

## ***Bacidina mendax* sp. nov., a new widespread species in Central Europe, together with a new combination within the genus *Bacidina***

**Paweł CZARNOTA and Beata GUZOW-KRZEMIŃSKA**

**Abstract:** *Bacidina mendax*, described here as a new lichen species, appears to be common and widespread, at least in Central Europe. Analyses of the ITS rDNA region and the morphology of specimens showed an intraspecific variation in the new taxon. It differs from *B. neosquamulosa* in the lack of a subsquamulose thallus, and from *B. caligans* in its longer and only slightly curved to apically hooked conidia and lack of a granular (sorediate) thallus. Since ITS rDNA data support the inclusion of *Bacidia pycnidata* Czarnota & Coppins in the genus *Bacidina*, a new combination is proposed.

**Key words:** Ascomycota, ITS rDNA, lichen taxonomy, lichenized fungi, molecular phylogeny

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### **Introduction**

More than 50 species have been found to belong to the genus *Bacidina* since it was first described by Vězda (1990). They were transferred to this genus (e.g. Vězda 1990; Wirth 1994; Ekman 1996a, 2004; Hauck & Wirth 2010; Farkas 2015) or were newly described (e.g. Farkas & Vězda 1993; Ekman 1996a; Cáceres 2007; Spribille *et al.* 2009; van den Boom & Sipman 2014) mainly based on morphological and anatomical characters, more particularly biatorine apothecia; excipular radiating hyphae with at least apically large, broadly ellipsoid to almost globose lumina ( $\pm$ paraplectenchymatous excipulum at least in an external portion); narrowly clavate asci with a distinct axial body that is basically conical but varies in height and width; and filiform, long, septate,

usually curved conidia (for more details see Ekman 1996a).

To date only a small proportion of validly described *Bacidina* species (10 species) have been included in phylogenetic analyses (Ekman 2001; Czarnota & Guzow-Krzemińska 2012) and consequently molecular affinities within the genus remain unresolved. Further approaches are needed to address this due to morphological variation which obscures the boundaries of many species. Such variability is found especially in representatives of *Bacidina* which are often labelled as *Bacidina neosquamulosa* (Aptroot & Herk) S. Ekman (or *Bacidia neosquamulosa* Aptroot & Herk), *Bacidina adastra* (Sparrius & Aptroot) M. Hauck & V. Wirth (or *Bacidia adastra* Sparrius & Aptroot) and *Bacidina caligans* (Nyl.) Llop & Hladún (or *Bacidia caligans* (Nyl.) A. L. Sm.). It was clear that the application of molecular data is necessary to understand relationships within this group of species and support potential nomenclatural innovations.

The main objectives of this study were to clarify species boundaries and relationships among specimens previously identified as *Bacidina neosquamulosa* or *B. caligans*, and to improve our understanding of the phylogenetic relationships within the genus *Bacidina*.

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## Materials and Methods

### Morphology and chemistry

All specimens examined were studied with a Zeiss Stemi DV4 stereomicroscope and a Zeiss Axiostar plus light microscope. Hand-cut apothecial sections mounted in water were prepared for measuring anatomical characters and photographs; at least 20 measurements were made for each diagnostic feature. We used 10% KOH to separate apothecial structures and to examine internal pigment reactions, and Lugol's iodine for the examination of ascus structure. The presence of secondary metabolites was studied using a standard TLC method with solvent C (Orange *et al.* 2001).

### Taxon sampling for DNA analyses

Specimens of *Bacidina* used for DNA analysis are listed in Table 1 and their GenBank Accession numbers are provided; these and other sequences downloaded from GenBank were used in the phylogenetic analyses. In total, 24 new sequences of ITS rDNA were obtained, including that for the newly described *B. mendax*. *Usnea fragiliscens* and *U. subantarctica* were used as an outgroup in the phylogenetic analyses.

### DNA extraction, PCR amplification and sequencing of the ITS rDNA region

DNA was extracted directly from pieces of thallus using a modified CTAB method (Guzow-Krzemińska & Węgrzyn 2000). DNA extracts were used for PCR amplification of the ITS rDNA marker using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). The 25 µl of PCR mix contained 1U of Taq polymerase (Thermo Scientific), 0.2 mM of each of the four dNTPs, 0.5 µM of each primer and 10–50 ng of genomic DNA. PCR amplifications were performed using a Mastercycler (Eppendorf) with the following programme: initial denaturation at 95 °C for 5 min followed by 35 cycles at 95 °C for 40 s, 54 °C for 45 s and 72 °C for 1 min, and a final elongation step at 72 °C for 10 min. PCR products were visualized on agarose gels in order to determine DNA fragment lengths. Subsequently, 5 µl of PCR product was treated with 10 units of Exonuclease I and 1 unit of FastAP<sup>TM</sup> Thermosensitive Alkaline Phosphatase enzymes (Thermo Scientific) to degrade primers and dephosphorylate dNTPs. Treatment was carried out for 15 min at 37 °C, followed by a 15 min incubation at 85 °C to completely inactivate both enzymes. Sequencing of each PCR product was performed using MacroGen sequencing service ([www.macrogen.com](http://www.macrogen.com)).

### Sequence alignment and phylogenetic analysis

The newly generated ITS rDNA sequences were compared with those available in the GenBank database (<http://www.ncbi.nlm.nih.gov/BLAST/>) using a BLASTN search (Altschul *et al.* 1990) in order to confirm their identity. The ITS rDNA sequences were aligned with sequences of selected representatives of the genera

*Bacidina* and *Bacidia*, as well as *Biatora*, *Bilimbia*, *Toninia* and *Usnea* (GenBank Accession numbers provided in Fig. 1). Alignment was performed using MAFFT with the parameters set to default values as available from EBI (<http://www.ebi.ac.uk/Tools/msa/mafft/>) (Katoh *et al.* 2002) and followed with a selection of ambiguous positions that might not have been homologous using Gblocks 0.91b, implementing the options for less stringent parameters (i.e. to allow less strict flanking positions and smaller final blocks) (Castresana 2000; Dereeper *et al.* 2008).

The phylogenetic analyses were performed using PAUP\* 4.0b10 (Swofford 2001) with maximum parsimony (MP) as the optimality criterion. Heuristic searches were performed with 1000 random sequence additions and TBR branch swapping. Gaps were treated as missing and support for the branches was evaluated with a bootstrap method with 100 pseudoreplicates (Felsenstein 1985).

Maximum likelihood (ML) analyses were performed using RaxML HPC v.8 on XSEDE (Stamatakis 2014) under the GTRGAMMAI model on the CIPRES Science Gateway (Miller *et al.* 2010). Rapid bootstrap analyses were performed with 1000 bootstrap replicates.

The data were also analyzed using a Bayesian approach (MCMC) in MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003). The GTR+G+I model was selected based on analysis using MrModeltest 2.0 (Nylander 2004). A run with 5 000 000 generations employing four chains was selected and every 100th tree was saved. The initial 25% of trees were discarded as burn-in and a majority-rule consensus tree was calculated to obtain posterior probabilities (BA).

The phylogenetic tree was drawn using FigTree version 1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bootstrap supports (in MP and ML) above 75% and posterior probabilities above 0.90 (in BA) were indicated near the branches as these values were considered to be significant.

## Results

Twenty-four new ITS rDNA sequences were obtained from eight representatives from the genus *Bacidina* (including the newly described *B. mendax* Czarnota & Guz.-Krzem. sp. nov.) and from *Bacidia pycnidata* Czarnota & Coppins. Among them, *Bacidina brandii* (Coppins & van den Boom) M. Hauck & V. Wirth, *B. egenula* (Nyl.) Vězda and *Bacidia pycnidata* were sequenced for the first time (see Table 1 for the list of all newly generated sequences).

