



A new species of bird's nest fungi: characterisation of *Cyathus subglobisporus* sp. nov. based on morphological and molecular data

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Key words

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Abstract Recent collections of bird's nest fungi (i.e. *Crucibulum*, *Cyathus*, *Mycocalia*, *Nidula*, and *Nidularia* species) in northern Thailand resulted in the discovery of a new species of *Cyathus*, herein described as *C. subglobisporus*. This species is distinct by a combination of ivory-coloured fruiting bodies covered with shaggy hairs, plications on the inner surface of the peridium and subglobose basidiospores. Phylogenetic analyses based on ITS and LSU ribosomal DNA sequences using neighbour-joining, maximum likelihood and weighted maximum parsimony support *Cyathus subglobisporus* as a distinct species and sister to a clade containing *C. annulatus*, *C. renweii* and *C. stercoreus* in the *Striatum* group.

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INTRODUCTION

The genus *Cyathus* along with the genera *Crucibulum*, *Mycocalia*, *Nidula*, and *Nidularia* are known as the bird's nest fungi because of their small vase-shaped or nest-like fruiting bodies containing lentil-shaped or egg-like peridioles. *Cyathus* is the most speciose genus in the family Nidulariaceae (Agaricales). *Cyathus* is distinguished from the other three genera in the Nidulariaceae based on grey to black peridioles with funicular cords and peridia composed of three layers of tissues (Brodie 1975). Historically, *Cyathus* was monographed by Lloyd (1906) and Brodie (1975, 1984), and their species concepts, especially those of Brodie (1975), are followed by most mycologists (Liu & Li 1989, Ren & Zhou 1992, Yang et al. 2002, Chen et al. 2003, Zhou et al. 2004). Recognition of *Cyathus* species is based on morphological characters such as fruiting body shape, coverings and plications of peridia, anatomy of peridioles, and the size and shape of basidiospores (Brodie 1975).

Molecular phylogenetic studies based on rDNA sequence data including several *Cyathus* species and other gasteromycetous fungi (e.g., *C. striatus* in Hibbett et al. 1997, Hibbett & Thorn 2001 and *C. stercoreus* in Moncalvo et al. 2002) showed that *Cyathus* nested within the euagarics clade. The most recent treatment of the Agaricales by Matheny et al. (2006) based on sequence analyses of six loci included *Crucibulum laeve* and

Cyathus striatus as representatives of the Nidulariaceae. Their phylogenetic reconstruction indicated that the Nidulariaceae was sister to the Cystodermateae (represented by *Cystoderma amianthinum*). Together these two clades appear sister to the Agaricaceae s.l. but without bootstrap support. A phylogenetic study of the genus *Cyathus* by Zhao et al. (2007) using ITS and LSU ribosomal DNA sequence datasets, and based primarily on type and authentic specimens of 22 taxa of *Cyathus*, indicated that the genus was monophyletic and included three infrageneric groups recognisable by morphological characters.

In Brodie's monographs (Brodie 1975, 1984) eight species were known from southeast Asia: *C. cheliensis* and *C. olivaceobrunneus* from China; *C. crispus*, *C. ellipsoideus*, and *C. griseocarpus* from India; *C. elmeri* and *C. gracilis* from the Philippines, and *C. triplex* from the West Indies, the Philippines, and Thailand. Within the last 20 years, seven new taxa have been described from south China: *C. africanus* var. *latisporus* (Chen et al. 2003), *C. cornucopioides* (Ren & Zhou 1992), *C. lijiangensis* (Zhou et al. 2004), *C. luxiensis* (Chen et al. 2003), *C. megasporus* (Ren & Zhou 1992), *C. renweii* (Zhou et al. 2004), and *C. yunnanensis* (Liu & Li 1989). Based on morphological analyses of 48 *Cyathus* taxa, including 30 type specimens in Zhao et al. (2006), three *Cyathus* species were found to represent synonyms of existing species. *Cyathus cheliensis*, *C. gansuensis* (Yang et al. 2002), and *C. megasporus* were accepted as synonyms of *C. limbatus*, *C. pygmaeus*, and *C. poeppigii*, respectively.

There has been a recent interest in studies of basidiomycetes in Thailand, particularly around the area of the Mushroom Research Centre (Le et al. 2007a–c, Wannathes et al. 2007). Some *Cyathus* species have been reported previously from Thailand (Brodie 1975, Ellingsen 1982, Soyong 1994, Desjardin et al. 2004). However, no new species have been described from Thailand prior to this paper. In this study *Cyathus subglobisporus* sp. nov. is described and its phylogenetic position is investigated based on ITS and LSU rDNA sequence data.

