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Speciation of *Botryobasidium subcoronatum* (Basidiomycota) collected in Taiwan: morphology, mating tests, and molecular data

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Taiwan is a phytogeographically isolated area, with a rate of ca. 40% endemism in higher plants. In this study, the strong tendency towards genetic isolation and speciation of *Botryobasidium subcoronatum* (Basidiomycota) from Taiwan is evaluated in comparison with European specimens. Mating tests among *B. subcoronatum* specimens collected in Taiwan and Europe were performed. Mon-mon and di-mon matings confirm different intersterility groups or biological species. The biological species concept was compared with results from morphological comparison and molecular data from mtSSU rDNA and the nuclear ITS2 region. Three intersterility groups have been detected comprising European and Taiwanese strains from temperate and subtropical areas. MtSSU rDNA and the ITS2 rDNA sequences confirm conspecifity of *B. subcoronatum*.

Key Words——Basidiomycota; *Botryobasidium*; mating systems; rDNA sequence data; Taiwan.

Botryobasidium subcoronatum (v. Höhn. & Litsch.) Donk is the type species of the corticioid, saprophytic, homobasidioid genus *Botryobasidium* Donk emend. G. Langer. The original description of *B. subcoronatum* was based on material from Austria (Vienna) and Germany (Berlin). This cosmopolitan species typically has resupinate, smooth whitish, yellowish to pale ochraceous basidiocarps with hypochnoid hymenium. Basidia with basal clamps are normally 6-(8)-spored and suburniform when mature. Basidiospores are navicular, hyaline, thin-walled, inamyloid, uninucleate, and germinating with germ tubes. No secondary spores are produced. Hyphae are branched right-angled and clamped at all septa. Detailed descriptions and illustrations were given by Langer (1994). Conidial stages like those of other species of *Botryobasidium* (e.g., *B. aureum* Parmasto) are unknown from nature, but have been observed recently in vitro (Langer and Langer, 2000). Septal pores with continuous parenthesomes and absence of secondary spores indicate a systematic placement on the borderline between Homo- and Heterobasidiomycetes (Langer, 1994). This was also supported by molecular data (Langer, 1998). Earlier investigations with pure cultures of *B. subcoronatum* have shown that this species is heterothallic tetrapolar (Langer, 1994). Compatibility studies based on mon-mon or di-mon matings demonstrate that all tested European collections of *B. subcoronatum* are compatible with tester strains from northern Europe (Sweden) and central Europe (northern Alps, Germany). Intercompatibility of these collections was independent of climatical conditions of the habitat or the substrate (Langer, 1994).

Of the 49 hitherto known and accepted *Botryobasidium* species, 13 occur in Taiwan (Langer, 1994). In several field trips from 1988 to 1996, six specimens fitting the morphotype of *B. subcoronatum* were found (Figs. 1, 2). Additionally, a specimen with broad-navicular basidiospores labeled *B.* aff. *subcoronatum* was collected (Figs. 3, 4). In this investigation compatibility tests among the European and Taiwanese collections have been performed. Additional comparative morphological comparisons and molecular data of mtSSU rDNA and nuclear internal transcribed spacer (ITS2 DNA including partial 5.8 S rDNA) were used for the evaluation of the Taiwanese *B. subcoronatum* collections.

Materials and Methods

Botryobasidium subcoronatum collections examined from Taiwan: **FO 41059**, Taiwan, Provinz Nantou, northeast of Puli, Huisun recreation area, ca. 600–800 m alt., on *Pinus taiwanensis*, leg. F. Oberwinkler, G & E. Langer, 09 April 1989, det. G. Langer. **GEL 3304**, Taiwan, R.O.C., Tahsueh Shan, recreation area, secondary forest near bungalows, ca. 2400 m alt., leg. E. & G. Langer, C.-J. Chen, 13 April 1996, det. G. Langer. **GEL 3330**, Taiwan, R.O.C., Tahsueh Shan, entrance of the recreation area at Sau Lei mountain, secondary forest with *Cryptomeria japonica*, ca. 2000 m alt., leg. E. & G. Langer, C.-J. Chen, 14 April 1996, det. G. Langer. **GEL 3436**, Taiwan, R.O.C., Sheipa National Park, Kuanwu, forest ca. 500 m in direction of Le Shan, trail on the right

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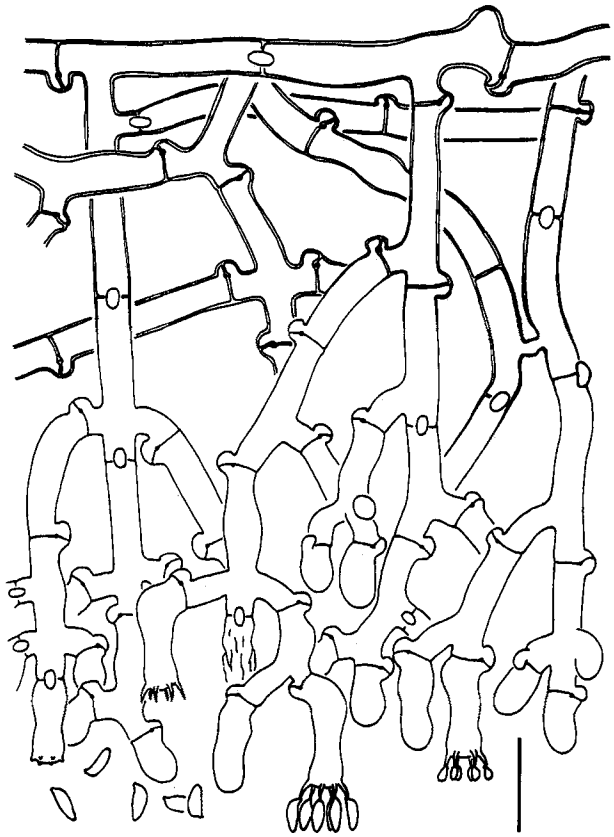

Fig. 1. *Botryobasidium subcoronatum*, specimen GEL 2061 from Austria. Cross-section of the entire basidiocarp showing hymenium with basidia and basidiospores. Basal hyphae have thickened walls; bar = 20 μm.

