



Identification of the new species *Comatricha macrospora* and two other recently recorded species of *Comatricha* from China

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Abstract

A new species (*Comatricha macrospora*) has been collected from the Changbai Mountain National Nature Reserve, Jilin Province, China. *Comatricha macrospora* has smaller sporocarps and larger spores (about 15–18 µm in diameter) than other species of *Comatricha* as well as a persistent peridium at the base of the sporotheca. In addition, two newly recorded species of *Comatricha*—*C. tenerrima* (M.A. Curtis) G. Lister and *C. afroalpina* Rammeloo—have been documented in China for the first time, based on material collected from northeast China and the campus of East China Normal University, Fuzhou City, Jiangxi Province. *Comatricha tenerrima* is characterised by fusiform long-stalked sporocarps and warted pinkish brown spores (about 7–8 µm in diameter), whereas *C. afroalpina* occurs on rotting logs and has spores marked by larger warts with an irregular reticulation at their base. Descriptions and scanning electron micrographs for these members of the genus *Comatricha* are provided. Phylogenetic analyses, based on small subunit ribosomal RNA sequences (SSUrRNA) of *Comatricha* and related genera, were carried out using Bayesian inference. These analyses confirmed the placement of the new species in the genus *Comatricha*.

Keywords: *Comatricha*, *Comatricha macrospora*, SEM, Stemonitidaceae, taxonomy

Introduction

Myxomycetes are common inhabitants of decaying plant material throughout the world. Within an ecosystem, myxomycetes are bacteria and some organism. When a plant dies, myxomycetes break down the dead plant matter. Consequently, these organisms are important for nutrient recycling. Myxomycetes are abundant in forested regions, where decaying logs, stumps and dead leaves provide a plentiful supply of potential substrates (Eliasson 2013, Rollins 2013, Stephenson & Stempen 1996). This taxonomic group of organisms consists of more than 1000 species worldwide (Li & Li 1989, Kirk *et al.* 2008).

The genus *Comatricha* was established by Preuss in 1851. Forty-one species of *Comatricha* have been reported throughout the world (Kirk *et al.* 2008, Lado 2005–2018). Only six species are known from China. These are *C. ellae* Härk. (Härkönen 1978), *C. reticulospora* Ing & P.C. Holland (Ing 1967), *C. laxa* Rostaf. (Rostafiński 1874), *C. nigra* (Pers. ex J.F. Gmel.) J. Schröt (Schröeter 1885), *C. pulchella* (C. Bab.) Rostaf. (Rostafiński 1876) and *C. clavicolumella* B. Zhang & Yu Li (Zhang & Li 2017). During an investigation of myxomycetes in northeast China in October 2017, specimens which could not be assigned to any known species were found on the bark surface of a dead log in the Changbai Mountain National Nature Reserve, Jilin Province, China. These specimens have been ascribed to a new species, which is easily distinguished from other known species of *Comatricha*. Meanwhile, two recently recorded species of *Comatricha*—*C. tenerrima* (M.A. Curtis) G. Lister (Lister 1919) and *C. afroalpina* Rammeloo (Heinemann & Rammeloo 1983)—have been described in China for the first time. Phylogenetic analyses have been performed using regions of the small subunit ribosomal RNA gene (SSU rRNA). According to the phylogenetic data and the morphological characteristics, the specimens have been categorised as belonging to a new species.

Materials and Methods

Morphological studies. The fruiting bodies and microscopic structures of the specimens were examined by light and scanning electron microscopy (SEM) (Martin and Alexopoulos 1969; Zhang & Li 2012). Permanent slides were mounted in Hoyer's medium (Martin & Alexopoulos 1969), after having been prepared according to Robbrecht (1974) by first dispersing the capillitia in a drop of 94% alcohol and determining the colour after one minute. The colour terms used are those in the *Flora of British Fungi: Colour Identification Chart* (Anonymous 1969). Observations and measurements of the morphological characteristics of the specimens were carried out using a stereomicroscope (20×) and an optical microscope (100×). Approximately ten sporocarps of each collection were measured. Measurements of approximately 20 spores and their ornamentation were made using an oil immersion objective. The sporocarps, capillitia and spores were measured using a Nikon dissecting microscope and a Zeiss compound microscope. Photographs were taken with a Leica DM2000 microscope. For SEM images, the sporocarps were attached to a holder, coated with gold using a Hitachi E-1010 sputter and examined with a Hitachi S-4800 SEM at 10 kV, located at the Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. The specimens have been deposited in the Herbarium of the Mycological Institute of Jilin Agricultural University (HMJAU).

