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Bionectria vesiculosa sp. nov. from Yunnan, China

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Abstract — A new species of *Bionectria* on decaying leaves is described from Xishuangbanna in southwestern China. It is distinctive in the brown perithecia, laterally collapsing when dry, a ring of large vesicular cells surrounding the subapical region of the perithecia, clavate asci with an apical ring, and fusiform, smooth-walled ascospores. Morphology and sequence analysis of ITS and 28S nrDNA support its taxonomic position as a new species in *Bionectria*.

Key words — Chelex-100, taxonomy

Introduction

The genus *Bionectria* Speg. (*Bionectriaceae*) is characterized by pale yellowish to orange perithecia that do not change color in 3% KOH or lactic acid, a smooth to warted perithecial wall of 1–3 layers, clavate asci with or without an apical ring, 2-celled ascospores with smooth, spinulose, warted or striate surface, and *Clonostachys* anamorphs. Members of *Bionectria* occur on woody and herbaceous plants or other fungi, and are mainly distributed in tropical and subtropical regions (Rossman et al. 1999, Schroers 2001). In connection with our work on the Chinese fungus flora, an interesting fungus was encountered that has brown perithecia with a ring of large cells at the subapical region. On the basis of the teleomorph morphology and sequence analysis of two nuclear ribosomal genes (nrDNA), its position in *Bionectria* is confirmed; its relationship with other species of the genus is discussed.

Materials & methods

The taxonomic treatments and methods of Rossman et al. (1999) and Schroers (2001) were followed for the morphological study. Water was used as the mounting medium

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Table 1. Sequences analyzed to determine relationships among species in *Bionectria*.

Species	ITS nrDNA		28S nrDNA	
	Collection no.	GenBank no.	Collection no.	GenBank. no.
Bionectria compactiuscula Schroers	CBS 913.97	AF358245	CBS 919.97	AF210690
B. coronata (Juel) Schroers	CBS 696.93	AF210667	CBS 696.93	AF210667
B. epichloe (Speg.) Schroers	CBS 101037	AF210675	CBS 101037	AF210675
B. grammicospora (Ferd. & Winge) Schroers & Samuels	CBS 209.93	AF210678	CBS 209.93	AF210678
B. grammicosporopsis (Samuels) Schroers & Samuels	CBS 115.67	AF210679	CBS 115.67	AF210679
B. levigata Schroers	CBS 948.97	AF210680	CBS 948.97	AF210680
B. ochroleuca (Schwein.) Schroers & Samuels	CBS 194.57	AF358249	CCFC 226708	AY686634
B. pityrodes (Mont.) Schroers	CBS 246.78	AF210673	CBS 102033	AF210672
B. ralfsii (Berk. & Broome) Schroers & Samuels	CBS 102845	AF358253	CBS 129.87	AF210676
B. rossmaniae Schroers	CBS 210.93	AF358227	CBS 211.93	AF210665
B. sesquicillii (Samuels) Schroers	CBS 180.88	AF210666	CBS 180.88	AF210666
B. setosa Schroers	CBS 834.91	AF210670	CBS 834.91	AF210670
B. vesiculosa	HMAS 183151	**HM050304**[a]	HMAS 183151	**HM050302**
B. zelandiae-novae Schroers	CBS 100979	AF358229	CBS 232.80	AF210684
Clonostachys divergens Schroers	CBS 967.73b	AF210677	CBS 967.73b	AF210677
C. miodochialis Schroers	CBS 997.69	AF210674	CBS 997.69	AF210674
C. phyllophila Schroers	CBS 685.96	AF210663	CBS 921.97	AF210664
Hydropisphaera erubescens (Roberge ex Desm.) Rossman & Samuels	HMAS 91779	FJ969800	HMAS 91779	GU075862
Ijuhya paraparilis (Samuels) Rossman & Samuels	HMAS 183506	FJ969801	HMAS 183506	**HM050303**

[a] Numbers in bold indicate the newly submitted sequences.

