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ARTICLE

Gelatinomyces siamensis gen. sp. nov. (*Ascomycota*, *Leotiomycetes*, *incertae sedis*) on bamboo in Thailand

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Abstract: *Gelatinomyces siamensis* gen. sp. nov., *incertae sedis* within *Leotiomycetes*, the Siamese jelly-ball, is described. The fungus was collected from bamboo culms and branches in Nam Nao National Park, Phetchabun, Thailand. It presents as a ping-pong ball-sized and golf ball-like gelatinous ascostroma. The asci have numerous ascospores, are thick-walled, and arise on discoid apothecia which are aggregated and clustered to form the spherical gelatinous structures. An hyphomycete asexual morph is morphologically somewhat phialophora-like, and produces red pigments. On the basis of phylogenetic analysis based on rRNA, SSU, and LSU gene sequences, the lineage is closest to *Collophora rubra*. However, ITS sequences place the fungus on a well-separated branch from that fungus, and the morphological and ecological differences exclude it from *Collophora*.

Key words:
Bambusa
Bambusicolous fungi
Collophora
Gelatinous ascostroma
Kao-niew ling
Siamese jelly-ball
Molecular phylogeny
Polyspored asci
Red pigments

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INTRODUCTION

Five specimens of a rarely encountered fungus were collected by N. S. from twigs of a bamboo ("Bong"; *Bambusa nutans*) in Nam-Nao National Park, Thailand, in August and September 2009–2011. The local name, "Siamese jelly-ball" or "kao-niew ling", recalls the dark, golf ball-like and gelatinous ascostromata, and it is claimed to be edible. It has only been found on bamboo, and was not seen on any other plants in the area. It occurs at 390–840 m, where the average temperature is generally less than at lower and flatter localities.

Numerous bambusicolous fungi have been reported, with the number of fungal genera reportedly greater in the tropical regions than other regions due to the higher number of bamboo species. More than 630 species of fungi are known from bamboo, most of which are ascomycetes; Eriksson & Yue (1998) discuss 587 names of pyrenomycetes described on bamboo, and approximately 200 species occur in south-east Asia (Hyde *et al.* 2002). However, few produce distinctive to sometimes very large ascostroma similar to those seen in the Thai fungus. *Daldinia bambusicola* (Ju *et al.* 1997) has a black, smooth surface, and relatively smaller ascostromata. *Engleromyces goetzei* produces very large ascostromata, up to 4.5 kg in weight, and *E. sinensis* is also considerably larger than *Gelatinomyces*; these two species appear to be confined to particular bamboo species normally found on very high mountains (Whalley *et al*. 2010). The hypocrealean fungi, *Ascopolyporus philodendrus* (Bischoff *et al.* 2005), *Moelleriella gaertneriana* (Chaverri *et al*. 2008), and *Mycomalus bambusinus* (Bischoff & White 2003), produce rather pale, smooth-walled or brain-like ascostromata and are probably associated with insects. *Munkia martyris*, *Neomunkia sydowii* and *Ustilaginoidea virens* are other hypocrealean fungi in the tribe *Ustilaginoideae* producing large asexual stromata on bamboo twigs but their relationships have not yet been resolved (Bischoff *et al*. 2005). In addition, *Shiraia bambusicola* (*Dothideomycetes*, *Pleosporomycetidae*) produces spectacular pinkish orange ascostromata (Liu *et al*. 2012). All taxa mentioned above have perithecoid or flask-shaped ascomata, 8-spored asci, ascospores that are not to several septate, may or may not have interascal filaments, and occur on living leaves or branches.

Since the Siamese jelly-ball fungus is distinct from any previously scientifically named fungus, it is described as a monotypic new genus here. It does, however, have some affiliation to *Collophora,* but molecular evidence supports its separation.

MATERIALS AND METHODS

Collecting and field sites

Five specimens were collected from *Bambusa nutans,* along creeks in Nam Nao National Park, Thailand, at an altitude of 390–840 m. The first specimen was found behind the Nam Nao Tourist Service area in late September 2009, and the next four specimens were from bamboo along the main road in August and September 2011. Bamboo branches or culms with specimens were cut off from the main culms, wrapped in newspaper and brought back to the Plant Pathology Laboratory, Faculty of Agriculture, Khon Kaen University, for isolation into pure culture. Dried reference specimens and living cultures have been deposited at the Khon Kaen University Culture Collection (KKUK), at Biotec Culture Collection (GESIASCO), CBS (CBS) and the Royal Botanic Gardens Kew (K; GESI).

Isolation, spore discharge, and germination

Two isolation techniques were employed to obtain pure cultures: tissue transplanting from parts of ascomata, and ascospores forced to eject directly from asci by exposing a piece of ascomata to incandescent light, Phillips 220V, 15W, for a few minutes. Ejected ascospores were collected on PDA plates or in sterile Petri dishes. The ejected ascospores on PDA plates were allowed to germinate directly to form colonies, while those in the empty Petri dishes were diluted in sterilized water and subsequently plated out on PDA plates to obtain single ascospore isolates. All white colonies forming within 3–4 d, with diffusible red pigment in the agar on the reverse side of these colonies were then selected and maintained for further study.

Morphological investigation

Fresh gelatinous ascostromata and thin sections of ascomata embedded in paraffin wax were examined by light microscopy (Olympus Model BX51 and DP21-LPT) equipped with anOlympus Nomarski Slider for Transmitted Light U-DICT (Olympus Model U-DICT) to record the detailed morphology of the sexual and asexual morphs. Slide cultures of representative isolates, from stroma, single ascus, and single ascospore isolations, were also examined microscopically for the development of asexual structures and for the production of crystals of insoluble pigment. Structures were mounted in water, and 30 measurements (at 1 000 ×) made of each feature. The 5th and 95th percentiles were defined for all measurements, and the extremes are given in parentheses, including the value of the mean ± SD and L/W ratio (Damm *et al*. 2008, Gramaje *et al*. 2012). To investigate discrete conidiomata production, water agar culture plates with sterile pine needles were placed under conditions defined by Gramaje *et al.* (2012) for 4–5 wk. Ascospores and conidia were suspended in distilled water then air dried on cellulose acetate filter paper (Sartorius Stedim Biotech, Bohemia, NY) for scanning electron microscopy. The samples were sputter-coated with a film of gold using a Polaron Range SC7620 sputter coater and examined under a LEO 145OVP scanning electron microscope.

DNA extraction, PCR amplification, DNA sequencing, and phylogenetic analysis

The genomic DNA of representative strains isolated from single asci and single ascospores was extracted from active growing mycelia on PDA plates using a cetyltrimethylammonium bromide (CTAB) protocol (Jeewon *et al.* 2004, Cai *et al.* 2006). The whole and partial sequences from three different regions of the rDNA molecules; the ribosomal small subunit (SSU), large subunit (LSU), and internal transcribed spacer (ITS), characterised by different rates of evolution, were amplified by PCR using primers having sequences and target regions shown in Table 1. Whole sequences of SSU were cloned using pGEM-T Easy Vector (Promega, Promega Corporation, Madison, WI) and *Escherichia coli* DH5α as a host. The amplification conditions were performed in a 50 µL reaction volume as follows: 1 × PCR buffer (Invitrogen™ Life Technologies, Foster, CA), 0.2 mM each dNTP, 0.3 µM of each primer, 1.5 mM $MgCl_2$, 0.8 units *Tag* DNA Polymerase (Invitrogen™ Life Technologies), and 100 ng DNA. PCR parameters for all the regions were as follows: initial denaturation at 94 ˚C for 3 min, 30 cycles of 94 ˚C for 1 min, 52 ˚C for 50 s, and 72 ˚C for 1 min, and final extension of 72 ˚C for 10 min. The PCR amplified products were examined by electrophoresis using 1 % agarose gel containing ethidium bromide (0.5 µg mL). The separated PCR products were then observed under short wavelength UV light. DNA sequencing was performed using the primers as mentioned above in an Applied Biosystems 3730 DNA Analyser at Macrogen Inc (#60-24, Gasan-dang, Geumchen-gu, Seoul, Korea). Since we could not assign or differentiate the fungus to known taxa, we used SSU, LSU and ITS for sequence comparisons and in BLASTn searches (www.ncbi.nlm.nih.gov). The rDNA sequences of the new fungus have been deposited in GenBank under accession numbers JX219377 and JX219378 (SSU), JX219381 and JX219382 (LSU), and JX219379 and JX219380 (ITS regions including 5.8S rDNA) for isolates KKUK1 and KKUK2, respectively.

