ALIQUANDOSTITIPITACEAE, A NEW FAMILY FOR TWO NEW TROPICAL ASCOMYCETES WITH UNUSUALLY WIDE HYPAE AND DIMORPHIC ASCOMATA

PATRIK INDERBITZIN, SARA LANDEVK, MOHAMED A. ABDEL-WAHAB, AND MARY L. BERBEE

In two short surveys of lignicolous, fruitbody-forming ascomycetes in Thailand and southern China, six species were found, of which five were new to science. Two fungi with affinity to the Dothideomycetes, one from Thailand and one from China, are described here in the new genus Aliquandostipite and included in the new family Aliquandostipitaceae. Aliquandostipite khaoyaiensis was found in a tropical rain forest in Thailand and A. sunyatsenii in a small stream in southern China. Both new species are closely related based on morphological and molecular characteristics and with uncertain affinity to other taxa of the Euscomycetes based on phylogenetic analyses of SSU rDNA sequences. The distinguishing features of the new species are the presence of both sessile and stalked ascomata side by side on the substratum and the widest hyphae known from ascomycetes.

Key words: Ascomycota; East Asia; fungal biodiversity; Loculoascomycetes; microfungi; phylogeny; taxonomy; tropical mycology.

Microfungi are rarely collected and poorly known. The majority of known microfungi belong to the Ascomycota, the largest phylum of the fungi with more than 30,000 described species (Hawksworth et al., 1995). Hawksworth et al. (1997) estimated that the total number of known Fungi constitute only 5% of the existing mycota. For the Ascomycota alone, that would leave 57,000 species to be discovered. This lack of knowledge of microfungal diversity greatly hampers our capability of correctly inferring and understanding many aspects of fungal biology and phylogeny.

Not surprisingly, taxonomic studies of microfungi from little explored areas regularly yield high numbers of undescribed species. For instance, in a monograph of the Coronophorales (Ascomycetes) from India, Subramanian and Sekar (1990) found that ten out of 23 fungi (43%) collected from the Western Ghats were new to science (Hawksworth, 1991).

On two short surveys of ascomycetous, fruitbody-forming microfungi on decaying wood in Thailand and southern China, we found a total of six species. Only one fungus could be named with certainty, and five seemed to be undescribed. The time invested in collecting decaying wood in the field and the amount of material returned to the laboratory for microscopic examination were minimal: less than 1 h was spent collecting and approximately half the volume of a large backpack of decaying wood was returned to the laboratory.

The number of existing Fungi worldwide has been estimated to 1.5 million species, based on the 1:6 ratio of vascular plants to fungi on the British Isles (Hawksworth, 1991). For a country like China, with ~27,000 species of vascular plants (Eriksson and Yue, 1988), and ~7000 species of Fungi (Tai, 1979), it follows that 155,000 Fungi or more than 90% of the mycota present in China are yet to be found. For Thailand, no comparable reliable data were available (R. Bandoni, personal communication, Ladner, British Columbia, Canada).

Two of the new fungi that we found, one from Thailand and one from China, were particularly interesting. They are described here as members of the new genus Aliquandostipite, placed in the new family Aliquandostipitaceae.

Initial examination of hyphae and ascomata (organs of sexual sporogenesis in ascomycetes) suggested the two new fungi were strikingly different from known ascomycetes. Both species of Aliquandostipite were characterized by the presence of hyphae that were five times wider than the widest hyphae known in ascomycetes. In ascomycetes in general, ascomata form superficially or immersed in the substrate, becoming superficial at maturity. In species of Aliquandostipite, however, ascomata seemed to be either borne by thick hyphae, which function as stalks, or were unstalked and erumpent from the substratum at maturity. The conventional, sessile ascomata and the stalked ascomata, resembling a tiny moss sporophyte more than a fungus, were present side by side on the substratum. By comparing the morphology of ascomata from nature and observing ascomata in culture, we investigated whether stalked and sessile forms represented dimorphisms within a species.

