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Kahakuloa operculispora, a new Hawaiian simple thalloid liverwort in a new genus and family, Kahakuloaceae (Fossombroniales)

A. VIRGINIA FREIRE[1,2], EMMET J. JUDZIEWICZ[1,2], D. CHRISTINE CARGILL[3], LAURA L. FORREST[4], S. ROBBERT GRADSTEIN[5], HANK L. OPPENHEIMER[6], ZACHARY PEZZILLO[6] & SOL SEPSENWOL[1]
[1]*Biology Department, Chemistry Biology Building, University of Wisconsin, Stevens Point, WI 54481-3897, USA*
calypogeia@gmail.com; https://orcid.org/0009-0002-0113-8856
ejudziew@uwsp.edu; https://orcid.org/0009-0004-4013-3980
ssepsenw@uwsp.edu; https://orcid.org/0009-0008-6939-2379
[2]*Herbarium Pacificum, Bernice Pauahi Bishop Museum, 1525 Bernice Street, Honolulu, HI, 96817, USA*
[3]*Australian National Herbarium CANB, Centre for Australian National Biodiversity Research (a joint venture between Parks Australia and CSIRO). GPO Box 1777, Canberra, ACT. Australia 2601*
Chris.Cargill@dcceew.gov.au; https://orcid.org/0000-0001-8390-3245
[4]*Royal Botanic Garden, 20A Inverleith Row, Edinburgh, EH3 5LR, Scotland*
l.forrest@rbge.ac.uk; https://orcid.org/0000-0002-0235-9506
[5]*Meise Botanic Garden, 1860 Meise, Belgium*
robbert.gradstein@adwgoe.de; https://orcid.org/0000-0002-3849-6457
[6]*Plant Extinction Prevention Program, Pacific Cooperative Studies Unit, University of Hawai'i, P.O. Box 909, Makawao, Hawai'i, 96768, USA*
henryo@hawaii.edu; https://orcid.org/0000-0002-9416-1889
pezzillo@hawaii.edu; https://orcid.org/0009-0005-7342-3738

Abstract

Kahakuloa operculispora, a new simple thalloid liverwort endemic to Hawai'i, so far only known from high elevation bogs in western Maui, is described and illustrated. The thallus has prominent reddish-purple rhizoids, uniseriate ventral slime papillae and lacks scales. The plants are dioicous; male plants have antheridia sunken in several rows of dorsal chambers, subtended by scales, while female plants have clustered archegonia enclosed by thick, barrel-shaped involucres that develop serially at the plant apex, before fertilization, and are arranged in a single dorsal row at maturity; perichaetial scales and pseudoperianths (defined as involucres developing after fertilization) are lacking. The capsules are spherical, 2−3 stratose, and dehisce irregularly; the spores are areolate and have a unique operculum-like disk on the proximal face. Molecular and morphological evidence supports placement of the plant in a new genus and family in the order Fossombroniales, suborder Fossombroniineae. *Kahakuloa* is the first liverwort genus endemic to Hawai'i, and Kahakuloaceae is the only known endemic Hawaiian land plant family.

Key words: Hawai'i, *Kahakuloa*, liverwort, Marchantiophyta, Fossombroniineae

Introduction

While visiting the Herbarium Pacificum at Bernice Pauahi Bishop Museum in November 2022, the first author encountered an unusual undetermined thalloid liverwort collected in 1980 by Derral R. Herbst (1934–2023) from the remote, montane Keahikauō Bog on Mauna 'E'eka, in western Maui, Hawai'i, in the West Maui Natural Area Reserve (Figure 1). The specimen consisted of female plants with broad, fleshy midveins and a thick, semi-erect, wavy thallus; the rhizoids were long and reddish-purple. They had several barrel-shaped involucres in a single dorsal row, enclosing small clusters of archegonia (Figure 2). No sporophytes or male plants were present. Then, on March 17, 2023, Zach Pezzillo and Hank Oppenheimer (working for the Plant Extinction Prevention Program) collected the same taxon from another bog on the flanks of Mauna 'E'eka, (Figures 3a, 3b), located just 1.5 km south of and about 300 m higher than the Herbst site in Keahikauō Bog. This specimen included both male and female plants, some with immature sporophytes and a few with dehisced capsules. The specimens could not be identified using the descriptions

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in Crandall-Stotler *et al.* (2009), Gradstein *et al*. (2001), Gradstein (2021), Lee & Gradstein (2021), or any other resource, and appeared to belong to an undescribed taxon. The purpose of this research was to elucidate the taxonomic position of this unknown Hawaiian liverwort, using both molecular analysis and morphological observations, and to describe it as a new taxon.

Material and Methods

Specimen collection

The type specimen was collected by Zachary Pezzillo, with Hank Oppenheimer, as part of the Plant Extinction Prevention Program, with automatic State permission to collect in the State Natural Areas on West Maui.

Field photography

Specimens collected on the flanks of Mauna ʻEʻeka were photographed *in situ* by Pezzillo, using a Canon MP-E 65mm f/2.8 1-5x Macro lens which was attached to a Canon EOS R5 camera. Adequate lighting was obtained by using a Canon MR-14EX II Macro Ring Lite which fired at 1/4 power. The exposure was set to 1/250 of a second; the aperture was set to *f*/7.1 to obtain the sharpest possible image; the ISO was at 250.

Light Microscopy

Specimens were studied in Hawaiʻi with an Olympus SZ51 dissecting scope with an AmScope LED-144B-ZK 144 PCS adjustable LED ring light, and with an AmScope B490B compound binocular microscope. Plants were photographed with an Olympus Tough TG-6 digital camera through ocular lenses of compound and dissecting microscopes.

In Stevens Point, Wisconsin, spores mounted in Brite floor cleaner (S.C. Johnson©) were studied with immersion oil (NA 1.5150) and photographed with a Leica microscope equipped with an ICC50W integrated digital camera. Six to seven through-focus images were captured of each subject and Z-stacked into a single in-focus image with Helicon Focus software (ver. 7.5.8).

