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SHORT COMMUNICATION

Cystotheca tjibodensis (Erysiphaceae, Ascomycota): rediscovery in Java after 90 years and first finding of anamorph

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Abstract *Cystotheca tjibodensis*, formerly known as *Lanomyces tjibodensis* (Perisporiales), is a fungus found in 1920 in Indonesia. This species, hitherto only known from its type collection, is now regarded as belonging to the Erysiphales. However, molecular data are still required to verify the taxonomic affinity. In March 2011, we rediscovered this fungus at Cibodas Botanical Garden, Java. Detailed characterizations of this tropical powdery mildew are reported in this study based on morphological and molecular examinations. The anamorph of this species that was not found in the type specimen is also reported in this study.

Keywords Indonesia · *Lanomyces* · Powdery mildew · rDNA ITS sequence · Taxonomy

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Cystotheca tjibodensis (Gäum.) Katumoto (Katumoto 1973), formerly known as *Lanomyces tjibodensis* (Gäumann 1922), is a tropical powdery mildew endemic in Indonesia and infecting *Castanopsis argentea*. This fungus was collected in Cibodas (Java Island) in 1920 by Gäumann (1922). It was first included in the family Perisporiaceae (sooty molds), not Erysiphaceae. Gäumann (1922), at the time of the establishment of *L. tjibodensis*, considered this fungus an intermediate between the Erysiphaceae and Perisporiaceae (Perisporiales). The genus *Lanomyces* Gäum. had been maintained in the family Perisporiaceae by Gäumann until 1949, although Hansford (1946) put it in the family Parodiellinaceae. Katumoto (1973) later re-examined the type specimen of the fungus and reported that it should be included in the Erysiphaceae, based on the morphological characteristics of its chasmothecia, asci, and haustoria. He considered the morphology of the chasmothecia with two easily separating peridium layers as the most important characteristic of the genus concerned. The 8-spored asci [Gäumann (1922) described asci with many ascospores] were also considered an important character. Katumoto (1973) pointed out that the description of "many ascospores per ascus" by Gäumann (1922) was based on a misinterpretation of the inner peridium cells of the chasmothecia because they were in an overlapped condition.

In order to clarify Katumoto's (1973) re-examination and reassessment, Takamatsu, one of the authors of the present report, has tried several times to determine the DNA sequence from the isotype specimen of *C. tjibodensis*, but all attempts failed, probably due to the age of this material. Therefore, fresh material was required for DNA sequencing, but *C. tjibodensis* was, unfortunately, known only from its type specimen collected 90 years ago. In March 2011, fresh materials of *C. tjibodensis* were