Initial observation suggested that species of Aliquandostipite belong to the Dothideomycetes (Eriksson and Winka, 1998), a group of ascomycetes formerly referred to as Loculoascomycetes. Members of this group are characterized by the presence of a functionally bilayered ascus wall developing in a lysogenic cavity, the centrum, within a compact hyphal body,

1 Manuscript received 18 November 1999; revision accepted 3 March 2000.

The authors thank Dr. L. L. P. Vrijmoed and Prof. E. B. G. Jones, Hong Kong, who organized field work in Thailand, and provided laboratory facilities at City University, Hong Kong; Mr. Somsak Sivichai, Bangkok, who supported field work in Thailand; Mr. Wong Kwang-wah, Zhongshan, and Mr. Lao Wen, Hong Kong, who organized field work in China, and provided the name of the collection site in China; Dr. Eduardo M. Leacho, Iloilo, who got his hands dirty collecting samples in China; Professors M. E. Barr Bigelow, Sidney, L. Ryvarden, Oslo, H. Clémenton, Lausanne, and O. E. Eriksson, Umeå, who commented on the fungi and informed about hyphal width; and Prof. and Mrs. R. Bandoni, Ladner, who made collecting in Canada possible, and commented on fungal diversity in Thailand.

A NSERC operating grant (principal investigator M. L. Berbee) is gratefully acknowledged. Expenses for field work were paid for by the authors.

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called the ascoma. Fungi of the Dothideomycetes have traditionally been separated into orders based on the morphology of their centrum tissue (Barr, 1987). The centrum tissue consists of asci containing sexual spores and sterile filaments, which sometimes are present intermingled among the asci. The presence or absence of the sterile filaments is taxonomically important.

Among the orders with bilayered ascus wall, the Dothideales lack sterile filaments in their centrum, while filaments are present in the Pleosporales and Patellariales (Barr, 1987). Ascomata of most orders of the Dothideomycetes open by a pore at maturity, through which the ascospores are released. In the Patellariales, however, the ascomata open by an apical cleft, and the outer wall recures, detaches from the hamathecium, and reveals the centrum.

In the latest system of classification, the Pleosporales and the Dothideales are united in the Dothideomycetes, but the affinity of the Patellariales is unclear (Eriksson and Wink, 1998). Morphology suggests that species of Aliquandostipite belong in the Pleosporales, but molecular evidence contradicts this placement.

In this paper, we describe two new species in the new genus Aliquandostipite, show that they are closely related based on morphological and molecular characters, and infer their phylogenetic relationship to the three orders of Dothideomycetes outlined above.

**MATERIALS AND METHODS**

*Collection, examination, and isolation of fungi—*Decaying branches were collected and stored in plastic bags until return to the laboratory. Fungi were located with an Olympus ZH10 or a Leica Wild M3Z stereomicroscope. Semithin cryosections of material embedded in Jung tissue-freezing medium were cut with a Jung CM 1500 cryostat. Cryosections and squash mounts of fungal material in water or glycerol were examined with an Olympus BH-2 or a Leica Leitz DMRB microscope. Photographs were taken with an Olympus C, 1 min at 56°C, and 1 min with a time extension of 4 sec per cycle at 60°C. Primers used for sequencing were NS2, NS3, NS4 (White et al., 1990), SL1, SL344 and SL887 (Landvik, 1996) for the SSU rDNA region, and primers ITS5 and ITS3 for the ITS region (White et al., 1990).

For A. sunyatsenii, parts of the SSU region and the ITS2 region of the rDNA gene were amplified with the primer pairs SL1/NS4 and ITS3/ITS4, respectively, and purified. PCR products were cloned following the instructions of the TOPO TA Cloning kit (Invitrogen, Carlsbad, California, USA). Ten bacterial colonies containing the respective PCR product insertions were transferred into 1.5-mL centrifuge tubes aliquoted with 1 mL of LB medium and 50 μg/mL ampicillin prepared according to the manufacturer’s instructions. The tubes were incubated at room temperature overnight and then centrifuged at 3000 rpm for 5 min. The supernatants were removed, and the bacterial pellets were resuspended in 200 μL TE buffer (White et al., 1990) and incubated at 95°C for 10 min. Two microliters of each bacterial suspension were used for a PCR reaction either with the primer pair SL1/NS2 or ITS1/ITS4. The PCR products were purified and sequenced, using the primers SL1 and ITS3 for the SSU and ITS products, respectively. All sequenced products were purified by ethanol precipitation (95% EtOH, 7.5 mol/L NaOAc) prior to processing by an ABI 377XL Automatic Sequencer (Perkin Elmer Corp., Norwalk, Connecticut, USA).

**Additional sequences obtained—*Tuberfia helicoma (Phill. & Flw.), Piriyazinski was collected on 26 February 1998 by M. A. Abdel-Wahab, at Pat Heung, New Territories, Hong Kong, on a decaying branch on the ground. A dried culture was deposited at the herbarium of the University of British Columbia, Vancouver, Canada (UBC F13877).