Specimens were studied at Canberra, Australia, with a Leica DMLS compound microscope and a Leica M60 dissecting microscope with a LED ring light. Whole plants were photographed with a Flexacam C1 camera and plant structures were photographed using a Flexacam C3 camera. Line drawings were made using drawing tubes attached to both microscopes.

Scanning Electron Microscopy

In Stevens Point, Wisconsin, spores were mounted on aluminum stubs with a thin layer of adhesive from a Quick-Coat "G" adhesive pen (Ted Pella, p/n 22310), then sputter-coated with 5 nm of 60:40 gold-palladium and imaged on a Hitachi S3400-IIN SEM in secondary-electron mode at an accelerating voltage of 5 kV. An SEM survey of stub-mounted spores was followed by a "rip and flip" technique, to study the reverse side of the same spores to be certain that the proximal disk is present in all spores.

Molecular Study

In Edinburgh, Scotland, total DNA was extracted from a single clean silica gel-dried thallus from the *Pezzillo 342* collection, using the Qiagen DNeasy® plant mini kit, following the manufacturer's instructions, with a 50 µl final elute. Quantification of genomic DNA using the Qubit High Sensitivity dsDNA assay kit gave a concentration of only 0.14 ng/µl, so PCR amplification was attempted for plastid loci *rbc*L, *psb*A-*trn*H, *atp*β, *rps*4, *rpo*C1 and *trn*L-*trn*F, and nuclear loci 26S and ITS2 (for primer and PCR details, see Forrest & Crandall-Stotler (2005) [*atp*β, *rbc*L, *rps*4, *trn*L-*trn*F, 26S], Cargill *et al.* (2017) [*rpo*C1] and Forrest & Hart (2023) [ITS2, *psb*A-*trn*H]). The PCR products were visualised on a 1% agarose Tris/borate/EDTA gel stained with Invitrogen SYBR™ Safe DNA Gel Stain and cleaned using Applied Biosystems ExoSAP-IT™ PCR Product Cleanup Reagent, before cycle sequencing using

Applied Biosystems BigDye™ Terminator v1.1 Cycle Sequencing Kit, diluted with MCLAB BDX64 dye enhancing buffer. Sequencing reactions were sent to the core DNA sequencing and fragment analysis facility at the University of Dundee, to be cleaned and run on a 3730 DNA Analyzer (Applied Biosystems ®). Bidirectional reads were combined and manually edited in Sequencher 5.4.6 ™ software (Gene Codes Corporation). An NCBI BLASTn search was used to identify the closest matches on GenBank. Sequences were also compared to in-house RBGE DNA barcode reference sequences for the simple thalloid liverwort genera *Sewardiella* Kashyap and *Sandeothallus* R.M.Schust., as these are not present in the NCBI database. To estimate phylogenetic placement, the dataset from Forrest *et al.* (2006) (comprising four of the sampled loci: *rbc*L, *rps*4, *psb*A, 26S) was edited to remove *nad*5 (which failed to amplify for the unknown species so could not be included), and to cut down outgroup sampling and sampling from within the complex thalloid and leafy liverwort lineages, leaving a total of 38 liverwort taxa; sequence data for the Hawaiian plant was then added to the dataset. A likelihood analysis was run using RAxML BlackBox (Kozlov *et al.* 2019), with 1000 rapid bootstrap inferences and a maximum likelihood (ML) search; maximum parsimony analyses were also run using PAUP* 4.0a169 (Swofford 2002) using a heuristic search strategy. Trees were rooted on Haplomitriopsida Stotl. & Crand.-Stotl. (*Haplomitrium* Nees and *Treubia* K.I.Goebel). Sequences for DNA plant barcode loci *rbc*L, *psb*A-*trn*H, *rpo*C1, *trn*L-*trn*F and ITS2 were deposited on the Barcode of Life database, BOLD http://www.boldsystems.org/; all sequence data was also deposited on the NCBI database GenBank under accession numbers 26S OR842236; ITS2 OR831214; *atp*β OR828561; *rbc*L OR828559; *rps*4 OR828562; *rpo*C1 OR828559; *psb*A-*trn*H OR828563; *trn*L-*trn*F OR828564.

Results and Discussion

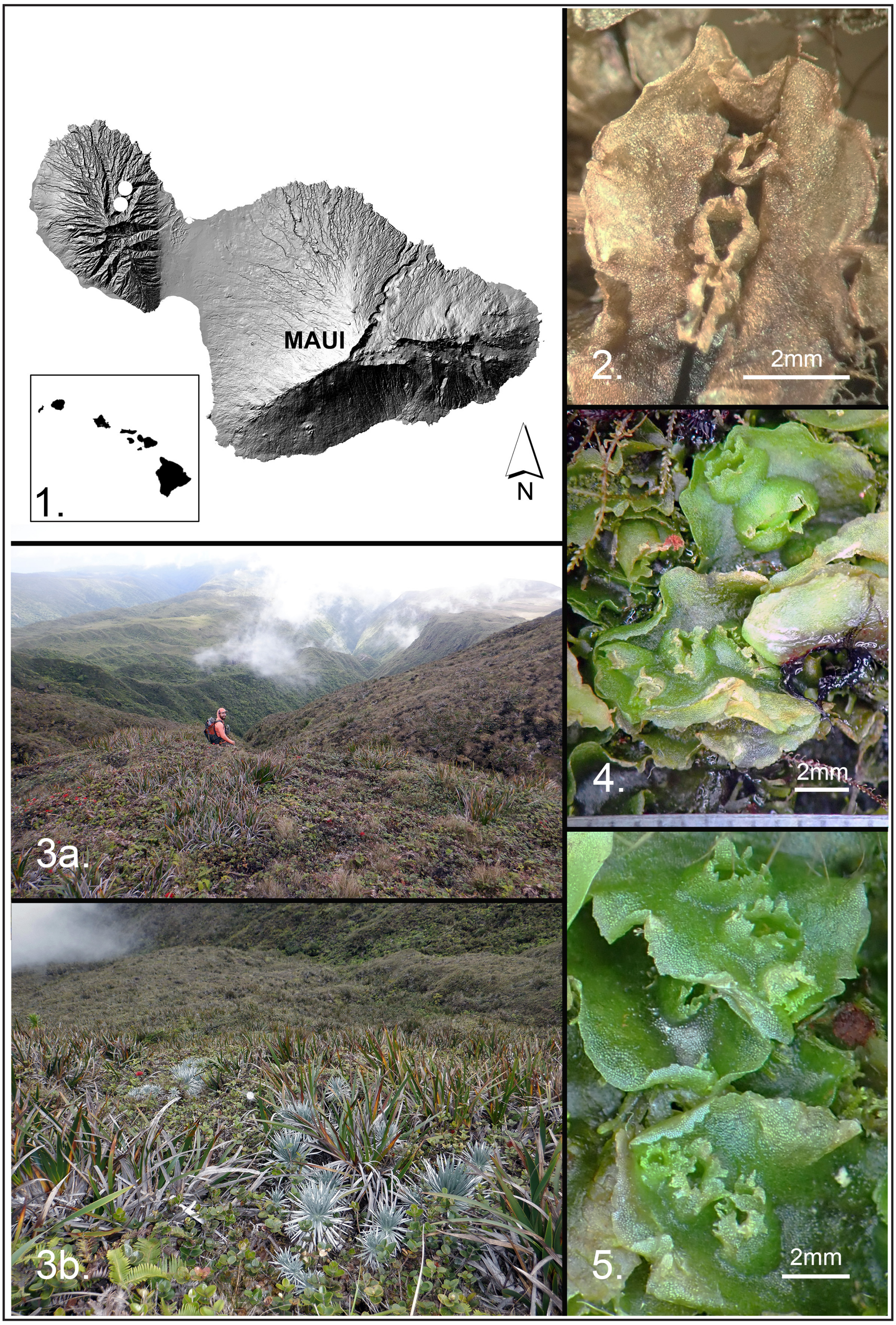
The following taxonomic analysis is based on the classification systems of Crandall-Stotler *et al*. (2009) and Söderström *et al*. (2016).

