The Journal of Microbiology (2011) Vol. 49, No. 5, pp. 862-866
DOI 10.1007/s12275-011-1479-1

NOTE

Sawadaea koelreuteriae comb. nov., a Powdery Mildew of *Koelreuteria paniculata*

Hyeon-Dong Shin* **and Mi-Jeong Park**

Division of Environmental Science and Ecological Engineering, College of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea

(Received September 23, 2011 / Accepted October 20, 2011)

A powdery mildew parasitizing *Koelreuteria* spp. was first described under the name *Uncinula koelreuteriae* Miyake and later transferred to the genus *Typhulochaeta*. Based on morphological and molecular data of several herbarium specimens collected from Korea, the generic placement of *Typhulochaeta* is discussed and *T. koelreuteriae* is combined in the genus *Sawadaea*. Redescription and epitypification of this species is provided hereby.

***Keywords*:** Erysiphales, fibrosin bodies, macro-conidia, micro-conidia, phylogenetic analysis

Powdery mildews (Ascomycotina, Erysiphales) are some of the world's most frequently encountered plant pathogenic fungi. They infect leaves, stems, flowers, and fruits of nearly 10,000 species of angiosperms, causing considerable economic loss (Braun *et al*., 2002; Glawe, 2008). *Koelreuteria paniculata*, native to eastern Asia including Korea, is popularly grown as an ornamental tree in temperate regions almost worldwide. Two species of powdery mildews have been reported on this tree, both of which known from China (Farr and Rossman, 2011), viz. *Erysiphe bulbouncinula* [≡ *Uncinula clintonii* var. *bulbosa*; *Uncinula bulbosa*; *Bulbouncinula bulbosa*] and *Typhulochaeta koelreuteriae* [≡ *Uncinula koelreuteriae*; *Erysiphe koelreuteriae*] (Miyake, 1913; Tai and Wei, 1932; Tai, 1936, 1946; Zheng and Chen, 1979; Braun, 1987; Braun and Takamatsu, 2000).

Occurrence of powdery mildew on *K. paniculata* was found in July 2010 at Anam-dong, Seoul, for the first time in Korea, and followed by findings at Sangam-dong and Gireum-dong of Seoul. The infections were observed continuously from July to November 2010 and widely at these three localities of Seoul. The specimens collected have been deposited at KUS (Herbarium, Korea University, Seoul, Korea) as KUS-F25041, F25458, F25488, F25489 and F25621.

Morphological characteristics of the fungal structures on fresh samples were investigated in brightfield- and DIC- light microscopy, using an Olympus BX51 microscope (Olympus, Japan) for measurements and a Zeiss AX10 microscope equipped with AxioCam MRc5 (Carl Zeiss, Germany) for photographs. The images H, I and J in Fig. 1 were taken through a dissecting microscope (model V12 equipped with AxioCam ICc3, Carl Zeiss, Germany). In each case, thirty measurements for structures with taxonomical value were made at ×400 magnification.

* For correspondence. E-mail: hdshin@korea.ac.kr; Tel.: +82-2-3290-3063; Fax: +82-2-921-1715

The conidial state of the powdery mildew agreed well with *Oidium* subgenus *Octagoidium* anamorphs of the genus *Sawadaea*, and the morphological characteristics found in the teleomorphic state of this fungus were in accordance with typical those of *Typhulochaeta koelreuteriae* described by earlier workers (Tai, 1936; Zheng and Chen, 1979; Braun, 1987), so that a generic affinity of *T. koelreuteriae* to the genus *Sawadaea* has to be assumed. To clarify this hypothesis a molecular analysis of this fungus was conducted. Type material of *T. koelreuteriae* (HMAS 44245) could not be included in the present study because this specimen was not available to us.

Among the five collections preserved in KUS, two (KUS-F25489, F25621) were used for the molecular phylogenetic analysis. Genomic DNA was extracted from mature chasmothecia following the method of Lee and Taylor (1990). Internal transcribed spacers of the ribosomal DNA (ITS rDNA) region were amplified using primers ITS5 (White *et al*., 1990) and P3 (Kusaba and Tsuge, 1995). The PCR amplicons were purified using a MultiScreen HTS™ PCR filter plates (Millipore, Ireland) and sequenced using an ABI Prism™ 377 automatic DNA sequencer with a BigDye™ cycle sequencing kit version 3.1 (Applied Biosystems, USA).