*Rhizophystus rufulum (Spreng. & Fr.) Petrak was collected on 16 May 1998 by M. A. Abdel-Wahab, at Pat Heung, New Territories, Hong Kong, on a decaying branch decorticated log on the ground. A dried culture was deposited at the herbarium of the University of British Columbia, Vancouver, Canada (UBC F13903). Molecular work was carried out as described above. DNA was isolated from a culture derived from a single ascospore, and the SSU rDNA was sequenced in both directions with the following primers: NS1, NS2, and cITS5, NS19 (Gargas and Taylor, 1992), SL344 and SL887, MB20 (Winka and Eriksson, 1998), MB1 (5’-GGA GTA TGG TCG CAA GGC TG-3’), MB2 (5’-GTG AGT TTC CCC GTG TTG AG-3’), MB3 (5’-ATG AGT TTC AAA GCA GCC-3’), and eBas3.

**Data analysis—**New sequences were assembled using AutoAssembler Version 1.4 (Applied Biosystems, Perkin Elmer Corp., Norwalk, Connecticut, USA). Thirty SSU rDNA sequences were retrieved from GenBank (Table 1). Most of them were longer than 1700 bp, with the exceptions of Sphaerophorus globosus and Botryosopheria rhodina (around 1650 bp), Peligeria neopolydactyla and Solorina crocea (1567 bp), and Tuberfia helicomyces (357 bp). Forty-five basepairs at the 3’-end of Lecanora dispersa were removed due to ambiguous alignment. The first 20 positions at the 5’-end of Aliquandostipite khaoyaiensis, the introns in T. helicoma and Monodictis castanea were excluded from analysis. Sequences were manually aligned using Se-Al Version 1.0 alpha 1 (Rambaut, 1999). Data sets were analyzed using PAUP Version 4.0b3 (Swofford, 2000) on a Power Macintosh G3 (Apple Computer, Inc., Cupertino, California, USA). Unless otherwise noted, default settings were used.

Most parsimonious trees (MPT) were generated in PAUP with 30 replicated heuristic searches and random taxon addition. Support for the branches was based on 500 bootstrap replicates. Most likely trees were found with 30 replicated heuristic searches with random addition of taxa; or with a heuristic search using the most likely of the parsimony trees as the starting tree. Support for the branches was based on 100 bootstrap replicates. The Kishino-Hasegawa test was used as implemented by PAUP Neighbor-joining analysis gen-
Establishment and definition of the new species Aliquandostipite khaoyaiensis Inderbitzin sp. nov.

Ascomata immersed-erumpent or superficial. Hamathecium comprising pseudoparaphyses. Asci bitunicate, fissitunicate. Mycelium visible on the substratum, comprising up to 50 μm wide hyphae, which may bear ascomata. Holotype species Aliquandostipite khaoyaiensis Inderbitzin sp. nov.


Sessile ascomata singly immersed to erumpent or superficial on old, decorticated branch lying on the ground in a tropical rain forest, globose to broadly ellipsoidal, 216–290 μm high, 220–344 μm wide, papillate, appearing pale brown when young or dark brown with age beneath stereomicroscope (Figs. 8, 9). Ascomal wall membranous, one-layered, in surface view pallid brown, forming a textura angularis-globulosa, in vertical section cells rounded to elongate (Figs. 8–10). Ascomal wall in basal part 6–16 μm thick, 1–2 cells wide, cells 3–15 μm in diameter, in apical part 13–31 μm thick, 2–4 cells wide.
Figs. 1–7. Stalked ascomata and hyphae of *Aliquandostipite khaoyaiensis*. Figs. 1–7. Features of stalked ascomata. **1.** Stalked ascomata on substrate. **2.** Mycelium on the substrate. Note thick, branching hyphae. **3.** Cluster of stalks bearing ascomata. Figs. 4–6. Vertical cryosection of stalked ascomata mounted in glycerol. **4.** Globose ascoma. **5.** Basal section: note the stalk formed by a single hypha with thick, refractive walls, and the widening apical segment of the stalk merging with the ascomal wall. **6.** Apical section: papilla and apically attached, branched and septate sterile filaments. **7.** Squash mount in glycerol of superficial hypha bearing an ascoma on a lateral branch. Scale bars: Figs. 1, 3 = 0.5 mm, Figs. 2, 4, 7 = 100 μm, Figs. 5, 6 = 50 μm. Figs. 1–3 in brightfield with stereomicroscope, Figs. 4–6 in Nomarski interference contrast, Fig. 7 in phase contrast.