The Hawaiian plant (Figures 2, 4−54) has a simple thallus (without air chambers or tissue differentiation), monomorphic rhizoids (without pegged rhizoids), 2−3 layered capsule walls and lacks archegoniophores, therefore placing it in class Jungermanniopsida Stotl. & Crand.-Stotl. There are two subclasses of simple thalloid liverworts within this class: the Metzgeriidae Barthol.-Began and Pelliidae He-Nygrén, Juslén, Ahonen, Glenny & Piippo. Since antheridia and archegonia occur on the dorsal surface of the thallus of the respective sexes rather than on lateral or ventral abbreviated branches as in Metzgeriidae, we place the Hawaiian plant in the Pelliidae. Members of this subclass are classified into three orders: Fossombroniales Schljakov, Pallaviciniales W.Frey & M.Stech and Pelliales He-Nygrén, Juslén, Ahonen, Glenny & Piippo.

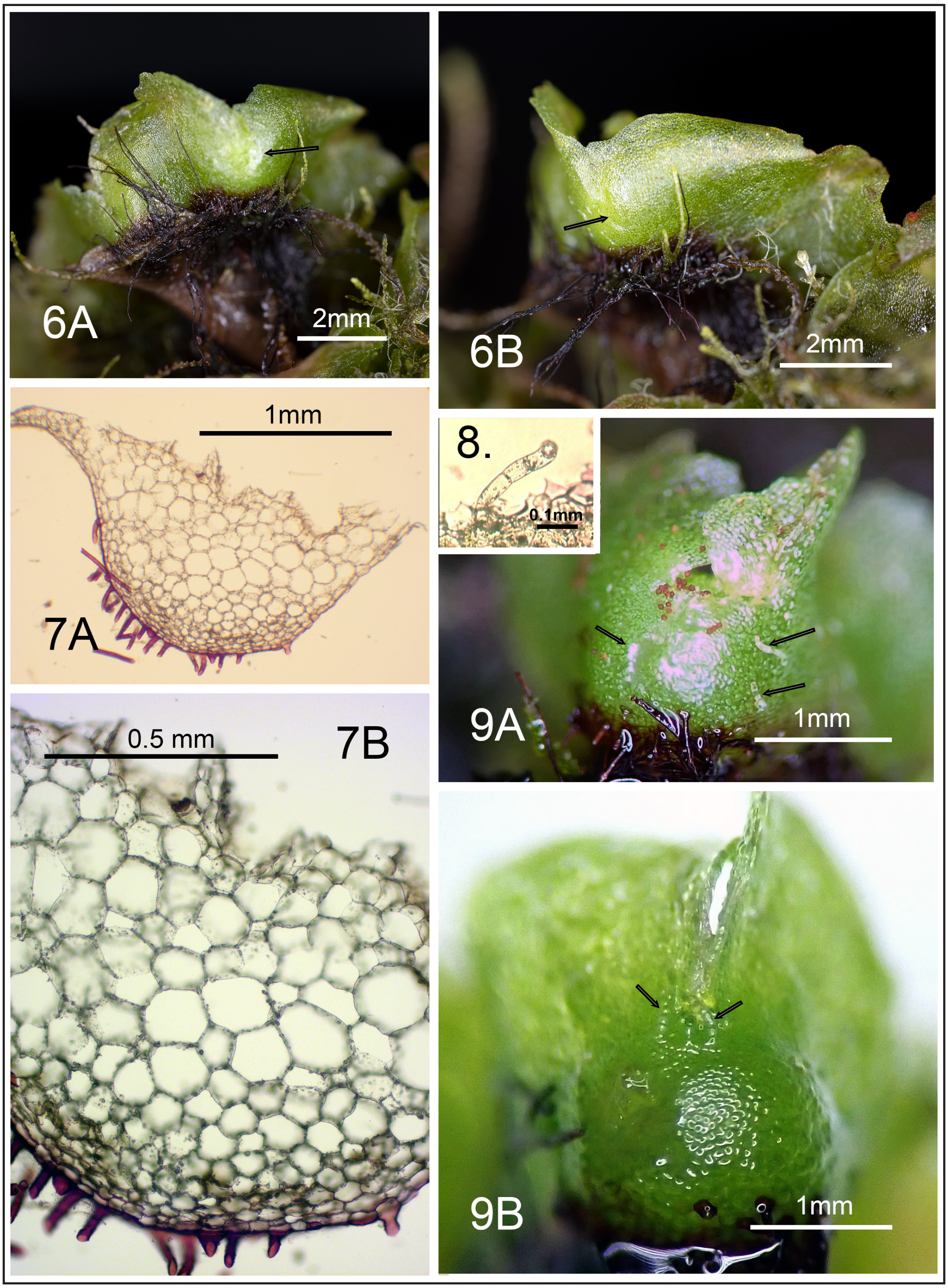
The Hawaiian plant lacks a central thallus strand (Figures 7a, 7b, 54) and has spherical capsules that open irregularly by several plates (Figures 18, 19, 22, 28, 30), and therefore is not congruent with placement in Pallaviciniales, which typically has a thallus midrib with a central strand, and elongate, 2−4-valved capsules. Neither does the Hawaiian plant appear to be congruent with placement in Pelliales, which has 4-valved capsules and a basal elaterophore. Instead, the reddish-purple rhizoids (Figures 10, 11, 12 and 13), the absence of a central vascular strand, and the spherical capsules with irregular dehiscence place the Hawaiian plant in Fossombroniales.

