

Early evolution of endoparasitic group in powdery mildews: molecular phylogeny suggests missing link between *Phyllactinia* and *Leveillula*

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Abstract: Of the 17 genera of the Erysiphaceae, only four genera (viz. *Leveillula*, *Phyllactinia*, *Pleochaeta* and *Queirozia*) exhibit (partly) endoparasitism. To investigate early evolution of this endoparasitic nature, we performed molecular phylogenetic analyses of powdery mildews belonging to the tribe Phyllactinieae collected in North and South America. The most ancestral taxa in the tribe Phyllactinieae belong to the *Pleochaeta/Queirozia* group, from which the genus *Phyllactinia* was derived. Finally, the truly endoparasitic genus *Leveillula* emerged from a part of *Phyllactinia*. The present study showed clear evolutionary polarity in the powdery mildews concerned (that is, partly endoparasitic group evolved from ectoparasitic group) and then a truly endoparasitic group emerged from a partly endoparasitic group. In addition, a group with distinctly dimorphic conidia proved to be basal in the Phyllactinieae, and a group without distinctly dimorphic conidia was derived from that group. The present analyses clearly showed that *Leveillula* derived from a part of the “Basal *Phyllactinia* group”. However, all sister taxa to *Leveillula* were distributed in North and South America. Because the putative geographic origin of *Leveillula* is assumed to be Central and Western Asia or the Mediterranean region, we postulate a missing link during the evolution of *Leveillula* from *Phyllactinia*. Based on the present phylogenetic studies and the new rules of the

International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012), the following new species and taxonomic re-allocations are proposed: *Phyllactinia bougainvilleae* sp. nov., *Ph. caricae* comb. nov., *Ph. caricicola* comb. nov., *Ph. durantae* comb. nov., *Ph. leveilluloides* sp. nov., *Ph. obclavata* comb. nov., and *Ph. papayae* comb. nov.

Key words: Erysiphaceae, Erysiphales, Phyllactinieae, *Pleochaeta*, *Queirozia*

INTRODUCTION

The fungi belonging to the Erysiphales, Ascomycota, are obligate biotrophs that cause diseases called powdery mildew on ca. 10 000 angiospermous plant species (Amano 1986). A total of 873 species covering 16 genera with sexual morphs and one genus with asexual morphs have been described worldwide (Braun and Cook 2012). All species belonging to this order are consistently phytoparasitic. In addition, 13 of the 17 genera exhibit ectoparasitism, in which most fungal structures are exposed on the surface of host plants, i.e. they form only haustoria, nutrient absorbing organs, in epidermal cells of plants. Conversely, four genera, viz. *Leveillula*, *Phyllactinia*, *Pleochaeta*, and *Queirozia*, exhibit (partly) endoparasitism. Of these four genera, only *Leveillula* has a truly endoparasitic nature by penetrating into host plants via stomata, developing haustoria in parenchyma cells, and elongating internal hyphae between host cells (Zheng et al. 2013). The remaining three genera exhibit partly endoparasitism: viz. they penetrate into host plants via stomata, form haustoria in parenchyma cells but do not form true internal hyphae (Itoi et al. 1962). These four genera form a distinct monophyletic group known as tribe Phyllactinieae (Mori et al. 2000), suggesting that the endoparasitic nature evolved from ectoparasitism by a single evolutionary event in the Erysiphales. Phylogenetic relationships within the Erysiphales have been studied in the last quarter-century by the molecular phylogenetic analyses (reviewed by Takamatsu 2004, 2013a, b; Braun and Cook 2012). Molecular phylogenetic relationships within the tribe Phyllactinieae have also been reported for *Phyllactinia* (Takamatsu et al. 2008) and *Leveillula* (Khodaparast et al. 2001, 2007, 2012; Voytyuk et al. 2009). However, information on the phylogeny of the basal group in the tribe Phyllactinieae including *Pleochaeta* and *Queirozia* is still lacking. In this study, powdery mildew specimens belonging to the tribe Phyllactinieae collected in

North and South America have been sequenced to investigate the early evolution of endoparasitic group in powdery mildews.

MATERIALS AND METHODS

Information on the specimens newly sequenced in this study, namely, host plants, locations of collections, voucher numbers and accession numbers of the nucleotide sequence databases (DDBJ, EMBL, and GenBank), is provided (TABLE I). All specimens are deposited in the herbarium of Mie University (MUMH). The nucleotide sequences of the 5'-end of the nuc 28S rDNA (including domains D1 and D2) and the nuc rDNA ITS1-5.8S-ITS2 (ITS) were determined by the procedure described by Takamatsu et al. (2013). To determine the sequence of *Phyllactinia leveilluloides*, we designed a new primer PH11 (5'-AGTCTTGTCAACTCGTGTA-3') that is specific to this species in the ITS1 region. A primer set PH11/NLP2 (Mori et al. 2000) was used to amplify the DNA region from the latter half of ITS to 5'-end of the nuc 28S rDNA. The sequences determined in this study were deposited in DNA Data Bank of Japan (DDBJ) under the accession numbers LC108830–LC108847. Newly determined sequences were aligned with other sequences of the Erysiphaceae using MUSCLE (Edgar 2004) implemented in MEGA6 (Tamura et al. 2013). Alignments were further manually refined using MEGA6 and deposited in TreeBASE (<http://www.treebase.org/>) under the accession number S18694. Phylogenetic trees were obtained from the data with maximum parsimony (MP) and maximum likelihood (ML) analyses. MP analyses were conducted by PAUP 4.0a146 (Swofford 2002) with the heuristic search option using tree bisection-reconstruction (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. Tree scores, including tree length, consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. The strength of internal branches of the resulting trees was tested with bootstrap (BS) analysis using 1000 replications with the stepwise addition option set as simple (Felsenstein 1985). The ML analysis was done with 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 query for the tree with highest likelihood. BS supports of 70% or higher are shown.

Topology tests were conducted against a hypothetical phylogeny by the Wilcoxon signed-ranks (Templeton 1983), Kishino-Hasegawa (KH, Kishino and Hasegawa 1989) and Shimodaira-Hasegawa (SH, Shimodaira and Hasegawa 1999) tests. A user-defined constraint tree was first drawn with Mesquite 2.75 (Maddison and Maddison 2011), and then the most parsimonious tree consistent with the constraint tree was found using the heuristic search using PAUP*. We compared the hypothetical tree with the most parsimonious tree to see whether our data can significantly reject the hypothetical tree using PAUP*.

RESULTS

nuc 28S rDNA tree.—Fourteen sequences of the nuc 28S rDNA determined in this study were combined with the sequences of the species of the tribe Phyllactinieae used in Takamatsu et al. (2008). Sixteen sequences of species of the tribes Erysipheae and Golovinomyceteae (Takamatsu et al. 2013, 2015) were used as outgroup taxa. This dataset consisted of 71 sequences and 616 characters, of which 172 (27.9%) characters were variable and 127 (20.6%) characters were informative for parsimony analysis. A total of 837 802 equally parsimonious trees with 551 steps were constructed by the MP analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal groups and branch lengths. One of the trees is shown (FIG. 1). ML analysis generated a tree topology almost identical to the MP tree, and only BS support values are shown on the MP tree.

