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FULL PAPER

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Taxonomic studies of nectrioid fungi in Japan. II: The genus *Bionectria*

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Abstract Based on the result of morphological and the phylogenetic analyses, three *Bionectria* (Bionectriaceae: Hypocreales) species are added to the mycobiota of Japan. Among them, one found in Okinawa and Kagoshima Prefectures is described as a new species, *Bionectria pseudostriatopsis* (anamorph: *Clonostachys pseudostriatopsis*). The other two, *B. grammicospora* and *B. sporodochialis*, are new records from Japan. Additional distribution records are given for *Bionectria* species hitherto known in Japan.

Key words Bionectriaceae · *Clonostachys* · Hypocreales · New species · Taxonomy

Introduction

In this article, we consider Japanese members of the genus *Bionectria* Spegazzini (Bionectriaceae, Hypocreales). The genus *Bionectria* has white to yellow perithecia, and most of the species have 1-septate ascospores and a *Clonostachys* Corda anamorph. The genus *Bionectria* has high ecological diversity. Some species are saprotrophic and some are necrotrophic or biotrophic, and many species are known as mycoparasites. Some of them are used as biocontrol agents (Schroers 2001).

The taxonomic status of *Bionectria*, which was proposed by Spegazzini (1919) based on a single species, *B. tonduzii* Speg., in the Hypocreales, has been controversial (Samuels et al. 1990). In previous studies, some species of *Bionectria* were distributed in the *Nectria ochroleuca* group (Samuels 1976; Rossman 1983; Schroers and Samuels 1997), *N. ralfsii* group (Samuels 1976), and *N. muscivora* group (Rossman 1983; Samuels 1988). Rossman et al. (1999) established a new family, Bionectriaceae, and several old genera, including *Bionectria*, which have been treated as a synonym of *Nectria* (*Nectria* sensu lato), were revived. Their taxonomic system was supported by molecular data (Rehner and Samuels 1994, 1995; Rossman et al. 2001; Schroers 2001).

Schroers et al. (1999a,b) described *Bionectria* and its anamorph in detail. Schroers (2000, 2001) classified the 35 *Bionectria* species having the *Clonostachys* anamorph and 9 other *Clonostachys* taxa based on detailed morphological and molecular data. In addition, Schroers divided the genus *Bionectria* into six newly distinguished subgenera, namely *Bionectria*, *Zebrinella*, *Astromata*, *Myronectria*, *Epiphloea*, and *Uniparietina*, based on morphology of stroma, structure of perithecial wall, habit of the perithecia on the natural substratum, ornamentation and septation of ascospores, and phylogenetic data.

Seven species of *Bionectria* have been known from Japan (Kimura 1979; Arie et al. 1987; Aoki et al. 1990; Sato et al. 1991, 1998; Schroers 2001) (Table 1). In this study, three species of *Bionectria,* including one new species, are newly added to the Japanese mycobiota based on their morphological and molecular phylogenetic analyses. In addition, comprehensive distribution records of hitherto known Japanese species in the genus *Bionectria* are given.

Materials and methods

Materials examined

Fresh specimens of the teleomorph and anamorph of the *Bionectria* and the single ascospore isolates were collected for the present study. All specimens were deposited in the Herbarium of Forest Mycology and Pathology (TFM), Forestry and Forest Products Research Institute (FFPRI), Tsukuba, Ibaraki, Japan.

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Table 1. List of the species of genus *Bionectria* (*Clonostachys*) and *Calonectria* used for molecular phylogenetic analysis

States		GenBank no.	Strain no.[a]	Country of origin	Substrate
Teleomorph	Anamorph				
Bionectria aureofulvella	*Clonostachys aureofulvella*	AF358182	CBS 200.93	Venezuela	Bark of *Polylepis sericea*
B. byssicola	*C. byssicola*	AF358154	CBS 365.78	Venezuela	Wood
B. capitata	*C. capitata*	AF358188	CBS 218.93	Japan	Bark
B. coronata	*C. buxi*	AF358215	CBS 696.93	France	Leaves of *Buxus sempervirens*
B. epichloë	*C. epichloë*	AF358209	CBS 101037	Japan	*Sasa* sp.
B. grammicospora	*C. grammicospora*	AF358206	CBS 209.93	French Guiana	Standing dead tree
B. grammicospora	*C. grammicospora*	AB237466	MAFF 239824	Japan	Bark of fallen twigs
B. grammicospora	*C. grammicospora*	AB237467	MAFF 239823	Japan	Bark
B. grammicosporopsis	*C. grammicosporopsis*	AF358204	CBS 115.87	New Zealand	Bark of *Metrosideros* sp.
B. ochroleuca	*C. rosea*	AF358167	CBS 406.95	France	Bark of *Salix* sp.
B. pityrodes	*C. pityrodes*	AF358212	CBS 102033	Mauritius	Bark
B. pseudochroleuca	*C. pseudochroleuca*	AF358173	CBS 191.94	French Guiana	Decaying palm
B. pseudostriata	*C. pseudostriata*	AF358183	CBS 119.87	Indonesia	Bark
B. pseudostriatopsis	*C. pseudostriatopsis*	AB237463	MAFF 239829	Japan	Bark of fallen twigs
B. pseudostriatopsis	*C. pseudostriatopsis*	AB237464	MAFF 239841	Japan	Bark of fallen twigs of *Cinnamomum camphora*
B. pseudostriatopsis	*C. pseudostriatopsis*	AB237465	MAFF 239827	Japan	Bark of fallen twigs
B. rossmaniae	*C. rossmaniae*	AF358213	CBS 210.93	French Guiana	Bark of twigs
B. samuelsii	*C. samuelsii*	AF358190	CBS 699.97	Venezuela	Bark
B. sesquicillii	*C. sesquicillii*	AF358214	CBS 180.88	Guyana	Twigs and lichen
B. solani	*C. solani*	AF358221	CBS 752.68	Germany	Bark
B. sporodochialis	*C. sporodochialis*	AF358149	CBS 101921	USA	Bark
B. sporodochialis	*C. sporodochialis*	AB237460	MAFF 239826	Japan	Bark of fallen twigs
B. sporodochialis	*C. sporodochialis*	AB237461	MAFF 239842	Japan	Bark of fallen twigs
B. sporodochialis	*C. sporodochialis*	AB237462	MAFF 239828	Japan	Dead bark of *Prunus jamasakura*
B. subquaternata	*C. subquaternata*	AF358207	CBS 107.87	Venezuela	Wood
Unknown	*C. divergens*	AF358191	CBS 967.73b	Germany	Soil
Unknown	*C. rogersoniana*	AF358189	CBS 582.89	Brazil	Soil
Calonectria ilicicola	*Cylindrocladium parasiticum*	AF333312	CBS 190.50	Indonesia	*Solanum tuberosum*

