Anthracophyllum sontraense (Omphalotaceae, Agaricales), a new species from Danang, Vietnam

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Abstract

During the collection of macrofungi resources in Son Tra Nature Reserve, Danang, Vietnam, a novel species of the genus *Anthracophyllum* living saprophytically on small shrubs and rotten wood of *Mussaenda frondosa* Linn in the forest at an elevation 368 m above the sea level was recorded. The phylogenetic analyses based on the combined ITS and LSU molecular markers placed the specimens within *Anthracophyllum* genus. Together with specific morphological characteristics, we introduced *A. sontraense* sp. nov. as a new species. *A. sontraense* was characterized by the pileus with the skewed bell shape when young, dark blue to midnight blue, 1.0–1.5 cm diam, stipeless, light pink lamellae, sparse, occasionally branching. Basidiospores broadly ovoid to subglobose, 6–7 × 5.5–6 µm. Molecular phylogeny analyses revealed that the new species formed a clade with *A. archeri* with strong support. In addition, *A. sontraense* formed a separate lineage which was closer related to *A. archeri* and *A. sinense* than *A. lateritium*. To our knowledge, this is the first record of *Anthracophyllum* fungus in Vietnam.

Introduction

Anthracophyllum is a genus of fungi in the family of Omphalotaceae in the order Agaricales. The genus was first established in 1879 by Cesati based on the samples collected by O. Beccari at the Royal Botanic Garden in Peradeniya, Sri Lanka[1]. All over the world, 14 species of the genus *Anthracophllum* have been recorded during the past 144 years: *A. beccarianum* Ces. 1879 (≡ *A. melanophyllum*)[1], *A. nigritum* (Lév.)[2,3], *A. dusenii* Henn.[4], *A. hasselmanni* Rick 1937 (MycoBank: 266843), *A. lateritium* (Berk. & M.A. Curtis), *A. discolor* (Mont.), *A. berteroi* (Mont.)[5], *A. paxilloides*[6], *A. andinum*[7], *A. proximum* (Berk. & Broome)[8], *A. archeri* (Berk.)[9], *A. glaucophyllum*, *A. pallidum*[10] and *A. sinense*[11]. Several species assigned in *Anthracophyllum* were earlier classified in the genus *Xerotus* such as *X. archeri*, *X. discolor*, *X. glaucophyllus*, *X. nigritus* and *X. proximus*. *Anthracophyllum* fungi are known to be distributed only in tropical regions including palaeotropical, neotropical, south American and Australasian[10].

The morphological characteristics of the species in the genus *Anthracophyllum* are easy to recognize by their pleurotoid pileus containing greenish, orange or reddish brown pigment with obvious striate, sparse lamellae, occasionally branches. The lamellae surface is pale or the same color as the pileus surface, sometimes creamy white, orange white, light pink. The basidiocarp has a rudimentary stipe or stipeless. Spores are normally ovo-ellipsoid or subglobose[1,10]. Normally, *Anthracophyllum* species live saprophytically on small shrubs or dead branches of big trees.

So far, most of the species in the genus *Anthracophyllum* have been recorded and classified by solely morphological methods. Up to now, only *A. lateritium*[12,13] and *A. archeri*[14] have been identified by both morphological and molecular methods. In addition, *A. sinense*, a very new species of *Anthracophyllum* accepted in January 2024, has also been recorded in China by those methods[11]. Recently, according to our previous study on the diversity of species composition of macrofungi belonging to the phylum Basidiomycota in Son Tra Nature Reverse (Danang city, Vietnam), 110 taxa belonging to 42 genera, 23

families, 10 orders and 3 classes of the phylum Basidiomycota have been recorded[15]. Among them, there is a specimen with some common morphological characteristics of *Anthracophyllum* but it also possesses some specific traits that are likely to be a new species. Therefore in this study, we used both morphological and DNA barcoding analyses to introduce a new species *Anthracophyllum sontraense* sp. nov. Molecular phylogeny analyses based on ITS (Internal Transcribed Spacer) and LSU (28S nuclear ribosomal large subunit rRNA gene) markers of the new species and its closest relates were then constructed. The identification key to the species of *Anthracophyllum* was also provided.

Results

Sample collection

In this study, samples with morphological characteristics which were typical of the genus *Anthracophyllum* were collected from Son Tra Nature Reserve at an elevation of 368 m. The mushroom was found on rotten branches of the plant *Mussaenda frondosa* Linn with the shape of a skewed bell (Fig. 1). The pileus was 1–1.5 cm in diameters, dark blue to midnight blue, pinkish white lamellae. The color of the pileus surface became darker when drying (Fig. 1A). At the maturity stage, the pileus spread out to a half shell shape, pinker lamellae (Fig. 1B).

DNA barcoding analysis

In this study, we used two different molecular markers including ITS and LSU to confirm the result of morphological classification. Firstly, the ITS fragment containing ITS1-5.8S-ITS2 of approximately 900 bp was amplified by using the primer pairs ITS5/ITS4B (Fig. 2B). Secondly, the LSU fragment containing 28S rDNA of approximately 1100 bp was amplified by primer pairs LROR/LR6 (Fig. 2A). Those PCR products were then purified and sequenced by single-end sequencing method for the sequence alignment and species identification.

The ITS, LSU sequences deduced from sequencing were aligned with reference sequences of related species on GenBank by Basic Local Alignment Search Tool (BLAST). The BLAST similarity search analysis indicated that the ITS sequence of our specimen exhibited a low similarity level of only 87% to the ITS sequences of both *A. lateritium* voucher UOC-DIMIA-D26 (KP757737) and *A. archeri* isolate AFTOL-ID 973 (DQ404387), respectively. However, the LSU sequence of our specimen exhibited a higher similarity level of 98% to the LSU sequences of both *A. lateritium* strain CULTENN4419 (AF261324) and *A. archeri* isolate AFTOL-ID 973 (AY745709), respectively. The ITS, LSU sequences of *Anthracophylum sontraense* sp. nov. were deposited to GenBank (NCBI) with the accession numbers of OR990596 and OR978578, respectively.

