Relationships among some members of the genus *Otidea* (*Pezizales*, *Pyronemataceae*)

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Relationships among some species of the genus *Otidea* were investigated based on sequence analyses of 28S rDNA, as well as morphological features. Phylogenetic trees generated using Neighbor joining, Maximum parsimony and Bayesian inference methods showed that *Otidea* is a monophyletic group. In general, apothecial shape is a reliable character in taxonomy, and apothecial color and paraphysis shape at apical portion are stable features at species level. Our morphological and molecular data indicate that *Flavoscypha* should be synonymized with *Otidea* and that *Otideopsis* is treated as a subgenus of *Otidea*.

Key words: Phylogeny, synonyms, taxonomy

Introduction

The genus *Otidea* with *O. onotica* as lectotype was established by Fuckel (1869-1870) for four species, *O. leporina*, *O. onotica*, *O. cochleata* and *O. abietina*. Large to medium-sized apothecia, cupulate fruitbodies with split or ear-shaped apothecia, and filiform paraphyses which are curved or capitate at the apex are diagnostic features of the genus by which species of *Otidea* are distinguished from other genera in the *Pezizales*. Besides the peculiar shapes of apothecia and paraphyses, other characters associated with the genus are: ectal excipulum of *textura angularis*, medullary excipulum of *textura intricata*, asci subcylindrical, nonamyloid, and ascospores elliptical, biguttulate and usually smooth-walled (Kanouse 1949; Dennis, 1968; Eckblad, 1968; Korf, 1972; Hansen and Knudsen, 2000). Previous studies on *Otidea* mainly focused on taxonomy and were based on morphological characters (Kanouse, 1949; Harmaja, 1976; Cao *et al*., 1990). Relationships among species were not thoroughly studied and molecular approaches have not been carried out to investigate the phylogeny of the genus. In our study, partial sequences of the

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large subunit rDNA were used to establish the phylogenetic relationships among species in the genus, as well as its relationship to morphologically similar genera *Flavoscypha* Harmaja (Harmaja, 1974) and *Otideopsis* B. Liu & J.Z. Cao (Liu and Cao, 1987). Our results indicated that a monophyletic *Otidea* is recognized and that *Flavoscypha* and *Otideopsis* are congeneric with *Otidea*.

Materials and methods

Materials used in this study are shown in Table 1. Thirteen taxa of *Otidea*, one of *Flavoscypha* and one in *Otideopsis* were chosen to explore relationships among species and taxa in the *Pyronemataceae* were also included in the phylogenetic analysis. *Tarzetta catinus* was chosen as outgroup. Genomic DNA was extracted from the dried herbarium specimens. The ascomata of herbarium specimens were firstly grinded in liquid nitrogen, and were extracted in CTAB isolation buffer according to the protocol of Doyle and Doyle (1987, 1990) as modified by Wittzell (1999). The 5′end of the large subunit rDNA was amplified using primers LROR (5′-ACCCGCTGAACTTAAGC) and LR3 (5′-TACTACCACCAAGATCT). Amplification was carried out in a 50 µl-volume reaction, with 1.25 units of Taq polymerase, 200 µM dNTPs and 1 µM each primer. The PCR program consisted of an initial denaturing step at 94°C for 60s, 35 amplification cycles (94°C for 30s, 50°C for 30s, 72°C for 30s), and a final step at 72°C for 6 min in a GeneAmp PCR System 2400 (Perkin Elmer). PCR product purification and DNA sequencing were performed by Songon Biotechnology Company and Genecore Biotechnology Company in Shanghai. Similar sequences also were retrieved from GenBank.

Sequences were aligned (under the default settings with a gap-opening penalty of 15.0 and a gap-extension penalty of 6.5 for pairwise and multiple alignments) with MEGA 3.0 (Kumar *et al*., 2004). The data matrix of 44 taxa and 554 characters was used for analysis, of which 316 were constant, 228 were variable, 151 were parsimony-informative sites and 77 were parsimony singleton sites. Three different optimality criteria, Neighbor joining, Maximum parsimony and Bayesian inference, were performed to infer phylogeny. The Neighbor joining tree was constructed with Kimura 2-parameter model and the bootstrap values were determined from 1000 replications by PAUP* version 4.0b10 (Swofford, 2002). Most parsimonious trees were conducted as heuristic search routines with 1000 random-addition sequences, gaps were treated as "missing data", and TBR branch swapping by PAUP* version 4.0b10. Support for the branching topologies was evaluated by bootstrap analysis derived from 1,000 replicates with 10 random addition replicates each. MrModeltest 2.2

Table 1. List of the sequences used in the phylogenetic analysis.