DNA extraction, PCR and sequencing. A CTAB rapid plant genome extraction kit (Demeter Biotech Co. Ltd., Changchun) was used to extract the total genomic DNA from dried specimens, according to the manufacturer's instructions with some modifications (Chen *et al.* 2015, 2016). The SSU rRNA region was amplified using the primer pairs SSU-R (TAGAGGGATGTGAAGTGCC) and SSU-F (AAGGAGCCGGTATCAAGTA) (Song 2013). The PCR procedure for the SSU rRNA was as follows: initial denaturation at 95°C for 3 min; followed by 34 cycles at 94°C for 40 s, 54°C for 45 s and 72°C for 1 min; and a final extension of 72°C for 10 min. The PCR procedure for the 28S rDNA was as follows: initial denaturation at 94°C for 1 min; followed by 34 cycles at 94°C for 30 s, 50°C for 1 min and 72°C for 1.5 min; and a final extension of 72°C for 10 min. The PCR products were directly sequenced using the same primers mentioned above at the Shenggong Genomics Institute, China.

Phylogenetic analysis.—Once the PCR sequences were generated, additional sequences were selected from GenBank, as listed in Table 1. The sequences were aligned with ClustalX (Thompson *et al.* 1997) and manually adjusted using BioEdit (Hall 1999). The sequences of *Echinostelium minutum*, *E. arboreum* and *E. coelocephalum* were obtained from GenBank and selected as the outgroup for the phylogenetic analysis based on the SSU rRNA datasets.

MrModeltest 2.3 (Nylander 2004) was used to determine the best-fit evolution model for the SSU rRNA datasets for the Bayesian inference. The Bayesian inference was calculated with MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), assuming a general time reversible (GTR) model of DNA substitution and an inverse-gamma distribution rate variation across the substitution sites. Four Markov chains were run for 1,000,000 generations until the split deviation frequency value <0.01. The burn-in value corresponding to the Markov chains was set to discard the first 25% of the trees and the remaining trees served to calculate the majority rule consensus tree.

The phylogenetic profile of the trees was visualised using Treeview (Page 1996). All branches that received bootstrap supports of greater than 95% in the Bayesian inference were considered significantly supported.

TABLE 1. Taxa information and GenBank accession numbers of the sequences used in this study.

Species	Sample no.	Country	GenBank accessions no. SSU rRNA
<i>Echinostelium minutum</i> de Bary	ATCC 22345	Switzerland	AY842034.2
<i>E. arboreum</i> H.W. Keller & T.E. Brooks	ATCC 66095	Switzerland	AY842030.2
<i>E. coelocephalum</i> T.E. Brooks & H.W. Keller	ATCC MYA-2964	Switzerland	AY842033.2
<i>Comatricha macrospora</i> B. Zhang & Yu Li	HMJAU-M1066!	China	SUB3594089
<i>C. nigra</i> (Pers. ex J.F. Gmel.) J. Schröt.	AMFD155	Switzerland	DQ903683.1
<i>C. pseudoalpina</i> G. Moreno, H. Singer, A. Sánchez & Illana	MM 23892	Switzerland	DQ903673.2
<i>C. sinuatocolumellata</i> G. Moreno, H. Singer, A. Sánchez & Illana	MM 24348	Switzerland	DQ903684.1
<i>Diacheopsis metallica</i> Meyl.	AMFD108	Switzerland	DQ903670.1

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TABLE 1. (Continued)