Söderström *et al*. (2016) circumscribed the order Fossombroniales to include five families (six genera) in two suborders: the monotypic Calyculariineae He-Nygrén, Juslén, Ahonen, Glenny & Piippo which includes the Calyculariaceae He-Nygrén, Juslén, Ahonen, Glenny & Piippo with the genus *Calycularia* Mitt. and Fossombroniineae R.M.Schust. which includes the monotypic Makinoaceae Nakai with the genus *Makinoa* Miyake; the monotypic Allisoniaceae Schljakov with the genus *Allisonia* Herzog; the monotypic Fossombroniaceae Hazsl. with the genus *Fossombronia* Raddi, and the Petalophyllaceae Stotl. & Crand-Stotl. with the genera *Petalophyllum* Nees & Gottsche ex Lehm. and *Sewardiella* (Figures 55–60).

Placement of the Hawaiian plant in the Fossombroniales is confirmed by the maximum likelihood (Figure 61) and maximum parsimony (not shown) analyses, which both showed it resolving with other members of the Fossombroniineae. Relationships within the order are not well resolved, however, with the Hawaiian plant sister to a poorly supported clade containing *Makinoa*, *Allisonia*, *Petalophyllum* and *Fossombronia*, while the position of *Calycularia* is also unsupported, sister to a lineage containing the Fossombroniineae and Pallaviciniales. Phylogenetic relationships within the Pelliidae remain to be confirmed using high throughput sequencing methods combined with reduced representation techniques, for example with the GoFlag 451 flagellate land plant probe set (Breinholt *et al.* 2021) as used in Bechteler *et al*. (2023).



FIGURES 1–5. 1. Locations of *Kahakuloa operculispora* in West Maui, found only in two bogs on Mauna ʻEʻeka in the Kahakuloa Stream watershed in the West Maui Natural Area Reserve – Kahakuloa Section. Map prepared by H. Oppenheimer. **2.** The Derral R. Herbst collections (*6609, 6609.*1, BISH), made in 1980 from Keahikauō Bog, 1.5 km from ʻEke Bog. The plants have large involucres in a uniseriate dorsal row and long purple rhizoids, as in *Pezzillo 342*. **3a.** Collector Zach Pezzillo in bog habitat of plants, West Maui Natural Area Reserve, Kahakuloa Section, el. 1,280 m. **3b.** Bog habitat; associates visible include *Metrosideros, Machaerina, Dicranopteris,* and *Argyroxiphium* species. **4.** Female plants with thick, semi-erect to erect wings and several uniseriate involucres. Note shortly emergent capsule (above). **5.** Female plants with several involucres sequentially produced in a single dorsal row. The involucres lack subtending scales and are barrel-shaped with a broad base and narrower mouth with laciniate lobes. Photographs: 2 by A.V. Freire of dried plant of *Herbst 6609* (BISH); 3a–3b by H. Oppenheimer; 4–5 by A.V. Freire of *Pezzillo 342* (BISH).



FIGURES 6–9. 6a, 6b. Thalli are thick with a prominent midrib that is rounded and fleshy at the apex (arrows). The margins of the thallus wings are unistratose and lighter in color to hyaline. The purple rhizoids have a complex association with other liverworts, cyanobacteria, fungi, and algae. **7a, 7b.** Transverse section through a male thallus showing the lack of a central vascular strand. In 7a note the multistratose thallus wings tapering towards the margin. In 7b note the sunken antheridial chambers subtended by scales (above) and the strata of smaller cells below. **8.** Filamentous hair with slime papilla at the tip of a 4-celled stalk, on the ventral side of thallus. **9a, 9b.** Frontal view of thallus. Slime papillae are present laterally and sub-apically, below the thallus wings and above the rhizoids (arrows). Photographs: 6a, 6b "*in situ*" by Z. Pezzillo *342* (BISH); 7a, 7b by D.C. Cargill of *Pezillo 342* (CANB); 8, 9a and 9b by A.V. Freire of *Pezzillo 342* (BISH).

Below, we compare the Hawaiian plant (Figures 2, 4–54) with all genera of Fossombroniales (Figures 55–60), with special attention to major morphological similarities and differences to help clarify its position. While the plant is distinct from all other genera in unique spore characteristics (Figures 33–46), those will be discussed in detail after the description of the species. We also briefly discuss the results of NCBI GenBank matches of the DNA sequence reads using the BLASTn algorithm, with particular focus on members of the Fossombroniales.