In the dataset, 51 ITS rDNA sequences were analyzed; of 417 characters, 233 were constant and 128 were parsimony-informative. 113 equally parsimonious trees of 491 steps were generated (MP) and the majority-rule consensus tree was created (data not shown). Trees of similar topologies

TABLE 1. Voucher information and GenBank Accession numbers for the new ITS rDNA sequences used in the phylogenetic analysis, including the holotype of *Bacidina mendax* sp. nov. named as *B. neosquamulosa* in GenBank.

Taxon	Collection information	Collection reference no.	GenBank Accession number
<i>Bacidina arnoldiana</i>	Portugal, Madeira, N of Funchal, Ribeiro Frio, trail to the west, to Mirador de Balcões, on shaded steep rock in a small gorge, alt. 900 m, 27 April 2012, <i>P. v. d. Boom</i> (hb. v. d. Boom); morphotype with white apothecia	<i>v. d. Boom</i> 47546a	KX239021
<i>B. arnoldiana</i>	as above; morphotype with grey apothecia	<i>v. d. Boom</i> 47546b	KX239020
<i>B. brandii</i>	Belgium, Limburg, NNW of Neerpelt, NW of Grote Heide, Hageven, nature reserve, 51°16'16"N, 5°25'70"E, on stump of <i>Quercus</i> sp., 14 May 2010, <i>P. v. d. Boom</i> (hb. v. d. Boom)	<i>v. d. Boom</i> 44815	KX239025
<i>B. brandii</i>	Poland, Carpathians, Western Beskidy Mts, Gorce Mts, 0.5 km N of Rozdziele glade by a tourist trail, 49°33'13"N, 20°05'38"E, alt. 1150 m, on horizontal surface of spruce stump in upper mountain spruce forest, 28 Oct. 2011, <i>P. Czarnota</i> (GPN)	<i>Czarnota</i> 7599	KX239029
<i>B. caligans</i>	The Netherlands, Noord-Brabant, Son, Gentiaan, Arafura 16, stones of terrace in garden, 15 March 2008, <i>P. v. d. Boom</i> (hb. v. d. Boom)	<i>v. d. Boom</i> 39590	KX239017
<i>B. caligans</i>	Germany, Mecklenburg-Vorpommern, Isle of Rügen, Jasmund, NW of Sagard, along road 0.5 km E of Polchow, 54°32'83"N, 13°31'60"E, roadside trees and shrubs, on bark of <i>Sambucus</i> sp., 2 Sept. 2010, <i>P. v. d. Boom</i> (hb. v. d. Boom)	<i>v. d. Boom</i> 44973	KX239024
<i>B. egenula</i>	Poland, Carpathians, Western Beskidy Mts, Gorce Mts, Poręba Wielka Village, the Wodzicki's manor park, 49°36'54.054"N, 20°03'59.910"E, alt. 520 m, on sandstone wall, 28 Aug. 2016, <i>P. Czarnota</i> (GPN)	<i>Czarnota</i> 8304	KY379235
<i>B. egenula</i>	as above but 49°36'54.462"N, 20°03'59.802"E	<i>Czarnota</i> 8314	KY379233
<i>B. egenula</i>	as above, another specimen	<i>Czarnota</i> 8337	KY379234
<i>B. egenula</i>	Czech Republic, Distr. Beroun, Český kras, old limestone quarry, c. 1 km W of Karlštejn Town, 49°55'53"N, 14°09'04"E, alt. 230 m, on vertical shaded limestone wall, 20 April 2008, <i>P. Czarnota</i> 5302 (GPN)	<i>Czarnota</i> 5302	KX239019
<i>B. inundata</i>	Poland, Carpathians, Western Beskidy Mts, Gorce Mts, Gorce National Park, forest section no. 148, alt. 900 m, on saxicolous bryophytes over shaded sandstone rocks by stream, 13 Oct. 2007, <i>P. Czarnota</i> (GPN)	<i>Czarnota</i> 5266	KX239022
<i>B. mendax</i>	Poland, Kotlina Sandomierska basin, Plaskowyż Kolbuszowski plateau, Budki settlement N of Głogów Małopolski Town, 50°10'20"N, 21°55'18"E, on bark of roadside <i>Populus nigra</i> , 28 April 2012, <i>P. Czarnota</i> (GPN)	<i>Czarnota</i> 7406	KX239023
<i>B. mendax</i>	Czech Republic, S Bohemia, Distr. Pisek, c. 1.2 km W of Oslov, 49°24'00.6"N, 14°11'35.4"E, alt. 415 m, on dry branches of <i>Quercus</i> sp., 15 May 2010, <i>Z. Palice</i> (PRA)	<i>Palice</i> 13638	KX239028
<i>B. mendax</i>	Czech Republic, W Bohemia, Distr. Karlovy Vary, Doupovske hory Mts, Ostrov-Vojkovice, 0.5 m WNW of railway station, alt. 380 m, on bark of <i>Fraxinus excelsior</i> , 16 April 2009, <i>ř. Malíček &amp; Z. Palice</i> (hb. Malíček)	<i>Malíček</i> 1769	KX239016
<i>B. mendax</i>	Poland, Pojezierze Olsztyńskie lakeland, Wichrowo forest division, forest section no. 363f, on bark of young <i>Populus tremula</i> in spruce-pine-deciduous forest, 6 Sept. 2011, <i>P. Czarnota</i> (GPN)	<i>Czarnota</i> 7403	KX239035
<i>B. mendax</i>	Poland, Wysoczyzna Ciecchanowska plateau, Gašocin Village, 52°44'38.5"N, 20°42'42.1"E, on bark of <i>Sambucus nigra</i> at the edge of alder forest, 26 Dec. 2009, <i>D. Kubiak</i> (OLTC)	OLTC-L-3396	KX239031
<i>B. mendax</i>	HOLOTYPE (GPN)	<i>Czarnota</i> 4888	JN972444
<i>B. neosquamulosa</i>	Poland, Wybrzeże Słowińskie coastland, Dąbkowice settlement, 54°20'26.8"N, 16°14'59.7"E, on bark of roadside <i>Fraxinus excelsior</i> , 6 Aug. 2009, <i>D. Kubiak</i> (OLTC)	OLTC-L-3221	KX239030
<i>B. neosquamulosa</i>	The Netherlands, Noord-Brabant, Son, Gentiaan, Arafura 16, on flowerpot in garden, 25 July 2008, <i>P. v. d. Boom</i> 41056 (hb. v. d. Boom)	<i>v. d. Boom</i> 41056	KX239026

TABLE 1 (continued).

Taxon	Collection information	Collection reference no.	GenBank Accession number
<i>Bacidia neosquamulosa</i>	The Netherlands, Noord-Brabant, SW of Valkenswaard, Westerhoven, old churchyard, on gravestones, on bark of <i>Syringa</i> , 18 Sept. 2010, P. v. d. Boom (hb. v. d. Boom)	v. d. Boom 44999	KX2239018
<i>B. neosquamulosa</i>	The Netherlands, Noord-Brabant, 20 km SSE of Eindhoven, Budel Village, churchyard, gravestones, on stump, 14 May 2010, P. v. d. Boom (hb. v. d. Boom)	v. d. Boom 44837	KX2239027
<i>B. phacodes</i>	Slovakia, Muranska planina, Martinovska dolina, 48°42'40.8"N, 19°59'40.4"E, alt. 505 m, on bark of old <i>Fagus sylvatica</i> , 30 Sept. 2009, P. Czarnota (GPN)	Czarnota 8264	KX2239036
<i>Bacidia pycnidiatata</i>	Poland, Góry Świętokrzyskie Mts, Pasma Zagórskie range, edge of forest from the side of Zagórze Village, on bark of <i>Quercus</i> sp., 9 Aug. 2007, A. Lubeč (KTC)	Lubeč s. n.	KX2239032
<i>B. pycnidiatata</i>	Czech Republic, Rychlebské hory near the border of Poland, W of Bila Voda Village, vicinity of worked-out quarry of marble 'Kukačka', 50°26'30"N, 16°53'24"E, alt. 360 m, on bryophytes over marble rock within mixed forest, 23 April 2004, P. Czarnota (GPN)	Czarnota 4157 HOLOTYPE	KX2239033
<i>B. pycnidiatata</i>	Czech Republic, S Moravia, Chřibý Mts, Distr. Uherské Hradiště, Medlovicé–Medlovický lom Nature monument, 49°03'01.8"N, 17°15'50.4"E, alt. 320 m, on bark of <i>Sambucus nigra</i> , 9 April 2010, J. Malíčėk, J. P. Haldá & A. Müller (hb. Malíčėk)	Malíčėk 2553 p.p.	KX2239034

were also generated using the maximum likelihood (ML; best tree likelihood LnL = -2895.180637) method and Bayesian approach (BA; harmonic mean was -3160.33). In the MrBayes analysis, the average standard deviation of split frequencies was 0.004069 and the average PSRF for parameter values was 1.00. The Bayesian tree is presented in Fig. 1 with added bootstrap supports from the MP and ML methods and posterior probabilities from the BA.