To construct the phylogenetic tree, the analysis was modified from Greif *et al.* (2007), instead of using two taxa, *Orbilia auricolor* and *Scutellinia scutellata* as outgroup, only *O. auricolor* was employed. The sequence data of the Siamese Jelly-ball were aligned by ClustalX2 with sequences of 60 species retrieved from GenBank (www.ncbi.nlm.nih.gov) representing different classes of ascomycete fungi, including *Arthoniomycetes*, *Dothideomycetes*, *Eurotiomycetes*, *Lecanoromycetes*, *Leotiomycetes*, *Lichinomycetes,* and *Sordariomycetes,* where both SSU and LSU sequences data were available (Table 2), and manually edited by MEGA 5.05. A data set comprising all known species of *Collophora* with available ITS sequences (Table 3) was used for comparison and the outgroups for this dataset were *Neobulgaria pura* and *Leotia lubrica*. A maximum parsimony analysis was conducted using PAUP v. 4.0b10 (Swofford 1998). A heuristic search was performed using parsimony as the optimality criterion. Gaps were treated as missing data. Starting trees were obtained at random via stepwise addition with tree-bisection-reconnection as the branch-swapping algorithm, and with the MulTrees option in effect. After 100 stepwise additional sequences were completed, confidence in the branches of the resulting trees was evaluated by bootstrap analysis

Table 1. PCR primers used for obtaining DNA sequences of the *Gelatinomyces siamensis.*

Name	Sequence (5'-3')	Target region[a]	Reference
NS1	GTAGTCATATGCTTGTCTC	SSU 20-38	White *et al.* (1990)
NS4	CTTCCGTCAATTCCTTTAAG	SSU 1150-1131	White *et al*. (1990)
SR8R	GAACCAGGACTTTTACCTT	SSU 732-749	Vilgalys & Hester (1990)
NS8	TCCGCAGGTTCACCTACGGA	SSU 1788-1768	White *et al.* (1990)
ITS4	TCCTCCGCTTATTGATATGC	Internal transcribed spacer (ITS) regions LSU 60-41	White *et al.* (1990)
ITS5	GGAAGTAAAAGTCGTAACAAGG	SSU 1744-1763	White *et al.* (1990)
NL1	GCATATCAATAAGCGGAGGAAAAG	Domain of large subunit (LSU) rDNA	O'Donnell (1993)
NL4	GGTCCGTGTTTCAAGACGG	D1/D2 domain of LSU rDNA	O'Donnell (1993)

[a] *Saccharomyces cerevisiae* numbering.

Table 2. Fungal taxa used for phylogenetic analysis with GenBank accession numbers for small subunit (SSU) and large subunit (LSU) sequences, including their main characteristics.

Ingroup	GenBank accession no. (SSU, LSU)	Origin (substrate, country)	Main characteristics	Reference
Class *Sordariomycetes*				
Cainia graminis	AF431948, AF431949	*Sesleria albicans*, France	Stromatic perithecial, unitunicate with pore, saprophytic, plant parasitic or endophytic	Lumbsch *et al*. (2005)
Chaetomium globosum	AB048285, AY346272	Indoor environment, Germany		Huhndorf *et al.* (2004), Okane *et al.* (2001)
Diatrype disciformis	DQ471012, DQ470964	Decayed wood, Netherlands		Spatafora *et al.* (2006)
Hypocrea americana	AY544693, AY544649	*Fomitopsis pinicola*, USA		Lutzoni *et al.* (2004)
Sordaria fimicola	AY545724, AY545728	Dung, Canada		Cai *et al.* (2006)
Xylaria acuta	AY544719, AY544676	Decayed wood, USA		Rogers (1984)
Xylaria hypoxylon	AY544692, AY544648	Downed rotting wood, USA		Spatafora *et al*. (2006)
Class *Leotiomycetes*				
Botryotinia fuckeliana	AY544695, AY544651		Apothecial or cleistothecial, unitunicate and inoperculate, saprophytic, plant parasitic, some species known only anamorphic i.e. *Collophora*	Hirschhauser & Frohlich (2007)
Bulgaria inquinans	DQ471008, DQ470960	Germany		Spatafora *et al*. (2006)
Collophora rubra	GQ154628, GQ154608	Wood necrosis close to pruning wound, South Africa		Damm *et al*. (2010)
Crinula calciiformis	AY544729, AY544680			Lutzoni *et al*. (2004)
Monilinia fructicola	AY544724, AY544683	Fruit, USA		Fulton & Brown (1997)
Neofabraea malicorticis	AY544706, AY544662	Apples, USA		Lutzoni *et al*. (2004)
Pezicula carpinea	DQ471016, DQ470967	*Carpinus caroliniana*, Canada		Spatafora *et al*. (2006)
Potebniamyces pyri	DQ470997, DQ470949	Cankered bark, USA		Spatafora *et al*. (2006)
Class *Lecanoromycetes*				
Diploschistes thunbergianus	AF274112, AF274095	Australia	Apothecial, unitunicate, rostrate asci, mostly lichenized	Lumbsch *et al*. (2005)
Lobaria scrobiculata	AY584679, AY584655	USA		Lutzoni *et al*. (2004)
Trapella placodioides	AF119500, AF274103	Wall, UK		Lumbsch *et al*. (2005)
Class *Lichinomycetes*				
Lempholemma polyanthes	AY548805, AF356691	USA	Apothecial, bitunicate, lichenized	Lutzoni *et al*. (2004)

Table 2. (Continued).

Ingroup	GenBank accession no. (SSU, LSU)	Origin (substrate, country)	Main characteristics	Reference
Peltula auriculata	DQ832332, DQ832330			Miadlikowska *et al*. (2006)
Peltula umbilicata	DQ782887, AF356689			Miadlikowska *et al*. (2006)
Class *Eurotiomycetes*				
Eremascus albus	M83258, AY004345	Dried fruit, UK	Cleistothecial, non-fissitunicate, saprophytic or plant parasitic	Berbee & Taylor (1992)
Eurotium rubrum	U00970, AY004346			Lumbsch *et al*. (2005a)
Penicillium expansum	DQ912698, AF003359	Fruit, USA		Seifert & Louis-Seize (2000)
Exophiala dermatitidis	DQ823107, DQ823100	Human, USA		James *et al*. (2006)
Glyphium elatum	AF346419, AF346420	Salix, USA		Lindemuth *et al*. (2001)
Ramichloridium anceps	DQ823109, DQ823102	Soil under *Thuja plicata,* Canada		James *et al*. (2006)
Class *Dothideomycetes*				
Order *Botryosphaeriales*				
Botryosphaeria ribis	DQ678000, DQ678053	*Ribes*, USA	Pseudothecial, fissitunicate asci, saprophytic or plant parasitic	Schoch *et al*. (2006)
Botryosphaeria stevensii	DQ678012, DQ678064	*Fraxinus excelsior*, Netherlands		Schoch *et al*. (2006)
Guignardia bidwellii	DQ678034, DQ678085	*Parthenocissus tricuspidata*		Schoch *et al*. (2006)
Order *Capnodiales*				
Catenulostroma abetis	DQ678040, DQ678092	*Abies*, Germany	Pseudothecial, fissitunicate asci, saprophytic or plant parasitic	Schoch *et al*. (2006)
Cercospora beticola	DQ678039, DQ678091	*Beta vulgaris*, Italy		Schoch *et al*. (2006)
Microxyphium citri	AY016340, AY004337	Fruit of *Citrus sinensis*, Spain		Lumbsch *et al*. (2005)
Mycosphaerella punctiformis	DQ471017, DQ470968	Dead fallen leaves of *Quercus robur*, Netherlands		Spatafora *et al*. (2006)
Scorias spongiosa	DQ678024, DQ678075	Aphid		Schoch *et al*. (2006)
Order *Dothideales*				
Aureobasidium pullulans	DQ471004, DQ470956	Fruit of *Vitis vinifera,* France	Pseudothecial, fissitunicate asci, pseudoparaphyses absent, mainly saprophytic	Spatafora *et al*. (2006)
Delphinella strobiligena	AY016341, AY016358	Cone of *Pinus halepensis,* Greece		Lumbsch & Lindemuth (2001)
Discosphaerina fagi	AY016342, AY016359	Leaf of *Populus*, UK		Lumbsch & Lindemuth (2001)
Dothidea ribesia	AY016343, AY016360	Cult of *Ribes,* Switzerland		Lumbsch *et al*. (2005)
Stylodothis puccinioides	AY016353, AY004342	*Viburnum lantana*, Switzerland		Lumbsch *et al*. (2005)
Order *Myriangiales*				
Cladosporium cladosporioides	DQ678004, DQ678057	Leaf of *Arundo*, England	Pseudothecial, globosa asci, non-ostiolar, saprophytic or plant parasitic	Schoch *et al*. (2006)
Davidiella tassiana	DQ678022, DQ678074	Human skin, Netherlands		Schoch *et al*. (2006)
Elsinoe centrolobi	DQ678041, DQ678094	*Centrolobium robustum*, Brazil		Schoch *et al*. (2006)

Table 2. (Continued).