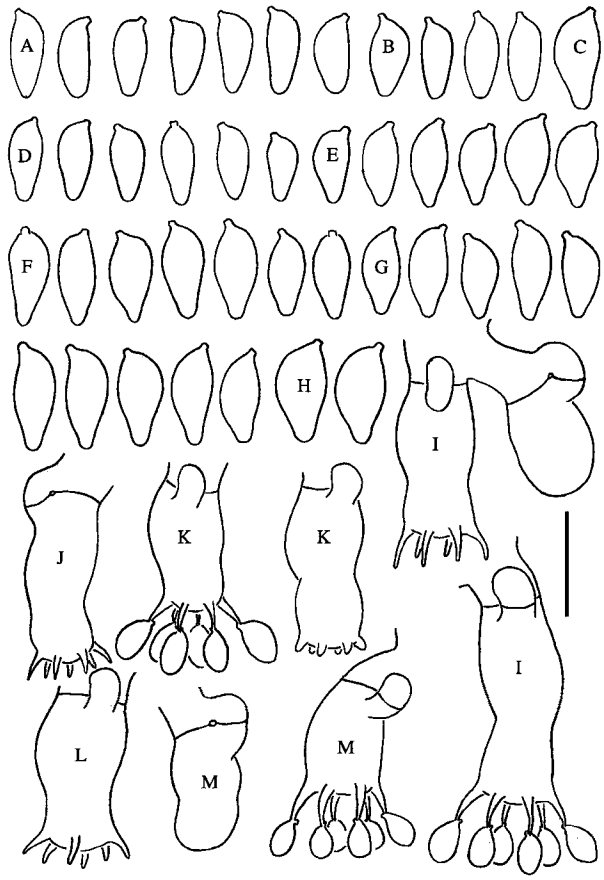

Fig. 2. *Botryobasidium subcoronatum*, typical basidiospores and basidia from different specimens; bar = 10 μm. A. GEL 2061, Austria. B. FO 41059, Taiwan, Huisun. C, L. GEL 3451, Taiwan, Kuanwu. D, J. 3474, Taiwan, Hsinchu. E, K. GEL 3436, Taiwan, Kuanwu. F, M. GEL 3304, Taiwan, Tahsueh Shan. G, I. GEL 3330, Taiwan, Tahsueh Shan. H. GEL 3384, Taiwan, Kuanwu.

side of the road, ca. 2100 m alt., leg. E. & G. Langer, C.-J. Chen, 19 April 1996, det. G. Langer. **GEL 3451**, Taiwan, R.O.C., Sheipa National Park, Kuanwu, forest ca. 500 m in direction of Le Shan, trail on the right side of the road, ca. 2100 m alt., leg. E. & G. Langer, C.-J. Chen, 19 April 1996, det. G. Langer. **GEL 3474**, Taiwan, R.O.C., Hsinchu, Shih-ba-chian shan, mixed forest with *Podocarpus* sp., *Pinus* sp., *Casuarina equisetifolia*, *Aralia* sp. etc., collected on wood of *Pinus* sp. under very dry conditions, ca. 50 m alt., leg. E. & G. Langer, 19 April 1996, det. G. Langer.

Botryobasidium aff. *subcoronatum*: **GEL 3484**, Taiwan, R.O.C., Sheipa National Park, Kuanwu, forest with *Taiwania cryptomerioides*, *Cryptomeria japonica*, and *Alnus formosana*, ca. 2000 m alt., on brown rotted coniferous wood, leg. E. & G. Langer, C.-J. Chen, 18 April 1996, det. G. Langer.

Botryobasidium subcoronatum collections examined from Europe: **FO 34634**, Germany, Bavaria, Weiler-Simmerberg, Obertrogen, Trogener Moor, leg. F. Oberwinkler, 25 September 1983, det G. Langer. **FCUG 1286-8**, MS-culture of NH 8656, Sweden, on wood of *Picea abies*, Halland, leg. et det. N. Hallenberg. **GEL 2061**, Austria, Tirol, Schattwald, Vils-Tal, Pfrontener Wald, Zwersberg, *Picea abies*/*Abies alba*-forest, ca.

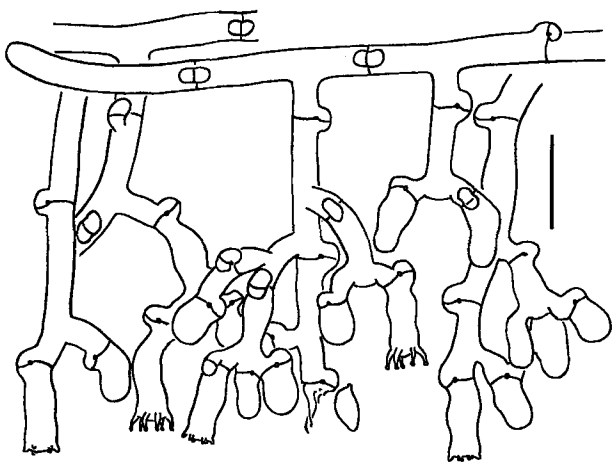

Fig. 3. *Botryobasidium* aff. *subcoronatum*, GEL 3384 from Taiwan. Cross-section of the entire basidiocarp showing hymenium with basidia and basidiospores; bar = 20 μm.

