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Re-evaluation of the morphological variability of *Microglossum viride* and *M. griseoviride* sp. nov.

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Abstract: Studies in *Microglossum viride* (Pers.) Gillet revealed that the name was used incorrectly for two similar but different taxa. Analyses of morphological, ecological and molecular (sequences of ITS and LSU region of rRNA gene) characters of type and voucher specimens of *M. viride* and related taxa resulted in delimitation and description of a new species, *Microglossum griseoviride* V. Kučera, Lizoň & M. Tomšovský. Lectotypes of *Geoglossum viride* Pers., and epitype of *Geoglossum viride* are designated. Species *Microglossum minus* Velen. and *Microglossum lutescens* Boud. are confirmed to be conspecific to *M. viride*.

Key words: Geoglossaceae, ITS, LSU, morphometrics, taxonomy

Introduction

The family Geoglossaceae traditionally included several genera, but according to recent studies it should accommodate within Geoglossomycetes only *Geoglossum*, *Trichoglossum*, *Sarcoleotia* (Schoch et al. 2009) and *Nothomitra* (Hustad et al. 2011). The genera *Microglossum*, *Neolecta*, *Mitrula*, *Cudonia*, *Thuemenidum*, *Spathularia* and *Bryoglossum*, traditionally placed within Geoglossaceae, are probably not closely related to the above four genera and should be treated in Leotiomycetes (Wang et al. 2006, Schoch et al. 2009).

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The generic name *Microglossum* was erected by Gillet (1879) for *Geoglossum viride* Pers. and *G. olivaceum* Pers. without indication of a type species. *Microglossum viride* was selected as the lectotype of the genus by Durand (1908). The name *Geoglossum viride* was first mentioned by Persoon 1794 and validly published in 1796. The name has been generally used for green earth tongues until now.

During examination of our collections of green earth tongues, we noticed some differences in the color of ascomata. Those collected on wet places in spruce forest (such as bank of a stream and a spring) were green-yellow (typical for *Microglossum viride*) but those in deciduous forests dominated by beech and oak were grayish green. Further morphological and molecular analyses proved that the latter collections represent a new species described in this paper.

Materials and methods

Morphological studies.—The macro-morphological characters were observed in fresh material. The micro-morphological structures were studied in dried material with the aid of a light microscope with oil immersion lens. Fragments of material were examined in 5% KOH, tap water, Melzer's reagent and a solution of Congo red in ammonia. Sizes of micro-morphological characters were estimated as means, plus and minus a standard deviation calculated from 30 measurements for each species (in parenthesis are 10 and 90 percentiles of measurements). Acronyms for herbaria follow Index herbariorum (Thiers continuously updated). Recent collections are georeferenced and the coordinates are in the WGS 84 system. All descriptions are based on studied collections.

Morphometric analyses.—Thirty-eight collections of the *M. viride* group were examined. The values of the characters used for morphometric study were taken from dried material (herbaria BRA, SAV, PRM, L, PC, HR). Studied collections represent two distinct morphotypes: one with yellowish green apothecia and another with grayish green apothecia. The characters employed for morphometric analyses correspond to those used for determination and distinguishing of *M. viride* (Durand 1908, Imai 1938, Mains 1955, Dennis 1968, Ohenoja 2000). Altogether 16 morphological characters were assessed: 12 quantitative and four derived ratios (Table I). All measurements were taken in material mounted in tap water. The aim of this assessment was to evaluate the morphological variation as well as the differences within the *M. viride* group. Both principal-component analysis (PCA; Sneath and Sokal 1973, Krzanowski 1990) and discriminant analysis (CDA, canonical

TABLE I. Morphological characters used in morphometric analyses with eingenvectors of principal component analysis (Prin 1, Prin 2, Prin 3; FIG. 1) and total canonical structure (Can 1, FIG. 2) of canonical discriminant analysis

Morphological character	Prin 1	Prin 2	Prin 3	Can 1
Ascus length	−0.003	**0.265**[a]	0.543	−0.058
Part of ascus without spores	−0.165	**0.245**	0.382	−0.070
Ascus width	−0.085	**−0.480**	0.137	**0.876**
Ascospore length	0.051	**−0.290**	0.408	**0.607**
Ascospore width	0.009	**−0.356**	0.378	**0.782**
Apical cell of paraphyse length	0.069	0.213	−0.038	−0.294
Apical cell of paraphyse width	0.025	−0.042	−0.148	−0.056
Fertile part of ascoma length	0.434	0.061	0.039	−0.257
Fertile part of ascoma width	0.419	0.095	0.001	−0.215
Sterile part of ascoma length	0.440	−0.121	0.101	0.030
Sterile part of ascoma width	0.409	0.037	−0.161	−0.272
Q value[b] of the fertile part	−0.106	**0.284**	−0.124	−0.322
Q value of the sterile part	0.026	0.068	0.285	0.127
Total high of ascoma	0.464	−0.055	0.072	−0.103
Q value of spores	0.054	0.153	−0.046	−0.353
Q value of asci	0.045	**0.490**	0.257	**−0.626**