Cells up to 22.5 μm in diameter (Figs. 8, 9). Cell walls of outermost cells up to 3.5 μm thick and refractive and the largest cells may protrude up to 8 μm (Fig. 9). Papilla ~50 μm high, 70 μm wide (Fig. 9). Hamathecium pseudoparaphyses, septate, sparsely branched, up to 3.5 μm wide (Figs. 8, 11). Asci 136–194 × 36–58 μm (166.67 × 45.57 μm on average, N = 30), eight-spored, clavate, bitunicate, fissitunicate, with thickened apical region, spores variably arranged, small peduncle observed at times (Fig. 11). Ascospores oval in outline, 49.6–70 × 12.8–20 μm (61.80 × 16.27 μm on average, N = 50), one-septate, constricted at the septum and there 11.2–16.8 μm wide (15.59 μm on average, N = 50), upper cell slightly longer and narrower than lower cell, smooth, pale brown, guttulate or not, sheathed (Figs. 11–13). Sheath first appressed to the wall, gradually expanding and detaching from the polar regions towards the septum, then balloon-like at the poles, finally surrounding the entire ascospore, ~150 × 50 μm (Figs. 12, 13).

Superficial mycelium: consisting of light to dark brown, up to 40 μm wide hyphae, septate every 40–100 μm, wall refractive in vertical section, 4–6 μm thick (Figs. 2, 7). In substrate repeatedly branching into narrower, finally ~2 μm wide hyphae. Single hyphae may bear ascomata (Figs. 1, 3).

Stalked ascomata: stalk up to 1.6 mm long and 42 μm wide, wall up to 15 μm thick, arising singly from superficial hypha (Fig. 7), or singly or gregariously from substrate (Figs. 1, 3). Apical segment of stalk broadening to up to 3 times the width of the one beneath, and comprising several rounded cells, ~25
μm in diameter in basal part, diminishing in size and merging with the peridium in the upper part (Figs. 4, 5). Ascomata globose to oval and then tapering towards the stalk, 140–320 μm high, 100–320 μm wide (Figs. 4, 5). Papilla up to 40 μm high, 110 μm wide (Fig. 6). Ascomal wall a textura angularis-globulosa in surface view. In vertical section 1–3 cells wide, in basal part ~10 μm thick consisting of hyaline, thin-walled cells, 15–25 μm thick in apical part, cell walls 1–4 μm thick and refractive (Figs. 4, 5). Asci 116–180 × 30–46 μm (146.60 × 39.17 μm on average, N = 30). Ascospores 54.4–66.4 × 12.8–20.8 μm (61.46 × 16.73 μm on average, N = 50), at the septum 12.4–17.6 μm wide (14.67 μm on average, N = 50).

Ascospores from sessile ascomata germinated on CMA petri dishes overnight in the dark at 25°C. Germination hyphae were up to 16 μm wide, constricted at the septa and there up to 10 μm wide. Germinated ascospores were transferred to PDA petri dishes and incubated on a laboratory bench exposed to artificial and day light, and darkness at night. After 5 wk, colonies measured 2–4 μm in diameter, and the mycelium was immersed in the agar and dark olive-brown to black. After 10 mo, colonies measured up to 4 cm in diameter. At the margin, the mycelium was immersed in the agar and comprised up to 20 μm wide hyphae. Towards the center, the mycelium was erumpent from the agar, forming a dark-brown, prosenchymatous stroma intermixed with agar. Stalked ascomata formed on the stroma. The stalks were up to ~500 μm long and 30 μm wide, bearing globose ascomata up to ~400 μm in diameter. Ascomata contained pseudoparaphyses and sterile asci. A culture was deposited at CBS.

Habitat and distribution: on decaying branch lying on the ground in tropical rain forest in Khaο Yai National Park, Thailand.


Establishment and description of the new species A. sunyatsenii sp. nov.—Aliquandostipite sunyatsenii Inderbitzin sp. nov. (Figs. 14–17). Etymology—after Dr. Sun Yat-Sen, a native of Zhongshan.

Ascomata globosa, ~350 μm diameter, papillata. Pseudo-
paraphyses septatae, ramosae, persistentes. Ascii elongati, \(\sim 145 \times 52 \mu m\). Ascosporae unisepatae, ellipsoidae, \(\sim 49 \times 20 \mu m\), appendiculatae. Mycelium praensae in pagina substra- \(to. Hyphae usque ad 50 \mu m latae, aliquando ascoma ferentem. Holotypos: in ligno emortuo, Wu Gui Shan, Z1.2 (UBC F13876).