Species	Sample no.	Country	GenBank accessions no. SSU rRNA
<i>Lamproderma sauteri</i> Rostaf.	AMFD208	Switzerland	DQ903674.2
<i>L. zonatum</i> Mar. Mey. & Poulain	MM 21644	Switzerland	DQ903672.1
<i>L. columbinum</i> Mar. Mey. & Poulain	F2	Germany	HQ687204.1
<i>L. columbinum</i>	179	Germany	HQ687203.1
<i>L. columbinum</i>	144	Germany	HQ687201.1
<i>L. columbinum</i>	132	Germany	HQ687200.1
<i>L. columbinum</i>	94	Germany	HQ687199.1
<i>L. columbinum</i>	90	Germany	HQ687198.1
<i>L. columbinum</i>	63b	Germany	HQ687197.1
<i>L. columbinum</i>	106	Germany	HQ687196.1
<i>L. puncticulatum</i> Härk.	162	Germany	HQ687202.1
<i>L. puncticulatum</i>	3	Germany	HQ687195.1
<i>L. puncticulatum</i>	172	Germany	HQ687194.1
<i>Macbrideola oblonga</i> Pando & Lado	—	Switzerland	DQ903682.1
<i>Meriderma aggregatum</i>	AMFD135	Switzerland	DQ903669.1
<i>M. aggregatum</i>	Now13395	Germany	KP967140.1
<i>M. fuscatum</i> (Meyl.) Mar. Mey. & Poulain	MM 24907	Switzerland	DQ903668.1
<i>M. fuscatum</i>	Ron378a	Germany	KP967139.1
<i>M. fuscatum</i>	MM38934	Germany	KP967126.1
<i>M. carestiae</i> (Meyl.) Mar. Mey. & Poulain	AMFD173	Switzerland	DQ903671.2
<i>M. cribrarioides</i> (Fr.) Mar. Mey. & Poulain	Ron668	Germany	KP967314.1
<i>M. cribrarioides</i>	Ron662b	Germany	KP967313.1
<i>M. cribrarioides</i>	LE299936	Germany	KP967284.1
<i>M. cribrarioides</i>	LE297281	Germany	KP967283.1
<i>M. echinulatum</i> (Meyl.) Mar. Mey. & Poulain	LE289595	Germany	KP967291.1
<i>M. echinulatum</i>	LE289560	Germany	KP967290.1
<i>M. carestiae</i> (Ces. & De Not.) Mar. Mey. & Poulain	sc22270	Germany	KP967279.1
<i>M. carestiae</i>	sc25378	Germany	KP967207.1
<i>M. carestiae</i>	HK120427-67	Germany	KP967206.1
<i>Stemonitopsis typhina</i> (F.H. Wigg.) Nann.-Bremek.	sc22550	Germany	KT358701.1
<i>St. typhina</i>	sc22723	Germany	KT358702.1

Results

Molecular phylogeny

The SSU dataset comprises 43 sequences representing 18 taxa. The most appropriate model for estimating the sequences within the dataset and employed in the Bayesian analysis was GTR + I + G. The Bayesian analyses revealed a topology with an average standard deviation of split frequencies = 0.006638. The Bayesian inference analyses generated trees with identical topologies.

Taxa in the order Stemonitaceae have been separated into several clades. These are *Meriderma echinulatum* (Meyl.) Mar. Mey. & Poulain, *M. carestiae* (Ces. & De Not.) Mar. Mey. & Poulain, *M. cribrarioides* (Fr.) Mar. Mey. & Poulain, *M. aggregatum*, *M. fuscatum* (Meyl.) Mar. Mey. & Poulain, *Diacheopsis metallica* Meyl., *Lamproderma columbinum* (Pers.) Rostaf. and *L. puncticulatum* Härk. All of these have been grouped into one clade with high branch supports. Two taxa represented by four sequences from *Lamproderma columbinum* and one sequence from *C. pseudoalpina* G.