[a] MAFF, MAFF Genebank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan; CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

Isolation

Methods for single ascospore isolation followed Hirooka and Kobayashi (2007). Monoascospore isolates were preserved at MAFF Genebank, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Morphological observation

Observation of teleomorph followed Hirooka and Kobayashi (2007). Colonies on oatmeal agar (OA) and potato dextrose agar (PDA; Kirk et al. 2001) grown for 2 weeks at 25°C in the dark were evaluated for growth rates and colony color. Color of the colonies observed from the surface and bottom was described according to Kornerup and Wanscher (1978). For observation of the *Clonostachys* anamorph, cultures on OA or PDA were used.

Phylogenetic analysis

Mycelia grown on PDA at 25°C were harvested after 1–2 weeks. Genomic DNA was extracted from lyophilized hyphae based on the method of O'Donnell et al. (1997), or with a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The *tub2* region (about 700 bp) of the β-tubulin genes was amplified with the primer pairs T1 and T224 (O'Donnell and Cigelnik 1997). Polymerase chain reaction amplification of the *tub2* region was performed with the TaKaRa ExTaq system (TaKaRa Bio, Otsu, Japan) with a first denaturation for 2 min at 95°C followed by 40 cycles of incubation for 35 s at 94°C, 55 s at 52°C, and 2 min at 72°C. Sequencing was conducted with the ABI-PRISM 377 DNA sequencing system (Applied Biosystems, Foster City, CA, USA) and DNA sequencing kit (Perkin-Elmer, Foster City, CA, USA) following the ABI protocol, by using the same primer pairs as described above.

Phylogenetic analysis was based on a segment of about 500 nucleotides including three introns and three exons in *tub2*. The length differences were compensated in the alignments by gaps. The sequence alignment and homology analysis were carried out using AssemblyLIGNTM 1.0.9c (Accelrys, San Diego, CA, USA) and the CLUSTAL W package with Mac Vector 6.5.3 (Accelrys) (Thompson et al. 1994). The aligned sequences were analyzed by the neighbor-joining method (Saitou and Nei 1987), using PAUP 4.0b (Swofford 1998). The distance matrix was calculated using DNADIST with the Kimura two-parameter method (Kimura 1980), and the topology was tested with 1000 bootstrap trials. The aligned sequences of 27 *Bionectria* species and the outgroup of *Calonectria* species, of which 20 sequences were drawn from GenBank, were analyzed (see Table 1).