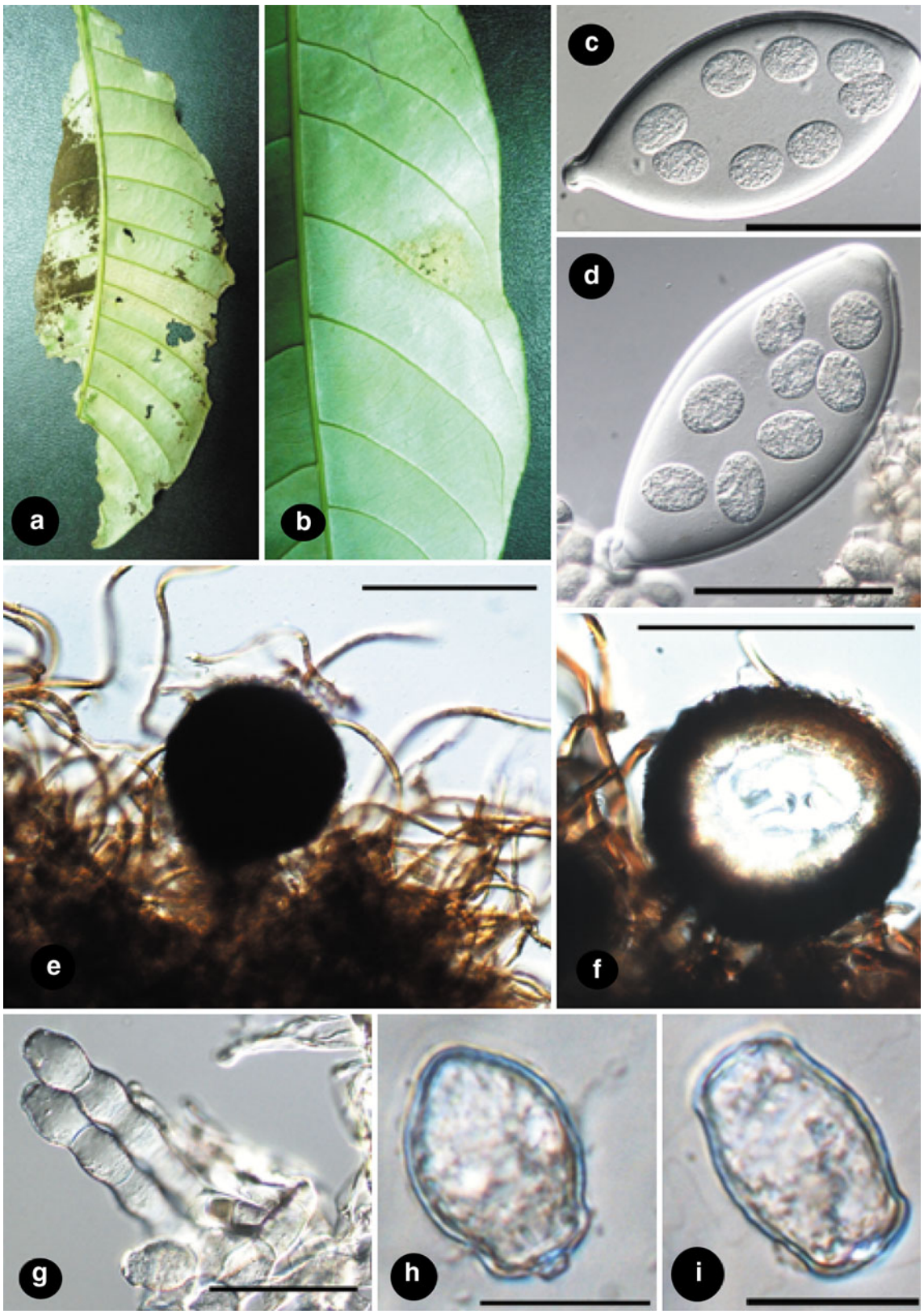

Fig. 1 *Cystotheca tjibodensis* on *Castanopsis argentea*: **a**, **b** symptoms; **c**, **d** asci with 8 ascospores; **e**, **f** chasmothecium; **g** conidiophores and catenate conidia; **h**, **i** barrel-shaped conidia. *Bars* **c**, **d** 30 μm; **e**, **f** 120 μm; **g** 30 μm; **h** 20 μm

collected from *Castanopsis argentea* at the type locality, i.e., Cibodas Botanical Garden (Java, Indonesia). The objectives of the present study were: (1) to obtain DNA samples of this fungus, and (2) to prove Katumoto's (1973) taxonomic conclusions by sequencing the DNA from fresh material.

