Occurrence of Anamorphic and Teleomorphic Stage of *Erysiphe palczewskii* (syn. *Microsphaera palczewskii*) on *Caragana arborescens* in the Czech Republic and Austria and its Morphological Characterisation

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Abstract

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During the summer of 2007 in a private garden in Smržice (district Prostějov, Czech Republic) symptoms of powdery mildew on *Caragana arborescens* were recorded. Until now at least two powdery mildew species (*Microsphaera trifolii* var. *trifolii* and *M. palczewskii*), both differing in some basic morphological features, have been recorded for the genus *Caragana*. Comparison of our measurements with previous descriptions of powdery mildew samples found in the Czech Republic on *Caragana arborescens* identified our species as *Microsphaera palczewskii* (newly *Erysiphe palczewskii*). This is probably the first detailed record of this species on *C. arborescens* in the Czech Republic. Fungus from Czech Republic was compared with the material collected in 2007 in Vienna (Austria). The aim of this paper was to provide a detailed comparative morphological characterisation of *E. palczewskii* anamorph and teleomorph found in the Czech Republic and in Austria. Serious reduction of the anamorph, caused by hyperparasitic fungus *Ampelomyces quisqualis*, was recorded on the sample from Austria.

Keywords: *Ampelomyces quisqualis*; *Caragana arborescens*; chasmothecia; conidia; *Erysiphe palczewskii*; *Microsphaera trifolii* var. *trifolii*; Siberian pea-shrub

The genus *Caragana* Fabr. (family Fabaceae) is comprised of 15 species (mainly shrubs) and their occurrence is limited to the temperate and tropical area of Eurasia (mainly Asia). *Caragana arborescens* Lam. (Siberian pea-shrub, Siberian pea-tree, Fabacea) is an ornamental deciduous shrub (native to Manchuria and Siberia) that can grow up to 6 metres tall but in practice seldom exceeds about 3 metres. In North Korea it is cultivated on a small scale for its medicinal roots, and in other countries (e.g. in Canada and USA) it is planted in windbreaks (Hanelt 2001). There

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are several known cultivars, e.g. Lorbergii (with linear leaves and fine texture), Nana (a dwarf form), Pendula (weeping form with stiffly arching branches), Pygmaea (dwarf with narrow leaves) and others (Hurych & Mikuláš 1973). From the viewpoint of garden and landscape architecture in the Czech Republic, *C. arborescens* belongs to the group of most frequently grown deciduous ornamental shrubs. According to available data, the frequency of occurrence of *C. arborescens* in the castle parks is about 28% (Hieke 1984). However, in Prague parks, *C. arborescens* belongs to one of the most frequently grown shrubs with a frequency of occurrence of over 90% (Dudych *et al.* 1975). Recently, this taxon has been considered as an important shrub well adapted to the conditions of the Czech Republic and is grown in groups of other shrubs in places where the conditions are not suitable for other shrubs and trees. As a soliterous shrubs are grown some cultivars (Pendula, Lorbergii and Walker) of *C. arborescens* (Pejchal 2007, unpubl.).

On representatives of the family Fabacea the occurrence of several powdery mildews is confirmed and they can be assigned to two genera – *Erysiphe* and *Microsphaera* (*Erysiphe* sect. *Microsphaera*) (Braun 1995; Braun & Takamatsu 2000). Recently, according to molecular studies (Braun & Takamatsu 2000; Mori *et a*l. 2000) the delimitation of genera *Microsphaera* and *Erysiphe* was re-evaluated; and now more valuable taxonomic features are considered such as anamorph stage (mainly the kind of conidia formation, which is same in both genera – Pseudoidium type). In contrast the shape of appendages of cleistothecia (chasmothecia), which strictly distinguish the previously separated genera *Erysiphe* (appendages simple) and *Microsphaera* (apex of the appendages dichotomously branched) is now not considered as an important taxonomical feature.