Descriptions

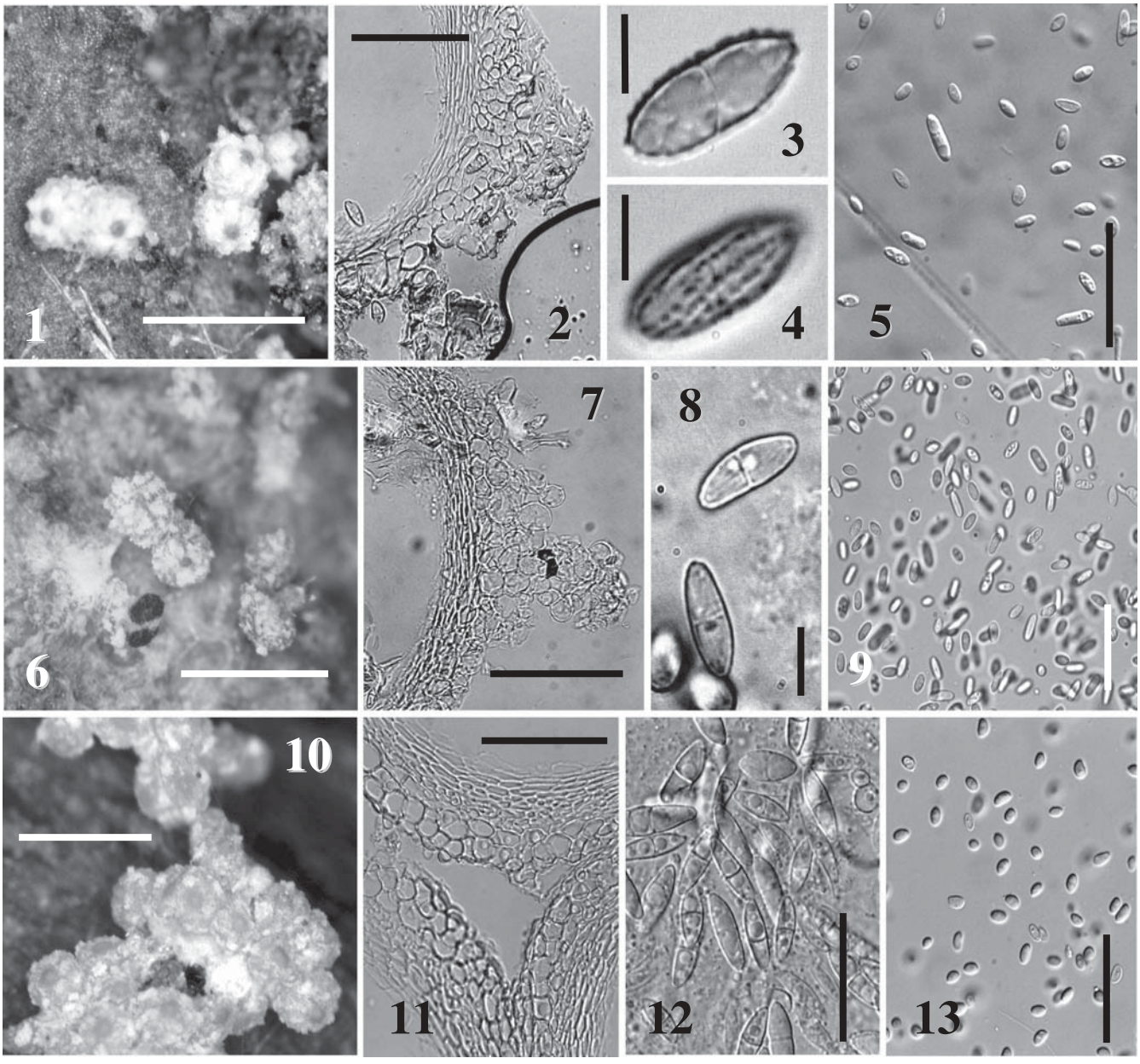
Bionectria Speg., Bol. Acad. Nac. Ci. 23: 563, 1919.
Type species: *B. tonduzii* Speg.

1. ***Bionectria pseudostriatopsis*** Hirooka & Tak. Kobay., sp. nov. Figs. 1–4, 14a–c

Stromata corticalis, erumpentia, contextu "textura epidermoidea" vel "textura angularis" composita. Perithecia ad stromate superficialia, sparsa vel paulo gregaria, subglobosa vel ovalia, 200–370 μm alta, 210–320 μm diametro, aurantiaca, ad apicem papillato-prominentia et ostiolata. Asci unitunicati, clavati, 51–82 × 9–13 μm, octospori. Ascosporae irregulariter distichae, ellipsoideae vel fusiformes, hyalinae, 15–21 × 5.5–7.5 μm, cum verruculis longitudinaliter dispositis obtectae.

Anamorph: ***Clonostachys pseudostriatopsis*** Hirooka & Tak. Kobay., anam. nov. Figs. 5, 14d–g

Conidiophora dimorpha. *Clonostachydis samuelsii* vel *C. graminicosporae* similis sed conidiophora primaria in vitro



Figs. 1–13. 1–5 *Bionectria pseudostriatopsis* (anamorph: *Clonostachys pseudostriatopsis*) (**1–4**, TFM FPH-7769; **5**, MAFF 239827). **1** Perithecia on natural substratum. **2** Section through lateral perithecial wall. **3** Ascospore in optical section. **4** Ascospore in surface view. **5** Conidia. **6–9** *B. grammicospora* (*C. grammicospora*) (**6–8**, TFM FPH-7773; **9**, MAFF 239823). **6** Perithecia on natural substratum. **7** Section through lateral perithecial wall. **8** Ascospores. **9** Conidia. **10–13** *B. sporodochialis* (*C. sporodochialis*) (**10–12**, TFM FPH-7763; **13**, MAFF 239828). **10** Perithecia on natural substratum. **11** Section through lateral perithecial walls. **12** Ascospores. **13** Conidia. *Bars* **1**, **6**, **10** 1000 μm; **2**, **7**, **11** 50 μm; **3**, **4**, **8** 5 μm; **5**, **9**, **12**, **13** 20 μm

