Molecular phylogeny of eastern Asian species of *Amanita* (*Agaricales*, *Basidiomycota*): taxonomic and biogeographic implications

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The phylogenetic relationships, taxonomy and biogeography of the genus *Amanita* with emphasis on eastern Asian species were estimated using sequence data from both internal transcribed spacer (ITS) regions and large subunit (nLSU) of nuclear ribosomal DNA. The separation of the two traditionally accepted subgenera was not well supported. Each section of *Amanita*, *Vaginatae*, *Caesareae*, *Phalloideae*, *Validae* and *Amidella* was supported as monophyletic by different methods of analysis and data sets of different regions. The monophyly of section *Lepidella* remained unclear. A few biogeographic and taxonomic implications were inferred: (1) few species of *Amanita* are widely distributed throughout eastern Asia, Europe and North America. Samples of some previously recognized disjunct species in the Northern Hemisphere were not monophyletic. Thus, the putative intercontinental disjunct distributions of these species were not supported in this study; (2) biogeographic relationships between *Amanita* of eastern Asia and Europe are relatively close and several taxa are common to both regions, while paired or closely related species between eastern Asia and North America are relatively common, but rarely have been confirmed as disjunct populations of the same species by molecular data yet. A number of species of *Amanita* in North America labelled with names based on European materials should be regarded as distinct species; (3) a few genetically cryptic species of *Amanita* in southwestern China need to be delimited; and (4) variations in colour and morphology of the fruit bodies in *A. parvipantherina* from different localities should be interpreted as modifications of environment.

Key words: *Amanita*, biodiversity, distribution, ITS, nLSU, phylogeny.

Introduction

Amanita is one of the better-known genera of basidiomycetes. Since Persoon established the genus in 1797, some infrageneric classifications based

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on morphology were proposed (e.g. Gilbert and Kuehner, 1928; Konrad and Maublanc, 1948; Singer, 1951, 1986; Corner and Bas, 1962; Moser, 1967; Bas, 1969; Yang, 1997). Molecular phylogeny studies on *Amanita* have been carried out based on ribosomal DNA sequence data from the large subunit (nLSU) of nuclear ribosomal DNA (Weiß *et al*., 1998; Drehmel *et al*., 1999) or from the internal transcribed spacer (ITS) region alone (Oda *et al*., 1999). Most studies supported the separation of the genus into two subgenera, *Amanita* and *Lepidella* (J.E. Gilbert) Veselý, in correspondence with spore amyloidity and other morphological and anatomic characters. However, these studies were inconsistent in their use of infrageneric classifications. For example, Weiß *et al*. (1998) separated the genus into two subgenera including seven sections, while Drehmel *et al*. (1999) divided the genus *Amanita* into two subgenera consisting of four sections covering seven subsections.

Most taxa of *Amanita* are known or suspected to form symbiotic associations with many trees or shrubs (e.g. Melin, 1925; Hacskaylo and Palmer, 1955; Bas, 1969; Singer, 1986; Cripps and Miller, 1995; Yang *et al*., 1999, 2000). Thus, data on distribution patterns of this genus will offer significant clues for understanding the evolution of this genus and the relationship between distributions of *Amanita* and their associated plants. In literature and herbarium records, many species of *Amanita* endemic to eastern Asia were labelled as names of American or European morphologically similar species (Yang and Doi, 1999; Yang, 2000a; Yang and Li, 2001; Yang *et al*., 2001; Yang, 2002; Yang and Zhang, 2002). Particularly in southwestern China (abbreviated as SW China), where the topography has been greatly changed in past geological times, various climates and soil types provide a wide range of environmental conditions for the growth of a great number of vegetation types. Thus, species of *Amanita* in SW China are abundant and diverse. Being dominant ectomycorrhizal partners with coniferous trees, for instance, *Suillus spraguei* from SW China has already been inferred geographically distinct (Wu *et al*., 2000). Whether species of *Amanita* in SW China and, in a broader range, in East Asia are distinctive have not been elucidated by DNA sequence date. Biogeographic relationships between *Amanita* of eastern Asia and other regions of the Northern Hemisphere based on molecular data have never been assessed before.

The aim of this study is to determine phylogenetic relationships of *Amanita* based on ribosomal DNA sequence from both the internal transcribed spacer (ITS) region and the large subunit (nLSU) of nuclear ribosomal DNA and try to shed new light on the taxonomy and biogeography of species of *Amanita* in eastern Asia at the same time.

Materials and methods

Taxon sampling

Specimens for DNA analysis were selected to include species of all sections of *Amanita* (Yang, 1997). Special emphasis was made on examples with morphological similarities and/or putative disjunctive distributions in the Northern Hemisphere. Four collections of *A. parvipantherina* with variations in colour and morphology were included in the analysis. Samples sequenced in this study were deposited in the Cryptogamic Herbarium of Kunming Institute of Botany of the Chinese Academy of Sciences (HKAS). The geographic localities and GenBank accession numbers of the samples together with scientific name were listed in Table 1. Other sequences were retrieved from GenBank, and their geographic localities and the GenBank numbers were listed in Table 2. Approximately half of the samples analyzed were collected from SW China. The corresponding author identified samples sequenced in the study. The concept of *A. manginiana sensu* W.F. Chiu follows that of Yang (1997). *Amanita pantherina* var. *lutea* W.F. Chiu (AF024468/HKAS 29627) is a synonym of *A. parvipantherina* (unpublished data of Yang). Names for two of the species are unavailable: they were labelled as *Amanita* sp. 1 and *A.* sp. 2. Authority names of subgenera, sections and of all lower levels are mentioned when used the first time except those available in Tables 1.

DNA isolation

Total DNA was obtained directly from dried specimens using a modified CTAB procedure of Doyle and Doyle (1987).

