

## **SEM studies on type specimens of two rare *Comatricha* taxa**

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The lectotype of *Comatricha brachypus* was examined with light and electron microscopy and compared with an isotype of *C. tenerrima* var. *macrospora*.

Key words: Myxomycota, scanning electron microscopy, taxonomy, type material.

The myxomycetes are a peculiar group of organisms forming together with dictyostelid slime molds a single coherent (monophyletic) group, whichs closest relatives are the amoebas (Protozoa), followed by the animals and the fungi (Baldauf *et al.*, 2000). Originally having been studied by botanists and mycologists, they are currently the focus of study of specialised investigators, the “myxomycetologists”.

Myxomycetes usually have small fructifications and very uniform spores with a spore ornamentation that is often very difficult to see clearly by light microscopy (LM). This situation frequently makes identification impossible. Scanning electron microscopy (SEM) with the application of the critical point drying technique makes it possible to observe spores in their native state, thus maintaining their complete spore ornamentation. It is therefore a basic tool in the revision of type material that has been described in years when optical microscopy was imprecise and the original diagnoses were quite incomplete. With SEM we can now obtain very valuable information that can be applied to resolving taxonomic problems in taxa that are difficult to distinguish and only occasionally collected. This is the case for the study described herein, which involved a revision of the type material of *Comatricha brachypus* (Meyl.) Meyl.

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## Materials and Methods

The examined specimens were obtained from the herbaria BR, LAU and AH.

Specimens were mounted in Hoyer's medium and studied with a Nikon microscope. SEM micrographs were produced with a Zeiss DSM-950. Spore measurements were made under the oil immersion objective and include surface structures such as spines or warts.

Twenty five spores of each specimen have been measured to determine spore size. Two microscopic slides have been examined of each specimen studied, except for type specimens, where only one slide has been examined. Spore diameters are expressed as ranges between smallest measured diameter and largest measured diameter. Values in brackets represent rare measurements (a maximum of two spores of 25 spores measured).

For ultramicroscopic studies the material was rehydrated in concentrated ammonium hydroxide (28 – 30 %) for 30 minutes, dehydrated in aqueous ethanol (70 %) for 30 minutes, fixed for 2 hours in pure ethylene glycol dimethyl ether (= 1,2-dimethoxymethane) and finally immersed in pure acetone for at least 2 hours followed by critical point drying and sputtering with gold-palladium.

## Taxonomy

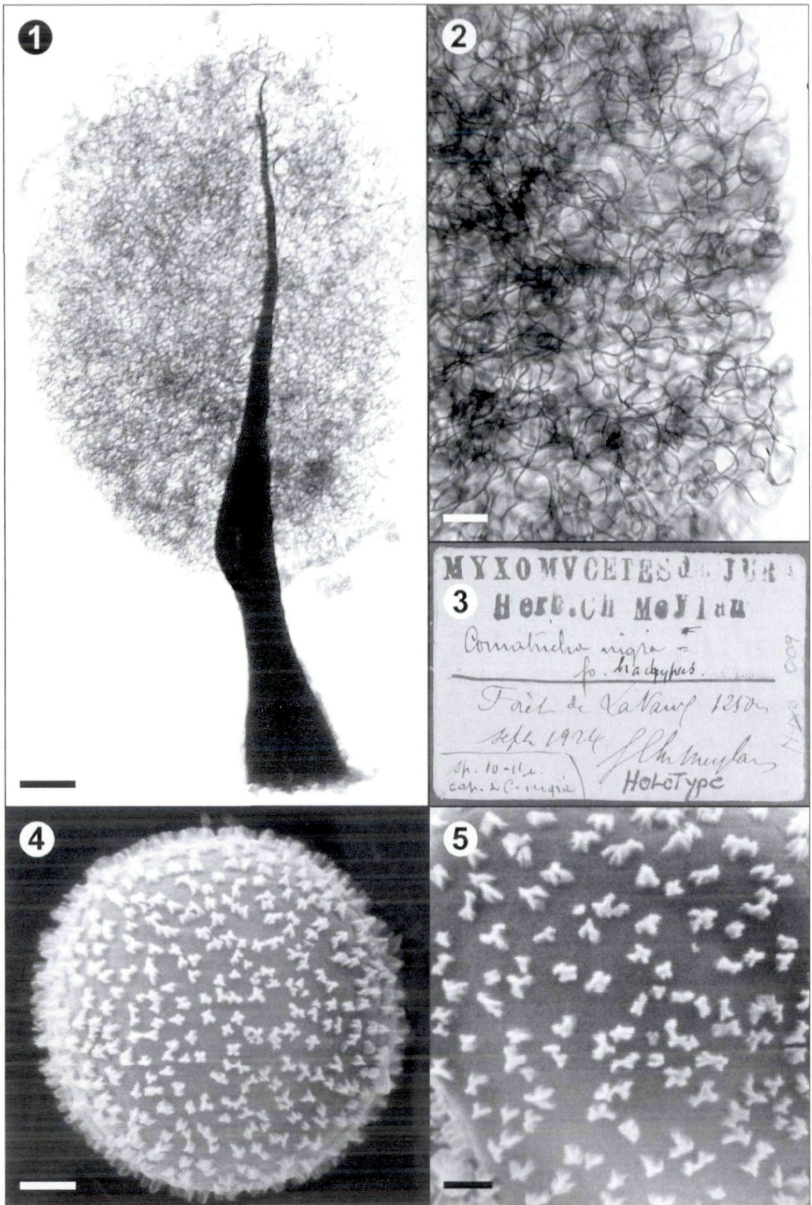
*Comatricha brachypus* (Meyl.) Meyl., Bull. Soc. Vaud. Sci. Nat., 57: 41. 1929. – Figs. 1 – 5.

