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Tubakia chinensis sp. nov. and a key to the species of the genus *Tubakia*

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Abstract: Braun, U., Bien, S., Hantsch, L. & Heuchert, B. 2014: *Tubakia chinensis* sp. nov. and a key to the species of the genus *Tubakia*. Schlechtendalia **28**: 23–28.

The new species *Tubakia chinensis*, collected on living leaves of *Castanea henryi* (*Fagaceae*) in China, Jiangxi Province, is described, illustrated and compared with other species of the genus *Tubakia*. *T. chinensis* is morphologically comparable with *T. japonica* and *T. seoraksanensis*, two Asian species characterised by having similar conidia, but differs from the two species in the size of conidia, conidiogenous cells and scutellum. An additional undescribed *Tubakia* found on *Castanea henryi* together with *T. chinensis* is briefly described, illustrated, discussed and tentatively referred to as *Tubakia* sp. The species assigned to *Tubakia* are discussed and keyed out.

Zusammenfassung: Braun, U., Bien, S., Hantsch, L. & Heuchert, B. 2014: *Tubakia chinensis* sp. nov. und ein Bestimmungsschlüssel für die Arten der Gattung *Tubakia*. Schlechtendalia **28**: 23–28.

Die neue Art *Tubakia chinensis*, gesammelt auf lebenden Blättern von *Castanea henryi* (*Fagaceae*) in China, Jiangxi Provinz, wird beschrieben, abgebildet und mit anderen Arten der Gattung *Tubakia* verglichen. *T. chinensis* ist morphologisch mit *T. japonica* und *T. seoraksanensis* vergleichbar, zwei asiatische Arten charakterisiert durch ähnliche Konidien, aber von beiden Arten durch die Größe der Konidien, konidienbildenden Zellen und des Scutellums zu unterscheiden. Eine weitere bisher unbeschriebene Tubakia-Art, die zusammen mit *T. chinensis* auf *Castanea henryi* gefunden wurde, wird kurz beschrieben, abgebildet, diskutiert und vorläufig als *Tubakia* sp. bezeichnet. Die zu *Tubakia* gestellten Arten werden aufgeschlüsselt.

Key words: *Ascomycota*, *Actinopelte*, pycnothyrium, new species, *Castanea henryi*, Asia.

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Introduction

During the course of mycological examinations in a forest biodiversity-ecosystem functioning experiment carried out in a subtropical forest site near Xingangshan, Jiangxi Province, China, numerous *Castanea henryi* leaves with distinct lesions and well-developed pycnothyria have recently been collected. The causal agent of the disease was readily identifiable as species of the genus *Tubakia* B. Sutton (Sutton 1973). A comparison with all species assigned to *Tubakia* revealed that a new, undescribed species is involved which is morphologically quite distinct from other species of this genus.

Material and Methods

Standard light microscopy (Olympus BX50) has been used to examine the leaf material (unstained samples mounted in distilled water, oil immersion, 1000×). The given size ranges of conidiophores, conidia and other structures are based on 30 measurements. Digital pictures were made with a ZEISS Axioskop 2 with ZEISS AxioCam HR and occasionally optimised with the software ZEISS AxioVision. Freehand drawings were carried out on the basis of microscopic preparations.

Results

Tubakia chinensis U. Braun, S. Bien & Hantsch, **sp. nov.** Figs 1–2

MycoBank, MB 809784.

Etym.: Epithet derived from China, the country of the type locality.

Diagnosis: Tubakiae japonicae similis sed conidiis minoribus, (20–)25–40 × 20–30 µm, parietibus hyalinis vel pallidis, cytoplasmatibus brunneolis et T. seoraksanensis paululum similis sed scutellis 135–200 µm diam., cellulis conidiogenis latioribus, 4–8 µm, hyalinis et conidiis multum maioribus, (20–)25–40 × 20–30 µm.