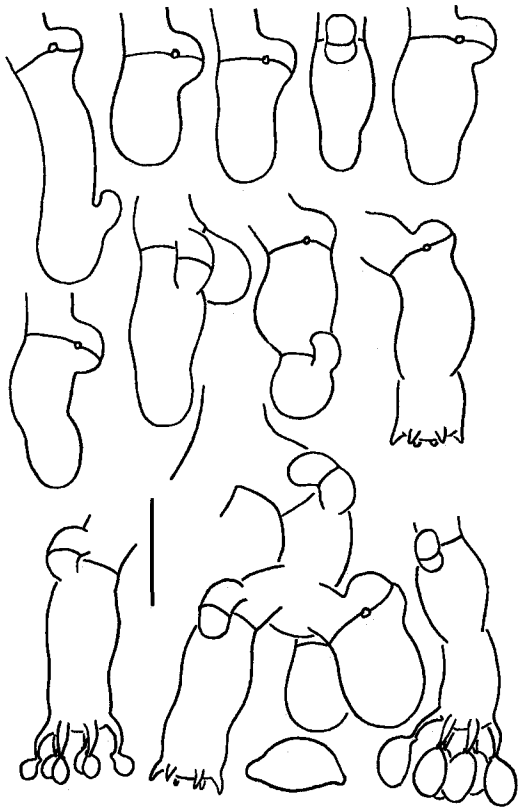

Fig. 4. *Botryobasidium* aff. *subcoronatum*, GEL 3384 from Taiwan, basidia of different ontogenetic stages and a typical basidiospore; bar = 10 μm.

1000 m alt., decayed wood of *Picea abies*, 15 September 1989, leg. et det. G. Langer. **GEL 2152**, Norway, Åkershus County, Nannestad, Tømte field station, ca. 300 m alt, on wood of *Pinus sylvestris* leg. N. Hallenberg, 19 September 1990, det G. Langer. **GEL 2160**, Norway, Åkershus County, Nannestad, Gardemoen Airfield, Ravine, ca. 200 m alt., growing on an old basidiocarp of *Inonotus radiatus*, leg. L. Ryvarden, 20 September 1990, det G. Langer. **GEL 2164**, Norway, Åkershus County, Nannestad, Gardemoen Airfield, Ravine, ca. 200 m alt., on frondose wood, leg. G & E. Langer, 20 September 1990, det G. Langer. **GEL 2276**, Germany, Bavaria, Oberallgäu, Unterjoch, Pfeiffermühle, ca. 1000 m alt., decayed wood of *Picea abies*, leg. et det. G. & E. Langer, 16 September 1991. **GEL 2280**, Germany, Bavaria, Oberallgäu, Unterjoch, Pfeiffermühle, ca. 1000 m alt., wood of *Alnus glutinosa*, leg. et det. G. & E. Langer, 16 September 1991. **GEL 2412**, Germany, Bavaria, Oberallgäu, Unterjoch, old bog close to Krumbach at the Austrian borderline, ca. 1100 m alt., on wood of *Picea abies*, leg. et det. G. & E. Langer, 16 September 1991. **GEL 2936**, Germany, Bavaria, Bavarian Forest, St. Oswald, Klosterforst, Freiamt Grafenau, *Fagus-Abies-Picea*-mixed forest, ca. 790 m alt., on coniferous bark, leg. N. Luschka, G. & E. Langer, 16 September 1994, det. G. Langer. **GEL 2943**, Germany, Bavaria, National Park Bavarian Forest, Sankt Oswald, Klosterforst, Freiamt Grafenau, ca. 790 m alt., mountainous mixed forest with *Picea abies*, *Fagus sylvatica* and *Abies alba*, on wood, leg. N. Luschka, G. & E. Langer, 16 September 1994, det. G. Langer. **GEL 4232**, Germany, Bavaria, National Park Bavarian Forest, on wood, leg. R. Kirschner, October 1996, det. G. Langer. **LY 10162**, France, Savoie, on *Picea*, leg. J. Boidin, August 1983 (Boidin et al., 1998).

Botryobasidium aureum: **GEL 2910**, Germany, Bavarian Forest, Zwiesel, nature reserve Mittelsteighütte, ca. 700–800 m alt., on brown-rotted wood, leg. N. Luschka, G. & E. Langer, 16 September 1994, det. G. Langer.

Botryobasidium intertextum: **DAOM 197881**, Canada, Nova Scotia, Grafton Lake, Kejimkujik National Park, on underside of a rotted oak (*Quercus* sp.) log, leg. J. H. Ginns, 19 September 1987, det. M. Cubeta. DNA-sequence obtained from Marc Cubeta, USA.

Botryobasidium isabellinum: **GEL 2108**, Germany, Baden-Württemberg, Black Forest, Würzbach, Bannwald Waldmoor-Torfstich and nature reserve Bruckmüsse, ca. 800 m alt, leg. G. Kost, K.-H. Rexer, 4 May 1990, det. G. Langer.

Botryobasidium grandisporum: **FO 40862**, Taiwan, province Taichung, Ama-Shan, southwest of Tah-Sueh-Shan, mountain forest with old trees of *Tsuga*, *Cryptomeria*, *Taiwania* and *Castanopsis*, ca. 2200 m alt., leg. F. Oberwinkler, E. and G. Langer, 7 June 1989, det. G. Langer.

Botryobasidium longisporum: **GEL 3321**, Taiwan, Tahsueh Shan, recreation area, secondary forest near the bungalows, ca. 2400 m alt., leg. E. and G. Langer, Ch.-J. Chen, 13 April 1996, det. G. Langer.

Specimens were examined from fresh, herbarium material and pure culture with a Zeiss Standard Lab 16 light microscope using phase optics and an Axioplan 2 light microscope using differential interference constrast. For analyzing hyphal texture, Phloxin B after application of potassium hydroxide (5%) or Cotton blue was used as stain.

Strains were isolated on malt yeast peptone agar (MYP) medium (Bandoni, 1972), modified by Langer (1994). Mating tests were performed in 90-mm plastic Petri dishes containing 20 ml of MYP agar (Langer, 1994).

Terms for climatic and altitudinal vegetation zones etc. follow the Flora of Taiwan 2nd edition ed. by Huang (1994).