Species belonging to *Bacidina*, including *B. mendax* sp. nov., form a monophyletic clade supported by bootstrap value (76 in ML) and posterior probability in BA (1.00) (Fig. 1). However, the position of *B. phacodes* (Körb.) Vězda, the type species of *Bacidina*, within the genus is not supported in all trees, and thus remains uncertain.

The morphologically variable specimens related to *B. mendax* (Figs 2 & 3) are grouped together, but without support (Fig. 1); they represent four different ITS haplotypes.

The four newly sequenced specimens of *B. egenula* form a highly supported lineage (100 in MP, 100 in ML and 1.00 in BA), but the sequence no. AF282095 from GenBank named *B. egenula* has no relationship to the clade mentioned above (Fig. 1). However, since it is nested within *Bacidina*, a provisional name *Bacidina* sp. has been adopted.

Six specimens of *B. arnoldiana* (Körb.) V. Wirth & Vězda form a moderately supported clade (80 in MP, 77 in ML and 1.00 in BA) (Fig. 1), as previously shown by Czarnota & Guzew-Krzemińska (2012); ITS rDNA data show that it is a highly variable species.

Three sequences from the specimens of *B. caligans* form a single highly supported clade (100 in both MP and ML methods and 1.00 in BA). Their haplotypes differ from each other in one to four positions. This species was found to be related to *B. inudata* (Fr.) Vězda (0.91 in BA) (Fig. 1).

Newly sequenced *Bacidia pycnidiatata*, represented here by three specimens collected in the Czech Republic and Poland, is placed within the genus *Bacidina* Vězda; we therefore propose to transfer *Bacidia pycnidiatata* to *Bacidina*. This species was found to be related to *B. inudata* and

*B. caligans*, and their relationship is supported in all optimization methods (88 in MP, 92 in ML and 1.00 in BA) (Fig. 1).

ITS rDNA sequences obtained from *B. neosquamulosa* represent two haplotypes that differ in a single position and form a monophyletic, highly supported clade (90 in MP, 96 in ML and 1.00 in BA) (Fig. 1); specimens are very variable in the morphology of apothecia and their thallus structure (Fig. 4).

Newly sequenced specimens of *B. brandii*, represented by two haplotypes that differ in four positions, were collected from widely spread geographical regions of Europe (see Table 1) and form a strongly supported monophyletic clade (100 in both MP and ML methods and 1.00 in BA). *Bacidina brandii* is most closely related to *B. sulphurella* (Samp.) M. Hauck & V. Wirth (0.97 in BA); both species are characterized by the dark, Arnoldiana-brown pigmented hypothecium.

Two sequences of *B. delicata* (Larbal. ex Leight.) V. Wirth & Vězda were downloaded from GenBank for this study. One of these, collected in Sweden (AF282097), seems to be closely related to *B. flavoleprosa* Czarnota & Guz.-Krzem. but the other, collected in France (JQ796854), seems to be related to *B. egenula*; both relationships are supported only in BA, 0.93 and 0.99 respectively (Fig. 1).

## Discussion

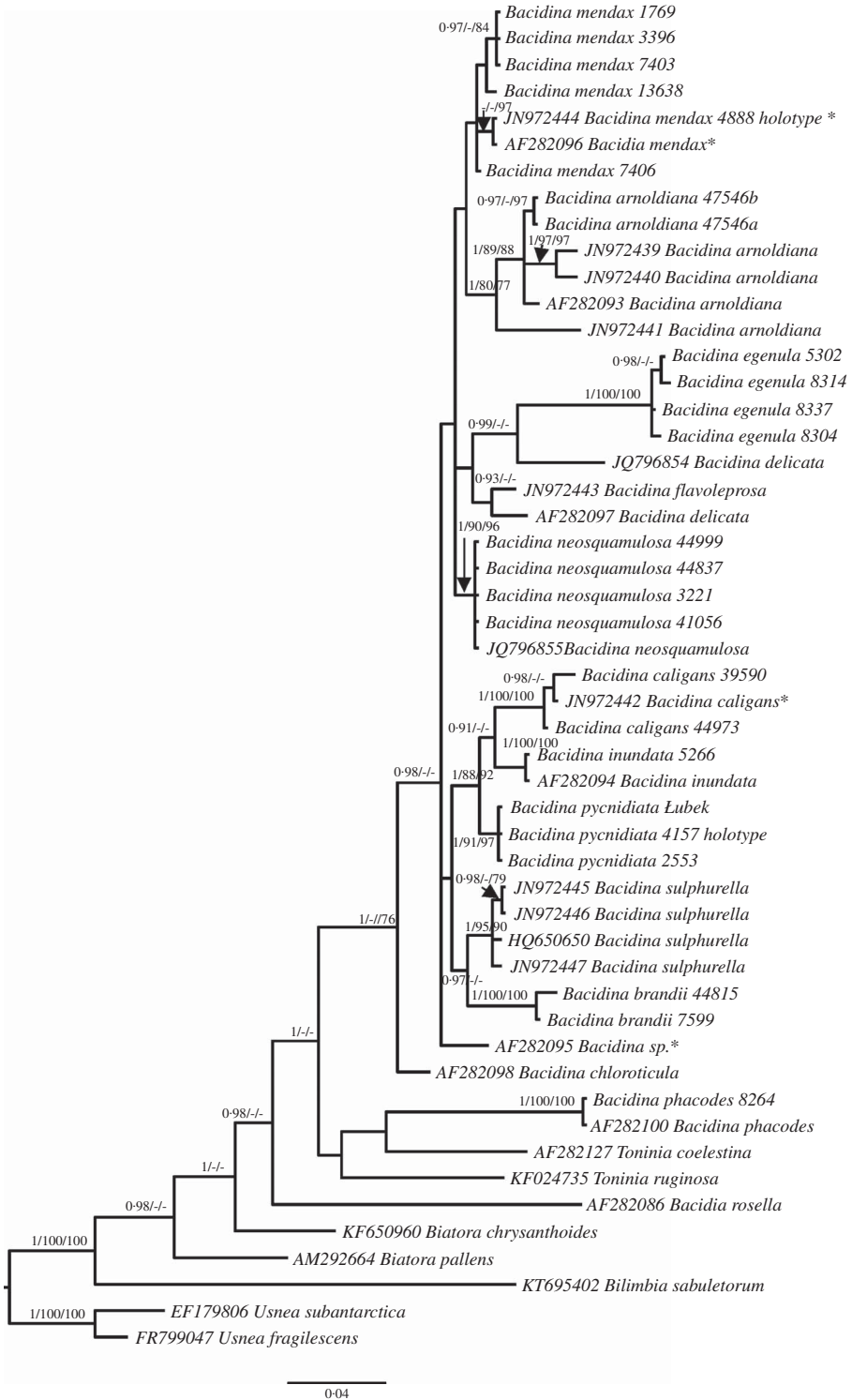
*Bacidina mendax* sp. nov. is a very variable taxon in its apothecial pigmentation as well as its thallus development; therefore earlier errors in its identification with other representatives of the genus *Bacidina* are understandable. Apothecia of this species are variously coloured (see Figs 2 & 3) but its conidia are uniformly filiform and often slightly hooked, as in *B. sulphurella* (Figs 2I & 3F); they are also variable in their length, even within one pycnidium. Molecular variability in *B. mendax* was also observed: within seven sequenced specimens referred to this species, four ITS haplotypes have been discovered, only three of which formed a poorly supported clade (84 in ML and 0.97 in BA) (Fig. 1). In several specimens of *B. mendax*, represented by, for