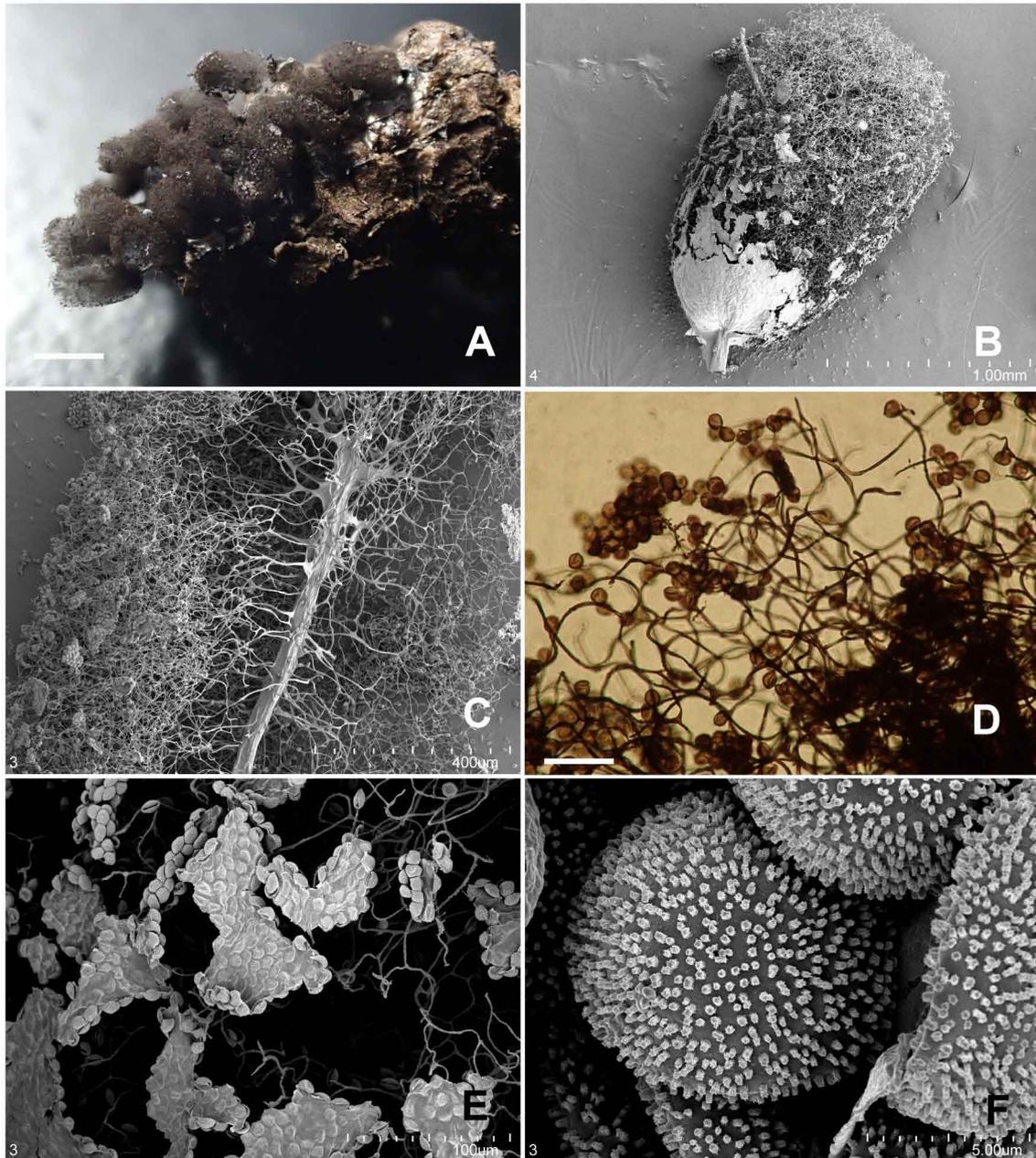


FIGURE 2. *Comatricha macrospora* B. Zhang & Yu Li (HMJAU-M1066!) A. Sporocarp growing on a log; B. Sporocarp as seen by SEM, C. Columella and capillitia; D. Capillitia and spores as seen by TL; E. Peridia and capillitia; F. Spores marked with spines. (Bar: A = 2 mm)

Discussion: About 42 species of *Comatricha* have been reported worldwide (Kirk et al. 2008, Lado 2001, 2005–18), of which nine species have reticulate spores and 33 species have spiny or verruculose spores. Of the accepted species, only *C. pseudoalpina* G. Moreno, H. Singer, A. Sánchez & Illana and *C. filamentosa* Meyl. are similar to *C. macrospora* in having a persistent peridium and sporothecae that are subcylindric and somewhat expanded in their upper portions. However, *C. pseudoalpina* has smaller spores (10–12 μm in diameter), larger sporothecae (1.5–2.5 mm) and warted capillitium threads. *Comatricha filamentosa* has an expanding capillitium that becomes entangled with the adjacent capillitia and minutely spinulose spores (10–13 μm in diameter).

Comatricha tenerrima (M.A. Curtis) G. Lister, in Lister, Guide Brit. Mycetoza, ed. 4, 39 (1919). Figure 3.