PCR amplification

The primers ITS4 and ITS5 (White *et al*., 1990) were used for amplification of the ITS region. Amplification primers for nLSU included LROR and LR6. Reaction volumes were 20 µl and contained 1.5 U of AmpliTaq DNA polymerase (Perkin-Elmer), Replitherm TM buffer, 1.5 mmol L^{-1} $MgCl_2$, 0.4 mmol L^{-1} dNTP, 0.1 µmol L^{-1} primer, 25-60 ng sample DNA. PCR was performed in a GeneAmp 9600 thermal cycler (Perkin-Elmer, Applied Biosystems). Cycling conditions were set as follows: initial denaturation at 97ºC for 4 min, 35 cycles of 30s at 94ºC, 1 min at 52ºC (in some cases at 55ºC), 1 min at 72ºC, and a final extension of 5 min at 72ºC. PCR amplification of nLSU followed the method of Weiß *et al*. (1998). PCR

Table 1. List of samples sequenced in this study.

Scientific Name	HKAS No.	Locality	GenBank accession No.	
			ITS	nLSU
Amanita altipes Zhu L. Yang *et al*.	*36609	Yunnan, China	AY436445	AY436487
A. atrofusca Zhu L. Yang	36610	Yunnan, China	AY436446	—
A. avellaneosquamosa (S. Imai) S. Imai	38300	Yunnan, China	AY436447	—
A. chepangiana Tulloss et Bhandary	34218	Sichuan, China	AY436450	—
A. aff. *citrina* (Schaeff.) Pers.	34170	Sichuan, China	AY436449	—
A. aff. *crocea* (Quél.) Singer	38461	Yunnan, China	—	AY436490
A. esculenta Hongo et I. Matsuda	34169	Sichuan, China	AY436451	—
A. excelsa (Fr.) Bertillon	31510	Tübingen, Germany	AY436453	AY436491
A. aff. *excelsa* (Fr.) Bertillon	32451	Sichuan, China	AY436452	—
A. exitialis Zhu L. Yang et T.H. Li	38162	Guangdong, China	AY436454	AY436492
A. flavipes S. Imai	36582	Yunnan, China	AY436455	—
A. flavoconia G.F. Atk.	34047	New Hampshire, USA	AY436456	—
A. fritillaria (Berk.) Sacc.	38331	Yunnan, China	AY436457	—
A. fuliginea Hongo	38129	Hunan, China	AY436458	—
A. griseofolia Zhu L. Yang	*38159	Yunnan, China	AY436448	AY436488
A. hemibapha (Berk. et Broome) Sacc.	38416	Yunnan, China	AY436460	—
A. jacksonii Pomerl.	34041	New Hampshire, USA	AY436461	—
A. liquii Zhu L. Yang *et al*.	*36611	Yunnan, China	AY436462	AY436493
A. manginiana sensu W.F. Chiu	38460	Yunnan, China	AY436463	—
A. orientifulva Zhu L. Yang *et al*.	*32522	Yunnan, China	AY436464	—
A. orientigemmata Zhu L. Yang et Y. Doi	38345	Yunnan, China	AY436465	AY436497
A. cf. *pantherina* (DC.: Fr.) Krombh.	26746	Yunnan, China	AY436466	—
A. parvipantherina Zhu L. Yang *et al*.	38297	Yunnan, China	AY436467	AY436499
A. parvipantherina Zhu L. Yang *et al*.	38340	Yunnan, China	AY436468	—
A. parvipantherina Zhu L. Yang *et al*.	38334	Yunnan, China	AY436469	AY436498
A. porphyria (Alb. et Schw.: Fr.) Fr.	31531	Schwarzwald, Germany	AY436471	AY436500
A. pseudogemmata Hongo	38371	Yunnan, China	AY436472	—
A. pseudovaginata Hongo	38323	Yunnan, China	AY436470	—
A. sepiacea S. Imai	38716	Yunnan, China	AY436473	AY436501
A. solitaria (Bull.: Fr.) Fr.	31459	Tübingen, Germany	AY436475	—
A. subfrostiana Zhu L. Yang	34551	Yunnan, China	AY436476	—
A. subjunquillea var. *alba* Zhu L. Yang	32665	Yunnan, China	AY436477	—
A. umbrinolutea (Gillet) Bataille	31451	Tübingen, Germany	AY436478	—
A. yuaniana Zhu L. Yang	29516	Yunnan, China	AY436479	—
A. sp. 1	38419	Yunnan, China	AY436474	AY436502
A. sp. 2	32523	Sichuan, China	AY436459	AY436494
Limacella glioderma (Fr.) Maire	31576	Tübingen, Germany	AY436480	—

Note: *Type material.

Table 2. List of samples whose sequences retrieved from GenBank.

Scientific name	Locality	Accession No. of ITS	Scientific name	Locality	Accession No. of nLSU	Scientific name	Locality	Accession No. of nLSU
Amanita abrupta	Japan	AB015685	*A. avellaneosquamosa*	China	AF024441	*A. longistriata*	Japan	AF024462
A. ceciliae	Japan	AB015694	*A. bisporigera*	USA	AF097385	*A. muscaria*	Germany	AF024465
A. citrina	Japan	AB015679	*A. brunnescens*	USA	AF097679	*A. muscaria*	USA	AF042643
A. citrina var. *grisea*	Japan	AB015680	*A. caesarea*	Italy	AF024443	*A. muscaria* var. *persicina*	USA	AF097367
A. flavipes	Japan	AB015696	*A. ceciliae*	France	AF024444	*A. pantherina*	Holland	AF024467
A. fulva	Japan	AB015692	*A. ceciliae*	USA	AF097372	*A. parvipantherina*	China	AF024468
A. hemibapha	Japan	AB015699	*A. chepangiana*	China	AF024445	*A. peckiana*	USA	AF042608
A. japonica	Japan	AB015684	*A. citrina*	German y	AF024446	*A. phalloides*	Germany	AF024469
A. longistriata	Japan	AB015678	*A. citrina*	USA	AF097377	*A. rhoadsii*	USA	AF097391
A. muscaria	Japan	AB015700	*A. citrina*	USA	AF097378	*A. rhopalopus*	USA	AF097393
A. pantherina	Japan	AB015701	*A. clarisquamosa*	China	AF024448	*A. solitaria*	Germany	AF024475
A. porphyria	Japan	AB015677	*A. cokeri*	USA	AF097399	*A. solitariiformis*	USA	AF097390
A. pseudoporphyria	Japan	AB015702	*A. excelsa*	German y	AF024449	*A. subglobosa*	China	AF024478
A. rubescens	Japan	AB015682	*A. fuliginea*	China	AF024454	*A. subjunquillea* var. *alba*	China	AF024479
A. rubrovolvata	Japan	AB015689	*A. fulva*	German y	AF024455	*A. vaginata*	Holland	AF024482
A. sp.	Japan	AB015687	*A. fulva*	USA	AF097373	*A. vaginata*	USA	AF097375
A. sychnopyramis f. *subannulata*	Japan	AB015690	*A. gemmata*	Holland	AF024457	*A. virgineoides*	China	AF024484
A. vaginata	Japan	AB015691	*A. gemmata*	USA	AF097371	*A. volvata*	USA	AF097388
A. virgineoides	Japan	AB015686	*A. hemibapha* var. *ochracea*	China	AF024458	*A. yuaniana*	China	AF024488
A. virosa	Japan	AB015676	*A. jacksonii*	USA	AF097376	*Limacella glishra*	USA	U85301
A. volvata	Japan	AB015681	*A. japonica*	China	AF024460	*L. glioderma*	Germany	AF024489