for microscopic examinations and measurements; and photographs were taken from water or cotton blue mounts (Stevens 1981). Continuous measurements of individual structures are based on 30 units except as otherwise noted. Specimen examined is deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

Chelex-100 was applied to extract genomic DNA from the dehydrated perithecia according to the method by Zhang et al. (2006) with modifications. Fifty perithecia were carefully collected from the substrate with a pair of forceps and rinsed in sterilized water. The perithecia were transferred into a 1.5 ml eppendorf tube, mixed with equal volume of quartz sand, and thoroughly ground with a glass pestle for 10 min. Then 200 µl 10%

w/v Chelex-100 chelating resin (Sigma) was added and mixed for 10 sec on a vortex. The tube was incubated at 56°C for 2 hr, mixed for 10 sec, and then incubated at 99°C for 10 min. After centrifuging at 12000 r/min for 10 min, the supernatant was transferred to another 1.5 ml tube filled with 4/5 volume of 100% pre-cooling isopropanol. The mixture was placed at –20°C overnight, and centrifuged at 12000 r/min for 15 min. After rinsing with 200 µl 75% ethanol, the precipitant was dried at room temperature and dissolved in 30 µl TE or ddH_2O as PCR template.

The ITS1-5.8S-ITS2 (ITS) and 28S regions of the nrDNA were amplified by using the primer pairs, ITS5–ITS4 (White et al. 1990), and LROR–LR5 (Rehner & Samuels 1994, Vilgalys & Hester 1990). The PCR reaction mixture (50 µl) contained 5.0 µl 10× PCR buffer, 3.0 µl $MgCl_2$ (25 mM), 2.5 µl sense primer (10 µM), 2.5 µl antisense primer (10 µM), 1.0 µl dNTP (10 mM each), 2.5 µl DNA template, 0.5 µl Taq polymerase (5.0 U/µl) (Bio Basic Inc.) and 33 µl ddH_2O. Reactions were performed on the 2720 Thermal Cycler (Applied Biosystems) with cycling conditions of denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C (ITS region) and at 55°C (28S region) for 30 s and elongation at 72°C for 60 s, with a final extension step at 72°C for 5 min to complete the reactions. Amplicon was purified with the PCR Product Purification Kit (Biocolor BioScience & Technology Co.) and sequenced with the ABI BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3730XL DNA Sequencer (SinoGenoMax Co. Ltd). The amplifying primers were served as sequencing primers. Final sequences were checked and edited manually by using BioEdit V.7.0.5 (Hall 1999). Sequences of the related species were retrieved from GenBank. Materials studied are listed in Table 1.

All sequences were aligned using ClustalX V.1.8 (Thompson et al. 1997), and the alignments were visually adjusted while necessary. A Neighbor-Joining tree was generated using MEGA 4.10 (Tamura et al. 2007) based on combined sequences of ITS and 28S genes with *Hydropisphaera erubescens* and *Ijuhya paraparilis* as outgroup taxa. Kimura 2-parameter was selected as the nucleotide substitution model, and gaps or missing data were pairwise deleted. Bootstrap method was performed with 1000 replicates to test phylogeny branch support.

Results and discussion

Bionectria vesiculosa J. Luo & W.Y. Zhuang, **sp. nov.** Figs. 1–2

MycoBank MB518120

Peritheciis subglobosis, 100–155 µm diam; ascis clavatis, 8-sporis, 35–47 × 3.5–7 µm; ascosporis fusiformibus, uniseptatis, 9.5–13 × 1.5–3 µm.

Holotype: China, Yunnan, Xishuangbanna, on decaying leaves of a dicotyledonous plant, W.-P. Wu & Y. Huang W2728b, 16 X 1999, HMAS 183151.

Etymology: The specific epithet refers to the vesicular cells forming a ring on the perithecial apex.