≡ *Stemonitis tenerrima* M.A. Curtis, Amer. J. Sci. Arts, ser. 2 6:352 (1848)

= *Stemonitis tenerrima* Berk. & M.A. Curtis, in Berkeley, Grevillea 2:69 (1873)

= *Comatricha argentinae* J.R. Deschamps, Bol. Soc. Argent. Bot. 15 (4):340 (1974)

= *Comatricha tenerrima* var. *macrospora* Rammeloo, Bull. Jard. Bot. Belg. 53(1/2):298 (1983)

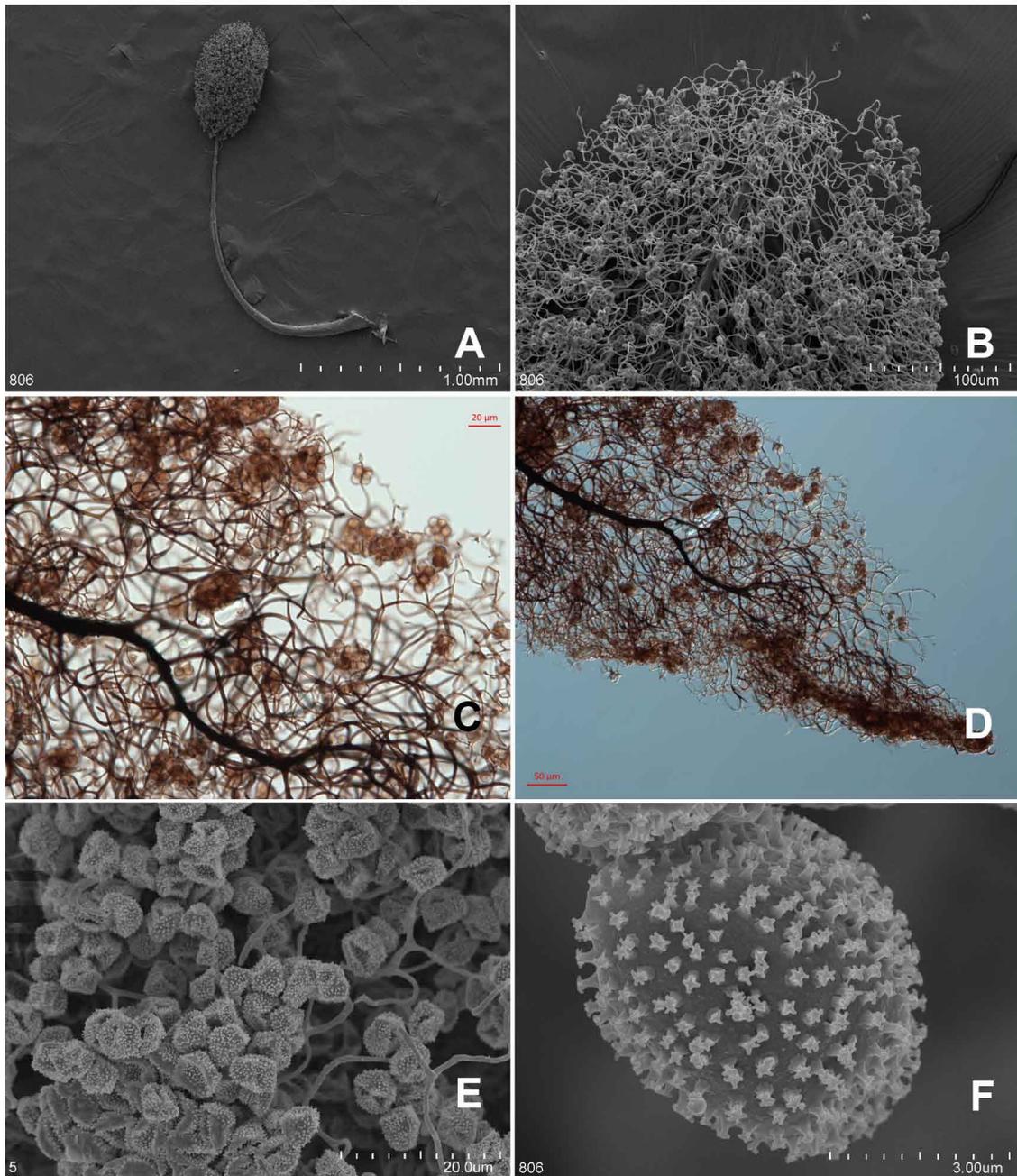


FIGURE 3. *Comatrixa tenerrima* (M.A. Curtis) G. Lister (HMJAU: M1058) A. Sporocarp as observed by SEM; B-D. Capillitium and spores as observed by SEM and TL; E-F. Spores marked with warts as viewed by SEM.