Ingroup	GenBank accession no. (SSU, LSU)	Origin (substrate, country)	Main characteristics	Reference
Myriangium duriaei	AY016347, DQ678059	*Chrysomphalus aonidium*, Argentina		Lumbsch & Lindemuth (2001)
Order *Pleosporales*				
Arthopyrenia salicis	AY538333, AY538339	Bark of *Salix*, Netherlands	Perithecoid pseudothecial, ostiolar, non-lichenized or lichenized with fissitunicate asci and pseudoparaphyses present	Lumbsch *et al*. (2005)
Cucurbitaria elongata	DQ678009, DQ678061	*Cytisus sessilifolius*, France		Schoch *et al*. (2006)
Dendrographa leucophaea	AY548803, AY548810			Lutzoni *et al*. (2004)
Lecanactis abietina	AY548805, AY548812			Lutzoni *et al*. (2004)
Neotestudina rosatii	DQ384069, DQ384107	Seed of *Cuminum cyminum* imported from India, Japan		Kruys *et al*. (2006)
Pleospora herbarum	DQ247812, DQ247804	Leaf of *Medicago sativa*, India		Schoch *et al*. (2006)
Setosphaeria monoceras	AY016352, AY016368			Lumbsch & Lindemuth (2001)
Trematosphaeria heterospora	AY016354, AY016369	Iris, Switzerland		Lumbsch *et al*. (2005)
Westerdykella cylindrica	AY016355, AY004343	Cow dung, Kenya		Lumbsch *et al*. (2005)
Order *Insertae sedis*, Family *Tubeufiaceae*				
Helicomyces lilliputeus	AY856942, AY856899	Rotten dicotyledonous wood, USA	Pseudothecial, fissitunicate	Tsui & Berbee (2006)
Helicomyces roseus	DQ678032, DQ678083	Submerged bark, Switzerland		Schoch *et al*. (2006)
Tubeufia cerea	AY856947, AY856903			Tsui & Berbee (2006)
Class *Arthoniomycetes*				
Arthonia dispersa	AY571379, AY571381	*Syringa vulgaris*, Sweden	Fissitunicate, mostly lichenized	Lumbsch *et al*. (2005)
Dendrographa leucophaea	AY548803, AY548810			Lutzoni *et al*. (2004)
Lecanactis abietina	AY548805, AY548812			Lutzoni *et al*. (2004)
Unknown				
Gelatinomyces siamensis		*Bambusa nutans,* Thailand	Apothecial, aggregated, embedded in gelatinous ball	This study
Isolate KKUK1	JX219377, JX219381			
Isolate KKUK2	JX219378, JX219382			
Outgroup Class *Orbiliomycetes*				
Orbilia auricolor	DQ471001, DQ470953	Soil, UK	Apothecial, non-fissitunicate	Spatafora *et al*. (2006)

(Felsenstein 1985) using 1000 replicates. The resultant tree was visualized using PAUP v. 4.0b10 (Swofford 1998). Additionally, Bayesian analysis using MrBayes version 3.2.1 which approximates posterior probabilities of clades using a Markov chain-Monte Carlo (MCMC) method (Huelsenbeck & Ronquist 2001) were performed. Four chains for a total of 2 500 000 generations for SSU and LSU datasets and 600 000 generations for ITS dataset with phylogenetic trees sampled every 100 generations were applied to all searches. The general time-reversible model with invariant sites and gamma distribution (GTR + I + Γ) were used. Scores in Baysian analyses were estimated as posterior probabilities calculated from the posterior distribution of trees excluding 25 % burn-in trees (Huelsenbeck & Rannala 2004). Nodes obtained from both analysis were considered well supported by bootstrap values greater than or equal to 70 % and posterior probabilities greater than or equal to 0.95 (Spatafora *et al.* 2007).

Table 3. Fungal taxa in the *Collophora* species, *Leotiomycetes* used for phylogenetic analysis with GenBank accession numbers for ITS sequences, including their main characteristics.

Species	GenBank accession no. (ITS)	Origin (substrate, country)	Main characteristics	Reference
Order Incertae Sedis, Family Incertae Sedis				
Collophora africana	GQ154570	*Prunus salicina*, South Africa	Hyphae carry short necks or mere collarettes that release conidia; discrete conidiomata present	Damm *et al.* (2010)
C. capensis	GQ154571 GQ154572 GQ154573 GQ154574	*Prunus salicina*, South Africa	As above	Damm *et al.* (2010)
C. hispanica	JN808840 JN808841 JN808842	*Prunus dulcis,* Spain	As above	Gramaje *et al.* (2012)
C. paarla	GQ154586	*Prunus salicina*, South Africa	As above	Damm *et al.* (2010)
C. pallida	GQ154578 GQ154580 GQ154582 GQ154584	*Prunus salicina*, South Africa	As above	Damm *et al.* (2010)
C. rubra	GQ154562 GQ154564 GQ154566 GQ154568	*Prunus salicina*, South Africa	As above	Damm *et al.* (2010)
Gelatinomyces siamensis Isolate KKUK1 Isolate KKUK2	 JX219379 JX219380	*Bambusa nutans* Thailand	Sexual morph present, asexual conidia produced on short and long conidiogenous cells	This study
Outgroup-*Leotiales*				
Leotia lubrica	GU222296			
Neobulgaria pura	HM051080			

RESULTS

The total number of characters in the SSU analysis was 2954, including gaps. All characters were given equal weight. The number of constant characters was 1122, and 1039 were parsimony-informative. The maximum parsimony analysis yielded a single tree with 4968 characters. The scores of the tree were as following; Consistency index (CI) = 0.623, Retention index (RI) = 0.468, Rescaled consistency index (RC) = 0.291, and Homoplasy index (HI) = 0.377. In the partial LSU sequence, the total number of characters from the alignment was 644 with gaps; 268 and 299 out of these 644 characters were constant and parsimony-informative, respectively. Only one tree was generated by maximum parsimony analysis from the LSU data set, with CI = 0.338, RI = 0.635, RC = 0.215, and HI = 0.622.

In the SSU tree, only members of one class of fungi grouped in the same clade as isolates KKUK1 and KKUK2. These belonged to *Leotiomycetes,* with a bootstrap score of 70 (Fig. 1). This suggests that the two isolates KKUK1 and KKUK2 are *Leotiomycetes.* To ascertain their closest sequenced relatives, the SSU sequences were also compared to those available in GenBank using the standard nucleotide-nucleotide BLAST program. Isolates KKUK1 (JX219377) and KKUK2 (JX219378) had the highest sequence similarity with *Collophora rubra* (GQ154628) at 97 %. The LSU tree gave a similar result, with the studied isolates KKUK1 and KKUK2 clustered in *Leotiomycetes,* with a bootstrap score of 72 (Fig. 3). BLASTn searches of SSU and LSU sequences of the isolates confirmed these results.

To determine whether the bamboo fungus was a *Collophora* species or not, an additional ITS tree was constructed; this had 638 characters, of which 380 were constant characters and 100 parsimony informative. The tree was parsimoniously constructed with *Neobugaria pura* and *Leotia lubrica* as outgroups (CI = 0.893, RI = 0.886, RC = 0.791, and HI = 0.107). KKUK1 and KKUK2 clustered together (bootstrap score = 100) and were separated from six *Collophora* species with bootstrap support at 99 (Fig. 5).