Species	Herbarium number	GenBank accession number
Aleuria aurantia (Pers.) Fuckel		AY544654
Flavoscypha cantharella (Fr.) Harmaja (as *O. concinna* Pers.) (1)		AF086590
F. cantharella (2)		AF086591
F. cantharella (3)		AF086592
F. cantharella (4)		AF086593
Geopyxis majalis (Fr.) Saccardo	HMAS* 71797	**DQ458804**
Melastiza contorta (Massee & Crossl.) Spooner & Y.J. Yao		AY500539
Otidea alutacea (Pers.) Massee (1)	HMAS 52742	**DQ443438****
O. alutacea (2)	HMAS 57844	**DQ443439**
O. alutacea (3)	HMAS 83563	**DQ443440**
O. alutacea (4)	HMAS 83559	**DQ443441**
O. alutacea (5)	HMAS 83560	**DQ443442**
O. alutacea (6)		AF086580
O. alutacea (7)		AF086582
O. alutacea (8)		AF086583
O. alutacea (9)		AF086585
O. cochleata (L.) Fuckel (1) (as *O. umbrina* Pers.)		AF086581
O. cochleata (2)		AF086584
O. cochleata (3)		AF086586
O. crassa W.Y. Zhuang (1)	HMAS 83570	**DQ443443**
O. crassa (2)	HMAS 83571	**DQ443444**
O. daliensis W.Y. Zhuang & Korf	HMAS 57688	**DQ443445**
O. grandis (Pers.) Rehm (1)	HMAS 51684	**DQ443446**
O. grandis (2)		AY789369
O. lactea J.Z. Cao & L. Fan	HMAS 61359	**DQ443447**
O. leporina (Batsch) Fuckel (1)	HMAS 83579	**DQ443448**
O. leporina (2)	HMAS 83568	**DQ443449**
O. onotica (Pers.) Fuckel var. *onotica* (1)		AF086577
O. onotica var. *onotica* (2)		AF086578
O. onotica var. *onotica* (3)		AF086579
O. onotica var. *onotica* (4)		AF335121
O. onotica var. *brevispora* W.Y. Zhuang	HMAS 43003	**DQ443450**
O. rainierensis Kanouse (1)		AF086597
O. rainierensis (2)		AF086598
O. rainierensis (3)		AF086599
O. sinensis J.Z. Cao & L. Fan	HMAS 61360	**DQ443451**
O. smithii Kanouse (1)		AF086574
O. smithii (2)		AF086572
O. smithii (3)		AF086575
O. tuomikoskii Harmaja (1)		AF086594
O. tuomikoskii (2)		AF086595
O. tuomikoskii (3)		AF086596
Otideopsis yunnanensis B. Liu & J.Z. Cao	HMAS 82166	**DQ443452**
Tarzetta catinus (Holmsk.) Korf & J.K. Rogers	HMAS 70355	**DQ458803**

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** Numbers in boldface indicating the newly submitted sequences.
The Tree BASE Matrix Accession Number is M2897

(Nylander, 2004) was used to select the substitution model for Bayesian inference analysis. Likelihood ratio tests indicated that GTR+G model was the

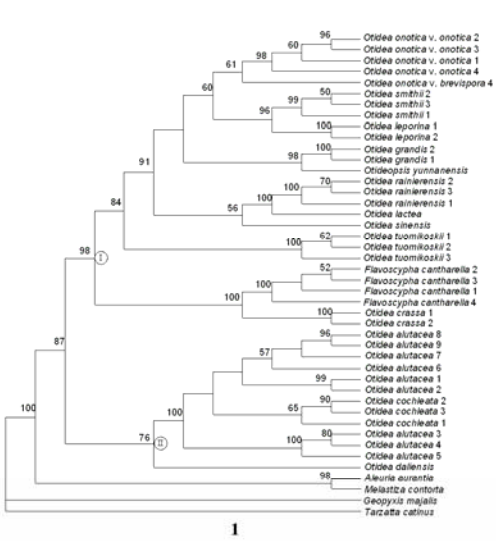
most appropriate for subsequent analysis. Model settings were: Base (base frequencies) = 0.2712, 0.1951, 0.2871, 0.2466; Nst (number of substitution types) = 6; Rmat (substitution-rate matrix) = 3.7705, 1.5903, 1.6435, 10.5306, 1; Rates = gamma shape (Shape = 0.4054); and Pinvar (proportion of invariable sites) = 0. MrBayes 3.04b4 (Huelsenbeck and Ronquist, 2001) software was used to reconstruct the Bayesian tree according to these conditions: (i) model of nucleotide substitution resulted from MrModeltest was used to reconstruct Bayesian trees; (ii) four Markov chains (number of generations = 1,000,000; frequency of sampling trees = one per 100 generations) were set according to specific conditions for running; (iii) data set stationary was reached at approximate generation 200000, thus the first 2000 trees were discarded ("burn in" of the chain); (iv) posterior probability of each node was estimated with the consensus option of 50% majority-rule. The posterior probability of the node is referred to as Bayesian support value.