DNA was extracted from small patches of mycelium grown on MYP. DNA was extracted using the method of Singer-Sam et al. (1989). Mycelium was incubated in a mixture of 500 μl of ultrapure water with 5% Chelex® 100 (BIO-RAD) at 65°C for 1 h, then at 90°C for 1 min. After centrifugation at 13,000 rpm, an aliquot of 25 μl was used for PCR. In the case of GEL 3384, herbarial material was used for DNA isolation following the methods described by Edwards et al. (1991) and Henrion et al. (1992). Although multiple trials were attempted, PCR failed in this sample.

Standard PCR used primers as described by White et al. (1990). Indentity of PCR products was controlled using 1.5% agarose gels. PCR products were cleaned from the PCR mix using QIAquick spin columns (QIAGen) following the producer's manual. DNA was precipitated with 3 volumes of ethanol for 1–24 h at −20°C, followed by 15 min. centrifugation at 4°C. The pellet was washed with 70% ethanol and dried for about 30 min at room temperature, then resolved in 30 µl of ultrapure water. Cycle sequencing was carried out with primers MS1/2 for mtSSU DNA and ITS1/3/4 for the regions ITS1 and ITS2. Cycle sequencing followed protocols published by Pohl and Maier (1995) when using a Direct Blotting Electrophoresis device with a 4% PAA gel. Blotted DNA on nylon membrane was visualized following the protocol of Kessler (1992). Moreover, an ABI 377 automatic sequencer was used following the protocols given by the manufacturer.

A total of 29 DNA sequences have been deposited in the EMBL sequence database: *B. longisporum* mtSSU, accession number AJ389797 (GEL 3321); *B. grandisporum* mtSSU, accession number AJ389798 (FO 40862); *B. isabellinum* mtSSU, accession number AJ389799 (GEL 2108); *B. subcoronatum* mtSSU, accession numbers AJ389800 (GEL 3436), AJ389801 (FCUG 1286), AJ389802 (GEL 2160), AJ389803 (GEL 2164), AJ389804 (GEL 2152), AJ389805 (LY 10162), AJ389806 (GEL 2280), AJ3898807 (GEL 2276), AJ3898808 (GEL 2412), AJ389809 (GEL 2936), and AJ389810 (GEL 3451); *B. subcoronatum* 5.8S/ITS2, accession numbers AJ389784 (FCUG 1286), AJ389785 (GEL 2152), AJ389786 (GEL 2160), AJ389787 (GEL 2061), AJ389788 (GEL 2412), AJ389789 (GEL 2280), AJ389790 (GEL 2936), AJ389791 (LY 10162), AJ389792 (FO 41059), AJ389793 (GEL 3474), AJ389794 (GEL 3304), AJ389795 (GEL 3436), and AJ389796 (GEL 3451). Sequences of *B. aureum*, accession number AJ389783 (GEL 2910), and *B. intertextum*, accession number AJ389782 (DAOM 197881), were also deposited. Two alignments have been deposited in the EMBL database under accession numbers ds40044 (mtSSU) and ds40043 (ITS2).

Nuclear ITS2 DNA sequences were aligned using CLUSTAL V (Higgins et al., 1992) with manual adjustment. PAUP 4.0b2 (Swofford, 1991) was used to calculate parsimony and maximum likelihood analysis. The number of informative characters was low compared with the numbers of available characters due to high homogenity of the investigated group. The mtSSU data matrix with 539 characters contained 20 informative sites, whereas ITS2 data with 431 characters showed 17 informative sites. Settings described by Hibbett et al. (1997) were used to calculate trees with the additional assumption option "gap=newstate" for parsimony analyes. Additionally, bootstrapping with 100 and 1000 replicates respectively was used to test robustness of trees.

Results

From all investigated specimens no anamorph was found in nature, but in pure culture conidial stages were produced (Fig. 5). Detailed information about these anamorphs was described in Langer and Langer (2000). Chlamydospores were absent in vivo and in vitro. The morphology of the six navicular-spored Taiwanese specimens fits the general description (Langer, 1994) of *B. subcoronatum*, but the basidiospores were slightly larger than described in the original description and that of the specimen GEL 2061 (compare also Table 1 and Fig. 1). A section through the basidiocarp of the typical Austrian specimen GEL 2061 is shown in Fig. 1. Isolated monospore cultures (MS-strains) of the Taiwanese strains and GEL 2276 proved to be clampless and monokaryotic. Secondary mycelia were clamped and dikaryotic.

Polarity tests performed with different single-spore cultures (MS-cultures) of GEL 3474 showed that this specimen was also heterothallic tetrapolar (hIV, Table 2) like the German specimen GEL 2276. The 10 tested MS-cultures with white cottony mycelium represented the following four mating types: Type I (GEL 3474-MS 3, 6, 7, 10, 11), which was compatible with Type II (GEL 3474-MS 4, 12, 15); and Type III (GEL 3474-MS 14), which was compatible with Type IV (GEL 3474-MS 17). Incompatible pairings between strains of the same mating type showed no clamp formation in the monokaryotic, white, cottony mycelium. Compatible pairings formed true clamps and the resulting mycelium was dikaryotic, turning from white to cream color. Hemi

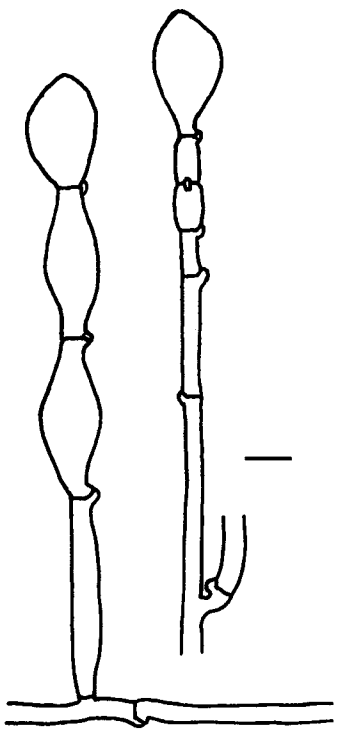

Fig. 5. *Botryobasidium subcoronatum*, specimen FO 41059 from Taiwan. Conidial stages in pure culture; bar = 10 µm.