Specimens were collected at Cibodas Botanical Garden, Bogor, West Java province, Indonesia, on 14 March 2011. We found both the teleomorphic and the anamorphic stages of this fungus. This is the first finding of the anamorphic stage of this fungus. Detailed morphological examinations were carried out as outlined for *Neoerysiphe* spp. in Heluta et al. (2010). Specimens were deposited at the National Museum of Nature and Science (TNS) and Mie University Mycological Herbarium (MUMH), Japan. Whole-cell DNA extraction of the teleomorphic stage and the anamorphic stage was performed separately, using the Chelex method (Walsh et al. 1991), as described in Hirata and Takamatsu (1996). The rDNA internal transcribed spacer (ITS) region including 5.8S rDNA was amplified using primers ITS5 (White et al. 1990) and p3 (Kusaba and Tsuge 1995) for the first amplification. The ITS5/p3 fragment was subjected to the second amplification using powdery mildew-specific primer sets ITS5/PM6 and PM5/p3 according to the procedure of Takamatsu and Kano (2001). The polymerase chain reaction (PCR) products obtained with primers ITS5/PM6 and PM5/p3 fragments were sent to SolGent (Daejeon, South Korea) for sequencing, using ITS1 and ITS4 (White et al. 1990) as sequence primers, respectively. Representative sequences determined in this study were deposited in DNA databases (DDBJ, EMBL, GenBank) under the accession numbers of JN807325–JN807326. Sequence alignments and phylogenetic analyses were carried out as described by Takamatsu et al. (2006). In addition to the two newly obtained ITS sequences, five others determined earlier in *Cystotheca* spp., as well as the ITS sequences of *Podosphaera* (twelve sequences) and *Sawadaea* (four sequences) retrieved from DNA databases, were included in the analysis. The alignments were deposited in TreeBASE (http://www.treebase.org/) under the accession number of S11971.

Teleomorph (Figs. 1a–f, 2)—colonies chocolate brown. Aerial hyphae long filiform, 5–7 µm wide, brown to dark brown. Chasmothecia (96–)105.5–139(–151) µm in diam. Appendages absent. Asci (98–)105–166(–191) × (71–)66.5–83(–88) µm, 8-spored, globose to ellipsoid. Ascospores (20–)23–35(–35.5) × (15.5–) 16–27(–32) µm, ellipsoid to subglobose. Host: *Castanopsis argentea* A.DC. (Fagaceae).

Anamorph (Figs. 1g–i, 3)—mycelium, hypophyllous, in grayish-white patches, persistent; hyphae branched, usually straight to somewhat sinuous, 3–6 µm wide, septate, hyaline, smooth, thin-walled. Appressoria indistinct. Conidiophores arising from superficial hyphal mother cells, terminal to lateral, almost in the middle of the mother cell, erect, straight, (94–)101–147(–158) × (10.5–)11–13.5(–15) µm, producing conidia in chains with sinuate edge. Foot cells (12–)19–37(–61) × (9–)11–14(–19) µm, cylindrical, straight to mostly somewhat curved to distinctly sinuous. Conidia broadly ellipsoid-ovoid, (22.5–)24.5–30(–33) × (16.5–)18–22(–23) µm, with fibrosin bodies but not conspicuous. Germ tubes not observed.

The two ITS sequences from the teleomorph and anamorph of *C. tjibodensis* were aligned with 21 sequences of *Cystotheca*, *Podosphaera*, and *Sawadaea* (tribe Cystotheceae) retrieved from DNA databases. Of the 496 total characters used in this analysis, 346 characters were constant, 18 characters were variable but parsimony-uninformative, and 132 characters were parsimony-informative. The best parsimonious tree generated by the maximum parsimony analysis was obtained in 310 steps (consistency index 0.697, retention index 0.873, rescaled consistency index 0.608) (Fig. 4). The three genera used in this analysis formed the respective monophyletic clades with strong bootstrap

