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A taxonomic and phylogenetic study of the *Golovinomyces biocellatus* complex (Erysiphales, Ascomycota) using asexual state morphology and rDNA sequence data

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Abstract The *Golovinomyces biocellatus* complex consists of powdery mildew (Erysiphales) species restricted to hosts of the family Lamiaceae. Previous authors used minor morphological features of the sexual state and host range data to split the complex. The data, however, were not sufficient to define a convincing species concept. Our taxonomic study is based on molecular phylogenetic and asexual state morphology data. For morphological studies, mainly features of the asexual morph (conidiophores, conidia, germination patterns) were studied using light and scanning electron microscopy. Detailed line drawings of asexual state features are provided. For phylogenetic analyses, two markers (rDNA: ITS, LSU) of 64 specimens were applied. The phylogeny resulted in two major clades. Clade I consists of specimens with Lamiaceae hosts and three specimens of *Verbena*. Clade II consists of two sister groups, the first (IIa) with *Salvia* spp. and the second (IIb) with *Lycopus europaeus* (the type host *G. biocellatus*) and *Glechoma*. Clades I and IIb and two subclades of IIa with *Salvia* hosts are characterized by specific morphological traits (differences in conidiophore length, conidial shape, width, and germination patterns). Based on these data, we suggest to consider specimens of clades I (including specimens on *Verbena*) and IIb and the two subclades of IIa as distinct species, namely *G. monardae*, *G. biocellatus*, *G. salviae*, and *G. neosalviae* sp. nov. A key for the identification of species based on asexual state features is provided. The results are discussed with respect to host range, jumps, co-evolutionary aspects, and distribution patterns.

Keywords Erysiphales · Lamiaceae · Anamorph morphology · Phylogeny · Host range · Host jump

Introduction

Golovinomyces biocellatus (Ehrenb.) Heluta (Erysiphales) as described by Braun and Cook (2012) is a species with worldwide distribution and broad host range. Hosts are mainly species and genera of the subfamily Nepetoideae (tribe Mentheae) within the family Lamiaceae, but some host genera, namely *Ajuga*, *Teucrium* (Ajugoideae), *Scutellaria* (Scutellarioidae), *Stachyopsis* (Lamieae), and *Stachys* (Stachydaeae), are members of other subfamilies (Braun and Cook 2012; Lamiaceae classification after Bendiksby et al. 2011; Walker et al. 2004). This species is the only member of the genus *Golovinomyces* on the Lamiaceae. Several authors (e.g., Jaczewski 1927; Blumer 1933; Nagy 1977) tried to split the species because of many biologically specialized strains and based on differences in fruiting body size: according to Blumer (1933), the fungus on *Salvia* has smaller fruiting bodies (chasmothecia) than strains on other host genera (91–110 μm vs. 118–135 μm).

The latter feature, however, could not be confirmed by us in this study. We measured fruiting bodies on *Salvia officinalis* and *S. lavandulifolia* with up to 135 μm diam. (unpublished). Jaczewski's (1927) measurements did not confirm Blumer's (l.c.) results either. The sexual state was described well

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(although often rarely formed), whereas previous descriptions of the asexual morph were generally scarce. Most authors, including Ehrenberg (1821) in his protologue, did not provide any information on conidiophores and conidia.

Major information on the *G. biocellatus* complex was provided by Takamatsu et al. (2013) in their comprehensive phylogenetic analysis of the genus *Golovinomyces*. In this study using ITS and 28S rDNA sequences as markers, the authors confirmed close evolutionary relationships of *Golovinomyces* species and their host plants, with one clade containing *G. biocellatus* on various species of the Lamiaceae and on *Verbena*, a species of the family Verbenaceae which is closely related to Lamiaceae. The authors, however, could not confirm a special position of *Salvia* specimens within the complex. In fact, *Salvia* specimens were placed in two different subclades of the *G. biocellatus* clade (Takamatsu et al. 2013).

In order to decide whether the *G. biocellatus* complex has to be maintained or needs to be split into several taxa, additional taxonomically relevant information is required. In the following, we focused on more phylogenetic information from rDNA sequence data and on morphological descriptions of the asexual morph, since the sexual morphs (chasmothecia) are little differentiated and barely diagnostic (in *Golovinomyces*, they are rather uniform and useful for taxonomic purposes only to a very limited extent).

Materials and methods

Molecular analyses

Whole-cell DNA was extracted from mycelia using the chelex method (Walsh et al. 1991), as described by Hirata and Takamatsu (1996). The 5′-end of the 28S rDNA (including the domains D1 and D2) and internal transcribed spacer (ITS) regions were amplified by polymerase chain reaction (PCR) using the respective primer pairs: PM3 (Takamatsu and Kano 2001) and TW14 (Mori et al. 2000) for 28S, PM5/ITS4 for ITS fragment 1, and ITS5/PM6 (Takamatsu and Kano 2001) for ITS fragment 2. For some specimens, *Golovinomyces*-specific primers PM5G (5′-GACCCTC CACCCGTGT-3′) and PM6G (5′-CGAGCCCCAACA CCAA-3′) were used instead of PM5 and PM6, respectively. KOD FX Neo DNA polymerase (Toyobo, Japan) was used in the PCR according to the manufacturer's protocol. The amplicons of the 28S rDNA and ITS regions were sent to Solgent Co. Ltd. (Daejeon, South Korea) for direct sequencing using primer pairs of NL1 (Mori et al. 2000) and NLP2 (Hirose et al. 2005) for the 28S region, and ITS1 and ITS4 (White et al. 1990) for the ITS region. New sequences determined in this study were deposited in the DNA Data Bank of Japan (DDBJ) under the accession numbers LC076800–LC076842.

These sequences were aligned with other sequences of *Golovinomyces* retrieved from DNA databases using MUSCLE (Edgar 2004) implemented in MEGA6 (Tamura et al. 2013). Alignments were further manually refined using the MEGA6 program and were deposited in TreeBASE (http://www.treebase.org/) under the accession number S18141. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) methods. MP analysis was performed in PAUP 4.0a144 (Swofford 2002) with heuristic search option using the tree bisection reconnection (TBR) algorithm with 100 random sequence additions to find the global optimum tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analysis using 1000 replications with the step-wise addition option set as simple (Felsenstein 1985). Tree scores, including tree length, consistency index (CI), retention index (RI), and rescaled consistency index (RC), were also calculated. The ML analysis was done using raxmlGUI (Silvestro and Michalak 2012), under a GTRGAMMA model. The BS supports and trees were obtained by running rapid bootstrap analysis of 1000 pseudo-replicates followed by a search for the tree with the highest likelihood.

Morphology

Specimens of mainly German origin (and a few others from Armenia, Hungary, and the USA) from KR, HAL, and PUL were studied. For light microscopy, both fresh (most specimens from KR, PUL) and dried (specimens from HAL, M, ZT, specimens KR-M-0043412 and KR-M-0043413 on *Verbena*, specimen KR-M-0018634 on *Salvia pratensis*) asexual structures of specimens were examined in tap water mounts using an Olympus BH2 and Zeiss Axioskop 2 Plus. Pertinent features were measured at magnifications of 400× and 1000× and documented by line drawings of fresh material. From each specimen, up to 25 conidia were measured. To induce conidial germination, the method of Schmidt and Scholler (2002) was applied. Fresh conidia were spread on a microscope slide and placed in a Petri dish with moist cellulose tissue. The closed Petri dishes were incubated at room temperature and exposed to daylight through a north-facing window for 24 h. For scanning electron microscopy (SEM), fresh fungus material with host tissue was submerged for two hours in 2 % glutaraldehyde buffered in a 0.1 M K_3PO_4 and 0.1 M Na_3PO_4 solution at pH 6.8. After 3× rinsing in the same buffer, samples were submerged in 1 % osmium tetroxide prepared with the phosphate buffer as well. After briefly rinsing in buffer, the material was dehydrated in an ethanol column of 30 %, 50 %, 70 % up to 100 % and dried with a critical point dryer. Sori were mounted with coloidal silver on metal blocks and coated with gold-palladium in a Hummer 1 sputter

coater. Samples were studied with a JEOL JSM-840 scanning electron microscope with digital image acquisition at 5 kV accelerating voltage.