Calycularia (Calyculariineae, Calyculariaceae) (Figure 60) contains two species, *C. crispula* Mitt. from Mexico, Central America, East Africa and Tropical Asia, and *C. laxa* Lindb. & Arnell from Alaska, western Canada, and Eurasia (Konstantinova & Mamontov 2010, Daniels *et al*. 2014, Printarakul *et al*. 2020, Lee & Gradstein 2021). The morphological characters for *Calycularia* listed below are taken from Crandall-Stotler *et al*. (2009) and the papers cited above. *Calycularia* shares with the Hawaiian plant a broad midrib that projects ventrally and tapers gradually towards the wing margins; both are dioicous, with female plants more robust than the males; both have slime papillae; antheridia in dorsal rows covered by scales; shoot calyptrae; and short setae subtending non-valvate, spherical capsules that dehisce into irregular segments. Unlike *Calycularia*, the Hawaiian plant lacks ventral scales on the female thallus and the slime papillae are at the apex and on the sides of the ventral portion of the midrib (Figures 8–11), rather than on ventral scale margins as in *Calycularia*; has dark reddish-purple rather than yellowish, brown, or colorless rhizoids (Figures 10–13); has antheridia sunken in thallus chambers, rather than superficial (Figures 7, 21–24, 50–53); and has clustered archegonia without scales but enclosed within involucres (Figures 15, 16), the latter structure not being present in *Calycularia*. The Hawaiian plant lacks a pseudoperianth (formed after fertilization) that is present in *Calycularia*. Finally, the spores of the Hawaiian plant are areolate (Figures 34–38 and 40–46) rather than echinate or with long-cylindrical projections. *Calycularia* is not a close match to the Hawaiian plant in BLASTn searches for the sampled molecular markers (Table 1).

TABLE 1. Sequence similarity of DNA sequence reads from the Hawaiian plant to genera of the Fossombroniales, based on BLASTn searches of the NCBI database, GenBank, on 6th July 2023; the *Sewardiella* data is based on sequences generated from a 2013 collection by J.G. Duckett.

Locus	***Calycularia***	***Makinoa***	***Fossombronia***	***Petalophyllum***	***Sewardiella* (match to RBGE)**	***Allisonia***
26S	992/1025 bases; 97%	996/1025 bases; 97%	1000/1033 bases, 97%	992/1023 bases; 97%	not present on GenBank	996/1023 bases; 97%
***atp*β**	989/1140 bases; 87%	975/1138 bases; 86%	980/1148 bases; 85%	981/1136 bases; 86%	not present on GenBank	998/1138 bases; 88%
ITS2	no significant match	not present on GenBank	354/426 bases; 83%	349/430 bases; 81%	385/459 bases; 84% match	349/420 bases; 83%
***psb*A-*trn*H**	585/707 bases; 83%	466/513 bases; 91%	475/516 bases; 92%	483/515 bases; 94%	578/682 bases; 85% match	604/707 bases; 85%
***rbc*L**	1008/1127 bases; 89%	1001/1130 bases; 89%	1010/1130 bases; 89%	993/1130 bases; 88%	not present on GenBank	1014/1131 bases; 90%
***rpo*C1**	not present on GenBank	not present on GenBank	654/788 bases; 83%	not present on GenBank	not present on GenBank	not present on GenBank
***rps*4**	456/556 bases; 82%	428/517 bases; 83%	405/499 bases; 81%	449/562 bases; 80%	not present on GenBank	454/555 bases; 82%
***trn*L-*trn*F**	310/435 bases; 71%	309/427 bases; 72%	321/449 bases; 71%	431/620 bases; 70%	not present on GenBank	317/430 bases; 74%

Makinoa (Fossombroniineae, Makinoaceae) (Figure 59) is known from a single species, *M. crispata* (Steph.) Miyake, found in eastern temperate and tropical Asia, and Papua New Guinea (Miyake 1899, Crandall-Stotler *et al*. 2009). Morphological characters are taken from Miyake (1899), Schuster (1992), Crandall-Stotler *et al*. (2009), and Choi *et al*. (2023). *Makinoa* shares with the Hawaiian plant uniseriate ventral hairs tipped with slime papillae; colored rhizoids (although in *Makinoa* they are reddish-brown rather than reddish-purple); sunken antheridia (but in *Makinoa* they lack scales and are clustered and protected by a flap of thallus tissue, rather than scattered along the dorsal midrib and subtended by scales); and archegonia in small dorsal clusters protected by a well-developed involucre (but the involucre in *Makinoa* is only a flap). Unlike *Makinoa*, the Hawaiian plant is dioicous, bright green rather than bluish green, has a conspicuous midrib, a shoot calyptra rather than a coelocaule (a calyptra that is thick and fleshy throughout), and a spherical rather than cylindrical capsule that dehisces irregularly rather than by a single slit. *Makinoa* is not a close match to the Hawaiian plant in BLASTn searches for the sampled molecular markers (Table 1). The maximum likelihood tree (Figure 61) places the Hawaiian plant in the same clade as *Makinoa crispata* although the relationship lacks bootstrap support.