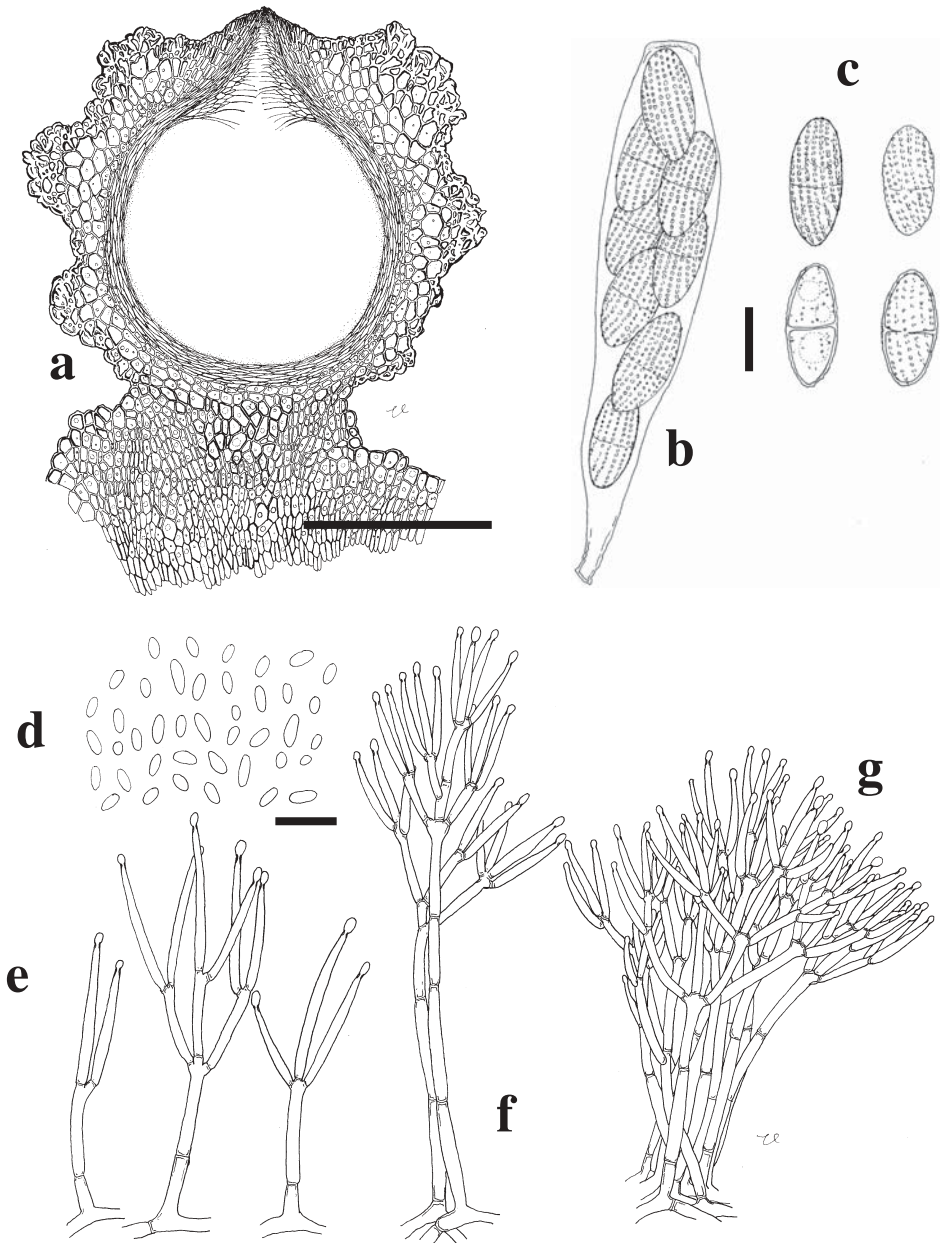


Fig. 14. *Bionectria pseudostriatopsis* (anamorph: *Clonostachys pseudostriatopsis*) (**a–c**, TFM FPH-7769; **d–g**, MAFF 239827). **a** Median section of perithecium. **b** Ascus with eight ascospores. **c** Ascospores with warts arranging in striate surface. **d** Ellipsoidal conidia**. e** Primary verticillum-like conidiophores. **f** Secondary conidiophores with long stipes. **g** Secondary conidiophores with short stipes. *Bars* **a** 100 µm; **b**, **c** 10 µm; **d–g** 20 µm

rariora et ramuli conidiophorum primariorum divergentes et non compressi. Conidia ellipsoidea, symmetrica, 3–16 × 2–4 µm, apice utrinque rotundata, hyalina, laevia.

Etymology: pseudostriat + -opsis; indicates similar to the *Bionectria pseudostriata*.

Holotypus: On bark of fallen twigs, Kunigami-son, Okinawa Pref. (Okinawa Island), January 21, 2003, by Y. Hirooka (Y.H.) (teleomorph: TFM FPH-7769; anamorph: TFM FPH-7770; culture: MAFF 239827).

Stromata formed in epidermal layer of outer bark, erumpent through the epidermis, "textura epidermoidea" to "textura angularis." Perithecia usually gregarious in groups of 5–10, with conspicuous basal stroma, subglobose to oval, 200–370 µm in height and 210–320 µm in diameter, constricted at the orange papilla, collapsing laterally when dry, whitish-orange, without any color reaction to KOH and LA, warted to rough; perithecial warts white or pale yellow, 50 µm high, largest in the upper part of the perithecia or around the ostiole; cells of perithecial warts angular to globose, 6–15 µm in size. Perithecial wall 35–60 µm thick, composed of two layers; outer layer 21–30 µm wide, angular to globose cells, with uniformly 5- to 10-µm thickened walls, with vacuoles; inner layer 9–20 µm thick, composed of hyphal to fusiform cells, becoming progressively thinner toward the perithecial locule. Asci narrowly clavate to clavate, 51–82 × 9–13 µm, with small refractive ring at apex, with 8 spores in two rows. Ascospores elliptic to fusioid, hyaline, 2-celled, 15–21 × 5–7.5 µm, with warts in longitudinal lines.