[a] Characters mentioned in text appear in boldface.
[b] Q value means ratio length to width of mentioned structures.

discriminant analysis; Klecka 1980) were used for morphometric analysis. PCA based on average value of characters per each population (specimen) and the correlation matrix between the characters was performed to reduce the multidimensionality of the original character space and to display the variation pattern along the first three components extracting most of the variation. Results from PCA analysis were tested by the CDA analysis based on the character averages. Additional exploratory data analyses (mean values, standard deviations, percentiles for each character) were computed. The SAS statistical package (SAS Institute 2000) was used for all analyses.

Molecular study.—Nine specimens of the *Microglossum viride* group, *M. olivaceum*, *M. rufescens* and *M.* cf. *nudipes*, were selected for molecular analyses. DNA was isolated from dried fungal material using the DNeasy Plant Mini Kit (Qiagen). DNA fragments encompassing the ITS and LSU regions of rRNA genes were amplified with these primer combinations: ITS1/ITS4 or ITS5/ITS4Asco (White et al. 1990, Nikolcheva and Barlocher 2004) and LR0R/LR6 (Moncalvo et al. 2000).

DNA was amplified with PCR as in Tomšovský et al. (2010); amplification reactions were carried out with a Mastercycler thermo-cycler (Eppendorf). Amplicons were custom-purified and sequenced at Macrogen (Seoul, Korea). The sequences were deposited in the NCBI Nucleotide Sequence Database (TABLE II). The ITS and LSU datasets were enriched with sequences of *M. viride* (specimen from RBG Kew K[M]90199; GenBank number EU784375) and *M. olivaceum* (specimen number FH-DSH97-103; GenBank AY789397 and AY789398) published by Brock et al. (2009) and Wang et al. (2005).

The sequences were aligned in MAFFT, 7, (http://www.ebi.ac.uk/Tools/msa/mafft/) with the Q-INS-i option (Katoh and Toh 2008). The aligned ITS dataset was 510 bp, that of LSU was 1193 bp. The introns (probably group I introns) revealed within LSU sequences of specimens *M. rufescens* (SAV 9921) and *M.* cf. *nudipes* (SAV 10024) were excluded from the LSU dataset. Therefore, the final LSU dataset was 1017 bp. The sequences of *Leotia lubrica*, strain ZW-GEO54-Clark (sequences AY789348, AY789349; Wang et al. 2005) were selected as outgroup. The partition-homogeneity test with heuristic search ($P = 0.440$) conducted in PAUP* (Swofford 2003) indicated that it was suitable to combine the ITS and LSU data in one alignment of 1526 bp.

The analysis was conducted with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Likelihood settings from best-fit model (GTR+I+G) were selected by AIC in MrModeltest 2.3 (Nylander 2004). Markov chains initiated from a random tree were run 2 000 000 generations with samples taken every 100th generation. The burn-in value (200 000 generations) was estimated in Tracer 1.5 (Rambaut and Drummond 2007). The additional maximum likelihood (ML) analysis was run in Garli 2.0 (Zwickl 2006). The GTR+I+G substitution model was selected and bootstrap branch support values (BP) were estimated under the maximum likelihood criterion with 500 replicates.

RESULTS

Morphometric analyses.—The ordination diagram of the PCA analysis (FIG. 1) based on 16 morphological characters shows two groups that are separated along the second axis with slight overlap. With this axis are correlated the following characters in descending order: Q value of asci, ascus width, ascospore width, ascospore length, Q value of the fertile part of ascoma, ascus length, and part of ascus without spores (TABLE I). No other groupings along the first

TABLE II. Herbarium and GenBank accession numbers of the sequenced specimens

Species	Herbarium specimen no.	ITS sequence GenBank accession no.	LSU sequence GenBank accession no.
Microglossum griseoviride, holotype	SAV 9920	KC595249	KC595250
M. griseoviride	SAV 10699	KC595261	KC595262
M. cf. *nudipes*	SAV 10024	KC595259	KC595260
M. rufescens	SAV 9921	KC595257	KC595258
M. olivaceum	SAV 9902	KC595251	KC595252
M. olivaceum	SAV 9967	KC595255	KC595256
M. viride, epitype	SAV 10249	KC595253	KC595254
M. viride	SAV 10697	KC595265	KC595266
M. viride	SAV 10698	KC595263	KC595264

and third axes are apparent. The histogram of CDA (FIG. 2) based on the same matrix of characters shows clear separation without overlap between two groups resulting from PCA analysis. The characters that contributed most to separation are the same as revealed in PCA (TABLE I). The results of exploratory data analysis of investigated collections (FIG. 3) showed that these two groups represent a distinct species (*Microglossum viride*, *M. griseoviride* sp. nov.), which differ in several characters that overlap in extreme values only. The best characters for differentiation of these two taxa are ascus width, ascospore length, ascospore width and Q value of asci.