Table 1. Data of the investigated Taiwanese and selected additional *Botryobasidium subcoronatum* specimens.

Specimen	Spores Basidia	Substrate	Location	Altitude	Climate
original description	5–7 × 2.5–3.5 16–18 × 6–8	wood, bark, old polypores	Austria Germany	ca. 175–250 m ca. 32–50 m	temperate
GEL 2061	6.5–8 × 2.5–3(–3,5) 20–25(–30) × 7–9	*Picea abies*	Austria/Alps	ca. 1000 m	temperate
GEL 2276	(7)–8–9.5 × 3–3.5 (12)–14–22 × 7–8.5	*Picea abies*	Germany/Alps	ca. 1000 m	temperate
GEL 2280	(7)–8–9 × 3.5–4 15–20 × 6–7	*Alnus glutinosa*	Germany/Alps	ca. 1000 m	temperate
FO 41059	7–8 × 3–3.5 —	*Pinus taiwanensis*	T, Huisun	ca. 600–800 m	subtropical
GEL 3474	6–7.5 × 3 15–20 × 7	*Pinus*	T, Hsinchu	ca. 50 m	(sub)tropical
GEL 3304	7.5–9 × 3.5–4 10–14 × 7–8	*Hymenochaete*	T, Tahsueh Shan	ca. 2400 m	temperate
GEL 3330	8–10 × 3–4.5 15–20 × 6–7	*Cryptomeria japonica*	T, Tahsueh Shan	ca. 2000 m	temperate
GEL 3436	7–8 × 3–4 10–15 × 7–8	wood	T, Kuanwu	ca. 2100 m	temperate
GEL 3451	6–9 × 3–4.5 15–16 × 7–8	wood	T, Kuanwu	ca. 2100 m	temperate
GEL 3384	8–9 × 4,5–5 18–22 × 6–7	coniferous wood	T, Kuanwu	ca. 2000 m	temperate

T = Taiwan, sizes in μm

Table 2. Polarity tests of monokaryotic *Botryobasidium subcoronatum* strains obtained from specimen GEL 3474, Taiwan, Hsinchu.

MS	3	6	7	10	11	13	4	12	15	14	17
3	—	—	—	—	—	—	+	+	+	-HA	-HB
6		—	—	—	—	—	+	+	+	-HA	-HB
7			—	—	—	—	+	+	+	-HA	-HB
10				—	—	—	+	+	+	-HA	-HB
11					—	—	+	+	+	-HA	-HB
13						—	+	+	+	-HA	-HB
4							—	—	—	-HB	-HA
12								—	—	-HB	-HA
15									—	-HB	-HA
14										—	+
17											—

hIV
I: 3, 6, 7, 10, 11, 13
II: 4, 12, 15
III: 14
IV: 15

Mon-mon tests; MS = monokaryotic, monospore or single spore culture; — = incompatible mating between strains of the same mating type, no clamp formation, mycelium monokaryotic, white and cottony; + = mating between strains of two compatible mating types, clamp formation, mycelium dikaryotic, turning to cream color; HA = hemicompatible A- mating, no clamp formation, mycelium mostly monokaryotic, white and cottony; HB = hemicompatible B- mating, sometimes formation of false clamps in the confrontation zone, mycelium mostly monokaryotic, sometimes with irregular content of nuclei per cell, both strains strongly inhibited, production of crystals and phenolic-like substances.

compatible A-pairings (HA) showed no clamp formation. Mycelium of both donor strains remained monokaryotic, white, and cottony. Usually one of the pairing partners was inhibited by the other. Hemicompatible B-pairings (HB) sometimes produced false clamps in the confrontation zone. The mycelium was mostly monokaryotic but sometimes cells exhibited an irregular number of nuclei. Both donor strains were strongly inhibited, indicated by reduced growth rate in comparison to incompatible or compatible donors and by production of crystals and brownish, phenolic-like substances.

Compatibility tests of monokaryotic strains representing the four mating types of the Taiwanese strain GEL 3474 (Type I: GEL 3474–10,12; Type II: GEL 3474–4,15; Type III: GEL 3474–14; Type IV: GEL 3474–17) with the German tester strains GEL 2276-MS 4 (A_1B_1), GEL 2276-MS 6 (A_2B_2), GEL 2276-MS 7 (A_1B_2), GEL 2276-MS 9 (A_2B_1) (Langer, 1994), were found to be incompatible in all cases (Table 3).

Di-mon tests between European and Taiwanese strains were also found to be incompatible (Tables 4, 5). Compatibility would have been accepted if at least one of the di-mon pairings with the four mating types produced a Buller phenomenon (Quintanilha, 1938). In the case of the dikaryotic donor GEL 2276 comprising the mating types (A_1B_1)+(A_2B_2), only the compatible monokaryotic testers GEL 2276–4 (A_1B_1) and GEL 2276–6 (A_2B_2) showed dikaryotization and clamp formation. This so-called "half compatible" di-mon mating was expected and theoretically described by Esser and Kuenen (1965) and Langer (1994). Strain GEL 2061 gave a positive di-mon reaction with all four tester strains. This completely compatible reaction indicated the presence of different alleles of the mating factors in *B. subcoronatum*. A single-spore culture of GEL 3304 from the Taiwanese high mountains also showed incompatiblity with the GEL 2276-MS-tester (Table 3). Pairings between temperate specimens from the high mountains were compatible, because there were positive di-mon matings between GEL 3304–6 from Tahsueh Shan and the strains of GEL 3330 (Tahsueh Shan, 2000–2400 m alt.), GEL 3436 and GEL 3451 (Kuanwu, ca. 2100 m alt.). These four collections

Table 3. Compatibility tests (mon-mon tests) of monokaryotic *Botryobasidium subcoronatum* strains obtained from specimen GEL 3474, Taiwan, Hsinchu and GEL 3304 from Tahsueh Shan with the German monokaryotic tester-strains GEL 2276. MS–4, 6, 7, 9 representing the four matings Types I–IV.