Colony on PDA attaining a diameter of 30–40 mm in 7 days at 25°C, cottony, white to light yellow; reverse pale

yellow. Colony on OA attaining a diameter of 25–35 mm in 7 days at 25°C, cottony, white to buff, sometimes sporodochial conidial masses produced; reverse buff. Conidiophores dimorphic. Primary conidiophores *Verticillium*-like, rare, sometimes not observed; stipes 30–70 µm long, 1.5–2.5 µm wide; branching parts 16–40 µm high; phialides borne in whorls of 2–3, 18–35 µm long, 1–2 µm wide, with minute collarette, each producing a small, hyaline drop of conidia. Secondary conidiophores broadly penicillate, scattered on the agar surface or arising from strands of aerial hyphae, phialides rarely adpressed; stipes variable, long or short, arising from the tissue of sporodochial aggregates; metulae 13–25 × 2–3 µm; phialides in loose whorls of 2–4, straight, flask-shaped or cylindrical, without a visible collarette, 13–22 µm long, 1–3 µm wide. Conidia produced in round heads, watery, transparent on primary conidiophores, small columns on secondary conidiophores, forming dome-like slimy masses, white, later light yellow in sporodochia; hyaline, ellipsoidal, almost straight, symmetrical, without a visible hilum, 3–16 × 2–4 µm. Perithecia not produced in culture.

Specimens and isolates examined: On bark of fallen twigs of *Cinnamomum camphora* (L.) J. Presl (Japanese name: Kusunoki), Kikai-cho, Oshima-gun, Kagoshima Pref. (Kikai Island) October 21, 2003, by Y.H. (teleomorph: TFM FPH-7767; anamorph: TFM FPH-7768; culture: MAFF 239841); on bark of fallen twigs, Kikai-cho, Oshima-gun, Kagoshima Pref. (Kikai Island) October 21, 2003, by Y.H. (teleomorph: TFM FPH-7765; anamorph: TFM FPH-7766; culture: MAFF 239829).

Note: *Bionectria pseudostriatopsis* is thought to be a subtropical fungus, because it has been collected only in Okinawa and Kagoshima Prefectures, the southern parts of Japan. *Bionectria pseudostriatopsis* resembles *B. byssicola* (Berk. & Broome) Schroers & Samuels (anamorph: *Clonostachys byssicola* Schroers) in forming whitish and large perithecial wall warts (Figs. 1, 2, 14a), but differs in having striate ornamentation of the ascospore surface (Figs. 3, 4, 14b,c). *Bionectria pseudostriatopsis* is also similar to *B. pseudostriata* Schroers (*C. pseudostriata* Schroers) (Schroers 2001), which is the only species having ascospore warts arranged in longitudinal lines. However, *B. pseudostriatopsis* clearly differs from *B. pseudostriata* in warted perithecia, paler color of perithecia, larger ascospores, and two-layered perithecial wall. This is the second species of genus *Bionectria* having ascospore warts arranged in longitudinal lines.

Clonostachys pseudostriatopsis is characterized by its short stipes of primary conidiophores (Fig. 14e) and ellipsoidal conidia (Figs. 5, 14d). Conidial morphology of *C. pseudostriatopsis* is similar to that of *C. samuelsii* Schroers and *C. grammicospora* Schroers & Samuels. However, *C. pseudostriatopsis* differs from *C. samuelsii* in having dimorphic conidiophores and obclavate conidiogenous cells, and from *C. grammicospora* in having shorter stipes of primary conidiophore (50–100 × 3.5–6.5 µm in *C. grammicospora*) and longer metulae of secondary conidiophore (7.5–11 × 2.2–3.2 µm in *C. grammicospora*). *Clonostachys pseudostriatopsis* differs from *C. pseudostriata* in having verticillate-like conidiophores, much shorter stipe of primary conidiophores (50–300 × 3.5–6 µm in *C. pseudostriata*), longer metulae of secondary conidiophores (9–15 × 2.5–3.5 µm in *C. pseudostriata*), larger (3.6–8 × 2–3.8 µm in *C. pseudostriata*), and ellipsoidal conidia.

All three isolates of *B. pseudostriatopsis* (AB237463, AB237464, and AB237465) belonged to a single clade within the subgenus *Bionectria* with a strong support. Although *B. pseudostriatopsis* and *B. pseudostriata* (AF358183) were in the same clade, it was not strongly supported, indicating the difference of the species (Fig. 16).

2. ***Bionectria grammicospora*** (Ferd. & Winge) Schroers & Samuels, Sutd. Mycol. 46: 154, 2001. Figs. 6–8, 15a–c

≡ *Nectria grammicospora* Ferd. & Winge in Raunk., Bot. Tidsskr. 29: 11, 1908.

≡ *Creonectria grammicospora* (Ferd. & Winge) Seaver, Mycologia 1: 192, 1909.

Anamorph: ***Clonostachys grammicospora*** Schroers & Samuels in Schroers, Sutd. Mycol. 46: 154, 2001. Fig. 9

Mycelium visible around perithecia or host. Perithecia solitary to gregarious in groups of 5–20, superficial or erumpent, with basal stroma, subglobose, 230–370 µm in height and 230–380 µm in diameter, constricted at the orange papilla, collapsing laterally when dry, whitish-orange to light orange, without any color reaction to KOH and LA, warted; perithecial warts white or pale yellow, 20–60 µm high; cells of perithecial warts globose, 7–20 µm in size, with evenly thickened walls. Perithecial wall 25–47 µm in thickness, composed of two layers. Cells of the stroma "textura intricata" or "textura epidermoidea." Asci clavate, 43–92 × 7–13 µm, without apical structure, with 8 spores in two rows. Ascospores elliptical, 1-celled, 10–15 × 4–5 µm, striate.