Leaf spots amphigenous, subcircular to angular-irregular, 0.5–5 mm diam., at first dingy greenish to greyish green, later brownish to dingy greyish brown, finally grey to greyish white, with narrow darker margin, brown to reddish brown, finally darker, often slightly raised or

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limited by veins. Conidiomata (pycnothyria) epiphyllous, small lesions with a single pycnothyrium, larger ones with up to 18, punctiform, black, circular or subcircular when viewed from above, superficial, easily removable, scutellate, fixed to the leaf by a central columella. Scutella convex, 135–200 µm diam., membranous, somewhat translucent (conidia more or less visible beneath the scutellum when viewed from above), with a central hyaline or pale disc, 15–20 µm diam., giving rise to radiating hyphae, cells (5–)8–15(–20) × 3–8 µm, medium brown, thick-walled (–1 µm), smooth, usually one to three times bifurcating, either only at the periphery or deeply cleft, ultimate branchlets with obtuse or often pointed tips. Conidiophores reduced to conidiogenous cells, arising from the underside of scutella around the columella, radiating downward and towards margin, subcylindrical, subclavate, ampulliform, mostly attenuated towards the tip, straight to slightly curved, 10–20 × 4–8 µm, hyaline or subhyaline, thin-walled, smooth, with a single terminal locus, monoblastic. Conidia solitary, globose, subglobose or broadly ellipsoid-obovoid, (20–)25–40 × 20–30 µm, length/width ratio 1.1–1.4, wall 0.7–1.2 µm wide, hyaline or pale, smooth, cell content pale brownish, sometimes somewhat granular, apex and base broadly rounded, with inconspicuous to conspicuous basal hilum, somewhat peg-like when conspicuous, about 4 µm wide and 1 µm high, with delicate frill. Microconidia not observed.

Holotype: China, Jiangxi Province, Xingangshan, subtropical forest site of the BEF-China Project, 29.1250° N, 117.9085° E, on living leaves of *Castanea henryi*, *Fagaceae*, 8 Sep. 2013, S. Bien (HAL 2674 F).

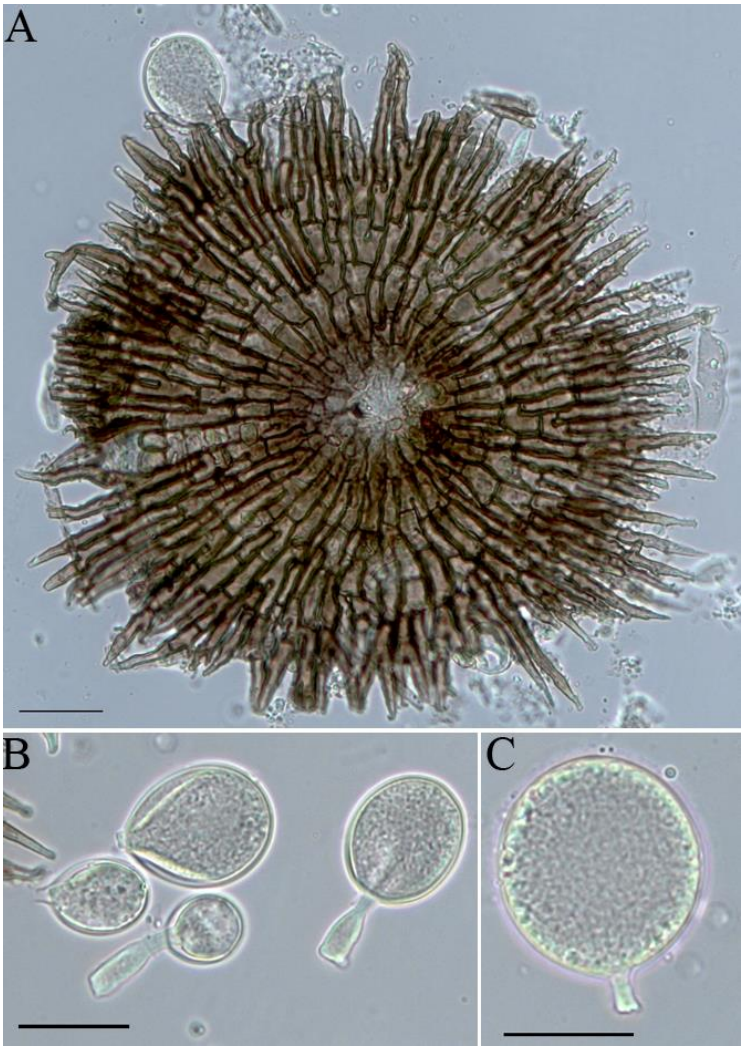


Fig. 1: *Tubakia chinensis*, A – Pycnothyrium, scutellum viewed from above, B – Conidia, shed and still attached on conidiogenous cells, C – Mature conidium with remnant of conidiogenous cell. Bars: 20 µm. Micrographs made by B. Heuchert.