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MATERIALS AND METHODS

Morphological studies

Macromorphological characters of freshly collected material were documented as follows. Colour terms follow Kornerup & Wanscher (1978). Peridioles were sectioned by hand, mounted in distilled water, and examined using a light microscope. Spore statistics include average dimensions \pm SD; Q, the quotient of spore length and spore width in any one spore; and Q_m , the mean of Q-values \pm SD. Duplicates are deposited in the BIO-TEC Bangkok Herbarium (BBH), Bangkok, Thailand, the H.D. Thiers Herbarium (SFSU) at San Francisco State University, San Francisco, California, USA, and the herbarium of the Mushroom Research Centre (MRC), Chiang Mai, Thailand.

Molecular phylogenetic studies

DNA extractions were made from gleba using a commercial DNA extraction kit (E.Z.N.A. Forensic Kit, D3591-01, Omega Bio-Tek). PCR reactions performed with primer pairs LROR and LR5 and ITS4 and ITS5. Sequencing protocols follow those of Zhao et al. (2007).

Newly generated sequences (ITS and LSU sequences from the new species), and those retrieved from GenBank (20 ITS and 19 LSU sequences; Table 1) were initially aligned using Clustal X with default settings (Thomson et al. 1997). Manual

adjustments were made in BioEdit v. 7.0.4 and gaps were introduced to improve alignments. The ITS and LSU alignments were separately submitted to TreeBASE (accession number: SN3455). All sequences used in this study were derived from type or paratype specimens or from authentic material determined by us or by H.J. Brodie (cf. Zhao et al. 2007).

Phylogenetic analyses were performed using PAUP v. 4.0b10 (Swofford 2003). Heuristic searches of the ITS, LSU, and ITS+LSU datasets were performed separately under three optimality criteria: weighed parsimony (WP), maximum likelihood (ML), and neighbour-joining (NJ). Unordered characters, random taxon addition sequences, gaps treated as missing data, and tree bisection-reconnection (TBR) branch swapping were used in all analyses. For weighted maximum parsimony, maxtrees was limited to 5 000 trees with 1 000 replications. The weighted parameters were produced using Stmatrix (François Lutzoni & Stefan Zoller, Duke University) as described in Miadlikowska et al. (2002). The best nucleotide substitution models for maximum likelihood were chosen by using MrModeltest v. 2.2 (Nylander 2004). Bootstrap values (BS) were obtained from 1 000 replicates. Unconstrained trees (WP, ML, and NJ trees) were compared in PAUP using Kishino-Hasegawa and

Table 1 Taxon information and sequences retrieved from GenBank.

Taxa	Origin	GenBank accession numbers	
		ITS	LSU
<i>Crucibulum laeve</i>	China	DQ463357	AF336246
<i>Cyathus africanus</i> ¹	Tanzania	DQ463347	DQ463330
	China	DQ463340	–
<i>C. annulatus</i> ¹	Canada	DQ463351	DQ463332
<i>C. berkeleyanus</i>	China	DQ463355	–
<i>C. colensoi</i> ²	India	DQ463344	–
<i>C. crassimurus</i> ¹	Hawaii	DQ463350	–
<i>C. griseocarpus</i> ¹	India	–	DQ463324
<i>C. guandishanensis</i> ¹	China	–	DQ463329
<i>C. helenae</i> ¹	Canada	–	DQ463334
<i>C. hookeri</i>	China	DQ463346	–
<i>C. jiayuguanensis</i> ¹	China	DQ463341	DQ463325
	China	DQ463342	DQ463328
<i>C. lanatus</i> ¹	USA	–	DQ463337
<i>C. olla</i> f. <i>olla</i> ²	Canada	DQ463345	DQ463327
<i>C. olla</i> f. <i>anglicus</i> ¹	USA	–	DQ463326
<i>C. olla</i> f. <i>brodiensis</i>	China	DQ463343	–
<i>C. pallidus</i>	China	DQ463356	DQ463336
<i>C. poeppigii</i>	China	–	DQ463339
<i>C. pygmaeus</i>	China	DQ463348	DQ463335
<i>C. renweii</i> ¹	China	DQ463352	DQ463333
<i>C. setosus</i> ¹	Jamaica	DQ463349	DQ463331
<i>C. stercoreus</i>	China	DQ463354	DQ463338
<i>C. subglobisporus</i> sp. nov. ¹	Thailand	EF613553	EF613554
<i>C. triplex</i>	China	DQ463353	–
<i>Cystoderma amianthinum</i>	–	AY207195	DQ192177
<i>Nidula niveotomentosa</i>	China	DQ463358	DQ463323

¹ Refers to the type specimen.