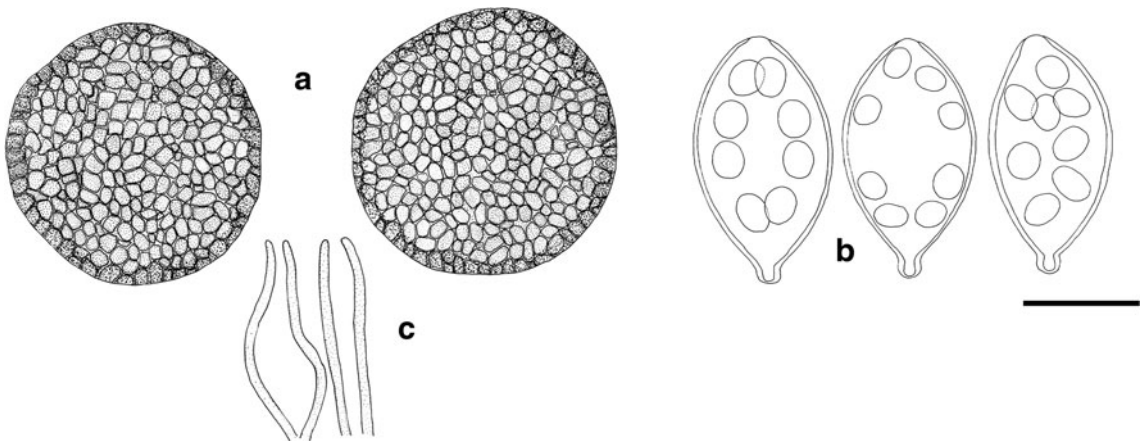

Fig. 2 Line drawing of *Cystotheca tjibodensis* (teleomorph): **a** chasmothecium; **b** asci with 8 spores; **c** aerial hyphae. *Bar* 50 µm

