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Antrodia cinnamomea reconsidered and *A. salmonea* sp. nov. on *Cunninghamia konishii* in Taiwan

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Abstract. *Antrodia cinnamomea* is reinstated as the correct name for a basidiomycete consistently associated with *Cinnamomum kanehirai*. *Antrodia salmonea* nov. sp., causing a brown heart rot of *Cunninghamia konishii* in Taiwan, is described and illustrated. *Antrodia cinnamomea* and *A. salmonea* are morphologically similar but can be separated by pore surface color of basidiomata and host preferences. Moreover, pairings between monokaryons of *A. cinnamomea* and those of *A. salmonea* demonstrate that these two fungi have different mating systems.

Keywords: *Antrodia camphorata*; *Antrodia cinnamomea*; *Antrodia salmonea*; Polypores; Taiwan.

Introduction

Cunninghamia konishii Hayata (Cunninghamieae) is a coniferous tree and endemic to Taiwan, growing in broad-leaved and coniferous forests at altitudes between 1,300 and 2,800 m in the central and northern parts of the island. A brown heart rot associated with resupinate, salmon-pink basidiomata in the empty rotten trunk of *Cu. konishii* has vernacularly been called Shiang-Shan-Chih. This fungus is similar to *Antrodia cinnamomea* T. T. Chang & W. N. Chou (Chang and Chou, 1995), which bears the vernacular name Niu-Chang-Chih and has only been collected from the endemic aromatic tree *Cinnamomum kanehirai* Hayata (Lauraceae) in Taiwan, but it has a different color on the pore surface of its basidiomata. The basidiomata of *A. cinnamomea* have medicinally been used for treatments of food and drug intoxications, diarrhea, abdominal pain, hypertension, skin itching, and cancer. Both species have a strong bitter taste, believed to indicate the presence of effective medicinal ingredients. Therefore, it is said that Shiang-Shan-Chih can substitute for Niu-Chang-Chih. In the present study, monokaryons (single basidiospore isolates) of the two species were paired to demonstrate that they have incompatible mating systems. A new name, *Antrodia salmonea*, is therefore given to the fungus associated with *Cu. konishii*.

Materials and Methods

Observation of Morphology

Descriptions of basidiomatal characters were based on fresh and dried specimens. Free-hand thin sections of basidiomata were mounted in two reagents for microscopic studies: a 3% KOH solution was used to rehydrate the thin sections, and Melzer's reagent (IKI) was used to detect amyloidity and dextrinoidity.

Cultural Studies

The methodology of Stalpers (1978) was followed for the cultural studies. The cultures were obtained from basidiomata and grown at 25°C on malt extract agar (MEA: 2% malt extract, 2% glucose, and 2% Bacto agar) and potato dextrose agar (PDA). The response to various temperatures was determined by measuring the linear growth of colonies on agar plate incubated at temperatures of 12, 16, 20, 24, 28, 32 and 36°C for 14 days. Petri dishes with an 85-mm internal diameter, which contained 20-25 ml agar media were inoculated with a piece of mycelium at the center, kept at 25°C, and macroscopically examined after 2 and 6 weeks of incubation. Separate slides prepared from various parts of the colonies were mounted in 3% KOH for observations by light microscopy.

Isolations of Monokaryons and Pairing Tests

Basidiomata from two collections of *A. cinnamomea* (TFRI B496 from Tona, Kaohsiung and TFRI B502 from Alishan, Chiai) and from one collection of *A. salmonea* (TFRI B492 from Sheng-Kuaug, Taichung) were used for isolating monokaryons. Pieces of pore surface were cut from fresh basidiomata, placed into a test tube with sterile distilled water, and mechanically stirred for 5 min to obtain a basidiospore suspension. The basidiospore suspension was spread onto modified MEA (0.1% malt extract, 2% glucose, and 2% Bacto agar) plates, and monokaryons, i.e., colonies resulting from single basidiospores, were transferred to MEA after incubation at 24°C for 7-10 days.

Pairings among the monokaryons were made by placing small pieces of mycelia (2 mm in diameter) at a distance of 1 cm on MEA plates. Dikaryosis and common B

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heterokaryosis of the paired monokaryons were confirmed by the appearance of clamp connections and pseudoclamps, respectively, under a microscope.

