Phylogeny of *Tremella* and cultiviation of *T. fuciformis* in Taiwan

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Abstract: *Tremella fuciformis* is a jelly fungus, so called Silver Ear, and very common in the world. It has been used as medicine in China, and is also recognized as natural food in Taiwan. Because of its values in medicine and economy, it is worth understanding its phylogeny and cultivation. Phylogenic grouping can be achieved by studying fungal morphology and the large submit ribosomal DNA sequences. Comparing morphological characters and molecular phylogenies, *Tremella* species can be divided into five phylogenetic groups, i.e. Aurantia, Foliacea, Fuciformis, Indecorata, and Mesenterica group. The novel technical cultivation of Silver Ear uses two isolates, *T. fuciformis* and its host *Annulohypoxylon archeri* (=*Hypoxylon archeri*), to obtain a rich yield.

1. Introduction

The fungal flora of Taiwan has been investigated since 1904, has been studied increasingly up to 2013, and is now estimated to comprise some 1,276 genera with 5,396 species, including subspecies and synonyms. The number of Taiwanese species accounts for 8.3 % of the the global record. The fungal diversity plays an important role in the study of fungal evolution, cultivation and even bio-resources for further application. The phylogeny in the genus *Tremella* and the novel cultivation of silver ear mushroom in Taiwan, *T. fuciformis*, are discussed in this paper.

Since the planning of a nation-wide research project of "Fungal Flora of Taiwan", which is supported by the National Science Council of Taiwan since 1983, the floristic survey of Heterobasidiomycetes has been neglected and very little progress was made owing to the absence of qualified taxonomic specialists of this fungal group in the country. Although fungi are common ingredients for Asian food (e.g. *T. fuciformis, Lentinula edodes, Flammulina velutipes*), scientific research on their agriculture began quite late. Because of the scanty literature on tropical fungi in Asia, the process of the inventory is slow. Therefore, it was desirable to begin with a monograph of a genus as a basic contribution to Taiwanese mycological research.

This study aims to investigate *Tremella* and related *Sirobasidium* species including type specimens. Additionally, other important herbarium material had be studied to understand the morphological differences which fructifications show in natural habitats. Investigating the association with hosts in symbiotic, parasitic and saprobic interactions will provide essential information for adequate culturing conditions. While the association with other fungi is common among *Tremella* species, it is not clear whether it is mycoparasitism in each case (Bandoni 1987). Mycoparasitism of *Tremella* on Corticiaceae

and/or Stereaceae are partially well studied, however, field observations of interactions of *Tremella* with Ascomycetes have yet not been emphasized.

The genus *Tremella* has been treated to some degree by Looney (1933), Bandoni (1957) and Donk (1966). Members of the genus *Tremella* (type: *T. mesenterica* Retz.: Fr.) have the largest and quite heterogeneous basidiocarps in the family Tremellaceae and comprise allegedly mycoparasitic species growing on the hymenium of Aphyllophorales, in basidiocarps of Dacrymycetales, on perithecia and/or stromata of Ascomycetes, as well as on lichens. A description of *Tremella* Pers. sensu lato is: jelly-like basidiocarps, soft to firm-gelatinous when moist, tremulous, pulvinate or cerebriform to foliose or lobed, flat or horny when dry; basidia globose to ovoid or pyriform to capitate or clavate, longitudinally and/or obliquely cruciate-septate; sterigmata cylindrical, with or without swollen apex; basidiospores subglobose to oval, germinating by budding or repetition, occasionally by germ tubes; generally growing on dead wood, associated with or without other fungi. In addition, tropical species of *Tremella* predictably include numerous undescribed species (Bandoni 1995). Within the Tremellales four families are described, and many of the genera are monotypic:

Tremellales Rea 1922
- Tremellaceae Fries 1822
  - *Bulleromyces* Boekhout & Fonseca 1991 (1 sp.)
  - *Holtermannia* Sacc. & Trav. 1910 (5 spp.)
  - *Sirotrema* Bandoni 1986 (3 spp.)
  - *Tremella* Pers. 1794 (valid names >80 spp.)
  - *Trimorphomyces* Bandoni & Oberwinkler 1983 (1 sp.)
  - *Xenolachne* Rogers 1947 (2 spp.)
- Sirobasidiaceae Möller 1895
  - *Sirobasidium* Lagerh. & Pat. 1892 (8 spp.)
  - *Fibulobasidium* Bandoni 1979 (1 sp.)
- Tetragoniomycetaceae Oberwinkler & Bandoni 1981
  - *Tetragoniomyces* Oberwinkler & Bandoni 1981 (1 sp.)
- Phragmoxenidiaceae Oberwinkler 1990
  - *Phragmoxenidium* Oberwinkler (1 sp.)

The genus *Sirobasidium*, with basidia in chains, was erected by Patouillard and Lagerheim in 1892, comprising *S. albidum* and *S. sanguineum*. Since then only eight species were published.

An important part of the life history in *Tremella* is a yeast stage. Basidia bear haploid basidiospores which germinate by budding or repetition in vitro. On the surface of fresh basidiocarps in nature, the secondary spores may also

germinate by budding or repetition (Bandoni 1971, 1987). Compatible cells produce conjugation tubes in response to pheromones (Bölker & Kahmann 1993, Boekhout et al. 1991, Hirata et al. 1980, Miyakawa et al. 1984, 1985a, 1985b, Okuda et al. 1981) and, after conjugation, the dikaryotic phase is initiated. Cells which fail to conjugate may revert to budding (Bandoni 1965, 1987). The pheromones of *T. mesenterica* were isolated and identified as decapeptides (Sakagami et al. 1981). Pheromones of the closely related species *T. brasiliensis* (Möller) Lloyd were identified by Ishibashi et al. (1984) and were found to be similar, namely tremerogens (Bölker &Kahmann 1993). Dikaryotic conidial development often precedes or accompanies basidial development, e.g. in *T. mesenterica*. It is also possible that some of the conidia, developing in basidiocarps are haploid, e.g. in *T. vasifera* C.-J. Chen (1998).

In earlier studies, biochemistry and physiology were often used for comparing related species. Conjugation was first shown to be regulated by complementary pheromones secreted by the A1 and A2 strains of *T. mesenterica* (Bandoni 1965). Nakase and Komagata (1971) reported that G+C content of the DNA of *Tremella* species fell within the range of the genus *Cryptococcus*. Based on similar long-chain fatty acid compositions, Smit et al. (1988) endorsed a possible close relationship between *C. laurentii* and *T. encephala*.

Since Dillenius (1741) created the genus *Tremella*, many mycologists have contributed to its taxonomy, however, it is still not settled since most of the type specimens are lost and the original descriptions and illustrations of numerous taxa are obscure and confusing. Most of the taxa were not provided with descriptions of the hymenium and subhymenium, inner parts of the basidiocarps, sterile surfaces close to the substrate, as well as of conidial stages. Basidia and spore measurements alone cannot provide enough information for the identification of species. Moreover, redundant and inconsistent descriptions and confused terminology impede systematic conclusions.

2. Materials and methods

2.1. Morphology and phylogeny

2.1.1. Morphology observation

Zeiss Lab16 light microscope with phase optics was used for illustrations. Most of illustrations were scaled with 10 μm, basidiospores with 5 μm, in double magnification.

2.2. DNA sequencing

2.2.1. DNA preparation

According to differences of fresh cultures and dry specimens, two distinct methods were performed. The Chelex method modified from Singer-Sam et al. (1989) and Walsh et al. (1991) was typically used for DNA extraction of cultures. Because it supported a very quick, convenient and efficient procedure, all genomic DNA from cultures was obtained under this method. However, the dry material was not adapted to be used by the Chelex method. The liquid nitrogen method, therefore, following the procedures described by Edwards et al. (1991) and Henrion et al. (1992) with modifications was used for DNA extraction of herbarium material. The primers NL1/NL4 (O'Donnell 1993, Boekhout et al. 1995) in pairs were selected for performing the polymerase chain reaction (PCR) (Mullis & Faloona 1987, White et al. 1990) to amplify the 5' end region of the large subunits (LSU).

2.2.2. Cyclic and autofluorence sequencing

The Perkin Elmer ABI PRISM™ Dye Terminator Cycle Sequencing Kit and autofluorence sequencer ABI 373A for sequencing the cyclic PCR product were used following the dideoxynucleotide chain termination method (Sanger et al. 1977).

