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Crassitunica tubakii gen. et sp. nov, a New Taxon in the Family Sclerotiniaceae *s. lat.*

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Abstract *Crassitunica tubakii* gen. et sp. nov. is proposed to accommodate a single species previously recognized under incorrectly identified name, *Lambertella brunneola*. Recent molecular phylogeny revealed it is no longer a member of *Lambertella*, but appropriate taxonomy for this fungus has not been proposed. Here, we propose a new genus to accommodate the fungus for its unique morphological features, supported by a molecular phylogenetic analysis based on ITS rDNA sequence.

Keywords: *Aucuba japonica*, ITS rDNA, *Lambertella brunneola*, Rutstroemiaceae, stroma, taxonomy.

Introduction

The genus *Lambertella* had been an intriguing, heterogenous genus containing c. 60 species, being the largest genus in the family Rutstroemiaceae until Zhao *et al.* (2016) proposed a strict generic circumscription based on morphology and molecular phylogenetic data. The genus *Lambertella* is now defined by having textura prismatica ectal excipulum composed of brown colored cells, and ascospores turning brown in the ascus before discharge. As a result of their revision, some species previously treated in *Lambertella* need to be disposed to other genera.

The fungus misidentified as *Lambertella brunneola* (Pat.) Le Gal. by Korf (1958) was one such species. The first specimen was collected and identified in Japan by R. P. Korf and isolated by K. Tubaki (Korf, 1958) and the culture was deposited to the Institute for fermentation, Osaka (currently Biological Resource Center, National Institute of Technology and Evaluation (Tokyo, Japan, https://www.nite.go.jp/en/nbrc/cultures/index.html). The specimen was later examined by Dumont (1971) who revised the genus *Lambertella*. Dumont (1971) concluded that the specimen was not *L. brunneola*, but he did not dispose the fungus to any known taxa. Therefore, the taxonomic position of the fungus remained uncertain. Zhao *et al.* (2016) repeatedly collected this fungus, and provided morphological and molecular phylogenetic information. It was treated as "*Lambertella* sp. 2" in their analysis (Zhao *et al.*, 2016). They clearly demonstrated that the fungus in question is distincct from *Lambertella s. str*., but they hesitated to establish a new taxon because there was no species with close phylogenetic or morphological affinity. In spite of five years of our continuous survey of Helotiales since then, we still have not found appropriate genus for the present fungus.

Meanwhile, Tubaki's isolate of the fungus has

been used in applied science studies and referred to incorrectly as *Lambertella brunneola* (Tayone, 2013). If the fungus is left misidentified or unidentified, further confusion may be brought up in future research projects. To avoid this, we establish a new genus and species for the isolate misidentified as *L. brunneola*.

Materials and Methods

Methods for isolation, culture, and observation followed Zhao *et al.* (2016). Description of the color codes followed the Pantone color code adopting CYMK system referring to a Pantone color bridge (Anonymous, 2005). Culture for DNA extraction to sequence procedure followed Zhao *et al.* (2016), and obtained sequences were deposited to DDBJ. Sequences for internal transcribed spacer (ITS) and 5.8S ribosome sequences were obtained using ITS1F and ITS4 (White *et al.*, 1990) as primers in polymerase chain reaction. Isolates were deposited in Biological Resource Center, National Institute of Technology and Evaluation (NITE-BRC, Tokyo, Japan, https://www.nite.go.jp/en/nbrc/cultures/index.html). Extracted DNA were deposited to the Center for Molecular Biodiversity Research, National Museum of Nature and Science. Coordinates followed WGS84 datum.

Descriptions

Crassitunica tubakii **Yan J.Zhao & Hosoya** gen. et sp. nov.

[Figs. 1–3]

Holotype. TNS-F-44021 (ex-type culture NBRC 114959), on decaying leaves of *Aucuba japonica* Thunb. Meijijingu Shrine, Yoyogi, Shibuya-ku, Tokyo, Japan (35.673767, 139.700775), 2011-XI-8, col. T. Hosoya.