Results and Discussion

Antrodia cinnamomea Reconsidered

Antrodia cinnamomea has red, red-orange to light cinnamon basidiomata that have received great attention in Taiwan primarily due to their potential to cure cancer and other illnesses. This fungus is known only from *Ci. kanehirai*. The teleomorphic, anamorphic, and cultural features of *A. cinnamomea* can be found in Chang and Chou (1995). Wu et al. (1997), after studying the holotype of *Ganoderma camphoratum* M. Zang & C. H. Su (as "*comphoratum*" in the protologue), combined the epithet *camphoratum* with the genus *Antrodia* to form *A. camphorata* (M. Zang & C. H. Su). S. H. Wu, Ryvarden & T. T. Chang and synonymized *A. cinnamomea* with it. As already pointed out by Wu et al. (1997), the holotype of *G. camphoratum* is composed of two heterogeneous elements belonging to two different fungal taxa: one is a *Ganoderma* species, and one is *A. cinnamomea*. ICBN Article 9.12 (Greuter et al., 2000) gives a clear indication that *G. camphoratum* must remain attached to the part corresponding most nearly with the original description or diagnosis. The protologue of *G. camphoratum* (Zang and Su, 1990) unambiguously indicates that the *Ganoderma* element is what, in all likelihood, the naming authors intended to describe. This is further corroborated by their illustrations of echinulate basidiospores and their placement of the fungus in the genus *Ganoderma*. It should be noted that the *Ganoderma* element in the original material is entirely limited to the basidiospores attached to the surface of the *Antrodia* basidiomata. In any case, we consider that *G. camphoratum* and *A. camphorata* are *nomen confusum* and should no longer be used. Furthermore, the epithet "*camphoratum*" is misleading due to the fact that the host tree of *A. cinnamomea* is *Ci. kanehirai* rather than *Ci. camphora* (L.) Presl. *Antrodia cinnamomea* is hereby resurrected to stand as the correct name for the fungus associated with *Ci. kanehirai*.

Specimens examined. **TAIWAN.** Taipei, on unknown substrate, Nov 1978, C. H. Su (HKAS 22294, LECTOTYPE [designated here] of *Ganoderma comphoratum*), TAIWAN, Hsin-chu, on rotten trunk of *Cinnamomum kanehirai*, Oct 1992, T. T. Chang TFRI 119 (TAIF, HOLOTYPE of *Antrodia cinnamomea*).

Taxonomy and Cultural Descriptions of Antrodia salmonea

Antrodia salmonea T. T. Chang et W. N. Chou, sp. nov. (Figures 1, 2)

Etymology. *Salmonea*, the color of the pore surface.

Basidiomata perennia, resupinata, effuso-reflexa vel plus minusve triquetra, elongata vel semicircularia, interdum subpendentia et irregularia, base latis lateralibus praedita, suberosa vel lignea, sapore valde amaro; superficies glabra, concentrice zonata, sulcata, strato resinaceo flavo, luteo vel subcinnamomeo, tum subnigricanti vel fuscanti, sed saepe supra areas juniores marginales immutato praedito. Pori rotundati vel angulares, 4-7 per mm; pagina pori salmonea ubi vegeta, cremea vel bubalina ubi vetus. Contextus calceus, cremeus vel subamygdalinus. Tubi usque ad 40 mm longi, paginae pori concolores. Systema hypharum dimiticum; hyphae generativae fibulis praeditae, 2-4 μm latae; hyphae skeletales hyalinae vel subbrunneae, usque ad 4.5 μm latae, subamyloideae. Basidia clavata, 4 sterigmatibus et fibulis basali praedita, 12-15 × 3-5 μm. Basidiosporae cylindraceae, leviter flexae, 3.5-5.0 × 1.5-2 mm, hyalinae, leaves, IKI^-.

Basidiomata perennial, resupinate, effused-reflexed to more or less triquetrous, elongated to semicircular, sometimes subpendant and irregular, strongly adnate to host substrate by a broad lateral base, margin sterile, corky to woody, with strongly bitter taste; upper surface glabrous, concentrically zonated, sulcate, with a resinous layer yellow, orange yellow to light cinnamon, becoming brown or blackish, but often with color unchanged on younger marginal areas; margins obtuse, deflexed, undulate. Pores round to angular, 4-7 per mm; pore surface salmon-pink when fresh, becoming cream-colored to buff-colored when aged. Context white, cream-colored to salmon-pink; tubes