Phylogenetic analysis

The sequences of ITS and LSU of the new species in this study were combined and used for the construction of the phylogenetic tree. The combined sequences of 1706 characters (containing gaps)

including 808 bp of ITS and 898 bp of LSU from species in different genera of the family Omphalotaceae were used for phylogenetic analysis. As shown in Fig. 3, the new species *A. sontraense* sp. nov. formed a distinct clade with *Anthracophyllum archeri* AFTOL-ID 973 with strong bootstrap support of 94%. The consensus Maximum Likelihood tree based on all available ITS sequences of *Anthracophyllum* species in GenBank database indicated that *A. sontraense* was closely related to *A. archeri* and *A. sinense* than *A. lateritium* with strong bootstrap support of 90% (Fig. 4).

Taxonomy

Anthracophyllum sontraense P.T. Tran *sp. nov.*

MycoBank: MB852025

Figure: 1, 5, 6

Etymology: "*sontraense*" reffers to the name of Son Tra Nature Reserve, Danang, Vietnam.

Ecology: Living parasitically, then saprophytically causing brown rot on branches of *Mussaenda frondosa* Linn in Son Tra Nature Reserve at an evelation between 368–412 m with a typical tropical climate.

Holotype: VIETNAM, Danang City, Son Tra Nature Reserve, 16º05¢50²N 108°12'45²E, elevation 368 m, on small shrubs and rotten wood of *Mussaenda frondosa* Linn, May, 2022, P.T. Tran (Voucher: M34295).

Description:

Pileus 1.0–1.5 cm diam, skewed bell shape when young, half shell shape when mature, convex, smooth, non-viscid, velutinose, dark blue to midnight blue, darker when dry. *Stipe* absent, sessile. *Basidiocarp* attached directly to the substratum. *Pileus trama* thin and soft (Fig. 1).

Lamellae thin, 1 mm thick, pinkish white when young and pinker when mature, sparse, radiating from a basal point, occasionally branching, edge concolorous, serrated and breakable when dry. Lamellae pinker at the basal point and gradually faded to the edge of the cap. Lamellae unequal in length, 7–9 through lamellae and 4–8 branched lamellae (lamellulae).

Basidiospores broadly ovoid to subglobose, (5.5-)6-6.5-7(-7.5) × (5-)5.5-6-6.5(-7) µm, thin-walled, smooth, hilar appendix obvious, hyaline, some with oleaginous contents (Fig. 5, 6). Spore-print light brown. *Basidia* broadly cylindrical, (24-)28–34–39(–41) × (4-)5–7-9(-10) µm, with 2-4 sterigmata, some with oleaginous contents, di- to tetrasporic (Fig. 6). *Sterigmata* subulate (2–)3–5 × 1–1.5(–2) µm. *Chailocystidia* (26–)29–37–49(–54) × (3–)5–7–9(–10) µm, hyphoid cylindric to subfusoid, clavate, fusiform, with oleaginous contents, hyaline, thin-walled. *Hyphae* 1–3 µm diam, slightly inflating up to 3–5 µm, smooth and hyaline, thin-walled, blue in KOH.

Keys

1. Spores with hilar appendix prominent……..2

1. Spores with hilar appendix not prominent……5

2. Spores broadly ovoid to subglobose……..3

2. Spores ellipsoid or elongate ellipsoid……4

3. Spores 6–7 ´ 5.5–6 µm, thin-walled. Basidia 28–39 × 5–9 µm. Lamellae sparse, occasionally branching, 7–9 through lamellae and 4–8 branched lamellae. Pileus 1–1.5 cm diam, skewed bell, half shell shape. Stipe absent, sessile……*Anthracophyllum sontraense*

3. Spores 8–14 ´ 5–9 µm, thin-walled. Basidia 30–45 × 6–12 µm. Pileus 1.5–4 cm diam, flabelliform to semicircular, convex to applanate, surface rugulose, irregularly to radially sulcate. Stipe rudimentary or absent, sessile……*Anthracophyllum sinense*

4. Spores ellipsoid, 8.5–11 ´ 5.5–6.5 µm. Basidia 35–40 ´ 8–11 µm. Lamellae 6–9 through-lamellae, radiating from a basal point, fuscous brown to cocoa-brown. Pileus 1–2.5 cm diam, sessile, conchiform to semiorbicular convex……*Anthracophyllum discolor*

4. Spores elongate ellipsoid, 10–12.5 ´ 5.5–6.5 µm. Lamellae are quite strongly interveined. Pileus more than 1 cm diam……*Anthracophyllum proximum*

5. Spores hyaline or stained brownish……..6

5. Spores oleaginous contents ……..9

6. Spores hyaline, ovoid to ellipsoid……7

6. Spores hyaline or staining brown. Pileus convex to applanate ……8

7. Spores 7.5–11 ´ 5–8 µm. Basidia 20–50 ´ 7–10 µm. Lamellae 5–9 through-lamellae. Pileus 1–3 cm, reniform to short flabelliform, convex; surface pale pinkish cream to reddish brown, smooth. Stipe rumidentary, up to 3 mm long……..*Anthracophyllum archeri*

7. Spores globose to ovoid, ellipsoide, 8–14 ´ 8–10 µm. Basidia 50–75 ´ 5–14 µm. Lamellae less than 5 through-lamellae. Pileus 0.6–0.8 cm diam, sometimes clustered, orbicular either kidney-shaped or shell-shaped. Stipe lateral, very short ……*Anthracophyllum pallidum*

8. Spores ovoid to ellipsoid, 8.5–11 ´ 6–7.5 µm. Lamellae 9–12 through lamellae. Basidia 35–45 ´ 8–11 µm. Pileus 1–3 cm diam, sessile, semiorbicular to reniform, surface brownish or brick-red……*Anthracophyllum melanophyllum*