Note: Data of ITS are from Oda *et al.* (1999). Data of nLSU with accession number 'AF02' initial are from Weiß *et al.* (1998), with 'AF09' or 'AF04' initials and U85301 are from Drehmel *et al.* (1999).

products were purified with Watson's purification kit (Watson, China) prior to being sequenced.

DNA sequencing

The purified PCR products were sequenced in an ABI PRISM Bigdye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS (Perkin-Elmer, Norfolk, Connecticut). Reactions and programs were chosen according to recommendation of the handbook, with slight modification in some cases. Same primers as described above for PCR were used for the sequencing reactions.

Alignment and phylogenetic analyses

DNA sequences were edited and aligned with SeqMan and Megalign (DNASTAR Package), and manually modified where necessary. Ambiguous positions were excluded from the matrix. Gaps were treated as missing data. All unambiguous characters and character-transformations were weighted equally. Neighbor-joining (NJ) analysis and maximum-parsimony (MP) analysis were performed with PAUP version 4.0b10 (Swofford, 2003) for the ITS and the nLSU data sets respectively. All of the trees were obtained by running the heuristic search with tree-bisection-reconnection (TBR) branch swapping and up to 1000 random-addition sequence replications. To assess the relative support for each clade, bootstrap values were calculated from 100 replicate analyses with the heuristic search strategy and random addition sequence of the taxa.

Results

Analysis of the ITS data set

Limacella glioderma was included and designated as outgroup. ITS sequence data of 58 samples were analyzed. The final alignment of ITS sequences consisted of 732 bp. The genetic divergence within ingroup ranged from 0 to 38 percent.

MP analysis

In maximum parsimony analysis, 149 characters were constant, 39 were variable, and 544 were parsimony-informative. The strict consensus of 60 most

parsimony trees of 2880 steps, CI = 0.4417, RI = 0.7239, RC = 0.3197, was shown with bootstrap values (≥ 50%) in Fig. 1.

The results suggested the major clade 1A within *Amanita* contained one weekly supported clade (3A), which corresponded to subgenus *Amanita*. Within subgenus *Amanita*, except *A. pseudogemmata*, which is very distinct morphologically from other species within the section *Amanita* by its truncate to subtruncate bulb on the stipe base, other members were well supported as a monophyletic clade (4A). Within clade 4A, two well-supported subclades were resolved: one subclade (5A) corresponded to section *Amanita*; the other contained two subclades (6A and 6B), which corresponded to section *Vaginatae* (Fr.) Quél. and section *Caesareae* Singer ex Singer respectively. Within subclade 6A, three species, *A. ceciliae* (Japan), *A. griseofolia* and *A. liquii*, possess a friable volva, cluster together with a bootstrap value of 78 percent. Clade 1B and 2C corresponded to sections *Amidella* (J.-E. Gilbert) Konrad et Maubl. and *Phalloideae* (Fr.) Quél., and both were strongly supported. Clade 2B, which corresponded to section *Validae* (Fr.) Quél., contained one moderate supported subclade (8A) and one well supported subclade (8B). Clade 3B contained one poorly supported subclade (7A), which corresponded to section *Lepidella*, and one well-supported subclade (7B), which consisted of the species pair *A. manginiana sensu* W.F. Chiu and *A. pseudoporphyria* of the section *Phalloideae*.

The material of *Amanita* from SW China showed genetic divergence from those with morphological similarities from Europe or North America, and even from Japan. Example pairs were: *Amanita* aff. *excelsa* (SW China) and *A. excelsa* (Germany); *A. flavipes* (SW China) and *A. flavipes* (Japan); *A.* aff. *citrina* (SW China) and *A. citrina* (Germany). The sister relationship of *A.* aff. *citrina* (SW China) and *A. citrina* (Japan) was well-supported; and the two formed a monophyletic clade with *A. citrina* from Germany with a bootstrap value of 94 percent. Three samples of *A. parvipantherina* were significantly joined together, and their sequences were nearly the same.