Molecular analysis.—The aligned dataset combining ITS and LSU sequences comprised 1526 positions including 1272 constant, 180 parsimony-informative, 102 singleton and 74 autapomorphic sites. Maximum likelihood analyses yielded trees with the likelihood values: ln = −3610.194854 in ML analysis (Garli) and −3714.7378 (MrBayes, the mean value).

The phylogenic analyses confirmed remarkable morphological differences between *Microglossum viride* and *M. griseoviride* previously revealed by PCA and CDA. The two species form two sister groups within one, well supported clade while another proximal clade includes *M. olivaceum*, *M. rufescens* and *M.* cf. *nudipes* (FIG. 4).

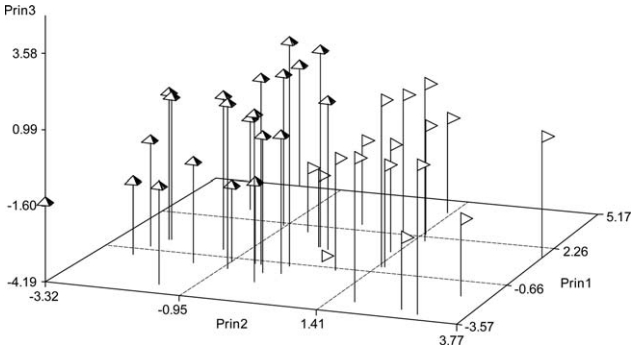

FIG. 1. Principal component analysis of *Microglossum viride* (triangles) and *M. griseoviride* (flags) collections based on 16 morphological characters (see TABLE I). First three axes explain 26.49%, 19.02% and 14.56% of the total variation respectively.

TAXONOMY

Microglossum viride (Pers.: Fr.) Gillet, Champignons de France, Discom. 1:25 (1879) FIGS. 5A, B.

≡ *Geoglossum viride* Pers., Observ. mycol. 1:39 (1796); lectotype: L 0110965 (Persoon collection) designated here; epitype: SAV 10249 (Czech Rep., Jablonec nad Nisou, Sep 2010), GenBank Nos. KC595253, KC595254, designated here.

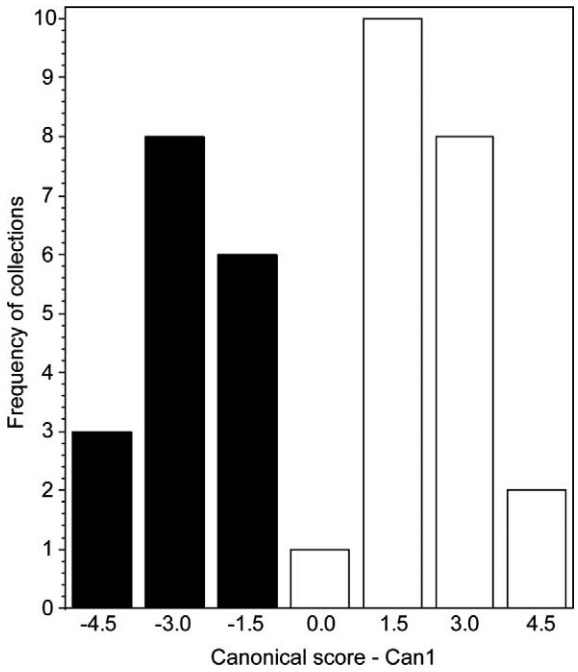

FIG. 2. Canonical discriminant analysis of *Microglossum viride* (white) and *M. griseoviride* (black) collections based on 16 morphological characters (see TABLE I).

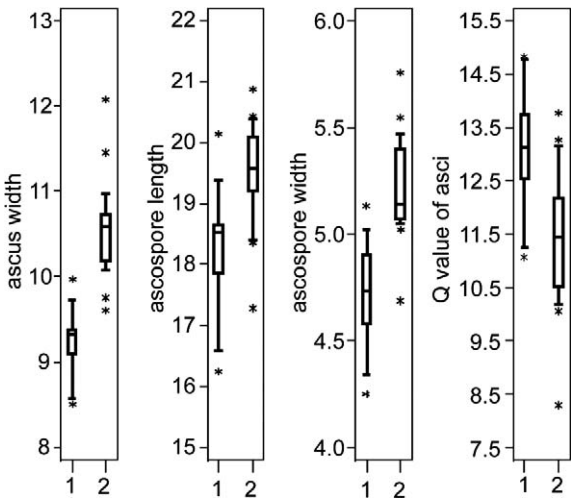

FIG. 3. Variation of selected morphological characters of examined collections (in µm): 1. *Microglossum griseoviride*, 2. *M. viride*. Rectangles define 25th and 75th percentiles, horizontal lines indicate median, whiskers are from 10–90th percentiles and asterisks indicate extreme values.