2.2.3. Neighbor Joining alignment

DNA sequences were corrected by using SeqEd (version 1.0.3, Applied Biosystems), and initially aligned using the PHYLIP (Felsenstein 1993). Phylogenetic analysis according to Neighbor Joining method (Saitou & Nei 1987, Li & Graur 1991) was performed with the DNAdist and NEIGHBOR modules of PHYLIP version 3.51c (Felsenstein 1993). Bootstrap analysis (Felsenstein 1985) with 100 alignment replicates was applied to the Neighbor Joining method by using SeqBoot and CONSENSE from PHYLIP.

3. Cultivation of *Tremella fuciformis*

3.1. Mixed cultures

Because *T. fuciformis* is a mycoparasite on *Annulohypoxylon archeri* (Berk.) Y.M. Ju, J.D. Rogers and H.M. Hsieh (Hsieh et al. 2005), adequate mother spawn requires both, host and parasite. They were cultured together in the same tube or petri dish medium, usually potato dextrose agar, in a so called "dual culture" method to observe the mycelial interaction.

A mixture of agar or liquid mother spawn usually can be used to inoculate the sawdust bran substrate with 60–65 % moisture content in bottles at 22–24 ℃ until the formation of primordia for primary spawn. The mixed culture age influences the yield of silver ear mushroom; therefore the period of day 6,10,14 and 18 after inoculation in substrate used for spawn has been used.

3.2. Temperature for primordia

In order to find out the optimum temperature for best primordia growth, 18, 20, 22, 24 and 26 °C have been applied. To insure a proper combination of *Tremella* and *Annulohypoxylon*, the culture has been crumbled and mixed thoroughly before spawning.

3.3. Substrate and fructification

In general, sawdust and rice bran (or wheat bran) have been used as substrates to cultivate *T. fuciformis* in Taiwan although cottonseed hull has been reported to get higher yields in China. The substrate formulation was softwood sawdust : rice or wheat bran : gypsum or lime : sucrose = 78:20:1:1, water content approximately 60 %. Fill Substrate into the polypropylene and polyethylene (PP) bottles, sterilize at 121℃ for 4 hours, cool down in a cold room over night until the temperature of the bottle center is lower then 28℃, take a small portion of mixed spawn, inoculate at 22–24℃ until the mycelium fills up the substrate, then move the bottle to the fruiting room at optimum temperature for fructification.

4. Results and discussion

4.1. Morphology

4.1.1. Morphology

Because morphological characters are insufficient, additional biochemical (Slodki et al. 1966), physiological (Burt & Cazin 1976, 1982), and molecular markers (Hanson & Wells 1991) might be helpful for understanding the phylogenetic relationships in *Tremella*. So far, few molecular data from *Tremella* species have been published. Only some 5.8S and 18S ribosomal sequences were used for comparison with data from other taxa in the Tremellales or for estimating the systematic position of the Tremellales within basidiomycetes (e.g. Blanz & Unseld 1987, Jung et al. 1995, Kwon-Chung et al. 1995, Mitchell et al. 1992, Swann & Taylor 1993, Walker & Doolittle 1982). Therefore, additional phylogenetic studies, using detailed morphological and molecular data, are necessary for analyzing the relationships of *Tremella* species.

Based on micromorphology, eleven new *Tremella* taxa were proposed, including nine species collected in Taiwan and two in Germany, namely *Tremella giraffa* C.-J. Chen, *T. cerebriformis* C.-J. Chen, *T. flava* C.-J. Chen, *T. fuscosuccinea* C.-J. Chen, *T. griseobrunnea* C.-J. Chen, *T. neofoliacea* C.-J. Chen, *T. nivalis* C.-J. Chen, *T. resupinata* C.-J. Chen, *T. taiwanensis* C.-J. Chen, *T. tropica* C.-J. Chen and *T. vasifera* C.-J. Chen (Chen 1998). In addition, fourteen *Tremella* and one *Sirobasidium* species were studied morphologically in detail (Chen 1998).

4.1.2. Description and illustrations of *Tremella fuciformis*

Tremella fuciformis Berkeley 1856 (Figs. 1–8)
Jour. Bot. & Kew Misc. 8: 277.
=*Nakaiomyces nipponicus* Y. Kobayasi 1939

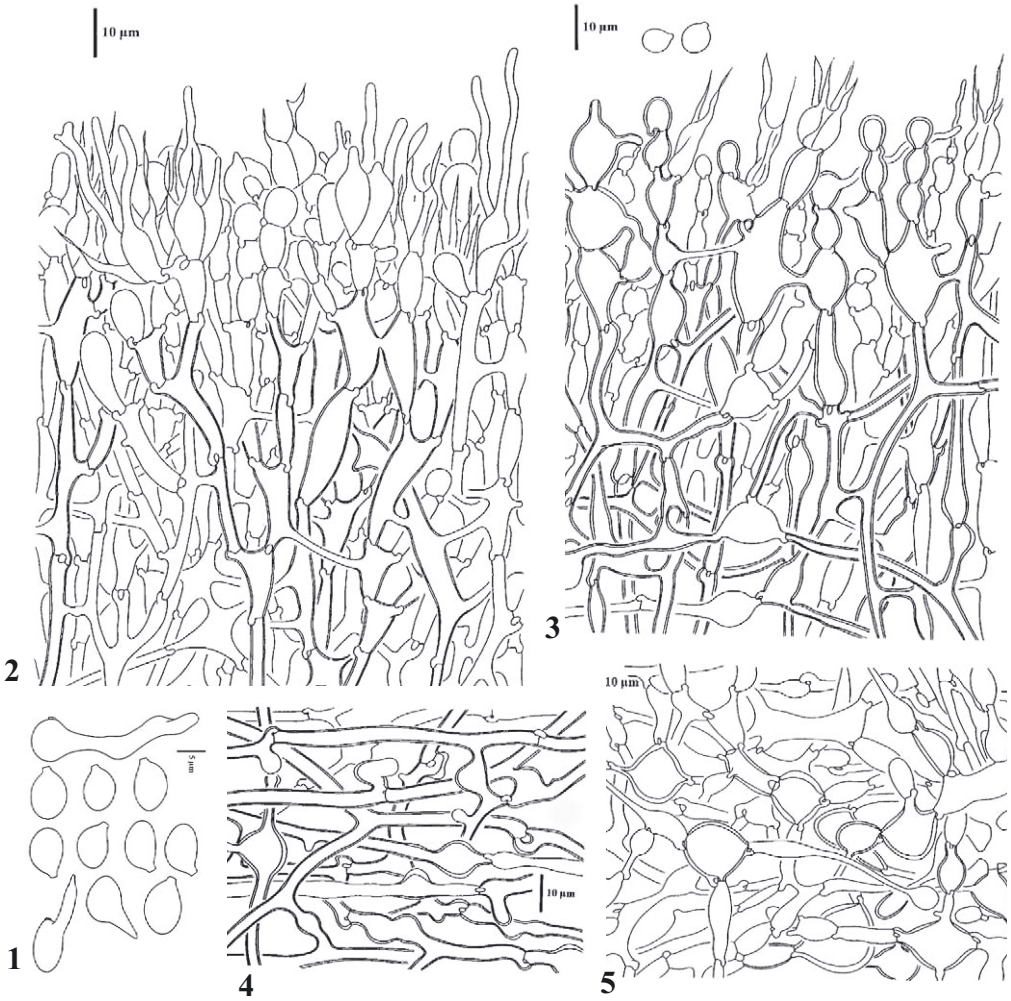
Macromorphology

Basidiocarps foliose, the lobes caespitose, lobed or forked, or with margins incised or crenate or sometimes entire, crisped, undulate, firm-gelatinous, white, up to 5 cm ø and 3 cm high, dry becoming pale whitish yellow; lobes usually very thin, generally associated with *Hypoxylon* sp. or *Annulohypoxylon* sp.

Micromorphology

Basidia: probasidia initials typically clavate, mostly proliferation from swollen and short hyphae, sometimes through the basidial clamps; mature basidia predominantly subglobose to ellipsoid, some globose or narrow clavate, (9–)11–13(–17) × (7–)8–10(–11) µm [Q=(1.00–)1.20–1.86(–2.29)], longitudinally, obliquely or diagonally cruciate-septate, mostly 4-, occasionally 2- or 3-spored; sterigma up to 50 × 2 µm, apically swollen up to 4–5 µm.

Spores: broadly ellipsoid to ellipsoid, 7–8(–9) × (4–)5–6(–7) µm [Q=(1.14–)1.27–1.55(–1.70)], smooth, hyaline, germinating by budding, repetition or sometimes germ tubes.