8. Spores oblong ellipsoid to ovo-ellipsoid, 6.5–9.5 ´ 3.5–5 µm. Basidia 27–33 ´ 6–8 µm. Lamellae 11–12 through-lamellae, radiating from a basal point, greyish-brown to fuscous brown. Pileus 0.5–2 cm, subreniform to flabelliform or orbicular. Stipe rudimentary or absent……*Anthracophyllum nigritum*

9. Spore broadly ovoid to subglobose, 11.5–16 ´ 10–13 µm. Basidia 50–80 ´ 11–15 µm. Lamellae 12–15 through-lamellae. Pileus 2–3 cm diam, reniforrn, convex to applanate, surface pale buffy brown to cinnamon-brown, stipe 4–12 ´ 1–2 mm……..*Anthracophyllum andinum*

9. Spores ovo-ellipsoid……..10

10. Spores 7–10 ´ 4.5–7 µm. Basidia 38–70 ´ 7 µm. Lamellae in 3 series with at least 9 principal ones in the bigger basidiomes. Pileus 1.5–2.5 cm diam, fan-shape. Stipe absent…… *Anthracophyllum glaucophyllum*

10. Stipe small to rudiment, 0.5–3 mm……11

11. Spores 9–12 ´ 5.5–7 µm. Basidia 33–40 ´ 7.5–9 µm. Lamellae 15-17 through-lamellae, subdecurrent, purplish umber drying cocoa-brown. Pileus 0.5–3.5 cm diam, sessile or nearly so, conchoid to reniform, convex to applanate. Stipe small to rudiment 1–2.5 mm ……..*Anthracophyllum beteroi*

11. Lamellae less than 13……12

12. Spores, 6.5–8 ´ 4–5 µm. Lamellae 8–10 through-lamellae. Pileus 0.5–2 cm diam. Stipe conspicuous up to 3 mm. Basidia 20–25 ´ 5.5–6.5 µm……*Anthracophyllum paxilloides*

12. Spores 9.5–15 ´ 5.5–8 µm. Lamellae 9–12 through-lamellae, but not interveining. Basidia 35–40 ´ 8–9 µm. Pileus 0.5–2 cm diam, semiorbicular to reniform, surface reddish brown. Stipe absent or very reduced lateral, 0.5–1 mm……..*Anthracophyllum lateritium*

Discussion

According to morphological analyses, *A. sontraense* possesses common characteristics of *Anthracophyllum*[1]. It has smooth, pleurotoid pileus, stipeless, sessile; sparse lamellae, occasionally branches, 7–9 through lamellae; subglobose to broadly ovoid basidiospores, hilar appendix prominent. In comparison with the species *A. archeri* which has bigger pileus (1–3 cm), surface pinkish cream to reddish brown; lamellae bright brick red to brownish; broadly ellipsoid basidospores and stipe rudimentay, up to 3 mm long[1]. However, *A. sontraense* sp. nov. was characterized by its dark blue pileus, skewed bell shape with smaller diameter of 1–1.5 cm, pinkish white lamellae, basidiospores usually subglobose, stipe absent. Therefore, in this study, in addition to macroscopic and microscopic morphological analyses, we also conducted the DNA barcoding method to confirm our findings.

Around the world, there is an increasing number of DNA barcoding studies using molecular markers such as ITS (Internal transcribed spacer), LSU (Large subunit, 28S nrDNA), SSU (Small subunit, 18S nrDNA) and some other protein coding genes for the identification of fungal species. According to a survey in the Journal of Natural Products (ISSN: 0163–3864), in 15 years-period from 2000 to 2015, 22% of articles

published using the ITS marker to identify fungal species. However, if only counted in the last 6 years from 2010 to 2016, this rate has increased to 42%. Publications based on solely morphological methods decreased from 31% in 2000–2015 to 8% in 2010-2016[16]. The ITS molecular marker is a segment of DNA in the nucleus containing 3 regions ITS1, 5.8S and ITS2 with sizes ranging from 0.45–0.80 kb. ITS1 and ITS2 are two DNA regions that have had quite large changes (mutations) during evolution, so they are suitable for the identification at the species-level. For the classification at higher taxonomic levels such as genus, family or even higher levels such as order, class, and phylum, the appropriate molecular markers are LSU and SSU, respectively[16].

As recommended by Schoch et al.[17], the combination of the ITS and LSU markers can also be valuable for fungal classification at the species level. In this study, the over 98% similarity in LSU sequences of our specimen and reference sequences of *Anthracophyllum* species from GenBank database revealed that the new species is assigned to the genus *Anthracophyllum* with strong support. For the ITS marker, there are several universal ITS primers used for amplifying different segments of ITS region[16], however, this study used ITS5/ITS4B which is the specific primer pair for the classification of basidiomycetes[18]. The lower similarity level of only 87% between the ITS sequence of our specimen and reference sequences of *Anthracophyllum* species in GenBank database strongly supported our hypothesis that the specimen was a new species of the genus *Anthracophyllum*.

The phylogenetic analyses based on the combined ITS and LSU sequences in this study revealed that the new species *A. sontraense* sp. nov. formed a distinct clade with *A. archeri* and it separated clearly with other genera of the family Omphalotaceae. The close relationship between *A. sontraense* and *A. archeri* in the ITS based phylogenetic tree also supports for the above result. However, due to the limitation of available sequences for *Anthracophyllum* species in GenBank, the phylogenetic trees in this study were constructed by only a few reference taxa which have both ITS and LSU sequences.

In this recent study, based on the morphology and phylogeny evidences, we introduced *A. sontraense* sp. nov. as a new species in the genus *Anthracophyllum* and it is the first record of *Anthracophyllum* species in Vietnam as well. However, future research on the taxonomy of macrofungi, especially on species of genus *Anthracophyllum* should perform DNA barcoding analyses to improve the resolution of the phylogenetic tree and the accuracy of taxonomic studies. In addition, species of *Anthracophyllum* are known to contain compounds with antioxidant, antimalarial, antibacterial activities and cytotoxic effects[19,20]. Thus, further studies should be conducted to identify and characterize the active compounds from the new species recorded in this study.