Sessile ascomata singly erumpent from decorticated branch immersed in small stream, rounded, 300–400 \(\mu m\) in diameter, papillate, ostiolate, light to dark brown, membranous (Fig. 14). Ascomal wall one-layered, 25–40 \(\mu m\) thick, 2–5 cells wide, forming a textura globulosa-angulares in surface view. Outer-most cells rounded to elongate, up to 30 \(\mu m\) in diameter, some protruding up to 13 \(\mu m\) above surrounding cells (Fig. 14), inner cells elongate and laterally compressed, cell walls 1–5 \(\mu m\) thick, refractive (Fig. 14). Cells at the base and towards papilla dark pigmented at times. Ostiole apically lined by elongate cells, \(\sim 10 \times 5 \mu m\) (Fig. 14). Pseudoparaphyses persist- \(ent, septate, branched, \sim 2.5 \mu m\) wide. Ascii originating from a cushion-shaped ascosogenous tissue at the base of the asco- mata, 128–193 \(\times\) 45–57.5 \(\mu m\) (145 \(\times\) 51.5 \(\mu m\) on average, \(N = 20\)). When young, saccate with thick-walled apex, ocular chamber and short stalk, completely filled by ascospores and void of elongate when mature, bitunicate, fissitunicate, eight- \(spored. Ascosporae straight or slightly curved (39–) 46–52 \(\times\) 16–23 \(\mu m\) (49 \(\times\) 19.5 \(\mu m\) on average, \(N = 30\)), one-septate up to 5 \(\mu m\) above or 4 \(\mu m\) below the middle (0.3 \(\mu m\) above on average, \(N = 30\)), upper hemisphere up to 3 \(\mu m\) wider than lower hemisphere (1 \(\mu m\) wider on average, \(N = 30\)), constrict- \(ed at the septum, light brown, heavily guttulate (Figs. 16, 17). Two helmet-shaped appendages are present on either side of both upper and lower poles, tending to unite over the respective pole (Fig. 17).

Superficial mycelium dark brown, up to 35 \(\mu m\) wide, sep- \(tate at intervals of 35–45 \(\mu m\), carrying single presumptive ascoma primordia at times (Fig. 15). Connections between ses- \(sile ascomata and superficial hyphae seen.

Stalked ascoma: one stalked ascoma was found, \(\sim 350 \mu m\) in diameter, originating from the apex of a concolorous stalk (Fig. 15). Stalk separtate at intervals of 30–40 \(\mu m\), thick-walled (up to 7.5 \(\mu m\)), 50 \(\mu m\) wide and 0.5 mm long, at the base branching into 15-\(\mu m\)-wide hyphae. Asci contained were 137.5–142.5 \(\times\) 45–62.4 \(\mu m\), and ascospores 50–52.5 \(\times\) 17.5–20 \(\mu m\) (both \(N = 4\)) (Fig. 15).

Ascospores failed to germinate in culture.

Habitat and distribution: on decaying branches immersed in a small stream at Wu Gui Shan, near Zhongshan, Guangdong Province, People’s Republic of China.

Specimen examined: Z1.2, holotype (UBC F13876), on decaying branch at Wu Gui Shan, 15 km south of Zhongshan, Guangdong Province, China, 11 November 1998, leg. Eduardo M. Leaño and P. Inderbitzin.

Molecular data—New sequences obtained—From the fol- \(lowing species, new SSU rDNA sequences were obtained and submitted to GenBank: Aliquandonistipta khaoyaensis (GenBank GBAN-AF201453), 1739 bp corresponding to posi- \(tions 17–1716 of Saccharomyces cerevisiae Meyen ex E. C. Hansen from GenBank (GBAN-V01335), A. sunyatsenii (GenBank GBAN-AF201454), 440 bp corresponding to posi- \(tions 130–571 of S. cerevisiae, Tubeufia helicoma (GBAN- \(AF201455), 2110 bp corresponding to positions 65–1690 of S. cerevisiae, and Rhizidhysteron rufulum (GBAN- \(AF201452), 1616 bp corresponding to positions 51–1665 of S. cerevisiae. In T. helicoma, introns of 81 and 402 bp were present at positions 467 and 565, respectively. From the fol- \(lowing taxa, sequences from the ITS rDNA-region were ob- \(tained and submitted to GenBank: 548 bp of ITS1, 5.8S rDNA, and ITS2-region of A. khaoyaensis (GenBank GBAN- \(AF201728), and 395 bp of the 5.8 rDNA and ITS2-region for A. sunyatsenii (GenBank GBAN-AF201727). The latter two sequences were too divergent to be aligned.