Colony on PDA attaining a diameter of 32–34 mm in 7 days at 25°C, cottony, white to pale yellow; reverse white (MAFF 239823), pale yellow (MAFF 239824). Colony on OA attaining a diameter of 32–34 mm (MAFF 239823), 35–42 mm (MAFF 239824) in 7 days at 25°C, cottony, white to pale yellow, sometimes sporodochial conidial masses produced (MAFF 239823); reverse white to pale yellow. Conidiophores monomorphic or dimorphic. Primary conidiophores, when present, narrowly penicillate, mostly arising from aerial mycelium, monocillate to verticillate, with divergent branches and phialides; stipes 55–125 µm long, 4–5 µm wide at base; branching part 20–50 µm high; phialides borne in whorls of 2–4, 15–50 µm long, 2–4 µm wide. Secondary conidiophores, when present, broadly verticillate, branches divergent; metulae 9–17 × 3–5 µm; phialides rarely adpressed; in loose whorls of 2–5, straight or slightly curved, flask-shaped, without a visible collarette, 7–21 µm long, 1.5–4 µm wide. Conidia hyaline, ellipsoidal, with a slightly tapering apex, 5–10 × 2–3.5 µm, rarely with a visible hilum. Perithecia not observed in culture.

Specimens examined: On bark, Akiyu-cho, Taihaku-ku, Miyagi-shi, Miyagi Pref., August 4, 2004, by Y.H. (teleomorph: TFM FPH-7773; anamorph: TFM FPH-7774; culture: MAFF 239823); on bark of fallen twigs, Rifu-cho, Miyagi-ku, Miyagi Pref., August 5, 2004, by Y.H. (teleo-

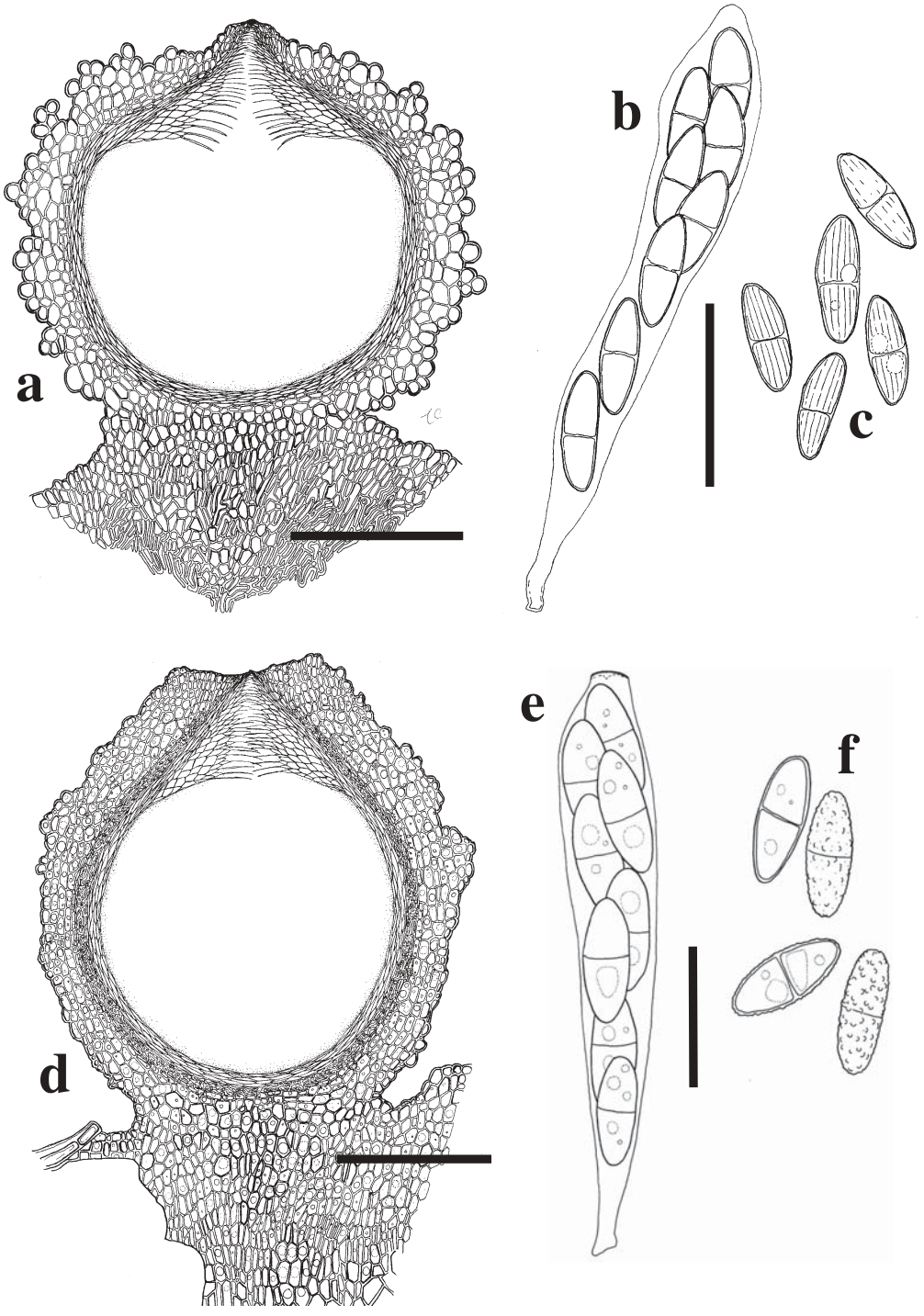


Fig. 15. *Bionectria grammicospora* (TFM FPH-7773; **a–c**) and *B. sporodochialis* (TFM FPH-7763; **d–f**). **a** Median section of perithecium. **b** Ascus with eight ascospores. **c** Ascospores with striate. **d** Median section of perithecium. **e** Ascus with eight ascospores. **f** Ascospores with or without tuberculate surface. *Bars* **a**, **d** 100 µm; **b**, **c** 20 µm; **e**, **f** 10 µm