Results

Phylogenetic analyses

A total of 44 ITS and 28S rDNA sequences, including 40 from lamiaceous hosts and four from *Verbena* spp., were determined in this study. These sequences were aligned with 20 *Golovinomyces* sequences retrieved from DNA databases. The ITS + 28S rDNA combined dataset consists of 64 sequences and 1277 characters, of which 69 (5.4 %) characters were variable and 66 (5.2 %) characters were informative for parsimony analysis. A total of ca. 3×10^5 equally parsimonious trees with 84 steps (CI = 0.9524, RI = 0.9896, RC = 0.9425) were constructed by the MP analysis. The tree topologies were mostly consistent among the trees, except for branching orders of the terminal branches and branch length. One of the trees is shown in Fig. 1. ML analysis generated a tree topology almost identical to the MP tree. Thus, only BS values of the ML analysis are shown in Fig. 1. The 59 sequences from the *G.*

Fig. 1 A phylogenetic tree of the *Golovinomyces biocellatus* complex inferred from the combined dataset of the rDNA ITS regions and the divergent domains D1 and D2 sequences of the 28S rDNA. This tree is a phylogram of one of the 485,103 most parsimony trees with 84 steps, which was found using a heuristic search by PAUP*. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. Bootstrap (BS) (≥70 %) values by the maximum parsimony (MP) and maximum likelihood (ML) methods are shown on the respective branch

ITS + 28S rDNA
64 sequences
1277 characters
TL = 84
CI = 0.9524
RI = 0.9899
RC = 0.9428
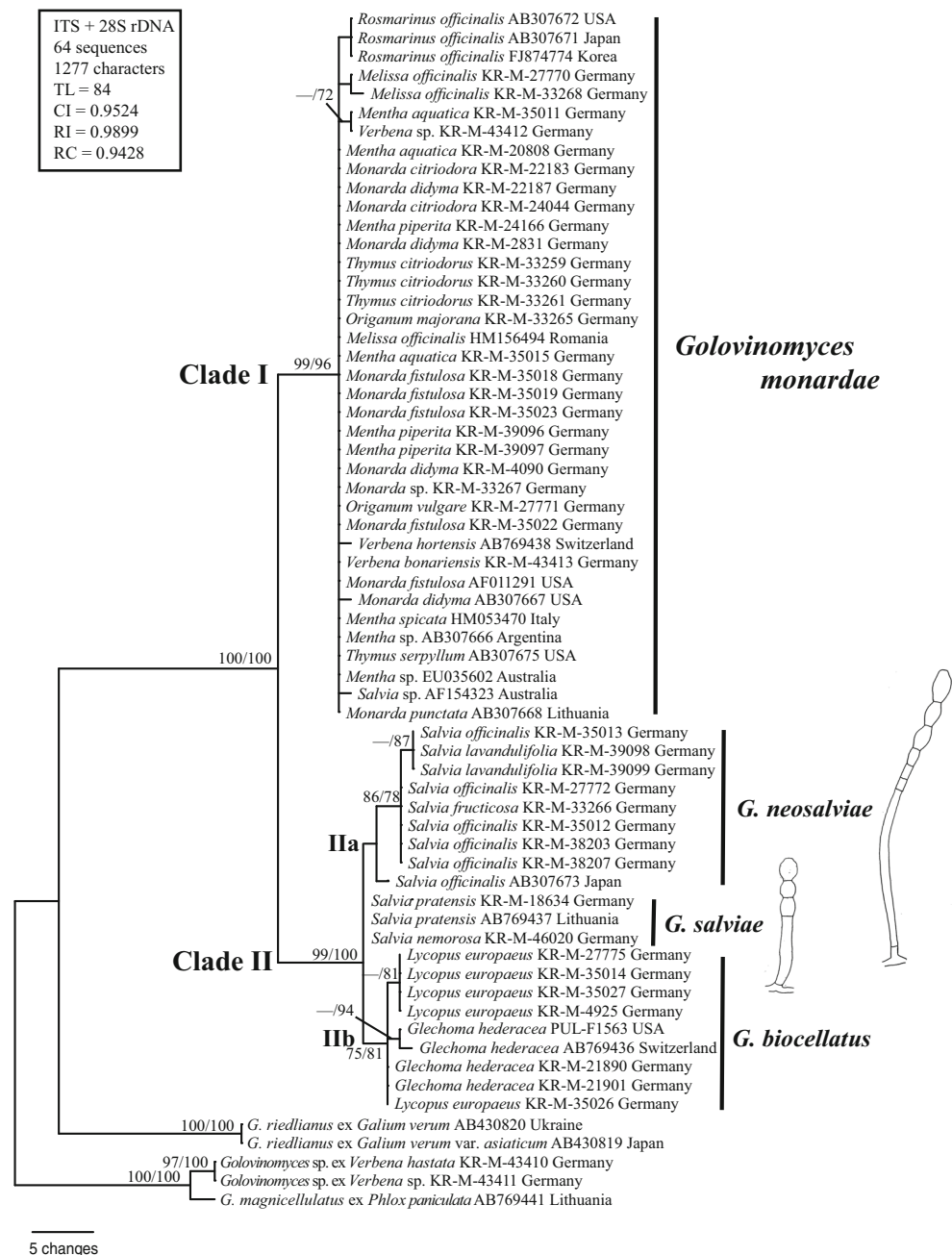
biocellatus complex are divided into two distinct large clades. Clade I consists of 37 sequences from the specimens on *Melissa*, *Mentha*, *Monarda*, *Origanum*, *Rosmarinus*, *Thymus* (Lamiaceae), and *Verbena* (Verbenaceae), and one sequence from a specimen on *Salvia* collected in Australia. This clade is homogeneous, and supported by strong BS supports (MP = 99 %, ML 96 %). Clade II is also supported by high BS values (MP = 99 %, ML = 98 %) and divided into two subgroups, IIa and IIb. Subgroup IIa is a paraphyletic group composed of specimens on *Salvia* spp. This group further splits into four small groups. Subgroup IIb consists of specimens on *Lycopus* and *Glechoma* and forms a distinct clade (MP = 75 %, ML = 81 %). Two sequences from the materials on *Verbena* did not belong to the *G. biocellatus* complex, and form a clade with *G. magnicellulatus* with high BS supports (MP = 100 %, ML = 100 %).

Taxonomy

Clades and subgroups resulting from our phylogeny (Fig. 1) are supported by features of the asexual morphs. Therefore,

we suggest to place species on *Melissa*, *Mentha*, *Monarda*, *Origanum*, *Rosmarinus*, *Thymus* (Lamiaceae), and *Verbena* (Verbenaceae) of clade I in a separate species, *G. monardae*. Specimens of subgroup IIb with host species of the genera *Lycopus* and *Glechoma* represent *G. biocellatus* s. str. Subgroup IIa consists of Old World specimens on *Salvia*. Major morphological differences (conidiophore length) between specimens on *S. fruticosa*, *S. lavandulifolia*, *S. officinalis*, and those on *S. pratensis* and *S. nemorosa* are supported by the taxonomy of the hosts. Both groups also form a distinct clade; however, the two clades have no strong bootstrap support. Despite low bootstrap support, but based on obvious morphological and host differences, we propose two species on *Salvia*, *G. salviae* comb. nov. and *G. neosalviae* sp. nov.

The following descriptions refer to asexual and partly sexual morphs of accepted species within the *G. biocellatus* complex:

Golovinomyces monardae (G.S. Nagy) M. Scholler, U. Braun & Anke Schmidt, **comb. nov.** (Figs. 2 and 3)
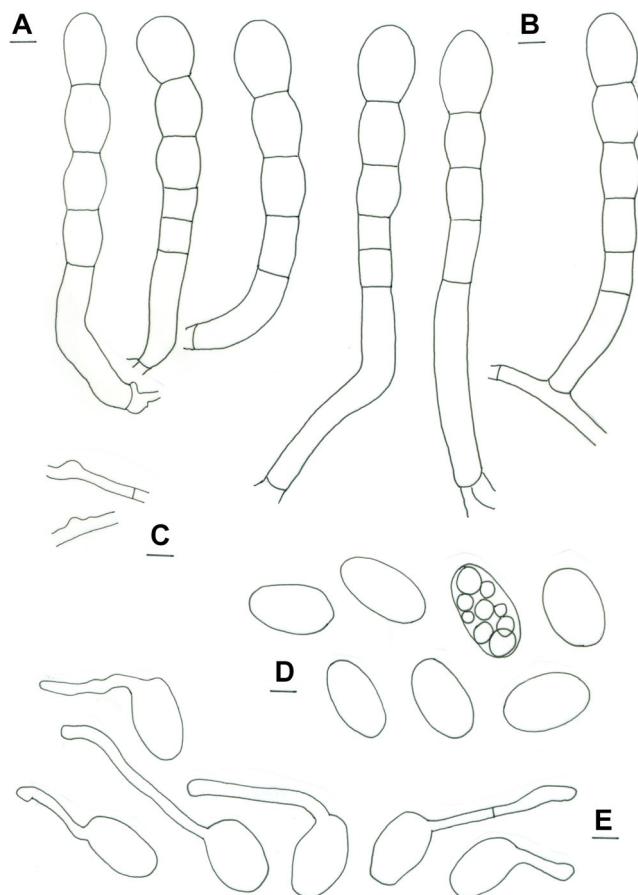
MycoBank, MB815612