SSU rDNA Sequences of A. khaoyaensis and A. sunyatsenii were more similar to one another than to any other sequence. Their overlapping region of 438 unambiguous sites differed in 1.8% of the sites. Among the other taxa included in the analy- \(ses, the homologous region in Botryosphaeria ribis was most similar to taxa of Aliquandostipta, differing from A. khaoy- \(aensis in 4.5% of the sites. Aliquandostipta khaoyaensis and A. sunyatsenii clustered together with high bootstrap support (Fig. 18).

Since a complete SSU rDNA sequence of a member of the Tubeufiaceaæ was not available from GenBank, we sequenced the SSU rDNA region of T. helicoma. This new sequence dif- \(fered in 0.8% of the sites from the homologous partial se- \(quence of T. helicomyces retrieved from GenBank (Table 1). This was comparable to the degree of variability in homolo- \(gous regions of other closely related taxa of the Dothideo- \(mycetes included in this study, such as Setosphaeria rostrata and Pleospora herbarum, which differed in 0.8% of the sites. On the other hand, the homologous regions of the most di- \(vergent taxa in the Dothideomyctes in this study, P. herba- \(rum and Aureobasidium pullulans, differed by 7.2%. Tubeufia helicoma clustered with the 357 bp sequence of T. helicomyces with bootstrap support (Fig. 18).

Similarly, a complete sequence of the SSU rDNA region of a member of the Patellariales was not available, and so we sequenced the gene from R. rufulum. The phylogenetic place- \(ment of R. rufulum inferred in this study agreed with results of previous studies (Winka and Eriksson, 1998) and confirmed the identity of our new sequence. A fragment of R. rufulum retrieved from GenBank (GBAN-U20506) differed from ours in the overlapping region of 1046 bp in five (0.48%) sites, and another sequence (GenBank GBAN-AF164375) differed from ours in 11 (1.03%) of the 1063 overlapping sites. The latter two fragments differed in 12 (1.15%) of the 1046 overlapping sites. As a comparison, Pleospora herbarum and Setosphaeria rostrata (Fig. 18), differed in 0.36% of 1046 sites.

Phylogenetic analyses—The new SSU rDNA sequences of A. khaoyaensis, A. sunyatsenii, T. helicoma and R. rufulum were aligned with 30 sequences retrieved from GenBank, us- \(ing Boletus satanas as an outgroup (Table 1). Hence, the data matrix contained 34 taxa and 1799 characters. The alignment was submitted to TreeBase. Ambiguous sites were excluded from analyses. These included 72 characters in the following positions: 35–39, 99–105, 141–160, 452–458, 1336–1361, 1484–1490. Of the remaining 1727 characters 1255 were constant, 211 of the variable characters were parsimony uninformative, and 261 were parsimony informative. Characters were weighted equally, gaps were ignored.

 Parsimony and likelihood trees inferred from a data matrix with ambiguous sites included were not significantly different from the analyses with ambiguous sites excluded, based on a Kishino-Hasegawa test (\(P > 0.05\). However, 88 MPTs were found (data not shown). For this reason, ambiguous sites were excluded in all the following analyses.
Fig. 18. Most likely tree (−ln likelihood = 8198). Bootstrap support percentages are shown above the branches. The first numbers are likelihood bootstrap percentages based on 100 replicates, and the second numbers are parsimony bootstrap percentages based on 500 replicates. Only bootstrap percentages higher than 50% in both analyses were included. For groups relevant to this study, bootstrap percentages are given in boldface and neighbor-joining bootstrap percentages (based on 500 replicates) are given last in the series of numbers. Branches of Dothideomycetes and Patellariales are in boldface. Species for which new sequences were obtained are also in boldface. Note the well-supported clades of Aliquandostipite spp. and Tubeufia spp., clustering outside the clade comprising Rhytidhysterion rufulum and Pleosporales.
In 30 heuristic searches using parsimony, two MPTs, each requiring 964 steps, were found (Consistency Index = 0.623, Retention Index = 0.661). The two MPTs differed in the arrangement of taxa within a clade of the Pleosporales: Leptosphaeria maculans appeared as sister taxon to either Septoria nodorum or Cucurbitaria elongata. The overall tree topology agrees with results from other authors (Winka and Eriksson, 1998). The most likely MPT (ln likelihood = 8204) differed from the most likely tree in Fig. 18 by the rearrangement of branches receiving less than 50% bootstrap support: Chaetomium elatum was sister taxon to the Chaetothyriales, the Lecanorales formed a sister group to the Peltigerales, and Aureobasidium pullulans, Dothidea insculpta, Coccodinium bartsi, and Aliquandostipite were sister group to the remainder of the Dothideomycetes.