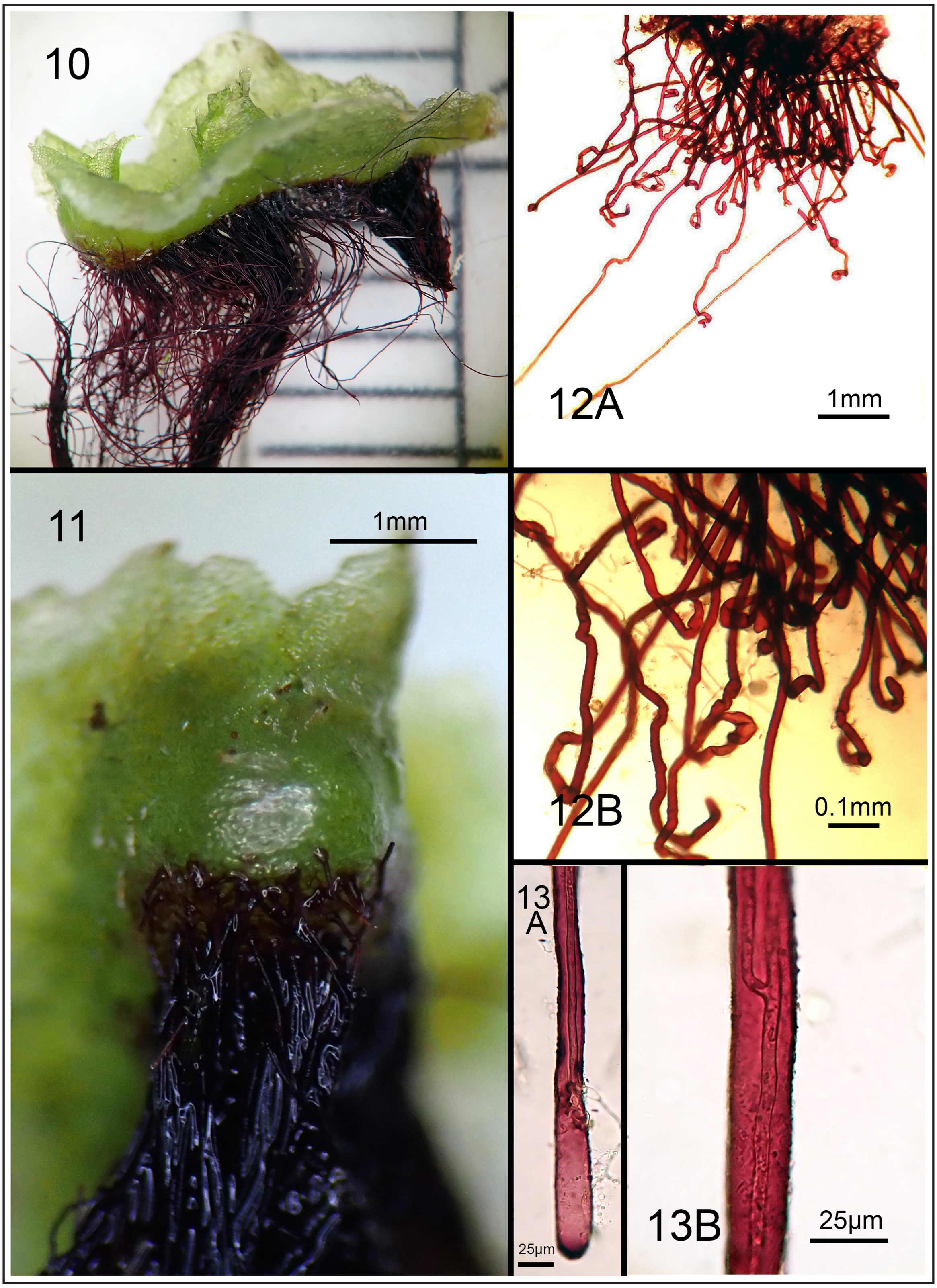
Fossombronia (Fossombroniineae, Fossombroniaceae) (Figure 58), a cosmopolitan genus of over 60 species (Cargill 2001, Freire 2002, Crandall-Stotler *et al*. 2009, Söderström *et al*. 2016), and the Hawaiian plant have in common reddish-purple rhizoids, shoot calyptras, and spherical capsules with irregular dehiscence (although the capsules of Hawaiian plants dehisce in long rectangular plates). Unlike *Fossombronia*, the Hawaiian plant is not a leafy-like segmented thallus; has sunken antheridia rather than on the thallus surface; has clustered archegonia enclosed by involucres that develop before fertilization, rather than naked and scattered on the dorsal surface without involucres; and does not have a caulocalyx that develops after fertilization. *Fossombronia* is the only other genus in the Fossombroniales known to occur in Hawaiʻi; Krayesky *et al.* (2005) cites specimens of *F. pusilla* (L.) Nees from Maui, and the first two authors have examined herbarium specimens of that species from Kauaʻi, and live plants on Maui and Hawaiʻi Island. *Fossombronia* is not a close match to the Hawaiian plant in BLASTn searches for the sampled molecular markers (Table 1).

Petalophyllum (Fossombroniineae, Petalophyllaceae) (Figure 57) is a genus of several species with a scattered distribution in the Mediterranean and Atlantic region of Europe, southeastern United States, southern Australia, and Aoteoroa/New Zealand. Morphological characters of *Petalophyllum* are taken from Crandall-Stotler *et al*. (2002), Crandall-Stotler *et al*. (2009), and Stotler & Crandall-Stotler (2017). *Petalophyllum* and the Hawaiian plant are both dioicous and heterothallic, with male plants being somewhat smaller than female plants; moreover, both have gametangia dispersed on the dorsal midrib; archegonia clustered and enclosed by a pre-fertilization structure (however, in *Petalophyllum*, this structure is underdeveloped and enlarges only after fertilization and is therefore a pseudoperianth); shoot calyptras (although these are described as “thin” by Crandall-Stotler *et al.* (2002) and Crandall-Stotler (2017)); and spherical capsules of irregular dehiscence. Unlike the Hawaiian plant, *Petalophyllum* has dorsal lamellae, multicellular ventral scales, hyaline or brownish rather than reddish-purple rhizoids, superficial antheridia rather than sunken in thallus chambers, and archegonia with perichaetial scales. While *Petalophyllum* may develop geotropic tubers, these were not observed in the Hawaiian plants. *Petalophyllum* is not a close match to the Hawaiian plant in BLASTn searches for the sampled molecular markers (Table 1).

Sewardiella (Fossombroniineae, Petalophyllaceae) is a rare Indian endemic known from just one species, *S. tuberifera* Kashyap (Figure 55). Morphological characters of *Sewardiella* are taken from Cargill (2001), Kashyap (1915) and Pant *et al*. (2023). Both the Hawaiian plant and *Sewardiella* are dioicous, but in *Sewardiella* male and female plants are similar in size, and both have a thick midrib with multistratose wings that taper towards the thallus margin. Unlike the Hawaiian plant, *Sewardiella* has superficial rather than sunken antheridia, pseudoperianths, a true calyptra, and reddish ventral scales. While DNA sequence data for *Sewardiella* are not currently (September 22, 2023) included in the GenBank database, the Royal Botanic Gardens Edinburgh has sequence data for two DNA barcode markers, to which the sequence data for the Hawaiian plant was compared; neither are a close match to the Hawaiian plant (Table 1).