Fig. 2 *Golovinomyces monardae* on *Monarda fistulosa* (KR-M-0035022). **a** Conidiophores (epiphyllous). **b** Conidiophores (on stems). **c** Appressoria. **d** Conidia, one with oil drops. **e** Germinated conidia. Bar = 10 μm (light microscopy drawing by A. Schmidt)
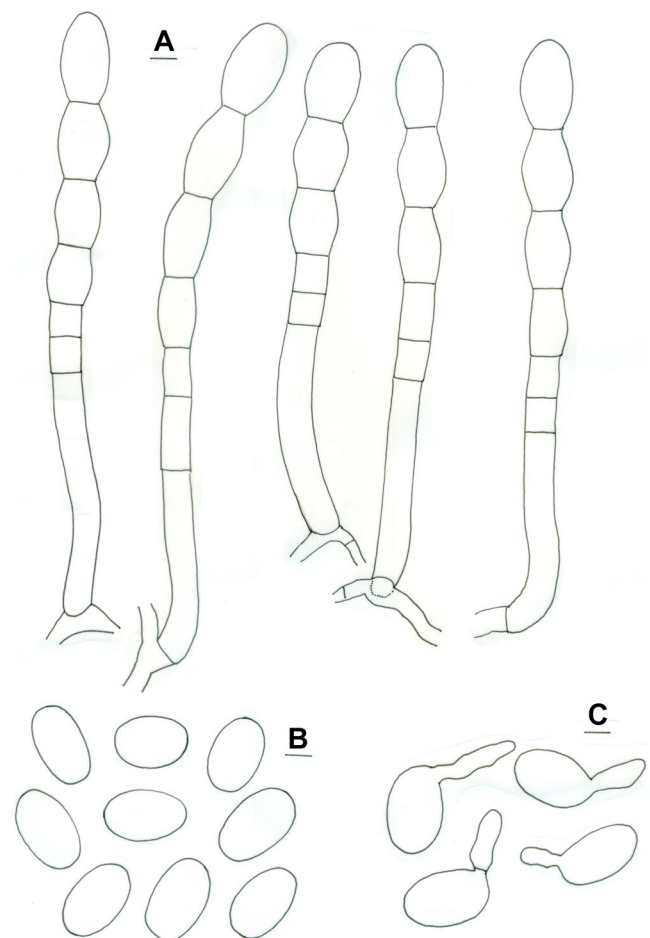

Fig. 3 *Golovinomyces monardae* on *Mentha × piperita* (KR-M-0024166). **a** Conidiophores (epiphyllous). **b** Conidia. **c** Germinated conidia. Bar = 10 μm (light microscopy drawing by A. Schmidt)

Basionym: *Erysiphe monardae* G.S. Nagy, Phytopathol. Z. 88: 285 (1977); holotype: Hungary, Budapest, garden of the University of Horticulture, on *Monarda didyma* L., 26 Aug. 1976, G.S. Nagy (HAL 2919 F).

≡ *E. biocellata* var. *monardae* (Nagy) U. Braun, Zentralbl. Mikrobiol. 137: 316 (1982).

= *E. cichoracearum* f. *menthae* Jacz., Karmany opredelitel' gribov. Vyp. 2: 217 (1927).

Mycelium more (e.g., on *Monarda*) or less (e.g., on *Origanum*) conspicuous, on stems and leaves, amphigenous, but more evident on the upper leaf surface, initially in patches, later effuse, often around leaf veins, whitish; hyphae hyaline, 4–8 μm wide; hyphal appressoria slightly or distinctly nipple-shaped, developing rarely in May to June, more frequently from July to August; conidiophores 50–145 μm long and 9–14 μm wide, partly widening from base to top, rarely about central on mother cell, mostly arising about 1/3–2/3 towards one end of the mother cell, no difference between epiphyllous and hypophyllous conidiophores, foot-cell 40–100(−140) μm long, mostly curved at the base, basal septum of the foot-cell partly raised above the junction with the hyphal mother cell, foot-cell followed by 0–3 shorter cells, forming catenescent conidia; conidia mostly ellipsoid, partly doliiform, swelling up in water, particularly in width, (24.4−)30−38(−46) × (16.4−)18–22(−24) μm, length/width ratio 1.5–2.0 (average 1.7); germ tubes subapically inserted, mostly without septum, rarely one-septate in short germ tubes, germ tubes mostly short to moderately long, sometimes also long, 11–84(−192) × 3.5–8.5 μm, terminating simply or in a club-shaped appressorium.

Sexual morph: see Braun and Cook (2012: 305), under *Golovinomyces biocellatus* (chasmothecia of *G. monardae* indistinguishable).

Specimens studied

On Lamiaceae: On *Melissa officinalis* L.: Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, flower-pot, 10 Jun. 2010, leg. A. Schmidt (KR-M-0027770) (GenBank LC076811); Baden-Württemberg, Karlsruhe, Grünwinkel, Silcherstr. 25, garden, 25 Jul. 2011, leg. M. Scholler (KR-M-0033268) (GenBank LC076821). On *Mentha aquatica* Ehrenb.: Germany, Schleswig-Holstein, Lübeck, Lauerholz, Wesloer Moor, 18 Sep. 1999, leg. A. Schmidt (KR-M-0020808) (GenBank LC076804); Schleswig-Holstein, Ostholstein near Malente between Kellersee and Krummsee, lakeside, 18 Sep. 2012, leg. A. Schmidt (KR-M-0035015) (GenBank LC076826); Mecklenburg-Vorpommern, Klein Schmölen, Schmölener Brack, bank, 20 Oct. 2012, leg. A. Schmidt (KR-M-0035011) (GenBank LC076822); Mecklenburg-Vorpommern, Klein Schmölen, Schmölener Brack, bank, 20 Oct. 2012, leg. A. Schmidt (KR-M-0035016). On *Mentha × piperita* L. '*chocolate*': Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, two flower-pots, 17 Jun. 2009, leg. A. Schmidt (KR-M-0024166) (GenBank LC076810); Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, flower-pot, 22 May 2014 (asexual state, KR-M-0039096) (GenBank LC076835) and 19 Jun. 2014 (teleom.; KR-M-0039097) (GenBank LC076836), leg. A. Schmidt. On *Monarda citriodora* Cerv. ex Lag.: Germany, Baden-Württemberg, Konstanz, botanical garden, 25 Jul. 2000, leg. H. Jage (KR-M-0022183) (GenBank LC076807); Sachsen-Anhalt, Halle (Saale), botanical garden, 02 Aug. 2004, leg. U. Braun (KR-M-0024044) (GenBank LC076809). On *Monarda didyma* L.: Armenia, Yerevan, botanical garden, 03 Sep. 1952, leg. S. Simonyan (HAL 1040 F); USA, Wisconsin, Sauke County, Parfrey's Glen, 03 Oct. 1956, H.C. Greene (HAL 1105 F); Germany, Bezirk Rostock, 1980, Neukloster, leg. H. Henker (HAL 1106 F); Baden-Württemberg, Isny, Großholzleute, Gasthof Adler, flowerbed, 28 Jun. 2008, leg. M. Scholler (KR-M-0002831) (GenBank LC076800); Baden-Württemberg, Konstanz, botanical garden, 25 Jul. 2000, leg. H. Jage (KR-M-0022187) (GenBank LC076808); Baden-Württemberg, Isny, Großholzleute, Gasthof Adler, flowerbed, 28 Jun. 2008, leg. M. Scholler (KR-M-004090) (GenBank LC076802). On *Monarda*-hybrid '*Prärienacht*': Germany, Hamburg, Klein-Flottbek, botanical garden, 16 Jul. 2011, leg. A. Schmidt (KR-M-0033267) (GenBank LC076820). On *Monarda fistulosa* L. '*Blaustrumpf*': Germany, Schleswig-Holstein, Herzogtum Lauenburg, Groß Grönau, An der Gärtnerei 1, Aeschlimann nursery, flower-pot, 27 Jun. 2012 (asexual state; KR-M-0035018) (GenBank LC076827) and 09 Sep. 2012 (teleom.; KR-M-0035019) (GenBank LC076828), leg. A. Schmidt, on *Monarda fistulosa* L. '*Schneewittchen*': Schleswig-Holstein, Herzogtum Lauenburg, Groß Grönau, An der Gärtnerei 1, Aeschlimann nursery, flower-pot, 27 Jun. 2012 (asexual state; KR-M-0035022) (GenBank LC076829) and 11 Sep. 2012 (teleom.; KR-M-0035023) (GenBank LC076830), leg. A. Schmidt. On *Origanum majorana* Moench: Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, flower-pot, 12 Jul. 2011, leg. A. Schmidt (KR-M-0033265) (GenBank LC076818). On *Origanum vulgare* L.: Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, flower-pot, 29 Jun. 2010, leg. A. Schmidt (KR-M-0027771) (GenBank LC076812). On *Thymus × citriodorus* (Pers.) Schreb. ex Schweigg.: Germany, Schleswig-Holstein, Ostholstein, Schürsdorf, Sandenredder 13, Rahlf nursery, flower-pot, 2 Aug. 2011 (asexual state; KR-M-0033259) (GenBank LC076815) and 25 Sep. 2011 (teleom.; KR-M-0033260) (GenBank LC076816), leg. A. Schmidt. On *Thymus × citriodorus* (Pers.) Schreb. ex Schweigg. '*Variegata*': Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, flower-pot, 2 Aug. 2011, leg. A. Schmidt (KR-M-0033261) (GenBank LC076817).