Germany	Taiwan						
	3474–10 (I)	3474–12 (I)	3474–4 (II)	3474–15 (II)	3474–14 (III)	3474–17 (IV)	3304–6
2276–4	—	—	—	—	—	—	—
2276–6	—	—	—	—	—	—	—
2276–7	—	—	—	—	—	—	—
2276–9	—	—	—	—	—	—	—

—=incompatible mating between strains, no clamp formation, mycelium monokaryotic; MS=monospore culture.

Table 4. Compatibility tests (di-mon tests) of dikaryotic *Botryobasidium subcoronatum* strains obtained from the Taiwanese specimens GEL 3474, FO 41059, GEL 3304, GEL 3330, GEL 3436, GEL 3436, and GEL 2061 (Austria), GEL 2276 and 2280 (Germany) with the monokaryotic tester-strains GEL 2276 MS–4, 6, 7, 9.

Origin	DS (A_1B_1)+(A_2B_2)	MS			
		GEL 2276–4 (A_1B_1)	GEL 2276–6 (A_2B_2)	GEL 2276–7 (A_1B_2)	GEL 2276–9 (A_2B_1)
G	GEL 2276	+	+	—	—
G	GEL 2280	+	—	+	+
G	GEL 2060	+	+	+	+
T	GEL 3474	—	—	—	—
T	FO 41059	—	—	—	—
T	GEL 3304	—	—	—	—
T	GEL 3330	—	—	—	—
T	GEL 3436	—	—	—	—
T	GEL 3451	—	—	—	—

+=compatible di-mon mating, dikaryon and clamp formation in the former monokaryotic mycelium; —=incompatible di-mon mating, no dikaryon and clamp formation in the monokaryotic mycelium; MS=monospore culture; DS=dikaryotic culture, G=Germany, T=Taiwan.

Table 5. Compatibility tests (di-mon tests) of dikaryotic *Botryobasidium subcoronatum* strains (DS) obtained from the Taiwanese specimens GEL 3474 (Hsinchu), FO 41059 (Huisun) GEL 3304, GEL 3330 (Tahsueh Shan); GEL 3436, GEL 3436 (Kuanwu), GEL 2060 (Austria), GEL 2276 (Germany) and other representative strains from Europe with the Taiwanese monokaryotic strains (MS) GEL 3474 MS-10/11, 4/15, 14, 17.

Origin, DS	MS						
	3474-10	3474-11	3474-4	3474-15	3474-14	3474-17	3304-6
A, 2060	—	—	—	—	—	—	—
N, 2152	/	/	—	—	/	—	/
N, 2164	/	/	—	—	/	—	/
G, a, 2276	—	—	—	—	—	—	—
G, a, 2280	/	/	—	—	/	—	/
G, a, 2412	—	—	—	—	—	—	—
G, bf, 2936	—	—	—	—	—	—	—
G, bf, 2943	/	/	—	—	/	—	/
G, bf, 4232	—	—	—	—	—	—	—
G, FO 34634	—	—	—	—	—	—	—
F, LY 10162	—	—	—	—	—	—	—
T, 3474	?	—	+	?	—	—	—
T, FO 41059	/	/	+	+	/	+	/
T, hm, 3304	—	—	—	—	—	—	+
T, hm, 3330	—	—	—	—	—	—	+
T, hm, 3436	—	—	—	—	—	—	+
T, hm, 3451	—	—	—	—	—	—	+

Taiwanian II

Taiwan. I

+=compatible di-mon mating, dikaryon and clamp formation in the former monokaryotic mycelium; —=incompatible di-mon mating, no dikaryon and clamp formation in the monokaryotic mycelium; /=not tested; A=Austria, G=Germany; F=France; N=Norway; T=Taiwan; a=Alps; bf=Bavarian forest; hm=high mountain.

were incompatible with the two paired strains from lower altitudes, GEL 3474 (Hsinchu, 50 m alt., tropical to subtropical) and FO 41059 (Huisun, 600–800 m alt., subtropical).

Mitochondrial small subunit rDNA (mtSSU rDNA) analyses confirmed monophyly of all *B. subcoronatum* strains using *B. grandisporum* G. Langer, *B. isabellinum* (Fr.) Rogers and *B. longisporum* G. Langer as sister groups for calculations. The sequences including primers MS1-MS2 were 536 bp long and were homogeneous in all *B. subcoronatum* strains. Consistency index in all calculated trees from mtSSU data was 0.979, homoplasy index was 0.021. Within the genus *Botryobasidium* sequences were generally homogeneous in the mtSSU rDNA at species rank (data not shown). The sequence of *B. aureum* was very similar to *B. subcoronatum*, same length, differing only in two bases. From primer MS1, these were in the positions 252 (*B aureum*: 5′AG**G**GT3′, *B. subcoronatum*: 5′AG**A**GT3′) and 327 (*B aureum*: 5′CT**C**AG3′, *B. subcoronatum*: 5′CT**G**AG3′). A parsimony bootstrap analysis with 100 replicates (Fig. 6A) confirmed conspecificity of *B. subcoronatum*. Although collections LY 10162 (Europe) and FO 41059 (Taiwan) were incompatible in mating tests, they clustered in one *B. subcoronatum* clade with a bootstrap value of 100% (Fig. 6A).

The ITS2 was a more variable region. The sequence including the primers ITS2-ITS4 had a length of 408 bp in the case of GEL 2061. Maximum likelihood was applied to this data set to find the best tree (Fig. 6B) among a set of different collections from intersterility groups shown in Fig. 7. *B. aureum* and *B. intertextum* were used as outgroups. The best tree scored -Ln likelihood=1086.05587 and was used to choose among tree topologies when showing parsimony results in Fig. 6D.