Until now, at least two powdery mildew species – *Microsphaera trifolii* (Grev.) U. Braun var. *trifolii* and *Microsphaera palczewskii* Jaczewski, both differing in some morphological features have been recorded to occur on the genus *Caragana* (Braun 1995). Appendages in *M. trifolii* var. *trifolii* can be 2–6 times as long as the cleistothecial diameter and the species has larger cleistothecia (90–150 µm in diameter) and larger conidia (37 × 18 µm) than in *M. palczewskii*. Appendages of *M. palczewskii* are only 1–2 times as long as the cleistothecial diameter and are 5–8 times more closely branched.

The occurrence of *M. palczewskii* was first reported from Asia (China, Kazakhstan, Russia, Turkmenistan), and since then Europe (Belarus, Germany, Hungary, Latvia, Lithuania, Poland, Rumania, Russia (European part), Slovakia, Spain, Sweden, Ukraine) and North America (in several states of USA (Idaho, Washington, North Dakota, Minnesota and Alaska)) and Canada (Braun 1995; Heluta & Minter 1998; Nischwitz & Newcombe 2003; Glawe & Laursen 2005; Glawe *et al.* 2006; Vajna 2006). Interestingly, it is only in recent decades that *M. palczewskii* has spread from Asia into Europe (Huhtinen *et al.* 2001) and it may have been recently introduced into North America as is suggested by areas in southern Canada and the northern United States in which *C. arborescens* remains unaffected by powdery mildew (Nischwitz & Newcombe 2003; Glawe *et al.* 2006). Heluta and Minter (1998) described the following species as hosts of *M. palczewskii: Caragana arborescens, C. boisii, C. brevispina, C. decorticans, C. fruticosa, C. manchurica, C. microphylla, C. mollis, C. spinosa, C. ussuriensis* and *Robinia pseudoacacia*.

Recently, first serious powdery mildew infections on *C. arborescens* were recorded in the Czech Republic, and the causal species were described as *Erysiphe palczewskii* (Jacz.) U. Braun and S. Takamatsu (Lebeda *et al.* 2008). The aim of this paper was to provide a detailed comparative morphological characterisation between *E. palczewskii* anamorph and teleomorph found in the Czech Republic with that found in material collected in 2007 in Austria.

MATERIAL AND METHODS

Samples of *Erysiphe palczewskii* collected and described from the Czech Republic (Lebeda *et al.* 2008) and a sample from Austria (Lebeda, unpubl.) were used for morphological characterisation of the anamorphic and teleomorphic state. For microscopic characterisation of the pathogen, three powdery mildew samples were collected altogether. These were, *Caragana arborescens* (CSU – collected on *C. arborescens* cv. Lorbergii, CSS – collected on *C. arborescens* cv. Pendula (both from the Czech Republic; Tables 1 and 2), and A – collected on *C. arborescens* cv. Pendula at the end of September 2007 in Vienna (Botanical Garden, Agricultural University in Vienna, Austria; Table 2)).

Pieces of severely infected leaves were used for evaluation by light microscopy (Olympus BX-60). For the observation of morphological features of anamorph (conidia and conidiophores) the modified method of Shin (2000) with lactic acid and fuchsin was used. The presence of fibrosin bodies in conidia was assessed by mounting of fresh conidia in 3% potassium hydroxide (Lebeda 1983). The sexual stage (cleistothecia, chasmothecia) was inspected without any staining. For statistical analyses (means, standard deviations and range), 50 (resp. 100) measurements of each of the characteristics were made where possible. The anamorph stage was not analysed for the Vienna sample because of superficial mycelium (conidiophores and conidia) shrivelling due to being collected at the end of the vegetation season.

RESULTS

Symptoms of powdery mildew on *Caragana arborescens*

During the summer of 2007 powdery mildew symptoms on *Caragana arborescens* cv. Pendula were recorded in a private garden in Smržice (district Prostějov, Czech Republic). White sporulating mycelium covered both upper and under sides of leaves (Figures 1A,B). On both sides of leaves cleistothecia (chasmothecia) were also found (Figures 1G,H). In the same garden the occurrence of powdery mildew symptoms on *C. arborescens* cv. Lorbergii was also recorded, however with less cleistothecia on the leaves. Although the work is concentrated upon describing powdery mildew on *Caragana arborescens* in the Czech Republic, measurements of *Erysiphe* (*Microsphaera*) *palczewskii* collected on *C. arborescens* cv. Pendula in Botanical Garden in Vienna (Austria) were completed also. On the samples of leaves of *C. arborescens* with powdery mildew symptoms collected in September 2007 only the teleomorph state was found on the leaves. The anamorph was nearly completely reduced by other microflora (saprophytes and also hyperparasitic fungus *Ampelomyces quisqualis*).