morph: TFM FPH-7771; anamorph: TFM FPH-7772; culture: MAFF 239824).

Note: *Bionectria grammicospora* (anamorph: *Clonostachys grammicospora*) is newly added to the Japanese mycobiota. This fungus is characterized by its conspicuous stroma, almost globose cells in the perithecial walls, striate ascospores (Figs. 7, 8, 15a,c), and divergently branched secondary conidiophores. Morphologically, the specimen TFM FPH-7773 agreed well with previous description of this fungus (Schroers 2001). The specimen TFM FPH-7771 showed some differences from the description of Schroers (2001) and with the specimen TFM FPH-7773, in the inconspicuously stroma, angular cells occasionally observed in the perithecial wall, and adpressed secondary conidiophores. Nevertheless, our two specimens, TFM FPH-7771 (AB237467) and TFM FPH-7773 (AB237466), showed only two nucleotides difference in *tub2* and fell in the same clade with the previously known sequence of *B. grammico-*

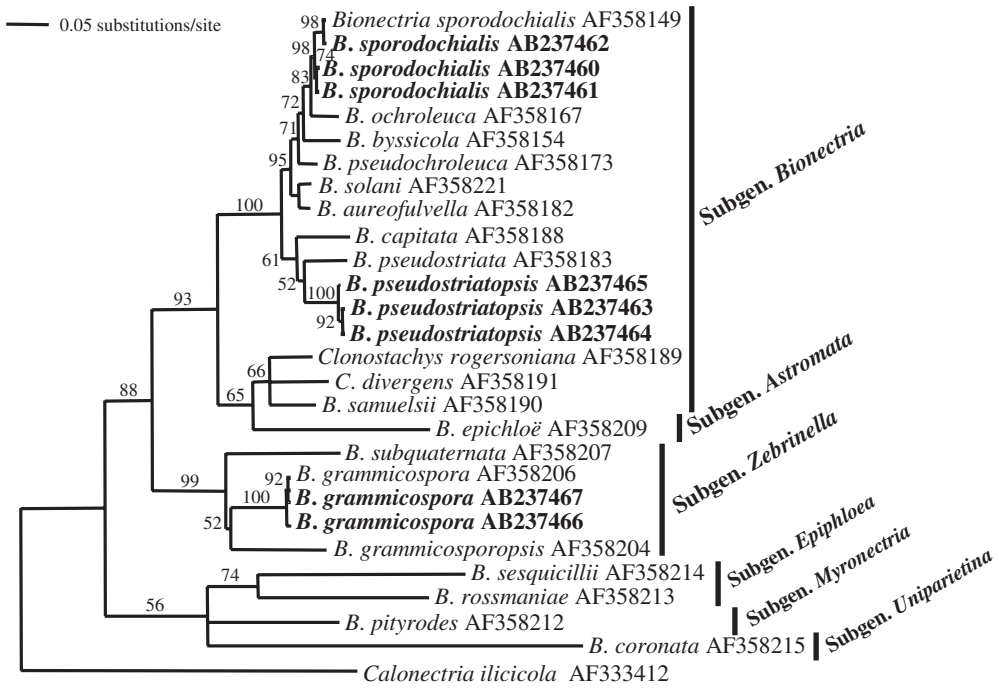


Fig. 16. Phylogenetic tree for subgenera in genus *Bionectria* (*Bionectria*; *Astromata*; *Zebrinella*; *Epiphloea*; *Myronectria*; *Uniparietina*) and present three species by neighbor-joining analysis of the *tub*2 sequences rooted with a sequence of *Calonectria ilicicola* AF333412 as an outgroup taxon. *Numbers above nodes* represent bootstrap intervals from 1000 replications. Sequences were retrieved from the DDBJ, EMBL, and GenBank databases under the accession numbers indicated. *Bionectria sporodochialis* and *B. pseudostriatopsis* were nested in the clade of subgenus *Bionectria*, and *B. grammicospora* was nested in the clade of subgenus *Zubrinella*

spora (AF358206) (Schroers 2001) with strong support in phylogenetic analysis (Fig. 16). Our results show that *B. grammicospora* and its anamorph have morphological variation of stroma, perithecial wall cells, and secondary conidiophores.