= *Microglossum lutescens* Boud., Bull. Soc. Mycol. France 12:14 (1896); lectotype: PC 0084332 (Fond des Aulnes, Sep 1891), designated here.

= *Microglossum minus* Velen., Monogr. Discom. Bohem. (Prague) 1:375 (1934); lectotype: PRM 148610 (Czech Rep., Rokycany, Aug 1925), designated here.

= *Leptoglossum alabamense* Underw., Bull. Torrey Bot. Club 24:82 (1897); type studied by Durand (1908).

[= *Mitrula lutescens* (Boud.) Massee, Ann. Bot. 11:271, Pl.13, Fig. 77 (1897), nom. illeg.]

Ascocarps (6–)10–30(–52) mm high, clavate, stipitate, slender, solitary, also in small (3–5) or large (up to 30) clusters. Fertile part (2–)5–13(–20) × (0.5–)1–4(–10) mm, mainly flattened, lanceolate, sometimes spoon-shaped or truncate, pea green, yellow green, olive green, glabrous, in wet condition almost viscid, when dry the entire ascoma dark green. Fertile part usually covers one-third to one-half of the ascoma. Sterile part (4–)8–18(–35) × 0.5–2 mm, delimited from the fertile part, cylindrical, yellow-green with dark green scales, green with dark green scales or olivaceous, always with more or less distinct scales, usually concolorous with fertile part, with dark tint at the base, viscid when moist and young, (109 ascomata examined). Asci (84–)106–134(–175) × (8–)9.5–12(–20) µm, mean Q value of asci = 11.35, clavate, apex rounded in some cases slightly narrowed, eight-spored, arising from simple septa, biseriate above, uniseriate below, pore bluing in Melzer's reagent. Spores (11–)18–22(–25) × (4–)5–7 µm, elliptical-oblong, sometimes slightly curved, ends obtuse, hyaline or with several lipid bodies, real septa not seen. Paraphyses filiform, branched, sometimes anastomosed basally to form H-like bridges, greenish in upper part, the apical cells often with greenish ''cap,''