On Verbenaceae: On *Verbena bonariensis* L.: Germany, Brandenburg, Oranienburg, Schloßgarten, 26 Aug. 2009, leg. V. Kummer (KR-M-0043413) (GenBank LC076842). On *Verbena* sp.: Germany, Brandenburg, Potsdam, botanical garden, 27 Sep. 1996, leg. V. Kummer (KR-M-0043412).

The following specimens on *Verbena* cannot be assigned to a species known to occur on *Verbena* according to Braun and Cook 2012 (*Golovinomyces monardae*, *G. verbenae* (Schwein.) Heluta) and do not belong to the *G. biocellatus* complex (see outgroup in Fig. 1). Morphologically, these differ from the latter two species by a higher number of conidiophore cells (4–6 vs. 1–3):

On Verbenaceae: On *Verbena hastata* L.: Germany, Brandenburg, Potsdam, Botanical Garden, 25 Sep. 2003, leg. V. Kummer (KR-M-0043410) (GenBank LC076839). On *Verbena* sp.: Germany, Brandenburg, Oberspreewald-Lausitz, market place, 9 Sep. 2002, leg. V. Kummer (KR-M-0043411) (GenBank LC076840).

Golovinomyces biocellatus (Ehrenb.) Heluta, Ukrayins'k. Bot. Zhurn. 45: 62 (1988) emend. M. Scholler, U. Braun & Anke Schmidt (Figs. 4, 5, and 6)
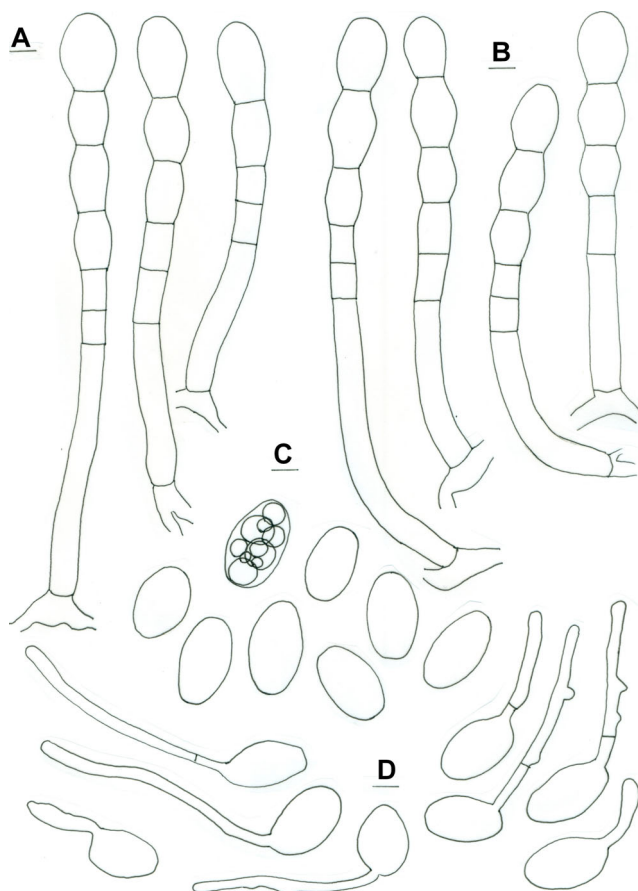

Fig. 4 *Golovinomyces biocellatus* on *Lycopus europaeus* (KR-M-0035014). **a** Conidiophores (epiphyllous). **b** Conidiophores (hypophyllous). **c** Conidia, one drawn with oil drops. **d** Germinated conidia. Bar = 10 μm (light microscopy drawing by A. Schmidt)
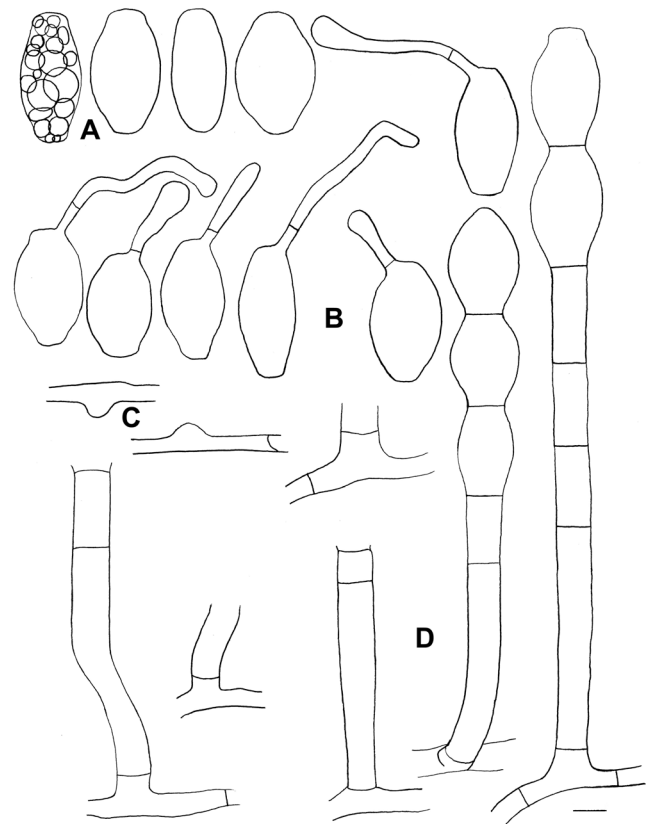

Fig. 5 *Golovinomyces biocellatus* on *Glechoma hederacea* (PUL F1563). **a** Conidia, one drawn with oil drops. **b** Germinated conidia. **c** Hyphal appressoria. **d** Conidiophores. Bar = 10 μm (light microscopy drawing by M. Scholler)

≡ *Erysiphe biocellata* Ehrenb., Nova Acta Phys.-Med. Acad. Caes. Leop.-Carol. Nat. Cur. 10: 211 (1821); lectotype [designated by Braun (1987)]: Ehrenberg (1821: Pl. 13, original drawing).

≡ *Erysibe biocellata* ("*biocellaris*") (Ehrenb.) Link, Sp. pl. 4, 6(1): 109 (1824).

≡ *Erysiphe communis* f. *biocellata* (Ehrenb.) Fr., Syst. Mycol. 3: 239 (1829).

= *Oidium erysiphoides* Fr., Syst. Mycol. 3: 432 (1832).

≡ *Euoidium erysiphoides* (Fr.) Y.S. Paul & J.N. Kapoor, Indian Phytopathol. 38: 762 (1985), nom. inval. (ICN, Art. 33.3).

≡ *Euoidium erysiphoides* (Fr.) Y.S. Paul & J.N. Kapoor, Indian J. Mycol. Pl. Pathol. 17: 302 (1987), nom. inval. (ICN, Art. 33.3).

= *E. cichoracearum* f. *lycopi* Jacz. (Jaczewski 1927: 216).