Ascomata on white subiculum, perithecial, solitary or gregarious up to 3 in a group, superficial, subglobose, 110–160 µm high, 100–155 µm diam., laterally collapsing when dry, pale yellow when young, and reddish brown to brown at

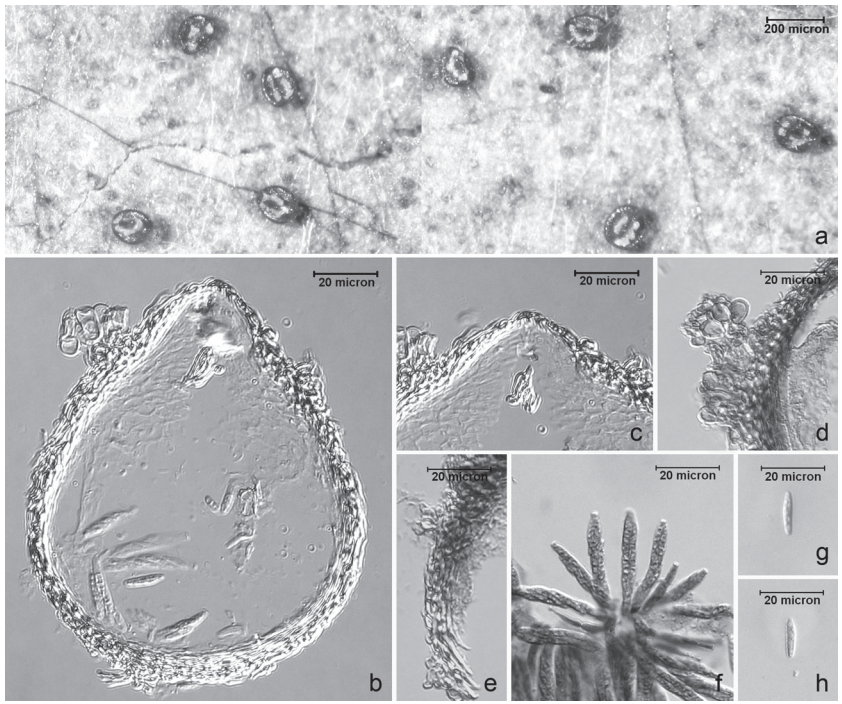

Fig. 1. *Bionectria vesiculosa* (HMAS 183151). a. Ascomata on natural substrate; b. Median section of an ascoma; c. Median section through apical portion of an ascoma; d, e. Structure of ascomatal wall at subapical portion showing vesicular cells; f. Asci with an apical ring; g, h. Ascospore.

maturity, not changing color in 3% KOH or lactic acid, surface smooth, with a ring composed of large cells or a ring of wart-like structures surrounding subapical region; coronate ring 5–26 μm high, pale yellow, cells vesicular, 2–12 × 2–7 μm, cell walls 0.5–1 μm thick. Ascomatal wall 7–15 μm thick, of two layers; outer layer 5–10 μm thick, cells angular, 5–10.5 × 3–5.5 μm, cell walls 0.5–2 μm thick; inner layer 2–6 μm thick, cells flattened, 6–11.5 × 1–3 μm, cell walls 0.5–1.5 μm thick. Asci clavate, 8-spored, with an apical ring, 35–47 × 3.5–7 μm (n = 50). Ascospores fusiform, uniseptate, not constricted at septum, hyaline, smooth, with 6–9 guttules, biseriate, 9.5–13 × 1.5–3 μm (n = 50).

Anamorph: Unknown.

Notes: Morphologically, the perithecial anatomy and negative reactions to KOH and lactic acid of the new species indicate its position in *Bionectria*. Unlike any other species of the genus, a crown-like ring composed of large vesicular cells is present at the subapical region of the perithecia. *Bionectria vesiculosa* is somewhat similar to *B. setosa* in having brown perithecia less than 200 μm in

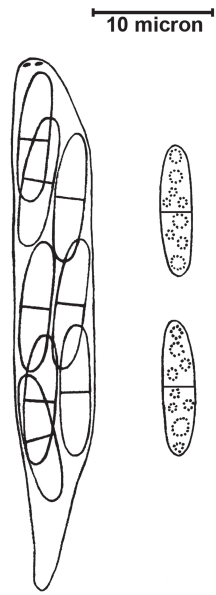

Fig. 2. Ascus and ascospores of *Bionectria vesiculosa* (HMAS 183151).