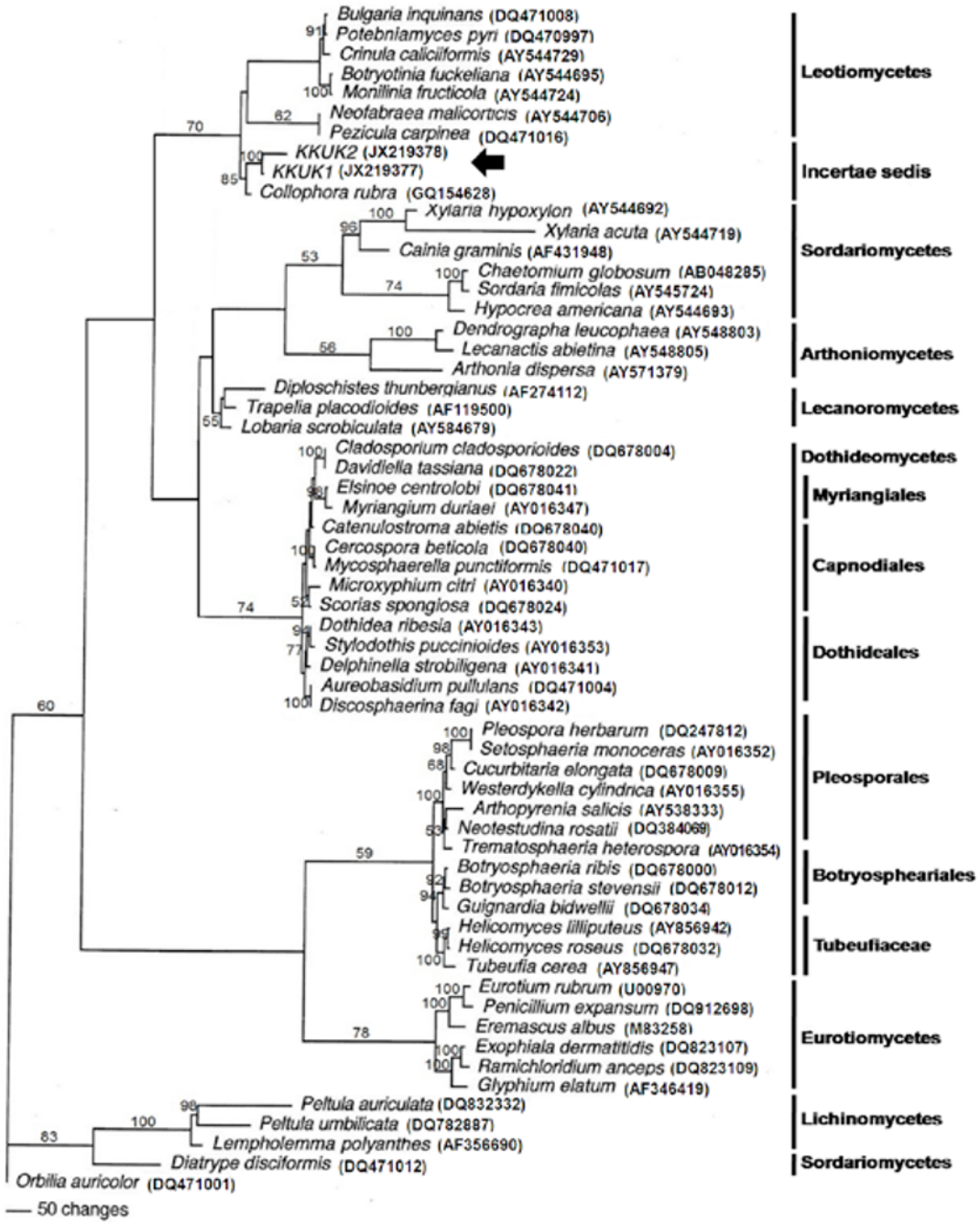

Fig. 1. Phylogenetic tree from maximum parsimony analysis based on SSU sequences showing the position of *Gelatinomyces siamensis* isolates KKUK1&2 (arrow), which is grouped closely in *Leotiomycetes*. Bootstrap support values > 50 % are shown above branches.

The SSU and LSU trees inferred by Bayesian analysis gave phylogenetic relationship results similar to those employed by maximum parsimony, although the topologies of the trees were different (Figs 2, 4). The KKUK1 and KKUK2 isolates were grouped in *Leotiomycetes*, with posterior probability values of 1.0. Similarly, the additional tree inferred from ITS information using the same dataset employed in Fig. 5 produced a tree with similar topology and support values which indicated that *Collophora* species were clustered separately from KKUK1 and KKUK2.

On the basis of the DNA sequence analyses from the SSU, LSU, and ITS regions, and the sexual and asexual characters of the fungus, we conclude that Siamese jelly-ball, found on bamboo in Nam Nao National Park, Thailand, is new to science and represents a previously undescribed genus and species, named *Gelatinomyces siamensis* here.

TAXONOMY

Gelatinomyces Sanoamuang, Jitjak, Rodtong & Whalley, **gen. nov.**
MycoBank MB804026

Diagnosis: *Ascostromata* ball-shaped, ping pong ball-sized, gelatinous, dark coloured when mature, with red pigmentation

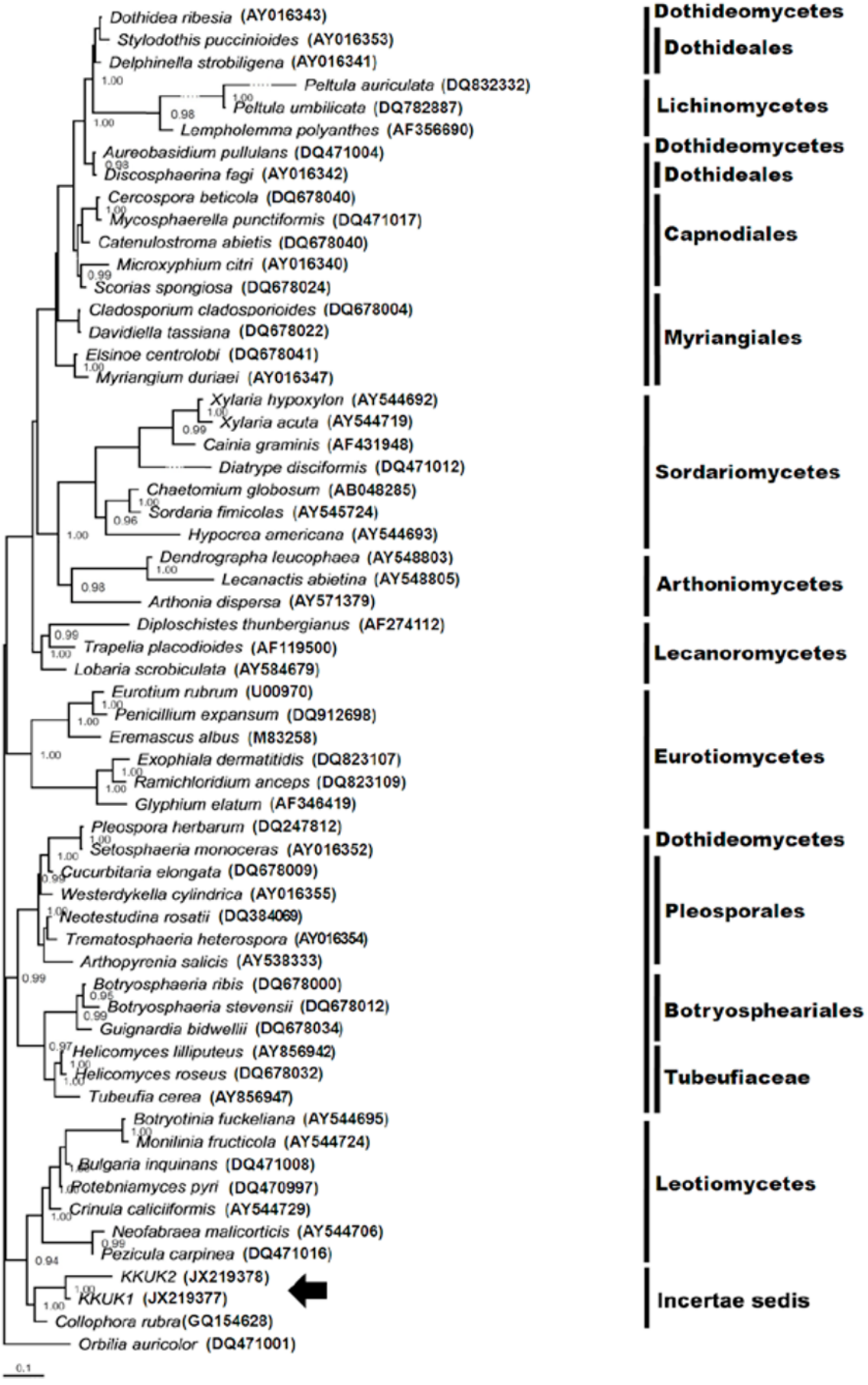

Fig. 2. Phylogenetic tree obtained from Bayesian analysis inferred from SSU sequences showing phylogenetic relationship among fungal species selected from *Ascomycota* and *Gelatinomyces siamensis* isolates KKUK1&2. Posterior probability values ≥ 0.95 yielded from a Bayesian analysis shown at nodes. *Gelatinomyces siamensis* is grouped in *Leotiomycetes* (arrow).