In a parsimony analysis a consistency index of 0.915 and a homoplasy index of 0.085 were calculated in all 100 trees. A tree was selected to show phylogeny according to best maximum likelihood as described above (Fig. 6D). A bootstrap was calculated together with *B. intertextum* (inconstant clamps, narrow navicular basidiospores) and *B. aureum* (clampless, navicular to subcylindrical basidiospores) as outgroups (Fig. 6C). The latter species can be distinguished distinctly by its morphology and sequence from *B. subcoronatum* specimens. As expected, all *B. subcoronatum* strains were monophyletic due to the overall homogeneous sequence. The Taiwanese strains were clustered with the French strain LY 10162 in the bootstrap tree (61%). All Taiwanese strains were characterized by the replacement of thymine by cytosine in position 147 (Taiwanese strains: 5′CC**C**GC; European strains: CC**T**GC). The compatible, temperate high mountain strains (GEL 3304, GEL 3436, and GEL 3451) also formed a monophyletic group.

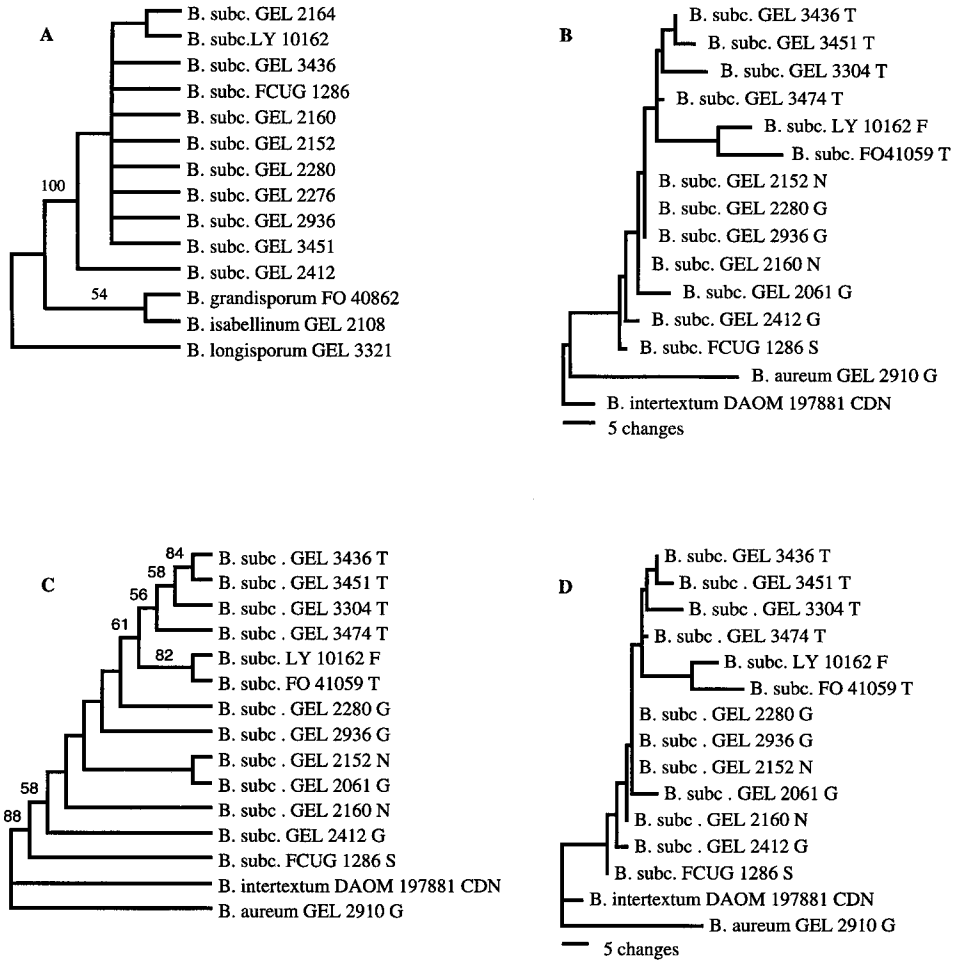

Fig. 6. Cladistic analysis of mtSSU and ITS2 rDNA including partly 5.8S rDNA data. Discussion see text.
A. Parsimony bootstrap tree with 100 replicates of mtSSU rDNA with TBR branch swapping, addition sequence simple and MulTrees on. Only bootstrap values $\geq 50\%$ shown. B. Maximum likelihood tree of partly 5.8S and ITS2 rDNA. Best tree of six trees with tree scores: -Ln likelihood = 1086.05587, consistency index = 0.9149, homoplasy index = 0.0851, consistency index excluding uninformative characters = 0.7143, homoplasy index excluding uninformative characters = 0.2857, retention index = 0.7333, rescaled consistency index = 0.6709. C. Parsimony bootstrap tree of partly 5.8S and ITS2 rDNA with TBR branch swapping, addition sequence random with 100 replicates 100, and MulTrees on. Only bootstrap values $\geq 50\%$ shown. D. Selected parsimony tree of 100 equal parsimonious trees using partly 5.8S and ITS2 rDNA with TBR branch swapping after 100 random sequence additions and MulTrees on. Tree scores: Consistency index = 0.915, homoplasy index = 0.085, retention index = 0.733, rescaled consistency index = 0.671.

Within this group the two different locations were also reflected by sequence differences (e.g., positions 331 and 333, in GEL 3304 from Tahsueh Shan: 5'CGGGC3'; in GEL 3435 and 3451 from Kuanwu: 5'CCGTC3'). The compatible strains GEL 3474 and FO 41059 surprisingly were not clustered together.

Discussion

The morphological characters of the Taiwanese collections FO 41059, GEL 3474, GEL 3304, GEL 3330, GEL 3435, and GEL 3451 described here fit the species concept of *B. subcoronatum* well, but they have slightly larger spores. Basidiospores were still navicular and, in the case of the tropical to subtropical specimens FO 41059 and GEL 3474, were within the upper range of the European specimens. It seemed reasonable, therefore, that FO 41059 clustered together with an incompatible European strain (LY 10162) when using the ITS 2 region for calculations. But considering the low number of informative characters, FO 41059 and LY 10162 may have been clustered due to stochastic reasons. Mating tests showed a large European intersterility group with several populations, but there was no confirmation of monophyly in this group from the ITS2 data. All Taiwanese strains, despite their forming of two different intersterility groups, shared a common character in the ITS2 region (position 147) which separated them from the European strains. The intersterility group comprising temperate high mountain specimens with slightly larger spores formed their own clade using ITS2 data. A separate biological species, therefore, could be support-

ed by molecular data. Moreover, the different locations of the high mountain strains Kuanwu and Tashue Shan were reflected by the ITS2 sequence data. The morphospecies of *B. subcoronatum* containing the investigated Taiwanese specimens was confirmed by ITS2 and mtSSU rDNA data.