3. ***Bionectria sporodochialis*** Schroers, Stud. Mycol. 46: 92, 2001. Figs. 10–12, 15d–f

Anamorph: ***Clonostachys sporodochialis*** Schroers, Stud. Mycol. 46: 92, 2001. Fig. 13

Mycelium visible around perithecia or host. Perithecia superficially gregarious in groups of up to 50, with conspicuous basal stroma, globose to subglobose, 200–350 µm in height and 200–300 µm in diameter, constricted at the dark orange papilla, not collapsing laterally when dry, yellowish-orange to reddish-orange, without any color reaction to KOH and LA, almost warted to rough; perithecial warts white or pale yellowish, rarely inconspicuous, 50 µm high; cells of perithecial warts mainly angular, 6–12 µm in size, with unevenly thickened walls. Perithecial walls 25–48 µm in thickness, composed of three layers. Cells of the stroma angular to globose. Asci narrowly clavate to clavate, 45–80 × 5–12.5 µm, with broadly rounded apex or furnishing inconspicuous ring, with 8 spores in two rows. Ascospores elliptical to broadly elliptical, 1-septate, hyaline, 9–13 × 3–4 µm, warted.

Colony on PDA attaining a diameter of 30–45 mm in 7 days at 25°C, cottony, white; reverse white to pale yellow. Colony on OA attaining a diameter of 30–50 mm in 7 days at 25°C, cottony, white to buff, sometimes sporodochial conidial masses produced; reverse white to buff. Conidiophores dimorphic. Primary conidiophores *Verticillium*-like, rare, arising from aerial mycelium, 2–3-branched, with divergent branches and phialides; stipes 70–150 µm long, 2–4 µm wide at the base; phialides in whorls of 2–3, or solitary at lower levels, straight, cylindrical, tapering towards the tip, with minute collarette, 20–37 µm long, 2–3 µm wide at base, each producing a small, hyaline drop of conidia. Secondary conidiophores broadly penicillate, branches of conidiophores in young sporodochial pustules divergent, in developed sporodochia adpressed; metulae 12–18 × 1.5–2.5 µm; phialides in loose whorls of 2–4, almost adpressed; straight, without a visible collarette, 10–30 µm long, 1.5–2.5 µm wide at the base. Conidia hyaline, slightly curved, distally broadly rounded, with a laterally displaced hilum, 3–8 × 1.5–3 µm.

Specimens examined: On dead bark of *Prunus jamasakura* Sieb. ex Koidz. (Japanese name: Yamazakura), Takakuma Forest Research Station of Kagoshima University, Tarumizu, Kagoshima Pref., August 26, 2002, by Y.H. (teleomorph: TFM FPH-7763; anamorph: TFM FPH-7764; culture: MAFF 239828); on bark of fallen tree, Noyamakita Park, Musashi-murayama-shi, Tokyo, June 15, 2003, by T. Tokiwa and Y.H. (teleomorph: TFM FPH-7759; anamorph: TFM FPH-7760; culture: MAFF 239826); on bark of fallen tree, Okutama-cho, Nishitama-gun, Tokyo, November 20, 2003, by Y.H. (teleomorph: TFM FPH-7761; anamorph: TFM FPH-7762; culture: MAFF 239842).

Note: *Bionectria sporodochialis* (anamorph: *Clonostachys sporodochialis*) is newly added to the Japanese mycobiota. Morphological characteristics of all specimens agreed well with those of Schroers' description (Schroers 2001). In our collections, the specimen TFM FPH-7761 differed from the previous description in the smooth perithecial walls and reddish-orange perithecia. However, its anamorph produced sporodochial conidiophores typical of *C. sporodochialis*. The *tub2* sequence from this specimen

Table 2. Distribution of *Bionectria* species hitherto known in Japan

Species of *Bionectria*	Locality[b,c]	References for the Japanese species
Bionectria byssicola (*Clonostachys byssicola*)[a]	**Miyagi**, Tokyo **(2)**, **Kanagawa (3)**, **Yamanashi**, **Mie**, **Kagawa**, **Kochi**, **Fukuoka (3)**, **Kumamoto**, **Kagosima**	Schroers 2001
B. capitata (*C. capitata*)	**Miyagi**, **Tokyo**, Gunma, Yamanashi, **Kochi**, **Kagosima**	Schroers 2001
B. compactiuscula (*C. capitata*)	Tochigi, Ibaraki, Nagano, **Mie**	Schroers 2001
B. epichloë (*C. epichloë*)	Gunma, **Okinawa**	Schroers 2001
B. oblongispora (*C. oblongispora*)	Iwate, **Tokyo**	Schroers 2001
B. ochroleuca (*C. rosea*)	**Miyagi (2)**, Ibaraki **(2)**, Tokyo, **Kanagawa**, Niigata **(2)**, **Shizuoka**, Siga, **Mie**, **Kyoto**, Tokushima, **Kochi (4)**, **Kumamoto**, **Kagoshima**	Aoki et al. 1990; Arie et al. 1987; Kimura 1979; Sato et al. 1991, 1998; Schroers 2001
B. pseudostriata (*C. pseudostriata*)	Chiba, **Tokyo**, **Kanagawa**, **Kagawa**, **Fukuoka (2)**, **Okinawa (2)**	Schroers 2001

[a] Anamorphic name in parentheses
[b] The names of localities described by bold font indicate newly recorded localities of hitherto known Japanese species
[c] Numbers of specimens are given in parentheses

(AB237461) and other two Japanese specimens clustered in the same clade with the previously known sequence of *B. sporodochialis* (AF358149) with strong support (see Fig. 16).

Distribution of *Bionectria* species hitherto known in Japan

Based on the published reports and from the collected specimens of the authors, *Bionectria* species were newly found to be distributed in various parts of Japan (Table 2). Among them, *B. byssicola* (*Clonostachys byssicola*) and *B. ochroleuca* (*C. rosea*) were collected at high frequency.

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