Tribe Phyllactinieae formed a monophyletic group with strong BS supports (MP = 100%, ML = 100%). Of the four genera in this tribe, *Pleochaeta* and *Queirozia* formed a paraphyletic group at the base of the tribe Phyllactinieae. *Phyllactinia* and *Leveillula* formed a distinct monophyletic group together with strong BS supports (MP = 100%, ML = 100%). *Leveillula* formed a nested clade with *Ph. adesmiae* Havryl. within the *Phyllactinia/Leveillula* clade (MP = 83%, ML = 84%). The unusual long-branch leading to *Ph. adesmiae* suggests that the placement of this sequence is a result of long-branch attraction (Page and Holmes 1998). Thus, we constructed a constraint tree based on the hypothesis that *Leveillula* spp. except for “cf. *Leveillula clavata*” would form a clade without *Ph. adesmiae* and compared it with the best MP tree. As a result, the hypothesis tree needed only one step longer compared with the best tree and was not rejected significantly by both the Wilcoxon signed-ranks test (MP method) and KH and SH tests (ML method). Thus, it is tenable that *Leveillula* spp. forms a monophyletic group. “cf. *Leveillula clavata*” is a powdery mildew occurring on poinsettia (*Euphorbia pulcherrima*) in 2005 on the Japanese Bonin islands (Horie et al. 2006), and similar to *L. clavata* Nour in its conidial shape. However, this fungus was not included in the *Leveillula* clade and situated at the very base of the tribe Phyllactinieae.

ITS tree.—Thirteen ITS sequences determined in this study were combined with the sequences of the tribe Phyllactinieae used in Takamatsu et al. (2008). *Golovinomyces orontii* (Castagne) Heluta and *Erysiphe heraclei* DC. were used as outgroup taxa on the basis of Mori et al. (2000). This dataset consisted of 38 sequences and 794 characters, of which 82 characters at the end of ITS2 region were deleted from the analysis for an

TABLE I. Hosts, fungal species, vouchers, collection places and DNA database accession numbers of the sequences determined in this study

Host family	Host plant	Fungal species	Voucher No.	Country of collection	ITS	28S
Apocynaceae	<i>Rhabdadenia madida</i>	<i>Phyllactinia</i> sp.	MUMH3723/OLP245	Brazil	LC108841	LC108841
Bignoniaceae	<i>Spathodea campamulata</i>	<i>Phyllactinia obclavata</i>	MUMH4619/1748	Panama	—	LC108846
Bignoniaceae	<i>Handroanthus impetiginosus</i>	<i>Phyllactinia obclavata</i>	MUMH1876	Argentina	LC108832	LC108832
Bignoniaceae	<i>Handroanthus impetiginosus</i>	<i>Phyllactinia obclavata</i>	MUMH1883	Argentina	—	LC108833
Bignoniaceae	<i>Handroanthus heptaphyllus</i>	<i>Phyllactinia obclavata</i>	MUMH3144	Argentina	—	LC108838
Bignoniaceae	<i>Handroanthus heptaphyllus</i>	<i>Phyllactinia obclavata</i>	MUMH3152	Argentina	—	LC108839
Bignoniaceae	<i>Handroanthus serratifolius</i>	<i>Phyllactinia obclavata</i>	MUMH3725/VIC26551	Brazil	LC108843	—
Euphorbiaceae	<i>Euphorbia pulcherrima</i>	cf. <i>Leveillula clavata</i>	MUMH3797	Japan	LC108845	LC108845
Cannabaceae	<i>Celtis sinensis</i>	<i>Pleochaeta shiraiana</i>	MUMH1742	Japan	LC108831	LC108831
Cannabaceae	<i>Celtis tala</i>	<i>Pleochaeta polychaeta</i>	MUMH3040	Argentina	LC108835	LC108835
Cannabaceae	<i>Celtis tala</i>	<i>Pleochaeta polychaeta</i>	MUMH3086	Argentina	LC108836	LC108836
Cannabaceae	<i>Celtis tala</i>	<i>Pleochaeta polychaeta</i>	MUMH3110	Argentina	—	LC108837
Cannabaceae	<i>Cavica papaya</i>	<i>Phyllactinia caricicola</i>	MUMH3722/VIC22169	Brazil	LC108840	—
Fabaceae	<i>Adesmia volckmannii</i>	<i>Phyllactinia adesmiae</i>	MUMH1938	Argentina	LC108834	LC108834
Fabaceae	<i>Prosopis strombulifera</i>	<i>Pleochaeta prosopidis</i>	MUMH1691	Argentina	LC108830	LC108830
Fagaceae	<i>Quercus potosina</i>	<i>Phyllactinia leveilluloides</i>	MUMH6549/HAL2917F	Mexico	LC108847	LC108847
Onagraceae	<i>Ludwigia peruviana</i>	<i>Phyllactinia</i> sp.	MUMH3724/DJS284	Brazil	LC108842	—
Verbenaceae	<i>Duranta erecta</i>	<i>Phyllactinia durantae</i>	MUMH3726/VIC26557	Brazil	LC108844	—