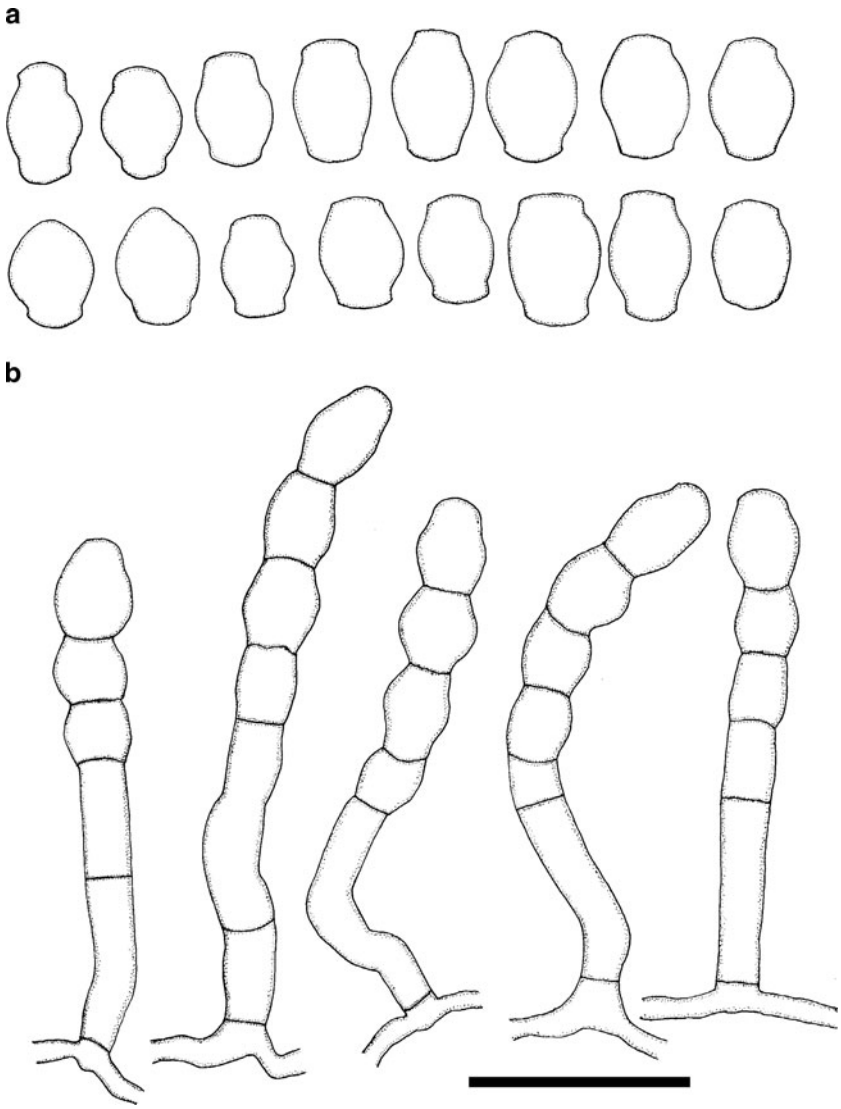

Fig. 3 Line drawing of *Cystotheca tjibodensis* (anamorph): **a** conidia; **b** conidiophores. *Bar* 50 μm

supports (≥92%). The two sequences of *C. tjibodensis* from the anamorph and teleomorph were identical to one another and were clearly included in the group of *Cystotheca*.

Until 1931, the genus *Lanomyces* had been included in the Perisporiaceae based on the intramatrical hyphae and the lack of the anamorphic stage. Clements and Shear (1931) tabulated the genus *Lanomyces* in the indexing key to the genera of Erysiphaceae along with *Sphaerotheca* Lév. and *Podosphaera* Kunze. Katumoto (1973) mentioned that the genera *Cystotheca* Berk. & M.A. Curtis and *Lanomyces* are morphologically indistinguishable, because both genera share similar characteristics such as intramatrical hyphae, aerial hyphae, and chocolate-brown to purplish-brown color of the colonies. Based on the ascogenous stage, the genera *Lanomyces* and *Cystotheca* were also indistinguishable by having two easily separating layers with a coriaceous outer and membranaceous inner one (Katumoto 1973). Katumoto (1973) has also emphasized that the characters mentioned above are not so essential in differentiating *Lanomyces* (Perisporiaceae) from the genera of Erysiphaceae, as intramatrical hyphae were also known in the genera *Phyllactinia* Lév., *Pleochaeta* Sacc. & Speg., and *Leveillula* G. Arnaud. Thus, Katumoto (1973) insisted that the genus *Lanomyces* should be included in the family Erysiphaceae based on the morphological characteristics mentioned above.

During the course of the present study, we found both the anamorphic and the teleomorphic stages of *C. tjibodensis* on *Castanopsis argentea* in the type locality, and determined the DNA sequences of the ITS region of the ribosomal DNA in both stages of the fungus. Our phylogenetic analysis (Fig. 2) showed that the ITS sequence of the anamorph is identical to that of the teleomorph, confirming that the anamorph found in Cibodas belongs to *C. tjibodensis.* The finding of the anamorphic stage with catenate conidia with a sinuate edge line also confirms that

