

# Phylogenetic and morphological notes on *Uleobryum naganoi* Kiguchi *et al.* (Pottiaceae, Musci)<sup>1</sup>

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*Uleobryum naganoi* Kiguchi *et al.*, endemic to Japan with a limited number of known locations, is newly reported from Shikoku, western Japan. Through careful examination of fresh material, rhizoidal tuber formation is confirmed for the first time. The phylogenetic position of this cleistocarpous moss is also assessed on the basis of maximum likelihood analysis of *rbcL* gene sequences. The current position in the Pottiaceae is supported and a close relationship to *Ephemerum spinulosum* is suggested.

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## Introduction

The genus *Uleobryum* is a small genus comprising only three species (Kiguchi *et al.* 1996; Zander 1993, 1994; Zander & Dixon 1999), with a distribution range in the West Indies, Mexico, Peru, Brazil, Australia, and Japan. The genus is characterized by its small size, a costa with two stereid bands and elongate adaxial epidermal cells, spheroidal cleistocarpous capsules with a hyaline seta, transparent capsule walls of which cells are mamillate, with slightly thickened free walls, and conic-campanulate calyptrae that are roughened apically (Zander 1993). It has been placed in the Pottiaceae since its establishment as an independent genus by Brotherus (1906). *Uleobryum naganoi* Kiguchi *et al.* has been described as endemic to Japan by Kiguchi *et al.* (1996), and is characterized by (1) linear-lanceolate leaves, (2) stomata on the middle of the upper half of the capsule, and (3) unlobed calyptrae. The systematic position of cleistocarpous mosses is equivocal when conclusions are limited to morphological characters, particularly when major characters

are missing. Molecular phylogenetic studies have been made to clarify the phylogenetic position of some mosses lacking peristomes (Goffinet & Cox 2000; Goffinet *et al.* 1998, 2002; Hedderson *et al.* 2004; Stech 1999). These researches demonstrate that molecular data provide us with information useful to better understand such enigmatic cleistocarpous mosses. In this paper, rhizoidal tuber formation for *Uleobryum naganoi* is newly reported and the phylogenetic position of this species is also discussed on the basis of ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) gene sequences with maximum likelihood analysis, together with a report of new habitat in Japan.

## Materials and methods

Total DNA was extracted from fresh material using the modifications of a phenol-chloroform method (Tsubota *et al.* 1999). PCR amplifications and direct sequence analyses were carried out using standard techniques. Details and further information for DNA extracting protocols, amplification primers, PCR protocols and those of sequencing reactions are described in Tsubota *et al.* (1999, 2000, 2002).

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Fifty six moss species were included in the analyses and the sequences were manually aligned. Outgroup taxa were selected based on the phylogenetic hypothesis proposed by Magombo (2003) and Tsubota *et al.* (2003).

A phylogenetic tree was constructed using maximum likelihood criteria (ML; Felsenstein 1981). An NJ tree was obtained using Njdist in MOLPHY 2.3b3 (Adachi & Hasegawa 1996) with HKY85 model (Hasegawa *et al.* 1985), and the best ML tree was searched using NucML in MOLPHY with the NJ tree as a start topology for a local rearrangement algorithm.

New *rbcL* sequences were determined for four species: \**Bryoxiphium norvegicum* subsp. *japonicum* (AB194720), \**Uleobryum naganoi* (AB194717), *Weissia controversa* (AB194718) and \**Ephemerum spinulosum* (AB194719). Three species with an asterisk were shown as an entire sequence 1,428 bp long. A data matrix was prepared for analyses of phylogenetic relationships of the

haploleptoid mosses including some diploleptoid mosses. For the analyses, a total of 1,210 sites (84.73% in 1,428 bp of the entire sequence) of 56 *rbcL* sequences were used.

Information on the specimens from which DNA was extracted with the accession numbers is given below:

*Bryoxiphium norvegicum* subsp. *japonicum* (Berggr.) Á.Löve & D.Löve, AB194720, Japan, Honshu, Tottori-ken, Hino-gun, Koufu-cho, Mt. Karasugasen, 1,200 m alt., June 18, 2000, Leg. S. Nogawa in hb. H. Deguchi 36501 (HIRO).