The raw sequences of *T. koelreuteriae* were edited using the DNASTAR computer package version 5.05 (Lasergene, USA). For the phylogenetic analysis, 34 reference sequences of *Sawadaea* spp. were retrieved from GenBank. *Podosphaera tridactyla* (AB000943) and *P. xanthii* (D84387) were used as outgroup taxa based on a previous study (Hirose *et al*., 2005). The sequences were aligned with the ClustalX program (Thompson *et al*., 1997) and then the alignment was manually edited in MacClade 4.05 (Maddison and Maddison, 2002). Maximum parsimony (MP) analysis was conducted using a heuristic search with 100 random sequence additions and tree bisection-reconnection (TBR) branch swapping algorithm in PAUP version 4b10 (Swofford, 2002). All nucleotide substitutions were unordered and equally weighted, and align-

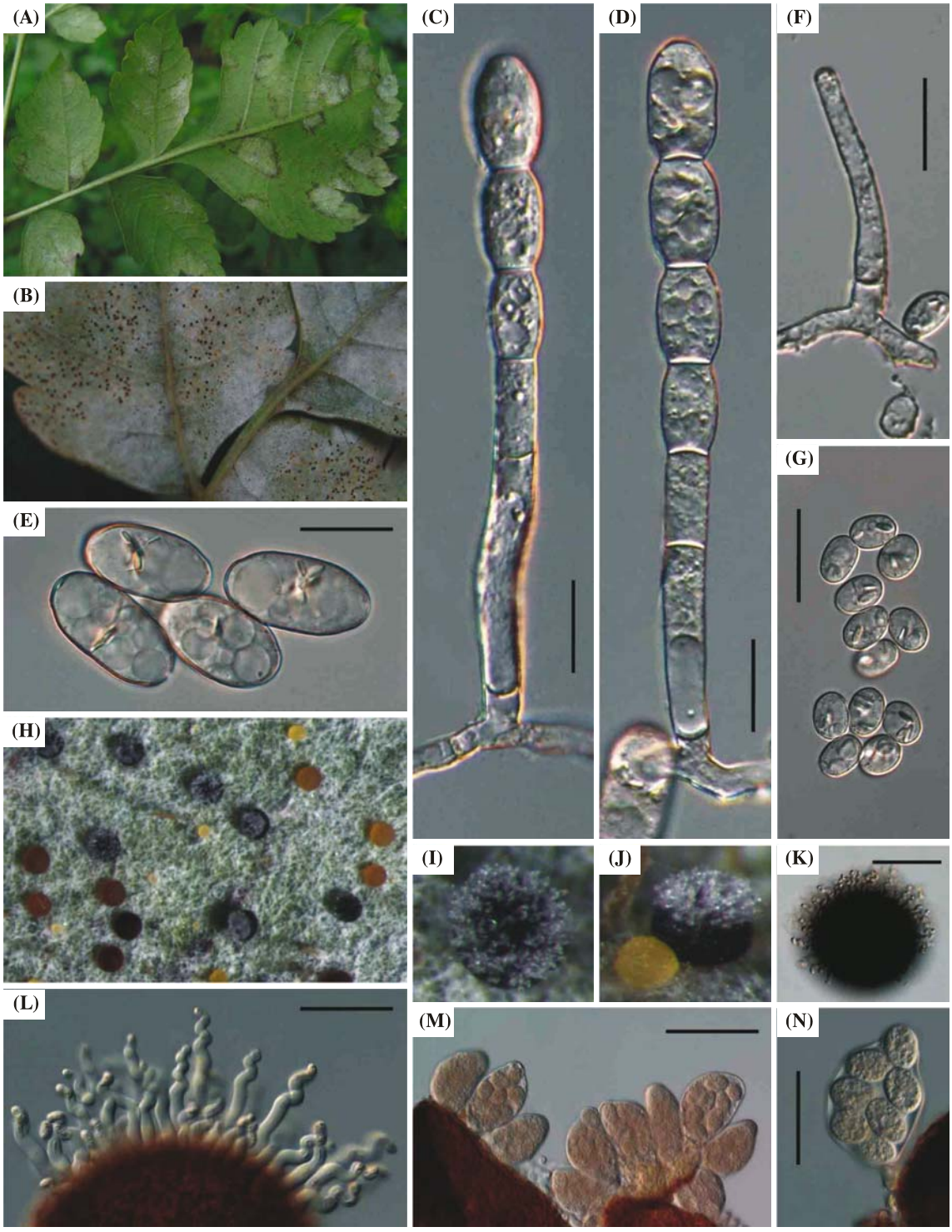

Fig. 1. Powdery mildew infections of *Koelreuteria paniculata* by *Sawadaea koelreuteriae*. (A) Symptoms on the lower leaf surface. (B) Numerous chasmothecia formed on the lower leaf surface. (C-D) Macro-conidiophores. (E) Macro-conidia. (F) Micro-conidiophore. (G) Micro-conidia. (H) Chasmothecia scattered on the lower leaf surface. (I-K) Close-up view of chasmothecia. (L) Details of chasmothecial appendages. (M) Asci from a chasmothecium. (N) Ascus containing 8 ascospores. Bar=20 μm for C-G, 50 μm for L and N, and 100 μm for K and M.