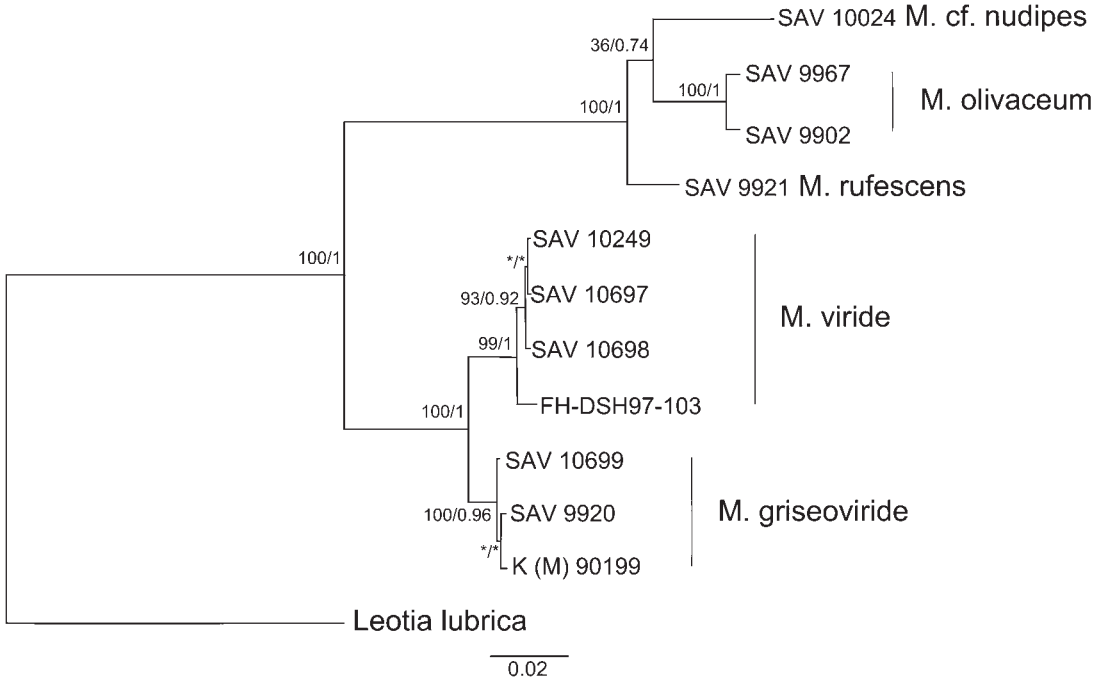

FIG. 4. ITS-LSU rDNA based phylogram obtained from Bayesian inference depicting relationships among *Microglossum* species. The numbers from left to right refer to percentage bootstrap values of maximum likelihood and posterior probabilities of the Bayesian analysis. Values less than 50% are not shown. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site.

FIG. 5. Ascomata of *M. viride* and *M. griseoviride*. A. *Microglossum viride* (SAV 10249), Photo J. Gaisler; B. *M. viride* (SAV 10696), Photo J. Gaisler; C. *M. griseoviride* (SAV 10497), Photo I. Hlavatý; D. *M. griseoviride* (SAV 10534), Photo V. Kunca; Bar = 2 cm.

pyriform (up to 5 µm) or filiform (up to 2 µm), forming brownish yellow-green epithecium.

Habitat: On the ground in shady humid forests, on wet old forest roads, on the bank of small springs in among liverworts (*Pellia epiphylla*), or in wet meadows near forest, on slightly acidic to acidic stands under *Alnus* sp., *Populus tremula*, *Picea abies*.

Collections examined: LECTOTYPE: EUROPE?, in sylvis ad vias, CH. Persoon (L 010965). EPITYPE: CZECH REPUBLIC: Jizerské hory Mountains, Jablonec nad Nisou, S periphery of town, 180 m N of ski tow Dobrá voda, 545 m, 50°42′44.6″N, 15°9′44.04″E, bank of small stream with liverwort *Pellia* sp., under *Alnus* sp. and *Picea abies*, granite bedrock, slight NNW slope, 9 Sep 2010, V. Kučera, J. Gaisler, V. Kautman (SAV 10249). OTHER COLLECTIONS: EUROPE?, CH. Persoon (L 010964, as *Geoglossum viride*). FRANCE: ad latera viarum umbrosarum (solo arenoso) Fond des Aulnes, 11 Sep 1891, E. Boudier (PC 0084332: lectotype of *Microglossum lutescens* Boud.; PC 0084330, PC 0084331, PC 0084342, PC 0084343, PC 0084344, PC 0084329); Pyrénées-Atlantiques, Arette, Col de Labays, 1375 m, 43°00′06.8″N, 00°43′56.3″W, between mosses and *Pellia*?, in forest of *Fagus sylvatica* and *Abies alba*, 11 Oct 2005, J. Hernanz, J. Fernandes (SAV 10702). CZECH REPUBLIC: Rokycany, Aug 1925, K. Cejp, (PRM 148610: lectotype of *Microglossum minus* Velen.);

Prachatice distr., Želnava, Q 7149, bank of the small meadow stream, 24 Sep 2003, M. Vašutová, V. Pouska (SAV 6985); Trutnov distr., Horní Staré Město, Horní výšina, ca. 2 km SW from the village, 500 m, Q 5461 a, 50°34′48″N, 15°51′51″E, mixed forest, 22 Sep 2001, J. Kováč, (HR 66143); Jizerské hory Mountains, Josefův Důl, 250 m SSE of Nature Reserve Jedlový důl, 625 m, 50°47′7.23″N, 15°14′39.56″E, mossy bank of the Jedlová stream, under *Fagus sylvatica*, *Betula* sp. and *Acer* sp., granite bedrock, S slope, 30 Aug 2012, J. Gaisler (SAV 10697, GenBank KC595265, KC595266); Jizerské hory Mountains, Jablonec nad Nisou, S periphery of town, 180 m N of ski tow Dobrá voda, 545 m, 50°42′44.6″N, 15°09′44.04″E, bank of small stream with liverworts *Pellia* sp., under *Alnus* sp. and *Picea abies*, granite bedrock, slight NNW slope, 9 Sep 2010, V. Kučera, J. Gaisler, V. Kautman (SAV 10704, SAV 10603); Jizerské hory Mountains, Hraničná, 600 m S of granite quarry, 525 m, 50°45′42.88″N, 15°09′21.59″E, bank of small stream, in liverworts *Pellia* sp. and moss *Polytrichum commune*, forest with *Picea abies*, *Alnus* sp., granite bedrock, 9 Sep 2010, V. Kučera, J. Gaisler, V. Kautman (SAV 9972); Jizerské hory Mountains, Kateřinky, Mordová rokle, 550 m, 50°48′2.64″N, 15°05′25.92″E, bank of stream, under *Fagus sylvatica*, granite bedrock, 9 Sep 2010, V. Kučera, J. Gaisler, V. Kautman (SAV 9970, SAV10204); Jizerské hory Mountains, Mníšek, Amerika, 400 m, 50°49′50.55″N, 15°02′17.76″E, bank of small stream, in liverworts *Pellia* sp., forest with *Alnus* sp., *Fraxinus* sp., granite bedrock, 10 Sep 2010, V. Kučera, J. Gaisler, V. Kautman (SAV 9961, SAV 10267); Chrudim distr., Sečská vrchovina Hills, Nasavrky, Nová Ves, ca. 400 m SE from the village, 530 m, Q 6160 d, 49°49′26′N, 15°49′32″E, bank of the stream, soil with mosses, 14 Oct 2010, L. Tmej, S. Flekrová, T. Tejklová (HR 86385). SWEDEN: Dalsland, Bengtsfors distr., Rollsbyn, 59°11′50,2″N, 12°13′17,8″E, wet grassland with mosses, under a tree, 12 Sep 2012, V. Kučera, I. Kautmanová, V. Kautman (SAV 10698, GenBank KC595263, KC595264). USA, SOUTH CAROLINA: in sylvis humidis, Curtis 2990, ex herb. JM. Berkeley, 1879 (K (M) 169301: holotype of *Mitrula lutescens* Berkeley & Curtis = *Microglossum rufum* (Schwein.) Underw.). CHINA, Sichuan, 1997, D. Hibbett (FH 00290421: originally identified as *M. viride* but later published as *M. olivaceum* [Wang et al. 2005], DSH 97-103, GenBank AY789397, AY789398).