Figs. 1–5: from wild specimens: Fig. 1: Basidiospores, three of them germinating with germ tubes. Fig. 2**:** Part of hymenium with basidia of different developmental stages, thin- and thick-walled hyphae with clamps and anastomoses. Fig. 3: Part of hymenium of basal part of a mature basidiocarp close to the substrate with collapsed basidia, catenulate cells, thin- and thick-walled hyphae with clamps, anastomoses, as well as two basidiospores. Fig. 4: Structure of the inner part of basidiocarp. Note that hyphae are swollen partly, wrinkled and thick-walled. Fig. 5: Structure in the inner part of the basidiocarp close to the substrate. Note that many swollen cells conjugate with neighboring cells.

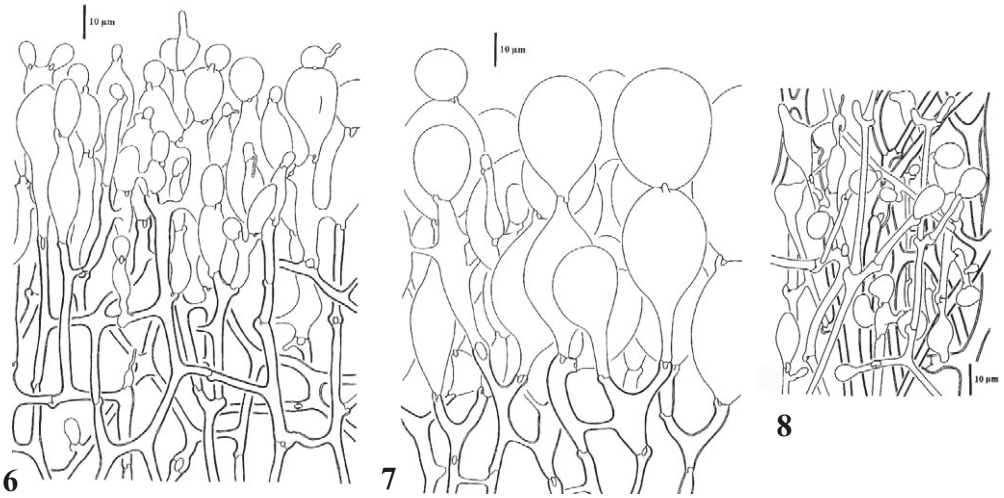
Conidia: absent, but some clamped and catenulate cells looking like conidia, globose to broadly ellipsoid, mostly 8–11 × 6–10 µm [Q=1.0–1.23(–1.40)], abundant in basal parts close to the substrate, occasionally appearing in the hymenium on the surface of basidiocarps.

Vesicles: absent.

Swollen cells: in the inner part of basidiocarps, shape variable, mostly subglobose to oval, 9–14(–18) × 7–12(–15) µm [Q=(0.90–)1.12–1.43(–2.12)]; the terminally and subterminally swollen cells of commercial basidiocarps on the sterile surface close to the substrate existing, terminal cells subglobose to oval, occasionally pyriform with short stalks, (15–)35–52 × (15–) 24–37 µm [Q=(1.00–)1.08–1.46(–1.73)] (measurements including stalks), stalks up to 4–6 µm long; subterminal cells, mostly citriform, tapering both sides, occasionally oval to capitate, 35–50 × 18–30 µm [Q=1.35–2.14(–1.78)] (measurements including stalks), stalks up to 17 µm long.

Hyphidia: absent.

Hyphae: in the inner part of basidiocarps mostly 2–4 µm ø, sometimes up to 6 µm ø; abundant ball-like hyphae close to clamps, frequently anastomosing with one to three neighboring cells, like a network; in the subhymenium mostly 4–7 µm ø, close to the substrate up to 12 µm ø; in the commercial material, hyphae in the hymenium and subhymenium variable in shape, mostly 7–20 µm ø.



Figs. 6–8: Cultivated specimens. Fig. 6: Cultivated specimen. Part of hymenium with one mature basidium, several terminally and subterminally swollen cells, haustoria producing single hyphae, thick-walled hyphae and anastomoses. Fig. 7: Part of sterile surface of the basidiocarps close to the substrate. Note that many terminally and subterminally swollen cells are in chains. Fig. 8: Cultivated specimen. Structure in the inner part of basidiocarp with several terminal cells, one haustorium, thin- to thick-walled hyphae and anastomoses.

Haustoria:	abundant in basal parts close to the substrate, rarely in mature hymenium, clamped, haustorial hyphae often branched.
Habitat and substrate:	on *Hypoxylon* sp. or *Annulohypoxylon* sp. growing on decayed stems of *Prunus* sp. (Sakura) and decayed wood of Fagaceae.
Type locality:	Panuré, Brazil.
Distribution:	tropic, subtropic and temperate zones.

Remarks

Tremella fuciformis is probably one of the most beautiful fungi growing in subtropical and tropical areas, or even temperate zones. It was first found in Brazil but has developed to an artificially cultivated species in Taiwan, China and some other countries in Asia. It is clearly associated with Ascomycetes in the field, especially *Hypoxylon* spp. or *Annulohypoxylon* spp., however, unlike other *Tremella* mycoparasites in basidiocarps of Basidiomycetes, the real host relationship of this group is still not investigated.

The basidiocarps are firm. Even soaking in 5 % KOH solution does not easily release the hymenial structure. This is apparently due to the numerous anastomoses in the subhymenium. Similar structures can be seen in *Tremella flava*. These species have three similarities: 1) the hyphae are frequently forming anastomoses, particularly in the subhymenium, 2) as soon as basidiospores were discharged, the basidium will collapse, 3) ecologically they are always associated with *Hypoxylon* sp. or *Annulohypoxylon* sp. in nature.

Tremella fuciformis has two substantial characters in the inner part of basidicarps and subhymenium: 1) the hyphae are often swollen towards the clamps, and then forming predominately anastomoses with neighboring hyphae; 2) the numerous anastomoses, typically present in the subhymenium, make such basidiocarps very stout. The artificially cultivated basidiocarps are slightly different in structures. Particularly the very big swollen cells in chains are present on the sterile surface of basal parts of basidiocarps close to the substrate.

Olive (1958b) reported from *T. fuciformis* collected in Tahiti, that their basidiocarps are quite often parasites on a sphaeriaceous or phomaceous fungi. Nevertheless most of their host-parasite relationships have not been clarified until now. When cultivating *T. fuciformis* artificially, the culture is always mixed with mycelia of *Hypoxylon* sp. or *Annulohypoxylon* sp., inoculated into logs or plastic bags. The real host relationships should be investigated by transmission electron microscopically with hosts and parasites in pure culture interactions.

Nevertheless, the interactions of haustorial and host hyphae cannot be seen clearly microscopically in situ. By checking the structures of hyphae in the substrate or between asci and *Tremella* hyphae, interactions cannot be observed. The branched haustorial hyphae can be found in the substrate.

Furthermore, Olive found that this species has vesicles in the hymenium. This could not be observed in my study. The *Penicillium*-like imperfect stage in the life cycle of *T. fuciformis* reported by Chen and Hou (1979) was probably only a contamination.

Kobayasi (1939) erected a new genus, *Nakaiomyces*, which he found to resemble *Tremella* in every respect except for the presence of dark "setulae". It is obvious from his illustrations that the material he described was infected with imperfect fungi. Olive (1958b) therefore believed *Nakaiomyces* is not a valid genus because some of his own collections from Tahiti had the same infection.

The structures of cultivated *T. fuciformis* are moderately different from typical basidiocarps in nature. For example, although the basidiocarps are well developed, the basidia are rarely mature, reversely most of them are remaining at young stage or becoming aborted cells. Therefore, basidiospores are hard to be found. It is conceivable that such cultivated basidiocarps growing in a green house will lose the ability of their progenitors. Meanwhile, many swollen cells are found in the basal parts of basidiocarps close to the substrate, which cannot be found in nature.

4.2. Molecular phylogeny

Relationships inferred from ribosomal DNA sequences compared with those based on morphological characters provide the information for adequate delimitation of taxa. Large subunit (LSU) 25S rDNA sequences are potentially useful in taxonomic, ecological and evolutionary studies. The results of LSU and ITS phylogenies compared with morphological data of *Tremella* spp. strongly suggest that LSU rDNA sequences are better suited for interpreting their interspecific phylogeny.

Molecular phylogenetic evaluations yielded five groups in the genus *Tremella* (Fig. 9). These groups correspond largely to clades in phylogenetic studies on *Tremella* by Bandoni and Boekhout (2011) and Tremellomycetes by Millanes et al. (2011) and Weiss et al. (2014).