Morphological and ultrastructure studies followed the methods by Zhuang (2005). Apothecia were rehydrated and sectioned by a freezing microtome at the thickness of 25-30 μm. Measurements were taken from sections mounted in cotton blue-lactophenol solution and from squash mounts. For SEM studies of the spore surface morphology, a portion of hymenium was cut and stuck directly on a stub. The materials were coated with gold-palladium and observed with SEM (FEI Quanta 200).

Results

Three phylogenetic trees, Neighbor joining, Maximum parsimony and Bayesian inference, are shown in Figs 1-3. With *Tarzetta catinus* as outgroup, two Clades were recognized in the Neighbor joining tree (Fig. 1). Bootstrap analysis indicated a moderate level of support (87%) for relationship between the two clades recognized within *Otidea*, *Flavoscypha* and *Otideopsis*. The first clade (Clade I) composed of most *Otidea* species, including *O. onotica* (type species of the genus), *O. leporina*, *O. smithii*, *O. grandis*, *Otideopsis yunnanensis*, *O. sinensis*, *O. lactea*, *O. rainierensis*, *O. tuomikoskii*, and *O. crassa*, as well as *Flavoscypha cantharella*. *Flavoscypha cantharella* grouped

Figs 1-3. Phylograms derived from the 28S rDNA partial sequences of 44 taxa. **1.** Neighbor joining tree is constructed using the Close-Neighbor-Interchange method. Bootstrap values are determined from 1000 replications. **2.** Strict consensus tree of 8 equally parsimonious trees, using heuristic searches with 1000 replicates and random-addition-of-taxon option. Bootstrap values are determined from 1000 replications. Tree length = 487, CI = 0.657, HI = 0.3429, RI = 0.8586. **3.** Bayesian inference tree constructed by MrBayes with GTR+G model. Bayesian support values of the 50% majority-rule consensus tree for sampled Bayesian trees are marked at nodes supported by more than 50%. Illustrations of fruitbodies are from Boudier (1905-1911).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Otidea onotica v. onotica 3