Mycobank number for the genus: MB838477

Mycobank number for the species: MB838478

Stroma substratal, visible as blackened zones on leaf veins and petioles of host. **Apothecia** superficial, solitary or gregarious, short-stipitate, occurring mainly on leaves, sometimes on petioles and branches of the host; disc flat to convex, 0.5–2.5 mm in diameter when dry; hymenium beige to pale brown (Pantone 7502PC = C0 M8 Y33 K10) when fresh, becoming paler when dry; receptacle cream to off white (Pantone warm gray 2C = R213 G210 B202), floccose; stipe concolorous with receptacle, becoming black towards the base, furfuraceous. **Ectal excipulum** textura prismatica, pale brown, composed of thick-walled, brick-shaped cells, 3–6 × 6–10 µm, tightly packed and arranged almost perpendicular to the surface, ending up to hyphal protrusions (hairs) in the outermost layer; at the upper portion of the margin, cells becoming thinner walled, arranged almost parallel to the surface. **Medullary excipulum** textura intricata, hyaline, smooth, loosely interwoven, separate hyphae of 2.5–3.5 µm wide. Hairs mostly cylindrical, sometimes tapered to the apex, thin-walled, 1–2 septate, 4 µm thick at the base, 6–15 µm long; apical cells mostly blunt, sometimes irregularly protruding. **Asci** 85–132.5 × 8.8–12 µm, cylindrical clavate, 8-spored, arising from croziers; apex flattened, thickened; pore faintly stained by Melzer's reagent, becoming more clearly stained after KOH pretreatment. **Ascospores** 11.3–13.8 × 4.5–6.3 µm (12.2 ± 0.7 × 5.4 ± 0.5 µm on average ± SD, n = 33), uniseriate or irregularly biseriate, ellipsoid, aseptate, mostly with 1–2 large guttules; becoming light brown, 1-septate and thick walled when germinated, one of the ascosporous cell sometimes darker than the other, or one cell remaining hyaline; membranaceous structure sometimes observed around the germinating ascospores. **Paraphyses** equal to or slightly exceeding the asci by 7.5–15 µm, filiform, septate, simple or branched near the base, frequently expanded at the apex of 2.5–3.5 µm wide, sometimes equipped with granulate resinous matter.

Cultural characteristics: **Colonies** on PDA fast growing, covering the whole plate after 4 weeks incubation, circular, flocculent, initially beige, becoming grayish to pale brown in age; aerial hyphae gray, cottony, partly developed; margin