The cluster of the Mesenterica group, including 15 *T. mesenterica* strains, one *T. tropica* and two *T. taiwanensis* strains, is strongly supported (97 %) by bootstrap. Fifteen *T. mesenterica* strains are nearly identical (90 %). Two *T. taiwanensis* strains clustered at the same linkage (100 %) corroborating a strong resemblance.

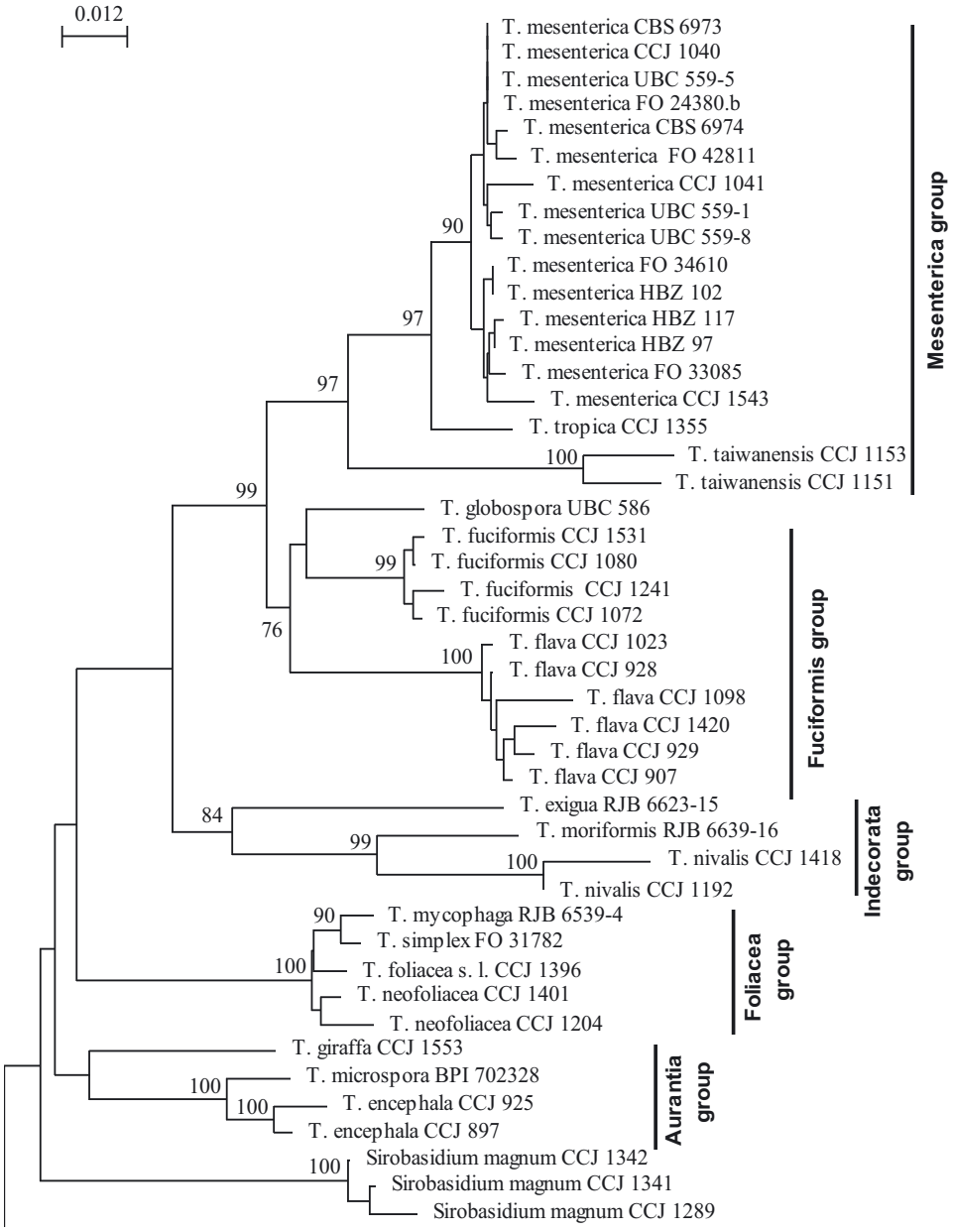


Fig. 9: Neighbor Joining tree of LSU rDNA sequences for 46 strains of *Tremella* species and 3 strains of *Sirobasidium magnum* with *Holtermannia corniformis* as the outgroup. Five groups of *Tremella* are separated: Mesenterica, Fuciformis, Indecorata, Foliacea and Aurantia, based on 1000 bootstrap replicates. Three strains of *S. magnum*, representing an individual cluster, are included in this analysis.

The Fuciformis group, including four *T. fuciformis* strains, six *T. flava* strains and one *T. globospora*, is only weakly supported (76 %), and comprises two subgroups containing four *T. fuciformis* strains (99 %) and six *T. flava* strains (100 %) *Tremella globispora* seems to be only distantly related to the subgroup of *T. fuciformis*. Except for *T. globispora*, all other species in this group are growing on stromata of *Hypoxylon*-like fungi.

Tremella exigua, T. moriformis and *T. nivalis* belonging to the Indecorata group are supported with a bootstrap of 84 %. Two *T. nivalis* strains collected in Kuanwu Forest and Tahsueh Shan Forest in Taiwan are identical (100 %). *Tremella moriformis* RJB 6639-16 is a sister of *T. nivalis* (99 %).

The Foliacea group, consisting of *T. foliacea, T. neofoliacea, T. mycophaga* and *T. simplex*, is strongly supported (100 %). *Tremella mycophaga* and *T. simplex*, mycoparasites of *Aleurodiscus* spp., cluster together (90 %).

Species growing on *Stereum* spp., including two *T. encephala* strains and the type of *T. microspora*, are strongly supported (100 %). Two strains of *T. encephala* from Taiwan are identical (100 %). The type of *T. giraffa* appears in a sister position to *T. encephala*, thus can be included in the Aurantia group in this sampling. Three *S. magnum* strains cluster together (100 %).

4.2.1. AURANTIA group: basidiocarps heterogeneously mixed with basidiomycetaceous-host hyphae up to the subhymenium, basidia mostly pyriform to capitate, subhymenial structure loose, haustoria abundant. Morphologically the Aurantia group is characterized by five main features:

1) basidiocarps large, conspicuous to lacking (i.e. *Tremella* in *Dacrymyces*).
2) hymenial and subhymenial structures moderately loose.
3) haustoria abundant, haustorial hyphae slender, rarely branched.
4) host hyphae intermixing in the inner part, or even in the subhymenium of the parasite.
5) conidia, if existing, normally monokaryotic.

Many *Tremella* species are known as parasites of basidiomycetaceous fungi, except *T. compacta, T. steidleri, T. boraborensis* and *T. loculata*, which are not reported to be mycoparasitic. Even though they are not mentioned to be parasites, they are assumably belonging to this group. Besides *T. encephala* and *T. microspora*, used in this study for molecular analyses, *T. aurantia* is found in this clade (Bandoni & Boekhout 2011, Millanes et al. 2011, Weiss et al. 2014) and also *T. aurantialba* clusters in this group (Weiss et al. 2014). *Tremella giraffa* is not supported in the Aurantia clade (Fig. 2, Bandoni &

Boekhout 2011), and it clusters with lichen parasites, group III, in a cladogram of Millanes et al. (2011). The following species are suggested to belong to the Aurantia group:

T. aurantia Schw.: Fr. 1822
T. aurantialba Bandoni & Zang 1990
T. aurantiolutea Lowy 1978
T. australiensis Lloyd 1912
T. boraborensis Olive 1958a
T. compacta Möller 1895
T. encephala Schw.: Fr. 1822
T. giraffa Chen 1998
T. loculata Bandoni 1957
T. microspora Lloyd 1920
T. obscura (Olive) Christiansen 1954
T. occultifuroidea Chen & Oberwinkler 1999
T. penetrans (Hauerslev) Jülich 1983
T. polyporina Reid 1970
T. ramarioides Zang 1992
T. spectabilis Möller 1895
T. steidleri (Bres.) Bourdot & Galzin 1928
T. subencephala Bandoni & Ginns 1993
T. versicolor Berk. & Broome 1854

4.2.2. FOLIACEA group: basidiocarps foliose and brown, basidia mostly oval, subhymenium with numerous anastomoses, terminally swollen cells in the inner parts of basidiocarps, haustoria rare. According to detailed studies of *T. vasifera, T. neofoliacea, T. fuscosuccinea* and *T. foliacea* sensu lato, four important characters can be summarized for this group:

1) basidiocarps brownish to reddish brown, conspicuously foliose.
2) hymenial and subhymenial structures firm, hyphae heavily anastomosing.
3) hymenium without hyphidia.
4) host dependency not clear in nature.