Otidea onotica v. onotica 1
Otidea onotica v. onotica 4
Otidea onotica v. brevispora 4
Otidea smithii 2
Otidea smithii 3
Otidea smithii 1
Otidea leporina 1
Otidea leporina 2
Otidea grandis 2
Otidea grandis 1
Otideopsis yunnanensis
Otidea rainierensis 2
Otidea rainierensis 3
Otidea rainierensis 1
Otidea lactea
Otidea sinensis
Otidea tuomikoskii 1
Otidea tuomikoskii 2
Otidea tuomikoskii 3
Flavoscypha cantharella 2
Flavoscypha cantharella 3
Flavoscypha cantharella 1
Flavoscypha cantharella 4
Otidea crassa 1
Otidea crassa 2
Otidea alutacea 8
Otidea alutacea 9
Otidea alutacea 7
Otidea alutacea 6
Otidea alutacea 1
Otidea alutacea 2
Otidea cochleata 2
Otidea cochleata 3
Otidea cochleata 1
Otidea alutacea 3
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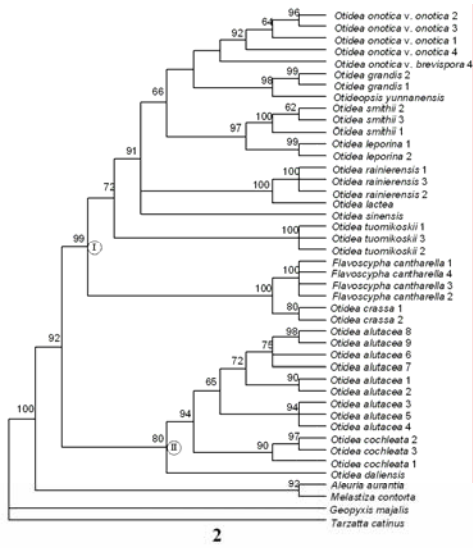
Otidea daliensis
Aleuria aurantia
Melastiza contorta
Geopyxis majalis
Tarzatta catinus
1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Otidea onotica v. onotica 4
Otidea onotica v. brevispora 4
Otidea grandis 2
Otidea grandis 1
Otideopsis yunnanensis
Otidea smithii 2
Otidea smithii 3
Otidea smithii 1
Otidea leporina 1
Otidea leporina 2
Otidea rainierensis 1
Otidea rainierensis 3
Otidea rainierensis 2
Otidea lactea
Otidea sinensis
Otidea tuomikoskii 1
Otidea tuomikoskii 3
Otidea tuomikoskii 2
Flavoscypha cantharella 1
Flavoscypha cantharella 4
Flavoscypha cantharella 3
Flavoscypha cantharella 2
Otidea crassa 1
Otidea crassa 2
Otidea alutacea 8
Otidea alutacea 9
Otidea alutacea 6
Otidea alutacea 7
Otidea alutacea 1
Otidea alutacea 2
Otidea alutacea 3
Otidea alutacea 5
Otidea alutacea 4
Otidea cochleata 2
Otidea cochleata 3
Otidea cochleata 1
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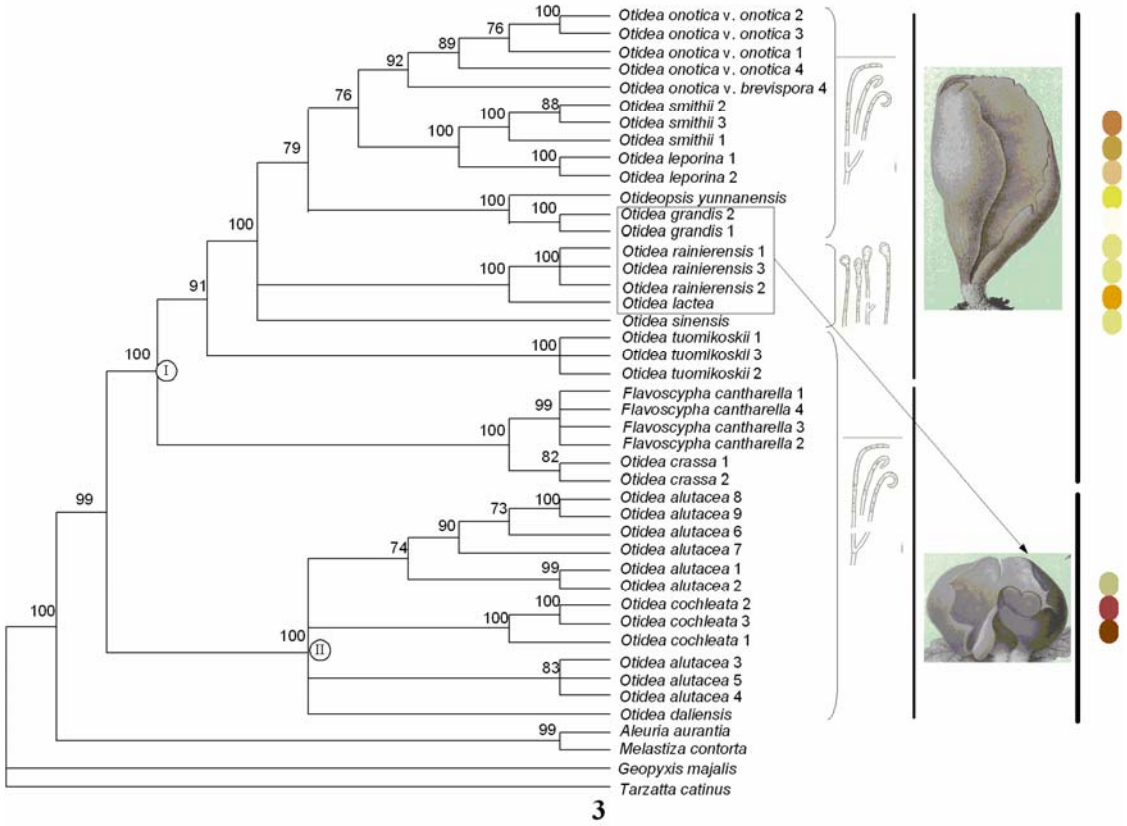
Geopyxis majalis
Tarzatta catinus
2