NJ analysis

Neighbor-joining analysis of sequences yielded a topology (Fig. 2) similar to that from the MP analysis. The NJ tree differed from MP tree (Fig. 1) in the following aspects. First, including *A. pseudogemmata*, all the species of section *Amanita* formed a weekly supported clade (5A); Secondly, within the section *Vaginatae*, three species, *A. ceciliae* (Japan), *A. griseofolia* and *A. liquii*, failed to cluster together. Thirdly, the *Phalloideae* species pair *A. manginiana sensu* W.F. Chiu and *A. pseudoporphyria* together with other

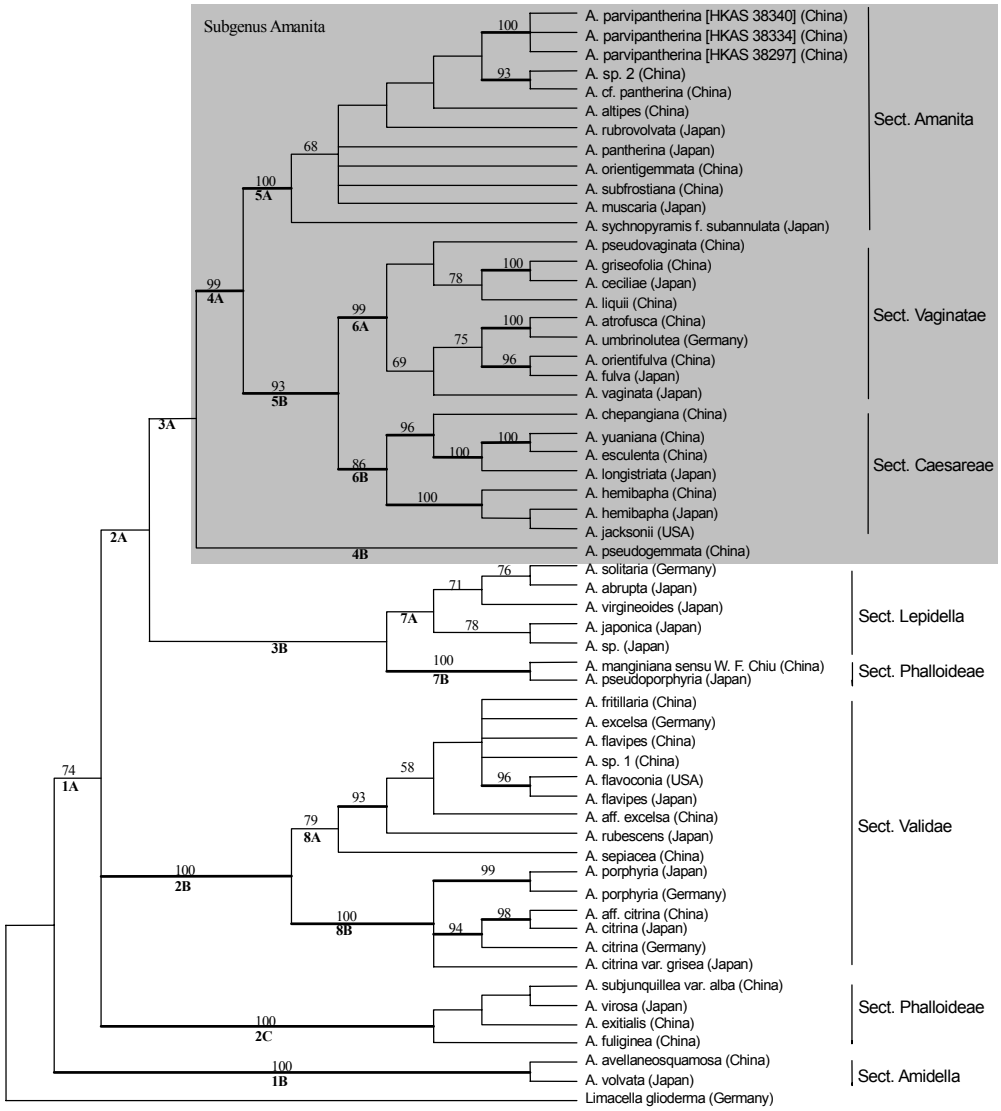

Fig. 1. Strict consensus of the most parsimonious trees generated for the genus *Amanita* based on ITS sequence data. Numbers above each internode are the percentage of 1000 bootstrap replicas supporting that binary partition (value ≥ 50%). The localities of specimens are showed after the Latin names.