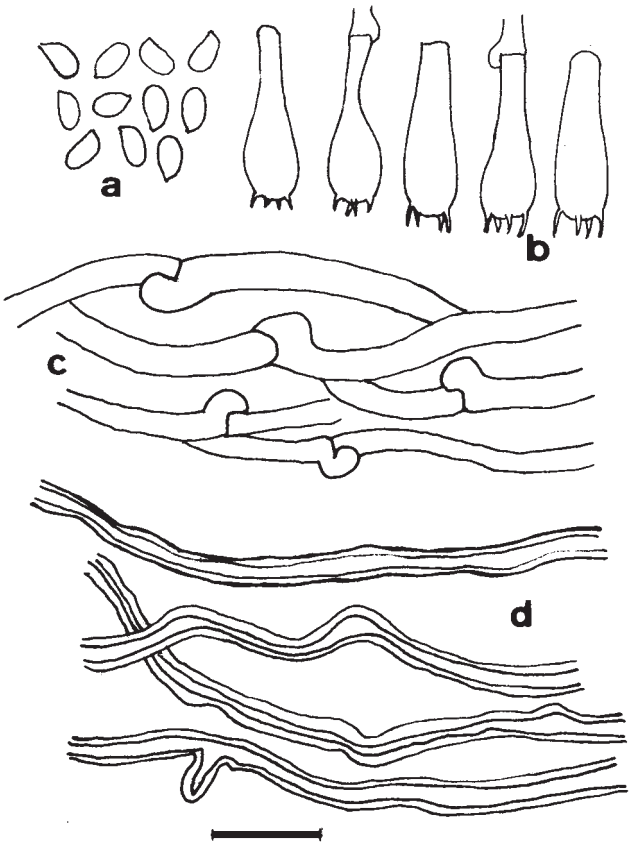

Figure 1. *Antrodia salmonea*. a, Basidiospores; b, Basidia; c, Generative hyphae; d, Skeletal hyphae. Bar = 10 μm.

Figure 2. Basidiomata of *Antrodia salmonea*.

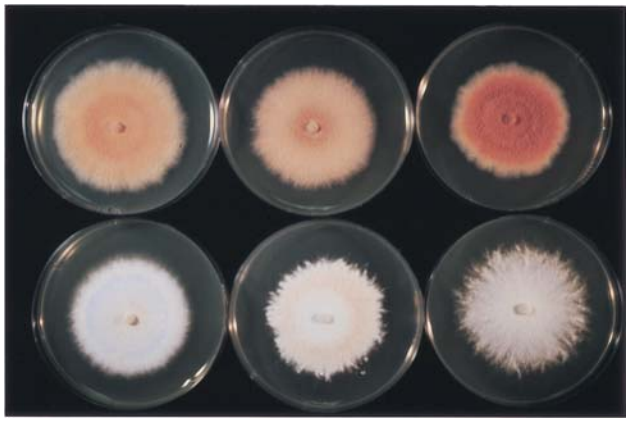

Figure 3. Colonies of *Antrodia cinnamomea* (upper) and *A. salmonea* (lower) growing on MEA plates for 3 weeks at 25°C.

up to 40 mm long, not stratified, concolorous with pore surface. Hyphal system dimitic; generative hyphae 2-4 µm wide, with clamp connections; skeletal hyphae hyaline to light yellow, up to 4.5 µm wide, weakly amyloid. Basidia clavate, 12-15 × 3-5 µm, with 4 sterigmata and a basal clamp. Basidiospores cylindrical, slightly bent, 3.5-5 × 1.5-2 µm, hyaline, smooth, IKI$^-$.

Cultures and anamorph. Colonies on PDA and MEA at 25°C growing slowly, attaining 1.2-1.6 mm/d, covering plates in 6 weeks, white, cream-colored to salmon-pink, with appressed to submerged, distant hyphae at the advancing zone, overlain with downy, cottony to woolly aerial hyphae, producing a peach-like smell; reverse uncolored. Generative hyphae with clamp connections, producing arthroconidia as well as chlamydospores. Arthroconidia rod-shaped, 3.5-9 × 2.5-3.5 µm. Chlamydospores ellipsoid to oblong, 12-18 × 4-7 µm. Specialized hyphae including only unbranched or rarely branched skeletal hyphae. On the basis of the tannic and gallic acid medium test (Davidson et al., 1938), the fungus is considered to form a brown rot, in agreement with our observation of a rotten trunk. The optimal temperature range for growth is 24-28°C, with the minimal temperature 12°C and the maximal temperature 32°C. The species code after Stalpers (1978) is as follows: 9, (11), 13, 14, (15), 17, 21, 22, 30, 31, 33, (35), 36, 39, 44, 45, 46, 52, 53, 83, 84, 85, 90.