Microscopic observation of the pathogen

The morphological features of this fungus may be summarised as follows. Mycelium was superficial, white (Figures 1A,B); hyphae branched, 5–7 µm wide; appressoria slightly lobed. Conidia were cy-

Table 1. Basic characteristics of anamorph stage of *Microsphaera* (*Erysiphe*) *palczewskii* collected in the Czech Republic

	Conidia			Conidiophore	
	length (µm)	width (µm)	L/W ratio	length (µm)	foot-cell length (µm)	number of distal cells
	mean ± SD (min–max)					
E. palczewskii isolate CSU (Smržice, CR)	$26.2^{a*} \pm 4.03$ (20–37.59)	$11.5^{a} \pm 1.1$ (8.75–13.75)	2.28 ± 0.4 (1.6–3.14)	67.9 ± 8.3 (47.5–92.5)	$27.5^{b} \pm 4.7$ (15–40)	2.74 ± 0.43 (2–3)
E. palczewskii isolate CSS (Smržice, CR)	$29.3^{b} \pm 3.5$ (21.25–37.5)	$13^{b} \pm 1.2$ (8.75–17.5)	2.27 ± 0.3 (1.54–3.14)	66.25 ± 10.4 (45–87.5)	$25.35^{a} \pm 4.47$ (17.5–32.5)	2.7 ± 0.45 (2–3)
F value	33.26	68.58	0.11	0.8	5.33	0.19
Mean of both samples of *E. palczewskii* (Smržice, CR)	27.9 ± 3.7 (20–37.5)	12.33 ± 1.45 (8.75–17. 5)	2.28 ± 0.3 (1.54–3.14)	67.1 ± 9.46 (45–92.5)	26.4 ± 4.7 (15–40)	2.72 ± 0.44 (2–3)
Microsphaera palczewskii (Braun 1995)	(21–37)	(11–19)				

*Scheffé's Multiple-Comparison test (^{a} and ^{b} = different letters express the statistical significance, $P = 0.05$)