Otidea onotica v. onotica 2
Otidea onotica v. onotica 3
Otidea onotica v. onotica 1
Otidea onotica v. onotica 4
Otidea onotica v. brevispora 4
Otidea smithii 2
Otidea smithii 3
Otidea smithii 1
Otidea leporina 1
Otidea leporina 2
Otideopsis yunnanensis
Otidea grandis 2
Otidea grandis 1
Otidea rainierensis 1
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Otidea lactea
Otidea sinensis
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Otidea alutacea 1
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Otidea cochleata 2
Otidea cochleata 3
Otidea cochleata 1
Otidea alutacea 3
Otidea alutacea 5
Otidea alutacea 4
Otidea daliensis
Aleuria aurantia
Melastiza contorta
Geopyxis majalis
Tarzatta catinus
3

with *Otidea* species in Clade I with 98% bootstrap support. *Otidea grandis* and *Otideopsis yunnanensis* grouped together with 98% bootstrap support, which are close to *O. onotica*, *O. smithii* and *O. leporina* with a low bootstrap support (50%). The remaining species, *O. daliensis*, *O. cochleata* and *O. alutacea*, formed the second Clade (Clade II) with 76% bootstrap support. Within Clade II, *O. cochleata* and *O. alutacea* received a strong support of 100%, whereas, *O. daliensis* joined them with a relatively weak support (76%).

The Maximum parsimony analysis yielded 8 equally parsimonious trees and the strict consensus tree is shown in Fig. 2 (Tree length = 487, Consistency Index = 0.657, Homoplasy Index = 0.3429, Retention Index = 0.8586). Two clades (Clades I, II) were recognized with 92% bootstrap support, which are supported respectively by 99% (Clade I) and 80% (Clade II). The components of species in each clade are exactly the same as that shown in the Neighbor joining tree. A higher support was given to *Otidea* and its closely related taxa of *Otideopsis* and *Flavoscypha* in the Maximum parsimony tree than in the Neighbor joining tree (92% vs. 87%). A different tree topology was detected between the Neighbor joining tree and Maximum parsimony tree among *O. alutacea* from different sources.

Most major clustering patterns revealed by the Bayesian inference tree were similar to those in the Neighbor joining tree. The Bayesian tree agreed with separation of the two clades (Clades I, II) with 99% bootstrap value (Fig. 3).

Discussion

Recognition of a monophyletic Otidea

The main morphological characters, apothecia shape and hymenium colour, shape of paraphysis apices and spore surface morphology, have long been used as criteria to distinguish species of *Otidea* in traditional taxonomy (Kanouse, 1949; Dennis, 1968; Cao *et al*., 1990).

Phylogenetic analysis of 28S rDNA sequences suggest that *Otidea* is a monophyletic genus, which is indicated by the high bootstrap support (87%, 92% and 99%) in the three phylogenetic trees in which two sister groups (Clades I, II) exist. Clade I comprises taxa possessing the ear-shaped, broadly ear-shaped or spoon-shaped, or less commonly truncate apothecia. It is represented by the lectotype species of the genus, *O. onotica*, and most taxa investigated, *O. leporina*, *O. smithii*, *O. grandis*, *Otideopsis yunnanensis*, *O. sinensis*, *O. lactea*, *O. rainierensis*, *O. tuomikoskii* and *O. crassa*, as well as *Flavoscypha cantharella*. The relationship between *O. onotica* and the other two species sharing similar apothecial shape, *O. leporina* and *O. smithii*, lacks

good bootstrap support (Figs 1-2); only the Bayesian inference tree obtains a 92% bootstrap value (Fig. 3). Those with truncate to discoid apothecia, including *O. alutacea*, *O. cochleata* and *O. daliensis*, group together and form the Clade II with 76%, 80% and 100% bootstrap values (Figs 1-3). In general, apothecial shape is a reliable character at species level in taxonomy.