Scattered stalked sporocarps which are 1.5–3 mm in total height, with black stalks that represent about 3/4 of the total height. Fusiform sporotheca are pale red or brownish-pink. The slender columella often reaches the top of the sporotheca. The capillitium is pale red-brown, flexuous, branched and anastomosed, tapering outwards with few or no larger branches that are looped at the periphery. Pale lilac-brown spores are minutely roughened with pale verrucous protrusions; the top of the verrucous protrusions have 5–8 regular lobate-like warts, visible in SEM, that are 7–8 µm in diameter.

Specimens examined: CHINA. Liaoning Province, Laotudingzi National Nature Reserve. 12 September 2010, Zhang2015051405 (HMJAU-M1058); Jilin Province, Jiaohe City, 12 July 2015, Zhang2015122301 (HMJAU-M1059); Sichuan Province, Mianning County, Yihai scenic spot, 7 July 2013, Zhang2015111806 (HMJAU-M1061); Guizhou Province, Maolan core area in Libo County, 19 July 2014, Zhang2015060806 (HMJAU-M1062); Guizhou Province, Maolan core area in Libo County, 17 July 2014, Zhang2015060912 (HMJAU-M1063); and Shanxi Province, Zhuoshui County, 21 July 2014, Zhang2015102904 (HMJAU-M1064).

Discussion: *Comatricha tenerrima* is widely distributed throughout the world, but this is the first time it has been reported from China. The specimen (HMJAU-M1059) collected from Jiaohe City, Jilin Province (about 5–6 μm in diam.) has smaller spores (about 5–6 μm in diam.) than the holotype (about 7–8 μm in diameter), while the specimens (HMJAU-M1064) collected from Zhuoshui County, Shanxi Province, have smaller sporocarps than the holotype. Specimens (HMJAU-M1061) were collected from Mianning County, at a Yihai scenic spot located at an elevation of 2980 m.

Comatricha afroalpina Rammeloo, Bull. Jard. Bot. Belg. 53(1/2):297 (1983). Figure 4.

Scattered sporocarps are 1–2 mm in total height. The red-brown stalk is 0.5–1 mm tall. The sporothecae are spherical and dark brown. A tapered columella reaches almost to the apex in the smaller sporocarps. An abundant capillitium peripherally s an incomplete irregular network with numerous free ends. Grey-brown spores are regularly verruculose with dispersed groups of larger dark warts, which sometimes form an incomplete reticulum; the spores are 8–10 μm in diameter.