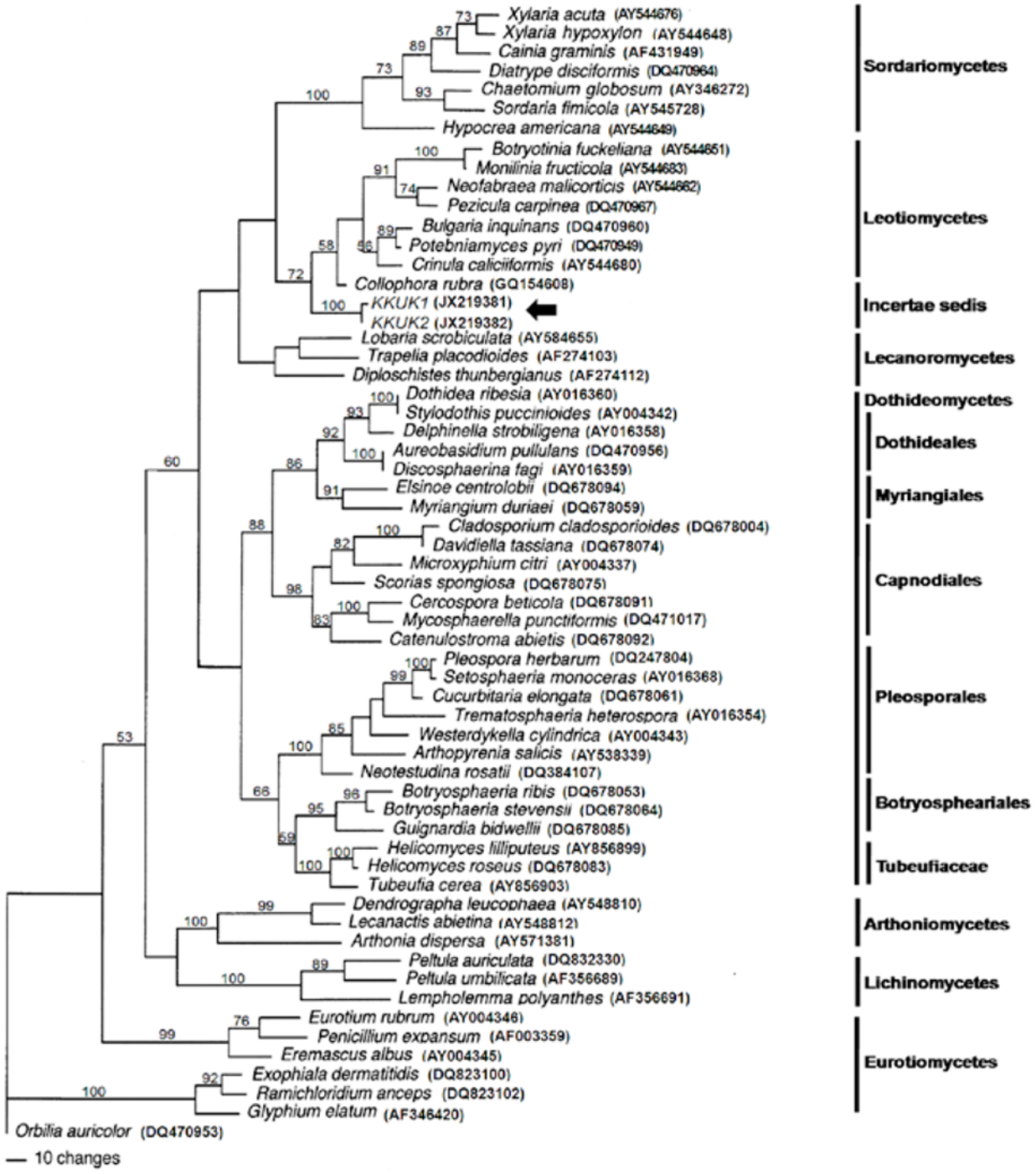

Fig. 3. Phylogenetic tree from maximum parsimony analysis based on LSU sequences showing the position of *Gelatinomyces siamensis* isolates KKUK1&2 (arrow), which clusters very close to *Leotiomycetes*. Bootstrap support values > 50 % are shown above branches.

inside. *Ascomata* apothecia, aggregated, containing thick-walled multi-spored asci.

Etymology: Recalling the gelatinous nature of the ball shaped ascostromata, and *myces* = fungus.

Type: *Gelatinomyces siamensis* N. Sanoamuang *et al*. 2013.

Description: *Stromata* present, pale grey to dark coloured, soft gelatinous in texture. *Ascomata* apothecia, aggregated but well separated, translucent, pale grey, convex or cushion-shaped, ± globose or pulvinate when young, later becoming brown-black to black and discoid, flattened or slightly depressed when mature. *Apothecia* sessile, the exciple dark and gelatinous, well-developed, ± glabrous. *Hymenial layer* composed of interascal filaments, asci, and with a gelatinous layer at the surface; interascal tissue poorly developed, composed of simple, branched paraphyses. *Asci* cylindrical, tapered at the base, without an operculum or any opening characters at the tip, non-amyloid apical ring, multi-spored, persistent. *Ascospores* minute, hyaline, globose to ovoid shaped with smooth walls.

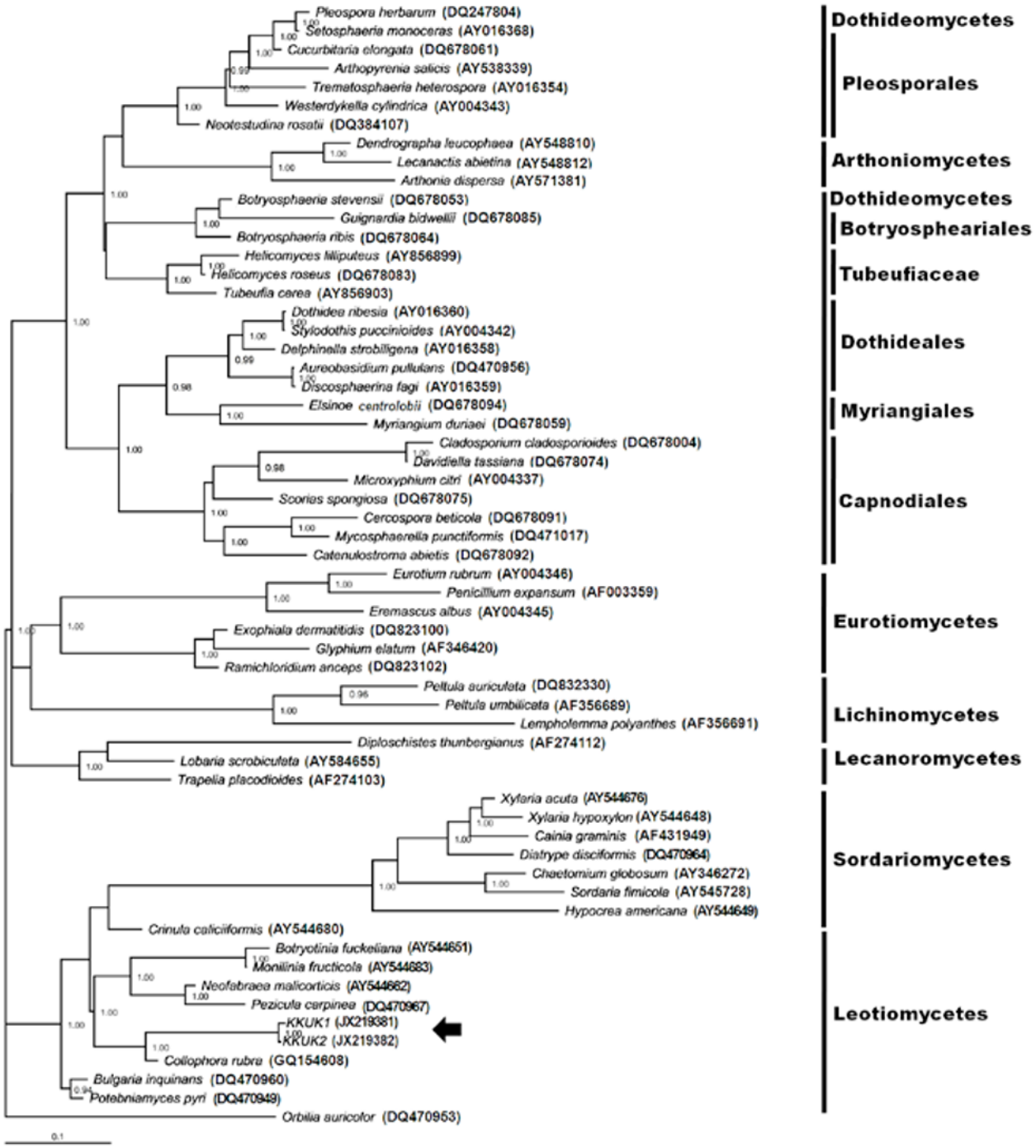

Fig. 4. Phylogenetic tree obtained from Bayesian analysis inferred from LSU sequences showing phylogenetic relationship among fungal species selected from Ascomycota and *Gelatinomyces siamensis* isolates KKUK1&2. Posterior probability values ≥ 0.95 shown at nodes.