Apothecial colour is a relatively stable feature at the species level. *Otidea onotica* and taxa with yellow pigmentation, except for *O. lactea* in which the apothecia are cream-coloured, form the Clade I. The colour of the hymenium is a diagnostic feature which distinguishes *O. leporina* with dull yellow-brown to brown-yellow hymenium and concolorous exterior from *O. smithii* having deep vinaceous brown hymenium. Of the two species having very similar apothecial shape, *O. alutacea* with woody brown apothecia can easily be separated from *O. cochleata* of dark-coloured fruitbodies. *Otidea onotica* var. *onotica* possessing yellowish to dull orange hymenium with rosy tints is consistent in different collections. Closely associated with this variety, *O. onotica* var. *brevispora* is similar in many aspects, except for the smaller ascospores.

Otidea lactea, *O. rainierensis* and *O. sinensis* sharing capitate paraphysis apices are associated. *Otidea sinensis* clusters with the former two species with a relative low support (56%) in the Neighbor joining tree and with bootstrap values below 50% in both the Maximum parsimony tree and Bayesian tree (Figs 1-3). Paraphysis shape at apical portion is a stable feature at species level and a useful character in taxonomy, but seems not phylogenetically important.

In accordance with the morphological features, our molecular data support strongly the placements of *Otideopsis yunnanensis* and *Flavoscypha cantharella* in the genus *Otidea*. The former species is close to *O. grandis* and the latter is related to *O. crassa*. The close relationship between *O. grandis* and *O. yunnanensis* has strong support (98%, 98% and 100%) and is first revealed in this study. Species of *Otidea* are mostly smooth-spored, as defined by Dennis (1968), however, these two are the only known species of the genus possessing ornamented ascospores, a yellowish hymenium, and a brown exterior. *Otidea grandis* has truncate apothecia and *O. yunnanensis* has spoon-shaped to more or less truncate ones. Spore surface morphology is consistent at species level and possibly of phylogenetic value. *Otidea cantharella* and *O. crassa* are closely related (Figs 1-3) and congeneric, their differences in spore shape and size, hymenium colour, and apothecial shape are interspecific variations.

What we currently call *O. alutacea* is possibly a species complex. Samples under that name from different sources are together with diverse bootstrap support (Figs 1-3). *Otidea daliensis* with typical discoid and very dark apothecia, even though grouped with *O. alutacea* and *O. cochleata* having truncate fruitbodies, is possibly not closely related to the latter species.

Placement of Otideopsis yunnanensis

Otidea grandis and *Otideopsis yunnanensis*, the type species of the genus *Otideopsis*, group together in Clade I with very high bootstrap support (Figs 1-3). These two species are more closely related to each other than to any other *Otidea* species used in this study. When the morphology of *Otideopsis* and *Otidea* are compared, the only character which distinguishes *Otideopsis* from most species of *Otidea* is the ornamented ascospores; whereas, the presence of fine ornamentation on the spore surface has already been shown in *O. grandis* (Kanouse, 1949), which was obviously overlooked by the authors when the genus was established (Liu and Cao, 1987). Both species share common morphological features, such as ascospores with ornamentation (as compared to smooth-walled in other *Otidea* species), hymenium with a yellow tint and dark-coloured receptacle surface (Figs 4-5), strongly pustulate (Figs 6-7), and similarly curved paraphysis apices. What distinguishes the two taxa are spore size; shape of spore markings, as irregular crests in *O. grandis* and spines in *O. yunnanensis* (Figs 8-9); number of spore guttules, biguttulate in the former and with two or more guttules in the latter; and length of apothecial stipes, short in *O. grandis* and longer in *O. yunnanensis* (Figs 4-5). In our opinion, the above distinctions should be treated at species level instead of generic rank.

Low support at the node connected with other *Otidea* taxa, ranging from below 50% in the Neighbor joining tree and Maximum parsimony tree to 79% in Bayesian tree (Figs 1-3), indicates separation of *O. grandis* and *O. yunnanensis* from the other species of *Otidea*. When morphological features are concerned, these two species appear to be the only species in the genus producing spore ornamentations and having a bright-coloured hymenium and very contrasting, dark receptacle surface. We distinguish them at subgeneric rank. A new taxonomic status for *Otideopsis* is here proposed.

Otidea subgenus ***Otideopsis*** (B. Liu & J.Z. Cao) W.Y. Zhuang & C.Y. Liu, **stat. nov.**

≡ *Otideopsis* B. Liu & J.Z. Cao, Journ. Shanxi Univ. 1987(4): 70, 1987.