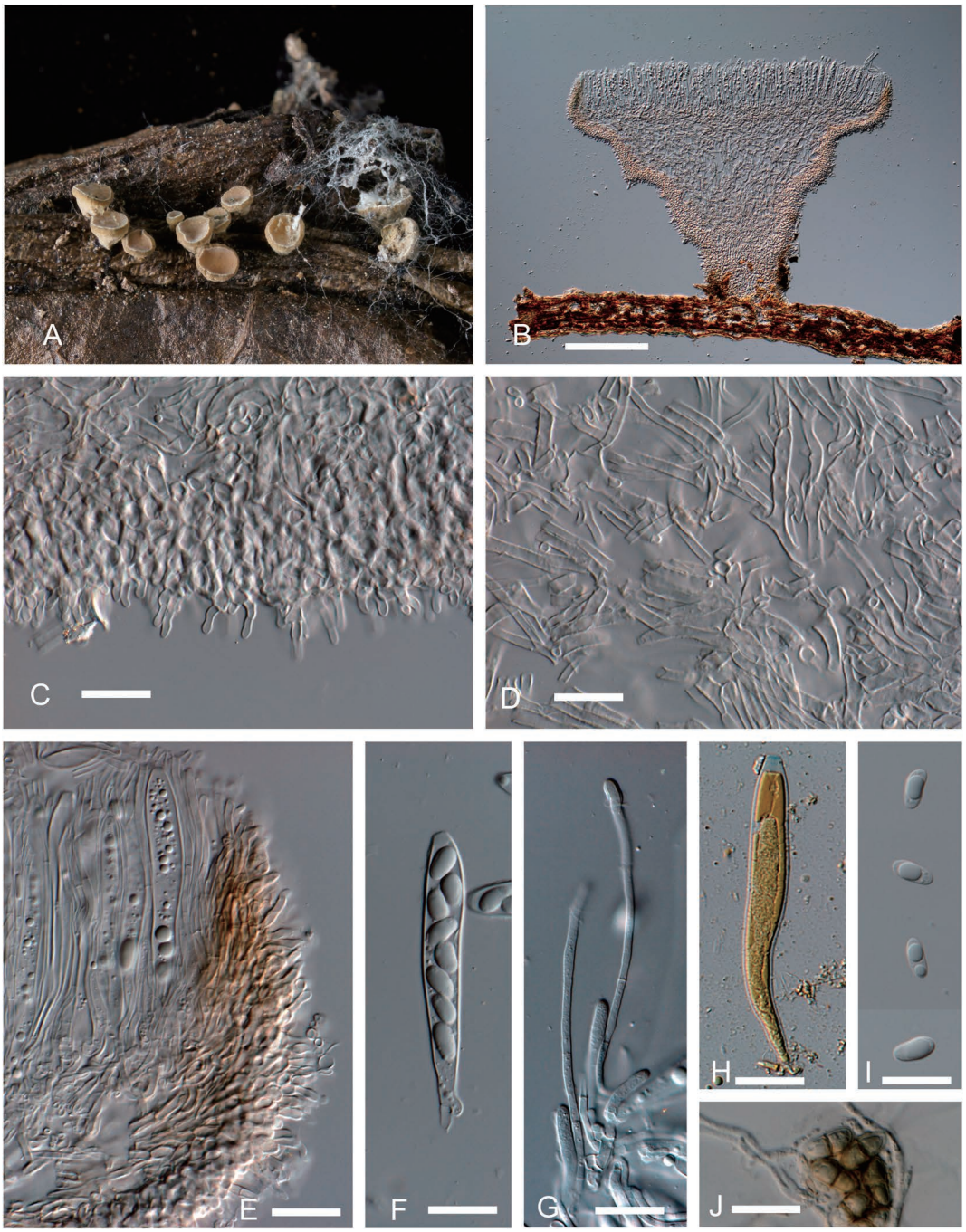

Fig. 1. *Crassitunica tubakii* (TNS-F-44021). A. Fresh apothecia on decaying leaves of *Aucuba japonica*. B. Vertical section of an apothecium. C. Close up of ectal exipulum. D. Close up of medullary excipulum. E. Close up of ectal excipulum at the margin. F. Ascus. G. Paraphyses. H. Reaction of ascal apex to Melzer's reagent. I: Ascospores. J: Germinating ascospores. Scales: B = 200 μm, C–J = 20 μm.