example, the designated holotype (Czarnota 4888), the thallus is well developed, thick, and composed of dense, scurfy warts (Fig. 2 A-C & G) with, usually, completely- or semi-immersed pycnidia (Fig. 2H). Other specimens (represented by, for example, Maliček 1769) have a negligible or poorly developed thallus composed of separated, small warts which never form a thick crust (Fig. 3B & C). Since the molecular data we generated are insufficient to support the description of some infraspecific taxa within *B. mendax* or to show some other phylogenetic relationships, we propose to keep the species in a broad sense. Despite its variability, thallus structure can be used as a key feature in the separation of *B. mendax*, in a broad sense, from a similar species, *B. neosquamulosa* (see Figs 2–4), which forms  $\pm$  coralloid to palmate thallus warts never found in *B. mendax*.

The new, more complete phylogenetic analysis of the genus *Bacidina* and additional taxonomic studies presented in this work showed that some representatives of the genus had been determined erroneously. Specimen Czarnota 4888 (GenBank no. JN972444), which was believed to represent *B. neosquamulosa* (Czarnota & Guzow-Krzemińska 2012), belongs to a separate lineage regarded as representing the new *B. mendax*. This specimen has been selected as the holotype of *B. mendax*. Moreover, as the haplotype of the holotype was found to be identical to the sequence obtained from *B. caligans* (AF282096) downloaded from GenBank, we suggest that this specimen also represents *B. mendax* and should be labelled as such (Fig. 1).

*Bacidina adastr*a (GenBank no. JN972442), mentioned in the analysis by Czarnota & Guzow-Krzemińska (2012), is nested here within the lineage of *B. caligans* and renamed as this species. This specimen has small, yellowish punctiform soralia and poorly developed apothecia and was consequently regarded as an immature specimen of *B. adastr*a.

Two sequences of *B. delicata* downloaded from GenBank are probably not the same species as they are not nested in the same monophyletic clade (Fig. 1). It would appear that at least one of them does not represent



this species but the solution to this problem requires further investigation.

The placement of the specimen obtained from GenBank as *B. egenula* (Ekman 3003, GenBank no. AF282095) is questionable. We sequenced four other specimens of *B. egenula* and they are not related to the sequence of the GenBank specimen. Our revision of this collection (Norway, Hordaland, Sund, Sotra, Golta, UTM WGS84: 32V KM 787 822, on brick, on the ground, in a pasture, alt. 20 m, 9.3.1997, Ekman 3003, hb. BG, L-65567) showed that it represents another species. It is characterized by short, ±straight, bacilliform to nearly fusiform ascospores, 14–18 × 2.5–3.5 µm (not acicular and >25 µm in length, as in *B. egenula*), needle-like, straight or only slightly curved (not sigmoid to strongly curved), 3–5-septate conidia 25–30(–35) × 1.5–2.0 µm, small patches of crustaceous, ±irregularly areolate (not granular; without goniocysts) thallus and colourless to slightly yellowish hypothecium (not Arnoldiana-brown pigmented). Such characters may resemble those found in *Lecania subfuscula* (Nyl.) S. Ekman but comparative studies to confirm this hypothesis have not been carried out.

Analyses of the newly sequenced *B. egenula* specimens may shed new light on the taxonomy of this species. All sequenced specimens from Poland (Czarnota 8304, 8314 & 8337) have a more or less Arnoldiana-brown pigmented hypothecium characterizing *B. egenula*, while the Czech collection (Czarnota 5302; see Table 1) has a completely colourless hypothecium and is therefore tentatively named *Bacidia viridescens* (A. Massal.) Norman since all its characters correspond well with the widely accepted description of this species (e.g. Coppins & Aptroot 2009; Wirth *et al.* 2013). The hypothecium pigmentation usually differs between these two species (Coppins & Aptroot 2009). Considering this result, the real

relationship between *Bacidina egenula* and *Bacidia viridescens* remains unclear and should be resolved in further phylogenetic studies based on additional samples of specimens thought to be *B. viridescens*.

One specimen of *Bacidina brandii* collected in Poland was used for the phylogenetic analyses. Rejecting the erroneously published previous record (Czarnota 2016), the specimen presented here confirms the occurrence of this species in Poland and the Carpathians.

Our analyses showed that species belonging to the genus *Bacidina* require further study. Some relationships within the genus are still unresolved and some species, such as *B. delicata*, should be analyzed in greater detail.

## Taxonomy

### *Bacidina mendax* Czarnota & Guzew.-Krzem. sp. nov.

MycoBank No.: MB 821694

Differing from *B. neosquamulosa* in the lack of a subsquamulose thallus and from *B. caligans* in its longer and only slightly curved to apically hooked conidia and lack of granular (sorediate) thallus.

Type: Slovakia, Považský Inovec, Tematiske Kopce, between Lúka Village and Tematísky hrad Castle, 48°39'52"N, 17°53'56"E, alt. c. 230 m, on roadside *Acer platanoides*, 22 April 2006, P. Czarnota 4888 (GPN—holotype; UGDA—isotype).

(Figs 2 & 3)

*Thallus* inconspicuous to distinct, crustaceous, straw-coloured to bright green, composed of minute, merged granules to form a thin, scurfy, uneven crust or small warts. *Photobiont* chlorococcoid, with large, globose cells up to 17 µm diam.

*Apothecia* 0.2–0.7 mm diam., constricted at the base, variable in colour, from whitish and flesh-coloured throughout, beige, pinkish-buff,

FIG. 1. Bayesian tree based on ITS rDNA data for *Bacidina* spp. *Usnea fragilesceus* and *U. subantarctica* are the outgroup taxa. Support values are displayed near the branches in the following format: posterior probabilities >0.90 / bootstrap percentage for MP (maximum parsimony) >75 / ML (maximum likelihood) >75. Species names are followed by their GenBank Accession numbers, apart from newly sequenced specimens of *Bacidina* spp. where the names are followed by herbarium collection numbers. \* = taxa that were labelled with other names in GenBank (i.e. JN972444 as *B. neosquamulosa*, AF282096 as *B. caligans*, AF282095 as *B. egenula* and JN972442 as *B. adastrata*).