- ≡ *Comatricha nigra* var. *brachypus* "brachypes" Meyl., Bull. Soc. Vaud. Sci. Nat., 56: 71. 1925.
- ≡ *Stemonitopsis brachypus* (Mey.) Y. Yamam., Myxomycete Biota Japan: 627. 1998.

Original description of *Comatricha nigra* var. *brachypus* (Meylan 1925). – Aspect des formes ovoïdes et courtes de *C. laxa*, soit: sporanges ovoïdes, à partie large en bas, de 1 à 1.5 mm de hauteur, portés par un stipe très court, de 0.2 à 0.4 mm, bruns. Capillitium de *C. nigra*. Spores brunes, de 10 à 11 µm. La Vaux, 1250 m, septembre 1924.

Original description of *Comatricha brachypus* (Meylan 1929). – Plasmodium blanc laiteux. Sporangies elliptiques ou ovoïdes de 1 à 1.5 mm de hauteur sur 0.7 à 1 mm de largeur, brun foncé, portés par un stipe toujours plus court que le sporange et mesurant de 0.2 à 0.5 mm. Columelle atteignant en général les  $\frac{3}{4}$  ou les  $\frac{2}{5}$  de la hauteur du sporange. Capillitium dense, formé de filaments sinueux, comme celui de *C. nigra*. Spores 10 – 11 µm, finement verruqueuses. Maturité: septembre, octobre.

Description: The type material is very well conserved in a matchbox on remains of liverworts growing on dead wood, glued to a piece of cardboard that can be removed from the box. The type material consists of about 50 sporangia.



**Figs. 1–5.** *Comatricha brachypus* (holotype): **1.** Sporangium (bar = 0.1 mm). **2.** Detail of capillitium (bar = 30  $\mu$ m). **3.** Lid of herbarium box where the type is kept. **4.** Spore (bar = 2  $\mu$ m). **5.** Detail of spore ornamentation (bar = 1  $\mu$ m).



Sporangia gregarious to crowded, stalked, 1–1.5 mm total height. Sporotheca broadly ellipsoidal to ovoid, 0.5–1.2 × 0.5–1 mm, lilac brown. Peridium entirely evanescent (i.e. not remaining partly as a basal cup). Stalk 0.2–0.5 mm high, smooth, flattened and widening towards the base, blackish brown. Columella occurring as a continuation of the stalk and concolorous, rostrate, reaching almost to the apex of the sporotheca where it sometimes becomes sinuous. Hypothallus membranous, hyaline, common to several sporangia. Capillitium dense, sinuous (resembling that of *Comatricha nigra*), branched and anastomosed, without main branches, arising from the length of the columella, threads 1–2 µm wide, uniformly reddish brown. Spore-mass violaceous brown, light violaceous by LM, (9.0–)10.0–11.0 µm in diam, with abundant, regularly distributed warts. By SEM spore ornamentation is formed by small and narrow baculae that converge obliquely, forming pyramid or “tripod-like” structures with part of the base more or less hollow, sometimes constituting irregularly crested structures.