Microglossum griseoviride V. Kučera, Lizoň, M. Tomšovský sp. nov. FIG. 5C, D.
MycoBank MB803402

Ascocarps (4–)8–32(–48) mm high, clavate, stipitate, scattered, solitary, also in small (3–5) or larger (up to 15) clusters. Fertile part (2–)4–16(–28) × (1–)2–3(–6) mm, mace-shaped, cylindrical, clavate, truncate or lanceolate, vertically grooved sometimes almost lacunose, glabrous, olive green to grayish green, when dry similar to fresh color. Fertile part usually one-third to two-thirds of the ascoma. Sterile part (2–)4–18(–30) × 0.5–3 mm, sharply delimited from the fertile part, cylindrical, sometimes flexuous, olive green to grayish green, concolorous with fertile part or paler, sometimes with whitish base, distinctly squamulose in upper part (68 ascomata examined). Asci (90–)105–139(–219) × 8–10 µm, mean Q value of asci = 13.04, cylindrical to clavate, apex rounded and narrowed, eight-spored, arising from simple septa, biseriate above, uniseriate below, pore blue in Melzer's reagent. Spores (14–)16–20(–23) × 4–5 µm, usually slightly curved or sigmoid, oblong-clavate, ends obtuse, hyaline or with several lipid bodies, real septa not seen. Paraphyses filiform, branched, sometimes in basal part anastomosed, green colored in upper part, the apical cells often with green ''cap'', pyriform (up to 5 µm) or filiform (up to 2 µm), forming gray-green epithecium.

Holotype: SLOVAKIA: Revúcka pahorkatina Mountains, Revúca town (SAV 9920, GenBank KC595249, KC595250).

Habitat: On the ground in forests (not among liverworts), on margins of old forest roads, on neutral or only slightly acidic soil under *Quercus* sp. and *Fagus sylvatica*.