Mycelium not conspicuous, on stems and leaves, amphigenous, rarely in patches, mostly effuse, often around leaf veins, thin, whitish to grayish; hyphae hyaline, 4–6 μm wide; hyphal appressoria not frequently formed, nipple-shaped; conidiophores 70–145 μm long and (8–) 10–12(–14) μm wide, partly increasing from base to top, erect or curved at the base, rarely about central on mother cell, mostly arising about 1/3–2/3 or 1/4–3/4 towards one end of
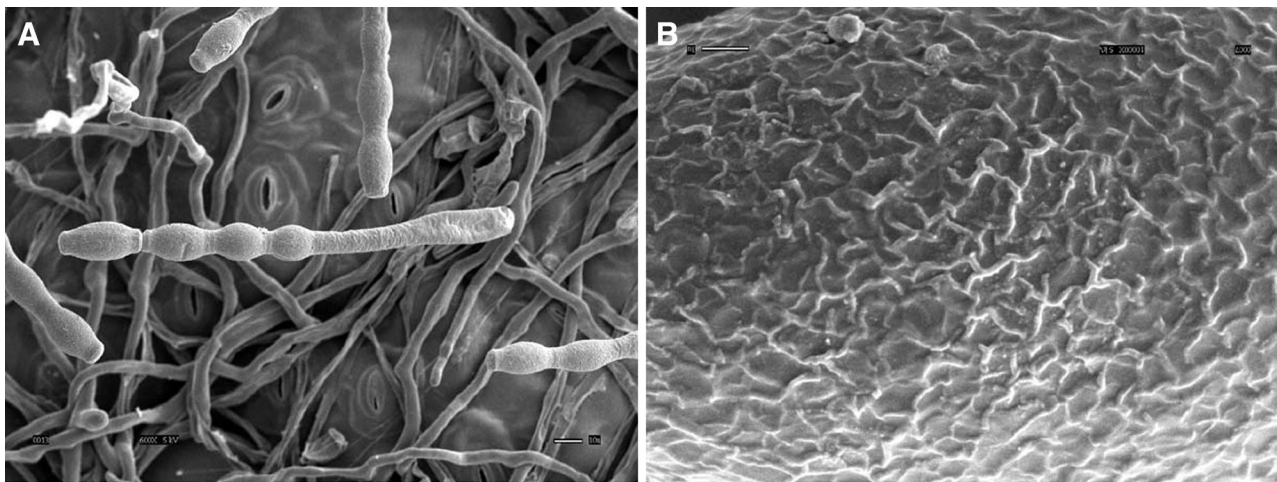
Fig. 6 *Golovinomyces biocellatus* on *Glechoma hederacea* (PUL F1563). **a** Conidiophore. **b** Conidial surface with almost reticulate ridges (SEM pictures by M. Scholler)

the mother cell, no difference between epiphyllous and hypophyllous conidiophores, foot-cell, longest cell of conidiophore, 55–100(−130) μm long, basal septum of the foot-cell partly raised above the junction with the hyphal mother cell, foot-cell followed by 0–3 shorter cells, forming catenescent conidia; conidia predominantly doliiform to limoniform, occasionally ellipsoid, 28–36(−43) × (18–)19–24(−26) μm, length/width ratio 1.3–2.0 (average 1.6), surface (SEM) polygonal, almost reticulate ridges; germ tubes subapically inserted, non- to one-septate (in relation about 1:1), mostly short to moderately long, sometimes also long, 24–72(−156) × 2.4–6.0 μm, terminating simply or in a club-shaped appressorium.

Sexual morph: see Braun and Cook (2012: 305).

On *Glechoma hederacea* L.: USA., Indiana, West Lafayette, Purdue University, greenhouse near Lilly Hall, 17 Sep. 2001, leg. M. Scholler (PUL F1563) (GenBank KX229701); Germany, Schleswig-Holstein, Lübeck, Israelsdorf, Holunderweg 5, garden, 04 Jul. 2007, leg. A. Schmidt (KR-M-21890) (GenBank LC076805); Niedersachsen, near Wulmstorf, Röttiger-barracks, way to the former military training area, 25 Aug. 2007, leg. A. Schmidt (KR-M-0021901) (GenBank LC076806). On *Lycopus europaeus* L.: Germany, Baden-Württemberg, Karlsruhe, Schloßpark, edge of the pond, 09 Jul. 2010, leg. A. Schmidt (KR-M-0027775) (GenBank LC076814); Schleswig-Holstein, Lübeck, St. Gertrud, city park, lakeside, 26 Aug. 2012, leg. A. Schmidt (KR-M-0035014) (GenBank LC076825); Schleswig-Holstein, Lübeck, St. Gertrud, city park, lakeside, 26 Aug. 2012 (asexual state; KR-M-0035026) (GenBank LC076831) and 14 Oct. 2012 (teleom.; KR-M-0035027) (GenBank LC076832), leg. A. Schmidt.

Fig. 7 *Erysiphe labiatarum* f. salviae (*Golovinomyces salviae*) on *Salvia pratensis* (Holotype of *G. salviae*, LEP 130001). **a** Leaves. **b** Chasmothecia. **c** Ascospores (light microscopy picture by P. Gannibal)
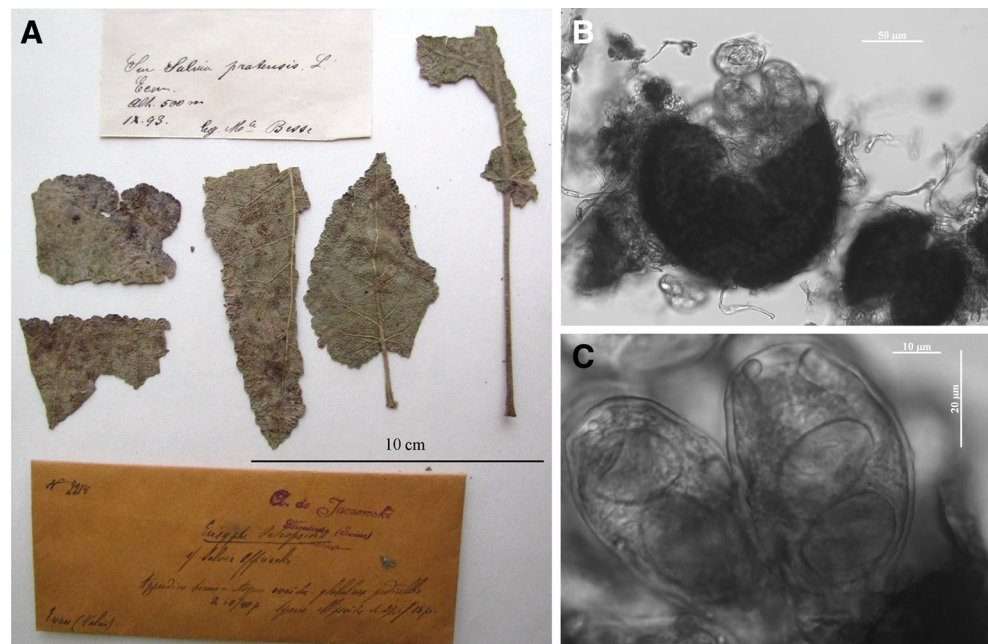
Golovinomyces salviae (Jacz.) M. Scholler, U. Braun & Anke Schmidt, **comb. nov.** (Figs. 7, 8, and 9)

MycoBank, MB815613

Basionym: *Erysiphe labiatarum* f. *salviae* Jacz., Karmanyi opredelitel' gribov. Vyp. 2. Muchnisto-rosyanye griby: 162 (1927) (type hosts: *Salvia glutinosa* L., *S. nutans* L. *S. pratensis* L., *S. silvestris*, *S. verticillata*, *S. virgata*, *S.* sp.); lectotype* (designated here, MycoBank, MBT203828): On *S. pratensis*, Switzerland, Valais (Wallis), Riddes, Ecône, Sep. 1893, 500 m, leg. Abbé Besse (LEP 130001 ex herb. A. de Jaczewski 2214). *Note: Jaczewski (1927) introduced f. *salvia* for collections on several *Salvia* species, but without specification of the type material. He cited particular hosts and localities in Russia and mentioned that this forma also occurs in west Europe. The lectotype on *Salvia pratensis* is a collection from Jaczewski's herbarium collected before 1927, which is accordingly qualified for a lectotypification.

≡ *E. salviae* (Jacz.) S. Blumer, Beitr. Krypt.-Fl. Schweiz 7(1): 273 (1933).

? = *Oidium hormini* Farneti, Atti Ist. Bot. Univ. Pavia 7: 255 (1902).

= *Oidium verbenacea* Pass., in Thüm., Mycoth. Univ., Cent. VIII, No. 789, Klosterneuburg 1877; lectotype (designated here, MycoBank, MBT203829): On *Salvia verbenaca*, Italy, Parma, May 1876, G. Passerini, Thüm., Mycoth. Univ. 789 (M-0014092).
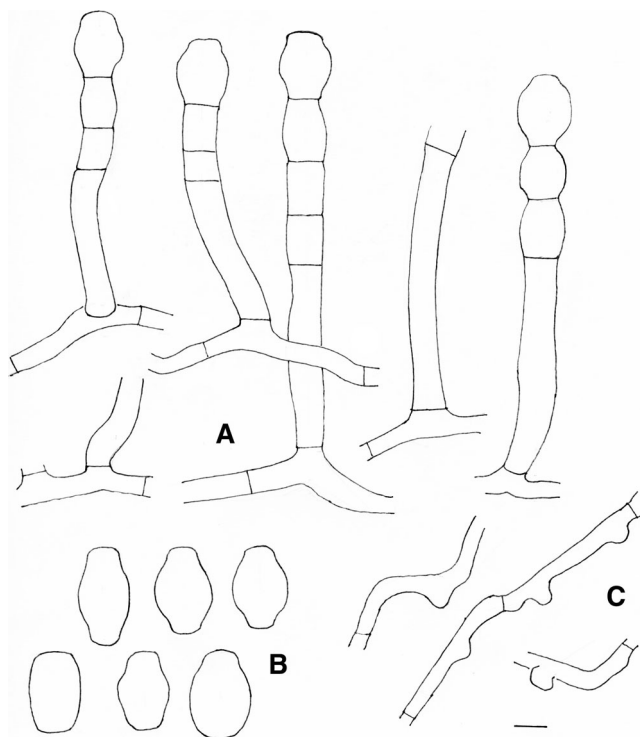