*Uleobryum naganoi* Kiguchi *et al.*, AB194717, Japan, Shikoku, Kagawa-ken, Ayauta-gun, Ayakami-cho, Nagara Lake, ca. 100 m alt. [associated with *Ephemerum spinulosum* Bruch & Schimp.], Dec. 13, 2003, H. Sato 377 (HIRO).

*Weissia controversa* Hedw., AB194718, Japan, Honshu, Hiroshima-ken, Higashi-hiroshima-shi, ca. 200 m alt., July 3, 2003, H. Sato 161 (HIRO).

*Ephemerum spinulosum* Bruch & Schimp.,

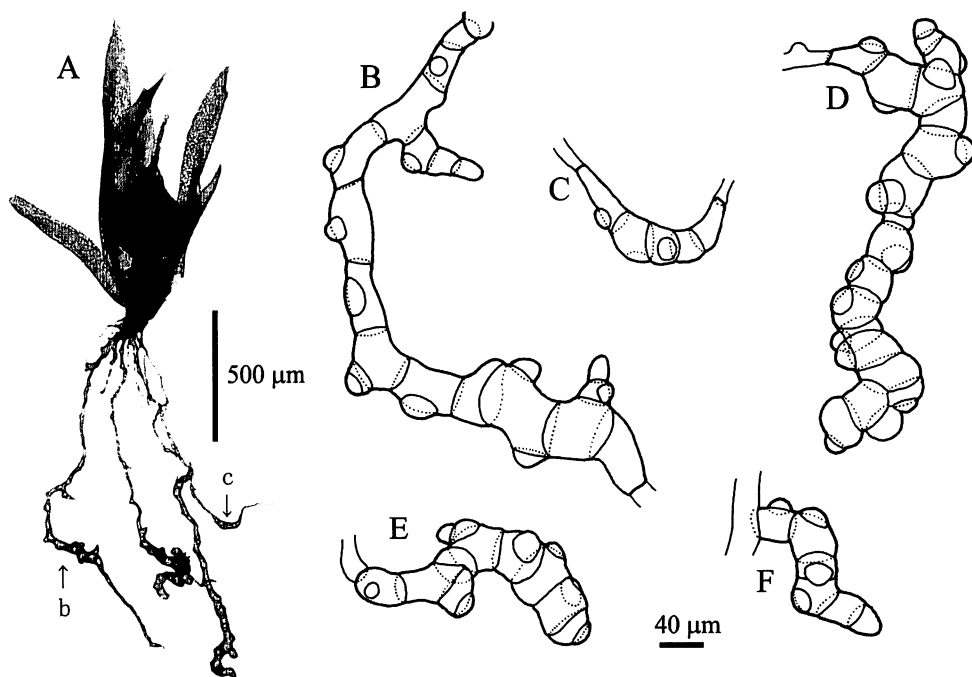


Fig. 1. Rhizoidal tubers of *Uleobryum naganoi* Kiguchi *et al.*

A. Plant with a capsule and rhizoids. B-F. Rhizoidal tubers (B corresponding to b in A; C to c). Photographed and drawn from H. Sato 377.

AB194719, Japan, Shikoku, Kagawa-ken, Ayauta-gun, Ayakami-cho, Nagara Lake, ca. 100 m alt., Dec. 13, 2003, H. Sato 378 (HIRO).

The other fifty two sequences were obtained from the DNA databases, and the accession num-

bers of OTUs were shown in parentheses in Fig. 2.