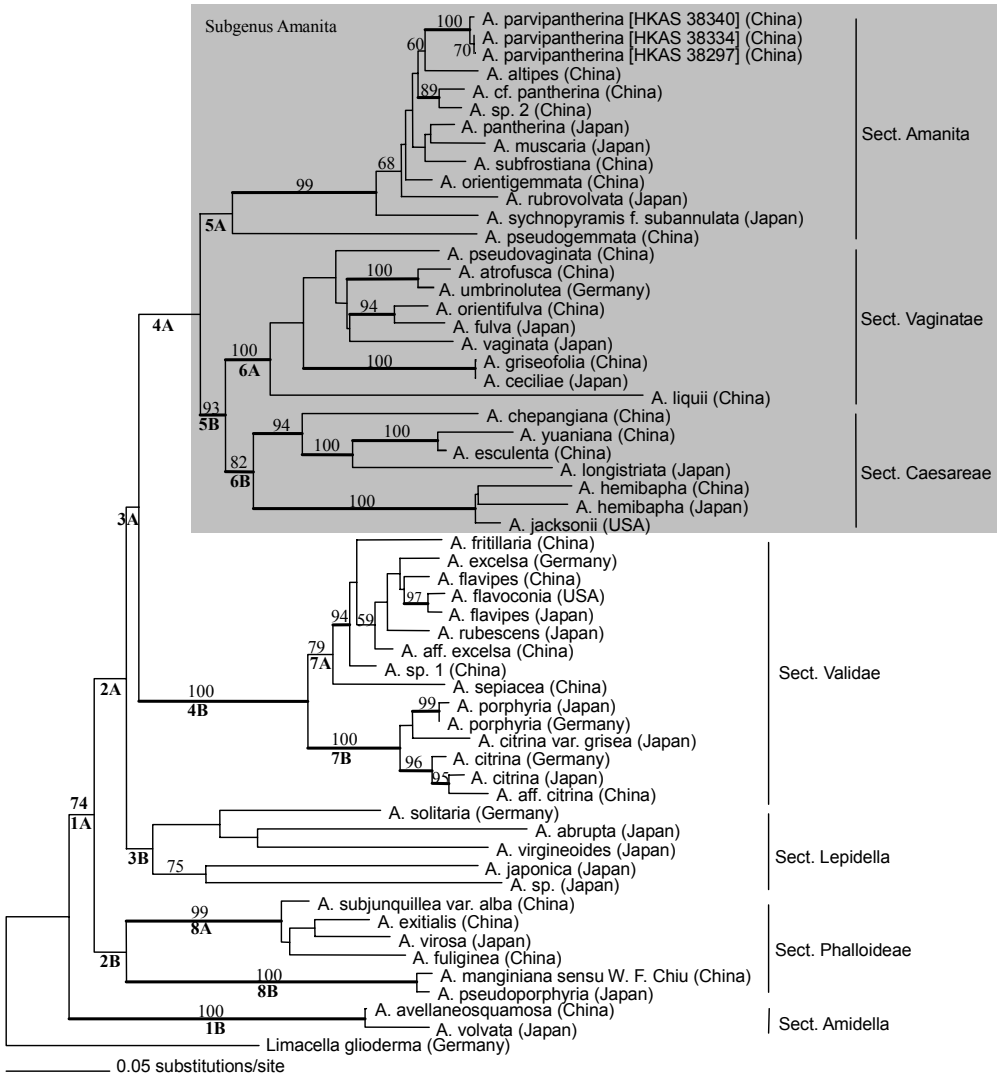

Fig. 2. Neighbor-joining tree generated for the genus *Amanita* based on ITS sequence data. Numbers above each internode are the percentage of 1000 bootstrap replicas supporting that binary partition (value ≥ 50%). The localities of specimens are showed after the Latin names.

members of section *Phalloideae* were grouped as a clade but with poor support (less than 50% bootstrap).

Analysis of the nLSU data set

The nLSU data of *Limacella glioderma* and *L. glishra* as outgroups were analyzed together with the nLSU sequences of *Amanita*. After excluded the ambiguous positions, the data matrix of nLSU region consisted of 902 characters. Sequence divergence within ingroup ranged from 0 to 13 percent. In maximum parsimony analysis, 590 characters were constant, 95 were variable, and 217 were parsimony-informative characters. Parsimony analysis yielded 12 most parsimonious trees (tree length = 988 steps, CI = 0.4109, RI = 0.6983, RC = 0.2869). Bootstrap frequencies less than 50% were not shown.

Parsimony analysis of the nLSU data set (Fig. 3) weakly supported the monophyly of the two major clades (1A and 1B) within *Amanita*, which correspond to subgenera *Amanita* and *Lepidella* as recognized by others (Weiß *et al*., 1998; Drehmel *et al*., 1999).

Within subgenus *Amanita*, three clades (2A, 4A and 4B) corresponding to the sections *Amanita*, *Vaginatae* and *Caesareae* were well-supported, and sections *Vaginatae* and *Caesareae* formed sister groups with a low bootstrap value.

Two weakly supported clades (5A and 5B) were recovered in subgenus *Lepidella*: one of which corresponds to section *Lepidella* (clade 5B); the other three sections, *Validae* (clade 7A), *Phalloideae* (clade 7B) and *Amidella* (clade 6B), grouped together. Different from the analyses of ITS region, sections *Validae* and *Phalloideae* formed sister groups linking with *Amidella* at the base. The monophyly of sections *Validae*, *Phalloideae* and *Amidella* was well supported.

Most of the samples from Europe and North America under the same names were poorly paired [viz., *A. gemmata* (Holland) and *A. gemmata* (USA); *A. ceciliae* (France) and *A. ceciliae* (USA); *A. fulva* (Germany) and *A. fulva* (USA); *A. vaginata* (Holland) and *A. vaginata* (USA); *A. citrina* (Germany) and *A. citrina* (USA)]. Same as the analyses of ITS region, three samples of *A. parvipantherina* formed a close cluster.

The topologies of neighbor joining tree (Fig. 4) and maximum likelihood tree (not shown) were similar to that of parsimony. The distinct discrepancy was that sections *Vaginatae* and *Caesareae* were not sister group in the neighbor joining tree.

