

Taiwanofungus, a polypore new genus

Sheng-Hua Wu¹, Zhi-He Yu², Yu-Cheng Dai³, Cheng-Tau Chen¹, Ching-Hua Su⁴,
Lung-Chung Chen⁵, Wei-Che Hsu⁶, and Guang-Yuh Hwang⁶

1. Department of Botany, National Museum of Natural Science, Taichung, Taiwan 404, R.O.C. (Email: shwu@mail.nmns.edu.tw)

2. College of Biological Sciences, Yangtze University, No. 88 Jingmi Rd., Jingzhou 434025, Hubei, China

3. Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

4. Department of Microbiology and Immunology, Taipei Medical University, Taipei, Taiwan, R.O.C.

5. Department of Plant Pathology, National Chunghsing University, Taichung, Taiwan, R.O.C.

6. Department of Life Science, Tunghai University, Taichung, Taiwan, R.O.C.

(Accepted: December 3, 2004)

ABSTRACT

Taiwanofungus Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su is proposed as a new genus of lignicolous polypore, and *Ganoderma camphoratum* M. Zang & C.H. Su is designated as its generic type. New combinations for two species of *Taiwanofungus* are presented: *T. camphoratus* (M. Zang & C.H. Su) Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su, and *T. salmoneus* (T.T. Chang & W.N. Chou) Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su. *Taiwanofungus camphoratus* is known in Taiwan as “niu-chang-chih”. *Taiwanofungus* shares many common characters with *Antrodia* and *Antrodiella*. Phylogenetic analysis based on sequence data derived from LSU rDNA indicates that *Taiwanofungus camphoratus* neither clusters with *Antrodia*, nor with *Antrodiella*. *Taiwanofungus camphoratus* is known only occurring in Taiwan and on trunk of *Cinnamomum kanehirai* Hay. This fungus is well known by its medicinal uses and high economic value. *Taiwanofungus camphoratus* was first named *Ganoderma camphoratum* in 1990. *Antrodia cinnamomea* T.T. Chang & W.N. Chou was presented for the same species in 1995. *Antrodia camphorata* (Zang & Su) Sheng H. Wu, Ryvarden & T.T. Chang, new combination proposed in 1997 based on *Ganoderma camphoratum*, has been widely applied to this fungus.

Key words: *Antrodia camphorata*, *A. cinnamomea*, *Ganoderma camphoratum*, new genus, polypore, Taiwan, *Taiwanofungus camphoratus*.

Introduction

Taiwanofungus camphoratus is one of the most valued polypore fungi used for medicinal purposes. It has been famous in Taiwan for several decades, and is said to have several medicinal uses including a curative effect on cancer (Wu *et al.*, 1997). This polypore has been known in Taiwan as “niu-chang-chih” (牛樟芝), or simply as “chang-chih” (樟芝). *Taiwano-*

fungus camphoratus has resupinate-reflexed, yellowish orange to brownish orange fruiting body, which is very bitter in taste. This fungus specifically occurs on a Taiwan endemic tree, *Cinnamomum kanehirai* Hay. This native host species is becoming scarce, leading to the difficulty in finding “niu-chang-chih” in the field. “Niu-chang-chih” is very expensive in Taiwan, due to host specificity and scarcity in nature, and the difficulty of artificial cultivation for

getting its fruiting body.