ment gaps were treated as missing data. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap (BS) replication using the above MP settings. The consistency index (CI), retention index (RI), and rescaled consistency index (RC) were calculated for all the most parsimonious trees. The resulting trees were viewed using TreeViewX program (Page, 1996).

The complete ITS rRNA gene region of two Korean specimens of *K. paniculata* consists of 464 nucleotides. Including two outgroup taxa, the final alignment of 38 sequences exhibits 488 characters, of which 360 are constant, 91 are parsimony-informative, and 30 variable characters are parsimony-uninformative. MP analysis resulted in 25032 equally most parsimonious trees with a tree length of 191 steps (CI= 0.7539, RI= 0.8753, RC= 0.6599), one of which is presented in Fig. 2. Two sequences of the complete ITS rRNA gene region from the powdery mildew on *K. paniculata* are identical to each other. The ITS sequences obtained in this study were deposited in GenBank with accession numbers JN171866 and JN171867.

***Sawadaea koelreuteriae* (I. Miyake) H.D. Shin & M.J. Park, comb. nov.** **Fig. 1**

MycoBank MB 563314

Basionym: *Uncinula koelreuteriae* I. Miyake, Bot. Mag. Tokyo 27: 39 (1913)

Synonyms: *Erysiphe koelreuteriae* (I. Miyake) F.L. Tai, Bull. Chinese Bot. Soc. 2: 16 (1936); *Typhulochaeta koelreuteriae* (I. Miyake) F.L. Tai, Bull. Torrey Bot. Club 73: 125 (1946)

Mycelium on leaves, occasionally on rachis and herbaceous stems, hypophyllous, but also epiphyllous in case of infections on young leaves, white, in patches or effuse, evanescent to persistent; *hyphae* straight to sinuous, septate, branched, thin-