Material examined. – *Comatricha brachypus* (Meyl.) Meyl.: SWITZERLAND, Canton Vaud, Fôret de La Vaux, near Sainte Croix, 1250 m, on dead wood, Sep 1924, leg. C. Meylan, holotype in LAU (without herbarium number).

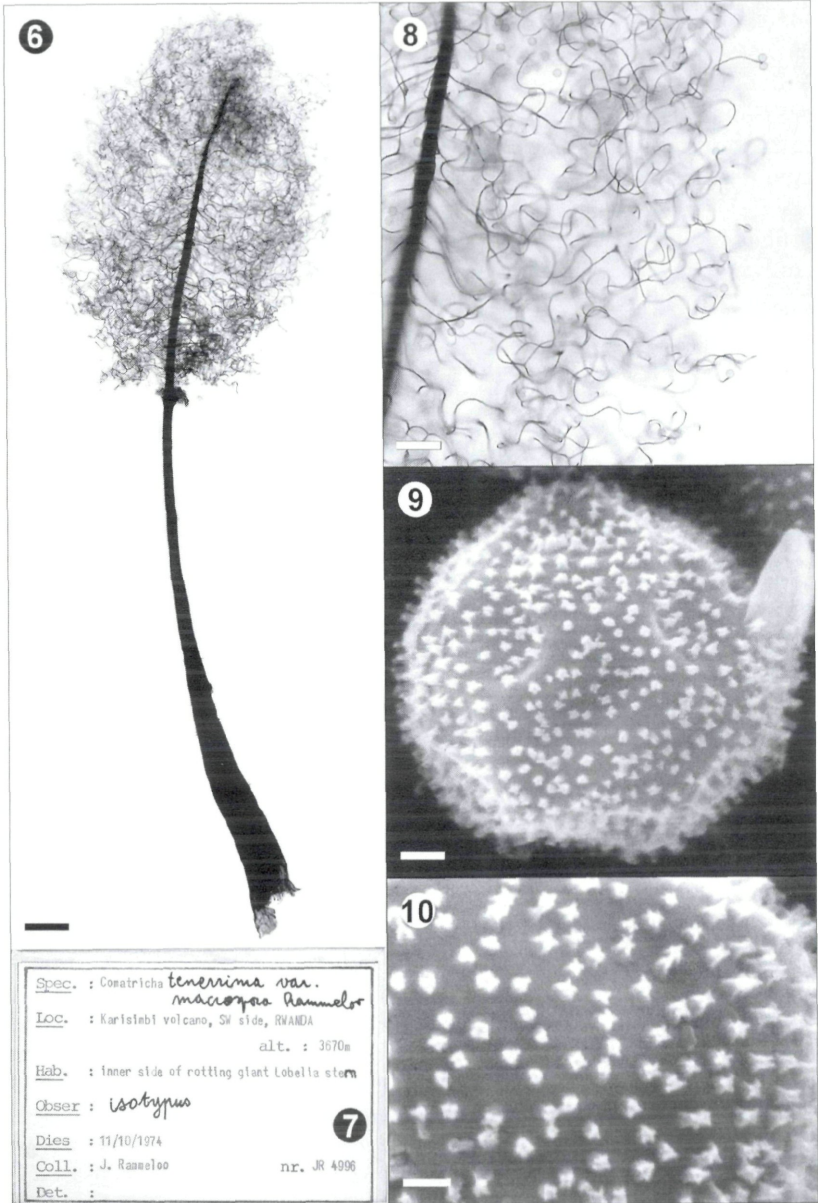
*Comatricha tenerrima* (M.A. Curt.) G. Lister var. *macrospora* Rammeloo, Bull. Jard. Bot. Belg., 53 (1/2): 298. 1983. – Figs. 6–10.

Latin diagnosis. – Ob sporocystas minores stipite brevior et capite globoso obscurioreque, capillitium minus differentiatum atque sporas majores a var. *tenerrima* removenda.

Description: The type material is in very good condition and is attached to a piece of cardboard. The entire collection consists of five pieces of wood, most of which are covered by numerous, well-developed sporangia.