Colonies slow-growing, white, moist at first then becoming dry with age, lacking aerial mycelium. *Conidiophores* hyaline, of two types, either with very short conidiogenous cells on hyphal cells, or longer conidiogenous cells arising at branching points where a septum forms. *Conidia* vary in shape and size at first, aggregated in masses around hyphae on the agar surface, becoming ovoid, minute, and powdery with age. No discrete conidiomata observed on sterilized pine needles on the surface of water agar.

Gelatinomyces siamensis Sanoamuang, Jitjak, Rodtong & Whalley, **sp. nov.** MycoBank MB804027 (Figs 6–7)

Diagnosis: Stromata gelatinous, ball shaped, 3-4 cm diam, surface with many discoid ascomata, aggregated but separate, pale greenish to pinkish grey, becoming black when mature, a band of red pigmented in the interior. *Asci* clavate with a short stipe, unitunicate in structure, multispored. *Ascospores* tiny, globose to slightly ovoid. *Asexual morph Phialosphora*-like, conidia produced on very short conidiogenous cells on hyphal cells and also on longer

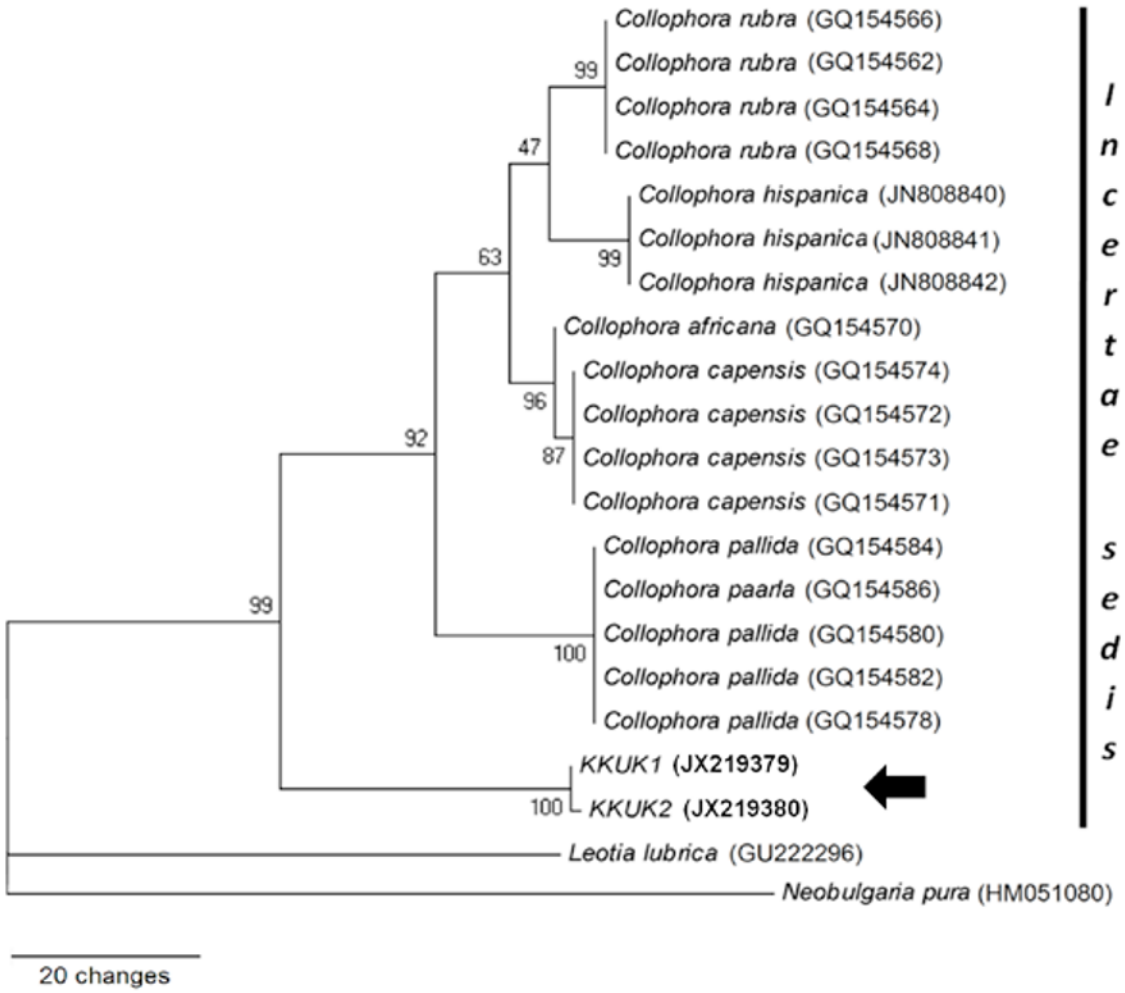

Fig. 5. Phylogenetic tree from maximum parsimony analysis based on ITS sequences showing the position of *Gelatinomyces siamensis* isolates KKUK1&2 (arrow) which clusters very close to *Collophora* spp. Bootstrap support values > 50 % are shown above the branches.

conidiogenous cells. *Colonies* white, but with a distinctive red pigmented underside, the red pigment diffusing into agar.

Etymology: Named after the country of origin.

Type: **Thailand:** *Phetchabun Province*: Nam Nao National Park, on bamboo culms and branches, 11 Sept. 2011, *Sanoamuang* (KKUK – **holotype**; KKUK1, 2, 3, 4…. 100 – ex-holotype cultures; Biotec Culture Collection codes: Gesiasco 6, 11, 18, and 19; CBS unique codes: CBS 135071, 135072, 135073 and 135074; K – Gesi01, 02 and 03 – isotypes).

Description: Stromata, 3–4 cm across, pale grey to brown black, soft and highly gelatinous, inner tissue repeatedly folded, up to golf-ball size when fresh, dark to black, hard and sclerotium-like when dry; 300–560 discoid ascomata aggregated, but separate, embedded in the surface of a single gelatinous stromatic ball. *Ascomata* apothecia, usually 100–200 µm tall and 340–600 µm diam in surface view, translucent, greyish green, sometimes pale pink, convex or cushion-shaped when young, ± globose or pulvinate, brown-black to black, discoid, flattened or slightly depressed when mature, sessile. *Hymenium* dark and gelatinous, well-developed, the exciple is smooth, interascal ascal tissues poorly developed, composed of simple, branched paraphyses. *Asci* (79.5–)84.5–175(–178) × (15–)15.5–31(–31.5) µm, clavate, tapered at the base, without an operculum or any opening structures at the tip, apical ring non-amyloid, multispored, persistent, thick walled but unitunicate in structure, 1–3 µm (av. 1.5 ± 0.5 µm) measured at the central part of asci, slightly thickening towards the tip, penetrating through the gelatinous layer covering asci to forcibly eject ascospores. *Ascospores* hyaline, globose to ovoid, smooth-walled, 2–2.5 × 1.5–2.5 µm, mean ± SD = 2.2 ± 0.25 × 1.8 ± 0.19 µm, L/W=1.2:1.

