictus*. Scale Bar: 60  $\mu\text{m}$ . 6 sporangiophores on stolons *Absidia spinosa*. Scale Bar: 35  $\mu\text{m}$ . 7 sporangium of *Absidia spinosa*. Scale Bar: 25  $\mu\text{m}$ . 8 columellae of *Absidia spinosa*. Scale Bar: 50  $\mu\text{m}$ . 9 conjugation of gametangia of *Absidia spinosa*. Scale Bar: 25  $\mu\text{m}$ . 10 zygospore of *Absidia spinosa*. Scale Bar: 40  $\mu\text{m}$ .

*Actinomucor elegans* was isolated from the hawthorn rhizosphere. Colonies are fast-growing, fluffy, first colorless, then grayish. Hyphae colorless, septate, with few rhizoids. Sporangiohores pale brown, straight, sometimes with granular content, intensely branched. Sporangia spherical,

varying sizes: from 40-60 to 60-100  $\mu\text{m}$  in diameter. After dehiscence of sporangium the columella seems colorless, slightly conical (Fig. 13).

*Piptocephalis arrhiza* was isolated from the apricot rhizosphere, together with species of the genus *Mucor*, it is their parasite. Hyphae uncolored, very thin, with rare septa; stolons absent. Merosporangiophores (Fig. 14) brownish, thin, repeatedly dichotomously branched; merosporangia simple, with 2–4 spores. Merosporangiospores pale brownish, smooth, cylindrical, (3.5–5.0  $\times$  2.5–3.0)  $\mu\text{m}$ . Zygosporangium (Fig. 15) yellow, then rusty-brown, spherical with the star-shaped protrusions, 30.0–40.0  $\mu\text{m}$  in diameter.

*Rhizopus stolonifer* was isolated from the spruce rhizosphere. Colonies are fast-growing, fluffy, brownish-gray. Rhizoids and stolons brown, transparent, well expressed (Fig. 16). Sporangiohores brown, erect, simple or branched, curved, bearing sporangia terminally. Sporangia dark brown to black, spherical, apparently subglobose after maturity, 100–200  $\mu\text{m}$  in diameter; columella brownish to brown, globose (Fig. 17), 90–125  $\mu\text{m}$  in diameter. Sporangiospores pale brown, spherical or ellipsoidal, (5.0–10.5  $\times$  5.9–8.5)  $\mu\text{m}$ . Zygosporangia were not observed.



**Figs 11–17** – Species of the genera *Cunninghamamella*, *Actinomucor*, *Piptocephalis* and *Rhizopus*. 11 conidia of *Cunninghamamella echinulata*. Scale Bar: 20  $\mu\text{m}$ . 12 vesicles on branches. Scale Bar: 20  $\mu\text{m}$ . 13 columellae of *Actinomucor elegans*. Scale Bar: 50  $\mu\text{m}$ . 14 merosporangia of *Piptocephalis arrhiza*. Scale Bar: 20  $\mu\text{m}$ . 15 zygosporangium of *P. arrhiza*. Scale Bar: 35  $\mu\text{m}$ . 16 rhizoids, stolons and sporangiophores of *Rhizopus stolonifer*. Scale Bar: 120  $\mu\text{m}$ . 17 columella of *R. stolonifer*. Scale Bar: 50  $\mu\text{m}$ .