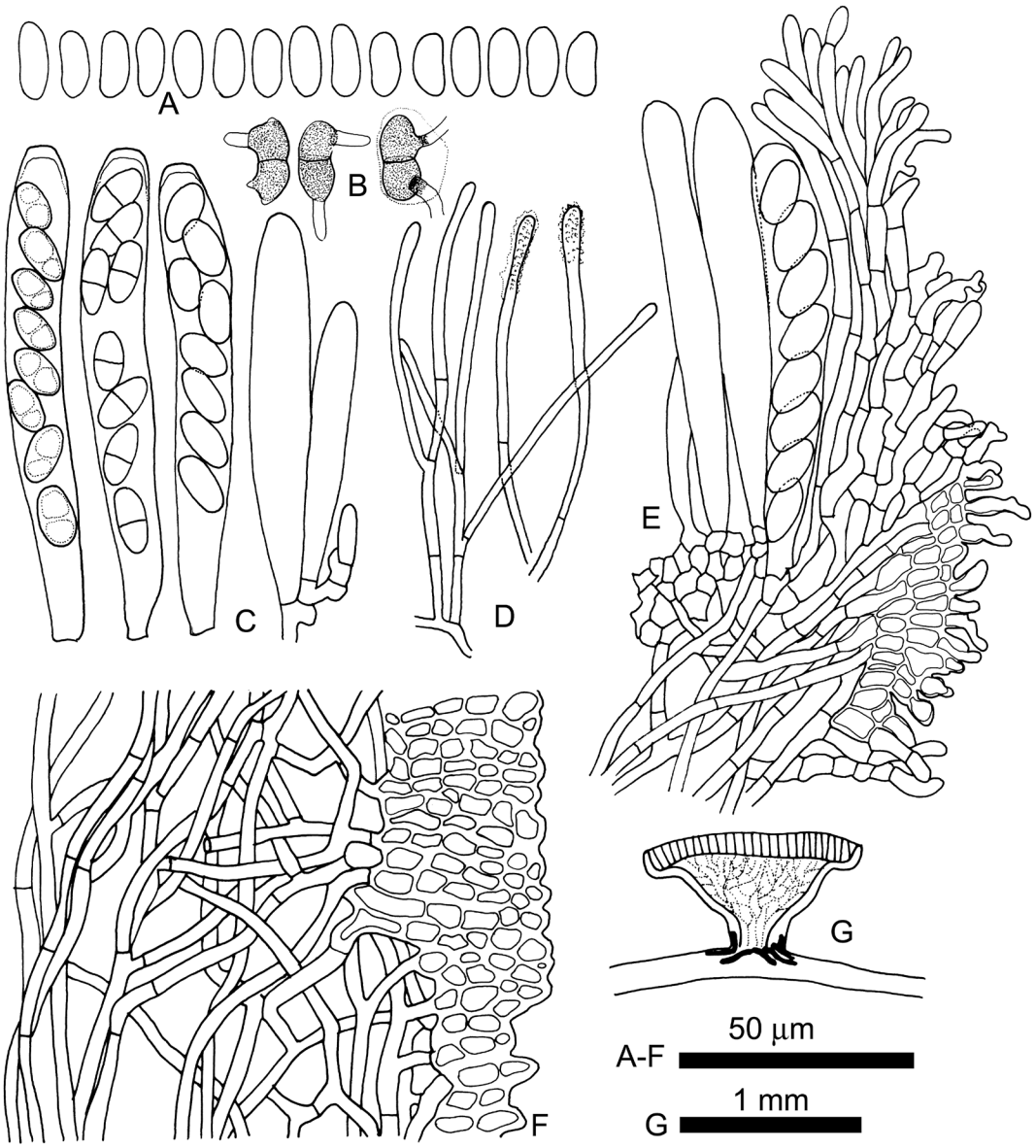

Fig. 2. *Crassitunica tubakii*. A. Young ascospores. B. Ascospores tuning brown, one-septate before germination. The dotted line shows membranaceous structure sometimes observed on germination. C. Asci and ascospores. One at the right shows the continuous corzier formation at the base. D. Paraphyses. Resinous materials observed at the apices. E. Vertical section of the apothecium at the margin. The ectal excipulum composed of thick-walled cells with short protrusion. F. Lower ectal excipulum. Thick-walled cells at the outside and inner tissue composed of sparsely intricated cells. G. Schematic drawing showing the outline of the apothecial structure. The black lines showing the developed stroma.

entire. Reverse brown, filamentous; rind becoming distinct after 8 weeks incubation, delimiting irregular portions of the agar, composed of a single layer of cells with walls pigmented to various extent, epidermoid in face view.

Etymology. The generic name refers to the thick-walled ectal excipulum. The specific epithet refers to the name of a Japanese researcher