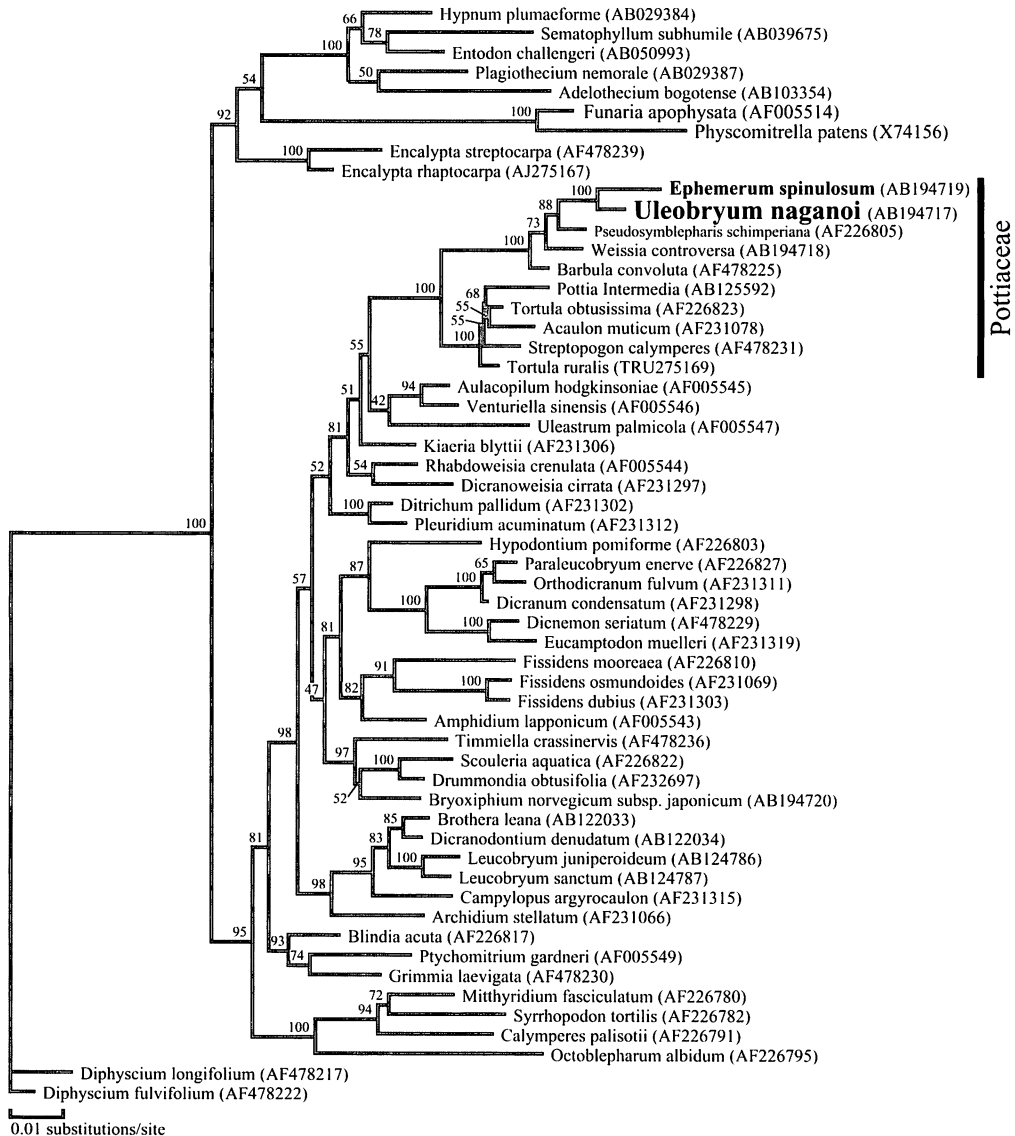


Fig. 2. Phylogenetic position of *Uleobryum naganoi*. The maximum likelihood tree of the aligned *rbcL* gene sequences, 1,210 bp of 56 *rbcL* gene sequences (HKY85 model [Hasegawa *et al.* 1985];  $2\alpha/\beta = 4.865$ ;  $\ln L = -9120.08 \pm 392.04$  by NucML); and the clades within the Pottiaceae clade. The numbers in parentheses show accession numbers of each OTU. The horizontal length of each branch corresponds to the estimated number of nucleotide substitutions. The root is arbitrarily placed on the branch leading to *Diphyssium longifolium* (AF478217) and *D. fulvifolium* (AF478222).