Methods

Sample collection

Fresh samples of *Anthracophyllum* sp. were collected from Son Tra Nature Reserve, Danang city, Vietnam (16°05'50"N 108°12'45"E; elevation 368 m above the see level, on small shrubs and rotten wood of

Mussaenda frondosa Linn). Specimens were dried in an electric dryer for 24 hours with the first 6 hours at 45^{o}C, the next 6 hours at 42^{o}C and the last 12 hours at 40^{o}C. The dried specimens were kept in plastic bag with an appropriate amount of silica gel particals and then deposited at The University of Danang and at International University, Vietnam National University Ho Chi Minh City under the voucher name M34295.

Macroscopic and microscopic analyses

The morphological characteristics of fruiting bodies were photographed directly at the collecting site and the habitat of the species was recorded while observing the fungus at the sampling area. The macromorphological descriptions of fruiting bodies based on the methods of Kiet[21] and Liao et al.[22]. Dried specimen samples were treated with 5% KOH and then dyed with 1% ammoniacal Congo red solution for the microscopic morphology analysis[23].

Scanning electron microscopy (SEM)

The dried fruiting bodies (basidiocarps) were cut into slices and affixed to the SEM holder using NEM conductive carbon adhesive tape (Nisshin, Japan). Following that, the sample was coated with nanogold (Jeol, Japan) for 2 minutes using Smart Coater (Jeol, Japan). Subsequently, the samples were analyzed using the SEM JSM IT 100 (Jeol, Japan) for imaging. The focus height was set at 1 mm, and the voltage was adjusted to 5kV. Images were captured at various magnifications.

DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from the dried basidiocarps following the protocol of Plant genomic DNA extraction mini kit (Favorgen, Taiwan). Two different molecular markers including the internal transcribed spacer (ITS) and the large subunit nuclear ribosomal RNA (LSU) were amplified by polymerase chain reaction (PCR) using the primer pairs ITS5/ITS4B and LROR/LR6, respectively (Table 1). The PCR mixture and PCR parameters were adapted from Nguyen et al.[24]. The PCR products were checked in 0.8% agarose gel and then sent to 1st BASE (Malaysia) for single-end sequencing by forward primers as shown in Table 1. The nucleotide sequences obtained from the sequencing company were first edited by MEGA version 11.0.13[25] and then compared to the references of ITS, LSU sequences retrieved from GenBank database (NCBI, USA).

Table 1
Molecular markers and primers used in this study

Marker	Primer	Primer sequence (5' – 3')	References
ITS	ITS5	GGAAGTAAAGTCGTAACAAGG	Raja et al.[16]
	ITS4B	CAGGAGACTTGTACACGGTCCAG	Gardes & Bruns[18]
LSU	LROR	ACCCGCTGAACTTAAGC	Raja et al.[16]
	LR6	CGCCAGTTCTGCTTACC	Raja et al.[16]

Phylogenetic analyses

The ITS, LSU sequences from *Anthracophyllum* sp. nov. and its related species downloaded from GenBank (Table 2) were aligned using the MUSCLE algorithm. The phylogenetic trees were constructed based on the combination of ITS and LSU sequences or by LSU sequences only using the Maximum Likelihood method. The reliability of the tree was estimated by the bootstrap method. All the evolutionary analyses were conducted in MEGA 11[25].

Table 2
Species information and GenBank accession number for ITS, LSU sequences used for phylogenetic analyses. The new species and its sequences are in bold.

Species	Voucher/Strain	GenBank accession no	
		ITS	LSU
Anthracophyllum archeri	AFTOL-ID 973	DQ404387	AY745709
Anthracophyllum archeri	TFB3511_TENN50049	DQ444308	-
Anthracophyllum lateritium	IBUG BF71	OP546336	-
Anthracophyllum lateritium	IBUG BF72	OP546337	-
Anthracophyllum lateritium	TENN62043 halotype H1	FJ596892	-
Anthracophyllum lateritium	TENN62043 halotype H2	FJ596891	-
Anthracophyllum lateritium	TFB4043_TENN50256	DQ444309	-
Anthracophyllum sontraense sp. nov.	**M34295**	**OR990596**	**OR978578**
Anthracophyllum sinense (Omphalotaceae sp. WY-2022a)	HFJAU12000	ON711250	-
Anthracophyllum sp.	BCC18695	KC190495	-
Anthracophyllum sp.	Biocode09-407	MZ996997	-
Anthracophyllum sp.	LE-BIN 3312	OL839224	-
Anthracophyllum sp.	TYY2021-6-1	OK586733	-
Anthracophyllum sp.	TYY2021-8-13	OL998876	-
Connopus acervatus	MO 377662	MT735148	MT735148
Gymnopanella nothofagi	SGO 163624	KT906426	KT906426
Gymnopanella nothofagi	SGO 163625	KT906425	KT906425
Gymnopus omphalinoides	GDGM 78318	NR_184932	NG_088179
Gymnopus tiliicola	HMJAU 60304	OM030282	OM033392
Marasmiellus bicoloripes	CAL 1524	KY817233	NR_164019
Marasmiellus griseobrunneus	CAL 1752	MK660191	MK660192
Marasmius curreyi	BRNM 714676	FJ936152	FJ917614
Marasmius hobbitorum	SP 445484	NR_177121	NG_088031
Micromphale brevipes	TFB14606	KY026753	KY026753

Species	Voucher/Strain	GenBank accession no	
		ITS	LSU
Mycetinis applanatipes	SFSU DED6628	KY696775	KY696775
Mycetinis scorodonius	CBS251.48	MH867884	MH867884
Neonothopanus cystidiosus	HMJAU 48222	NR_186961	NG_228862
Neonothopanus hygrophanus	HMJAU 48223	MW298685	MW250231
Neonothopanus nambi	ACL251	KJ206982	KJ206956
Omphalotus japonicus	TNS Kasuya B817	KJ395102	KJ395105
Omphalotus olearius	AFLTOL-ID 1718	DQ494681	DQ470816
Rhodocollybia badiialba	UBC F16283	EU486446	EU486446
Rhodocollybia butyracea	UBC F16294	EU486454	EU486454
Hygrophorus cossus	-	AY548963	AY548963

Declarations

Competing interests

The authors declare no competing interests.