² Indicates that H.J. Brodie determined the specimen.

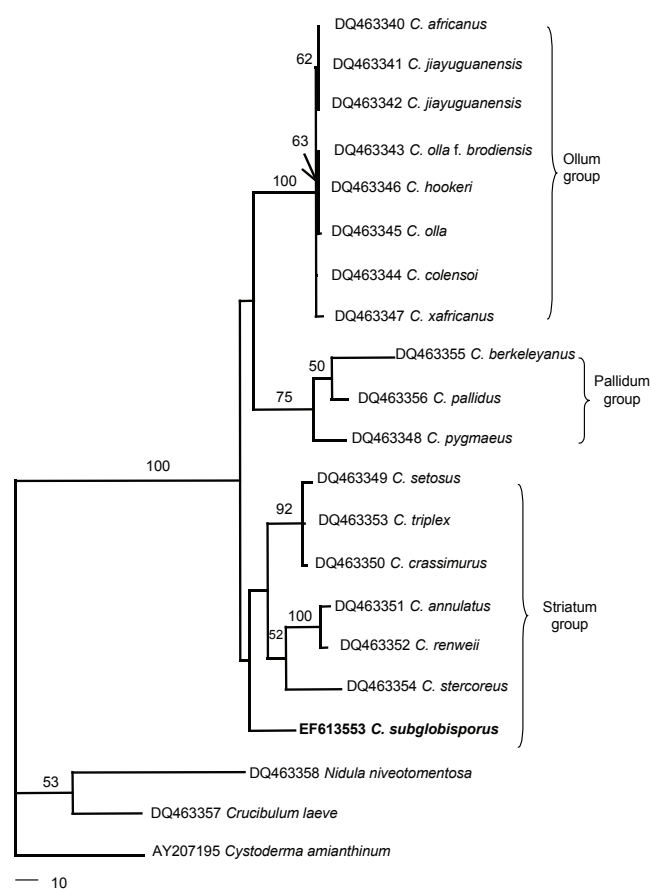


Fig. 1 Phylogenetic relationship of *Cyathus* inferred by maximum likelihood analysis of ITS rDNA sequences. Bootstrap support (BS) values above 50 % are shown.

Shimodaira-Hasegawa tests (Kishino & Hasegawa 1989). Trees were viewed in TreeView v. 1.6.6 (Page 1996) and exported to graphics programmes.

The informal infrageneric group names Ollum, Pallidum, and Striatum in *Cyathus* follow the phylogenetic nomenclature established by Zhao et al. (2007). Although these names constitute improper Latin and do not match the specific epithets olla, pallidus, and striatus, they were established by Zhao et al. (2007) to distinguish the clades from similarly named infrageneric groups used by Brodie (1975), viz., Striatus, Pallidus, and Olla, that contain different subsets of species.

RESULTS

DNA alignment and phylogeny

The ITS dataset consisted of 776 characters of which 371 characters were constant, 109 variable characters were parsimony-uninformative, and 171 characters were parsimony-informative. One hundred and twenty four characters were ambiguous and were excluded. The sequences represent 16 *Cyathus* species (18 strains), *Crucibulum laeve*, and *Nidula niveotomentosa*, while *Cystoderma amianthinum* was used as the outgroup for

rooting purposes based on the previous result that *C. amianthinum* (Cystodermateae) is sister to Nidulariaceae (Matheny et al. 2006). In all phylogenies under different optimality criteria (NJ, ML, and WP), the genus *Cyathus* is monophyletic with 100 % bootstrap and all trees have similar topologies. ITS results indicate that *Cyathus* species were partitioned into three main clades as shown by Zhao et al. (2007). Our new taxon, *Cyathus subglobisporus*, belongs to the Striatum group, albeit with weak statistical support (Fig. 1).

The LSU dataset consisted of 797 characters, of which 661 characters were constant, 56 variable characters parsimony-uninformative, and 70 characters parsimony-informative. Ten characters were excluded. The LSU dataset includes 16 *Cyathus* species (17 strains), *Crucibulum laeve*, and *Nidula niveotomentosa*. *Cystoderma amianthinum* was chosen as the outgroup for rooting purposes. Phylogenies (NJ, ML, and WP) show that *Cyathus* is monophyletic with 100 % bootstrap support, and that *C. subglobisporus* clusters with *C. setosus*, and together they are sister to the Pallidum group but this relationship was not statistically supported (Fig. 2).