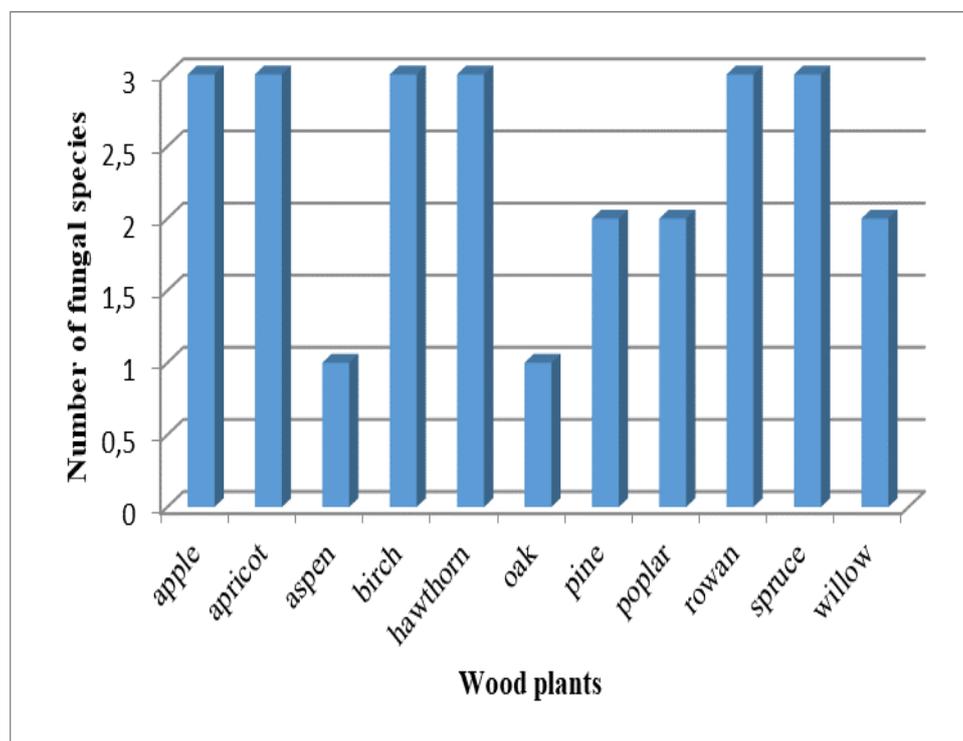
Eight species of Zygomycetes are found in the rhizosphere of mixed forest trees, three species are found in the soil of spruce forests (Table 5). Two species are common to mixed and spruce forests.

**Table 5** Prevalence of fungal species (%) in different habitats (- – the species is absent, + – accidental species, occurrence frequency – A<30%, ++ – typical species, A>30%, +++ – dominant species, A>50%)

Fungal species	Mixed and small-leaved forest			Coniferous forest		
	1400–1700 m a.s.l.	1700–2000 m a.s.l.	2000–2300 m a.s.l.	1600–1900 m a.s.l.	1900–2200 m a.s.l.	2200–2500 m a.s.l.
	<i>Absidia spinosa</i>	+++	+++	+	++	-
<i>Actinomucor elegans</i>	+	-	-	-	-	-
<i>Cunninghamella echinulata</i>	+	-	-	-	-	-
<i>Mucor mucedo</i>	+++	+++	++	+++	-	++
<i>Mucor fuscus</i>	-	+	-	-	-	-
<i>Mucor silvaticus</i>	-	+	-	-	-	-
<i>Mucor strictus</i>	-	+	-	-	-	-
<i>Piptocephalis arrhiza</i>	+	-	-	-	-	-
<i>Rhizopus stolonifer</i>	-	-	-	-	+	-

The greatest number of soil fungi species is typical for mixed and small-leaved forest at the altitude range 1400–2000 m above sea level, much less of them in higher ranges (Table 5). *Absidia spinosa* and *Mucor mucedo* are dominant for these altitudes despite the fact that they are observed at all studied altitudes, except for coniferous forest for the interval 1900-2200 m above sea level.

The number of soil Zygomycetes varies in the rhizosphere of different forest species. The greatest number of soil fungal species (3 species) is observed in the rhizosphere of apple, apricot, birch, hawthorn, rowan and spruce (Fig. 18).



**Fig. 18** – Distribution of soil Zygomycetes in the rhizosphere of different forest species

## Discussion

*Mucor* species are observed as the most prevalent species in rhizosphere of woody plants of Ile-Alatau Natural Park. Intensive development of *Mucor* fungi is associated with a high content of

poorly decomposed organic substances in the soil (Dyakov 2006, Berseneva et al. 2008). So, if in the rich forest soils to 25-40% of propagules of the considered fungi are *Mucor* fungi, then in the soils of arable land their number does not exceed 12-15% (Classen 2003). Some species are predominantly found in coniferous forest soils of Minsk upland, (Belomesyatseva & Shabashova 2004), in the rhizosphere of forest ecosystems of the European part of Russia (Kurakov & Semenova 2016), in the rhizosphere of various plants of the city of Lahore (Samina et al. 2017), in the rhizosphere of larch-spruce forests in the central part of the Dzhagdy ridge (Shumilova 2013), in the soils of Ukraine (Minter & Dudka 1996) and Poland (Budziszewska et al. 2010, Ruskiewicz-Michalska et al. 2012, 2016). While *Mucor racemosus*, *M. nodosus*, and *M. mucedo* are found mainly in agricultural soils (Mirchink 1988).