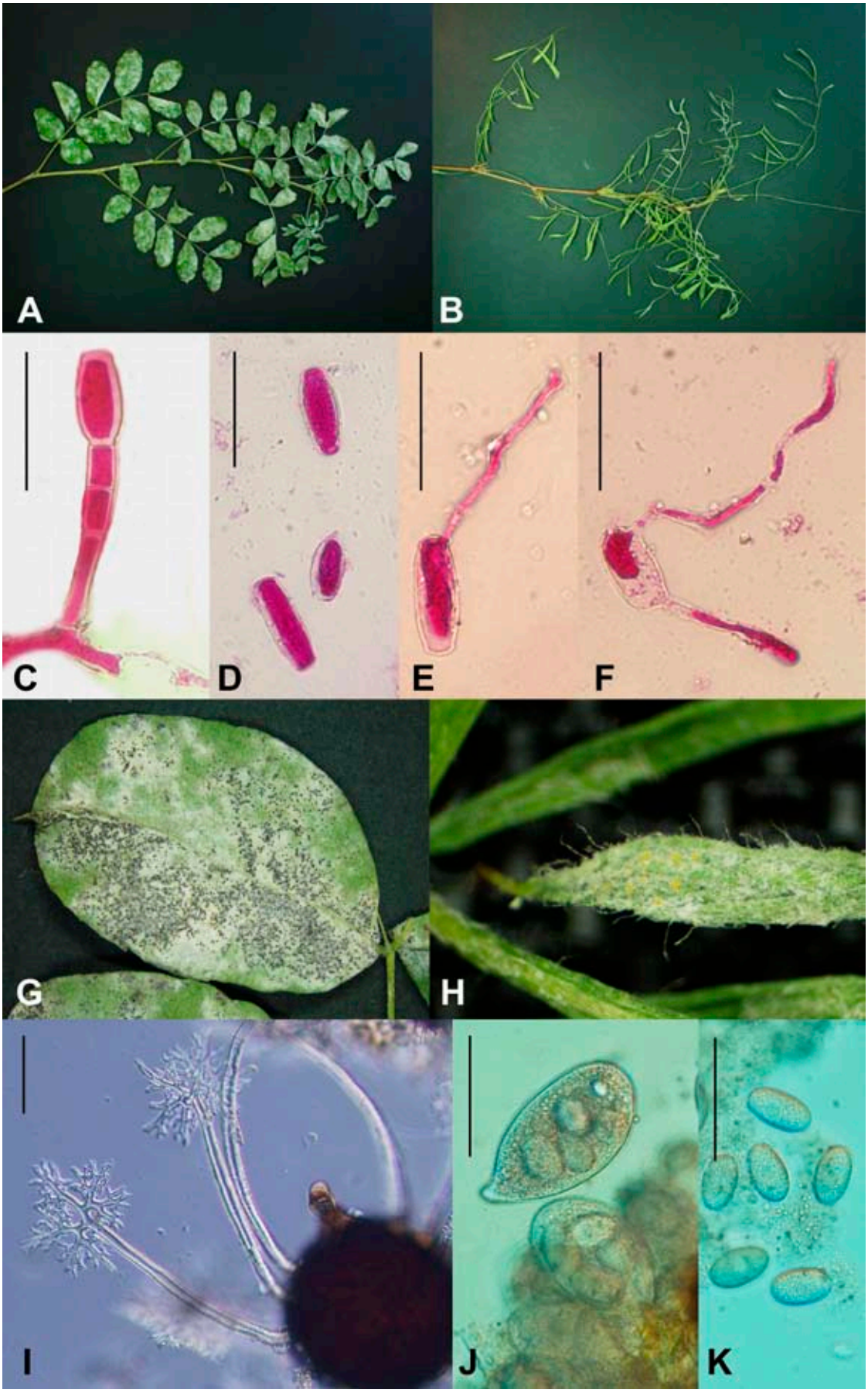
A
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Figure 1. (A) Pustules and sporulating mycelium of *E. palczewskii* on upper side of leaves of *C. arborescens* cv. Pendula; (B) Sporulating mycelium of *E. palczewskii* on of *C. arborescens* cv. Lorbergii leaves; (C) Foot-cell, conidiophore and conidia formation of *E. palczewskii* (bar = 50 μm); (D) Cylindrical conidia of *E. palczewskii* (bar = 50 m); (E–F) Germinating conidia of *E. palczewskii* (bar = 50 μm); (G–H) Mycelium and cleistothecia (chasmothecia) of *E. palczewskii* on upper side of the leave of *C. arborescens* cv. Pendula (G) and cv. Lorbergii (H) leaves; (I) Dichotomously branched appendages (apex) on *E. palczewskii* cleistothecia (bar = 50 μm); (J–K) Asci (J) and ascospores (K) of *E. palczewskii* (bar = 50 μm)

lindrical, lacking fibrosin bodies (Figure 1D), length 20–37.5 μm (mean 27.9 μm), width 8.75–17.5 μm (mean 12.33 μm), shape index 1.54–3.14 (mean 2.28), with germ tube arising from the end of the conidium (Figures 1E,F). Conidiophores were erect, 45–92.5 μm (mean 67.1 μm) long, foot-cells straight, 15–40 μm (mean 26.4 μm) long, followed by 2–3 distal cells (mean 2.72); conidia were formed singly (Pseudoidium type) (Table 1; Figure 1C).