The combined ITS and LSU sequence dataset consisted of 1573 characters of which 1045 characters were constant; 171 variable characters were parsimony-uninformative, 223 charac-

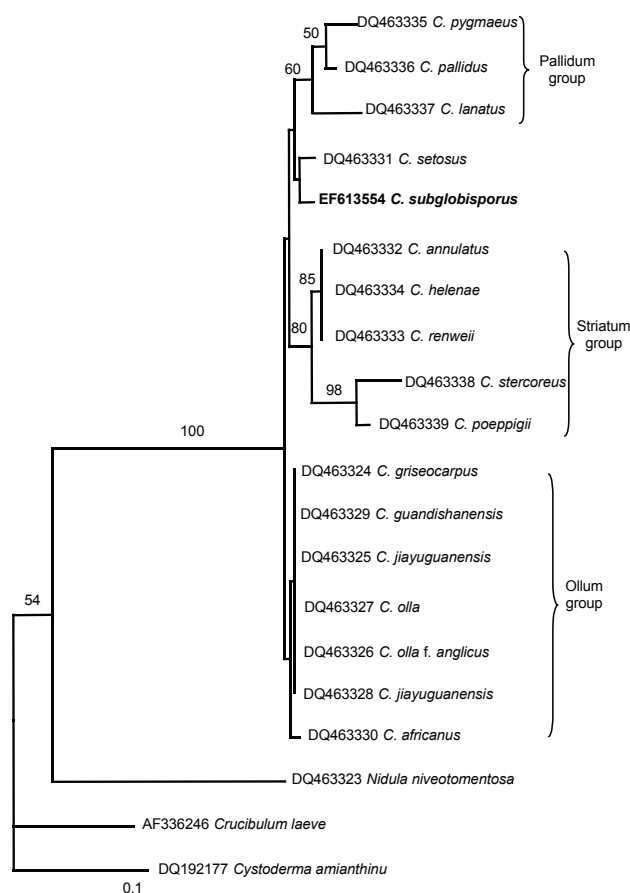


Fig. 2 Phylogenetic relationship of *Cyathus* inferred by maximum likelihood analysis of LSU rDNA sequences. Bootstrap support (BS) values above 50 % are shown.

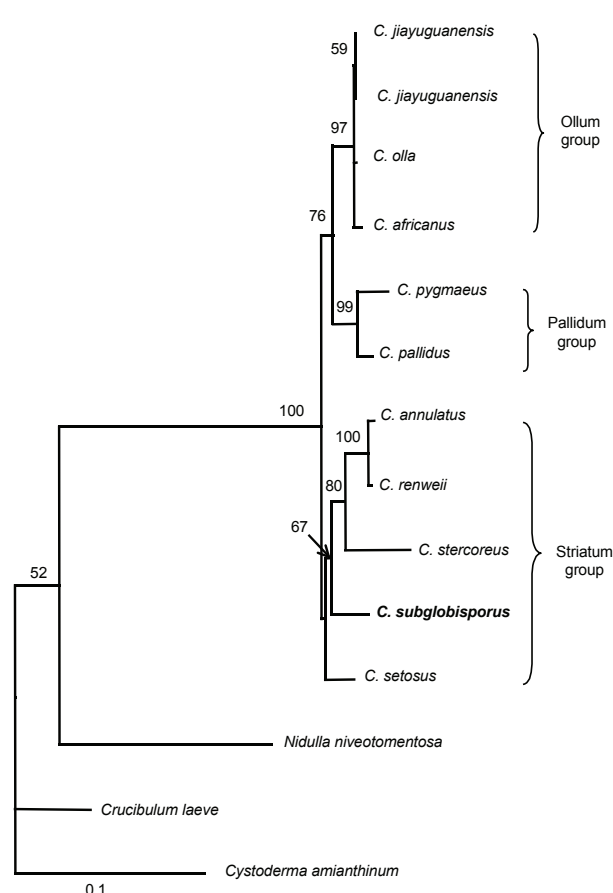


Fig. 3 Phylogenetic relationships of *Cyathus* inferred by maximum likelihood analysis of combined ITS and LSU rDNA sequences. Bootstrap support (BS) values above 50 % are shown.

ters were parsimony-informative, and 134 characters were excluded. This dataset represents 10 *Cyathus* species (11 strains; all taxa for which both ITS and LSU data were available), *Crucibulum laeve*, and *Nidula niveotomentosa*. Kishino-Hasegawa and Shimodaira-Hasegawa tests among NJ, ML, and WP indicated that the ML tree was the best tree (Fig. 3). *Cyathus* is monophyletic with 100 % bootstrap support, and *C. subglobisporus* is sister to *C. annulatus*, *C. renweii*, and *C. stercoreus* in the Striatum group with 67 % bootstrap support.