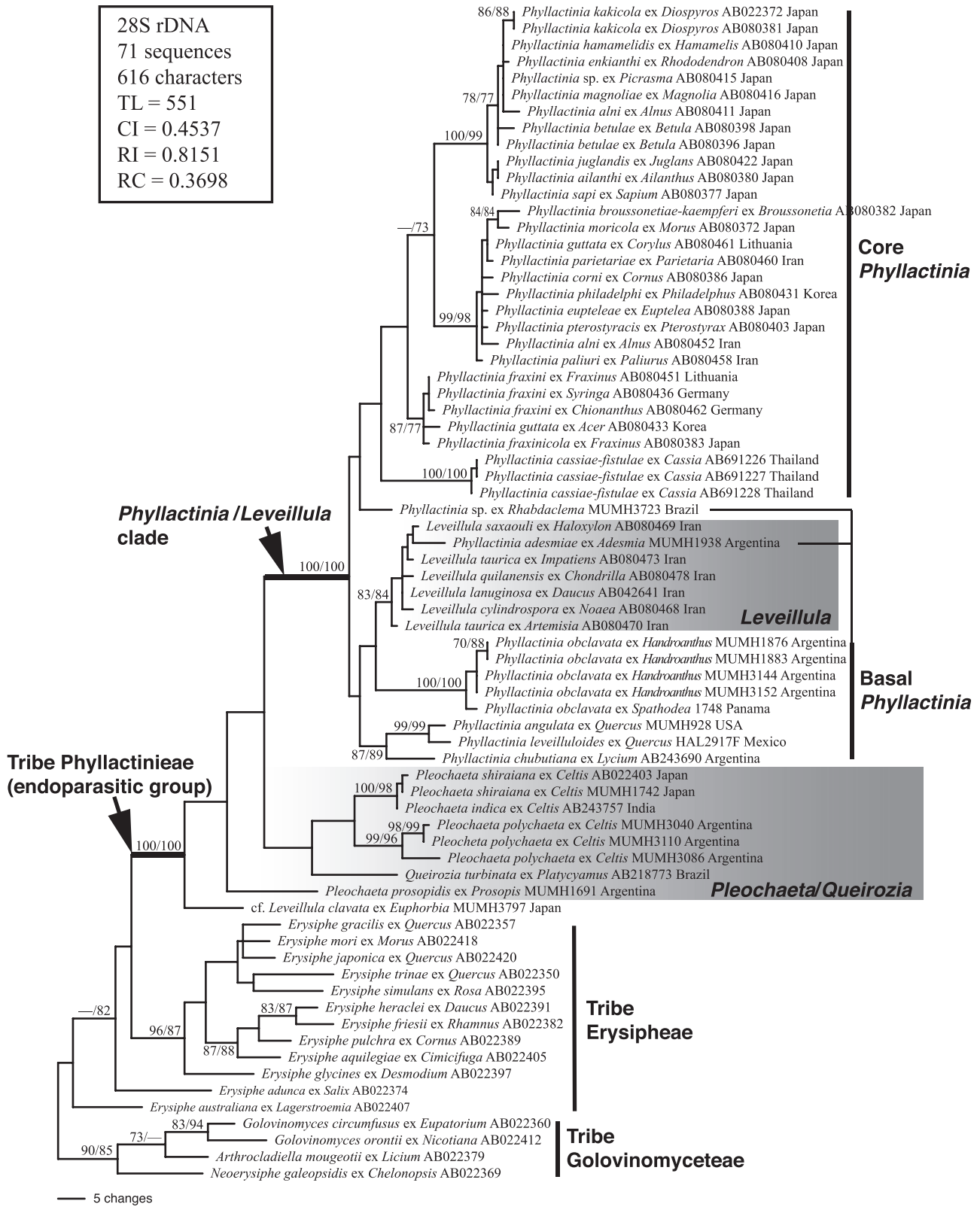


FIG. 1. Phylogenetic analysis of the divergent domains D1 and D2 sequences of the nuc 28S rDNA for 71 sequences from the tribes Phyllactinieae, Erysipheae, and Golovinomyceteae. This tree is a phylogram of one of the 837 802 most parsimonious trees with 551 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS ($\geq 70\%$) values by the maximum parsimony (MP) and maximum likelihood (ML) methods are shown on or under the respective branch.

ambiguous alignment. Of the remaining 712 characters, 388 (54.5%) characters were variable, and 261 (36.7%) characters were informative for parsimony analysis. A total of 21 equally parsimonious trees with 1108 steps were constructed by the MP analysis. Tree topologies were almost consistent among the trees, except for branching orders of the terminal groups and branch lengths. One of the trees with the highest likelihood value is shown (FIG. 2). ML analysis generated a tree topology almost identical to the MP tree, and only BS support values are shown on the MP tree.

The phylogenetic relationships based on the ITS sequences were mostly concordant with the 28S rDNA tree. Again, *Pleochaeta*, *Queirozia*, and “cf. *Leveillula clavata*” formed a paraphyletic group at the base of the tribe Phyllactinieae. *Phyllactinia* and *Leveillula* formed a monophyletic group with strong BS supports (MP = 100%, ML = 100%). *Leveillula* grouped in a subclade within the *Phyllactinia/Leveillula* clade (MP = 95%, ML = 90%). *Phyllactinia adesmiae* was a sister to the *Leveillula* clade (MP = 58%, ML = 71%). In addition, *Ph. bougainvilleae* on *Bougainvillea spectabilis* (Nyctaginaceae) collected in Mexico (Fernández-Pavía et al. 2014), and *Ph. obclavata* on *Handroanthus* (former *Tabebuia*) spp. grouped with the *Leveillula* and *Ph. adesmiae* sequences with strong BS supports (MP = 67%, ML = 90%).

TAXONOMY

According to the discontinuation of the dual nomenclature of fungi and the new principle “one fungus, one name” (Hawksworth 2011, Norvell 2011, Braun 2012), the anamorph-typified genus *Ovulariopsis* has to be considered a heterotypic synonym of the teleomorph-typified genus *Phyllactinia*. However, re-allocations of anamorph-typified species of these genera to teleomorph genera under the new rules are not subject to any automatism. Asexual morphs are morphologically often little differentiated, and accidental infections cannot be excluded. The morphology of certain anamorph species must be unequivocally in favor of a corresponding teleomorph genus, and the particular morphological traits have to support the justification of a species of its own. Data of phylogenetic analyses are the best proof for generic affinities and new species. The present paper includes the basic *Phyllactinia* spp., characterized by forming dimorphic, *Leveillula/Pleochaeta*-like conidia, and so the asexual morphs of these three genera are confusable. Therefore, phylogenetic confirmations of the generic affinity of the taxa concerned are particularly important. The present phylogenetic data confirmed that *Ovulariopsis caricicola* (= *Streptopodium caricae*), *Ov. durantae*, and *Ov. obclavata* (= *St. tabebuiae*) belong to a basal group

of species of the genus *Phyllactinia* and so must be re-allocated to the latter genus. Species of *Ovulariopsis* and *Phyllactinia* on *Carica papaya* are a case in point. Several species unequivocally differentiated morphologically clearly belonging to *Phyllactinia* are known and have been described, illustrated, and discussed in detail by Liberato et al. (2004) and Braun and Cook (2012). The available data justify the re-allocation of these species to *Phyllactinia*, although phylogenetic confirmations are not yet available. Therefore, we prefer to re-assess the whole complex, which helps to prevent taxonomic and nomenclatural confusion. Furthermore, two new *Phyllactinia* species are described only on the basis of their asexual morphs. Their affinity to this genus has been proven by molecular sequence analyses.

Phyllactinia bougainvilleae Fernández-Pavía, Rodríguez-Alvarado, Cabrera & U. Braun, sp. nov.
MycoBank MB815598

Typification: MEXICO: Michoacan, Morelia municipality, urban garden, on leaves of *Bougainvillea spectabilis* Willd. (Nyctaginaceae), Sep 2009, S. Fernández-Pavía, HAL 2588 F (**holotype**), EBUM 23687 (**isotype**). GenBank accession No.: ITS = KC556804.

Etymology: Epithet derived from the host genus.

Illustration: Fernández-Pavía et al. (2014:225, FIG. 1), as “*Bougainvillea* powdery mildew”.