Cleistothecia (chasmothecia) 67.5–112.5 μm (mean 91.5 μm), with 4–13 (mean 8.3) appendages dichotomously branched at the end of apex, 180–330 μm long (mean 241.5 μm), 1.8–4.1 times as long as the cleistothecial diameter, hyaline, mycelioid, thin-walled, smooth (Table 2; Figures 1G,H,I). Asci 3–8 (Figure 1J), 47.5–77.5 μm (mean 62.18 μm) long and 22.5–42.5 μm (mean 32.83 μm) wide, short-stalked, shape index 1.92 (1.26–3.22), with 3–8 ellipsoid-ovoid ascospores (Figure 1K), 17.5–27.5 μm (mean 20.84 μm) long and 8.75–16.25 μm (mean 11.88 μm) wide, shape index between 1.16–2.25 (mean 1.77) (Table 2). Specimens are preserved in the Department of Botany, Faculty of Science, Palacký University, Olomouc, Czech Republic.

DISCUSSION

Changes in the nomenclature and taxonomy of powdery mildews have been made based on recent molecular studies. Based on new SEM examinations as well as molecular data, Braun (1999) recognised *Erysiphe* s. str. (= *Erysiphe* sect. *Erysiphe*), the genus *Golovinomyces* (U. Braun) V.P. Gelyuta (= *Erysiphe* sect. *Golovinomyces* U. Braun), and introduced the new genus *Neoerysiphe* U. Braun (= *Erysiphe* sect. *Galeopsidis* U. Braun). Braun and Takamatsu (2000) confirmed the very close relationships between the genera *Erysiphe* s. str. (*Erysiphe* sect. *Erysiphe*), *Microsphaera* and *Uncinula,* which are connected by numerous intermediate taxa, which is strongly supported by results from examinations of nucleotide sequences of rDNA internal transcribed spacers (ITS) as well as by light and scanning electron microscopical features. Anamorphs of the three genera are uniform and belong in *Oidium* subgen. *Pseudoidium* Jacz. (Cook *et al.* 1997). According to Takamatsu *et al.* (1998, 1999) *Erysiphe* s. str. and *Microsphaera* do not group into separate monophyletic lineages, but form several small mixed clusters, which cannot be phylogenetically distinguished from each other. It turned out that the formation of branched ascoma appendages does not have any taxonomic value on a generic level. Thus it was not tenable to maintain *Microsphaera* as a separate genus, the genus had to be reduced to synonymy with *Erysiphe* s. str. *Erysiphe* emend. and is formally divided into sections for morphological (not phylogenetical) groups of species, viz. *Erysiphe* sect. *Erysiphe, E.* sect. *Microsphaera* and *E.* sect. *Uncinula.* The new combinations, which are necessary, were introduced by Braun and Takamatsu (2000). The former species *Microsphaera palczewskii* Jacz. was renamed to *Erysiphe palczewskii* (Jacz.) U. Braun and S. Takamatsu.

The samples of powdery mildew collected on two different cultivars of *Caragana arborescens* in the Czech Republic slightly differed in some morphological characteristics; conidia and cleistothecia size were larger in cv. Pendula, whilst the size of asci and number of appendages per cleistothecium was bigger in cv. Lorbergii. However, the main morphological features of powdery mildew on both cultivars were identical (Pseudoidium type of conidia formation, shape of conidia, shape of appendages). Comparison of our measurements with descriptions of *Microsphaera palczewskii* previously published (Braun 1995) identificated this powdery mildew fungus as *Microsphaera palczewskii* (newly *Erysiphe palczewskii*). Some morphological characteristics, mainly size of conidia and cleistothecia probably varied due to differing cultivars; e.g. samples collected in Hungary greatly differed in their size (Vajna 2006), on

Table 2. Basic characteristics of the teleomorph stage of *Microsphaera* (*Erysiphe*) *palczewskii* collected in the Czech Republic and Austria