Zang and Su (1990) firstly published this species using the name *G. comphoratum* M. Zang & C.H. Su. The junior author (C.H. Su) of *G. comphoratum*, was sometimes inquired by Taiwanese mycologists about reality of the type specimen, after its presentation. He replied that it represents a good specimen of "Niu-chang-chih". He thought the wrong placement of its generic placement was probably due to observation of contamination of some foreign "*Ganoderma* spores" adhering to the type specimen (Wu *et al.*, 1997). It is noted here that the illustration of the so-called "*Ganoderma* spores" shown in the protologue (Zang and Su, 1990), in reality do not fit that of *Ganoderma*. Wu *et al.* (1997) studied type specimen of *G. comphoratum*, confirmed that the fruiting body, bitter taste, hyphal elements, basidia and basidiospores all represent that of "niu-chang-chih", while neither found any *Ganoderma* basidiospores, nor any kind of spores like those in illustration of Zang and Su (1990). It means that the foreign "*Ganoderma* spores", if present on type of *G. comphoratum*, should be scarce. Chang and Chou (1995) published new species, *Antrodia cinnamomea* T.T. Chang & W.N. Chou, also based on "niu-chang-chih". Wu *et al.* (1997) studied type specimens of *G. comphoratum* and *A. cinnamomea*, confirmed both are conspecific, and proposed new combination: *Antrodia camphorata* (M. Zang & C.H. Su) Sheng H. Wu, Ryvariden & T.T. Chang, based on *G. comphoratum*. After 1997, *A. camphorata* has been widely adopted for naming this species. Morphological and molecular features of this study indicate that "niu-chang-chih" represent a genus of its own, accordingly new genus (*Taiwanofungus*) and new combina-

tions (*T. camphoratus* and *T. salmoneus*) are proposed in this paper.

Taxonomy

Taiwanofungus Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su, gen. nov.

Type species. *Ganoderma comphoratum* M. Zang & C.H. Su, Acta Bot. Yunnanica 12: 395. 1990.

Basidiocarpus perenni, resupinato-reflexus vel pileatus, amarus; superficies hymenialis porosa. Systema hypharum dimiticum vel trimiticum; hyphae generativae fibulis praeditae; hyphae skeletales leviter IKI+. Cystidia vulgo destituta. Basidia clavata, 4 sterigmatibus. Basidiosporae cylindriceae, leaves, tenuitunicatae, IKI–, CB–.

Etymology. from Taiwan + fungus, referring to the place the fungal taxon was found.

Basidiocarp perennial, resupinate-reflexed to pileate, taste bitter, woody. Hymenial surface poroid, yellowish, orange red or orange brown. Hyphal system dimitic to trimitic. Generative hyphae nodose-septate. Skeletal hyphae slightly IKI+. Basidia clavate, 4-sterigmate. Basidiospores cylindrical, smooth, thin-walled, IKI–, CB–. Arthroconidia and chlamydospores present in living culture.

Morphological characteristics such as the resupinate-reflexed to pileate basidiocarp, dimitic-trimitic hyphal system with nodose-septate generative hyphae and occasionally branched skeletal hyphae, clavate basidia, and smooth, thin-walled, IKI–, CB– cylindrical basidiospores, mostly correspond with *Antrodia* and *Antrodiella* among polypore genera. *Antrodia* is a brown-rotter while *Antrodiella* is a white rotter. *Taiwanofungus camphoratus* was

treated in *Antrodia* twice (Chang and Chou, 1995; Wu *et al.*, 1997), as its fruiting bodies were often associated with brown-rot wood, and test of living mycelium grown on tannic and gallic acid medium were indicative of negative (Chang and Chou, 1995; Chang *et al.*, 1997, Wu *et al.*, 2000). As this fungus has also been occurring on white-rot wood, there is no consensus about its rotten type. Further studies are required to clarify its rotten type. Generative hyphae are fairly abundant in context of *T. camphoratus*, while skeletal are dominant in *Antrodia*. *Taiwanofungus camphoratus* has slightly amyloid skeletal hyphae, which are not present in *Antrodiella* spp. In addition, both arthroconidia and chlamydospores are present in culture of *Taiwanofungus*, but not found in *Antrodia* and *Antrodiella*.

New combination for the type species of this new genus is also presented as below.

Taiwanofungus camphoratus (M. Zang & C.H. Su) Sheng H. Wu, Z.H. Yu, Y.C. Dai & Su, comb. nov. (Figs. 1 and 2)

Basionym: *Ganoderma comphoratum* M. Zang & C.H. Su, Acta Bot. Yunnanica 12: 395. 1990.

Antrodia camphorata (M. Zang & C.H. Su) Sheng H. Wu, Ryvarden & T.T. Chang, Bot. Bull. Acad. Sin. 38: 273. 1997.