Taxonomy

Cyathus subglobisporus R.L. Zhao, Desjardin, K. Soyong & K.D. Hyde, *sp. nov.* — MycoBank MB512024; Fig. 4

Peridium obconicum, crassum, 7–10 mm altum, ore 5–8 mm lato, extra pallide flavum vel pallide brunneum, pilis resupinatis et fasciculatis obtectum, intus griseum, argenteum, tenuiter sed distincte striatum; labium minute fimbriatum; epiphragma album, tenue; peridiola 1.5–2 mm diam, lentiformia, tunicam perpallide fuscam ferentia; cortex simplex; sporae subglobosae vel ellipsoideae, 13–18 µm longae, 12–16 µm latae.

Etymology. Refers to its subglobose spores.

Fruiting bodies clavate when young, then opening, extending and becoming obconic to infundibuliform, with relatively straight sides in side view; 7–10 mm high, 5–8 mm wide at the top, with the quotient of height by width 1–1.4; external peridium covered by hairs aggregated into shaggy or hirsute clusters, ivory, pale yellow or buff when young, sometimes with a hint of pale orange, then darkening to pale brown with age and the hairs remaining pallid. Inner peridium surface grey to brownish grey, darkening with age, distinctly plicate when young, becoming striate to smooth with age. *Epiphragm* membranous, white, covered by buff to pale orange-white hairs similar to those on the external surface of the peridium, cracking irregularly during maturation and disappearing or leaving a minutely fimbriate lip along the top edge of the peridium. Base of the fruiting bodies narrower than the rest of the fruiting body but lacking a distinct stipe. *Peridioles* 1.5–2 mm diam, lenticular, greyish brown to pale brown; peridiole covering composed of two layers: a black inner cortex layer, 15–25 µm thick, and a yellowish brown or dark brown outer tunica layer, 25–50 µm thick. *Basidiospores* 13–18 × 12–16 µm (av. = 15.8 ± 2.8 × 14.1 ± 2.1, Q = 1–1.31, Q_m = 1.12 ± 0.19, n = 50), subglobose or rarely broadly ellipsoid, hyaline, smooth, thick-walled (1.5–2(–3) µm). *Basidia* not observed. *Clamp connections* present.

Habitat — On rotten bamboo stems in moist forest.

Specimens examined. *C. annulatus*: CANADA, Alberta, Cypress Hills, above Elkwater lake, 8 Aug. 1968, H.J. Brodie, holotype DAOM 200366. — *C. berkeleyanus*: CHINA, Sichuan Prov., Chengdu, Nov. 1999, Tongxin Zhou, SWFC 20789. — *C. bulleri*: INDIA, West India, Guadeloupe, 8 Feb. 1966, H.J. Brodie, isotype BPI 727126. — *C. cornucopioides*: CHINA, Yunnan Prov., Luxi county, 7 July 1983, L.Z. Zhao, holotype, SWFC 20414. — *C. crispus*: GHANA, Bunsu, 17 June 1949, S.T. Hughes, holotype DAOM 200373. — *C. durus*: USA, Colorado, Denver, 1900, E. Bethel, isotype BPI 727135, 727134. — *C. griseocarpus*: INDIA, Manipur State, Ukhrul, 29 Aug. 1978, H.J. Brodie, holotype DAOM 200396. — *C. guandishanensis*: CHINA, Shanxi Prov., Guandishan, 11 Aug. 1987, M.C. Chang, holotype HMAS 81896. — *C. helenae*: CANADA, Alberta, 20 Aug. 1965, H.J. Brodie, holotype DAOM 200384. — *C. olivaceobrunneus*: CHINA, Yunnan Prov., Dali, 28 Aug. 1939, H.S. Yao, holotype HMAS 01518. — *C. pallidus*: CHINA, Guizhou Prov., Aug. 2000, S.K. Bai, SWFC 21160. — *C. setosus*: JAMAICA,