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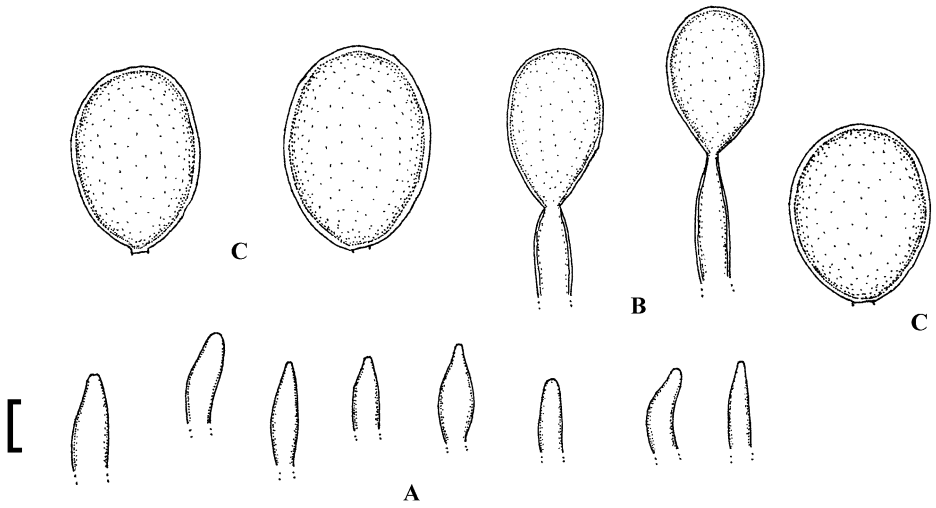


Fig. 2: *Tubakia chinensis*, A – Conidiogenous cells, B – Conidiogenous cells with attached conidia, C – Conidia. Bar: 10 µm. U. Braun del.

Tubakia sp.

Leaf spots amphigenous, subcircular to angular-irregular, 1–7 mm diam., sometimes oblong, up to 10 µm, medium brown on the upper leaf surface, paler below, finally with pale centre, pale brownish to ochraceous, margin indefinite or narrow and somewhat darker, occasionally surrounded by a narrow diffuse halo, yellowish to yellow-green. Conidiomata (pycnothyria) epiphyllous, up to about 15 per leaf spot, punctiform, scutellate, blackish. Scutella convex, 40–80 µm diam., membranous, barely translucent, with a central paler disc, 8–15 µm diam., giving rise to radiating hyphae, cells 4–15 × 2–5 µm, peripheral cells mostly somewhat broadening towards the margin, medium brown, thick-walled (–1 µm), smooth, up to three times bifurcating, either only at the periphery or deeply cleft, peripheral bifurcations mostly shallow, branchlets with obtuse to truncate tips. Conidiophores reduced to conidiogenous cells, arising from the underside of scutella around the columella, radiating downward and towards margin. Conidia solitary, globose to subglobose, 9–11 × 7–9 µm, length/width ratio 1.0–1.2, wall thin, 0.3–0.8 µm wide, hyaline or subhyaline, very pale greenish or faintly olivaceous, smooth, apex and base broadly rounded, basal hilum inconspicuous or with minute, not very conspicuous, delicate frill or peg. Microconidia not observed.

Material examined: China, Jiangxi Province, Xingangshan, subtropical forest site of the BEF-China Project, 29.1250° N, 117.9085° E, on living leaves of *Castanea henryi*, *Fagaceae*, 8 Sep. 2013, S. Bien (HAL 2675 F).