Mycelium on leaves, hemiendophytic, superficial mycelium mostly hypophyllous, with hyphae emerging through stomata. Hyphae hyaline, septate, thin-walled, 2–6 µm wide, frequently with approximately right angled branching. Hyphal appressoria numerous, distinct, oblong, hooked, sinuous and/or coral-like. Conidiophores consistently on top of mother cells and more or less midway between two septa, cylindrical, straight, unbranched, 140–252.5 (average 180) µm long and 7.5–9 µm wide, hyaline, thin-walled, smooth; foot-cells very long, 100–220 µm, cylindrical-filiform, but sinuous to spirally twisted at the very base just above the basal septum that is slightly elevated above the junction with the mother cell, foot-cells followed by 1–2 much shorter cells. Conidia formed singly, dimorphic, primary conidia lanceolate to ovoid-lanceolate, 62.5–87.5 (average 77.65) × 12.5–27.5 (average 20.85) µm, secondary conidia ellipsoid-subcylindrical, ends subtruncate, 55–77.5 × 7.5–15 µm, hyaline, with numerous oil-droplets, germ tubes arising near base or apex, single, short producing hooked and/or coral-like appressoria.

Additional specimen examined: MEXICO: Michoacan, Tarímbaro municipality, urban garden, on leaves of *B. spectabilis* (Nyctaginaceae), Jul 2009, S. Fernández-Pavía (EBUM 24362).

Host range and distribution: On *B. spectabilis*, Mexico.

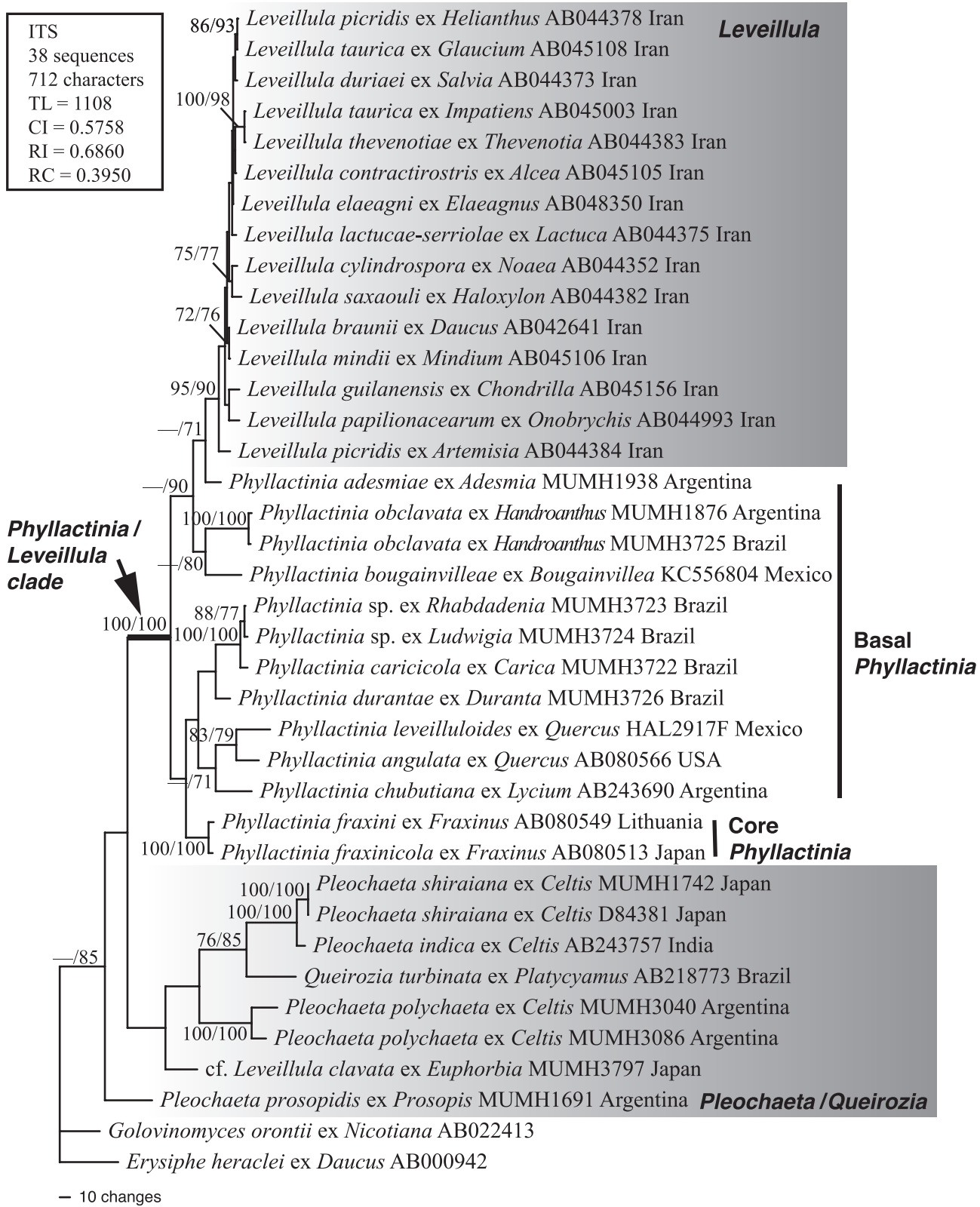


FIG. 2. Phylogenetic analysis of the nuc rDNA ITS1-5.8S-ITS2 for 38 sequences from the tribes Phyllactinieae and outgroup taxa. This tree is a phylogram of one of the 21 most parsimonious trees with 1108 steps, which were found using a heuristic search. Horizontal branch lengths are proportional to the number of substitutions that were inferred to have occurred along a particular branch of the tree. BS ($\geq 70\%$) values by the maximum parsimony (MP) and maximum likelihood (ML) methods are shown on or under the respective branch.