Molecular data of Bandoni and Boekout (2011), Millanes et al. (2011) and Weiss et al. (2014), support a cluster of *T. foliacea, T. neofoliacea, T. mycophaga* and *T. simplex*, the latter two most likely identical. Thus, the following species might fit to this group, referring to the characters given above:

T. anomala Möller 1895
T. aspera Coker 1920 emend. Bandoni (1957)
T. dahliana Henn. 1898
T. fimbriata Fr. 1822
T. foliacea sensu lato
T. foliacea Pers.: Fr. sensu Bandoni (1957)
T. frondosa Fr. 1822
T. fuscosuccinea Chen 1998
T. griseobrunnea Chen 1998
T. physalia Bandoni 1957
T. neofoliacea Chen 1998
T. undulata Hoffm. 1787
T. vasifera Chen 1998
T. wrightii Berkeley 1868

4.2.3. FUCIFORMIS group: basidiocarps predominantly associated with *Hypoxylon* ascocarps, basidia mostly oval, subhymenial hyphae with numerous anastomoses, terminally swollen cells in the inner parts of basidiocarps, haustorial hyphae heavily branched between the interface of parasites and hosts, haustoria rare. The following points are the common characters in the Fuciformis group. Many species in the group are very similar in most respects.

1) basidiocarps mostly foliose, whitish, yellowish or reddish.
2) basidiocarps frequently associated with fungi of Xylariaceae, i.e. *Hypoxylon* spp. and *Xylaria* spp.
3) subhymenial structure firm, hyphae with numerous anastomoses.
4) hyphidia lacking.
5) haustorial hyphae close to the substrate always branched.

Some species, i.e. *T. elastica* Zoll. & Moritzi 1844 sensu Boedijn 1940 and *T. flammea* Kobayasi 1939, cannot be assigned to the group of Fuciformis, because there is insufficient information from their descriptions and illustrations. Most types or authentic material have to be restudied, especially old and doubtful species. Alternatively, in some other species more details from illustrations and descriptions are available. For instance, the type of *T. dysenterica* Möller, restudied in detail by Bandoni and Oberwinkler (1983), is micromorphologically clearly understandable. *Tremella armeniaca* on *Xylaria* sp. (Bandoni et al. 1996) can be easily grouped. *Tremella samoensis* var. *boninensis* Kobayasi [=*T. boninensis* (Kobayasi) Ito & Imai] and *T. iduensis* are assumably belonging to the Fuciformis group according to the photographs of Kobayasi (1939). Besides *T. flava, T. fuciformis* and *T. globispora*, treated in this study

and by Millanes et al. (2011), Bandoni and Boekout (2011) and Weiss et al. (2014) included also *T. cinnabarina* in their phylograms.

T. armeniaca Bandoni & Carranza 1996 on *Xylaria* sp.
T. boninensis (Kobayasi) Ito & Imai 1940
T. cinnabarina (Mont.) Pat. 1900, according to Kobayasi (1974)
T. dysenterica Möller 1895
T. flava Chen 1998
T. fuciformis Berkeley 1856
T. globispora Reid 1970
T. grandibasidia Olive 1944
T. iduensis Kobayasi 1939
T. resupinata Chen 1998
T. reticulata (Berk.) Farl. 1908
T. tawa McNabb 1990 (Bandoni & Buchanan 1990)

4.2.4. INDECORATA group: basidiocarps predominantly associated with pyrenomycetes, basidia mostly pyriform to clavate, subhymenia loose, haustoria rare. Although there is a copious variety in color of basidiocarps, they are structurally very similar:

1) basidiocarps normally not more than 1 cm ø, gyrose or pulvinate, normally growing on pyrenomycetacous fungi.
2) basidia pyriform, capitate, or clavate, or mixed with oval ones.
3) basidia mostly with apical protuberances.
4) spores dorsiventrally compressed (broader than long).
5) subhymenial structure loose, hyphae with rare anastomoses.

Bourdot and Galzin (1928) studied some *Tremella* species in this group perfectly, i.e. *T. albida* and *T. moriformis*. In contrast to most other mycologists, showing only single basidia and few basidiospores, their illustrations clearly disentangle the real structure of basidial ontogeny in the hymenium. According to the above mentioned characters, the following species might be potential members of the Indecorata group. However, some of the available descriptions and illustrations are not clear enough for a final decision. Careful restudies of type material is urgently needed. In molecularly based cladograms, Millanes et al. (2011) and Weiss et al. (2014) also found *T. indecorata, T. moriformis* and *T. nivalis* clustering together.

T. acaciae Olive 1958b
T. albida Huds.: Fr. 1822 according to Bourdot & Galzin (1928)
T. bambusina Sacc. 1917 emend. Bandoni (1957)

T. carneoalba Coker 1920
T. clavisterigma Lowy 1964
T. durissima Lowy 1962
T. exigua Desm. 1847
T. fusispora Bourdot & Galzin 1923
T. indecorata Sommerf.: Fr. 1822 according to Bourdot & Galzin (1928)
T. lilacea Bandoni & Caranza 1996
T. moriformis (Fr.) Smith ex Berk. 1860 (Berkeley 1860)
T. neglecta Tul. 1871 according to Bandoni (1957)
T. nigrescens Fr. 1849 according to Bourdot & Galzin (1928)
T. nigrifacta Bandoni & Caranza 1996
T. nivalis Chen 1998
T. pulvinalis Kobayasi 1939
T. pyrenophila Trav. & Migl. 1914 according to Bandoni (1957)
T. ribrancensis Lowy 1982
T. subanomala Coker 1920 according to Bandoni (1957)
T. violacea Relh.: Fr. 1874 according to Bourdot & Galzin (1928)
T. virescens Bref. 1888 according to Bourdot & Galzin (1928)
T. volcanagua Lowy 1971

4.2.5. MESENTERICA group: basidiocarps gyrose to cerebriform, yellowish, basidia oval, hyphidia existing, subhymenial structure loose, terminal cells in the inner parts of basidiocarps lacking, haustoria abundant and haustorial hyphae heavily branched. Many species of *Tremella* with yellowish, reddish to orange basidiocarps are related to *T. mesenterica* morphologically. They share the following characters:

1) basidiocarps conspicuous, mostly larger than 1 cm and whitish yellow to orange yellow.
2) hymenial and subhymenial structure loose.
3) hyphidia in the hymenium present; terminally swollen cells in the inner part of basidiocarps lacking.

According to the above features, it is likely that some of the species listed below belong to this group. In molecular trees of Bandoni and Boekout (2011), Millanes et al. (2011) and Weiss et al. (2014), *T. mesenterica, T. tropica, T. brasiliensis* and *T. taiwanensis* cluster together. Apparently, *T. coalescens* is conspecific with *T. mesenterica* (Bandoni & Boekout 2011).

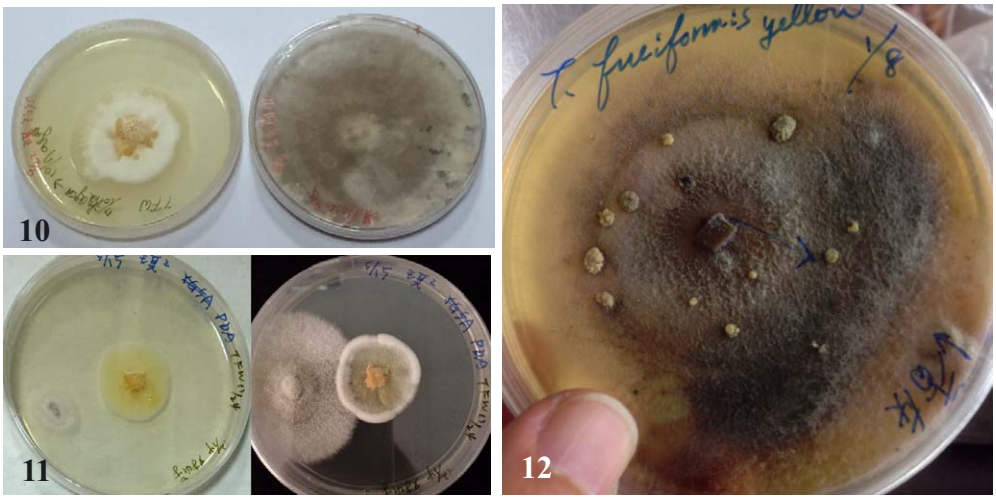
T. auricularia Möller 1895
T. brasiliensis (Möller) Lloyd 1922

T. cerebriformis Chen 1998
T. coalescens Olive 1958a
T. fibulifera Möller 1895
T. mesenterica Retz.: Fr. 1822
T. neofibulifera Kobayasi 1939
T. philippinensis Lloyd 1922
T. roseolutescens Bandoni & Carranza 1996
T. rubromaculata Lowy 1964
T. rufobrunnea Olive 1948
T. subrubiginosa Lowy 1976
T. taiwanensis Chen 1998
T. tropica Chen 1998
T. vesiculosa McNabb 1990 (Bandoni & Buchanan, 1990)

4.3. Cultivation of *Tremella fuciformis*

Tremella fuciformis is known to be a mycoparasite of *Hypoxylon* species (Chen 1998). Some of these species were reassigned to a new genus *Annulohypoxylon*, including *A. archeri*, the species routinely used in commercial cultivation (Stamets 2000, Hsieh et al. 2005).