The most likely tree of the two MPTs was used as the starting tree in a likelihood analysis, which yielded the same tree topology as 50 likelihood heuristic searches with taxa added by random stepwise addition. The most likely tree (ln likelihood = 8198) was 965 steps long, one step longer than the MPTs, and not significantly different from either MPT (P > 0.6). Clades supported by at least 50% of the bootstrap replicates in either most likely tree or MPTs were present in both trees.

Based on a Kishino-Hasegawa test, the neighbor-joining tree was significantly worse than the most likely tree (P < 0.05), and thus is not discussed here in detail.

Clades with relevance in this study and high likelihood, parsimony, and neighbor-joining bootstrap support include: Pleosporales, with 99, 95, and 87% support in the respective analyses, Pleosporales and R. rufulum with 95, 89, and 58% support, species of Aliquandostipite with 100, 92, and 100% support, and species of Tubeufia with 91, 73, and 83% bootstrap support (Fig. 18). In neither analysis did the Dothideales, i.e., Dothidea insculpta, Coccodinium bartsi, Aureobasidium pullulans, and species of Botryosphaeria, receive support as a monophyletic group. The Dothideomycetes consisting of Pleosporales and Dothideales formed a monophyletic group in both parsimony and likelihood analyses, receiving the highest support in the likelihood analysis with 63% of the bootstrap replicates. The morphologically related taxa in Pleosporales, Patellariales, Tubeufiales, and species of Botryosphaeria formed a monophyletic group in all analyses, with a maximum bootstrap support of 58% in the likelihood analysis.

Constraining species of Aliquandostipite to the Pleosporales yielded four trees that were 11 steps longer than the MPTs and significantly worse than either one of them (P < 0.05), as evaluated by the Kishino-Hasegawa test.

**DISCUSSION**

In the previous section, we gave evidence that the new species of Aliquandostipite have morphologically dimorphic ascomata within one species, that they are closely related based on morphological and molecular data, and distant from their presumptive closest relatives based on morphology.

In the following, we justify the inclusion of both new species in one genus, the establishment of the new genus Aliquandostipite and the new family Aliquandostipitaceae. Finally, we show that the stalked and sessile ascomata present side by side on the substratum belong to the same species and discuss another distinguishing feature of both new species, the unusually wide hyphae.

**Two new congeneric species**—Molecular evidence for a close relationship of species of Aliquandostipite included the high support that their clade received in phylogenetic analyses of the SSU rDNA sequences using different methods. Both neighbor-joining and maximum likelihood clustered A. khaoyaiensis and A. sunyatsenii together with 100% bootstrap support (Fig. 18). Bootstrap values obtained with parsimony support the Aliquandostipite clade with 92% (Fig. 18). Morphological characters common to both species of Aliquandostipite were a light-colored, one-layered ascomal wall, downward-growing sterile filaments, functionally two-layered ascocarps sparsely filled with ascospores at maturity, and one-septate, sheathed ascospores. The habitats and ecology of both species of Aliquandostipite were similar. They were found on old, decorticated branches in very humid and warm habitats: Aliquandostipite khaoyaiensis on branches lying on the ground of a tropical rain forest in Thailand, A. sunyatsenii on a branch immersed in a stream in subtropical southern China.

Hence, morphological and ecological characters supported SSU rDNA data and indicated a close relationship of A. khaoyaiensis and A. sunyatsenii. Even though the ITS rDNA sequences of the species of Aliquandostipite were too different to be aligned, the inclusion of both species into one genus seemed most appropriate at present.

**The new genus Aliquandostipite and new family Aliquandostipitaceae**—Species of Aliquandostipite did not group with any significant support with other taxa included in the phylogenetic analyses. In the most likely tree, the genus Aliquandostipite was sister group to the Dothideomycetes (Fig. 18). The Dothideomycetes comprise fungi traditionally placed in the Dothideales and Pleosporales. The morphological characters of species of Aliquandostipite, the presence of bitunicate ascii, and the presence of sterile filaments, both developing in a stroma, are consistent with a placement in the Dothideomycetes.