Antrodia cinnamomea T.T. Chang & W.N. Chou, Mycol. Res. 99: 756. 1995.

Specimens examined. Taiwan. Taipei, on trunk of *Cinnamomum kanehirai*, 1978 (HKAS 22294, holotype of *Ganoderma comphoratum*). Hsinchu, on trunk of *Cinnamomum kanehirai*, 1992, TFR1 119 (TAIF, holotype of *Antrodia cinnamomea*).

Chang and Chou (2004) recently published a new species, *Antrodia salmonea*, very allied to

T. camphoratus. All diagnostic features of *A. salmonea*, such as bitter taste of fruiting body, slightly amyloid skeletal, and presence of both arthroconidia and chlamydospores in culture, correspond with that of *Taiwanofungus*. New combination for this species is thus proposed as following.

Taiwanofungus salmoneus (T.T. Chang & W.N. Chou) Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su, comb. nov.

Basionym: *Antrodia salmonea* T.T. Chang & W.N. Chou, Bot. Bull. Acad. Sin. 45: 347–352. 2004.

Molecular analysis to elucidate taxonomic placement of *Taiwanofungus camphoratus*

Materials and methods

Phylogenetic analysis of this study was based on sequence data derived from LSU rDNA. Totally 26 fungal strains used in this study, as well as their origin and accession numbers, are pro-



Fig. 1. Basidiocarp of holotype of *Taiwanofungus camphoratus* (*Ganoderma comphoratum*). Image provided by Dr. Z. L. Yang.