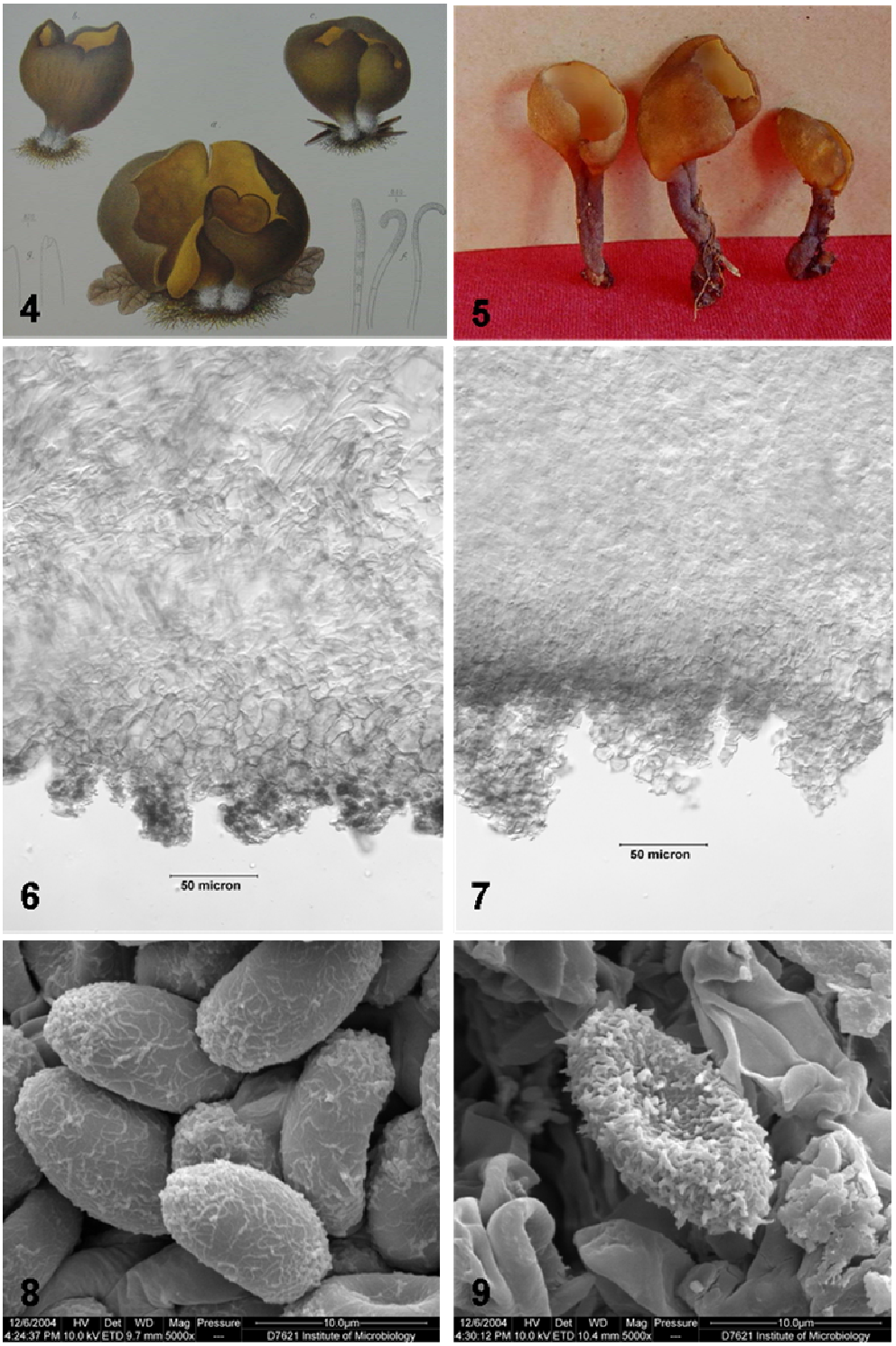
Type species: *Otideopsis yunnanensis* B. Liu & J.Z. Cao.

Currently recognized taxa of *Otidea* subgenus *Otideopsis*:

Otidea grandis (Pers.) Rehm

Otidea yunnanensis (B. Liu & J.Z. Cao) W.Y. Zhuang & C.Y. Liu, **comb. nov.**

≡ *Otideopsis yunnanensis* B. Liu & J.Z. Cao, Journ. Shanxi Univ. 1987(4): 70, 1987.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Figs 4-9. *Otidea* spp. **4.** Apothecia of *O. grandis*, from Boudier (1905-1911). **5.** Apothecia of *O. yunnanensis* (photographed by Z.L. Yang). **6.** Ectal excipular structure of *O. grandis*. **7.** Ectal excipular structure of *O. yunnanensis*. **8.** Spore surface morphology (SEM) of *O. grandis*. **9.** Spore surface morphology (SEM) of *O. yunnanensis*.