Collections examined: HOLOTYPE: SLOVAKIA: Revúcka pahorkatina Mountains, town Revúca, site called za Peklom, 325 m, 48°40′21.1″N, 20°07′06.8″E, mixed forest, on soil, under *Quercus* sp. and *Fagus sylvatica*, 3 Oct 2010, V. Kučera, V. Kautman (SAV 9920). OTHER COLLECTIONS: SLOVAKIA: Sv. Jur [Svätý Jur], in fageto, humi in muscis, 7 Sep 1846, J. Bolla (BRA CR 8941, as *Geoglossum viride)*; Eper. [Eperjes = Prešov], F. Hazslinsky, (BRA, as *Geoglossum viride*); Gebirgsväld, 1926, K. Mergel (BRA 3918, as *Geoglossum viride*); Kremnické vrchy Mountains, Kováčová, 400 m, fallow deer park, forest with *Quercus* sp. div, *Carpinus betulus*, 1 Sep 1999, S. Glejdura, S. Jančovičová (SLO 0008, as *Microglossum viride*); Javorníky Mountains, Kátlina, valley of the river, 535 m, 49°12′44.3″N, 18°17′27.7″E, forest with *Fagus sylvatica*, 5 Oct 2005, E. Pisarčíková (SAV 7073); Javorníky Mountains, Považská Bystrica, Kunovec, forest with *Fagus sylvatica*, *Carpinus betulus*, *Betula pendula*, 14 Sep 2010, J. Kianička, (SAV 10701); Tríbeč Mountains, Jelenec, forest with *Quercus* sp. div, 15 Sep 2010, I. Hlavatý (SAV 10497); Revúcka vrchovina Mountains, Revúca, Za Peklom, 325 m, 48°40′21.1″N, 20°07′06.8″E, mixed forest, on soil, under *Quercus* sp. and *Fagus sylvatica*, mixed forest, 15 Oct 2010, V. Kučera (SAV 10703); Dolný Kubín, mixed forest with *Carpinus betulus*, 16 Oct 2010, R. Rutkowski (SAV 10134); Štiavnické vrchy Mountains, Kysihýbeľ, forest with *Fagus sylvatica*, 20 Oct 2010, V. Kunca, (SAV 10534). CZECH REPUBLIC: Karlov pod Ještědem, margin of the National Reserve Karlovská Bučina, under *Fagus sylvatica*, 11 Sep 2010, Z. Egertová, M. Kříž (PRM 899609 as *Microglossum viride*); Chýnov, Dolní Hořice, National Reserve Pacova hora, 570 m, 49°25′54.8″N, 14°49′45.7″E, MTB 6555 c, forest with *Fagus sylvatica*, 24 Sep 2010, P. Špinar (PRM 899545, as *Microglossum viride*). FRANCE: Pyrenees atlantiques, Artte, 43°00′05.12″N, 00°44′00.92″W, between mosses, forest with *Fagus sylvatica* and *Abies alba*, 26 Sep 2004, J. Hernanz, (SAV 10705). GERMANY: Thuringia, ''NSG Aspenbusch'', southern

Erfurt, limestone, *Fagus* forest, 2 Oct 2010, F. Hampe, FH20101002-01, (SAV 10700). NORWAY: Veggeråsen, Andebu, Vestfold, 59°18′28.1″N, 10°06′24.0″E, forest with *Fagus*, in the path, 9 Sep 2012, T. Nakling Kristiansen (SAV 10699, GenBank KC595261, KC595262). UNITED KINGDOM, WALES: Caernarfonshire (Gwynedd), Coed Llyn Mair Nature Reserve, on mossy ground, 23 Sep 2001, D. Griffin (K(M)90199 as *Microglossum viride*, GenBank EU784375).

Additional collections used for molecular analyses: Microglossum olivaceum: SLOVAKIA: Nízke Tatry Mountains, Malužiná, settlement Michalovo, 775 m, 48°59′54.6″N, 19°45′04.8″E, Q 8470a, meadow near the house of Gašperik, 1 Sep 2010, V. Kautman (SAV 9902, GenBank KC595251, KC595252). *Microglossum olivaceum*: CZECH REPUBLIC: Jizerské hory Mountains, Machnín, Natural Reserve Hamrštejn, 50°47′09.73″N, 14°58′17.25″E, mixed forest with *Fagus sylvatica*, 10 Sep 2010, V. Kučera, V. Kautman, J. Gaisler (SAV 9967, GenBank KC595255, KC595256). *Microglossum rufescens*: SLOVAKIA: Malé Karpaty Mountains, Chtelnica, Plešivá hora, 48°34′09″N, 17°35′55.5″E, meadow, 12 Sep 2010, V. Kučera (SAV 9921, GenBank KC595257, KC595258). *Microglossum* cf. *nudipes*: SLOVAKIA: Stolické vrchy Mountains, Muránska Huta, Predná Hora recreation area, grassland, 13 Oct 2010, V. Kučera (SAV 10024, GenBank KC595259, KC595260).

DISCUSSION

Saccardo (1884) based his *Microglossum* (an illegitimate superfluous name) on *Geoglossum hookeri* Cooke that was later accepted in *Thuemenidium* Kunze. There are few names connected to green (or greenish) earth tongues published before Persoon's work, namely *Clavaria serpentina* O. F. Müll. (1776), *Clavaria viridis* Schrad. (Gmelin 1791) and *Clavaria mitrata* β [var.] *viridis* Holmsk. (1790). Their descriptions are short and insufficient and the identity of these species is dubious. These names are not available for any of taxa presented in this paper.