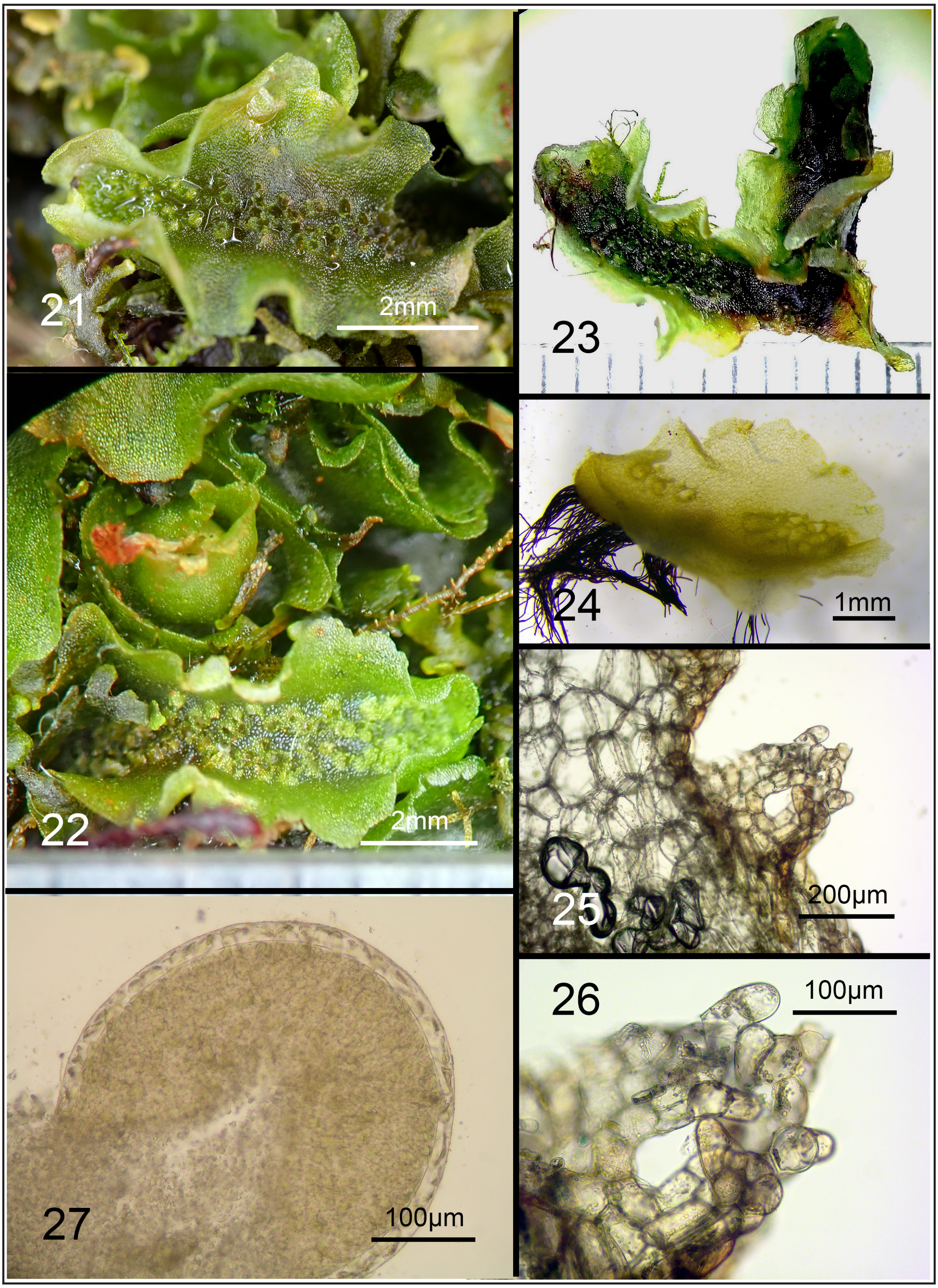
Finally, we compare the Hawaiian plant to the Aoteoroa/New Zealand endemic *Allisonia*, with the single species *A. cockaynei* (K.I.Goebel) R.M.Schust. (Figure 56). The morphological characters of *A. cockaynei* are taken principally from Schuster (1964) but also from Goebel (1906), Herzog (1941), Renzaglia (1982), and Crandall-Stotler *et al*. (2009). Both the Hawaiian plant and *A. cockaynei* have a prominent, ventrally projecting midrib with ventral hairs (slime papillae), elongate reddish-purple rhizoids, and spherical capsules that dehisce irregularly in long plates. They are both dioicous, but unlike the Hawaiian plant, *A. cockaynei* is homothallic rather than heterothallic; the mature ventral hairs are 3–4 cells rather than 6–8 cells long; the oil bodies are homogeneous, 1.8–2 × 4–6 µm and number 70–120 per cell, rather than granular to homogeneous, 4–6 µm in diameter and only 7–12 per cell; the antheridia are superficial rather than sunken in thallus chambers; the archegonia are in a single cluster enclosed by partially connate scales rather than in several clusters enclosed by solid, barrel-shaped involucres; a true calyptra is present, rather than a shoot calyptra; the capsule wall is 3–4(5) layers, rather than 2–3 layers thick; the setae are 20–30 mm rather than up to 5 mm long; and the spores are cristate and (40–)45–55 µm rather than areolate and (36–)37.5–41 µm in diameter, and have ridges 6–7 µm high rather than 1–2 µm high.

The genus *Allisonia* is one of the top matches using BLASTn for six out of the seven molecular markers with *A. cockaynei* sequence data available on GenBank (i.e., discounting *rpo*C1) (Table 2); however, the high levels of sequence divergence even in coding genes do not indicate a close taxonomic relationship between the taxa.