Fig. 8 *Golovinomyces salviae* on *Salvia pratensis* (KR-M-18634). **a** Conidiophores (epiphyllous). **b** Conidia. **c** Appressoria. Bar = 10 μm (light microscopy drawing from dried material by M. Scholler)
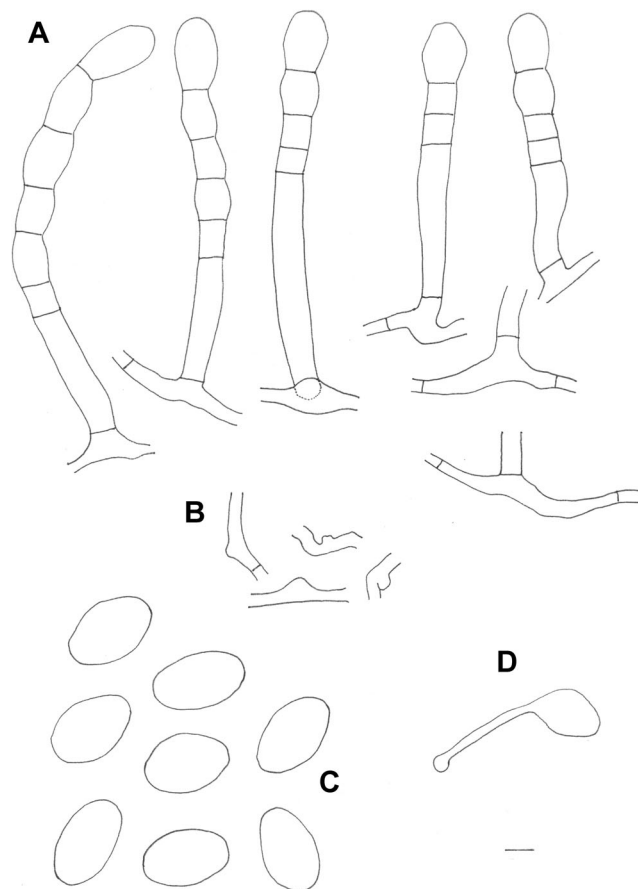

Fig. 9 *Golovinomyces salviae* on *Salvia nemorosa* (KR-M-46020). **a** Conidiophores. **b** Appressoria. **c** Conidia. **d** Conidium with germ tube. Bar = 10 μm (**a**, **b**, **d** from dried material, **c** from fresh material; light microscopy drawing by A. Schmidt)

? = *E. cichoracearum* f. *salviae* Koshk., Bot. Mater. Otd. Sporov. Rast. Bot. Inst. Komarova Akad. Nauk S. S. S. R. 14: 123 (1961) (type host *S. virgata*).

= *E. simplex* Heluta, Ukrayins'k. Bot. Zhurn. **43**(5): 53 (1986), nom. illeg. (nom. superfl.).

≡ *Golovinomyces simplex* (Heluta) Heluta, Ukrayins'k. Bot. Zhurn. **45**(5): 63 (1988).

Mycelium conspicuous, on leaves, hypophyllous or amphigenous, sometimes causing reddish spots epiphyllously, in distinct patches (later effuse), whitish to grayish; hyphae hyaline, 4–7(−10) μm wide; hyphal appressoria nipple-shaped to slightly lobed; conidiophores (50–)60–140 μm long and 8–15 μm wide (sometimes basal cell swelling up to 20 μm in the middle), erect or curved at the base, formed about 1/3–2/3 or 1/4–3/4 towards one end of the mother cell, basal septum of the foot-cell mostly raised 5–15 μm above junction with mother cell, 1–3(−4) cells, basal cell longest, 30–100(−120) μm, followed by 0–3 shorter cells, forming catenescent conidia; conidia doliiform, rarely cylindrical, (25–)30–36(−45) × (15–)20–24 μm, length/width ratio 1.3–1.7 (average 1.5); germ tube terminating in a club-shaped appressorium.

Sexual morph: Chasmothecia scattered to gregarious, subglobose, (75–)90–125 μm diam., peridium cells irregularly polygonal to daedaleoid, 5–25 μm diam., appendages numerous, arising from the lower half, mycelioid, simple, rarely branched, 0.5–2 times as long as the chasmothecial diam., 3–7 μm wide, septate, hyaline to pale brown, thin-walled, smooth or almost so, asci 5–13 per chasmothecium, broadly ovoid-saccate, 40–80 × 25–45 μm, subsessile to short-stalked, thin-walled, without distinct terminal oculus, 2-spored, ascospores ellipsoid, 17–28 × 10–17 μm, colorless.

On *Salvia pratensis* L.: Germany, Baden-Württemberg, Owingen, NSG an der Eyach N Weilerkirche, dry grassland, south slope, 19 Aug. 2005, leg. M. Scholler (KR-M-0018634) (GenBank LC076803). On *Salvia nemorosa* L.: Germany, Berlin, Kreuzberg, entrance to Marheinikeplatz 13, flowerpot, 04 Sep. 2015, leg. M. Scholler (KR-M-0046020) (GenBank LC100001).

Further specimens studied with light microscopy

On *Salvia pratensis* L.: Germany, Baden-Württemberg. Kreis Aalen, Goldberg near Goldburghausen, 23 Sep. 1967, H. & H. Doppelbaur (asexual state; M-0014107); Baden-Württemberg. Kreis Wangen, Großholzleute, 3 Sep. 1964, O. Klement (asexual state; M-0014106); Bavaria, Nördlingen, 19 Sep. 1955, K. Ruttmann (asexual state; M-14120); Bavaria, Schwaben, Landkreis Günzburg, Offingen, 25 Sep. 1968, H. & H. Doppelbaur (asexual state; M-0014118); Saarland, Habkirchen, Bliestal, 1 Sep. 2012, U. Richter (asexual state; HAL 2977 F).

On *Salvia nutans* L.: Ukraine, Melitopol, Altagir, 2 June 1984, leg. U. Braun (asexual and sexual state; HAL 1049 F).

On *Salvia verbenaca* L. Italy, Parma, May 1876, Passerini, Thüm., Mycoth. Univ. 8789 (asexual state; M-0014092).

On *Salvia verticillata* L.: Armenia, Stepanavan, Dendropark "Sosnyaki", 10 Sep. 1949, D. N. Babayan (asexual and sexual state; HAL 1048 F). Germany, Bavaria, Oberbayern, Landkreis Altöttingen, Burghausen, Alter Burgberg, 2 Sep. 1969, H. & H. Doppelbaur (asexual state; M-0014119). Romania, distr. Craiova, Oltenia, garden, 17 Oct. 1965, I. Comes et al., Flora Olteniae Exsiccata 542 (asexual and sexual state; M-0014108, ZT Myc 55370). Russia, Bashkortostan, Ufa, slope on the river Belaya with steppe vegetation, 5 Jul. 1977, U. Braun 514 (asexual and sexual state; HAL 1341 F). Switzerland, Cantoin de Vaud, between Montcherand and Orbe, 17 Sep. 1943, E. Mayor (ZT Myc 55369); Canton Neuchâtel, between Frensens and Vernéaz, 11 Jul. 1951, E. Mayor (ZT Myc 55365).