4.3.1. Influence of mixed cultures on the growth of *Tremella fuciformis*



Figs. 10–12: Fig. 10: Stock cultures on PDA petri dish. *Tremella fuciformis* (left) is whitish and *Annulohypoxylon archeri* (=*Hypoxylon archeri*) (right) is greyish. A mixture of the two cultures is used commercially for the production of *Tremella* fruiting bodies (Silver Ear mushroom). Figs. 11, 12: Two isolates of *T. fuciformis* and *A. archeri* grown together strongly stimulated silver ear mycelial formation.

Because *T. fuciformis* is a mycoparasite on *Annulohypoxylon archeri* adequate mother spawn requires both, host and parasite (Fig. 10). They are cultured together in the same tube or petri dish medium, usually potato dextrose agar, in a so called "dual culture" method. The aerial mycelia of *T. fuciformis* are stimulated by inoculating culture of *A. archeri* (Fig. 11). The result indicates that a mixed culture age of 10–14 days after inoculation is better for the spawn (Tab. 1). A huge portion of *Tremella* to *Annulohypoxylon* ratio is required in this approach. *Tremella fuciformis* is incubated at 25 ˚C until the colony reaches 1~2 cm in diameter, then a few hyphae of *A. archeri*, approximately 0.1–1 %, are inoculated at an opposite position to create a mixed culture (Chen & Huang 2000). Sometimes the primordial of silver ear can be induced on agar medium (Fig. 12). Stamets (2000) produced mixed two liters of liquid spawn of *T. fuciformis* by using 24 drops of *A. archeri*. In our previous study, we used mixed liquid spawn of *T. fuciformis* and *A. archeri* by 95:5 with an excellent result for fructification.

Tab.1: The influence of mixture spawn age to the harvest of silver ear mushroom

mixture spawn age after inoculation in substrate	mycelial diameter on the surface of bottle (mm)	average fresh weight of fruiting body (g)	average dry weight of fruiting body (g)
6	42±3	221±11	18±1.9
10	70±4	250±15	23±2.2
14	72±3	300±23	28±3.1
18	75±1	233±12	22±2.3

Note: the diameter of bottle is 76mm and the fruiting body was surveyed 40 days after inoculation at 24℃. Data are expressed as the mean values of hundred samples analysis ± S.D.

4.3.2. Influence of temperature on primordia

As soon as *T. fuciformis* and *A. archeri* grow together, primordial formation may be induced (Figs. 13–14). A mixture spawn is used to inoculate the sawdust bran substrate with 60–65 % moisture content in bottles until the formation of primordia for primary spawn (Figs. 15–16). The optimal temperature for forming primordia is between at 22–24 ˚C (Tab. 2). White mycelial globules and a yellowish brown exudate on top of the bottles seems to be an important indicator for primordium formation (Fig. 16). Formation of primordia ensures that the mixture of *T. fuciformis* and *A. archeri* is capable of producing *Tremella* fruiting bodies.

Tab. 2: The influence of temperature for the mycelial growth, exudate and primordia formation of silver ear mushroom.

temperature (℃)	mycelia diameter on surface (mm)	yellowish brown exudate (day)	primordia formation (day)
18	16	23	35
20	17	23	30
22	13	20	26
24	11	15	27
26	10	–	–

Note: "–" indicates no secretion and primordia formation after 35 days.

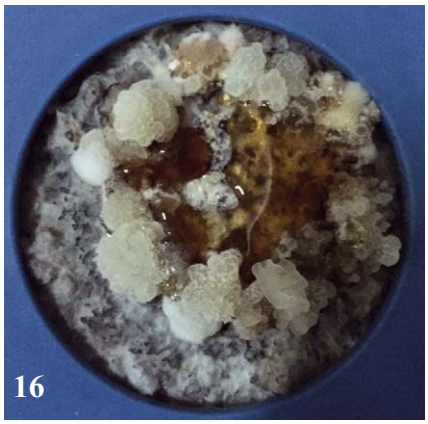


Fig. 13: Formation of primordia ensures that the mixture of *T. fuciformis* and *A. archeri* is capable of producing *Tremella* fruiting bodies. Fig. 14: Young fruiting bodies on the surface will be removed and the substrate thoroughly mixed befor spawning. Fig. 15: A small portion of mixed spawn in the bottle is taken and inoculated at 22–24 until the mycelial growth fills up the substrate. Fig. 16: White mycelial globules and yellowish brown exudates appear on the inoculation surface within a week. Fig. 17: To maintain the proper temperature, moisture, ventilation and illuminance is important to prevent abortion of primordia and to obtain fruiting bodies successfully.

4.3.3. Fruiting body formation

Too much of the exudate on the spawn has to be drained off. The growth conditions of mixed isolates during the period from the formation of the whitish mycelial globules to the development of fruit bodies are shown in Table 2. To prevent abortion of primordia and to obtain sufficient fruitingbodies, it is important to maintain the proper temperature, moisture, ventilation and illuminance (Tab. 3). Illuminance with 100–600 lux is essential for full fruit body development. Dark growing conditions have been reported as an alternative method (Oei 1996). Primordia appeared approximately two weeks after the formation of white mycelial globules. Mature fruiting bodies, ready for harvest, were produced within 30–40 days after inoculating mixed isolates (Figs. 17–18).

Tremella fuciformis is one of the major delicacy mushrooms in China. When in former times only mycelium was used to cultivate *T. fuciformis*, the yield was very low. The yeast stages, pseudohyphae or mycelia of *T. fuciformis* cannot degrade cellulose and lignin efficiently. Understanding the natural occurrence of *T. fuciformis* always together with *Hypoxylon* spp. or *Annulohypoxylon* spp. led to mixed isolate cultures with strongly improved basidiocarp development. Since mycelial growth of *T. fuciformis* is much slower than that of *A. archeri*, the culture portion of *T. fuciformis* to *A. archeri* ratio should be very high, up to 99 %, to ensure success.

Tab. 3: Growth conditions of silver ear mushroom.

	Mycelial growth in bottle after spawning	White mycelial globules formation (primordia)	Fruiting body development
Temperature	18–26℃	18–24℃	22–24℃
Relative humidity	70–75 % RH	85–95 % RH	90–100 % RH
Illuminance	–	–	100–600 lux
Ventilation	–	increase	2 times daily (30 min each)
Duration	10–17 days	15–23 days	30–40 days after inoculating mixed isolates

Fig. 18: Mature fruiting bodies of *T. fuciformis*, ready for harvest, are produced within 30–40 days after inoculating mixed spawn.

5. References

Bandoni, R.J. 1957: Taxonomic studies of the Tremellaceae. — Ph.D. dissertation of Iowa University.

Bandoni, R.J. 1965: Secondary control of conjugation in *Tremella mesenterica*. — Can. J. Bot. **43**: 627–630.

Bandoni, R.J. 1979: *Fibulobasidium*: a new genus in the Sirobasidiaceae. — Can. J. Bot. **57**: 264–268.

Bandoni, R.J. 1987: Taxonomic review of the Tremellales. — Stud. Mycol. **30**: 87–110.

Bandoni, R.J. 1986: *Sirotrema*: a new genus in the Tremelllaceae. — Can. J. Bot. **64**: 668–676.

Bandoni, R.J. & Buchanan, P.K. 1990: Two new species of *Tremella* from New Zealand. — New Zealand J. Bot. **28**: 451–454.

Bandoni, R.J. 1995: Dimorphic heterobasidiomycetes: taxonomy and parasitism. — Stud. Mycol. **38**: 13–27.