Considering all investigated data, we are of the opinion that *B. subcoronatum* is a morphospecies of worldwide distribution that shelters different intersterility groups. Three were detected in this study: a) the European group, with specimens from Austria, Germany, Norway, Sweden, and France; b) the temperate Taiwanese high mountain group (Taiwanese I) with specimens GEL 3304, 3300, 3436, 3451; and c) the subtropical Taiwanese II group of lower and submontane elevations with specimens GEL 3474 and FO 41059 (Fig. 7). The morphological differences among the Taiwanese intersterility groups were too weak for a discrete delimitation. In the case of the Taiwanese I group, found in the montaneous *Quercus* vegetation zone, the biological species concept and the one based on DNA data were congruent. Therefore, it might be useful to characterize this group as comprising sibling species. Monophyly of the Taiwanese II group is not supported.

Taiwan shelters a high number of endemic plant species (Huang, 1994: ca. 40%) and exhibits a high rate of speciation. It seems obvious that Taiwanese species endemism in general is more strongly indicated in temperate genera than tropical ones (Li, 1963). Speciation is also forced by several geographical barriers due to the central high mountain range and the isolated island situation. The rate of speciation is indicated within *Botryobasidium* by the high number of new morphospecies described from this island (Langer, 1994). Of the 49 *Botryobasidium* species known worldwide, seven taxa (14.3%) have been described from Taiwan. Four warm-temperate to cold-temperate species have been described as new from Taiwanese montane and upper montane to subalpine altitudinal zones. *B. grandisporum* and *B. sublaeve* G. Langer are typical of the temperate *Quercus* vegetation zone. *B. arachnoidium* G. Langer collected from *Tsuga chinensis* (Franchet) Pritz. ex Diels and *B. asterosporum* G. Langer collected from *Abies kawakamii* (Hayata) Ito occur in the upper montane to subalpine vegetation zone. Additionally, *B. longisporum* is a subtropical to temperate species appearing in both submontane and montane regions. The cold-temperate *B. arachnoideum* is similar to *B. subcoronatum* but differs in its ellipsoid to ovoid basidiospores (6–7 × 3–4 μm) and the scattered lack of clamps in basal hyphae. This species may be a result of a vicarious speciation from *B. subcoronatum*. Genetic differentiation of Taiwanese *B. subcoronatum* specimens is, however, linked to an enlargement of the basidiospores and small but significant differences in the mtSSU DNA and ITS2 regions. It is also expressed by complete sexual incompatibility be-

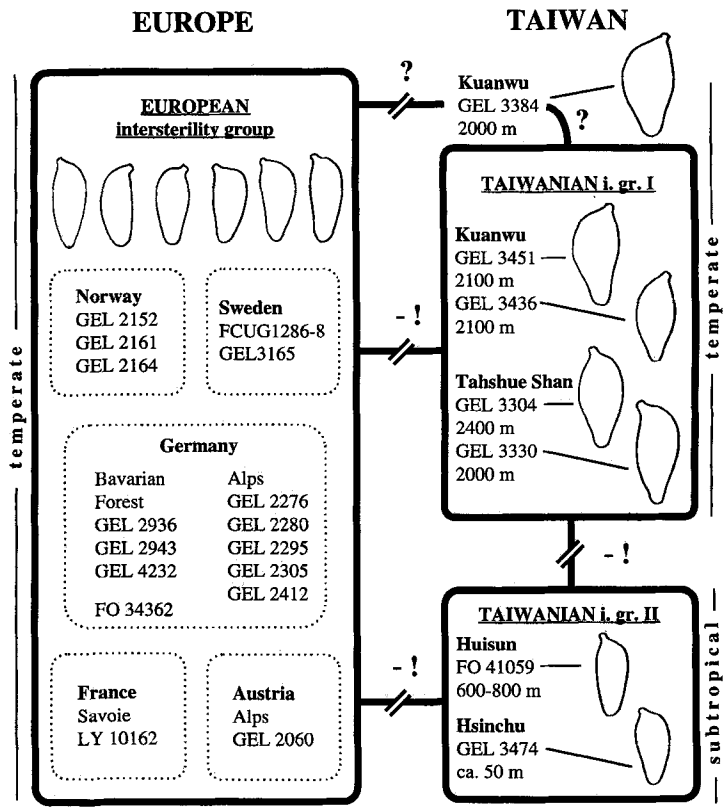

Fig. 7. Mating barriers and intersterility groups/siblings within *Botryobasidium subcoronatum*. (——//—— Incompatibility, indicating a mating barrier, compatibility and intersterility groups surrounded by bold rectangles).

tween the European and Taiwanese strains. Additionally, speciation is also manifested by a *B. subcoronatum*-like specimen collected in Kuanwu, ca. 2000 m alt., with distinctly broad-navicular basidiospores (8–9 × 4–5 μm, Figs. 2, 4). In light of this, the latter morphotype is easy to distinguish from typical *B. subcoronatum* (Fig. 1). Therefore, a new species epithet could be proposed; but on the one hand, there are no cultures available from GEL 3384 to test compatibility with the other *B. subcoronatum* strains from Kuanwu, and on the other hand, there are no sequence data to verify if GEL 3384 is molecularly similar to the other *B. subcoronatum* strains. We intend to use the label *B.* aff. *subcoronatum* for GEL 3384 until more specimens are collected and additional data are available.

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