Golovinomyces neosalviae M. Scholler, U. Braun & Anke Schmidt, **sp. nov.** (Figs. 10 and 11)

MycoBank, MB815614

Mycelium conspicuous, on stems and leaves, amphigenous, but mainly epiphyllous, initially in distinct patches, later effuse, covering the entire surface of the
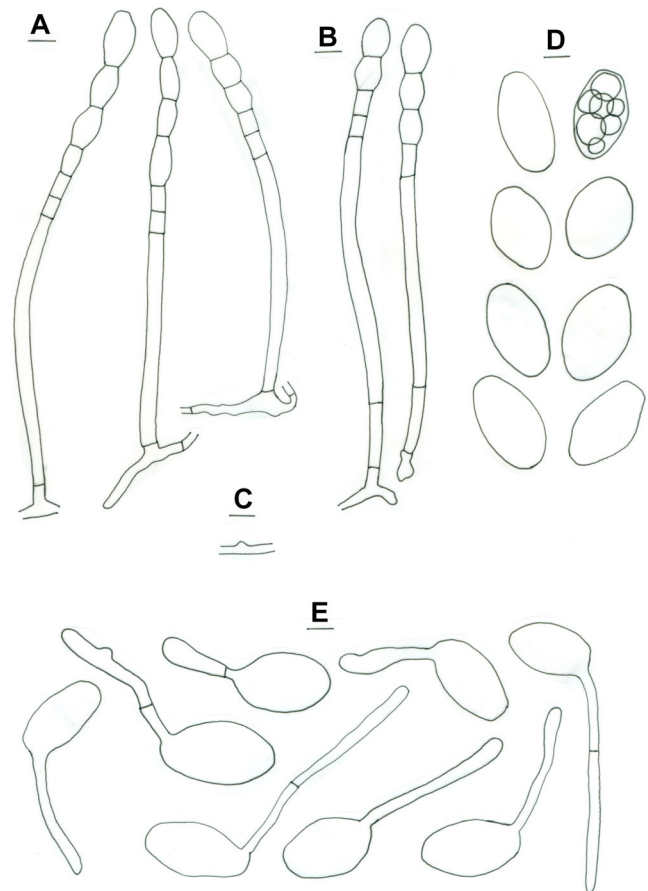
Fig. 10 *Golovinomyces neosalviae* on *Salvia officinalis* (KR-M-0035013; holotype). **a** Conidiophores (epiphyllous). **b** Conidiophores (hypophyllous). **c** Appressorium. **d** Conidia, one with oil drops. **e** Germinated conidia. Bar = 19.5 μm (**a**, **b**), bar = 10 μm (**c**, **d**, **e**) (light microscopy drawing by A. Schmidt)

leaves, whitish to grayish, infected leaves soon become yellowish and falling off already in July or August, after that fresh leaves growing without any infection; hyphae hyaline, 4–7 μm wide; hyphal appressoria not frequently formed, nipple-shaped to slightly lobed; conidiophores 140–400 μm long and (8–)9–13(–14.5) μm wide, often increasing from base to top, erect or curved at the base, central on mother cell or formed about 1/3–2/3 or 1/4–3/4 towards one end of the mother cells, no difference between epiphyllous and hypophyllous conidiophores, basal septum of the foot-cell always raised 7–25 μm above junction with mother cell, size and arrangement of cells very variable, mostly at the base with 1–3 cells between 45 and 75(–115) μm long, followed by the longest cell of 105–205 μm, followed by 0–3 shorter cells, or, rarely, foot-cell, longest cell of conidiophore, followed by 1–3 shorter cells, forming catenescent conidia; conidia distinctly doliiform to limoniform, rarely ellipsoid, swelling up in water, particularly in width, (28.5–)33–44(–47.5) × (20–)22–26(–28) μm, length/width ratio 1.2–2.2 (average 1.7); germ tubes subapically inserted, non- to
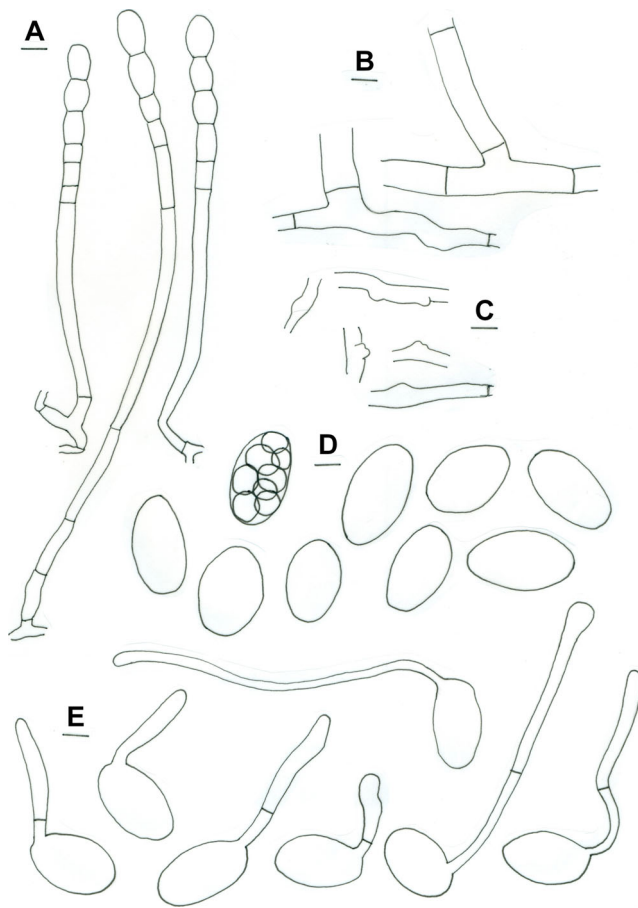
Fig. 11 *Golovinomyces neosalviae* on *Salvia fruticosa* (KR-M-0033266). **a** Conidiophores (epiphyllous). **b** Conidiophore bases. **c** Appressorium. **d** Conidia, one with oil drops. **e** Germinated conidia. Bar = 19.5 μm (**a**), bar = 10 μm (**b**, **c**, **d**, **e**) (light microscopy drawing by A. Schmidt)

one-septate (in relation about 1:2), mostly short to moderately long, sometimes also long, 18–96(−170) × 2.4–10.8 μm, terminating simply or in a club-shaped appressorium.

Sexual morph: Chasmothecia scattered to subgregarious, subglobose, 100–135 μm diam., peridium cells irregularly polygonal to daedaleoid, 7–30 μm diam., appendages numerous, arising from the lower half, mycelioid, simple, rarely branched, about as long as the chasmothecial diameter or shorter, 3–8 μm wide, septate, hyaline to pale brown towards the base, thin-walled, smooth or almost so, asci 5–10 per chasmothecium, broadly ovoid-saccate, subcylindrical or somewhat asymmetric, 50–80 × 25–45 μm, subsessile to distinctly stalked, thin-walled (to 1 μm), without distinct terminal oculus, 2-spored, ascospores ellipsoid to somewhat ellipsoid-ovoid, 18–35 × 12–18 μm, colorless.

Holotype: On *S. officinalis* L. 'Purpurascens': Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, two flower-pots, 04 Jun. 2012, leg. A. Schmidt (KR-M-0035013) (GenBank LC076824).

Further specimens studied

On *S. fruticosa* Miller: Germany, Baden-Württemberg, Karlsruhe, botanical garden, 06 Jul. 2011, leg. A. Schmidt (KR-M-33266) (GenBank LC076819). On *S. lavandulifolia* Vahl: Germany, Schleswig-Holstein, Ostholstein, Schürsdorf, Sandenredder 13, Rahlf nursery, two flower-pots, 07 Jun. 2014 (asexual state; KR-M-39098) (GenBank LC076837) and 03 Jul. 2014 (teleom.; KR-M-39099) (GenBank LC076838), leg. A. Schmidt. On *Salvia* cf. *officinalis* L.: Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, flower-pot, 10 Jun. 2010, leg. A. Schmidt (KR-M-27772) (GenBank LC076813). On *S. officinalis* L. 'Icterina': Germany, Schleswig-Holstein, Lübeck, Grootkoppel 6, Flora nursery, three flower-pots, 04 Jun. 2012, leg. A. Schmidt (KR-M-35012) (GenBank LC076823). On *S. officinalis* L.: Germany, Schleswig-Holstein, Lübeck, St. Gertrud, Grootkoppel 6, Flora nursery, flower-pot, 01 Jun. 2013, leg. A. Schmidt (KR-M-38207) (GenBank LC076834); Schleswig-Holstein, Herzogtum Lauenburg, Groß Grönau, An der Gärtnerei 1, Aeschlimann nursery, flower-pot, 04 Jun. 2013, leg. A. Schmidt (KR-M-0038206); Schleswig-Holstein, Lübeck, St. Gertrud, Wesloer Landstraße 72–74, OBI, flower-pot, 05 Jul. 2013 (asexual state; KR-M-0038203) (GenBank LC076833) and 03 Aug. 2013 (teleom.; KR-M-0038204), leg. A. Schmidt. Switzerland, Neuchâtel, garden, 15 Aug. 1944, leg. E. Mayor (ZT Myc 55367. 55369).