## Results

Rhizoidal tubers consist of a single, bifurcated or branched cell-row, tinged reddish-brown, which is similar in color to the rhizoids. The tuber cells are barrel-shaped, more or less isodiametric, ca. 20–55  $\mu\text{m}$  thick; each of them is usually accompanied by a small lenticular initial cell ca. 14–32  $\mu\text{m}$  in diameter. Initial cells began to develop into protonema in a few weeks after liquid cultivation in a petri dish at room temperature (20–25°C).

The ML tree was obtained by NucML (ln  $L = -9120.08 \pm 392.04$ ; Fig. 2). Four large clades corresponding to the Bryidae, Funariaceae, Encalyptaceae, and Dicranidae were resolved with a high BP support (100%, 100%, 100%, and 95%, respectively). Within the Dicranidae clade, the Pottiaceae–*Ephemerum* clade was resolved with strong support (100%). Within the Pottiaceae–*Ephemerum* clade, two subclades were resolved: the *Uleobryum*–*Ephemerum*–*Pseudosymblypharis*–*Weissia*–*Barbula* clade (100%) and the *Pottia*–*Tortula*–*Acaulon*–*Streptopogon* clade (100%). Within the former clade, *Uleobryum* has a close relationship with *Ephemerum* (100%). Homology values of the total *rbcL* gene sequences of *U. naganoi* and *E. spinulosum* proved to be identical with the value 98.39% (= 1,405 bp).

## Discussion

*Uleobryum* is a cleistocarpous moss, and the gametophytes show features characteristic of many species of the Pottiaceae, such as (1) obovate to linear lanceolate leaves with an acute tip, and (2) lamina cells that are densely papillate on both surfaces in the upper and middle parts of the leaves. The present results suggest a close relationship of *Uleobryum* with *Ephemerum*, and their inclusion in the Pottiaceae was strongly supported, as previously reported on the basis of the data on *rps4* gene sequences (Goffinet & Cox 2000; Goffinet *et al.* 2001; Hedderson *et al.* 2004). Hedderson *et al.* (2004) further inferred that some species of *Weissia* in the Pottiaceae also approach *Ephemerum*, i.e., species with reduced plant size, immersed sporophytes, and persistent protonemata.

The close relationship of *Ephemerum* with

those of the genera of the family Funariaceae and the systematic position of the genus in the order Funariales proved to be doubtful, judging from the present result and their dissimilar morphological characters. Hence *Ephemerum* should be transferred from the Ephemeraceae to the Pottiaceae as was done by Goffinet & Buck (2004).

The homology value 98.39% (= 1,405 bp) of *U. naganoi* and *E. spinulosum* suggested more detailed investigation is required with more species sampled from the Pottiaceae to obtain more precise phylogenetic information on the genera *Uleobryum* and *Ephemerum* within the Pottiaceae. *Uleobryum* was related to other cleistocarpous genera *Bryocephospora* and *Trachycarpidium* as previously suggested by Zander (1993, 1994).

Finally, populations of *Uleobryum naganoi* remain in restricted locations which include Kanagawa, the type locality, Shizuoka, and Saitama Prefectures of East Japan (Kiguchi *et al.* 1996; Iwatsuki & Suzuki 1998; Kiguchi 2003). A new location is first reported here from Shikoku, western Japan. The plants were growing on sandy soil mixed with gravel on the shore of a water reserve for irrigation, being associated with *Ephemerum spinulosum*.

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佐藤裕幸・坪田博美・山口富美夫・出口博則：ツチノウエノハリゴケの系統および形態について

西日本新産となるツチノウエノハリゴケについて仮根上結節を報告した。さらに葉緑体 *rbcL* 遺伝子の塩基配列に基づき系統解析を行なった結果、ツチノウエノハリゴケはセンボンゴケ科のクレードに含まれ、さらにカゲロウゴケと系統的に近い関係にあることが示唆された。