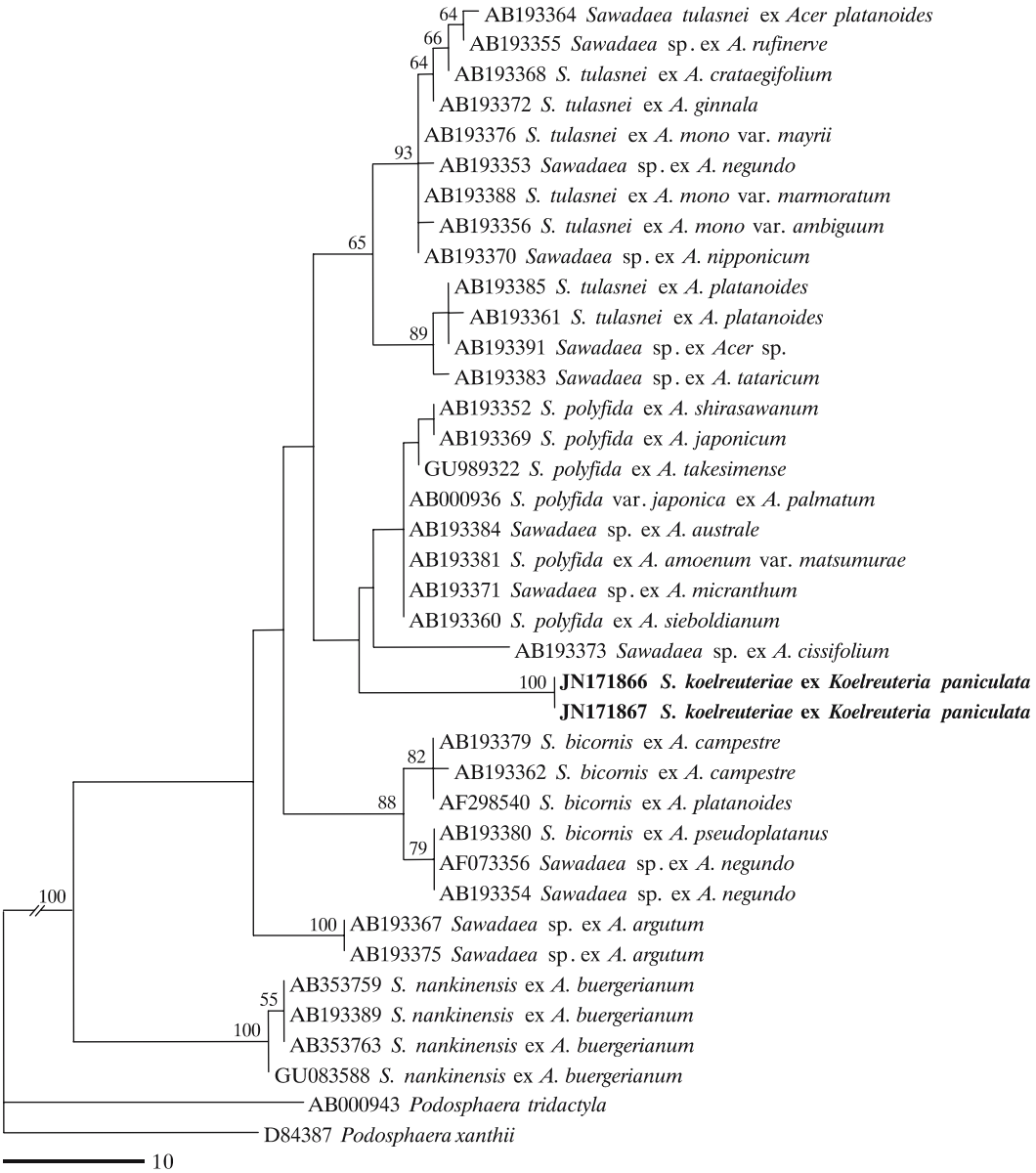

Fig. 2. One of 25032 equally most parsimonious trees resulting from a heuristic search with 100 random taxon additions and TBR branch swapping algorithm based on ITS rRNA gene sequence alignment of 36 *Sawadaea* isolates and two *Podosphaera* outgroup taxa. Scale bar indicates 10 character state changes. Numbers above the branches are bootstrap values (1000 replicates, values greater than 50% shown).