Fries (1821) adopted four varieties in *Geoglossum viride*, *G. viride* var. *aeruginosum*, *G. viride* var. *atrovirens*, *G. viride* var. *viscidum* and *G. viride* var. *gracile*, presumably representing only development stages. Berkeley and Curtis (Berkeley 1875) described the greenish *Mitrula lutescens* which, according to our examination of the type specimen, represents *Microglossum rufum* (Schwein.) Underw. Boudier (1896) used the same epithet for his *Microglossum lutescens*. Imai (1938) listed this name as a synonym of *M. viride* and we were able to confirm this by studying Boudier's type specimen. Boudier noted another green species (that represents our new species, *M. griseoviride*) but used the name *M. viride* for it. Later Massee (1897) introduced a superfluous name *Mitrula lutescens* (Boud.) Massee (a younger homonym of *M. lutescens* Berk. & M.A. Curtis). With regard to its description and illustration, it is clear that Massee's *Mitrula lutescens* is conspecific with *Microglossum viride* (Pers.) Gillet.

Durand (1908) and Imai (1941) studied type specimen of *Leptoglossum alabamensis* Underw. and said that it represents *M. viride*. Velenovský (1934) presented two green species: *M. minus* Velen. and *M. viride*. According to the original description, it seems that the fungus he named *M. viride* is a different taxon, probably related to *M. nudipes* Boud. It has relatively large ascomata with smooth stipe, the fertile part is longer than the sterile part and the size of asci is different. In comparison, the type specimen of his *Microglossum minus* is composed of young ascomata that belong to *M. viride*.

These illustrate that earth tongues with green ascomata have been typically identified as *Microglossum* (*Geoglossum*) *viride* ''forms'' with grayish tones and were not distinguished as different taxa. Our morphometric and molecular analyses show that such a ''form'' represents a distinct species described herein as *Microglossum griseoviride*. According to the morphometric analyses (PCA, CDA), *M. viride* and *M. griseoviride* are grouped in two separate clusters. The new species can be distinguished by width of asci (8–10 μm), length and width of spores (16–20 × 4–5 μm) and by the Q value of asci (mean Q = 13.04) that are different from *M. viride* (9.5–12 μm, 18–22 × 4–6 μm, mean Q = 11.35 respectively). Ascomatal pigments have not been studied in this group of Ascomycota but might introduce applicable characters for delimitation of taxa.

These two species differ also in ecology. While *M. viride* occurs in wetlands, among species of liverwort *Pellia* and other bryophytes in association with *Picea abies* and/or *Alnus* sp. and *Populus tremula*, *M. griseoviride* prefers less humid sites usually in forests with *Quercus* spp. and/or *Fagus sylvatica*. Measurements of the acidity of soil samples from two collecting sites showed pH 4.56 for *M. viride* and pH 5.29 for *M. griseoviride*. It seems that *M. viride* prefers more acidic soils, although the difference in pH is small. Further study of the relationship between pH and species preferences will be required. A green, probably closely related species *Microglossum rickii*, was described by Imai (1942) from Brazil. The original specimen was identified by Rick as *Geoglossum viride* (held at FH); it was distiguished as a new species *Microglossum rickii* by Imai (1941), but the name was validly published one year later (Imai 1942). We have not located and studied the type specimen, but based on its completely different habitat and known distribution this is likely to be a distinct taxon. Moreover, *M. rickii* has markedly smaller asci (65–75 × 9–10 μm) than the two European species (Imai 1942). Similarly small asci of *M. griseoviride* were

found in the lectotype of *Microglossum minus* Velen. (87 × 8 µm) but average value within specimen reached 100 × 12 µm.

The *Microglossum olivaceum* group (*M. olivaceum*, *M. rufescens*, *M. nudipes*, *M. fuscorubens*) includes taxa having ascomata with smooth stipe and represents the sister group of *Microglossum viride*. Baral (2000) who has extensively studied geoglossoid fungi, compiled a key for *Microglossum* that includes a species that is not validly published. He called it ''*Microglossum denunciatum*'' (but has only a single collection). Ascomata of this taxon have smooth stipes, and thus it might be a member of *M. olivaceum* group. During the study of collections at PC Baral marked one of the specimens as lectotype of *M. lutescens* Boud. We accepted his proposal and designated that specimen as lectotype in this paper. Our analysis includes also sequences (AY789397, AY789398) of a Chinese specimen (FH-DSH97-103) erroneously treated as *M. olivaceum* (Wang et al. 2005) that proved to be *M. viride*. Another GenBank sequence, EU784375, of specimen K(M)90199 represents our new taxon *M. griseoviride*.

All Slovak collections held in herbaria and published as *Microglossum viride* (e.g. Ripková and Kučera 2006) represent *M. griseoviride*. It is likely that the new species is, in some areas, more frequent than *M. viride*. Currently known distribution of *M. griseoviride* covers temperate Europe from France to Slovakia, southern Norway and southern Great Britain. The exact distribution of both species in Europe deserves further study.

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