Author Contribution

P.T.T. and T.T.N. designed experiments. Mushroom specimens were collected by P.T.T. and H.H.D. Specimens preparation and morphological analyses were proceeded by P.T.T., H.H.D., T.S.N. and U.T.D.H. Sample preparation and SEM imaging were conducted by T.T.T.P. and T.T.H.N. Molecular and phylogeny analyses were done by H.T.L.N. and T.T.N. Data were analyzed and the manuscript was wrote by P.T.T and T.T.N. All authors reviewed the manuscript.

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Data availability

The ITS and LSU sequences of *A. sontraense* sp. nov. are available on GenBank databases with the GenBank accession numbers: OR990596 and OR978578, respectively. Other data and analyses generated in the current study are included in this published article.

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Figures

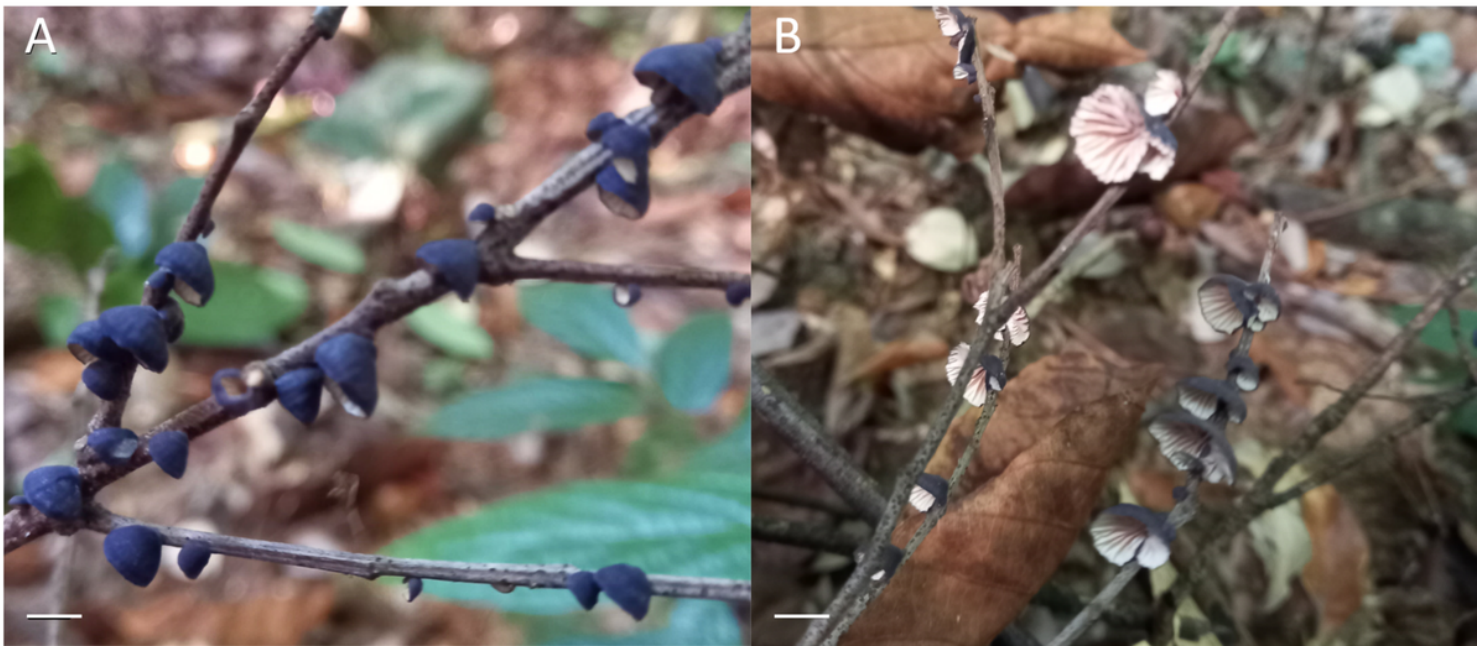

Figure 1

The basidiocarps of *A. sontraense* sp. nov. grown on rot branches of *Mussaenda frondosa* Linn. (A) Pileal surface of young basidiocarp; (B) Lamellae surface of mature basidiocarp. Bars: 1 cm.