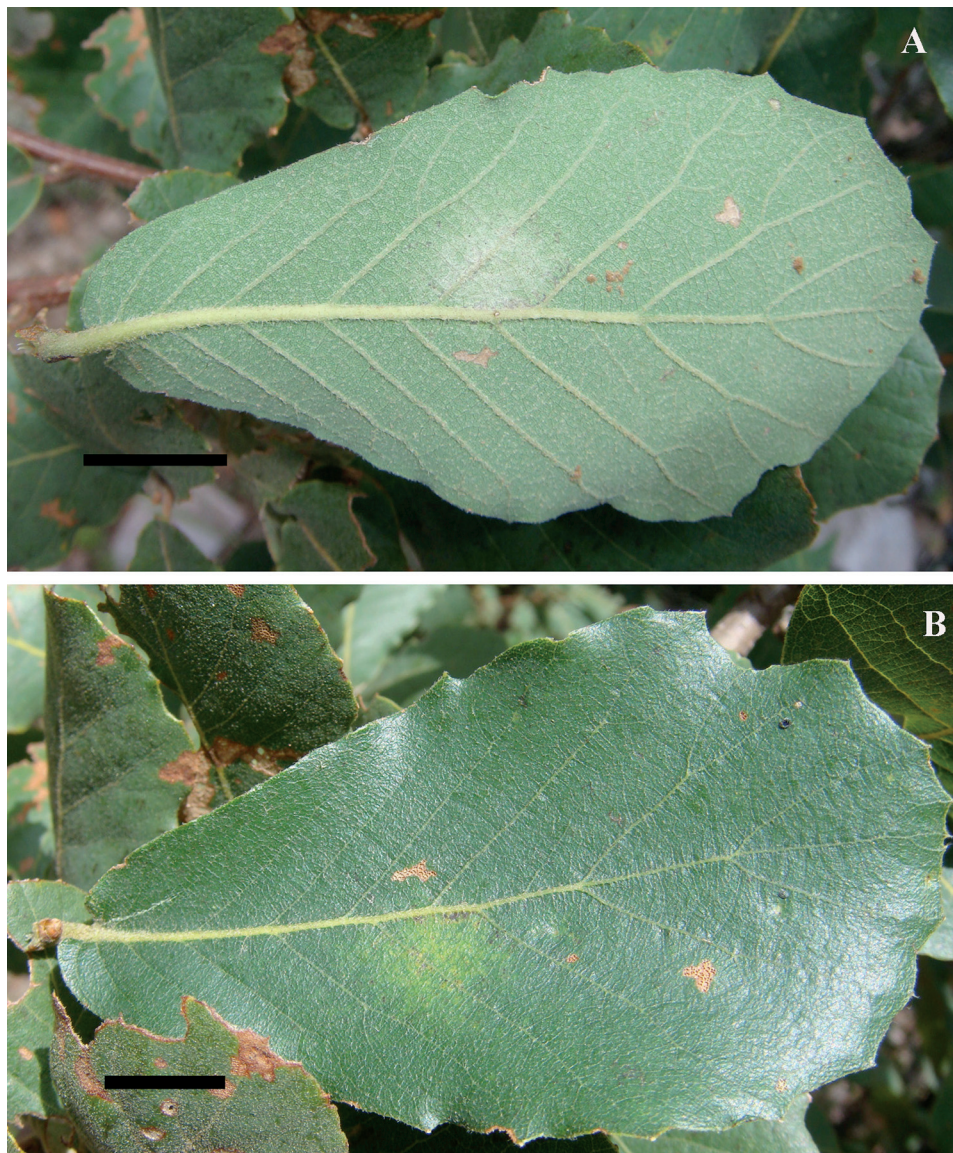


FIG. 3. *Phyllactinia leveilluloides*, symptoms on leaves of *Quercus potosina*. A. White mycelial colony on the lower leaf surface. B. Corresponding yellowish discoloration on the upper leaf surface. Bars = 1 cm. Photo by O. Moreno-Rico.

Notes: Fernández-Pavía et al. (2014) described and illustrated this species as “*Bougainvillea* powdery mildew” without formal introduction of a new species. A phylogenetic examination based on rDNA ITS data was performed, but the number of taxa and sequences included in the analysis was too small to yield unequivocal results for a final taxonomic conclusion. The present phylogenetic analysis based on a larger sampling yielded clear outcomes showing that the Mexican *Bougainvillea* powdery mildew belongs to an undescribed species in a basal group of *Phyllactinia* spp. *Phyllactinia bougainvilleae* is the first and only species of *Phyllactinia* on a host of the *Nyctaginaceae*.

Phyllactinia durantae (Liberato & R.W. Barreto) Liberato, R.W. Barreto & S. Takam., comb. nov.
Mycobank MB815599

Basionym: *Ovulariopsis durantae* Liberato & R.W. Barreto, Fungal Divers 18:97, 2005.

Illustration: Liberato & Barreto (2005:98–99, FIGS. 1–6), Braun & Cook (2012:286, FIG. 304).

Phyllactinia leveilluloides O. Moreno-Rico & U. Braun, sp. nov. FIGS. 3–5
Mycobank MB815600

Typification: MEXICO: Aguascalientes, San Jose de Gracia municipality, on leaves of *Quercus potosina* Trel. (Fagaceae), 5 Aug 2015, O. Moreno-Rico, HAL