	Cleistothecia			Asci				Ascospores		
	diameter (µm)	appendages length (µm)	length of appendages/diameter of cleistothecia	number of appendages	length (µm)	width (µm)	L/W ratio	length (µm)	width (µm)	L/W ratio
	mean ± SD (min–max)			mean ± SD (min–max)						
E. palczewskii isolate CSU (Smržice, CR)	89.37[a*] ± 7.87 (75–107.5)	243 ± 33.9 (180–330)	2.75 ± 0.46 (1.84–4.13)	8.72[b] ± 2.12 (4–13)	63.77[b] ± 5.45 (52.5–77.5)	33.32 ± 3.78 (22.5–42.5)	1.94 ± 0.32 (1.4–3.22)	21.07 ± 2.61 (17.5–27.5)	12.52[b] ± 1.75 (8.75–16.25)	1.69 ± 0.2 (1.16–2.11)
E. palczewskii isolate CSS (Smržice, CR)	93.68[b] ± 10.81 (67.5–112.5)	236.53 ± 26.22 (190–300)	2.62 ± 0.43 (1.95–3.96)	7.44[a] ± 1.98 (3–13)	57.77[a] ± 6.11 (47.5–72.5)	31.45 ± 3.15 (25–37.5)	1.86 ± 0.31 (1.26–2.6)	20.59 ± 1.34 (17.5–23.75)	11.19[a] ± 1.19 (10–13.75)	1.85[b] ± 0.2 (1.45–2.25)
F value	10.25	1.77	2.39	11.83	14.49	3.4	0.84	1.21	18.08	14.15
E. palczewskii isolate A (Vienna, Austria)	92.92 ± 9.2 (72.5–112.5)	239.35 ± 25.0 (200–300)	2.68 ± 0.45 (1.95–3.86)	6.59 ± 2.54 (3–9)	55.87 ± 4.12 (47.5–62.5)	33.93 ± 3.42 (30–42.2)	1.66 ± 0.19 (1.11–2.08)	18.08 ± 1.27 (16.25–20)	11.07 ± 0.96 (10–12.5)	1.64 ± 0.14 (1.4–2)
Mean of both samples of *E. palczewskii* (Smržice, CR)	91.5 ± 9.69 (67.5–112.5)	241.47 ± 31.77 (180–330)	2.71 ± 0.46 (1.84–4.13)	8.3 ± 2.18 (4–13)	62.18 ± 6.23 (47.5–77.5)	32.83 ± 3.72 (22.5–42.5)	1.92 ± 0.32 (1.26–3.22)	20.84 ± 2.11 (17.5–27.5)	11.88 ± 1.65 (8.75–16.25)	1.77 ± 0.21 (1.16–2.25)
Microsphaera palczewskii (Braun 1995)	(80–140)		(2×)	(5–12)	(45–80)	(25–40)		(14–27)	(8–15)	

*Scheffe's Multiple-Comparison test ([a] and [b] = different letters express the statistical significance, $P = 0.05$)

the other the hand description of *M.* (*Erysiphe*) *palczewskii* from North America (Nischwitz & Newcombe 2003) is very similar to our results. Similarly, the sample of *M.* (*Erysiphe*) *palczewskii* collected from the Botanical Garden in Vienna has nearly the same size of main features of the teleomorph stage as samples collected in the Czech Republic (Table 2).

There is an increasing number of reports on the occurrence of *M.* (*Erysiphe*) *palczewskii* from the various countries of Europe and also from North America. This fact confirmed the recent trend in the spreading of this fungus on *C. arborescens* throughout Europe and North America. For example, Nischwitz and Newcombe (2003) and Glawe *et al.* (2006) reported that in the northern United States and south Canada there are areas in which *C. arborescens* is unaffected by powdery mildew, however in other parts of the country the fungus progressively attacked *C. arborescens*. Also Huhtinen *et al.* (2001) described that though the first specimen of *M.* (*Erysiphe*) *palczewskii* was collected in south Finland in 1981 it has now been distributed throughout the country to all areas where *C. arborescens* is cultivated. This epidemic spread caused a total decline of the occurrence of *M. trifolii* (Grev.) U. Braun on *Caragana*. The last record of *M. trifolii* dates back to the year 1987. In the Czech Republic it was not confirmed until 2006 (Lebeda *et al.* 2008) and in Austria until 2007 (Lebeda, this paper), thus this is the first detailed description of *Microsphaera* (*Erysiphe*) *palczewskii* on *Caragana arborescens* from Central Europe. However, there is no detailed information about the distribution and disease severity of *M. (Erysiphe) palczewskii* in the Czech Republic and more detailed study is needed with the aim to reveal the progressive spreading of this disease.

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