Specimens examined. **TAIWAN.** Hsinchu County, Wufong, 2,000-2,500 m, on rotten trunk of *Cunninghamia konishii*, Apr 2002, T. T. Chang TFRI B147 (TAIF, HOLOTYPE); Taichung Country, Sheng-Kuang, 2,000-2,300 m, on rotten trunk of *Cu. konishii*, Nov 2002, T. T. Chang TFRI B492 (TAIF); Taichung County, Sheng-Kuaug, 2,000-2,300 m, on rotten trunk of *Cu. konishii*, Nov 2001, T. T. Chang TFRI 1005 (TAIF); Taichung County, Anmashan, 2200-2,500 m, on rotten trunk of *Cu. konishii*, Apr 2002, T. T. Chang TFRI 1041 (TAIF). The cultures were obtained from all the examined specimens.

Commentary. *Antrodia salmonea* is characterized by resupinate, effused-reflexed to pileate basidiomata with a salmon-pink pore surface and a strong bitter taste and by cylindrical basidiospores. *Antrodia cinnamomea* resembles *A. salmonea* but differs from the latter mainly in having a red-orange to light cinnamon pore surface (Chang and Chou, 1995). In addition, *Antrodia cinnamomea* is only from *Ci. kanehirai* whereas *A. salmonea* is with *Cu. konishii*. It should be noted that these two trees are endemic to Taiwan, thus indicating that the two *Antrodia* species are endemic as well. Although the fresh basidiomata of *A. cinnamomea* and *A. salmonea* smell like the wood of their respective hosts, when aged or dried up, they lose these odors presumably acquired from the hosts. *Antrodia salmonea* and *A. cinnamomea* have different colony colors (Figure 3), but they are otherwise similar in their cultural characteristics. It is worth noting that these two fungi produced arthroconidia and chlamydospores in culture. To our knowledge, these two structures have not been reported in the cultural studies of other *Antrodia* species (Lombard and Gilbertson, 1965; Nobles, 1965; Stalpers, 1978; Lombard, 1990).

The studies from pairing tests between *A. salmonea* and *A. cinnamomea* (see below), which revealed the incompatibility between their mating systems, reinforce our decision to treat the two fungi as distinct species.

Pairing Tests

Basidiomata from two collections of *A. cinnamomea* (TFRI B496 and TFRI B502) and from one collection of *A. salmonea* (TFRI B492) were used for isolating monokaryons, which resulted from single basidiospores. Twelve monokaryons from each collection were selected randomly and paired in all possible combinations. Results of pairings from monokaryons of TFRI B492 showed that the mating system of *A. salmonea* is heterothallic-tetrapolar, involving four mating types determined by two incompatibility factors A and B (Table 1). Similar results were obtained from pairing tests of TFRI B502 and TFRI B496 of *A. cinnamomea* (data not presented). In a heterothallic-tetrapolar mating system, only a dikaryon resulting from a pairing between two compatible monokaryons with different incompatibility factors A and B can produce clamp connections at the interaction zone. A pairing between two

Table 1. Pairings in all possible combinations between 12 monokaryons isolated from TFRI B492 of *A. salmonea*.

Isolate	2	5	10	12	1	4	7	11	8	3	6	9
2	–	–	–	–	–	–	–	–	B	+	+	+
5		–	–	–	–	–	–	–	B	+	+	+
10			–	–	–	–	–	–	B	+	+	+
12				–	–	–	–	–	B	+	+	+
1					–	–	–	–	+	B	B	B
4						–	–	–	+	B	B	B
7							–	–	+	B	B	B
11								–	+	B	B	B
8									–	–	–	–
3										–	–	–
6											–	–
9												–
Mating type	A_1B_1	A_1B_1	A_1B_1	A_1B_1	A_1B_2	A_1B_2	A_1B_2	A_1B_2	A_2B_1	A_2B_2	A_2B_2	A_2B_2

The isolate numbers are arbitrarily assigned. The "+" sign denotes the appearance of clamp connections (dikaryosis) at the interaction zone; the letter "B" denotes the appearance of pseudoclamp connections (common B heterokaryosis); and the "–" sign denotes no observed phenotypical changes.

monokaryons sharing only the incompatibility factor B (common B heterokaryosis) produces pseudoclamp connections at the interaction zone whereas one between two monokaryons either sharing only the incompatibility factor A (common A heterokaryosis) or sharing both incompatibility factors A and B does not interact phenotypically. Four monokaryons with different mating types from each of the three collections (TFRI B492, TFRI B496 and TFRI B502) were used for pairing tests to determine if the mating systems of *A. salmonea* and *A. cinnamomea* are compatible. The results showed that TFRI B496 and TFRI B502 share the same mating system, but, while an allele of the incompatibility factor B is present in both strains, no alleles of the incompatibility factor A are shared by the two collections. The genotype in TFRI B496 is herein designated as $A_1A_2B_1B_2$ and that in TFRI B502 as $A_3A_4B_2B_3$ (Table 2); B_2 is the only allele shared by the two collections. Completely incompatible pairings between TFRI B492, with the genotype designated as $A_XA_YB_XB_Y$, and either TFRI B496 or TFRI B502 were also shown in Table 2, further indicating that *A. cinnamomea* and *A. salmonea* should be considered distinct species rather than subtaxa of the same species.