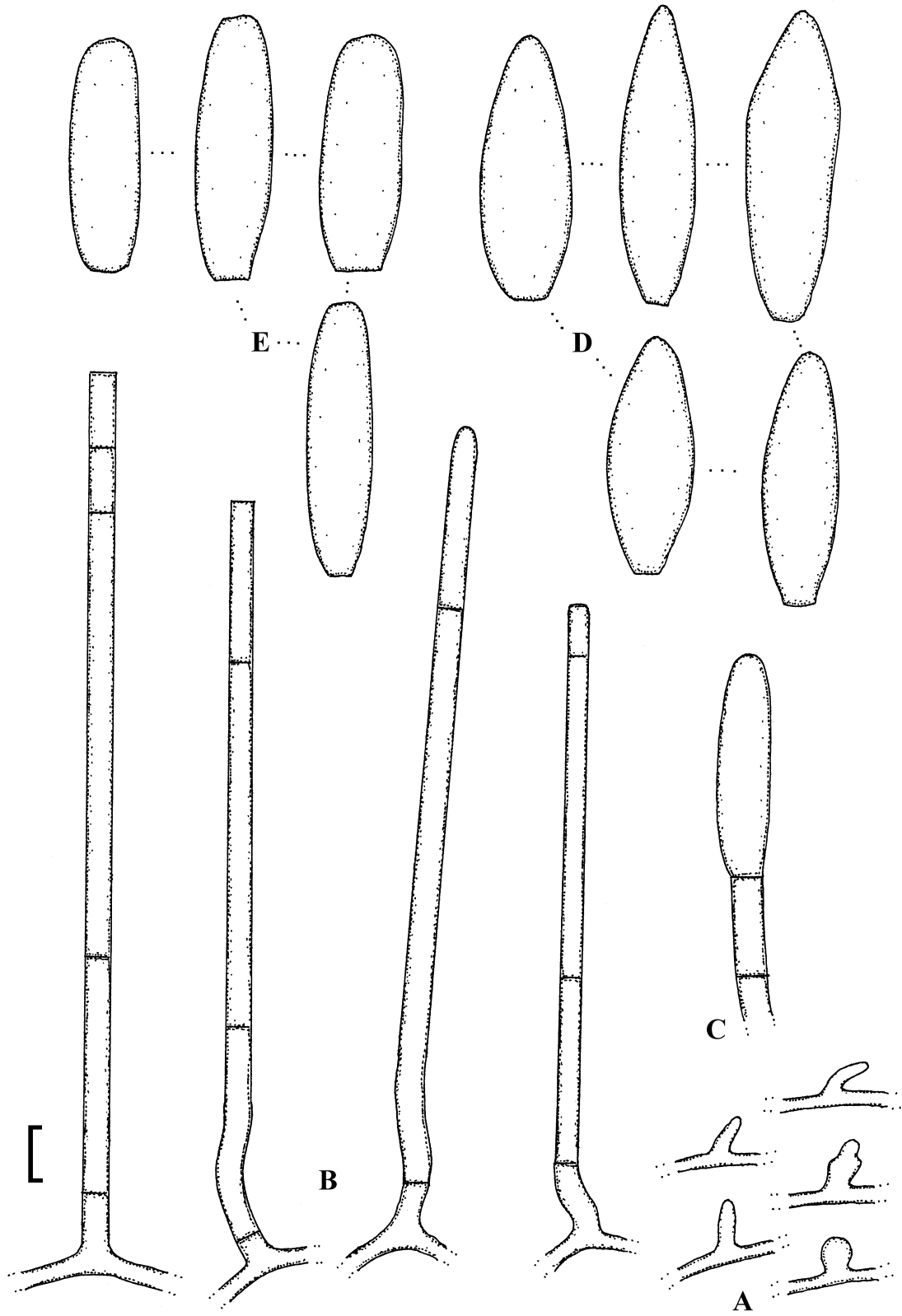


FIG. 4. *Phyllactinia leveilluloides*. A. Hyphal appressoria. B. Conidiophores. C. Conidiophore tips with conidium initial. D. Primary conidia. E. Secondary conidia. Standard bar = 10 μ m. U. Braun del.

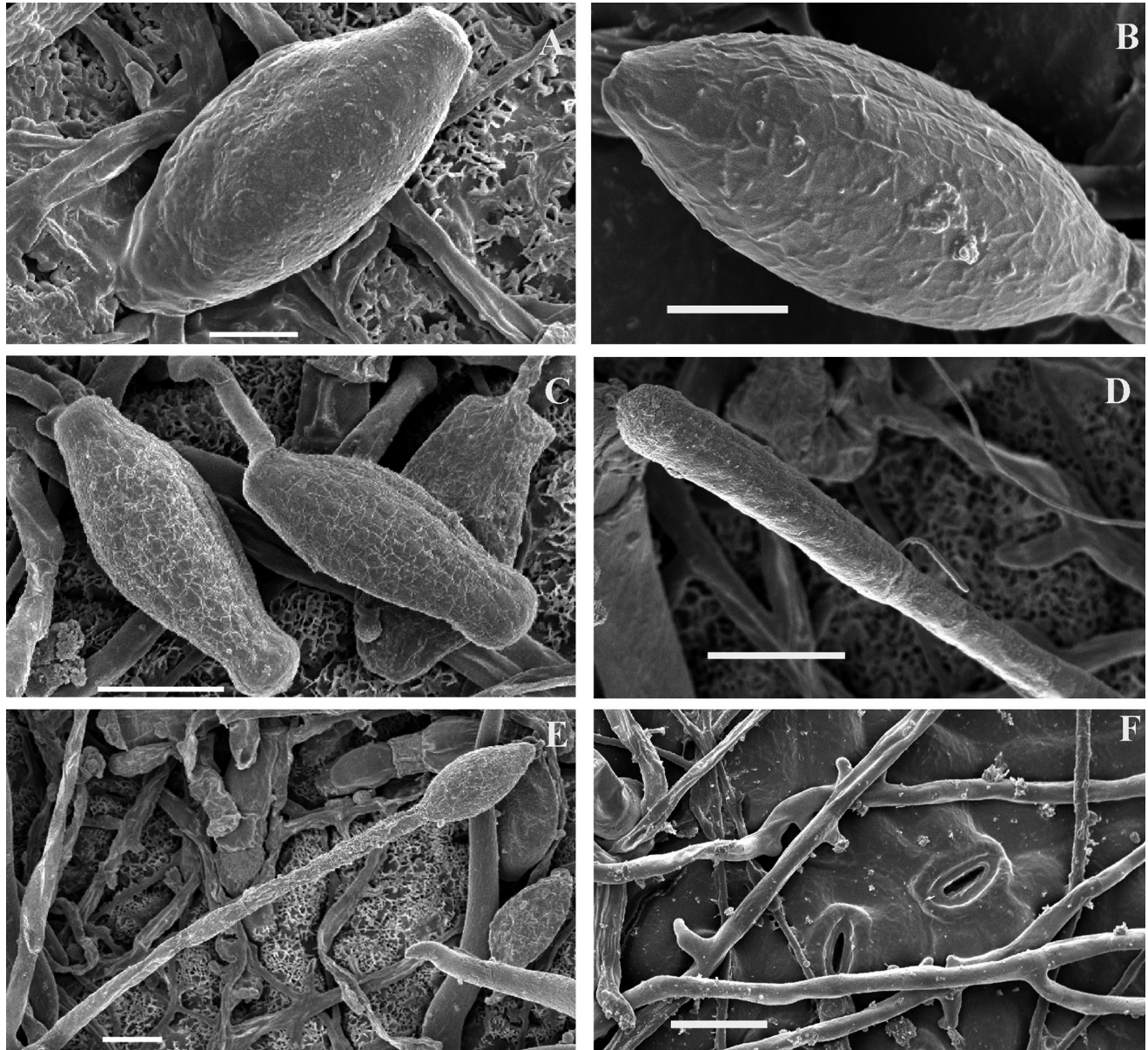


FIG. 5. *Phyllactinia leveilluloides*, SEM micrographs. A, B. Primary conidia, C. Secondary conidia. D. Conidium initial. E. Conidiophore with straight base and primary conidium. F. Hyphae with appressoria (C, E show severe wrinkling; A, B, D slight wrinkling). Bars: A, B, D = 10 μm ; C, E, F = 20 μm .

2917 F (**holotype**), MUMH 6549 (**isotype**). GenBank accession: ITS + 28S = LC108845.

Etymology: Epithet referring to the similarity of the dimorphic conidia to species of the genus *Leveillula*.

Colonies hypophyllous, effuse or in small to moderately large, thin patches, whitish, on the corresponding upper leaf surface often causing yellowish discolorations (FIG. 3). Mycelium internal and external; superficial mycelium consisting of loosely arranged to interwoven hyphae, straight to strongly sinuous, branched, 2–7 μm wide, septate, thin-walled, smooth. Hyphal appressoria solitary, short cylindrical and

straight to hooked, sometimes nipple-shaped to somewhat lobed (FIGS. 4A, 5F). Conidiophores arising from superficial hyphae, on top of mother cells, position between two septa usually not in the middle, occasionally branched near the base, erect, subcylindrical, straight throughout or curved-sinuous at the very base, 90–200 μm long, 5–8(–9) μm wide throughout or slightly wider toward apex, basal septum usually somewhat elevated, 5–18 μm from the junction with supporting hypha, arrangement of conidiophore cells variable, either with a very long foot-cell, 60–110 μm long, followed by 1–3 short cells, about 10–30 μm , or

a shorter foot-cell, about 35–55 μm long, followed by a longer cell, to 100 μm , and 1–3 short cells, 10–25 μm (FIGS. 4B, C). Conidia solitary, dimorphic; primary conidia broadly ellipsoid to ovoid, apex obtuse to somewhat pointed, base rounded to subtruncate, (40–)50–65 \times (15–)18–26 μm , on average 53 \times 21 μm , length/breadth ratio 1.9–3.0, on average 2.5 (FIG. 4D); secondary conidia subcylindrical to broadly ellipsoid, (40–)55–70 \times (15–)19–27 μm , on average 61 \times 22 μm , length/breadth ratio 2.3–3.2, on average 2.8 (FIG. 4E), germination not observed.