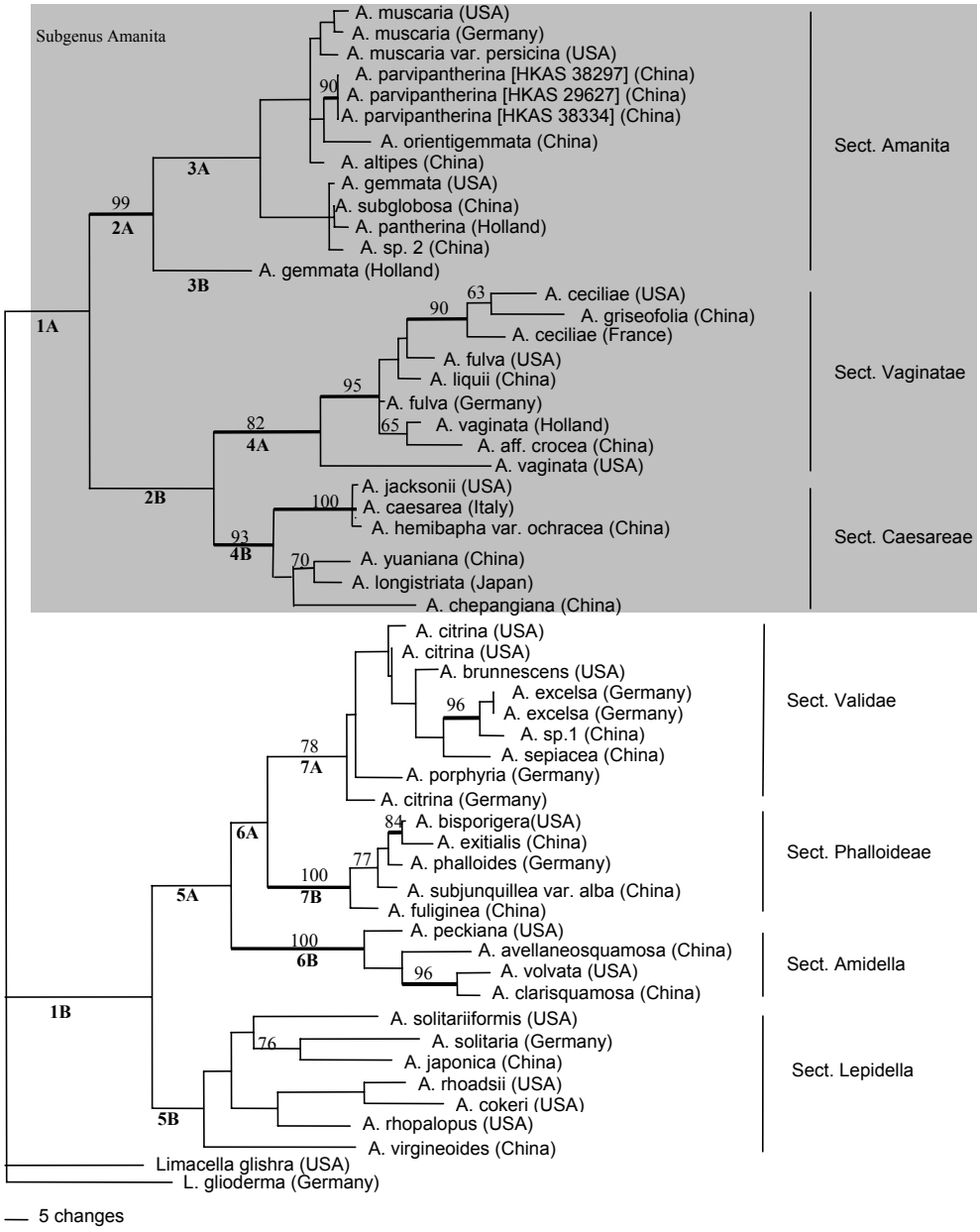

Fig. 3. Phylogram showed one of 12 most parsimonious trees of *Amanita* resulting from phylogenetic analysis of nLSU data. Numbers above each internode are the percentage of 1000 bootstrap replicas supporting that binary partition (value $\geq$ 50%). The localities of specimens are showed after the Latin names.

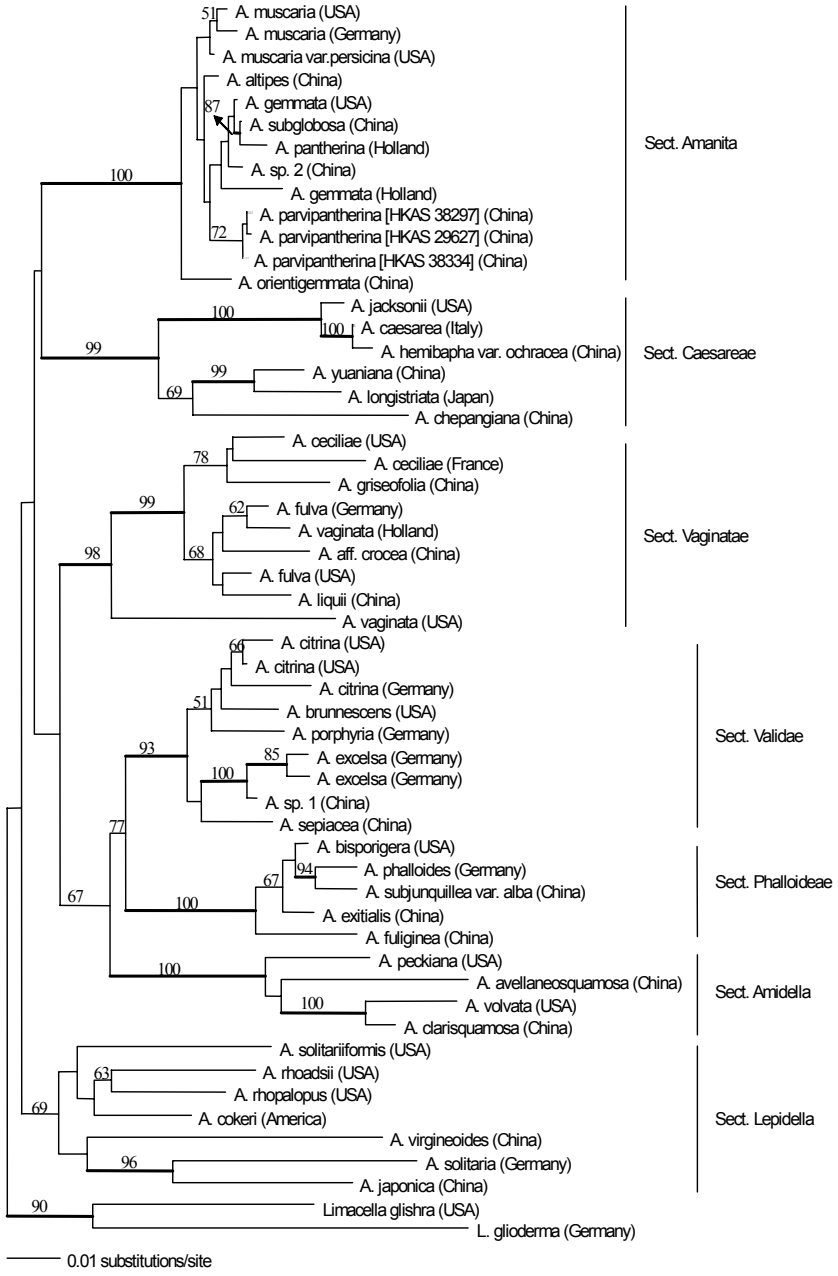

Fig. 4. Neighbor-joining tree of *Amanita* inferred from nLSU sequence data. Numbers above branches are bootstrap values. The localities of specimens are showed after the Latin names.

Discussion

Phylogeny of Amanita

No strong support on the taxonomic distinction of the two subgenera *Amanita* and *Lepidella* were inferred by phylogenetic analyses of the ITS and nLSU regions. At the section level, the different types of analysis and the data sets of different segments yielded groupings generally consistent with the *Amanita* system of Yang (1997).