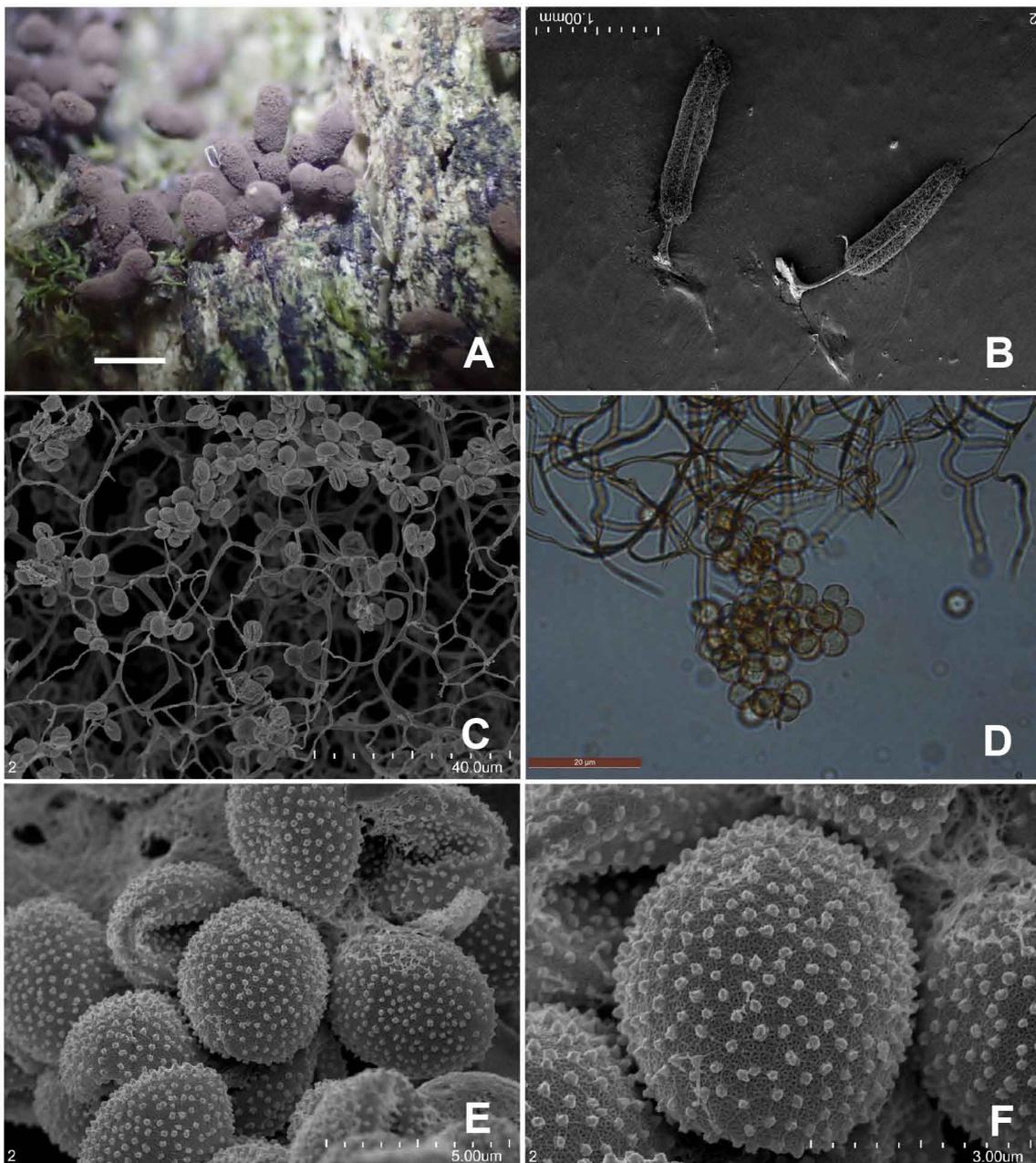


FIGURE 4. *Comatricha afroalpina* Rammeloo (HMJAU: M1006) A. Sporocarps occurring on a log; B. Two sporocarps as observed by SEM; C. Incomplete surface net and spores as observed by SEM; D. A portion of the spores and capillitium as viewed by TL; E-F. Spores marked with warts by as viewed by SEM. (Bar: A=1 mm)

Specimen examined: CHINA. Jiangxi Province, East China Institute of Technology, 17 June 2013, Zhang2016011102 (HMJAU-M1006).

Discussion: The genus *Comatricha* Preuss has been reported to contain 41 species, but only two species, *C. vineatilis* and *C. alpina*, are similar to *C. macrospora*. Like *C. macrospora*, *C. alpina* has a persistent peridium at the base of the sporotheca; meanwhile *C. vineatilis* is similar to *C. afroalpina* in having regularly verruculose spores dispersed in larger groups. *Comatricha vineatilis* (spore size: 6–7 µm in diameter) has smaller spores than *C. afroalpina* (spore size: 8–10 µm in diameter), whereas *C. alpina* (spore size: 11–12 µm in diameter) is a nivicolous species and has larger spores than *C. afroalpina*.

Key to species of *Comatricha* in China

1	Spores marked with reticulations	2
1	Spores marked with spinulose or warts	3
2	Ends of columella not enlarged	<i>C. reticulospora</i>
2	Ends of columella with a clavate expansion	<i>C. clavicumella</i>
3	Capillitium flexuous, looped	4
3	Capillitium rigid, not looped	7
4	Sporocysts globose, ovoid	5
4	Sporocysts cylindric, long ovoid or fusiformis	6
5	Stalk 2/3 the total height, peridium fugacious	<i>C. nigra</i>
5	Stalk small, peridium persisting at the base of the sporotheca	<i>C. macrospora</i>
6	Spores warted with some groups of darker warts	<i>C. afroalpina</i>
6	Spores warted	<i>C. ellae</i>
7	Stalk long, more than 3/4 of total height	<i>C. tenerrima</i>
7	Stalk short, no more than 1/2 of total height	8
8	Spores 6–9 µm in diam., spinulose	<i>C. pulchella</i>
8	Spores 8–11 µm in diam., marked densely warted	<i>C. laxa</i>

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