*Absidia spinosa*, isolated from the rhizosphere of apple, apricot, hawthorn, pine, poplar, rowan, spruce and willow, is the next prevalent fungus in the studied rhizosphere samples from Ile-Alatau Natural Park. Fungi of the genus *Absidia* behave like fungi of the genus *Mucor* (Dyakov 2006, Berseneva et al. 2008). This may explain the high occurrence of *Absidia spinosa* in the rhizosphere of spruce in the forests of Ile-Alatau Natural Park. Species of the genus *Absidia* are also characteristic of the soil of coniferous forests of the Russian Far East (Egorova 2009, 2011, Egorova & Kovaleva 2012, Egorova et al. 2013).

Genus *Cunninghamella* is usually found in the soils of subtropical and tropical zones. This fungus is not found or poorly represented in the soils of the North (Egorova 2009, 2011, Egorova & Kovaleva 2012, Egorova et al. 2013, Shumilova 2013, Kurakov & Semenova 2016, Samina et al. 2017). In Kazakhstan, the genus *Cunninghamella* is represented by only one species *Cunninghamella echinulata* found once in the hawthorn rhizosphere in the forests of Ile-Alatau Natural Park. The same species is found in India (as *C. echinata*) (Koilaraj et al. 1999). Another species *Cunninghamella elegans* is discovered in soils of Ukraine (Minter & Dudka 1996) and in dune, sand grassland of the Biebrza National Park, Poland (Ruskiewicz-Michalska et al. 2012).

*Actinomucor elegans* isolated from the hawthorn rhizosphere in the forests of Ile-Alatau Natural Park is also found in the soils of Ukraine (Minter & Dudka 1996) and European part of Russia (Kurakov & Semenova 2016).

The genus *Rhizopus* is considered as a thermophilic and is more often found in the soils of the Southern zone (Dyakov 2006). However, *Rhizopus stolonifer* is common for the rhizosphere of forest ecosystems of Ile-Alatau Natural Park as well as Ukraine (Minter & Dudka 1996), Poland (Ruskiewicz-Michalska et al. 2012, 2016), Russian Far East (Egorova 2009, 2011, Egorova & Kovaleva 2012, Egorova et al. 2013, Shumilova 2013), European part of Russia (Kurakov & Semenova 2016), Lahore city, Pakistan (Samina et al. 2017), and Arunachal Himalaya (Sharma et al. 2015), India, (Koilaraj et al. 1999).

Mycophilic fungus *Piptocephalis arrhiza* is isolated from the apricot rhizosphere in mixed forest of Ile-Alatau Natural Park. Species of the genus *Piptocephalis* are obligate parasites of the members of *Mucorales* and they are commonly found on forest litter or in soil. *Piptocephalis* spp. are characterized by the production of dichotomously branching sporophores with cylindrical uniseriate merosporangia (Benjamin 1959, Mukerji 1968, Kirk 1978, Ho 2003, 2004, 2006a, 2006b). Currently, 24 species are described (Index Fungorum 2018). Of the 24 species only one species *Piptocephalis arrhiza* is found in Kazakhstan, while 3 species are registered on the territory of Biebrza National Park (Poland): *Piptocephalis fimbriata*, *P. lepidula*, *Piptocephalis* sp. (Ruskiewicz-Michalska et al. 2012, 2016).

Thus, the species composition of the soil Zygomycetes of the Ile-Alatau Natural Park is distinguished by its originality, due to the variety of environmental conditions.

## Acknowledgements

This work was financed by the Project with title “Evaluation of the effect of micromycetes soil reserve on the restoration potential of the Zailisky (Trans-Ili) Alatau forests after fires”, IRN AP05131258.