Except for the stalked ascomata, all morphological features of species of Aliquandostipite are encountered in the Pleosporales, the light-colored ascomal wall suggesting a possible affinity with the family Tubeufiaceae (M. E. Barr, personal communication, Sidney, British Columbia, Canada). In phylogenetic analyses, however, species of Aliquandostipite did not cluster within the Pleosporales. Rhytidhysteron rufulum in the Patellariales, appeared as sister taxon to the Pleosporales, excluding both Tubeufia and Aliquandostipite. The genera Tubeufia and Aliquandostipite were as similar to other filamentous ascomycetes as to one another, and did not form a monophyletic group (Fig. 18). Constraining the genus Aliquandostipite to be within the Pleosporales yielded significantly worse trees than the most parsimonious tree (P < 0.05).

The Dothideales are defined morphologically by the absence of sterile filaments (Barr, 1987), and the presence of sterile filaments excludes Aliquandostipite from this group. In the likelihood analysis, the Dothideales did not cluster together (Fig. 18). In the parsimony analysis, Aureobasidium pullulans, Dothidea insculpta, and Coccodinium bartsi of the Dothideales and Aliquandostipite formed a monophyletic group without significant support. However, molecular data provided little support for membership of Aliquandostipite in the Dothideales, but poor resolution of branching order made a monophyletic relationship of Aliquandostipite and Dothideales impossible to exclude.

Hence, the genus Aliquandostipite could neither be included...
in the Dothideales nor in the Pleosporales. The lack of morphological and molecular affinity to taxa known to us justified the establishment of the new family Aliquandostipitaceae and the new genus Aliquandostipite for the two new species of A. khaoyaiensis and A. sunyatsenii.

Dimorphic ascomata and the widest hyphae in ascomycetes—Besides the characters mentioned above, the new family Aliquandostipitaceae is supported by the presence in both species of two unique features, distinguishing them from all other Euascomycetes. These are the widest hyphae reported in the ascomycetes, and the formation of both sessile and stalked ascomata side by side on the substratum.

Stalked ascomata are atypical among ascomycetes. In species of Aliquandostipite, stalked and sessile ascomata are present side by side on the substratum. Stalked ascomata are rounded to elongate and lack the flattened base of the sessile, dome-shaped ascoma. The stalks originate either directly from the substratum (Fig. 3) or from a superficial hypha (Fig. 7). In A. sunyatsenii, superficial hyphae were observed to be connected to sessile ascomata as well. Hence, both stalked and sessile ascomata may have issued from the same mycelium. This situation is identical to what is encountered in culture: Single ascospore isolates from sessile ascomata of A. khaoyaiensis produced both stalked and sessile ascomata.

Microscopic features of the centrum tissue and ascomal wall in stalked and sessile ascomata vary only to a degree to be expected within one species. In A. khaoyaiensis, the ascospores of both stalked and sessile ascomata are nearly identical in size and the dimensions of the asci clearly overlap, being on average 12% longer and 14% narrower in the sessile than in the stalked ascomata. In A. sunyatsenii, dimensions of asci and ascospores overlap as well, and their means are very close. A more detailed comparison is not possible, because of the fact that only few asci and ascospores from one stalked ascomata could be measured. Vertical sections of stalked and sessile ascomata in A. khaoyaiensis show the same type of sterile filaments, which are apically attached and seem to have grown downwards (Figs. 4–6, 8). The ascomal wall is one-layered and light colored, and the constituting cells are largest at the exterior of the ascoma, and diminish in size towards the inside. Some of the outermost cells in sessile ascomata of A. khaoyaiensis and A. sunyatsenii were observed to protrude up to 13 μm above the surrounding cells (Figs. 9, 14). This was not observed in stalked ascomata of A. khaoyaiensis. In A. sunyatsenii, stalked ascomata were not sectioned.

Species of Aliquandostipite produce the widest hyphae of any known ascomycete. The ascomal stalks, which are single hyphae, are up to 50 μm wide and 1.6 mm long. This is five times wider than the widest hyphae previously reported in the ascomycetes. So far, the widest hyphae in lignicolous ascomycetes were known from species in the genus Botryosphaeria, reaching a width of 10 μm (M. E. Barr Bigelow, personal communication, Sidney, British Columbia, Canada). In the lignicolous genus Wolfiporia, hyphae may in rare cases reach a width of 20 μm (L. Ryvarden, personal communication, University of Oslo, Norway). In lamellae of certain Basidiomycetes, 30-μm-wide hyphae are possible (H. Clémençon, personal communication, University of Lausanne, Switzerland).

The results of this study are surprising, in that two short surveys in geographically distant localities yielded two new, closely related species that cannot be placed in a known family. We hope these results will encourage further study of fungal diversity in little explored areas of the world.

LITERATURE CITED

BARK, M. E. 1987. Prodromus to class Lociolascornycetes. Published by the author, Amherst, Massachusetts, USA.