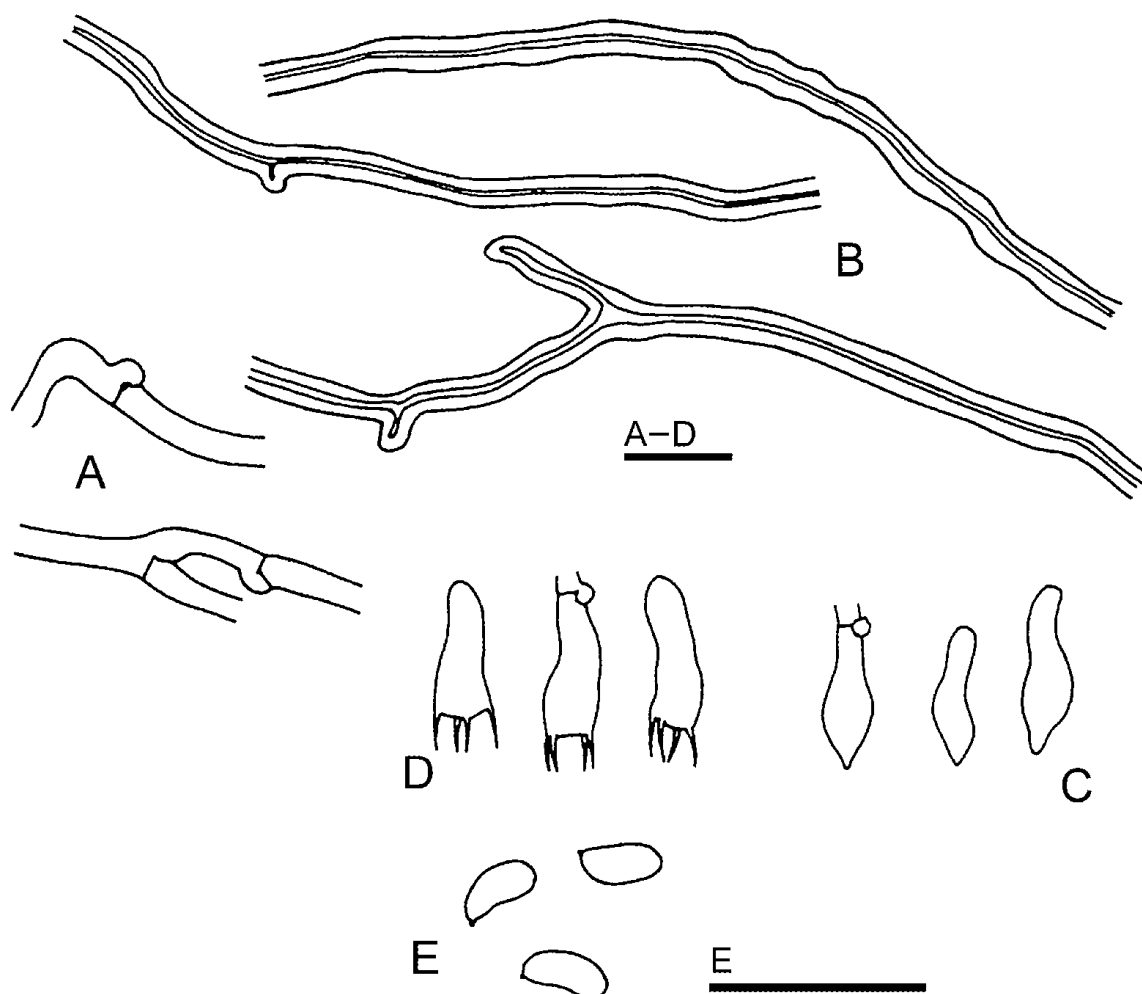


Fig. 2. Microscopic features of holotype of *Taiwanofungus camphoratus* (*Ganoderma camphoratum*). A. Contextual generative hyphae. B. Contextual skeletal hyphae. C. Cystidioles. D. Basidia. E. Basidiospores. Scale bars = 10 μ m.

vided in Table 1. Sequence data of twenty-one isolates are newly derived from this study, and the other five were from GenBank.

Mycelia were transferred from agar cultures to 100 mL liquid medium (2% Malt extract) and incubated on a rotary shaker (160 rpm) for 2–3 weeks at RT. DNA was isolated from freeze-dried or fresh cultured mycelia using Plant Genomic DNA Extraction Miniprep System (Viogene, Taiwan) according to the manufacturer's instructions. Approximately 980bp nucleotides of the 5' end of the larger subunit of the rDNA were amplified with a pair of primers: LROR and LR5 (Moncalvo *et al.*,

2000). Vio Taq polymerase (Protech, Taiwan) was used for PCR reactions and PCR condition was according to the protocol (Viogene, Taiwan). PCR products were purified with PCR-M Clean Up (Viogene, Taiwan) and both strand sequences were determined using the ABI PRISM *BigDye* Terminator Cycle Sequencing Ready Reaction kit on ABI 3730 DNA sequencer (Perkin Elmer, Applied Biosystems).

Alignments were performed using Clustal W program and adjusted by hand in Genedoc 2.5.000 (Nicholas *et al.*, 1997). Sequences were deposited to Genbank (Accession No AY333810-AY333846, AY826982-AY826984)

Table 1. Species, strains, and Genbank accession numbers used in this study.

Species	Strains	Accession No.
<i>Abortiporus biennis</i> *	FO37378	AJ406527
<i>Agaricus bisporus</i> *	SAR88/411	U11911
<i>Antrodia albida</i>	FCUG1100	AY333846
<i>Antrodia albida</i>	FCUG1396	AY333845
<i>Antrodia juniperina</i>	FP97452T	AY333839
<i>Antrodia juniperina</i>	WM284T	AY333838
<i>Antrodia malicola</i>	BCRC35452	AY333837
<i>Antrodia malicola</i>	MJL1167SP	AY333835
<i>Antrodia variiformis</i>	FP89848R	AY333828
<i>Antrodia variiformis</i>	FP90100SP	AY333827
<i>Antrodiella americana</i>	HHB4100SP	AY333825
<i>Antrodiella americana</i>	L3468R	AY333824
<i>Antrodiella romellii</i>	FP100215SP	AY333822
<i>Antrodiella romellii</i>	L9443SP	AY333821
<i>Antrodiella semisupina</i>	FCUG1216	AY333820
<i>Antrodiella semisupina</i>	FCUG960	AY333819
<i>Diplomitoporus lindbladii</i>	HHB5629-SP	AY333814
<i>Fomitopsis pinicola</i>	FP105760T	AY333811
<i>Fomitopsis pinicola</i>	FP98533T	AY333810
<i>Ganoderma applanatum</i> *	GEL4206	AJ406526
<i>Gloeoporus taxicola</i> *	KEW213	AF287861
<i>Grifola frondosa</i>	ACCC50289	AY826982
<i>Laetiporus sulphureus</i> *	DSH93-194	AF287870
<i>Trametes suaveolens</i>	FP-102529-T	AY826984
<i>Taiwanofungus camphoratus</i>	BCRC35398	AY333843
<i>Taiwanofungus camphoratus</i>	CWN01385	AY333841

* Sequences obtained from Genbank.