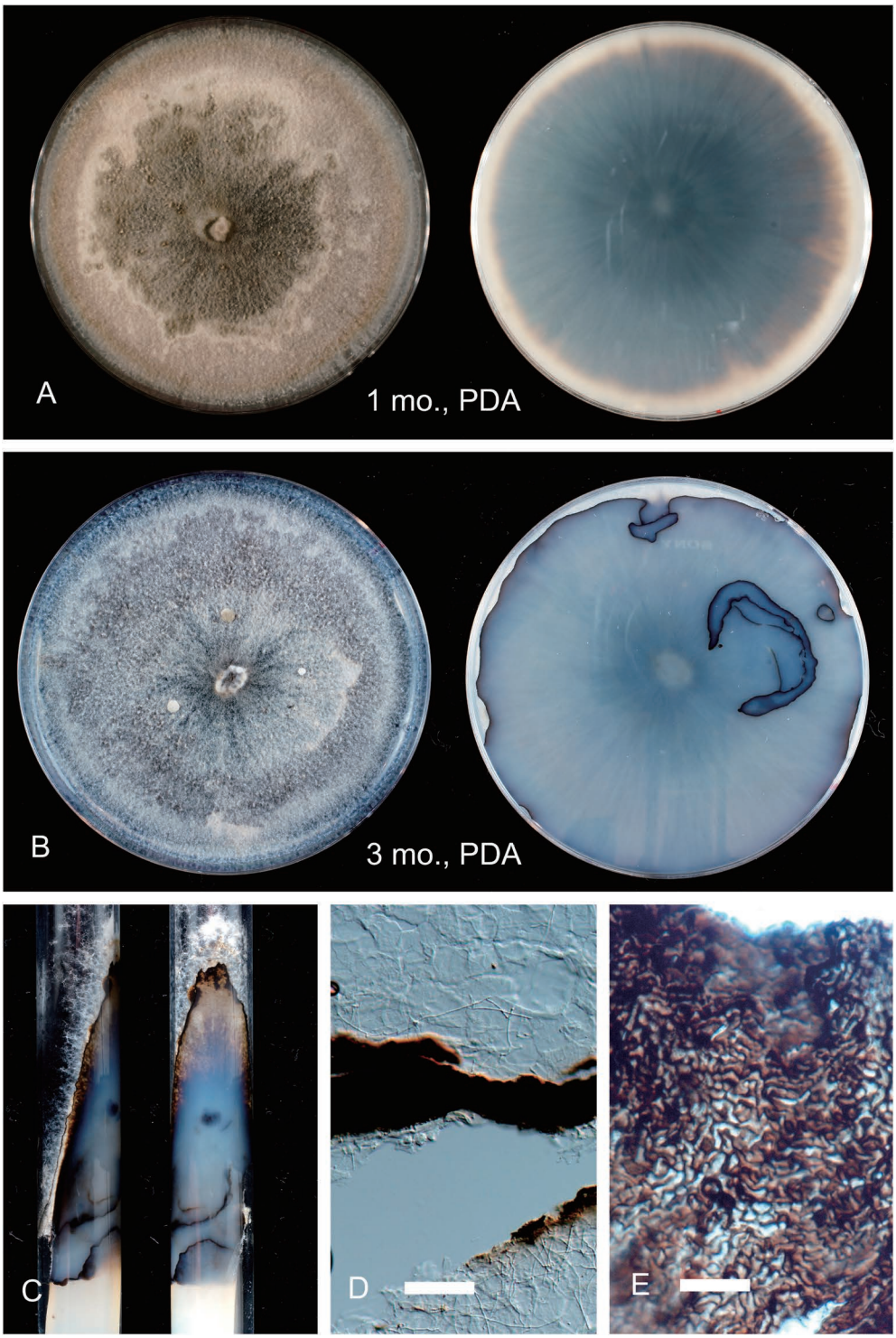

Fig. 3. Cultural characters of *Crassitunica tubakii* (NBRC 114959). A. Colony on potato dextrose agar (PDA, 20°C, 1 month). B. Colony on PDA (20°C, 3 month). C. Rind on PDA (20°C, 6 month). D. Vertical view of rind. E: Surface view of rind showing the epidermoid cells. Scales D–E = 20 µm.

who firstly found this species.

Other specimens examined. All except for TNS-F-36994 were on hanging decaying leaves of *Aucuba japonica.* Unless noted, collector is TH.