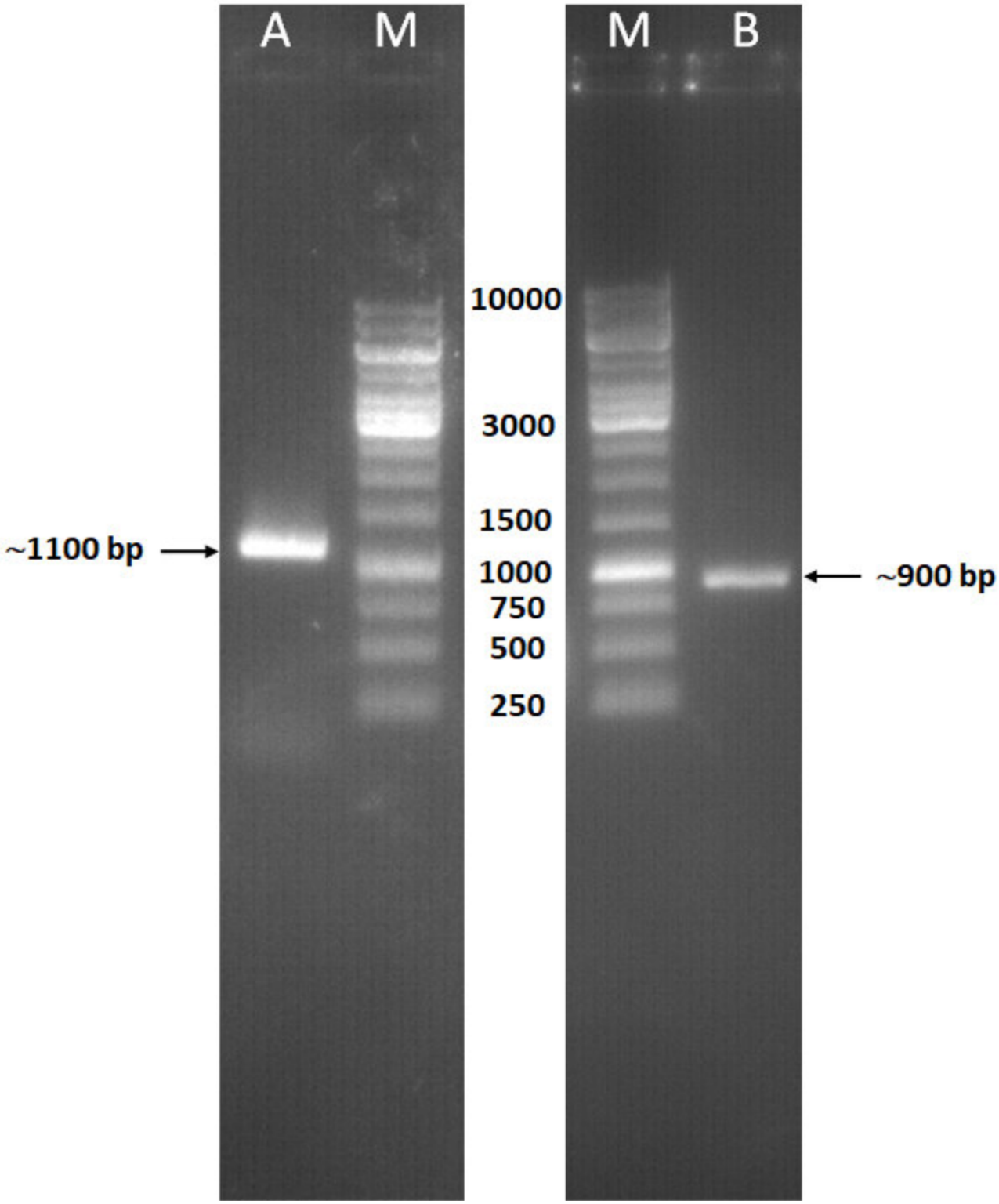

Figure 2

Electrophoresis of the LSU (A) and ITS (B) rDNA fragments amplified in this study on 0.8% agarose gel. M. 1kb DNA ladder (Thermo Scientific).

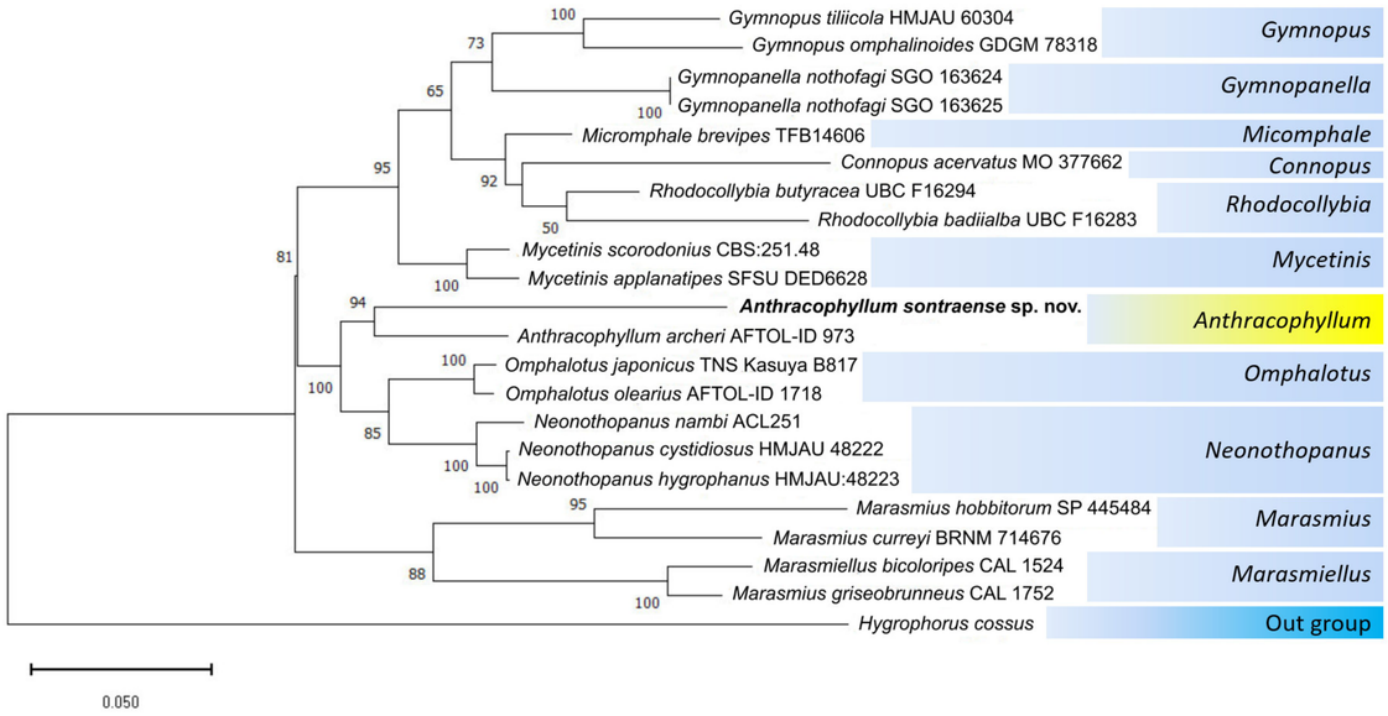

Figure 3

Maximum Likelihood tree based on combined ITS and LSU sequences of the new species and different species from allied genera. Numbers at the branches represent bootstrap percentages. The new species in this study is shown in bold. *Hygrophorus cossus* is used as out group.