Colonies slow-growing, white, moist at first then dry with age, lacking aerial mycelium. *Conidiophores* hyaline, of two types: (1) Conidiophores reduced to very short conidiogenous cells or conidiogenous pegs arising from hyphal cells, ~1 µm long; and (2) Longer conidiogenous cells, (10–)12.0–46.0(–48) × 2.0–3.0 µm, produced at the branching points where the septum appears. *Conidia* are single-celled and colourless. Conidia produced on very short conidiogenous cells on hyphal cells, vary in shape and size, (2.5–)3–11.5(–12) x 2.0–5.0 µm, mean ± SD = 6.29 ± 0.32 × 2.79 ± 0.25 µm, L/W=2.3:1, aggregated in masses around the hyphae or around the apex of,

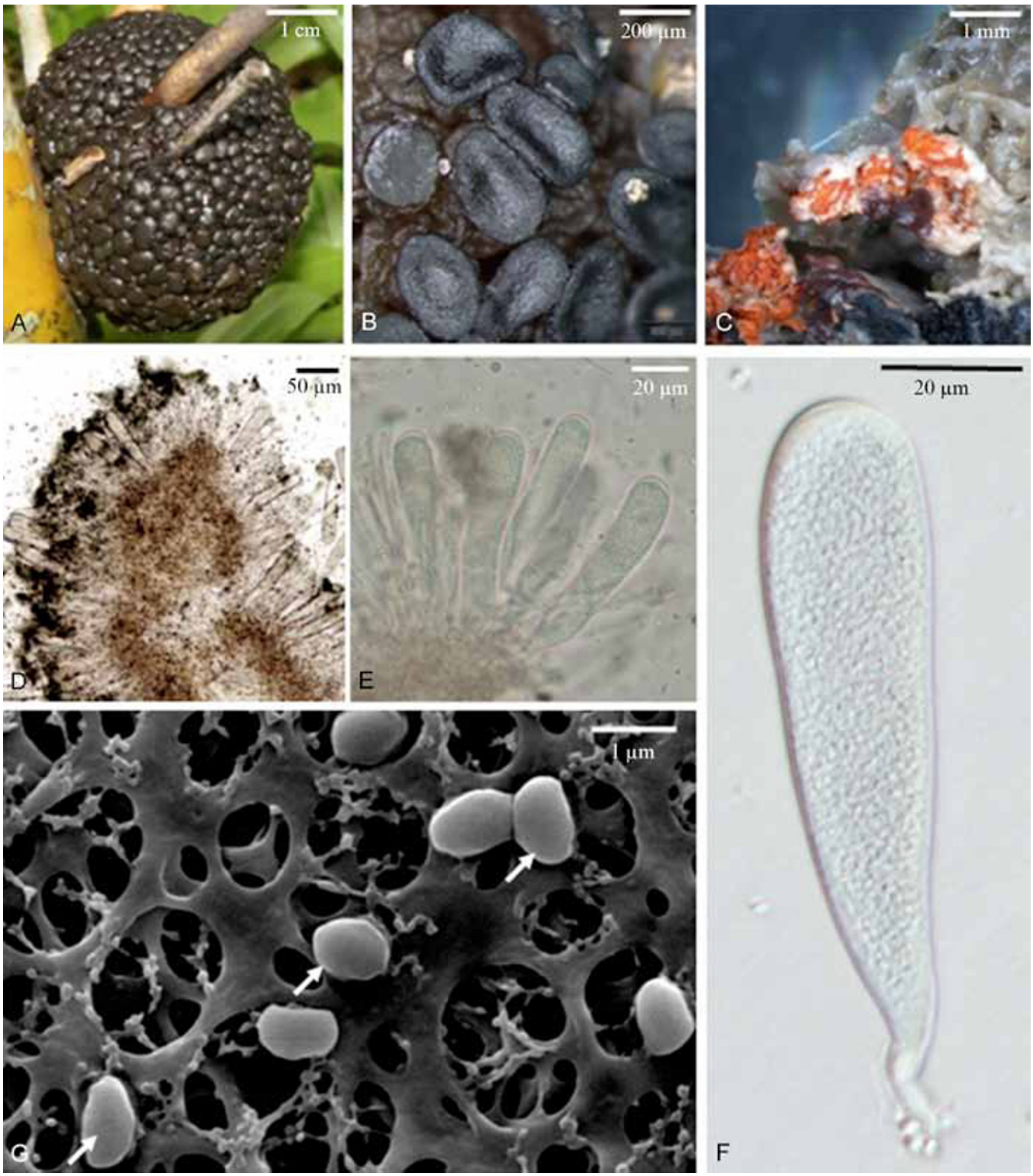

Fig. 6. Sexual morph of *Gelatinomyces siamensis* (A–C, F, G holotype; D, E isotype). **A.** Ascostromata. **B.** Apothecia. **C.** Red pigments accumulated inside ascostroma. **D.** Ascal arrangement on gelatinous apothecium covered by dark matter. **E.** Asci and paraphyses. **F.** Single ascus. **G.** Ascospores under scanning electron microscope (arrow).

annellide-like conidiogenous cells and on the agar surface from conidiogenous pegs. *Conidia* produced on the longer conidiogenous cells, nearly ovoid, 2.0–4.0 × 2–2.5 µm, mean ± SD = 2.27 ± 0.19 x 2.12 ± 0.16 µm, L/W=1.1:1, similar to conidia obtained from old cultures. Swollen hypha also present.

An unidentified red pigment is always associated with ascostromatic structures and the asexual morph in artificial culture. Patches of red pigment are accumulated inside the ascostroma, visible when cut. The red pigment appears in culture as both diffusible and water insoluble substances. The soluble red pigment stains the medium soon after the