## References

- Belomesyatseva DB, Shabashova TG. 2004 – Features of the species composition of soil micromycetes in coniferous forests of the Minsk upland. *Mycology & Phytopathology* 38(6), 1–7. [in Russian]
- Benjamin RK. 1959 – The merosporangiferous Mucorales. *Aliso* 4, 321–433.
- Berseneva OA, Salovarova VP, Pristavka AA. 2008 – Soil micromycetes of main natural areas. *News of Irkutsk state University. Series “Biology. Ecology”*. 1(1), 3–9. [in Russian]
- Budziszewska J, Boulahdjel A, Wilk M, Wrzosek M. 2010 – Soil zygomycetous fungi in Biebrza National Park (northeast Poland). *Pol Bot J*. 55(2). 391–407.
- Classen AT. 2003 – Community-level physiological profiles of bacteria and fungi. *Microbiol. Ecol. J*. 44. 319–328.
- Dyakov. 2006 – Fungi and plants. Moskva: Angle. [in Russian]
- Egorova LN. 2009 – Soil Zygomycetes (*Zygomycetes: Mucorales, Mortierellales*) of coniferous forests of the Russian Far East. *Mycology & Phytopathology* 43(4), 292–297. [in Russian]
- Egorova LN. 2011 – Soil fungi of “Bastak” nature reserve (Jewish Autonomy). *Mycology & Phytopathology* 45(6), 131–145. [in Russian]
- Egorova LN, Kovaleva GV. 2012. – Soil micromycetes of the nature reserve “Botchinsky” (Khabarovsk territory). *Mycology & Phytopathology* 46(2), 131–145. [in Russian]
- Egorova LN, Kovaleva GV, Aleshina OA. 2013 – Soil micromycetes of the nature reserve “Bolonsky” (Khabarovsk territory). *Mycology & Phytopathology* 47(4), 497–503. [in Russian]
- Ho HM. 2003 – The merosporangiferous fungi from Taiwan (III): three new records of *Piptocephalidaceae* (Zoopagales, Zygomycetes). *Taiwania* 48, 53–59.
- Ho HM. 2004 – The merosporangiferous fungi from Taiwan (IV): two new records of *Piptocephalis* (Piptocephalidaceae, Zoopagales). *Taiwania* 49, 188–193.
- Ho HM. 2006a – The merosporangiferous fungi from Taiwan (VI): two new records of *Piptocephalis* (Piptocephalidaceae, Zoopagales, Zygomycetes). *Taiwania* 51(3), 210–213.
- Ho HM. 2006b – A new species of *Piptocephalis* from Taiwan. *Botanical Studies* 47, 453–456.
- Index Fungorum. 2018 – Available from: <http://www.indexfungorum.org> (cited 2018 Dec 24)
- Kirk PM. 1978 – A new and unusual species of *Piptocephalis* (Mucorales). *Trans. Br. Mycol. Soc.* 70, 335–340.
- Kirk PM, Cannon PF, David JC, Stalpers JA. 2008 – *Ainsworth and Bisby's Dictionary of Fungi*. CAB International.
- Koilraj AJ, Marimuthu G, Natarajan K, Saravan S et al. 1999 – Fungal diversity inside caves of Southern India. *Current science*. 77(8), 1081–1084.
- Kurakov AV, Semenova TA. 2016 – Species diversity of microfungi in the forest ecosystems of southern taiga in the European part of Russia. *Mycology & Phytopathology* 50(6), 367–378. [in Russian]
- Minter DW, Dudka IO. 1996 – *Fungi of Ukraine. Preliminary checklist*. CAB International.
- Mirchink TG. 1988 – *Soil Mycology*. Moskva: MGU. [in Russian]
- Mukerji KG. 1968 – Fungi of Delhi: IV. A new species of *Piptocephalis* from India. *Mycologia* 60(2), 326–330.
- Pidoplichko NM, Mil’ko AA. 1971 – *Atlas of Mucorales fungi*. Kiev: Naukova dumka. [in Russian]
- Polyksenova VD, Khramtsov AK, Piskun SG. 2004 – *Methods of experimental study of microscopic fungi (Methodical instructions)*. Minsk: BSU. [in Russian]
- Ruszkiewicz-Michalska M, Balazy S, Chelkowski J, Dynowska M et al. 2016 – Preliminary studies of fungi in the Biebrza National Park (NE Poland). III. Micromycetes – new data. *Acta Mycol.* 50(2), 1067–1095.
- Ruszkiewicz-Michalska M, Tkaczuk C, Dynowska M, Sucharzewska E et al. 2012 – Preliminary studies of fungi in the Biebrza National Park (NE Poland). I. Micromycetes. *Acta Mycol.* 47(2), 213–234.

- Samina S, Tahreem M, Shabnum S, Farah K et al. 2017 – Micromycete diversity associated with the rhizospheres of plants from different polluted soils of Lahore, Pakistan. *Current Research in Environmental & Applied Mycology* 7(3), 193–202.
- Schwarzman SR. 1962 – Materials to history of mycoflora in Kazakhstan. Alma-Ata. [in Russian]
- Sharma D, Gosai K, Dutta J, Arunachalam A et al. 2015 – Fungal diversity of twelve major vegetational zones of Arunachal Himalaya, India. *Current Research in Environmental & Applied Mycology* 5(2), 101–119.
- Shumilova LP. 2013 – Microscopic fungi of mountain brown-taiga soils of Dzhagdy ridge. *Problems of ecology of the Upper Amur region* 15, 83–90. [in Russian]
- Watanabe T. 2002 – Pictorial atlas of soil and seed fungi: Morphologies of cultured fungi and key to species. CRC Press.