While there was evidence under the MP criterion showing close relationship between section *Vaginatae* and section *Caesareae*, each of the two clades as an individually monophyletic group was supported by an high bootstrap values, but there was no support between the two clades except in the MP and NJ analyses of the ITS region (Figs. 1, 2). In the works of Weiß *et al*. (1998), and Oda *et al*. (1999), the sections *Vaginatae* and *Caesareae* separated well. In Drehmel *et al*. (1999), the only one species of section *Caesareae*, *A. jacksonii*, was not well supported to be clustered with the other members of section *Vaginatae*. Moreover, the members of *Caesareae* are with annulus and clamp connections, while the species of *Vaginatae* are usually without the characters mentioned above. Thus, it seems reasonable to treat *Vaginatae* and *Caesareae* as two distinct sections. Tulloss (1994) reported a few species of section *Vaginatae* with clamps but without annulus. It would be interesting to include such species in the molecular phylogenetic analysis in the future (Weiß *et al*., 1998).

The monophylies of *Phalloideae* and *Amidella* were strongly supported by separate bootstrap analyses (Figs. 1-4). Although sections *Phalloideae* and *Amidella*, together with *Validae*, constituted a monophyletic group with a moderate support value in the Neighbor-joining analysis of nLSU sequence data (Fig. 4), such a relationship was not well supported in the parsimony analysis of the same data set (Fig. 3). In addition, *Phalloideae* and *Amidella* in the analyses of ITS sequence data were not supported as a monophyletic group (Figs. 1, 2). Taking the morphological features of the two groups into consideration, especially the attenuate lamellulae and the more or less bulbous base of the stipe with a limbate volva in *Phalloideae* in contrast to the truncate lamellulae and the non-bulbous base of the stipe with a saccate volva in *Amidella*, we are inclined to treat *Phalloideae* and *Amidella* as two separate sections.

The monophyly of section *Lepidella* was weakly supported in all the analyses. Therefore, this section might be a heterogeneous taxon as indicated only by nLSU data of three members of the section before (Weiß *et al*., 1998).

An example of the disjunct distribution of *Amanita* in the Northern Hemisphere is the well-known *A. muscaria*, which occurs widely in natural forests in Europe (e.g. Moser, 1967), North America (e.g. Jenkins, 1986) and temperate eastern Asia (e.g. Hongo, 1959). *Amanita hemibapha* from Japan and SW China and *A. jacksonii* constantly formed a well-supported monophyletic group in neighbor joining as well as in parsimony analysis of ITS dataset, and the homology of the ITS data of *A. hemibapha* (SW China) and *A. hemibapha* (Japan) is 95 percent, of *A. hemibapha* (Japan) and *A. jacksonii* is 95 percent, of *A. hemibapha* (SW China) and *A. jacksonii* is 93 percent. In both neighbor joining and parsimony analysis of nLSU dataset, *A. hemibapha* var. *ochracea*, *A. jacksonii* and *A. caesarea* were in one group, which supported by a high bootstrap value of 100 percent. The homology of the nLSU data of *A. jacksonii* and *A. caesarea* is 99 percent. All of *A. caesarea*, *A. hemibapha* and *A. jacksonii* have an orange or orange red pileus, a whitish stipe with yellow to orange squamules and a lobed saccate volva. In consideration of their morphological differentiations among the three taxa (Tulloss, 1998), they may be better regarded as different subspecies in different geographical locations.

By contraries, many other previously putative examples in *Amanita* turned out to be distinct taxa in different continents according to our molecular analysis. For example, *A. gemmata* was originally described from Europe (Fries, 1838), and then reported from North America (e.g. Coker, 1917; Jenkins, 1986) and eastern Asia (e.g. from Japan by Nagasawa and Hongo, 1985). Tulloss *et al*. (1995) pointed out that the name *A. gemmata* was applied to a number of distinct taxa in North America, and a part of *A. gemmata auct. amer*. from North America is *A. russuloides* (Peck) Sacc. Yang and Doi (1999) studied the material, on which *A. gemmata* was reported from Japan, and described it as a new species, viz. *A. orientigemmata*. Our molecular phylogenetic analysis showed that the so-called *A. gemmata* in North America and eastern Asia are distinct from that in Europe (Figs. 3, 4). Generally, the putative disjunct distributions of *Amanita* in the Northern Hemisphere were not well supported in this study. Analogous results for other groups of macrofungi were gained by Mueller *et al.* (2001). In the following, relationships between *Amanita* of SW China and Japan, of eastern Asia and Europe, of eastern Asia and North America, and of North America and Europe will be discussed.

Relationship between Amanita *of SW China and Japan*

Generally speaking, the flora of *Amanita* in SW China and in Japan is closely related and many species are common to both regions (Yang, 2000a). In our study, some sample pairs from the two regions probably were the same species or varieties of same species, e.g. *A. citrina* and *A. hemibapha*. The ITS sequences of *A. griseofolia* from SW China and '*A. ceciliae*' from Japan were the same. Therefore, the Japanese collection of '*A. ceciliae*' probably is a representative of *A. griseofolia*. Similarly, Japanese material labelled as *A. fulva* and *A. orientifulva* of SW China formed a distinct, well-supported clade in both analyses of ITS data (Figs. 1, 2) and showed little sequence divergence (4%). Thus, the Japanese sample of '*A. fulva*' probably is a representative of *A. orientifulva*. On the other hand, it was indicated in the analyses of ITS data set that a few species with morphological similarities in Japan and SW China may be different taxa. For example, collections of *A. flavipes* from SW China and Japan have significant differences in the ITS region. Collections from SW China (*A.* cf. *pantherina* and *A.* sp. 2) and Japan phenetically similar to *A. pantherina* were polyphyletic (Figs. 1, 2). Thus, cryptic taxa need to be delimited.