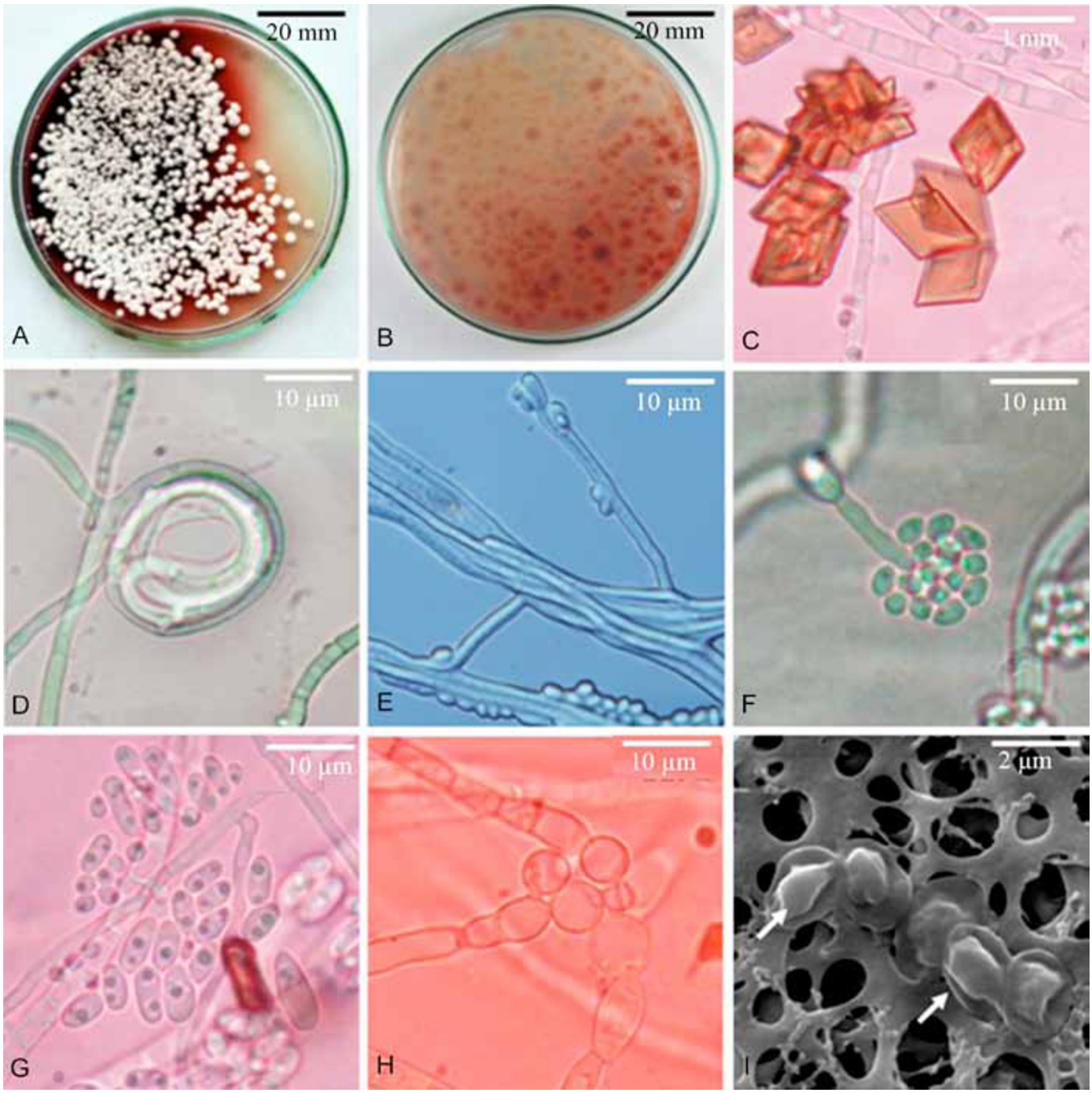

Fig. 7. Microscopic characteristics of the asexual morph of *Gelatinomyces siamensis* (ex-holotype). **A, B.** Fungal colonies on PDA producing red pigments into the media. **C.** Red crystals generated by mycelia. **D.** Hyphal coil. **E.** Hyphal pairing, condia from short conidiophores directed from mycelium. **F.** Conidia cluster at the apex of the tapered annellides and long, thick-walled, septate conidiophores. **G.** Conidia with internal inclusions. **H.** Swollen hypha. **I.** Dried conidia under scanning electron microscope (arrow).

establishment of the colony starting under the fungal colonies and covers the whole Petri dish within a week, whereas the insoluble red pigment appears as crystals on the surface of the fungal colony (Fig. 7a–c).

DISCUSSION

Bamboos are the only known habitat for a wide range of fungi, to which can be added *Gelatinomyces siamensis*. As mentioned in the Introduction, the majority of bamboo fungi reported to produce large stromata are in *Sordariomycetes* and *Dothideomycetes,* whereas molecular analyses show that *G. siamensis* belongs in *Leotiomycetes* (Figs 1–4), with bootstrap values of 70 and 72 in both SSU and LSU trees, respectively. Further, all the previously reported taxa with large stromata produce perithecioid structures immersed in ascostromatic tissue, whereas *G. siamensis* produces discoid apothecia on the surface of a gelatinous ascostromatic ball. The discoid apothecia are sessile or very short stalked, and contain thick-walled asci with numerous ascospores originating from the same level in a single layer

Table 4. Ecological and morphological characteristics of *Collophora* spp. in comparison to *Gelatinomyces siamensis.*

Characteristics	Species	
	Gelatinomyces	***Collophora***
Associated plant	Bamboo species	*Prunus* spp. and almond
Position	Attached to the point where bud breaks, culms or branches	Deep inside the heart of the wood, with heart rot symptom
Teleomorph	Apothecia aggregate in a ball-like cluster	Unknown
Red pigment crystalline in pure culture	Numerous, parallelogram or rhombus in shape	Absent, not mentioned
Conidiogenous pegs, intercalary	Present	Present
Conidiogenous cells at the septal point	Present	Absent
Swollen hypha as conidial mother cells	Present	Absent
Conidia	Various sizes and shapes but turn slightly ovoid in shape and minute in size when age	Consistency in shape and size

Table 5. Characteristics of *Collophora* spp. in culture media in comparison to *Gelatinomyces siamensis.*

Species	Spore size (µm)	Discrete conidiomata	Pigment	Endo-conidia	Sexual morph
C. africana	(2.5–)3.5–5.5(–8) × 1–2(–2.5) L/W = 3:1	Present	Red	Present	Unknown
C. capense	(4–)4.5–6.5(–9) × 1–1.5(–2) L/W = 3.7:1	Present	Red	Present	Unknown
C. hispanica	(2.5–)3.5–5(–6.5) × (1–)1.5(–2) L/W = 2.9:1	Present	Red	Present	Unknown
C. paarla	(3–)4–7.5(–11) × (0.5–)1–2(–3) L/W = 4.1:1	Present	Yellow, red	Present	Unknown
C. pallida	(2.5–)3–5(–7) × 1–1.5(–2) L/W = 3.5:1	Present	None	Present	Unknown
C. rubra	(3.5–)4–5.5(–8) × 1–2(–3.5) L/W = 3.2:1	Present	Red	Present	Unknown
G. siamensis	(2.0–)2.1–3.9(–4) × (2–)2–2.5(–2.5) L/W=1.1:1	Absent	Red	Absent	Apothecia

inside the apothecium. Branched and septate interascal filaments grow between the asci. In the parsimonious tree derived from SSU sequence data, *Leotiomycetes* diverged before *Dothideomycetes* and *Sordariomycetes.*

The ascus type is one of the essential morphological characters used to classify and identify ascomycete fungi. There is a wide range of ascus types, e.g. operculate, poricidal, non-poricidal, deliquescent, fissitunicate and rostrate, based on how ascospores are discharged (Bellemère 1994, Schoch *et al*. 2009). In *Gelatinomyces siamensis*, the ascospores are forcibly released when exposed under light through the thick-walled, and apparently multi-layered ascus which is functionally unitunicate. However, there is no evidence that the asci are operculate or porous. Therefore, the *G. siamensis* ascus is best termed rostrate because when discharging ascospores, the apical part of the ascus is broken to release the spores (Schoch *et al.* 2009).

In terms of the ascostromatal texture, the gelatinous nature of apothecia is one of the key characteristics mentioned by Wang *et al.* (2006a, b) to indicate membership of *Helotiaceae,* and is seen, for example, in *Ascocoryne, Ascotremella* and *Neobulgaria* (Seaver 1930, Petersen & Læssøe 2012). An additional feature recognized in this family is an endophytic lifestyle. However, *G. siamensis* does not appear to be endophytic as the ascostromata are superficially attached to the pole surface and are easily removed without any apparent damage to either the trees or the ascostromata. *Gelatinomyces siamensis* seems to be associated only with bamboo and its biological role requires further investigation.

Generally, the number of ascospores in an ascus is eight, whereas *G. siamensis* has numerous ascospores in a single mature ascus. Polyspored asci can originate as a result of one of several different mechanisms: fragmentation of eight originally multiseptate spores, repeated mitotic divisions following meiosis leading to numerous spores being then cut simultaneously from the ascus protoplast, or the direct formation of conidia from ascospores while still in the ascus (Hawksworth 1987, Raju 2002). Polyspory is a

diagnostic character in some families and genera in diverse classes and orders of ascomycetes, while in other cases it is phylogenetically informative only at the species level, arising in particular species within genera otherwise comprising 8-spored species. An example from the *Leotiomycetes* is *Thelebolus stercoreus* (de Hoog *et al*. 2005). This feature should, therefore, not be over-emphasized in the recognition of the genus *Gelatinomyces,* especially as the ontogeny of ascosporogenesis in this fungus has not yet been determined

As the phylogenetic trees obtained from SSU and LSU sequence data and BLASTn results hinted that *Collophora rubra* was the most closely related species, an ITS dataset containing various ITS sequences from all six known *Collophora* species was compiled (Table 3). Maximum parsimony and Bayesian analysis of these data confirmed that *Gelatinomyces siamensis* occupied an isolated position well-separated from the *Collophora* clade. The separation was supported by bootstrap scores of 99 (Fig. 5). As no sexual morph is currently known in any of the described *Collophora* species, we speculated that *G. siamensis* could be a sexual morph of *Collophora,* but this possibility is excluded by molecular and morphological comparisons.

Further, while bamboos are the natural habitat for *G. siamensis,* and the ascostromata can easily be detached from the poles, *Collophora* species live inside peach and almond trees and can be pathogenic. We attempted induction of conidiomata in our cultures, under conditions applied to *C. hispanica* (Gramaje *et al.* 2012). *Gelatinomyces siamensis* did not produce any discrete conidiomata, but only separate tiny conidia instead. In addition, the development of internal conidia inside hyphae, as seen in *Collophora*, did not occur. On the other hand, conidiogenous cells arose at septal points and swollen hypha were microscopically seen in *G. siamensis,* whereas *Collophora* species have not been shown to have either of these characteristics. A comparison of the significant features exhibited by *G. siamensis* and *Collophora* species is presented in Tables 4 and 5.

On the grounds of morphological characteristics and molecular phylogeny of the fungus, *G. siamensis* belongs in phylum *Ascomycota*, class *Leotiomycetes*, but cannot be referred to any accepted order at this time; i.e. it has to be treated as *incertae sedis* within the class. It is conceivable that future molecular data on this and other genera of *Leotiomycetes* might indicate that a new order is appropriate, but we consider that this would be premature at this time.

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