diam., two-layered perithecial wall, clavate asci with an apical ring, shape, size, septation and surface morphology of ascospores, and leaf-inhabiting. The latter differs in having smooth and thicker perithecial walls 20–30 µm thick and asci 45–53 × 6.5–9 µm (Schroers 2001). The new species also resembles *B. coronata* in the presence of a thin subiculum at the perithecial base, small, subglobose perithecia that are laterally pinched when dry, acute perithecial apex, shape and size of asci, shape, size and surface of ascospores, and foliicolous habit. *Bionectria coronata* differs in having pale yellow to yellowish orange perithecia, one-layered perithecial walls, 15–20 µm thick, with the outermost cell layer connected with a hyphal stroma, undulate setae surrounding the ostiole, asci lacking of an apical apparatus, and unicellular ascospores (Schroers 2001).

Seventeen related species of *Bionectria/Clonostachys* were selected to investigate the phylogenetic position of *B. vesiculosa*. As shown in Fig. 3, all species tested formed one monophyletic clade with 100% bootstrap support, which confirms the placement of the new species in *Bionectria*. The morphologically similar *B. coronata* appears to be only distantly related. *Bionectria pityrodes* and *B. setosa* form a poorly supported subclade with *B. vesiculosa* (Fig. 3). The morphological characteristics of these three species do not show much similarity (Schroers 2001).

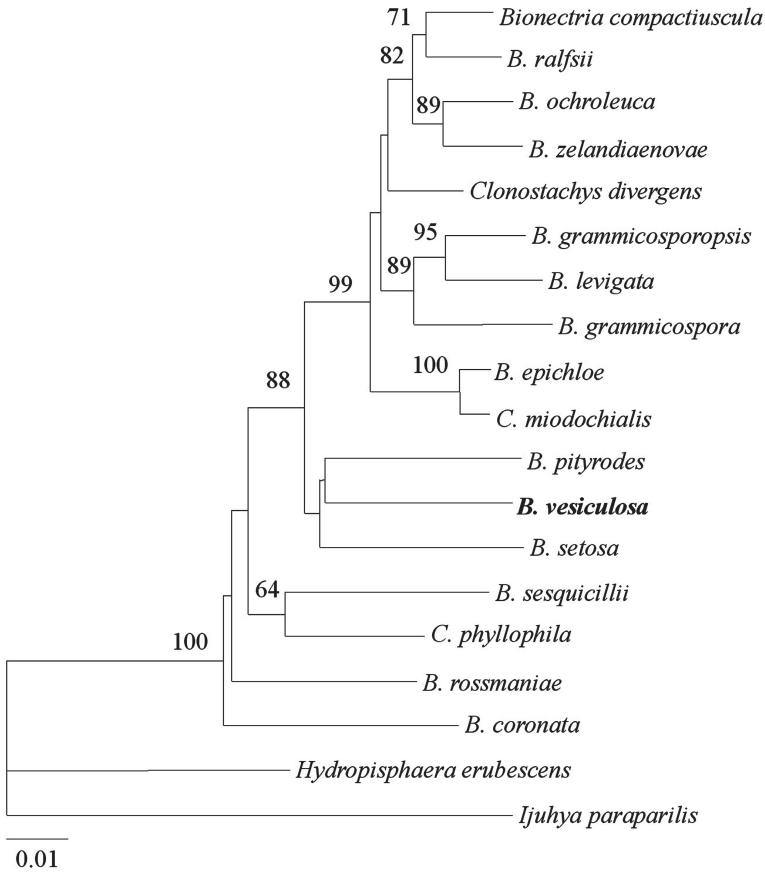

Fig. 3. Neighbour-joining tree based on combined sequences of ITS and 28S nrDNA, showing the relationships among some *Bionectria*/*Clonostachys* species. Bootstrap values ≥ 50% are noted above internodes.

In conclusion, both morphology and DNA sequence analysis support the recognition of *Bionectria vesiculosa* as a new species.

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