Sporangia scattered, 1.8–2.2 mm total height. Sporotheca globose to subglobose, 0.5–0.6 mm in diam, blackish brown. Peridium evanescent, leaving a collar at the base of the sporotheca. Stalk 0.5–1 mm high, smooth, cylindrical, widening somewhat towards the base, blackish brown. Columella occurring as a continuation of the stalk and concolorous, cylindrical, reaching to the apex of the sporotheca or nearly doing so. Hypothallus membranous, hyaline, discoid. Capillitium dense, sinuous, branched and anastomosed, without main branches, arising from all along the columella, threads 1–2 µm wide, uniformly blackish brown. Spore-mass dark violaceous brown, violaceous brown by LM, 8.0–10.0 µm in diam, with abundant, regularly distributed warts. By SEM spore ornamentation is composed of baculae with the





**Figs. 6 – 10.** *Comatricha tenerima* var. *macrospora* (isotype): **6.** Sporangium (bar = 0.1 mm). **7.** Herbarium box. **8.** Detail of capillitium (bar = 30  $\mu$ m). **9.** Spore (bar = 2  $\mu$ m). **10.** Detail of spore ornamentation (bar = 1  $\mu$ m).

apex divided into 3–4(–6) lobes giving it a more-or-less stellate appearance.

Material examined. – *Comatricha tenerrima* var. *macrospora* Rammeloo: RWANDA, Karisimbi volcano, SW side, 3670 m, inner side of a rotting giant *Lobelia* stem, 11 Oct 1974, leg. J. Rammeloo (JR 4996 in BR 1728, isotype).

### Discussion

Meylan (1925) originally described *Comatricha nigra* var. *brachypus* Meyl. from a collection made from dead wood in the forest of La Vaux (Switzerland) at 1250 m elevation. Meylan indicated from this variety to which – somewhat misleading – he refers to as a form: “Cette forme paraît si différente des formes connues de *C. nigra* qu’elle constitue peut-être une espèce autonome. De nouvelles récoltes et observations pourront seules en décider” (translation: The form seems so different from the known forms of *C. nigra* that it constitutes perhaps an autonomous species).

Several years later Meylan (1929) decided to consider this taxon an autonomous species. Subsequent authors such as Nannenga-Bremekamp (1991) and Kowalski (1975) have also considered it as an independent species, although Kowalski noted its similarity to *C. pulchella* (C. Bab.) Rostaf., “except for spore size”.

Martin & Alexopoulos (1969) regarded *Comatricha brachypus* as a synonym of *C. subcaespitosa* Peck (= *Stemonitopsis subcaespitosa* (Peck) Nann.-Bremek.) and concluded that there were “no significant differences when compared an authentic specimen of *C. brachypus* from Meylan with specimens of *C. subcaespitosa*”. Following our detailed study of the type material of *Stemonitopsis subcaespitosa*, we find this to be a different species due to its capillitium having a more-or-less complete surface net and possessing a spiny spore ornamentation (Moreno *et al.* 2004). More recently, Yamamoto & Nishikawa (1995) found in Japan a specimen they determined as *Comatricha* cf. *brachypus*, and three years later, Yamamoto (1998) made the combination *Stemonitopsis brachypus* (Meyl.) Y. Yamam., based on this material (the drawings are the same in both publications). The Japanese material shows longly cylindrical sporangia with a short stalk and capillitium threads connected perpendicularly to the columella and forming an internal three dimensional net and a dense, small-meshed, almost complete surface net, very different from the studied type material of *Comatricha brachypus* (see drawings in Yamamoto, 1998). We do not know further records of this species.

When the type of *Comatricha brachypus* is observed under a magnifying glass, the first impression one has is that he is looking at a specimen of *C. pulchella*, as already indicated by Kowalski (1975).

But when its microscopic characters are studied, notable differences between the two taxa are observed, because the capillitium of *C. brachypus* forms a sinuous and intricate net of filaments without differentiation into primary and secondary branches, while the capillitium of *C. pulchella* has thicker primary branches arising from the columella at right angles from which once again arise secondary branches in the form of thinner and sinuous threads that form a net (Lizárraga *et al.* 2003)

*Comatricha brachypus* also reminds one microscopically of *C. meandrispora* A. Castillo, G. Moreno, & Illana due to its rostrate columella that becomes abruptly narrower at the apex (Castillo *et al.* 1993).

*Comatricha brachypus* can be distinguished from the aforementioned species by studying its spore ornamentation by SEM, the latter consisting of small and narrow baculae that converge obliquely forming pyramid or “tripod-like” structures with part of the base more or less hollow, sometimes constituting irregularly crested structures. In *C. pulchella* the spore surface is covered by long, spiny baculae with excrescences in the middle part, as can be observed in the SEM photographs provided by Rammeloo (1983a), and in *C. meandrispora* spore ornamentation is subreticulate to reticulate.