Host range and distribution: On *Quercus potosina*, Mexico.

Notes: The conidia of *Phyllactinia leveilluloides* resemble *Leveillula* conidia in being obviously dimorphic, but the conidiophores are consistently formed as lateral branches of superficial hyphae as in *Phyllactinia* spp. Conidiophores arising from superficial hyphae are also known from some *Leveillula* spp., but in the latter genus they usually emerge through stomata (Braun and Cook 2012). In *Ph. leveilluloides*, the affinity of the new species to *Phyllactinia* has been confirmed by its position in the present phylogenetic tree. The resemblance between *Ph. leveilluloides* and asexual morphs of *Leveillula* species is not surprising because the present Mexican oak powdery mildew pertains to a basal group of *Phyllactinia* spp. clustering close to *Leveillula* spp. Several *Phyllactinia* spp. have been described on oaks, but all of them are distinct in having non-dimorphic conidia and deviating conidial shapes. The conidia of *Ph. angulata* (E.S. Salmon) S. Blumer are characteristically angular in outline, and *Ph. orbicularis* (Ehrenb.) U. Braun as well as *Ph. roboris* (Gachet) S. Blumer are characterized by clavate to somewhat spatuliform conidia (Braun and Cook 2012). Scanning electron microscopic characters are in general accordance with taxa belonging to tribe *Phyllactinioideae* (Braun and Cook 2012). Turgid conidia are irregularly verrucose (with irregularly scattered hemispherical warts, FIG. 5A). The pattern on the surface of wrinkled conidia corresponds with type C.2 in Braun and Cook (2012:28, angular, ex “verrucose”), which is also characteristic of taxa of the tribe *Phyllactinioideae* (FIG. 5B).

Phyllactinia obclavata (Wakef.) U. Braun & S. Takam., comb. nov.

MycoBank MB815601

Basionym: *Ovulariopsis obclavata* Wakef., Bull. Misc. Inf. Kew 7:238, 1920.

Synonym: *Streptopodium tabebuiae* Liberato & R.W. Barreto, Fungal Divers 18:100, 2005.

Illustration: Liberato & Barreto (2005:103, FIGS. 13–15), Braun & Cook (2012:290, FIG. 311).

***Phyllactinia* spp. on *Carica* spp.**

Phyllactinia caricae (Sawada) U. Braun, comb. nov.
MycoBank MB815602

Basionym: *Ovulariopsis caricae* Sawada, Bull. Dept. Agric. Gov. Res. Inst. Formosa 61:84, 1933.

Description and illustration: Braun & Cook (2012:286–287, FIG. 302).

Phyllactinia caricicola (U. Braun) Liberato, R.W. Barreto & S. Takam., comb. nov.

MycoBank MB815603

Basionym: *Ovulariopsis caricicola* U. Braun, in Braun & Cook, CBS Biodiversity Series 11:287, 2012.

Synonym: *Streptopodium caricis* Liberato & R.W. Barreto, Mycol. Res. 108:1186, 2004.

Illustrations: Liberato et al. (2004:1187, FIGS. 1–6), Braun & Cook (2012:286, FIG. 303).

Phyllactinia papayae (Van der Byl) U. Braun, comb. nov.

MycoBank MB815604

Basionym: *Ovulariopsis papayae* Van der Byl, Trans. Roy. Soc. South Africa 9:189, 1921.

Description and illustration: Braun & Cook (2012:291, FIG. 312).

Phyllactinia caricifolia Viégas

Descriptions and illustrations: Liberato et al. (2004:1187, FIGS. 1–6; 1189), Braun & Cook (2012:234–235, FIG. 223).

DISCUSSION

Phylogenetic relationships within the tribe Phyllactinieae have been reported by Takamatsu et al. (2008), Khodaparast et al. (2001, 2007, 2012), and Voytyuk et al. (2009). However, these reports referred only to core species of the respective genera: In other words, sequence data of basal taxa of the tribe Phyllactinieae were not included. In this study, species collected in North and South America have been examined and sequenced, showing that these sequences are situated at the base of the tribe Phyllactinieae. In addition, *Pleochaeta* species collected in Argentina have been sequenced for the first time. The phylogenetic trees based on these new sequences revealed that tribe Phyllactinieae forms a distinct monophyletic group, suggesting that these endoparasitic powdery mildew taxa diverged from a single ancestor.

Four *Pleochaeta* and one *Queirozia* species used in this analysis formed a paraphyletic group at the basal position of the tribe Phyllactinieae with a sequence of “cf. *Leveillula clavata*”. This result suggests that the *Pleochaeta/Queirozia* group is associated with the early evolution of endoparasitic powdery mildews. Fungal groups having appendages with circinate or uncinat tips are considered to be the most ancestral in the Erysiphales (Mori et al. 2000, Takamatsu 2013b). The genera *Pleochaeta* and *Queirozia* also have these types of

appendages. Thus the basal position of these genera is supported by their morphology. Among *Pleochaeta* species occurring on *Celtis*, the American species *Pl. polychaeta* (Berk. & M.A. Curtis) Kimbr. & Korf and the Asian/African species *Pl. shiraiana* (Henn.) Kimbr. & Korf and *Pl. indica* N. Ahmad, A.K. Sarbhoy & Kamal formed two separate subclades and then grouped in a larger clade, suggesting that an ancestral species of *Pleochaeta* on *Celtis* split on the two continents. This may be an example of disjunct distributions between America and Asia in the Erysiphales. We calculated the time of separation between the two groups with the molecular clock of the nuc 28S rDNA (Takamatsu and Matsuda 2004). The molecular clock suggested that the Asian *Pleochaeta* species were divided from the American *Pleochaeta* species ca. 30 million years ago (Ma) in the early Oligocene Epoch of the Paleogene Period. This age corresponds to the rapid global cooling of the Earth that began from the early Eocene (Tiffney 1985). This climatic deterioration may have affected the splitting of *Pleochaeta* species between America and Asia.