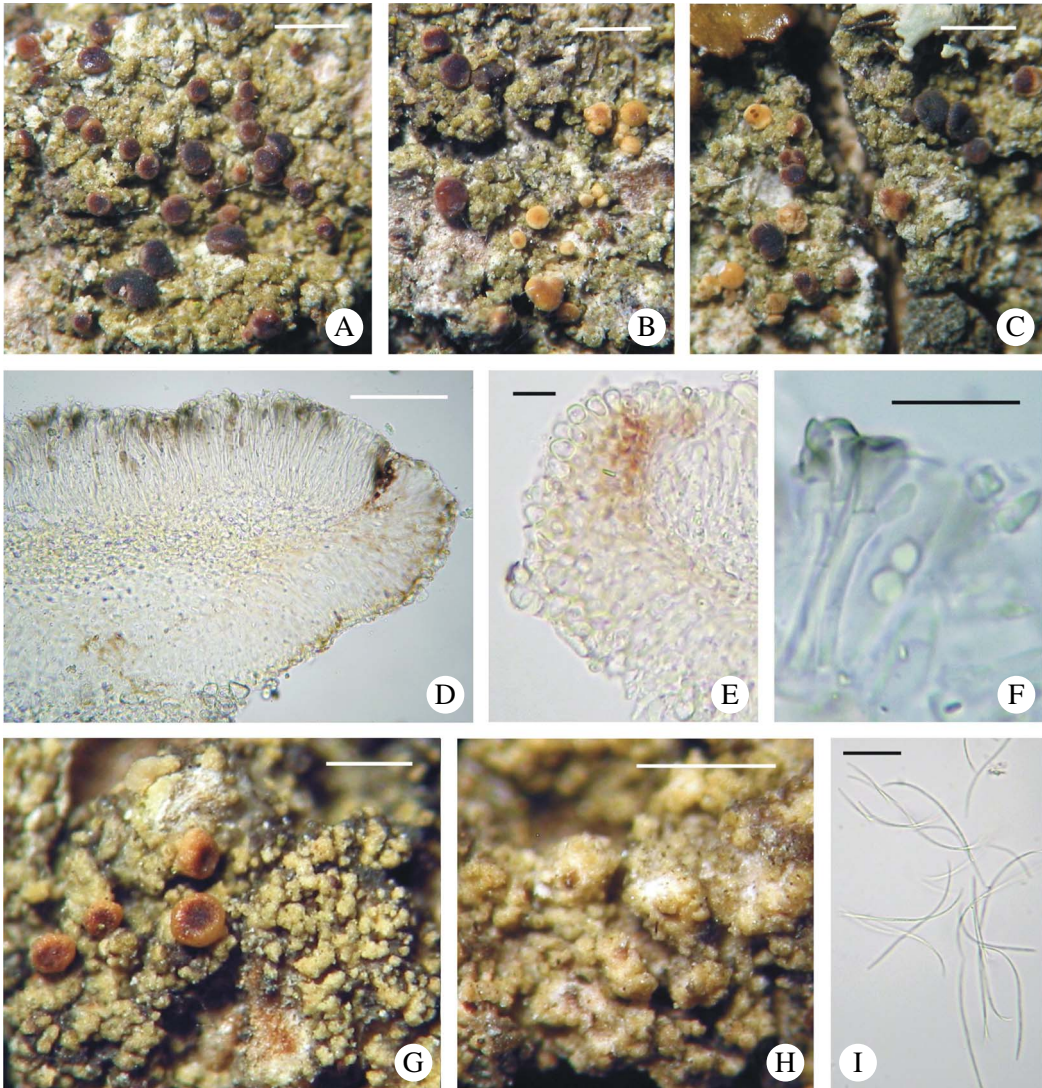


FIG. 2. *Bacidina mendax*. A–C & G, habitus of variously coloured morphotypes; D, section through apothecium; E, section through excipulum with external rows of globose, apical cells; F, paraphyses of the second type forming pigmented, vertical streaks (see species description); H, pycnidia; I, pycnospores. A–D & F, *Czarnota* 4888 (GPN—holotype, GenBank no. JN972444); E & G–I, *Czarnota* 7406 (GPN—paratype, GenBank no. KX239023).

Scales: A–C, G & H = 1 mm; D = 50  $\mu$ m; E & F = 10  $\mu$ m; I = 20  $\mu$ m. In colour online.

brownish to grey-brown and dark fuscous-brown, marginate at the beginning, later sometimes with margin excluded; *disc* concolorous, paler or darker than the margin; in the case of less coloured apothecia, margin usually at least partially extraneously dark-pigmented. *Excipulum* (30–)40–60(–70)  $\mu$ m

wide, composed of radiating hyphae 1.5–2.5  $\mu$ m in width, but 1–2 rows of cells of outermost part with lumina widening to 5–7  $\mu$ m; upper and outer part of excipulum pinkish orange, brown, fuscous brown and then K– or K $\pm$  pinkish to purplish or olive-brown, K $\pm$  intensifying; inner part of excipulum



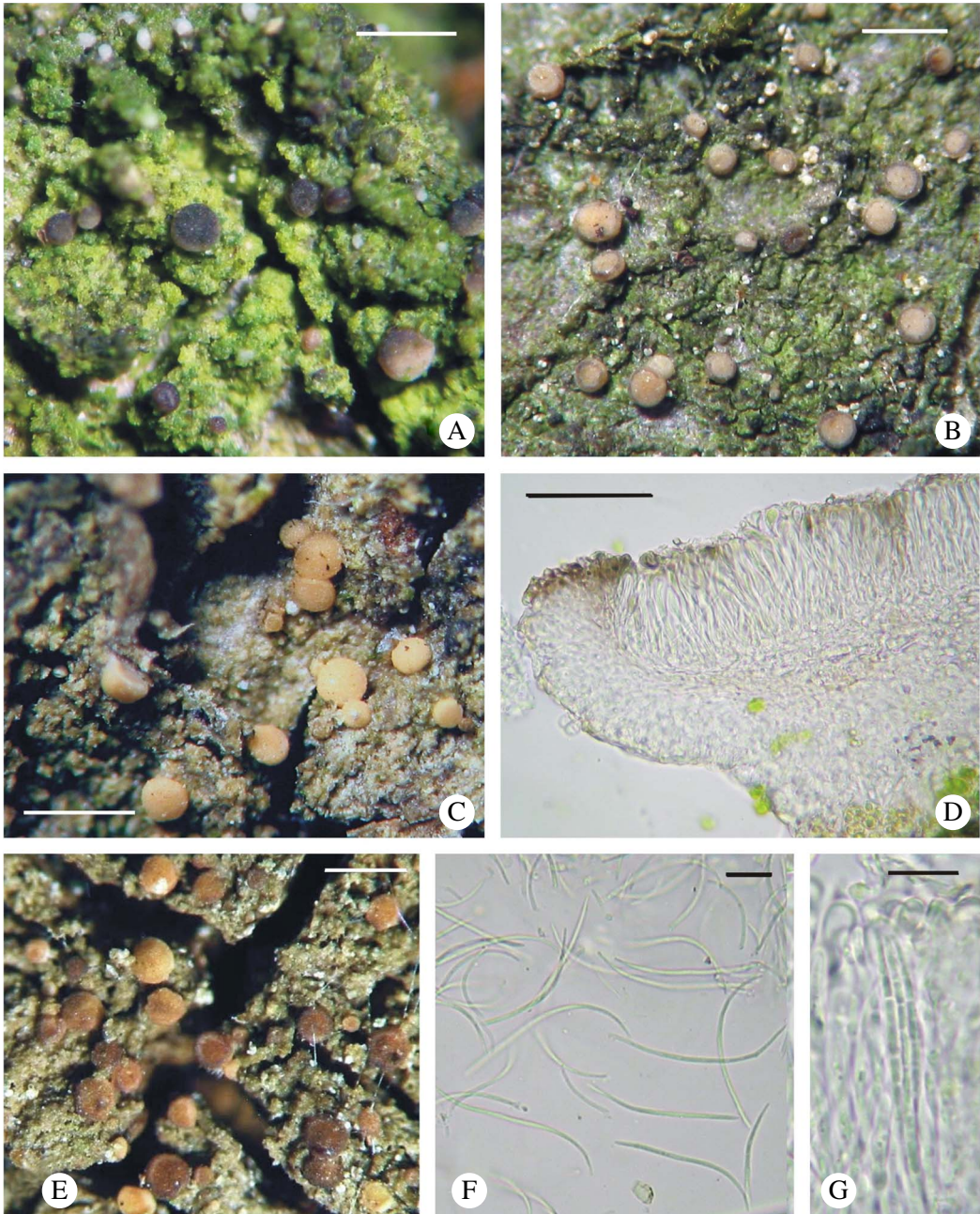


FIG. 3. *Bacidina mendax*. A–C & E, habitus of different morphotypes; D, section through apothecium; F, pycnospores; G, ascus with ascospores. A & D, *Kubiak* (OLTC-L-3396—paratype, GenBank no. KX239031); B, *Łubek* s. n. (KTC, loc. P1—paratype), C, *Maliček* 1769 (hb. Maliček—paratype, GenBank no. KX239016); E, *Palice* 13638 (PRA—paratype, GenBank no. KX239028); F, *Czarnota* 7403 (GPN—paratype, GenBank no. KX239035); G, *Łubek* s. n. (KTC, loc. M11—paratype). Scales: A–C & E = 1 mm; D = 50  $\mu$ m; F & G = 10  $\mu$ m. In colour online.