Bandoni, R.J. & Boekhout, T. 2011: *Tremella* Persoon (1794). — In Kurtzman, C.P., Fell, J.W. & Boekhout, T. (eds.): The yeasts, a taxonomic study, vol. **3**, fith ed., pp. 1567–1590. — Amsterdam, Boston, Heidelberg: Elsevier.

Bandoni, R.J. & Buchanan, P.K. 1990: Two new species of *Tremella* from New Zealand. — New Zealand J. Bot. **28**: 451–454.

Bandoni, R.J. & Oberwinkler, F. 1983: On some species of *Tremella* described by Alfred Möller. — Mycologia **75**: 854–863.

Bandoni, R.J. & Ginns, J. 1993: On some species of *Tremella* associated with Corticiaceae. — Trans. Mycol. Soc. Japan **34**: 21–36.

Bandoni, R.J. & Zang, M. 1990: On an undescribed *Tremella* from China. — Mycologia **82**: 270–273.

Bandoni, R.J., Carranza, J. & Bandoni, A.A. 1996: Four new species of *Tremella* (Tremellales: Basidiomycotina) from Costa Rica. — Rev. Biol. Trop. **44** (Suppl. 4): 15–24.

Berkeley, M.J. & Broome, C.E. 1854: Notices of British Fungi – XXXV. — Ann. Mag. Nat. Hist. ser. II, **13**: 396–407.

Berkeley, M.J. 1856: Decades of Fungi LXI–LXII: Rio Negro fungi. — Hooker's J. Bot. Kew. Gard. Misc. **8**: 272–280.

Berkeley, M.J. 1860: Outlines of British Fungology, pp. 286–292. — London: L. Reeve.

Berkeley, M.J. 1868. — J. Linn. Soc., Bot. **10** (46): 344.

Blanz, P.A. & Unseld, M. 1987: Ribosomal RNA as a taxonomic tool in mycology. p. 247–258. — In de Hoog, G.S., Smith, M.T. & Weijman, A.C.M. (eds.): The expanding realm of yeast-like fungi. — Amsterdam, Boston, Heidelberg: Elsevier.

Boedijn, K.B. 1940: Mycetozoa, fungi and lichens of the Krakatau group. — Bulletin du Bull. Jard. Bot. Buitenzorg, sér. 3, **16**: 358–427.

Boekhout, T., Fonseca, A. & Batenburg-van der Vegte, W.H. 1991: *Bulleromyces* genus novum (Tremellales), a teleomorph for *Bullera alba*, and the occurrence of mating in *Bullera variabilis*. — Antonie van Leeuwenhoek **59**: 89–93.

Boekhout, T., Fell, J.W. & O'Donnell, K. 1995: Molecular systematics of some yeast-like anamorphs belonging to the Ustilaginales and Tilletiales. — Stud. Mycol. **38**: 175–183.

Bölker, M. & Kahmann, R. 1993: Sexual pheromones and mating responses in fungi. — Plant Cell **5**: 1461–1469.

Bourdot, H. & Galzin, A. 1923: Heterobasidieae nondum descriptae. — Bull. Soc. Myc. Fr. **39**: 261–266.

Bourdot, H. & Galzin, A. 1928: Hyménomycètes de France. I. Heterobasidiés. — Sceaux: Losle-Saunier.

Burt, W.R. & Cazin, J.J. 1976: Production of extracellular ribonuclease by yeasts and yeastlike fungi, and its repression by orthophosphate in species of *Cryptococcus* and *Tremella*. — J. Bacteriol. **125**: 955–960.

Burt, W.R. & Cazin, J.J. 1982: Comparison of ribonucleases from *Cryptococcus laurentii* and *Tremella foliacea*. — Mycologia **74**: 66–76.

Chen, A.W. & Huang, N.L. 2000: Mixed-culture cultivation of *Tremella fuciformis* on synthetic logs. — The Mushroom Grower's Newsletter (2000): 1–3.

Chen, C.-J. 1998: The morphological and molecular studies in the genus *Tremella*. — Biblioth. Mycol. 174. — Stuttgart: J. Cramer.

Chen, P.-C. & Hou, H.-H. 1979: The life cycle of the white jelly mushroom, *Tremella fuciformis*. — Taiwan Mushrooms **3**: 19–27.

Chen, C.-J. & Oberwinkler, F. 1999: *Tremella ocultifuroidea* sp. nov., a new mycoparasite of *Dacrymyces*. — Mycoscience **40**: 137–143.

Christiansen, M.P. 1954: Tre ejendommelige *Tremella*-Arter: *Tremella mycophaga* Martin, *Tremella simplex* Jacks. & Martin og *Tremella obscura* (Olive) comb. N. Paavist i Danmark. — Friesia **5**: 55–64.

Coker, W.C. 1920: Notes on the lower Basidiomycetes of North Carolina. — J. Mitchell Soc. **35**: 113–181.

Dillenius, J.J. 1741: Historia muscorum, pp. 41 –55. — Oxford: Publisher unknown.

Donk, M.A. 1966: Check list of European Hymenomycetous Heterobasidiae. — Persoonia **4** (2): 145–335.

Edwards, K., Johnstone, C. & Thompson, C. 1991: A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. — Nucleic Acids Res. **19**: 1349.

Felsenstein, J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. — Evolution **39**: 783–791.

Felsenstein, J. 1993: PHYLIP – phylogeny inference package, version 3.51c. Computer programs distributed by the author. — Seattle: Department of Genetics, University of Washington.

Fries, E. 1822: Systema Mycologicum II, pp. 207–238. — Greifswald: Publisher unknown.

Hanson, L.C. & Wells, K. 1991: Characterization of three *Tremella* species by isozyme analysis. — Mycologia **83**: 446–454.

Hennings, P. 1898: Fungi Novo-guineenses. III. — Bot. Jahrb. Syst. Pflanzengeschichte und Pflanzengeographie **25**: 495–509

Henrion, B., Le Tacon, F. & Martin, F. 1992: Rapid identification of genetic variation of ectomycorrhizal fungi by amplification of ribosomal RAN genes. — New Phytol. **122**: 289–298.

Hirata, A.E., Tsuchiya, S.F. & Tanaka. K. 1980: An electron microscopic study on the mating tube formation in the Heterobasidiomycetes *Tremella mesenterica*. — Arch. Microbiol. **128**: 215–221.

Hsieh, H.M., Ju, Y.M. & Rogers, J.D. 2005: Molecular phylogeny of *Hypoxylon* and closely related genera. — Mycologia **97**: 844–865.

Hoffmann, G. F. 1787: Vegetabilia Cryptogama. 1: 1–42. — Erlangen: Publisher unknown.

Ishibashi, Y., Sakagami, Y., Isogai, A. & Suzuki, A. 1984: Structures of tremerogens A-9291-I and A-9291-VIII: peptidyl sex hormones of *Tremella brasiliensis*. — Biochemistry **23**: 1399–1404.

Ito, S., Imai, S. 1940: Fungi of the Bonin Islands V. — Trans. Sapporo Nat. Hist. Soc. **16**: 120–138.

Jung, S.J., Shin, Y.K., Joo, W.H. & Lee, J.D. 1995: Phylogenetic relationships of yeast-like fungi deduced from partial sequences of 18 S ribosomal RNA. — Korean J. Mycol. **23**: 310–317.

Jülich, W. 1983: Parasitic heterobasidiomycetes on other fungi. A key to the European taxa. — Int. J. Mycol. Lichenol. **1**:189–203.

Kobayasi, Y. 1939: On the genus *Tremella* and its allies from Japan. — Sci. Rep. Tokyo Bunrika Diagaku **4**: 1–26.

Kobayasi, Y. 1974: Three jelly fungi collected in New Guinea. — J. Jap. Bot. **49**: 1–4.

Kwong-Chung, K.J., Chang, Y.C., Bauer, R., Swann, E.C., Taylor, J.W. & Goel, R. 1995: The characteristics that differentiate *Filobasidiella depauperata* from *Filobasidiella neoformans*. — Stud. Mycol. **38**: 67–79

Li, W.-H. & Graur, D. 1991: Fundamentals of molecular evolution. — Sunderland, Massachusetts: Sinauer Associates.

Lloyd, C.G. 1912: Mycological Notes. No. **38**: 556.

Lloyd, C.G. 1920: Mycological Notes. No. **62**: 894–1098.

Lloyd, C.G. 1920: Mycological Notes. No. **64**: 991.

Lloyd, C.G. 1922–1925: Mycological Notes. scattered, pp. 1115–1360.