At first glance, the spore ornamentation of *Comatricha brachypus* is similar to that of *C. tenerrima* (M.A. Curtis) G. Lister. Rammeloo (1983a) studied the spore ornamentation of *C. tenerrima* var. *tenerrima* by SEM and provides a photo that shows spore ornamentation with a surprising resemblance. However, when both types of spore ornamentation are compared thoroughly, they can be distinguished on the basis of several characteristics: While in *C. brachypus* the ornamentation is formed by fusing baculae that appear as pyramid or “tripod-like” structures, *C. tenerrima* has solitary baculae with lobed apices (Lizárraga *et al.* 2005). Both types of spore ornamentation have a star-like appearance when seen from above (Figs. 4, 9).

Rammeloo (1983b) described a macrosporic variety of *Comatricha tenerrima*, *C. tenerrima* var. *macrospora* Rammeloo, basing himself on a single collection from Rwanda, the isotype of which we have studied. Rammeloo (1983a) gives further records of this variety collected several times in Rwanda, between 2350 – 3670 m elevation, on bamboo, leaves of *Dendrosenecio*, and on the inner side of an inflorescence of *Lobelia gigantea*. *Comatricha tenerrima* var. *macrospora* differs from *C. tenerrima* var. *tenerrima* by having smaller, more globose and darker sporothecae, a shorter stalk, a less differentiated capillitium and larger spores (8.0 – 10.8 µm in diam). Spore ornamentation is formed by baculae with an apex which is frequently divided into 6 lobes, in contrast to those of *C. tenerrima* var. *tenerrima* which have 3 – 4 lobes.



*Comatricha tenerrima* var. *macrospora* shares with *C. brachypus* a sinuous capillitium without main branches (*Sinuaria* type), and similar spore diameters, 10.0–11.0 µm in *C. brachypus*, and 8.0–10.8 µm in *C. tenerrima* var. *macrospora* (according to the authors). But *C. brachypus* can easily be distinguished by its broadly ellipsoidal to ovoid sporotheca, a short stalk that attains approximately 1/3 of the height of the sporotheca, a rostrate columella, the absence of a collar at the base of the sporotheca and a different spore ornamentation that has been described above. *Comatricha tenerrima* var. *macrospora* has globose to subglobose sporothecae, a long stalk that is approximately twice the height of the sporotheca, a more or less cylindrical columella and a collar at the base of the sporotheca as a remnant of the peridium.

The differences between the three taxa mentioned above are summarized in Tab. 1. The description of *Comatricha tenerrima* var. *tenerrima* follows Rammeloo (1983 a).

**Tab. 1.** – Differences among *Comatricha tenerrima* var. *tenerrima*, *C. tenerrima* var. *macrospora* and *C. brachypus*.

		<i>C. tenerrima</i> var. <i>tenerrima</i>	<i>C. tenerrima</i> var. <i>macrospora</i>	<i>C. brachypus</i>
<b>Sporangia</b>	Total height	3 – 4 mm	1.5 – 2 mm	1 – 1.5 mm
<b>Sporotheca</b>	Height	0.9 – 1.3 mm	0.5 – 0.6 mm	0.5 – 1.2 × 0.5 – 1.0 mm
	Form	± fusiform or long ovoid	globose to subglobose	broadly ellipsoidal to ovoid
	Colour	light brown, reddish or lilac	blackish brown	lilac brown
<b>Stalk</b>	Height	2 – 3 mm	0.5 – 1.0 mm	0.2 – 0.5 mm
<b>Spores</b>	Diameter	5.8 – 8.2 µm	8.0 – 10.8 µm	(9.0 –)10.0 – 11.0 µm
	Ornamentation by SEM	baculae with the apex divided into 3 – 4 lobes	baculae with the apex divided into 3 – 4(–6) lobes	baculae that converge obliquely forming pyramid or “tripod-like” structures

### Taxonomic treatment

- 1** Spores 6.0 – 8.0 µm in diam . . . . . *C. tenerrima* var. *tenerrima*
- 1\*** Spores 8.0 – 11.0 µm in diam . . . . . 2
- 2** Stalk higher than sporotheca, collar present at its base. Spore ornamentation (SEM) formed by baculae with lobed apex . . . . .  
. . . . . *C. tenerrima* var. *macrospora*
- 2\*** Stalk less than half the height of the sporotheca, without collar at its base. Spore ornamentation (SEM) formed by fusing baculae that form pyramid or “tripod-like” structures . . . . . *C. brachypus*

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