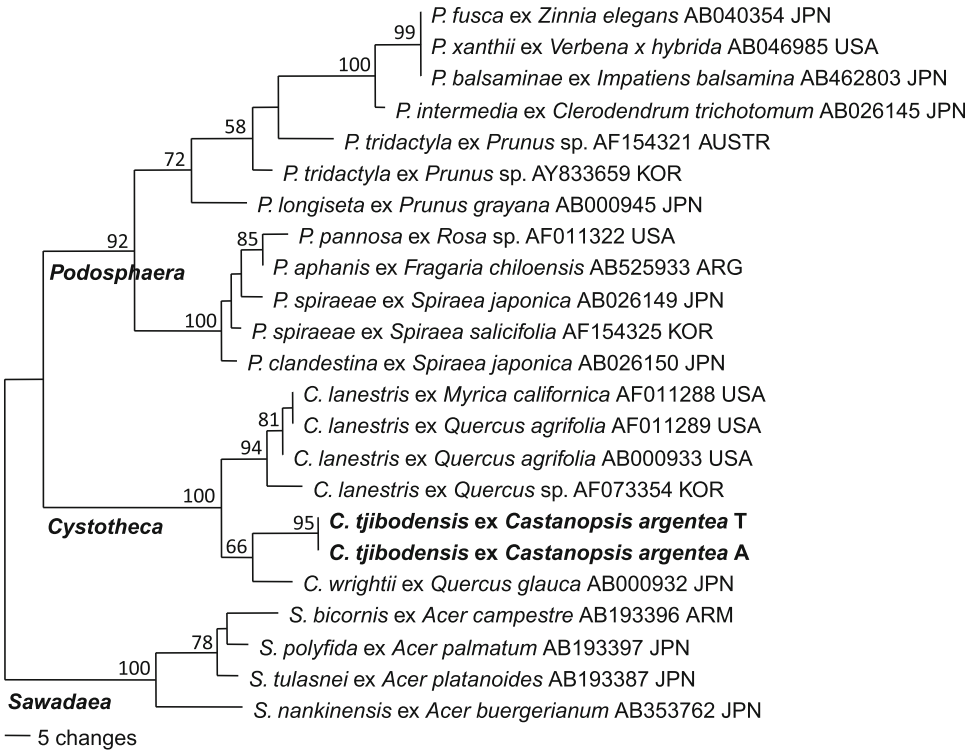

Fig. 4 Phylogenic tree of *Cystotheca tjibodensis* inferred from the rDNA internal transcribed spacer (ITS) sequences analysis using the maximum parsimony (MP) method. The percentage bootstrap support values (1,000 replications; ≥50%) are shown on the branches. *T* teleomorph, *A* anamorph

the fungus belongs to *Cystotheca* sensu Braun (1987). The *C. tjibodensis* forms a clade separate from a *C. wrightii*–*C. lanestris* clade. Morphologically, *C. tjibodensis* differs from *C. wrightii* by having intramatrical hyphae, long filiform aerial hyphae, lacking chasmothecial appendages, and having larger asci (80–100 × 70–80 μm vs. 60–75 × 45–60 μm in *C. wrightii*) (Katumoto 1973). In addition, *C. tjibodensis* is morphologically easily distinguishable from *C. lanestris* by having intramatrical hyphae, lacking chasmothecial appendages, and having larger asci (80–100 × 70–80 μm vs. 75–80 × 50–60 μm of *C. lanestris*). Both the morphological and the molecular data support the species status of *C. tjibodensis* and nicely strengthen Katumoto's earlier work on this fungus.

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References

Braun U (1987) A monograph of the Erysiphales (powdery mildews). Beih Nova Hedwigia 89:1–700

Clements FE, Shear CL (1931) The genera of Fungi. The H.W. Wilson Company, New York

Gäumann E (1922) Über die Entwicklungsgeschichte von Lanomyces, einer neuen Perisporiaceen Gattung. Ann Jard Bot Buitenzorg 32:43–63

Hansford CG (1946) The foliicolous Ascomycetes, their parasites and associated fungi. Mycol Pap 15:1–240

Heluta V, Takamatsu S, Harada M, Voytyuk S (2010) Molecular phylogeny and taxonomy of Eurasian *Neoerysiphe* species infecting *Asteraceae* and *Geranium*. Persoonia 24:81–92

Hirata T, Takamatsu S (1996) Nucleotide diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. Mycoscience 37:283–288

Katumoto K (1973) Notes on the genera *Lanomyces* GÄUM. and *Cystotheca* BERK. et CURT. Rept Tottori Mycol Inst 10:437–446

Kusaba M, Tsuge T (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

Takamatsu S, Kano Y (2001) PCR primers useful for nucleotide sequencing of rDNA of the powdery mildew fungi. Mycoscience 42:135–139

Takamatsu S, Matsuda S, Niinomi S, Havrylenko M (2006) Molecular phylogeny supports a northern hemisphere origin of *Golovinomyces* (Ascomycota: Erysiphales). Mycol Res 110:1093–1101

Walsh SP, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513

White TJ, Bruns TD, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide of methods and applications. Academic Press, San Diego, pp 315–322