colourless to,  $\pm$ straw-coloured towards the hypothecium and below hypothecium sometimes paraplectenchymatous, composed of  $\pm$ globose to elongate cells of 3–5(–7)  $\mu\text{m}$  wide lumina. *Hypothecium* to 60  $\mu\text{m}$  tall, colourless to  $\pm$ straw-coloured. *Hymenium* (40–)50–60 (–65)  $\mu\text{m}$  tall, colourless or sometimes with  $\pm$ pinkish to olive-brown, K– or K+ intensifying epihymenium as well as vertical streaks; pigment confined to walls of paraphyses and asci. *Paraphyses* of two types: first, simple, 1.5–2.0  $\mu\text{m}$  wide, straight to slightly curved with globose apical cells, slightly widening to 2.5(–3.0)  $\mu\text{m}$ ; second, irregular, stout, 3–4  $\mu\text{m}$  wide, sometimes forming vertical streaks, forked with pigmented globose to elongated apical cells, up to 6  $\mu\text{m}$  in width. *Asci* cylindrical to narrowly clavate, 7–9  $\times$  (25–)35–40 (–45)  $\mu\text{m}$ , *Lecanora*-type. *Ascospores* acicular, (25–)30–38(–40)  $\times$  1.2–1.5  $\mu\text{m}$ , 3–5(–6)-septate, straight.

*Pycnidia* pale-coloured throughout, globose,  $\pm$ immersed within the thallus warts, 0.15–0.30 mm diam. *Conidia* filiform,  $\pm$ straight to slightly curved (usually in upper half) or  $\pm$ hooked, (25–)35–55(–62)  $\times$  1.0–1.2  $\mu\text{m}$ , 3–6-septate.

*Chemistry.* No substances detected by TLC.

*Etymology.* ‘Mendax’, from its similarity with other related species.

*Remarks.* A provisional revision of material stored in several European herbaria showed that *Bacidina mendax* had already been collected in the past, but its darker morphs with a poorly developed thallus were usually identified as *B. caligans*, while collections forming a thicker, warted thallus were identified as *B. neosquamulosa*. Specimens with predominantly pale-coloured apothecia were also misidentified as *B. phacodes* or *B. delicata*. Indeed, morphological variation in *B. mendax* can be a source of taxonomic error, particularly since in the same collection pale brown to fuscous brown apothecia can grow together or, depending on the circumstances, can be almost entirely pale or dark brown. This extensive apothecial variability also determines the internal

pigmentation which is either very pale brown throughout or distinctly dark brown in the outermost part of the excipulum and hymenium and being confined to walls of excipular hyphae, apical parts of paraphyses or asci. The type of pigmentation does not seem to be a stable feature since it can often be represented by the fuscous brown, K $\pm$  purplish pigment or by grey-brown, brown to orange-brown, K+ intensifying or K– pigments, or a mixture of these pigments. For a detailed comparison of the main diagnostic features of *B. mendax* and several European representatives of the genus resembling different morphs of this species, see Table 2 and Figs 2–5.

*Distribution and habitat.* The new *Bacidina* species, reported here from several European regions, is probably widespread in the central-eastern part of the continent. It is a tolerant epiphyte found on deciduous trees and shrubs (*Acer*, *Betula*, *Carpinus*, *Fraxinus*, *Malus*, *Populus*, *Pyrus*, *Quercus*, *Salix*, *Sambucus* and *Tilia*), usually in mostly anthropogenic habitats including roadside trees in large cities, urban parks and on riversides, and solitary trees in rural landscapes, orchards and edges of woodland, as well as in natural, old-growth forests (such as strictly protected areas of the Białowieża National Park). *Bacidina mendax* occurs on trunks, twigs and branches of trees, usually in association with many lichens tolerant of both air pollution and nitrification (e.g. *Amandinea punctata*, *Lecania naegelii*, *Physcia adscendens*, *P. tenella*, *Scoliciosporum* spp. and *Xanthoria parietina*). On the other hand, in natural woodlands *B. mendax* was found to grow in association with some old-growth forest indicators such as *Biatora hemipolia*, *Bacidia laurocerasi*, *B. subincompta* and *Fellhanera gyrophorica*.

*Additional specimens examined.* **Czech Republic:** *Rychlebské hory Mts:* c. 0.5 km W of Bíla Voda Village, limestone quarry Kukačka, 50°26'29"N, 16°53'01"E, alt. c. 350 m, on *Salix* sp., 2004, *M. Kukwa* 3140 (UGDA-L-10926). *Středočeská pahorkatina* foothills, ‘Husova kazatelna’ hill, c. 1 km NE of Petrovice Village, 49°34'03"N, 14°21'49"E, alt. 500 m, on *Betula pendula*, 2008, *P. Czarnota* 5327 (GPN); Praha – Řepy, Hekova Street in front of supermarket ‘Plus’, 50°04'28"N, 14°18'18"E, alt. 350 m, on pollarded *Acer platanoides*,

TABLE 2. *A comparison of main diagnostic characters for Bacidina mendax and similar European corticolous representatives of the genus with colourless hypothecium.*

Species	<i>B. mendax</i>	<i>B. adastr</i>	<i>B. caligans</i>	<i>B. delicata</i>	<i>B. neosquamulosa</i>	<i>B. phacodes</i>
Thallus morphology	Inconspicuous to irregularly warted to form uneven crust, never sorediose	Sorediose to thickly leprose	Inconspicuous to scurfy granular-sorediose composed of goniocysts	Finely granular-sorediose composed of goniocysts	Isidiose, granular microsquamulose	Thin to irregularly warted to form uneven crust, never granular-sorediose
Apothecia						
Diam. (mm)	0.2–0.7(–1.0)	0.2–0.4(–0.7)	0.2–0.6(–1.0)	0.2–0.7	0.2–0.7	0.2–0.5
Colour	Various, flesh-coloured, beige, pinkish buff, brownish, grey-brown to dull fuscous brown, sometimes piebald	Various, pale pink to dark blue-black, often piebald	Various, beige-brown, reddish brown, brown to blackish brown, sometimes piebald	Whitish beige, beige to orange-beige	Various, pinkish buff, flesh-coloured, brown to dull grey-brown, sometimes piebald	Pale pink, flesh-coloured, pinkish buff
Margin	Paler than disc but in pale-coloured apothecia at least partially extraneously dark pigmented or excluded	Pinkish with aeruginose-blue or dark brown flecks	Usually darker than disc or excluded	Concolorous with disc	Usually darker than disc, in pale-coloured ascocarps paler than disc but at least partially extraneously dark pigmented	Concolorous with disc to slightly paler
Epihymenium	± Pinkish to olive-brown, K– or K+ dull grey-brown or sometimes partially K± purple brown	Colourless to slightly greenish grey or aeruginose-blue, K– or K+ dulling or orange-brown, K± purplish	Colourless to pale brown, K± pinkish to purple-brown	Colourless	Colourless, pale brown, olive-brown to dark grey-brown, K– or K± pinkish to dull brown with purple tinge	Colourless to pale yellow
Excipulum						
Tissue	Prosoplectenchymatous etc	Paraplectenchymatous	Prosoplectenchymatous	Prosoplectenchymatous	Prosoplectenchymatous	Prosoplectenchymatous
Hyphae	Branched, 1.5–2.5 µm, lumina widening to 5–7 µm towards outer edges	Isodiametric lumina 5–10 µm	Branched, 2–3 µm, lumina widening to 7 µm towards outer edges	Branched, 2–3 µm, lumina widening to 7 µm towards outer edges	Branched, 1.5–2.5 µm, lumina widening to 8 µm towards outer edges	Branched, 1.5–2.0 µm, lumina widening to 4 µm towards outer edges
Colour of upper and outer part	Slightly pinkish orange or brown to olive-brown, K+ dulling or fuscous brown sometimes partially K± purple brown	Colourless to greenish grey, aeruginose-blue, K– or K+ dulling or orange-brown, K± purplish	Brown, K± purple-brown	Colourless	Pale to dark brown, K± pinkish to dull brown with purple tinge	Colourless
Conidia						
Shape	Filiform, ±straight, slightly curved (usually in upper half) to ±hooked	Filiform, ±straight to slightly curved	Filiform, strongly curved	Filiform, strongly curved	Filiform, slightly curved or sometimes ±hooked	Filiform, ±straight
Size (µm)	(25–)35–55(–62) × 1.0–1.2	35–50 × 1.0–1.2	(25–)30–45(–50) × 1.0–1.5	20–40 × 1.0–1.5	(35–)40–50(–64) × 1.0–1.5	28–50(–60) × 1.0–1.5