Relationship between Amanita *of eastern Asia and Europe*

The ITS genetic distances between the samples of *A. porphyria* from Germany and Japan are relatively low (divergence = 0.5%), and the monophyly of the samples was strongly supported (Figs. 1, 2), which indicated that significant differentiations between the samples might have not occurred. The linkage of *A. umbrinolutea* (Germany) with *A. atrofusca* (SW China) was well supported in both most parsimony and neighbor-joining analysis of ITS data. *A. umbrinolutea* is distinguished from *A. atrofusca* by only its light-coloured pileus, stipe, lamellae and volva (Yang, 1997). *Amanita umbrinolutea* and *A. atrofusca* may be just two different subspecies in different geographical locations. As predicted, material of *A. citrina* from Germany and from eastern Asia (Japan and SW China) formed a monophyletic group on the most parsimonious tree (94% bootstrap) (Fig. 1) and the neighbor-join tree (96% bootstrap) (Fig. 2). The ITS sequence divergence between them is only 2.4-2.5 percent. The distinctions between specimens from Europe and eastern Asia are morphologically subtle. Thus, the Europe-eastern Asia disjunct distribution of *A. citrina* was not refuted in this study.

On the other hand, data of nLSU showed that *A. griseofolia* from SW China was different from the *A. ceciliae* from France. In China, *A. griseofolia*

was usually regarded as *A. ceciliae*, originally described from Europe, which differs from *A. griseofolia* by its much more robust fruitbody with a yellow-brown, reddish brown to grey-brown or olive-brown pileus covered with lighter coloured (greyish to brownish) volval remnants, white lamellae with white edges, and a relatively thicker stipe. Furthermore, the volval remnants at the base of the stipe of *A. ceciliae* often form a ring-zone above a strangulate region and a floccose, nearly cupulate structure at the very base of the stipe, and there are more filamentous hyphae in the volval remnants of European *A. ceciliae* (Yang *et al*., 2004).

In China, *A. parvipantherina* is usually regarded as *A. pantherina*, originally described from Europe (see Yang *et al.*, 2004). Analysis of nLSU sequences showed that the separation of *A. parvipantherina* from *A. pantherina* was justified (Figs. 3, 4).

All the cases above indicated that the biogeography relationship between *Amanita* of eastern Asia and Europe is relatively close and some taxa are common to both regions.

Relationship between **Amanita** *of eastern Asia and North America*

A relatively high morphological similarity between *Amanita* of eastern North America and eastern Asia was briefly summarized by Wu and Mueller (1997). Yang (2000a,b) has proposed some pairs of related *Amanita* taxa with such distribution patterns. The sister group relationships between *A. frostiana* (USA) and *A. subfrostiana* (SW China), *A. flavipes* (Japan) and *A. flavoconia* (USA), and *A. volvata* (USA) and *A. clarisquamosa* (SW China) were confirmed by molecular analyses in the studies of Weiß *et al*. (1998) and us. The relationship between the morphologically similar material of *A. flavipes* from SW China and *A. flavoconia* from the USA, was unresolved in the maximum parsimony tree (Fig. 1), and in the neighbor-join tree (Fig. 2), they were paraphyletic. The ITS genetic distances between the two were 4 percent, which was greater than that between the samples of Japanese *A. flavipes* and American *A. flavoconia* (1%). Similar results between the relationship *A. volvata* and *A. peckiana* from North America and *A. avellaneosquamosa* and *A. clarisquamosa* from eastern Asia were also obtained. That is, *A. volvata* and *A. clarisquamosa* were more closely related than to the other species (Figs. 3, 4). Thus, paired or closely related species of *Amanita* between eastern Asia and North America are relatively common, but rarely have been confirmed as disjunct populations of same species by molecular data yet.

Relationship between Amanita *of North America and Europe*

In the analyses of nLSU data, except the complex of *A*. *ceciliae*, no samples labelled as the same names (viz. *A*. *citrina*, *A*. *fulva*, *A*. *gemmata* and *A*. *vaginata*) from North America and from Europe were supported as monophyletic or sister group (Figs. 3, 4). Because all of the species mentioned above were originally described from Europe, the taxa from North America, labelled as such names should be regarded as different species.

Variations within A. parvipantherina

All of the analyses indicated that the samples HKAS 38297, 38340, 29627, and 38334 represent the same species, *A*. *parvipantherina* (Yang *et al.*, 2004), though differences in geographical localities, colour and morphology of fruit bodies were present among them. For example, growing under *Pinus armandii* at alt. 2900 m, HKAS 38297 had a brown to greyish pileus with greyish to grey, verrucose to subconical volval remnants, and a whitish shorter stipe 6 × 1-1.5 cm. HKAS 38340, 29627, and 38334 were collected in forests dominated by fagaceous plants (*e.g*. *Lithocarpus mairei*) and *P*. *armandii* at lower altitude (2200-2400 m) in a mountainous area at least 250 km away from HKAS 38297. The former two had yellowish to yellow pileus with grey to dirty white, verrucose to felty volval remnants, and a whitish to cream, longer stipe 7-15 × 0.5-1.5 cm; HKAS 38334 had a brownish yellow to yellowish brown pileus with darker disc and with grey to brownish grey, verrucose to subconical volval remnants, and a whitish, longer stipe 11-14 × 1-1.3 cm. Since the molecular data revealed nearly no differences among these samples (ITS sequences divergence ≤ 0.3% between them), variation in colour and morphology of fruit bodies should be interpreted as environmentally induced.

Concluding remarks

Our molecular analyses showed that many samples of *Amanita* with same names with wide distributions in the Northern Hemisphere formed polyphyletic groups. The large genetic distance among phenetically similar samples might due to morphological stasis as explained by Mueller *et al*. (2001) on one hand. On the other hand, some taxa with ‘similar morphology’ but great genetic distances are due to want of careful and detailed comparative morphological observations (Yang, 2000b). The traditional treatments of some species of *Amanita* common to different continents need to be verified.

This study provided some information on the phylogeny and biogeography of the genus *Amanita* in the Northern Hemisphere. Further sampling from these vast regions and the other parts of the world, and additional nuclear markers besides morphology may improve our understanding of the evolution and biogeography of *Amanita*.

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