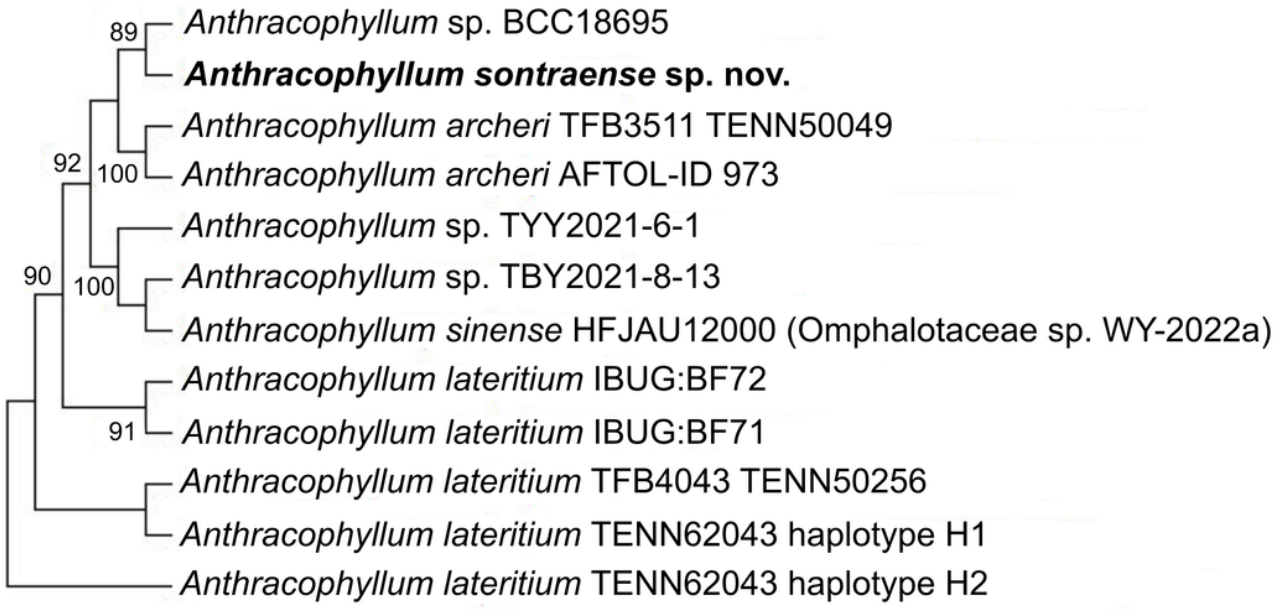

Figure 4

Maximum Likelihood consensus tree based on all available ITS sequences of *Anthracophyllum* species in GenBank database. Numbers at the branches represent bootstrap percentages. Bootstrap percentages lower than 50% are not shown. The new species in this study is shown in bold.