Looney, A.M. 1933: A morphological study of certain species of *Tremella*. — State Univ. Iowa Stud. Nat. Hist. **15**: 17–33.

Lowy, B. 1962: Contribucion al estudio de los Tremellales de la Argentina. — Lilloa **31**: 213–228.

Lowy, B. 1964: New species of Tremellales from Guatemala. — J. Elisha Mitch. Sci. Soc. **80**: 65–70.

Lowy, B. 1971: Flora Neotropica Monograph No. 6, Tremellales. — New York: Hafner Publishing Company, Inc.

Lowy, B. 1976: New Tremellales from Panama. — Mycologia **68**: 1103–1108.

Lowy, B. 1978: A new *Tremella* from Mexico. — Mycotaxon **7**: 218–220.

Lowy, B. 1982: New Tremellales from west Brazilian Amazon. — Mycotaxon **15**: 95–102.

Millanes, A. M., Diederich, P., Ekman, S. & Wedin, M. 2011: Phylogeny and character evolution in the jelly fungi (Tremellomycetes, Basidiomycota, Fungi). — Molec. Phylogen. Evol. **61**: 12–28.

Mitchell, T. G., White, T.J. & Taylor, J.W. 1992: Comparison of 5.8 S ribosomal DNA sequences among the basidiomycetous yeast genera *Cystofilobasidium*, *Filobasidium* and *Filobasidiella*. — J. Med. Veterin. Mycol. **30**: 207–218.

Miyakawa, T., Miyama, R., Tabata, M., Tsuchiya, E. & Fukui, S. 1985a: A study on the biosynthesis of tremerogen A-10, a polysioprenyl peptide mating pheromone of *Tremella mesenterica*, using an inhibitor of mevalonate synthesis. — Agric. Biol. Chem. **49**: 1343–1347.

Miyakawa, T., Miyama, R., Tabata, M., Tsuchiya, E. & Fukui, S. 1985b: Biosynthesis and secretion of tremerogen A-10, a polyisoprenyl peptide mating pheromone of *Tremella mesenterica*. — Eur. J. Biochem. **147**: 489–493.

Miyakawa, T., Kadota, T., Okubo, Y., Hatano, T., Tsuchiya, E. & Fukui, S. 1984: Mating pheromone-induced alternation of cell surface proteins in the Heterobasidiomycetous yeast *Tremella mesenterica*. — J. Bacteriology. **158**: 814–819.

Möller, A. 1895: Protobasidiomyceten. Vol. 8. — In Schimper, A.F.W. (ed.): Botanische Mittheilungen aus den Tropen. — Jena: G. Fischer.

Mullis, K.B. & Faloona, F.A. 1984: Specific synthesis of DNA in virto via a polymerase-catalyzed chain reaction. — Methods Enzymol. **155**: 335–350.

Nakase, T. & Komatgata, K. 1971: DNA base composition of some species of yeasts and yeast-like fungi. — J. Gen. Appl. Microbiol. **17**: 363–369.

Oberwinkler, F. & Bandoni, R.J. 1983: *Trimorphomyces*: a new genus in the Tremellaceae. — System. Appl. Microbiol. **4**: 105–113.

Oberwinkler, F. & Bandoni, R.J. 1985:. *Tetragoniomyces* gen. nov. and *Tetragoniomycetaceae* fam. nov. (Tremellales). — Canad. J. Bot. **59**: 1034–1040.

Oberwinkler, F., Bauer, R. & Schneller, J. 1990: *Phragmoxenidium mycophilum* sp. nov., an unusual mycoparasitic Heterobasidiomycete. — System. Appl. Microbiol. **13**: 186–191.

O'Donnel, K. 1993: Fusarium and its near relatives. — In Reynolds, D.R. & Taylor, J.W. (eds.): The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics, pp. 225–233. — Wallingford: CAB International.

Oei, P. 1996: Mushroom cultivation (with special emphasis on appropriate techniques for developing countries). — Leiden, the Netherlands: Tool Publications.

Okuda, T., Ikeda, A. & Maruyama, H.B. 1981: *Holtermannia corniformis*: physiological characterization of the yeast state and mating. — Trans. Mycol. Soc. Japan **22**: 153–163.

Olive, L.S. 1944: New or rare Heterobasidiomycetes from North Carolina–I. — J. Elisha Mitchell Sci. Soc. **60**: 17–26.

Olive, L.S. 1954: New or noteworthy species of Tremellales from the southern Appalachians–III. — Bull. Torrey Bot. Club **81**: 329–339.

Olive, L.S. 1958a: The lower Basidiomycetes of Tahiti–I. — Bull. Torrey Bot. Club **85**: 5–27.

Olive, L.S. 1958b: The lower Basidiomycetes of Tahiti (continued). — Bull. Torrey Bot. Club **85**: 89–110.

Patouillard, N. & Lagerheim, G. 1892: Champignons de L'Équateur. — Bull. Soc. Myc. Fr. **8**: 113–140.

Patouillard, N. 1900: Essai taxonomique sur les familles et genres des Hyménomycètes. — Lons-le-Saunier: Lucien Declume.

Rea, C. 1922: British Basidiomycetaceae. A handbook of the larger British fungi. — Cambridge: University Press.

Reid, D.A. 1970: New or interesting records of British Hymenomycetes, IV. — Trans. Br. Mycol. Soc. **55**: 413–441.

Rogers, D.P. 1947: A new gymnocarpous heterobasidiomycete with gasteromycetous basidia. — Mycologia **39**: 556–564.

Saccardo, P. A. 1910: Sylloge Fungorum **19**: 871.

Saitou, N. & Nei, M. 1987: The neighbor-joining method: a new method for reconstructing phylogenetic trees. — Mol. Biol. Evol. **4**: 406–425.

Sakagami, Y., Yoshida, M., Isogai, A. & Suzuki, A. 1981: Peptidal sex hormones inducing conjugation tube formation in compatible mating-type cells of *Tremella mesenterica*. — Science **212**: 1525–1527.

Sanger, F., Nicklen, S. & Coulson, A.R. 1977: DNA sequencing with chain terminating inhibitors. — Proc. Nat. Acad. Sci. **74**: 5463–5467.

Sawada, K. 1931: Descriptive catalogue of Formosan fungi. Part II. Order Ustilaginales. — Dept. Agr. Gov't Res. Inst. Formosana Rep. **2**: 1–172.

Singer-Sam, J., Tanguay, R.L. & Riggs, A.D. 1989: Use of Chelex to improve the PCR singal from a small number of cells. — Amplifications: A Forum for PCR Users, issue **3**: 11.

Slodki, M.E., Wickerham, L.J. & Bandoni, R.J. 1966: Extracellular heteropoly-saccharides from *Cryptococcus* and *Tremella*: a possible taxonomic relationship. — Can. J. Microbiol. **12**: 489–494.

Smit, E.J., Kock, J.L.F., van-der-Westhuizen, J.P.J. & Britz, T.J. 1988: Taxonomic relationships of *Cryptococcus* and *Tremella* based on fatty acid composition and other phenotypic characters. — J. Gen. Microbiol. **134**: 2849–2855.

Stamets, P. 2000: Growth parameters for gourmet and medicinal mushroom species. — In Stamets, P. (ed.): Growing gourmet and medicinal mushrooms (3rd ed.), pp. 402–406. — Berkeley, California: Ten Speed Press.

Swann, E.C. & Taylor, J.W. 1993: Higher taxa of Basidiomycetes: an 18S rRNA gene perspective. — Mycologia **85**(6): 923–936.

Walker, W.F. & Doolittle, W.F. 1982: Redividing the Basidiomycetes on the basis of 5S rRNA sequences. — Nature **299**: 723–724.

Walsh, P.S., Metzger, D.A. & Higuchi, R. 1991: Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. — BioTechniques **10**: 506–513.

Weiss, M., Bauer, R., Sampaio, J. P. & Oberwinkler, F. 2014: Tremellomycetes and related groups. — In McLaughlin, D.J. & Spatafora, J.W. (eds.): Systematics and

Evolution, pp. 331–355. — The Mycota **XII**, Part A., 2nd ed.. — Heidelberg, Berlin, New York: Springer.

White, T.J., Bruns, T., Lee, S.B. & Taylor, J.W. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. pp. 315–322. — In Innis, M.A., Gelfand, D.H., Sninsky, J.J. & White, T.J. (eds.): PCR protocols: A guide to Methods and Applications. — San Diego: Academic Press.

Zang, M. 1992: Endemic higher fungi with a note on China and adjacent areas. — Acta Botanica Yunnanica **14**: 385–400.

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