walled, 4-6(-7) μm wide; *hyphal appressoria* inconspicuous, rarely nipple-shaped; *macro-conidiophores* up to 130 μm long, arising from the upper part of mother cell centrally or not centrally, *foot-cells* cylindrical, straight, (24-)30-50 μm long, 7-9 μm wide, followed by 1(-2) shorter cells, basal septum often elevated up to 20 μm from the junction with the mother cell, developing macro-conidia in chains (catenescent) with crenate edge line; *macro-conidia* variable in shape, more or less cylindrical with angular outline forming an octagon or ellipsoid-ovoid to doliiform, fresh conidia 22-32(-38) μm long, 14-20 μm wide, length/width ratio 1.6-2.3, with conspicuous fibrosin bodies, *germ tubes* producing on perihilar position of the conidium, terminating in an unlobed or occasionally moderately lobed appressorium; *micro-conidiophores* 35-75 μm long, *foot-cells* 20-30 μm long, 3.5-5 μm wide, followed by 1-2 shorter cells, basal septum often elevated up to 8 μm from the junction with the mother cell, forming catenescent micro-conidia; *micro-conidia* broadly ellipsoidal to subglobose, 7.5-12 μm long, 5.5-8 μm wide, length/width ratio 1.2-1.5, with conspicuous fibrosin bodies. *Chasmothecia* scattered to somewhat gregarious, 150-190 μm diam., subglobose to hemispherical; *peridium cells* irregularly polygonal, inconspicuous, (6-)10-18 μm diam.; *appendages* numerous, about 50-200 in number on a chasmothecium, arising from the upper half of the chasmothecium, 25-55 μm long, 7.5-12.5 μm wide but somewhat variable, not branched, very occasionally 1(-2) times branched dichotomously, twisted, aseptate, becoming swollen and gelatinized in water, probably ejecting mucilaginous material, hyaline, apices circinate to hooked, becoming narrower; *asci* (7-)10-18 in a chasmothecium, broadly obovoid-saccate, 70-95 μm long, 35-45 μm wide, short-stalked, 8-spored; *ascospores* ellipsoid-ovoid or subglobose, 17-25 μm long, 15-18 μm wide, subhyaline to pale greenish yellow.

Epitypification (designated here): KOREA: Seoul, Seongbuk-gu, Gireum-dong, on living leaves of *Koelreuteria paniculata* Laxm., 14 Oct. 2010, H.D. Shin (KUS-F25489)

Additional specimens examined: Seoul, Anam-dong, 11 Jul. 2010, H.D. Shin (KUS-F25041); Seoul, Mapo-gu, Sangam-dong, 10 Oct. 2010, H.D. Shin (KUS-F25458); Seoul, Seongbuk-gu, Gireum-dong, 14 Oct. 2010, H.D. Shin (KUS-F25488); Seoul, Seongbuk-gu, Anam-dong, 6 Nov. 2010, H.D. Shin (KUS-F25621). All on leaves of *Koelreuteria paniculata*.

Up to now, a total of four species including *T. koelreuteriae* have been described in the genus *Typhulochaeta*, viz. *T. alangii*, *T. couchii*, *T. japonica* and *T. koelreuteriae* (Braun, 1987; Braun *et al*., 2002; Glawe, 2008). However, their anamorphs have not yet been described for any of the *Typhulochaeta* species. During the course of the present study, the anamorphic state of the genus *Typhulochaeta* has been first found in *T. koelreuteriae* (now *S. koelreuteriae*). Interestingly, it exhibits both macro-conidia and micro-conidia stages, an unique character of the genus *Sawadaea* among all members of powdery mildews. Therefore, it proved that *T. koelreuteriae* belongs to the *Sawadaea* anamorph type.

The present phylogenetic analysis of ITS rRNA gene sequences showed that *S. koelreuteriae* is positioned within the genus *Sawadaea*. In addition, this analysis revealed that *S. koelreuteriae* clade is a sister of a separate group which mostly accommodates *Sawadaea polyfida* isolates. However, the phylogenetic relationship between them was not significantly supported. It is assumed that *S. koelreuteriae* might have been diverged from a species of *Sawadaea* during the evolution of this genus. Presumably, a species of *Sawadaea* might have expanded its host range to *Koelreuteria paniculata* from *Acer* species. These two host genera, *Acer* and *Koelreuteria*, belong to the same family, *Sapindaceae* (Herrington *et al*., 2005). As a result of the adaptation to the new host, *S. koelreuteriae* might have acquired a different type of twisted appendage morphology derived from the unique types of *Sawadaea* species. In the previous phylogenetic analyses (Braun and Takamatsu, 2000; Takamatsu *et al*., 2008) the type species of *Typhulochaeta*, *T. japonica*, is placed within *Erysiphe* species. Therefore, it was believed that the genus *Typhulochaeta* belongs to the tribe *Erysipheae* based on the phylogenetic placement of the type species, *T. japonica* which is closely related to *Erysiphe* species. It is quite different from the present result that *T. koelreuteriae* belongs to another genus *Sawadaea*. For this reason, the ITS sequence of *T. japonica* was excepted from the present phylogenetic analysis because *Koelreuteria* powdery mildew showed 51.1% sequence homology when compared with the type species. As the anamorph of *T. japonica* is still unknown, a taxonomic revision of the type species and the genus still remains to be done. In further phylogenetic studies, it is necessary to determine whether the remaining species of *Typhulochaeta* could be transferred to *Sawadaea*, *Erysiphe* or other genus.