TNS-F-17633, Kikuchi Gauge, Kumamoto Pref., 2005-X-10; TNS-F-18101, Maruyama, Iryuda, Odawara-shi, Kanagawa Pref., 2002-XI-8, col. Yousuke Degawa; TNS-F-30018 (Culture NBRC 114958), The Fukiage Gardens in the Imperial Palace Grounds, Chiyoda-ku, Tokyo (35.686306, 139.752222), 2009-X-7; TNS-F-31044, Kazusa-kamatari, Kisarazu, Chiba Pref., 2003-VII-18; TNS-F-31157 (Culture NBRC 110760), Iryuda, Odawara-shi, Kanagawa Pref. (35.243305, 139.11711), 2005-VIII-18, col. Y. Degawa; TNS-F-36994 (culture NBRC 110762), on *Fatsia japonica* petiole, The Fukiage Gardens in the Imperial Palace Grounds, Chiyoda-ku, Tokyo (35.686036, 139.751053), 2010-X-5; TNS-F-40099, Meijijingu Shrine, Yoyogi, Shibuya-ku, Tokyo (35.6697222, 139.705556), 2011-XI-24, col. Y. Zhao; TNS-F-40126 (Culture NBRC 109885), The Fukiage Gardens in the Imperial Palace Grounds, Chiyoda-ku, Tokyo (35.685914, 139.751219), 2012-VI-20; TNS-F-40315 and TNS-F-40319, Institute of Nature Study, Meguro, Tokyo, 2016-XI-15; TNS-F-44244 (Culture NBRC 114960), Iryuda, Odawara, Kanagawa Pref. (35.241672, 139.119964), 2011-XI-12; TNS-F-44262 (Culture NBRC 114961), The Fukiage Gardens in the Imperial Palace Grounds, Chiyoda-ku, Tokyo (35.687317, 139.748667), 2011-XI-21; TNS-F-57191, Daiomatsu, Tsukuba, Ibaraki Pref., 1997-VII-2; TNS-F-190417, Government Forest Station, Asakawa, Tokyo, Honshu, 1957-XII-8, col. K. Aoshima, K. Tubaki *et al.*

Japanese name. Aoki-ba-nise-kinkaku-byo-take

Sequences registered. AB705246 (NBRC 110760), AB705247 (NBRC 114958), AB705245 (NBRC 110762), LC597363 (NBRC 114959), LC425046 (NBRC 114960), LC597362 (NBRC 114961) AB926080 (NBRC 109885); all sequences are for ITS-5.8S ribosomal RNA. AB926208 (NBRC 109885, RPB2). AB926163 (NBRC 109885, partial sequence for large subunit ribosomal RNA).

Notes. As Dumont (1971) suggested, the most characteristsic morphological feature of *Crassitunica tubakii* is the structure of ectal excipulum, composed of narrow, thick-walled cells vertically oriented to the surface and tightly compressed, having short protrusions. Such a structure is clearly different from *Lamberella s. str.*, supporting the unique position of the present fungus.

In the maximum likelihood analysis using combined LSU and RPB2 by Zhao *et al.* (2016), *C. tubakii* (cited as *Lambertella* sp. 2 in their paper) was situated at the base of strongly supported group composed of *Rutstroemia firma* (Pers.) P.Karst., *Poculum pseudosydowianum* Yan J.Zhao & Hosoya, *Lanzia pruni-serotinae* (Whetzel & W.L.White) M.P.Sharma & R.M.Sharma with unidentified species referred to *Lanzia* and *Moellerodiscus*. None of these genera showed resemblance to accommodate *C. tubakii*, and it was thought to be more appropriate to establish a new genus. This clade was clearly apart from *Lambertella s. str.*

All the sequences obtained in the present study were identical in barcoding region, indicating the species is well-defined in molecular aspect.

Crassitunica tubakii belongs to Rutstoremiaceae, a paraphyletic family recognized by Zhao *et al.* (2016), characterized by the presence of substratal stroma. However, in the more recent molecular analysis based on wider range of fungi and multiple genes (Johnston *et al*, 2019), the circumscription of Rutstroemiaceae is more vague and should be treated in Sclerotiniaceae *s. lat.*

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