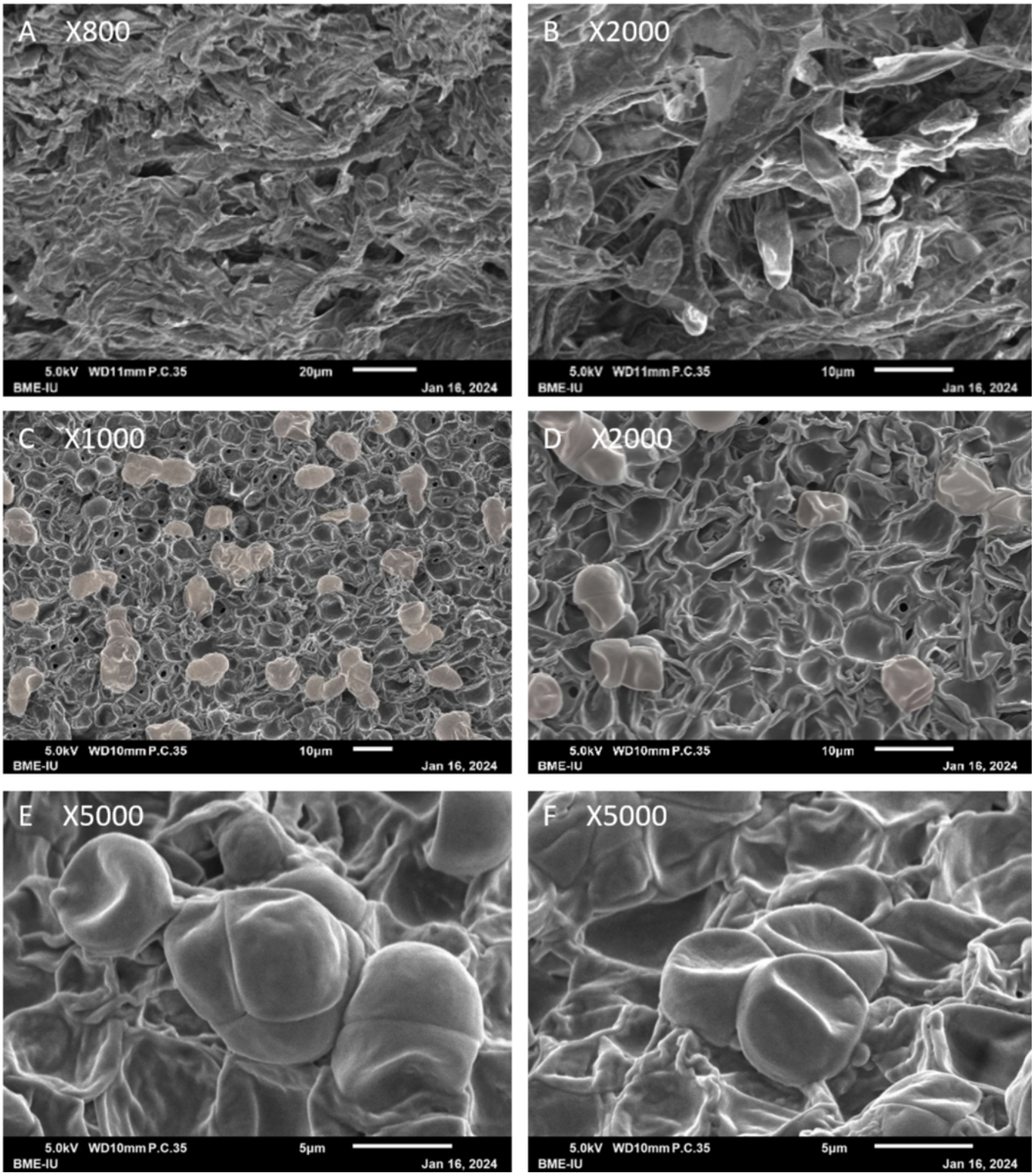

Figure 5

Scanning electron microscope images of basidiocarps. (A–B): Cap surface, showing mycelium structure with a mass of hyphae at 800X (A), 2000X (B); (D–E): Basiodiospores arrangement on mass of basidia at 1000X (D), 2000X (E); (G–H): Basiodiospores at 5000X from different view. Scales were indicated on the images.

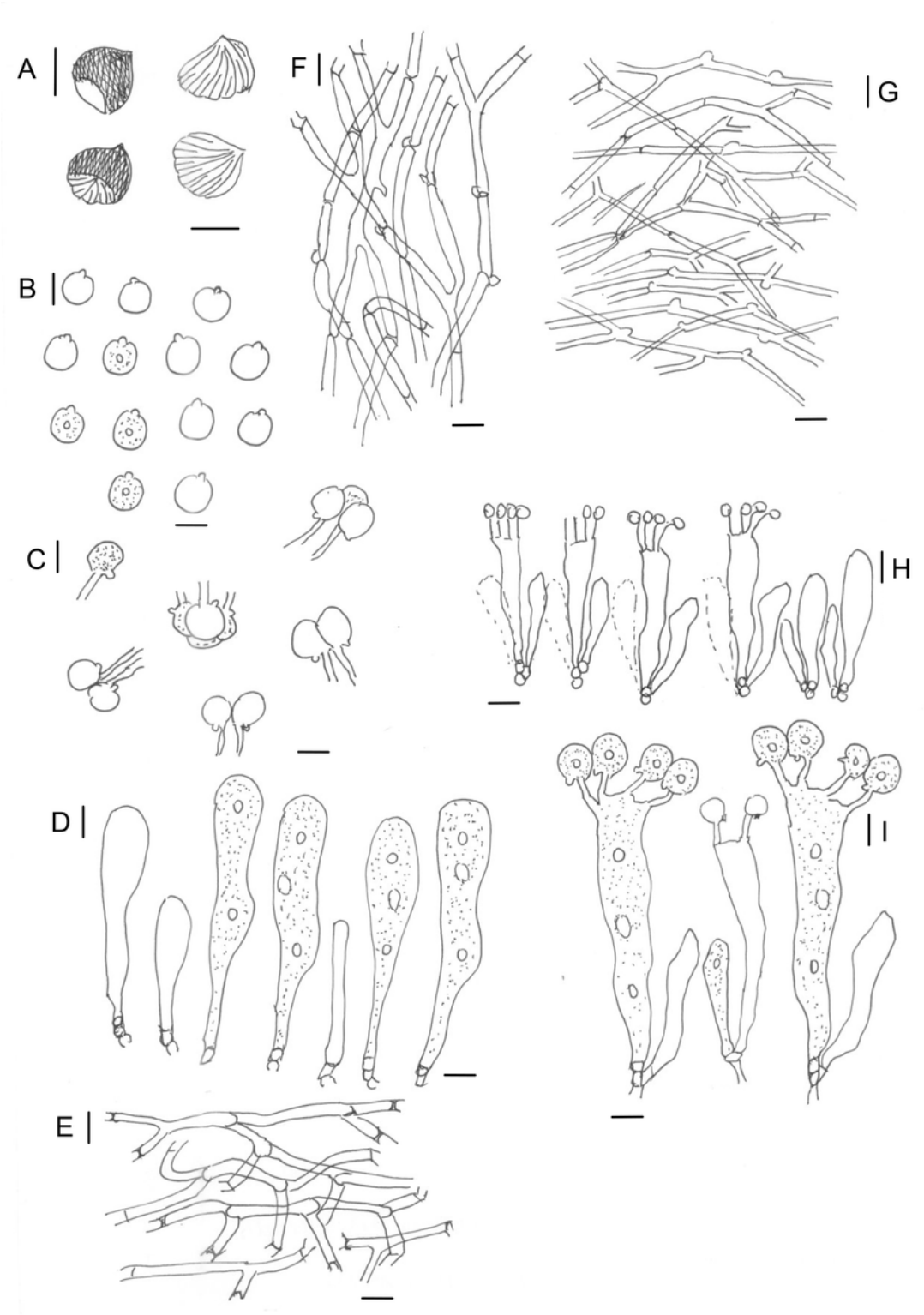

Figure 6

Macroscopic and microscopic structures of *Anthracophyllum sontraense* sp. nov. (A) Basidiocarps; (B) Basidiospores; (C) Basidiospores on sterigma; (D) Cystidium; (E) Pileipellis; (F) Trama; (G) Hyphae of context; (H) Hymenium; (I) Basidia with two to four basidiospores, one on the tip of each sterigma. Bars: (A) = 1 cm; (B-I) = 5 µm.