Circumscription of Otidea yunnanensis

The genus *Otideopsis* typified by *O. yunnanensis* was established based on a combination of the following characteristics: apothecia more or less otideoid, lack of carotinoid, excipulum of two layers, ectal excipulum of *textura globulosa* to *textura angularis*, medullary excipulum of *textura intricata*; asci subcylindrical, J– in Melzer's reagent; ascospores ellipsoid, guttulate and spinulose; and paraphyses filiform, "fusion at apical portion" but not forming epithecium (Liu and Cao, 1987). According to the original authors, *Otideopsis* can be distinguished from *Otidea* by the ornamented ascospores and paraphyses fusion at apex (Liu and Cao, 1987). When *O. yunnanensis* was chosen for the phylogenetic analysis, the morphology of the fungus was also studied in detail. Re-examination of type specimen of the species (HKAS 12150) and a recent collection from Lushui, Yunnan, China (HMAS 82166) revealed that the original description and illustration of the genus and *O. yunnanensis* needs emending. The receptacle surface of the fungus is not smooth as illustrated by the original authors but strongly pustulate. The paraphyses do not fuse at apical portion but are more or less aggregated with free ends.

Otidea yunnanensis

Apothecia broad fan-shaped to somewhat discoid with one side split down, medium- to long-stipitate, 2.5-4.5 cm high, 0.8-2 cm in diam, hymenium surface cream to yellow when fresh, receptacle surface brownish when fresh, stipe dark brown to blackish, 1.5-2.5 × 0.3-0.6 cm; ectal excipulum of *textura angularis*, surface strongly postulate, 64-77 μm thick excluding pustules, cells subhyaline to hyaline, pustules 25-75 μm high, cells in pustules light brown; medullary excipulum of *textura intricata*, 200-300 μm thick; subhymenium ca 50 μm thick; hymenium ca 255 μm thick; asci 8-spored, subcylindrical, J- in Melzer's reagent, ca 220-250 × 9.5-12.5 μm wide; ascospores ellipsoid, with spines on surface, with 2 large guttules and several small ones, uniseriate, 16.5-20 × 7.6-10 μm; paraphyses filiform and curved at apex, 2.5-3.8 μm wide at apex and 1.5-2 μm below.

Material examined : CHINA, Yunnan, Dulongjiang, on soil in forest, altitude 2200 m, D.C. Zhang, 30 August 1982, HKAS 12150 (holotype); Yunnan, Lushui, on soil in forest, altitude 2200 m, Z.L. Yang 3884, 27 July 2003, HMAS 82166.

Taxonomic position of Flavoscypha

Flavoscypha cantharella [≡ *Otidea cantharella* (Fr.) Quél., = *Otidea concinna*, (Häffner, 1994)] was excluded from *Otidea* and transferred to a new genus *Flavoscypha* Harmaja based on the "*Helvella*-like" ectal excipulum structure and presence of bright yellow pigmentation in apothecia (Harmaja, 1974). Relationships between *Flavoscypha* and *Otidea* are investigated in this study. Our results do not support the separation of *F. cantharella*, type species of *Flavoscypha*, from *Otidea*. Its position in Clade I of our phylogenic trees and its close-relation to *O. crassa* is well supported. The morphological character, "a deviating structure of the ectal excipulum of *Flavoscypha* somewhat similar to that of *Helvella*" stated by Harmaja (1974), is not a reliable feature at generic level since diverse structures have been found in taxa of *Otidea*. Structures of ectal excipulum of the genus vary from *textura angularis* in most species, through the small-celled *textura angularis* growing towards outside to form the club-shaped cells in *O. cochleata* (Zhuang, 2006), to the *Helvella*-like ectal excipulum in *O. felina* Pers. (Boudier, 1905-1910). Furthermore, the so-called "*Flavoscypha* ectal excipular structure" is not always associated with the bright-yellow pigmentation in fruitbodies. For example, the ectal excipular structure of *O. felina* is *Flavoscypha*-like, however, the apothecia colour of the fungus is not bright-yellow, but brown (Boudier, 1905-1910). *Flavoscypha* should be synonymized with *Otidea*.

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