Initials of BCRC are cultures from Bioresources Collection and Research Center, ROC (Taiwan). Initials of FCUG are cultures from Systematic Botany & Plant Ecology, Göteborg University, Sweden. Initial of CWN is culture from National Museum of Natural Science, R.O.C. (Taiwan). Initials of others are cultures from Center for Forest Mycology Research, United States Department of Agriculture, U.S.A.

and the aligned data matrix has been deposited to Treebase (Accession No S1216). Phylogenetic analysis was performed by the maximum-

parsimony method using the heuristic search algorithm of the Phylogeny Analysis Using Parsimony (PAUP*) program version 4.0b10 (Swofford, 2002) on Macintosh computer. Heuristic searches with 1000 random taxa stepwise addition sequences, TBR branch swapping and MAXTREES set to autoincrease, were performed. All transformations were considered unordered and equally weighted, with gaps treated as missing data. *Agaricus bisporus* was used as outgroup for rooting purpose. Relative robustness of clades was assessed by the bootstrap method using 1000 heuristic search replicates with 100 random taxa stepwise addition sequences and TBR branch swapping with MAXTREES set to autoincrease.

Results

Amplification of the 28S ribosomal DNA yielded fragment of approximately 980 bp. Missing data at the 5' and 3' end of the aligned sequences were removed from the analyses. Of the remaining 896 characters, 674 characters were constant, 85 variable characters were parsimony uninformative, and 137 were parsimony informative. The phylogenetic analysis with maximum parsimony resulted in 8 most parsimonious trees in two islands, each with a length of 416 steps. (consistency index, CI = 0.6202; retention index, RI = 0.7168). The 50% majority consensus of the 8 most parsimonious trees is shown in Fig. 3.

Two monophyletic groups are distinct in the phylogenetic tree (Fig. 1). A monophyletic *Antrodia* group consists of four studied species of *Antrodia* (*A. albida* (generic type), *A. juniperina*, *A. malicola* and *A. variiformis*) and *Fomitopsis pinicola*. They are all brown rot fungi, the node is shown a high level of boot-