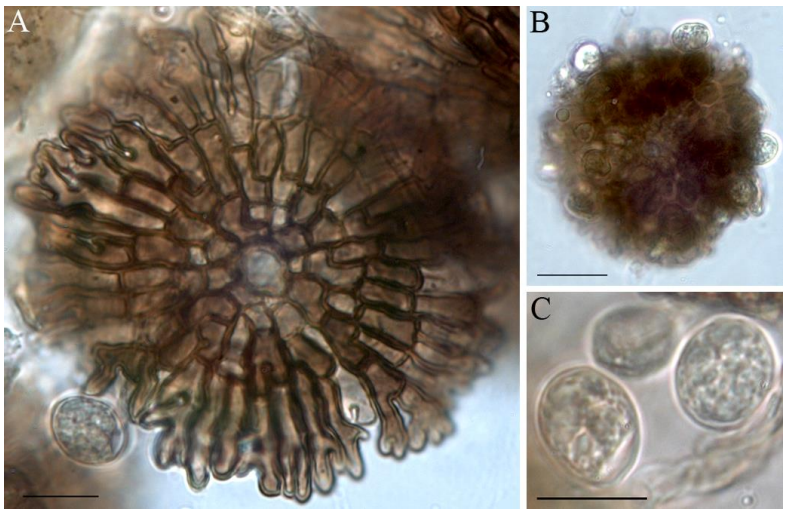


Fig. 3: *Tubakia* sp., A – Pycnothyrium, scutellum viewed from above, B – Base of a pycnothyrium below scutellum, C – Mature conidia. Bars: A and C = 10 µm, B = 20 µm. Micrographs made by B. Heuchert.

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Discussion

Saccardo (1913) introduced the genus *Actinopelte* Sacc. for a scutellate fungus found in Japan on *Castanea crenata* (= *C. pubinervis*). Yokoyama & Tubaki (1971) discussed the history of this genus in detail, published results of comprehensive examinations of Japanese collections, and described several new species. Since Saccardo's *Actinopelte* turned out to be illegitimate (later homonym of *Actinopelte* Stitzenb. 1861), Sutton (1971) introduced the replacement name *Tubakia* and reallocated all species recognised and treated in Yokoyama & Tubaki (1971). Two additional *Tubakia* species were described by Yun & Rossman (2011) and Harrington et al. (2012). A comparison of Chinese collections on *Castanea henryi* and all species assigned to *Tubakia* revealed that an undescribed, morphologically quite distinct species is involved. Scutellum size and shape, size and colour of conidia are the most important characters to distinguish species of *Tubakia*, a genus which is currently assigned to *Diaporthales* within *Ascomycota* (Yokoyama & Tubaki 1971, Yun & Rossman 2011). Shape and size of the central columella, emphasized as important feature for *Tubakia* species in Yokoyama & Tubaki (1971), are, however, less useful due to delicacy, difficulties to examine these structures in detail and unreliable historical data. *Tubakia castanopsidis* (T. Yokoy. & Tubaki) B. Sutton, *T. dryina* (Sacc.) B. Sutton, *T. iowensis* T.C. Harr. & D. McNew, *T. rubra* (T. Yokoy. & Tubaki) B. Sutton and *T. subglobosa* (T. Yokoy. & Tubaki) B. Sutton (Yokoyama & Tubaki 1971, Harrington et al. 2012) are characterised by having relatively small conidia with an average length below 15 µm. *Tubakia japonica* (Sacc.) B. Sutton (Yokoyama & Tubaki 1971) and *T. seoraksanensis* H.Y. Yun (Yun & Rossman 2011) are two deviating species with rather large conidia (length on average > 15 µm). *T. chinensis* belongs to the latter group of large-spored *Tubakia* species, but can be easily differentiated by traits of the scutellum and conidia. *T. japonica* has much larger, colourless conidia, 40–55 × 35–45 µm, and forms microconidia, 5–7 × 1.5–2 µm (Yokoyama & Tubaki 1971), and *T. seoraksanensis* differs in having smaller scutella, 90–160 × 90–130 µm, narrower conidiogenous cells, 14–22 × 3–5 µm, and obviously smaller conidia, 13–25 × 10–15 µm. The size of mature conidia of *T. chinensis* is not overlapping with size ranges in *T. japonica* and *T. seoraksanensis*. Based on these clear morphological differences, it is justified to introduce a new species of *Tubakia* for Chinese collections on *Castanea henryi* although cultures and molecular sequence data are not yet available. Moreover, an assignment of the new species to *Tubakia* is advised and justified, although the sexual morph of *T. dryina* has been referred to as *Dicarpella dryina* Belisario (Belisario 1991). However, *D. dryina* is not the type species of *Dicarpella* Syd. & P. Syd., and at least one species appears linked to a *Harknessia* Cooke anamorph (Cannon 2001). Relation and synonymy of *Dicarpella* and *Tubakia* are not yet settled.