The phylogenetic position of “cf. *Leveillula clavata*” on poinsettia was unexpected. *Leveillula clavata* was first described in 1950 based on a specimen collected in Kenya (Braun and Cook 2012). A powdery mildew occurred on poinsettia in Italy in 2005, and the causal fungus was identified as *L. clavata* based on its asexual morph (Garibaldi et al. 2006). A similar powdery mildew occurred on poinsettia in Japan in the same year and was considered an unidentified species although the asexual morph was similar to *L. clavata* (Horie et al. 2006), so that we tentatively call this fungus “cf. *Leveillula clavata*” in this report. However, the present analysis revealed that this fungus does not belong to the *Leveillula* clade and is placed at the very base of the tribe Phyllactinieae. Unfortunately, because the fungus could not be re-collected after 2006, we were not able to examine fresh material of this fungus. Siahaan et al. (2015) reported a powdery mildew with *Ovulariopsis* anamorph on poinsettia in Indonesia. This fungus belongs to the *Phyllactinia* group in phylogeny and clearly differs from the sequence of “cf. *Leveillula clavata*.” They proposed the new species *Ph. poinsettiae* Siahaan & S. Takam. for this fungus. Taxonomic re-examination of “cf. *Leveillula clavata*” is urgently required. Furthermore, recollection, re-examination and phylogenetic analyses of the genuine *L. clavata* are necessary. The identity and above all the connection between the described asexual and sexual morphs of this species are unproven and, due to the new collection from Japan, questionable. The described asexual morph fits better with *Phyllactinia* spp. than with common *Leveillula* spp. It cannot be excluded that *L. clavata* was based on two different powdery mildews

simultaneously occurring on poinsettia, above all since *Euphorbia pulcherrima* is of American origin, but *L. clavata* was based on cultivated material from Africa. *Leveillula clavata* has been recorded from Africa, Asia, and Europe (Braun and Cook 2012), but not from the region native to poinsettia, and all collections, except for type material, refer to the asexual morph. The ascomata might belong to one of the *Leveillula* spp. occurring on hosts of the Euphorbiaceae, and the asexual morph could be a separate species. According to Braun and Cook (2012), the name *Ov. erysi-phoides* Pat. & Har., described from Senegal on *Eu. balsamifera*, could be a potential candidate for the asexual phyllactinioid fungus on poinsettia.

Phyllactinia and *Leveillula* form a distinct clade derived from the *Pleochaeta/Queirozia* group in both the 28S and ITS trees. *Phyllactinia* species divided into two distinct groups. One group is paraphyletic and situated at the base of the *Phyllactinia/Leveillula* clade. The other group is monophyletic and derived from a part of the former group. The former group is classified as “Basal *Phyllactinia* group” because of its basal position in the phylogenetic tree, and the latter group as “Core *Phyllactinia* group” because this group includes most *Phyllactinia* species distributed worldwide. All “Basal *Phyllactinia* group” specimens were collected in North and South America and have distinct dimorphic conidia. In contrast, the “Core *Phyllactinia* group” does not form distinctly dimorphic conidia. The first split within the “Core *Phyllactinia* group” occurred between the Southeast Asian species *Ph. cassiae-fistulae* U. Braun & Y.S. Paul and *Ph. dalbergiae* Piroz. (data not shown) and other members of the “Core *Phyllactinia* group,” suggesting that Southeast Asia is associated with early evolution of the “Core *Phyllactinia* group”.

The genus *Leveillula* is the only genus in the Erysiphales that has true internal hyphae elongating between host cells. This genus forms a monophyletic group derived from the “Basal *Phyllactinia* group”. *Phyllactinia adesmiae* is sister to the *Leveillula* clade. These results suggest that an evolution from ectoparasitism to partly endoparasitism occurred at the base of the tribe Phyllactinieae, and then further evolution from partly endoparasitism to true endoparasitism occurred at the base of the *Leveillula* clade. Calibration of the evolutionary timing using molecular clock analysis suggests that the first split within *Leveillula* occurred later than 5 Ma (Takamatsu et al. 2008). The small amount of morphological divergence among *Leveillula* species may support the recent origin of this genus (Braun and Cook 2012). Uneven distributions of *Leveillula* species have been reported by Amano (1986) and Palti (1988), with the highest number of host species in Central and West Asia and the

Mediterranean region, and much smaller numbers of hosts in other regions. Considering the recent origin of *Leveillula*, the uneven distribution may indicate Central and West Asia and the Mediterranean region as the geographic origin of *Leveillula*. *Leveillula* spp. may be on expanding their geographic distributions to other regions of the world. It is curious that all members of the “Basal *Phyllactinia* group,” which is sister to the *Leveillula* clade, are distributed in North and South America but neither in Central and West Asia nor in the Mediterranean region.

Central and West Asia and the Mediterranean region may be associated with the evolution not only of *Leveillula* but also of *Blumeria*, *Golovinomyces*, and *Neoerysiphe* (Hirata 1975, Heluta et al. 2010, Takamatsu 2013a). Because all these genera are mainly parasitic to herbaceous plants, these areas may be associated with the early evolution of herb-parasitic powdery mildews. Overall, the evolutionary history of the Erysiphales is a history of host shifts from trees to herbs that occurred multiple times independently (Takamatsu 2013a). Of the four genera of the tribe Phyllactineae, three genera (viz. *Pleochaeta*, *Queirozia*, and *Phyllactinia*) are strictly tree-parasitic, and only *Leveillula* is herb-parasitic with a few exceptions. The monophyly of *Leveillula* suggests that host shift from trees to herbs occurred only once in the tribe Phyllactineae at the branch leading to the *Leveillula* clade. Of the five tribes of the Erysiphales, tribes Erysiphaceae, Cystothecaceae, and Phyllactineae contain both tree- and herb-parasitic taxa, and tree parasitic taxa are situated at the bases of the respective tribes. Conversely, there are almost no tree-parasitic taxa in the tribes Golovinomycetaceae and Blumeriaceae. *Golovinomyces* and *Neoerysiphe* belong to the tribe Golovinomycetaceae and *Blumeria* to the tribe Blumeriaceae. Central and West Asia was a region surrounded by the Turgai Strait and Tethys Sea in the Paleogene Period and vegetated by deciduous tree genera like *Pterocarya*, *Betula*, *Alnus*, *Salix*, *Nyssa*, and so forth (Tiffney and Manchester 2001). The subsequent Alpine and Himalayan orogenies shut off the southern source of moisture, allowing this area to become arid during the Miocene of the Neogene Period when the diversity of angiosperms decreased. Rapid climatic changes in this area may have caused extinction of the old tree flora and also of powdery mildews associated with this flora. These kinds of climatic change occurring in Central and West Asia and the Mediterranean region might have facilitated the evolution of herb-parasitic powdery mildew species.

In summary, the most ancestral group in the tribe Phyllactineae is the *Pleochaeta/Queirozia* group, from which the genus *Phyllactinia* was derived. Finally, the true endo-parasitic genus *Leveillula* evolved from a

part of *Phyllactinia*. Thus, the Phyllactineae shows a distinct hierarchy in phylogeny. The group with distinct dimorphic conidia appeared first in the Phyllactineae, from which the group without distinct dimorphic conidia was derived. The present analyses clearly show that *Leveillula* is derived from a part of the “Basal *Phyllactinia* group”. All sister taxa to *Leveillula* were distributed in North and South America. Because a putative geographic origin of *Leveillula* is assigned to Central and West Asia or the Mediterranean region, a missing link during the evolution of *Leveillula* from *Phyllactinia* has to be postulated.

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