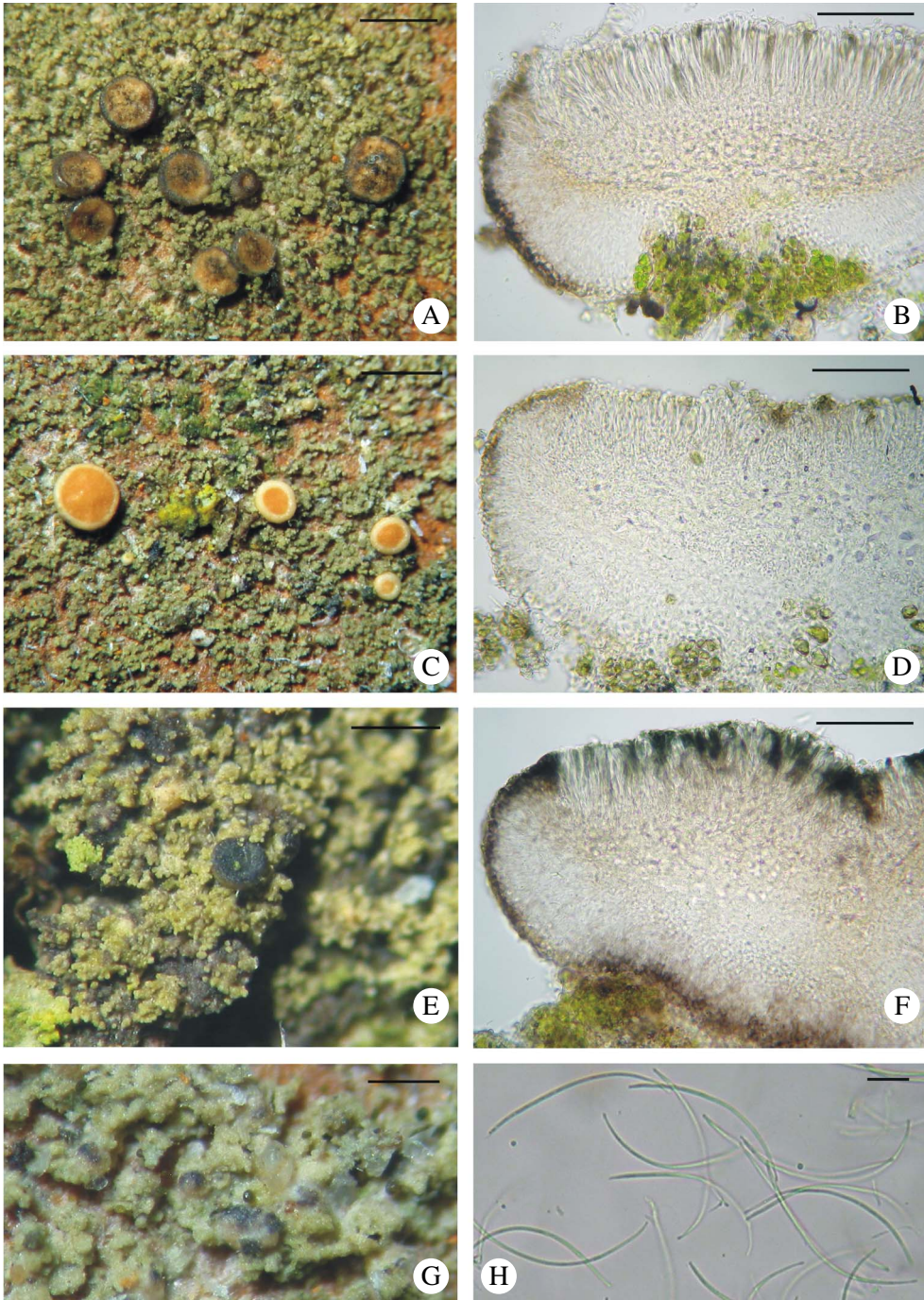


FIG. 4. *Bacidina neosquamulosa*. A, C & E, habitus of variously coloured morphotypes; B, D & F, sections through variously pigmented apothecia; G, pycnidia; H, pycnospores. A–D & G, *v. d. Boom* 41056 (hb. *v. d. Boom*, GenBank no. KX239026); E & H, *v. d. Boom* 44999 (hb. *v. d. Boom*, GenBank no. KX239018); F, *v. d. Boom* 44837 (hb. *v. d. Boom*, GenBank no. KX239027). Scales: A, C & E = 1 mm; B, D & F = 50  $\mu$ m; G = 0.5 mm; H = 10  $\mu$ m. In colour online.