9 Jan. 1966, H.J. Brodie, holotype DOAM 200815. — *C. subglobisporus*: THAILAND, Chiang Mai Province, Chiang Dao, gregarious on rotten bamboo stem, 22 July 2006, R.-L. Zhao, K.D. Hyde, H.-L. Hu, J.-N. Liu, W. Nilam & C. Ratchadawan, holotype BBH 18348, isotypes MRC 00800 and SFSU zrlc013. — *C. tianshanensis*: CHINA, Neimenggu, collection data unknown, Y.Z. Shang, SWFC 21157. — *C. yunnanensis*: CHINA, Yunnan Prov., 13 Sept. 1935, Q.W. Wang, holotype HMAS 17373.

DISCUSSION

At first glance the proposed new species looks like *Cyathus griseocarpus* (Brodie 1984), which is commonly encountered in northern Thailand. Both species share features of pale yellow or pale brown fruiting bodies, an external peridium covered by pale yellow hairs that aggregate into conic mounds, an inner peridium surface that is striate to plicate, and grey-toned peridioles. The subglobose basidiospores and pale brown peridioles of *C. subglobisporus*, however, can differentiate it from *C. griseocarpus*. The latter species possesses much smaller, more ellipsoid basidiospores (av. = 7.3 ± 0.7 × 5.3 ± 1.3 µm, Q_m = 1.4 ± 0.6, from holotype) and pale grey peridioles. The LSU tree (Fig. 2) clearly indicates that *C. griseocarpus* belongs to the Ollum group and is distantly related to *C. subglobisporus*.

Species of *Cyathus* whose fruiting bodies are pale yellow or pale brown and have a distinctly plicate inner peridium include *C. annulatus*, *C. berkeleyanus*, *C. bulleri*, *C. cornucopioides*, *C. crispus*, *C. durus*, *C. guandishanensis*, *C. helenae*, *C. pallidus*, *C. setosus*, *C. tianshanensis*, and *C. yunnanensis*. Only *C. bulleri*, *C. guandishanensis*, and *C. yunnanensis*, however, possess subglobose or globose spores. After comparison of their spore size, *C. subglobisporus* is distinct in possessing larger spores (av. = 15.8 ± 2.8 × 14.1 ± 2.1, Q_m = 1.12 ± 0.19) than those of *C. bulleri* (av. = 7.3 ± 1.7 × 6.8 ± 1.8 µm, Q_m = 1.08 ± 0.15, from isotype) and *C. guandishanensis* (av. = 11.6 ± 2.15 × 8.6 ± 1.4 µm, Q_m = 1.37 ± 0.46, from holotype), and smaller spores than those of *C. yunnanensis* (av. = 22.38 ± 3.6 × 18 ± 3, Q_m = 1.25 ± 0.42, from holotype). Of the latter three species sequence data was obtained successfully only from *C. guandishanensis*. The LSU tree (Fig. 2) clearly indicates that *C. guandishanensis* belongs to the Ollum group and is distantly related to *C. subglobisporus*.

The spores of *Cyathus olivaceobrunneus* (named after its olive-brown peridium) are similar to those of *C. subglobisporus* in shape and size, and the former species has been suspected to be a synonym of *C. poeppigii* (Brodie 1975). Examination of the type specimen of *C. olivaceobrunneus* showed the colour of its fruiting bodies to be much darker and to have longer spores (av. = 17.88 ± 8.75 × 13.2 ± 2, Q_m = 1.36 ± 0.34, from holotype) than those of *C. subglobisporus*. We were unable to generate quality sequence data from available material of *C. olivaceobrunneus* for comparison with *C. subglobisporus*.

The genus *Cyathus* was originally subdivided into seven groups based on morphological characters (Brodie 1975, 1984). Brodie's classification system is not supported by phylogenetic analysis of molecular data, and the recognition of only three infrageneric groups (Ollum, Pallidum, and Striatum) was established based on morphological and molecular data (Zhao et al. 2007). In this study, the combined ITS and LSU phylogenies indicate that *C. subglobisporus* belongs to the Striatum group.



Fig. 4 *Cyathus subglobosporus* (from holotype) a. Fruiting body in top view showing peridioles and plications on the inner surface of peridium; b. fruiting body in side view showing shaggy hairs; c. opening changes with the development of fruiting bodies; d. basidiospores. — Scale bars: a = 1.5 mm; b = 1.8 mm; c = 2 mm; d = 11 μ m.

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