Key to the recognized species of the *Golovinomyces biocellatus* complex and other *Golovinomyces* spp. on Verbenaceae and Lamiaceae based on features of the asexual morph:

On Verbenaceae (*Verbena* spp.):

1 Conidiophores curved at base2

1* Conidiophores not curved at base*Golovinomyces verbenae*

2 Conidiophore cells 4–6*G.* sp.

2* Conidiophore cells 1–33

3 Conidia sometimes below 16 μm wide*G. orontii s. lat.* (= *G. monardae*?)

3* Conidia almost never below 16 μm wide*G. monardae*

On Mentheae

1 On *Salvia* s. str. (*Salvia* clade 1 species, Walker et al. 2004), conidiophores either short (60–140 μm) or long (140–400 μm)2

1* Not on *Salvia* s. str., conidiophores 70–145 μm3

2 Conidiophores 60–140 μm long*Golovinomyces salviae*

2* Conidiophores 140–400 μm long*G. neosalviae*

3 Conidia mostly ellipsoid, conidial germ tubes non-septate, very rarely one-septate

G. monardae

3*Conidia mostly doliiform to limoniform, conidial germ tubes often one-septate

G. biocellatus

Discussion

Powdery mildew on lamiaceous hosts, nowadays belonging to *Golovinomyces*, were previously assigned to *Erysiphe cichoracearum* sensu *latissimo* (Salmon 1900, Jaczewski 1927) comprising most species of the current genus *Golovinomyces* ("*Erysiphe* sect. *Euerysiphe*" sensu Blumer 1967). Blumer (1933, 1967) split *E. cichoracearum s. lat.* into several species, recognized the corresponding powdery mildews on Lamiaceae as a distinct species, and reintroduced the name *Erysiphe biocellata*. However, Blumer (1933) excluded the powdery mildew on *Salvia* species, treated it as a separate species, and introduced the combination *Erysiphe salviae*, based on *E. labiatarum* f. *salviae* (Jaczewski 1927). Braun (1987) used a broad concept for the name *Erysiphe biocellata*, encompassing all corresponding collections on lamiaceous hosts, including those on *Salvia* spp., i.e., *E. salviae* was reduced to synonymy with *E. biocellata*, but without any discussion. Braun (1995) followed the broad concept of *E. biocellata*, but added a brief discussion. Braun and Cook (2012) maintained this broad concept, provided a more detailed discussion, and emphasized the necessity of comprehensive biological and molecular examinations for the expected further splitting of *G. biocellatus* into smaller taxonomic units. Hence, the present investigations aimed at clarifying the unresolved phylogeny and taxonomy of the *G. biocellatus* complex. The results are clear and convincing with respect to host range and morphology. Concerning the host range, we could not confirm *G. biocellatus s. lat.* on *Ajuga*, *Scutellaria*, *Stachys*, or *Teucrium*, which belong to host subfamilies other than Nepetoideae. The powdery mildews on these plants were often named *Golovinomyces biocellatus* (and listed accordingly in the recent literature, e.g., in Braun and Cook 2012; Klenke and Scholler 2015), but turned out to be *Neoerysiphe galeopsidis* (DC.) U. Braun. We think that species of the *G. biocellatus* complex are restricted to members of the Lamiaceae subfamily Nepetoideae tribe Mentheae, although occasional infections of plants of other Lamiaceae subfamilies, tribes, or related families such as Verbenaceae (see below) may occur. The phylogenetic data are unambiguously in favor of two well-supported clades, I and II (Fig. 1). Clade I including *Monarda* spp. and numerous other host plant species and genera is considered an own species for which the name *Erysiphe monardae* is available. This species differs from *G. biocellatus* in morphological traits of its asexual morphs. The sexual morphs (chasmothecia) of the two species are little differentiated and barely diagnostic, which is, however, not surprising since fruiting bodies in *Golovinomyces* are rather uniform and, for taxonomic purposes, only of very limited use. Taking these results into account, the new combination *Golovinomyces monardae* is introduced. This plurivorous species occurs, as far as known and examined, on diverse hosts of genera belonging to the Nepetoideae and is obviously able to infect *Verbena* spp., at least accidentally. Braun and Cook (2012) recognized two species of the genus *Golovinomyces* on *Verbena* spp., viz. *G. verbenae* (Schwein.) Heluta and the plurivorous *G. orontii* (Castagne) Heluta. Previous records of *G. orontii* (= *Erysiphe polyphaga*) on *Verbena* spp. possibly refer to *G. monardae*. An additional, possibly undescribed species characterized by up to six conidiophore cells was found within the framework of this study. It clusters outside the *G. biocellatus* complex in our phylogeny (Fig. 1). Provided that *G. monardae* is different from *G. orontii*, four species of *Golovinomyces* occur on *Verbena* (see key).

Golovinomyces on *Salvia* spp. represents a particular case. All sequences retrieved from *Salvia* powdery mildew included in the present analysis cluster adjacent to the *G. biocellatus s. str.* clade (clade IIa), but in any case in separate groups, i.e., not mixed with *G. biocellatus* sequences. There is no bootstrap support for the *Salvia* powdery mildew at the species level, at least by ITS and LSU data, but the conidiophores and conidia are quite distinct from those of *G. biocellatus* (see descriptions and key) and due to rather broad, doliiform–limoniform conidia, they are rather reminiscent of the asexual state of species belonging to *Golovinomyces* sect. *Depressi* (U. Braun) U. Braun (Braun and Cook 2012), which is, however, a non-phylogenetic section just based on morphology. Two groups within clade IIa may be distinguished, one with three related host plants (*S. officinalis*, *S. lavandulifolia*, *S. fruticosa*) and very long conidiophores, and another one with two related host plants (*S. pratensis*, *S. nemorosa*) with very short conidiophores. Although the two features are not supported by sequence data, we think that the morphological and the host range data suggest that powdery mildews on *Salvia* spp. should be better considered as two species of their own, which we call *G. salviae* (lectotype host *S. pratensis*) and *G. neosalviae* (type host *S. officinalis*). All host species belong to clade I in the *Salvia* phylogeny performed by Walker et al. (2004). ITS sequences are often not sufficient for a reliable resolution at the species level (Kovács et al. 2011; Kiss 2012). Additional markers are probably necessary. Furthermore, there are additional *Salvia* species reported to be hosts of *G. salviae* that have not yet been included in the phylogenetic analyses. Characters of the chasmothecia, above all the size of the fruiting bodies, are rather variable and were previously used for the differentiation between particular species in the *G. biocellatus* complex. Blumer (1933) and Heluta (1989) confined *E. salviae* (*E. simplex*) to collections with relatively small chasmothecia. Blumer (l.c.) included *Salvia glutinosa*, *S. pratensis*, and *S. verticillata* as hosts, whereas Heluta (1989) cited only *S. glutinosa* and *S. verticillata*, and assigned collections on *S. pratensis* and other hosts to *G. biocellatus*. However, based on Braun and Cook (2012) and our own examinations, the chasmothecial size is rather variable and barely applicable

for taxonomic purposes. The key to solve the taxonomic problems around *Salvia* powdery mildew lies in the asexual morph, which has been little and insufficiently examined until now. The biological specialization of *Salvia* powdery mildews is barely known. Laibach (1930) carried out inoculation experiments and stated that powdery mildew from *Salvia verticillata* was not able to infect *S. pratensis*, and with powdery mildew from *S. pratensis*, he could successfully infect *S. dumetorum*, *S. pratensis* × *sylvestris*, but not *S. glutinosa*, *S. verticillata*, *S. officinalis*, and other hosts, suggesting that biological specialization may occur among powdery mildew on *Salvia* spp., but modern examinations under verifiable conditions are not available. The study of the *Golovinomyces* species on *S. glutinosa*, which we could not examine molecularly, will be of particular interest because this plant belongs to *Salvia* clade III species and not clade I, as demonstrated in the phylogeny of Walker et al. (2004). Several herbarium specimens on *S. glutinosa* (only asexual morphs) have been examined [Germany, Baden-Württemberg, Geißkopf, 3 Sept. 1964, O. Klement 12993 (M-0014104); Bavaria, Rosenheim, between Gattern and Kraimoos, 25 Aug. 1934, H. Poeverlein (M-0014115); Bavaria, Rosenheim, near Aschau, 20 Aug. 1934, H. Poeverlein (M-0014116); Bavaria, Rosenheim, above Hohenaschau, 24 Aug. 1934, H. Poeverlein (M-0014117). Romania, Baile Herculeane, Valea Cernei, 8 Sep. 1962, E. Eliade (M-0014105). Switzerland, Canton Neuchâtel, Neuchâtel, Candolles, 21 Sep. 1945, E. Mayor (ZT Myc 55366)]. Conidiophores and conidia of all samples agree well with *G. salviae*, but since sequence data retrieved from *S. glutinosa* powdery mildew are not yet available, we refrain from a final taxonomic conclusion. Also, *Salvia* sp. from Australia hosting *G. monardae* (AF154323, see Fig. 1) may be a *Salvia* clade III species.

Due to the fact that we could not confirm *Ajuga*, *Stachys*, or *Teucrium* as hosts of *G. biocellatus s. lat.*, there is evidence that species of the *G. biocellatus* complex are host-specific and restricted to species of the Mentheae within the Lamiaceae (apart from occasional infections of *Verbena* spp.). The present analyses are, of course, just a first step in resolving problems and open questions around the *G. biocellatus* complex. Although the results are unequivocal, the morphological examinations of asexual morphs and phylogenetic data were mainly focused on Central European collections. A broader sampling is necessary for a more detailed verification of the distribution of the particular species.

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