The second *Tubakia* found on *Castanea henryi* together with type material and other collections of *T. chinensis* is characterised by having small pycnothyria and globose to subglobose conidia. Based on the small scutella, 40–80 µm diam., it belongs to *T. dryina* s. lat., which is undoubtedly a complex of several similar species. According to Harrington et al. (2012), Japanese specimens referred to *T. dryina* in Yokoyama & Tubaki (1971) do not belong to *T. dryina* s. str. However, *T. dryina* s. lat. differs from the Chinese fungus on *Castanea henryi* in forming microconidia and broadly ellipsoid to obovoid conidia. Due to globose to subglobose conidia, *T. subglobosa* on *Quercus glauca* in Japan is an additional comparable species, but can be easily ruled out as causal agent of this leaf-spotting disease on *Castanea henryi* by its much larger pycnothyria, 80–150 µm diam., and somewhat longer, hyaline to pale yellowish ochraceous conidia 10–13 × 9–11 µm. The Chinese *Tubakia* on *Castanea henryi* with small scutella and globose to subglobose conidia is probably a new, undescribed species. However, in contrast to *T. chinensis*, which is common and widespread around its type locality, the second small-spored *Tubakia* has hitherto only been found on a few leaves. This material is not sufficient for a formal taxonomic description of a new species and is not qualified to serve as holotype. Therefore, the material concerned is tentatively referred to as *Tubakia* sp., in anticipation of additional collections.

The species of *Tubakia* can be keyed out as follows.

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Key to *Tubakia* species based on pycnothyrial characters on leaves

1. Conidia large, length on average > 15 µm 2
1* Conidia smaller, length on average < 15 µm 4

2. Conidia very large, 40–55 × 35–45 µm, wall colourless; microconida present, 5–10 × 1–2 µm; on *Castanea crenata*, *C. mollissima* and *Quercus acutissima*, Asia (China, Japan, Korea) ..... *T. japonica*
2* Conidia much smaller, length < 40 µm, width < 30 µm, wall colourless to slightly pigmented or at least cell content pigmented; microconidia not formed 3

3. Conidia 13–25 × 10–15 µm; scutella 90–160 × 90–130 µm; conidiogenous cells 14–22 × 3–5 µm, hyaline to pale brown; on *Quercus mongolica*, Korea ..... *T. seoraksanensis*
3* Conidia much larger, (20–)25–40 × 20–30 µm; scutellum larger, 135–200 µm diam.; conidiogenous cells wider, 10–20 × 4–8 µm, hyaline; on *Castanea henryi* ..... *T. chinensis*

4(1) Scutella relatively small, 60–100 µm diam.; conidia 12–15 × 10–13 µm, at first hyaline, later pale yellowish brown to light orange yellow; microconidia formed, bacilliform, 8–10 × 1 µm; on *Quercus phillyreoides*, *Q. serrata*, Japan, Korea ..... *T. rubra*
4* Scutella larger, up to 150 µm diam. and/or conidia narrower (width < 10 µm); microconidia either broader, 1.5–2.5 µm, or not developed 5

5. Conidia globose to subglobose (scutellum either small, 40–80 µm diam., or relatively large, 80–150 µm diam.) or scutellum large, up to 180 µm diam., and conidia oblong to oblong-ellipsoid; microconidia lacking 6
5* Scutella small, 40–110(–135) µm diam.; conidia broadly obovoid to ellipsoid; microconidia developed 8

6. Conidia oblong to oblong-ellipsoid, much narrower, 12–13 × 7–8 µm, hyaline; on *Castanopsis cuspidata*, Japan ..... *T. castanopsidis*
6* Conidia globose or subglobose 7

7. Scutellum large, 80–150 µm diam.; conidia 10–13 × 9–11 µm, hyaline to pale yellowish ochraceous; on *Quercus glauca*, Japan ..... *T. subglobosa*
7* Scutellum small, 40–80 µm diam.; conidia 9–11 × 7–9 µm; hyaline or subhyaline; on *Castanea henryi*, China ..... *Tubakia* sp.

8(5) On *Castanea*, *Fagus* and *Quercus* spp., *Fagaceae*[1], Asia (Iran, Japan), Europe (Germany, Italy, Poland, Romania, Russia, Turkey, UK), North America (Canada, Mexico, USA) and New Zealand ..... *T. dryina*
8* On *Quercus macrocarpa*, North America, USA (pycnothyria on leaves morphologically barely distinguishable from those of *T. dryina*, but *in vitro* and genetically clearly distinct) ..... *T. iowensis*

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[1] North American records of *Tubakia dryina* on hosts of various other families, e.g. *Acer*, *Carya*, *Fraxinus*, *Nyssa* spp., etc. (Farr et al. 1989), are doubtful and unproven.

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