Table 2. Pairings in all possible combinations between 12 monokaryons from TFRI B496 and TFRI B502 of *A. cinnamomea* and TFRI B492 of *A. salmonea*.

Isolate		TFRI B496				TFRI B502				TFRI B492			
		1	2	3	4	1	2	3	4	1	2	3	4
TFRI B496	1	–	+	–	B	+	+	+	+	–	–	–	–
	2	+	–	B	–	B	+	B	+	–	–	–	–
	3	–	B	–	+	B	+	B	+	–	–	–	–
	4	B	–	+	–	+	+	+	+	–	–	–	–
TFRI B502	1					–	+	–	B	–	–	–	–
	2					+	–	B	–	–	–	–	–
	3					–	B	–	+	–	–	–	–
	4					B	–	+	–	–	–	–	–
TFRI B492	1									–	+	–	B
	2									+	–	B	–
	3									–	B	–	+
	4									B	–	+	–
Mating type		A_1B_2	A_2B_1	A_1B_1	A_2B_2	A_3B_1	A_4B_3	A_4B_1	A_3B_3	A_XB_Y	A_YB_X	A_XB_X	A_YB_Y

The isolate numbers are arbitrarily assigned. The "+" sign denotes the appearance of clamp connections (dikaryosis) at the interaction zone; the letter "B" denotes the appearance of pseudoclamp connections (common B heterokaryosis); and the "–" sign denotes no observed phenotypical changes.

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Literature Cited

Chang, T.T. and W.N. Chou. 1995. *Antrodia cinnamomea* sp. nov. on *Cinnamomum kanehirai* in Taiwan. Mycol. Res. **99:** 756-758.

Davidson, R.W., W.A. Campbell, and D.J. Blaiadell. 1938. Differentiation of wood-decaying fungi by their reactions on gallic or tannic acid medium. J. Agric. Res. **57:** 683-695.

Greuter, W., J. Mcneill, F.R. Barrie, H.-M. Burdet, V. Demoulin, T.S. Filgueiras, D.H. Nicolson, P. C. Silva, J. E. Skog, P. Trehane, N. J. Turland, and D. L. Hawksworth. 2000. International Code of Botanical Nomenclature (St Louis Code). Regnum Vegetabile 138. Königstein: Koeltz Scientific Books.

Lombard, F.F. 1990. A cultural study of several species of *Antrodia* (Polyporaceae, Aphyllophorales). Mycologia **82:** 185-191.

Lombard, F.F. and R.L. Gilbertson. 1965. Studies on some western Porias with negative or weak oxidase reactions. Mycologia **57:** 43-76.

Nobles, M.K. 1965. Identification of cultures of wood-inhabiting Hymenomycetes. Can. J. Bot. **43:** 1097-1139.

Stalpers, J.A. 1978. Identification of wood-inhabiting Aphyllophorales in pure culture. Stud. Mycol. **16:** 1-248.

Wu, S.H., L. Ryvarden, and T.T. Chang. 1997. *Antrodia camphoratum* ("niu-chang-chih"), new combination of a medicinal fungus in Taiwan. Bot. Bull. Acad. Sin. **38:** 273-275.

Zang, M. and C.H. Su. 1990. *Ganoderma comphoratum*, a new taxon in genus *Ganoderma* from Taiwan. Acta Bot. Yunnanica **12:** 393-396. (in Chinese).

恢復 *Antrodia cinnamomea* 名字和香杉上新種 *Antrodia salmonea*

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本文陳述恢復使用 *Antrodia cinnamomea*（牛樟芝）名字的理由及依據。同時，記述及圖示生長於香杉上之多孔菌 *Antrodia salmonea*（香杉芝）為新種，並以單核體菌絲株配對，證明上述兩種不能交配。*A. cinnamomea* 和 *A. salmonea* 形態上非常相似，僅子實體顏色及寄主有差異：*A. cinnamomea* 寄生於牛樟木材，其新鮮子實體血紅至肉桂紅，而 *A. salmonea* 寄生於香杉木材，其新鮮子實體淡粉紅色至淡黃色。

關鍵詞：牛樟芝；香杉芝；新種；多孔菌；台灣。