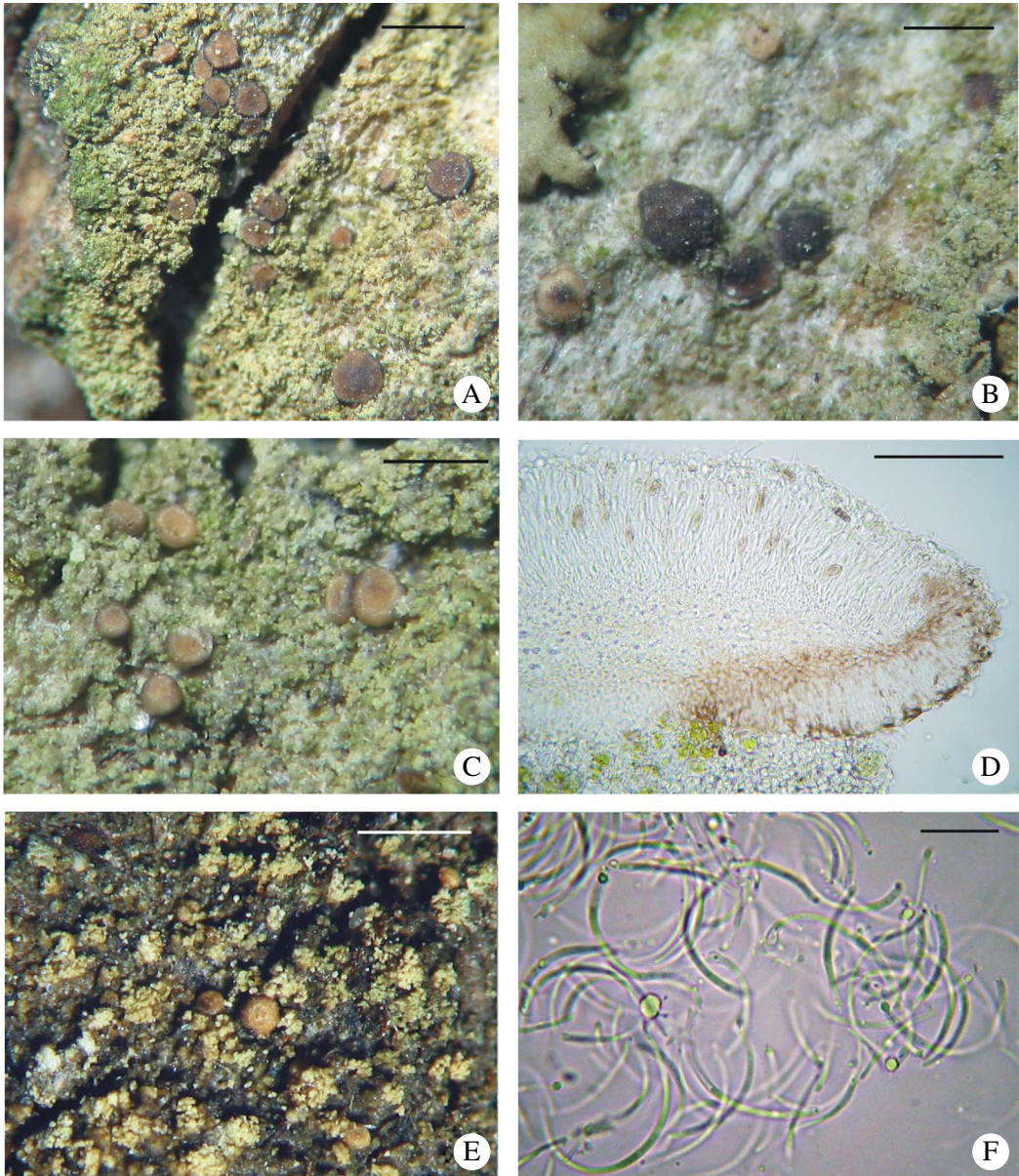


FIG. 5. *Bacidina caligans*. A–C & E, habitus of various morphotypes; D, section through apothecium; F, pycnospores. A–D, *v. d. Boom* 44973 (hb. *v. d. Boom*, GenBank no. KX239024); E & F, *Czarnota* 4961 (GPN, GenBank no. JN972442). Scales: A–C & E = 1 mm; D = 100  $\mu$ m; F = 10  $\mu$ m. In colour online.

2007, *Z. Palice* 11110 & *ř. Palicová* (PRA). *S Moravia*: Břeclav District, Lanžhot, Cahnov-Soutok National Nature Reserve, old-growth forest in flood plain, 7.5 km SSW of Břeclav Town, 48°39'09"N, 16°56'20"E, alt. 150 m, on log of *Fraxinus* within old-growth deciduous forest, 2014, *P. Czarnota* 7571 (GPN).—**Great**

**Britain:** *England:* V.C. 17, Surrey, London, Kew, Royal Botanic Garden, on *Quercus* sp., 2005, *M. Kukwa* 4568 (UGDA-L-12931).—**Poland:** *Pomerania:* Mierzeja Wiślana sandbar, Sobieszewska Island, 1.5 km N of Świbno by Vistula River, 54°20'42"N, 18°56'23"E, on riverside *Salix* sp., 2006, *M. Kukwa* 5171 (UGDA-L-13408); *Mierzeja*

Wiślana sandbar, between Piaski Village and Krynica Morska Town, forest by Zalew Wiślany Bay, 54°24'51"N, 19°33'41"E, on *Salix* sp., 2004, *M. Kukwa* 2966 (UGDA-L-11718); on *Sambucus nigra*, 2004, *M. Kukwa* 2960 (UGDA-L-11712). *Itawskie Lakeland*: Postolińska Struga Valley, c. 0.5 km NNW of Nowa Wieś Village, on twigs of *Salix* sp., 2004, *M. Kukwa* 3611 (UGDA-L-12339); *ibid.*, c. 1 km NE of Małki Village, on *Salix* sp. in a gravel pit, 2001, *M. Kukwa* 99 (UGDA-L-12514). *Fordońska Valley*: near Kiełp Village, close to 'Zbocza Płutowskie' Nature Reserve, 53°17'28"N, 18°22'50"E, on roadside *Salix* sp., 2004, *M. Kukwa* 3512 (UGDA-L-11187). *Wysoczyzna Ciechanowska* plateau, Gašocin Village, 52°44'38.5"N, 20°42'42.1"E, on *Sambucus nigra* at the edge of alder forest, 26 xii 2009, *D. Kubiak* (OLTC-L-3397 & 3398). *Równina Bielska* plain, Białowieża Primeval Forest, Białowieża National Park, forest section no. 256, 52°46'28"N, 23°51'37"E, on young *Carpinus betulus* in *Carici elongatae-Alnetum* community, viii 2014, *M. Kukwa* & *A. Łubek* s. n. (KTC; loc. P1); *ibid.*, 52°46'15"N, 23°51'58"E, on young *Quercus* sp., viii 2014, *M. Kukwa* & *A. Łubek* s. n. (KTC; loc. J5); *ibid.*, 52°46'18"N, 23°52'30"E, on *Fraxinus excelsior* in *Circeo-Alnetum* community, viii 2014, *M. Kukwa* & *A. Łubek* s. n. (KTC; loc. M11). *Beskid Mały Mts*: Kocierz Rychwałdzki Village, alt. 500 m, on roadside *Populus* sp., 10 v 1962, *Ĵ. Norwak* (KRAM-L-9455). *Kotlina Jasielsko-Krośnińska* basin, Podole Village by the Wisłok River, 49°38'37.3"N, 21°54'43.0"E, on riverside *Salix fragilis*, 2010, *P. Czarnota* 7244 (GPN). *Beskid Niski Mts*: Magurski National Park, forest section no. 158, close to Nieznajowa settlement, by Wisłoka River, on riverside *Salix alba*, alt. 435 m, 11 ix 2009, *E. Adamska*, *D. Bielec*, *Ĵ. Kozik* & *A. Łubek* (KRAP).—**Slovakia**: *Považský Inovec*: Tematíske Kopce, between Lúka Village and Tematísky hrad Castle, 48°39'52"N, 17°53'56"E, alt. c. 230 m, on roadside *Acer platanoides*, 2006, *M. Kukwa* 4992 (UGDA-L-13227—topotype). *S Moravia*: near Morava River, Horný les National Nature Reserve, 48°21'09"N, 16°51'47"E, alt. 150 m, on *Quercus robur* branch in old-growth forest in flood plain, 2014, *P. Czarnota* 7786 (GPN).—**Ukraine**: *Eastern Carpathians*: Ivano-Frankivsk Region, Rozhniativ District, 1.5 km W of Ivanivka Village, 48°53'09.8"N, 24°05'29.8"E, alt. 490 m, on *Quercus* sp. in young oak-hornbeam forest, a former wood pasture, 2015, *P. Czarnota* 8117, 8119, 8157, 8158, 8159 & 8160 (GPN).

## New combination

### ***Bacidina pycnidia* (Czarnota & Coppins) Czarnota & Guz.-Krzem. comb. nov.**

MycoBank No. MB 821695

Basionym: *Bacidia pycnidia* Czarnota & Coppins, *Lichenologist* 38: 407–408 (2006); type: Czech Republic, Eastern Sudetes, Rychlebské hory Mts, W of Bila

Voda Village, vicinity of worked-out marble quarry 'Kukačka' near the border of Poland, 50°26'18"N, 16°53'14"E, alt. c. 360 m, on bryophytes over marble rock within mixed spruce-ash forest, 23 April 2004, *P. Czarnota* 4157 (GPN—holotype; E, UGDA—isoatypes).

**Remarks.** Owing to the placement of *Bacidia pycnidia* within the monophyletic group of species that correspond by their characters to the conserved genus *Bacidina* (Ekman 1996b) (Fig. 1), *B. pycnidia* is transferred to *Bacidina*. Despite the nomenclatural problem related to a choice of *Bacidia phacodes* Körb. as the type species for the genus *Bacidina* (Vězda 1990), it is at least clear now that *B. pycnidia* is not nested within *Bacidia* s. str.

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