The authors are grateful to Uwe Braun of Martin-Luther University in Germany and two anonymous reviewers for their critical comments that improved the manuscript.

References

Braun, U. 1987. A monograph of the *Erysiphales* (powdery mildews). *Beih. Nova Hedwigia* 89, 1-700.

Braun, U., R.T.A. Cook, A.J. Inman, and H.D. Shin. 2002. The taxonomy of the powdery mildew fungi, p. 13-55. *In* R.R. Bélanger, W.R. Bushnell, A.J. Dik, and T.L.W. Carver (eds.), The Powdery Mildews: A Comprehensive Treatise. The American Phytopathological Society Press, St. Paul, USA.

Braun, U. and S. Takamatsu. 2000. Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (*Erysipheae*) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (*Cystotheceae*) inferred from rDNA ITS sequences – some taxonomic consequences. *Schlechtendalia* 4, 1-33.

Farr, D.F. and A.Y. Rossman. 2011. Fungal Databases, Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved 25th July 2011, from http://nt.ars-grin.gov/fungaldatabases/

Glawe, D.A. 2008. The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. *Annu. Rev. Phytopathol*. 46, 27-51.

Harrington, M.G., K.J. Edwards, S.A. Johnson, M.W. Chase, and P.A. Gadek, 2005. Phylogenetic inference in Sapindaceae *sensu lato* using plastid *mat*K and *rbc*L DNA sequences. *System. Bot.* 30, 366-382.

Hirose, S., S. Tanda, L. Kiss, B. Grigaliunaite, M. Havrylenko, and S. Takamatsu. 2005. Molecular phylogeny and evolution of the maple powdery mildew (*Sawadaea, Erysiphaceae*) inferred from nuclear rDNA sequences. *Mycol. Res.* 109, 912-922.

Kusaba, M. and T. Tsuge. 1995. Phylogeny of *Alternaria* fungi known to produce host-specific toxins on the basis of variation in internal transcribed spacers of ribosomal DNA. *Curr. Genet.* 28, 491-498.

Lee, S.B. and J.W. Tayler. 1990. Isolation of DNA from fungal mycelia and single spores, p. 282-287. *In* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White (eds.), PCR Protocols: A Guide to Methods

and Applications. Academic Press, San Diego, USA.
Maddison, D.R. and W.P. Maddison. 2002. MacClade4: analysis of phylogeny and character evolution. Sinauer Associates, Sunderland, UK.
Miyake, I. 1913. Studies in Chinese Fungi. *Bot. Mag.* (Tokyo) 27, 41-44.
Page, R.D.M. 1996. TREEVIEW: An application to display phylogenetic trees on personal computers. *Comput. Appl. Biosci.* 12, 357-358.
Swofford, D.L. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods). Version 4b10. Sinauer Associates, Sunderland, UK.
Tai, F.L. 1936. Notes on Chinese Fungi VI. *Bull. Chinese Bot. Soc.* 2, 16-28.
Tai, F.L. 1946. Further studies on the *Erysiphaceae* of China. *Bull. Torrey Bot. Club* 73, 108-130.
Tai, F.L. and C.T. Wei. 1932. Notes on Chinese Fungi II. *Sinensia* 3, 93-130.
Takamatsu. S., T. Ito, H. Yamamoto, and U. Braun. 2008. *Sawadaea nankinensis* comb. nov.: a powdery mildew fungus of *Acer buergeriana*. *Mycoscience* 49,161-167.
Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 24, 4876-4882.
White, T.J., T. Bruns, S.B. Lee, and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315-322. *In* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White (eds.), PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, USA.
Zheng, R.Y. and G.Q. Chen. 1979. Taxonomic studies on the genus *Bulbouncinula* of China. I. The establishment of the genus *Bulbouncinula* gen. nov. *Acta Microbiol. Sin.* 19, 375-378.