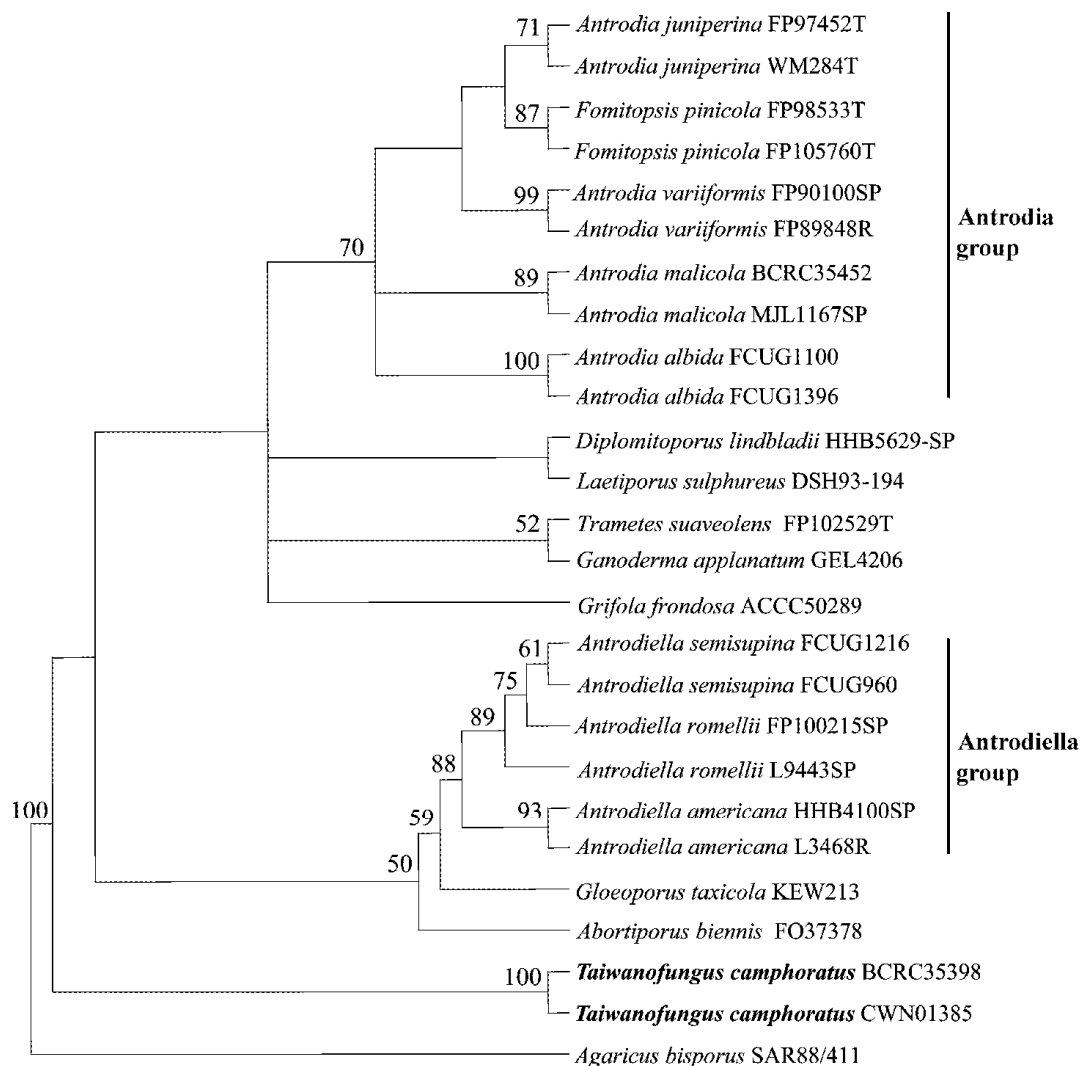


Fig. 3. Phylogram as obtained from MP analysis of all complete LROR-LR5 sequences. 50% Majority Consensus of 8 most-parsimonious trees (tree length = 416, CI = 0.6202, RI = 0.7168). *Agaricus bisporus* was used as an outgroup taxon to root the tree. Bootstrap percentages higher than 50% based on 1000 replications are shown on branches.

strap support (70%), and this group is supported. The second distinct monophyletic group encompasses three studied species of *Antrodiella* (*A. americana*, *A. romellii*, and *A. semisupina* (generic type)). This *Antrodiella* group is supported by bootstrap values of 88%. Evidence from this phylogenetic analysis does not show any close relationship between *Antrodiella* and *Antrodia*, though both genera bearing most similar morphological characteristics. Two strains of *Taiwanofungus camphoratus* neither cluster with *Antrodia* group, nor with

Antrodiella group, instead are shown to be distantly related with these two monophyletic groups.

Discussion

Among polypore genera, gross morphological features of *Taiwanofungus camphoratus* are most likely indicative to be *Antrodia* or *Antrodiella*. *Taiwanofungus* differs from *Antrodia* in having fairly abundant generative hyphae in context, and from *Antrodiella* by having

slightly amyloid skeletal hyphae and different rotten type. Moreover, this new genus is further distinguished from *Antrodia* and *Antrodiella* by having both arthroconidia and chlamydospores in culture. Phylogenetic analysis based on sequence data derived from LSU rDNA indicates that *Taiwanofungus* is neither allied to *Antrodia*, nor to *Antrodiella*. Molecular evidence shows that morphological resemblance among *Taiwanofungus*, *Antrodia* and *Antrodiella*, are merely superficial. Consequently, *Taiwanofungus* is proposed as a new genus of its own in this paper.

Acknowledgments

The authors are indebted to Dr. Chien-Hsien Kuo, for improving the manuscript. The authors are grateful to Prof. Wen-Hsui Hsieh and Prof. Chin-Hsiung Hung for reviewing the paper.

References

- Chang, T.T., W.H. Chiu, and J. Hua. 1997. The cultural atlas of wood-inhabiting Aphyllophorales in Taiwan (volume 2). Mycol. Monog. 11: 1–118.
- Chang, T.T. and W.N. Chou. 1995. *Antrodia cinnamomea* sp. nov. on *Cinnamomum kanehirai* in Taiwan. Mycol. Res. 99: 756–758.
- Chang, T.T. and W.N. Chou. 2004. *Antrodia cinnamomea* reconsidered and *A. salmonea* sp. nov. on *Cunninghamia konishii* in Taiwan. Bot. Bull. Acad. Sin. 45: 347–352.
- Moncalvo J.M., F.M. Lutzoni, S.A. Rehner, J. Johnson, and R. Vilgalys. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Syst. Bio. 49: 278–305.
- Nicholas K.B., Nicholas H.B. Jr, D.W. II. Deerfield. 1997. GeneDoc: analysis and visualization of genetic variation. EMBNEW.NEWS 4: 14.
- Swofford D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods) Version 4. Sinauer Associates, Sunderland, Massachusetts.
- Wu, S.H., W.N. Chou, Y.Z. Wang, and B.C. Wang. 2000. The cultural atlas of potentially edible and medicinal fungi in Taiwan. Mycol. Monog. 14: 1–139.
- Wu, S.H., L. Ryvarden, and T.T. Chang. 1997. *Antrodia camphorata* (“niu-chang-chih”), new combination of a medicinal fungus in Taiwan. Bot. Bull. Acad. Sin. 38: 273–275.
- Zang, M. and Q. Su. 1990. *Ganoderma camphoratum*, a new taxon in genus *Ganoderma* from Taiwan, China. Acta Bot. Yunnanica 12: 395–396.

多孔菌新屬，台芝屬 (*Taiwanofungus*)

吳聲華¹ 余知和² 戴玉成³ 陳成桃¹ 蘇慶華⁴ 陳隆鐘⁵ 徐唯哲⁶ 黃光裕⁶

1. 國立自然科學博物館植物學組，臺中
2. 長江大學生命科學院，荊州
3. 中科院瀋陽應用生態研究所，瀋陽
4. 臺北醫學大學醫學系微生物免疫學科，臺北
5. 國立中興大學植物病理學系，臺中
6. 東海大學生命科學系，臺中

摘 要

本文提出多孔菌新屬：台芝屬 (*Taiwanofungus*)，並指定 *Ganoderma comphoratum* 爲其模式種；另提出台芝屬兩新組合名：*Taiwanofungus camphoratus* (M. Zang & C.H. Su) Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su 以及 *T. salmoneus* (T.T. Chang & W.N. Chou) Sheng H. Wu, Z.H. Yu, Y.C. Dai & C.H. Su。 *Taiwanofungus camphoratus* 在臺灣被稱爲「牛樟芝」。台芝屬的形態特徵接近 *Antrodia* 及 *Antrodiella*。由 LSU rDNA 序列分析結果，顯示 *Taiwanofungus camphoratus* 與 *Antrodia* 和 *Antrodiella* 的親緣性並不接近。牛樟芝僅知於臺灣，且只生長於牛樟樹幹，因具醫療功效而著名並享有高經濟價值。牛樟芝以 *Ganoderma comphoratum* 之名首次發表於 1990 年，1995 年以 *Antrodia cinnamomea* 之名再次被發表。1997 年以 *G. comphoratum* 爲原名 (basionym) 而提出新組合名：*Antrodia camphorata*，此名自提出後曾廣爲學界及產業界採用。

關鍵詞：牛樟芝、多孔菌、新屬、臺灣。