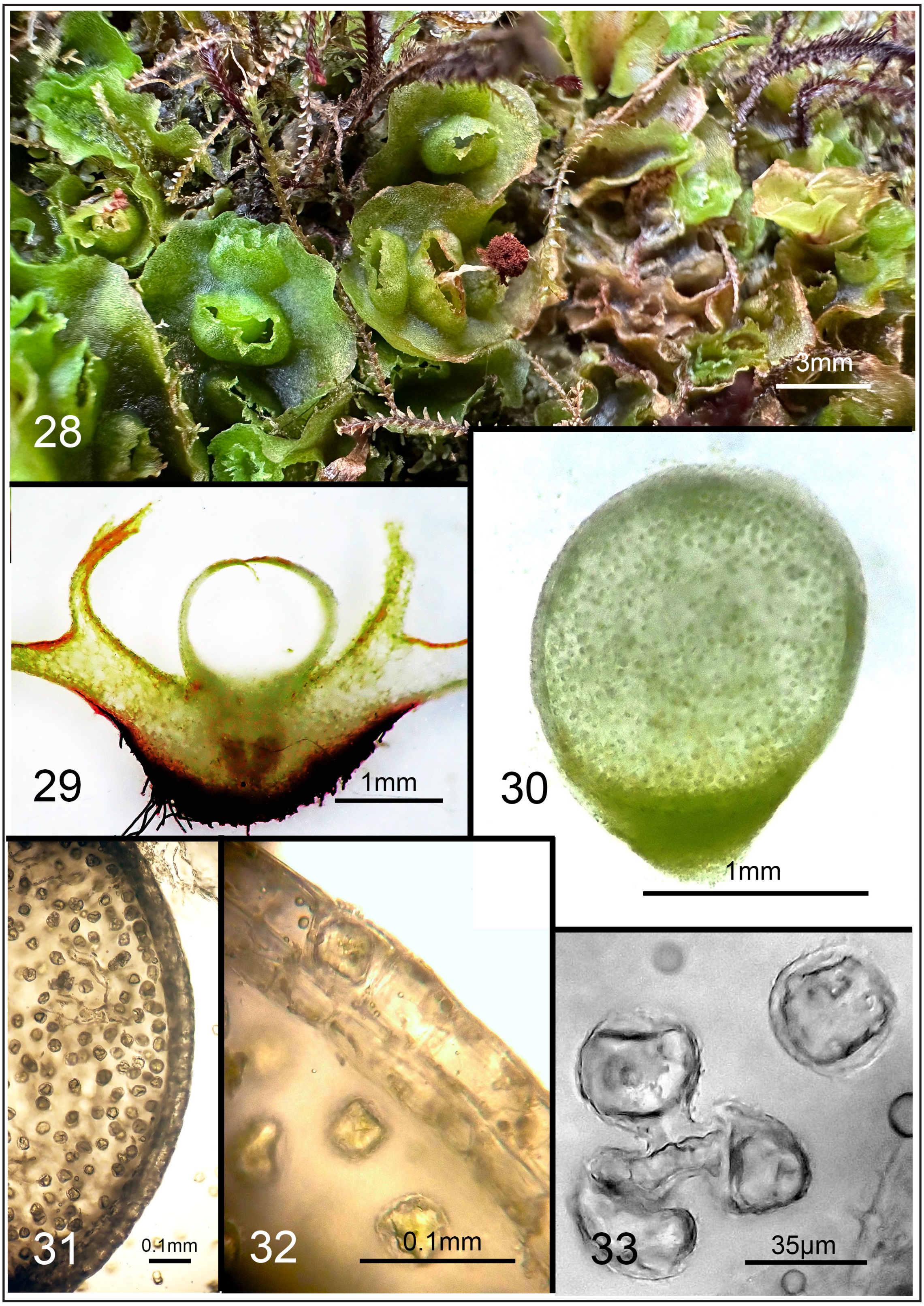


FIGURES 10−13. 10. Lateral view of female plant with a developing involucre at its tip (left; seen as a large, curved apical scale still open on the anterior end at this stage). A young, more developed and completely fused involucre is behind it, to the right. Note prominent reddish-purple rhizoids, up to 15 mm long (scale in mm). **11.** Plant frontal view. Note prominent dark purple rhizoids. **12a, 12b.** Elongate rhizoids; some are hooked and twisted, observed wrapping around soil particles, possibly to facilitate endophytic relations. In 12b. note the hooked and swollen rhizoid apices and the fungal hyphae associated with the rhizoids. **13a, 13b.** Rhizoids with endophytic fungus. In 13a. the hypha emerges from the rhizoid near its clavate apex. Presumably the fungal endophyte aids the plant with nutrient absorption. 13b. Branching hyphae growing within the rhizoid. Photographs by A.V. Freire of *Pezzillo 342* (BISH).

FIGURES 14−20. 14. Female plants are relatively robust and can be over 26 mm long and up to 9 mm wide. They frequently produce numerous involucres in a single row (uniseriate). Liverwort associates visible include *Bazzania* cf. *minuta*, *Kurzia mauiensis*, *Riccardia* sp., and *Symphyogyna* sp. (note male plant above center). **15, 16.** Involucres enclose small clusters of archegonia without perichaetial scales. **17.** Profile of involucre and a cross section of thallus with multistratose wings. The involucre is stout, with a laciniate mouth. Note the purplish pigmentation of the ventral side of the thallus midrib. **18.** Spherical immature capsule within involucre enclosed by a calyptra. **19.** Irregular capsule dehiscense. **20.** Orange-brown spores and elaters. Photographs 14−19 by A.V. Freire of *Pezzillo 342* (BISH). Photograph 20 taken "*in situ"* by Z. Pezzillo.



FIGURES 21–27. 21. Male plant with several rows of sunken antheridia and subtending scales. **22.** Female plant (above) with shortly emergent capsule; the pattern of capsule dehiscense is irregular; male plant (below) with dorsal antheridial scales. **23.** Male plants can be up to 16 mm long and up to 5 mm broad. Note its bifurcation and the sub-erect, undulate thallus wings. **24.** Young male plant with antheridia. **25, 26.** Cross section through male thallus showing the finger-like antheridial scales which surround the antheridial cavities. **27.** Antheridium with a single layer of jacket cells. Photographs: 21 taken "*in situ*" by Z. Pezzillo; 22, 23 by A.V. Freire of *Pezzillo 342* (BISH); 24–27 by D.C. Cargill of *Pezzillo 342* (CANB).



FIGURES 28–33. 28. Population with abundant community associates. The recently dehisced capsule (center) shows irregular dehiscence but still retains its spherical shape. Spores are brown rather than orange-brown as in other capsules. Note the immature spherical capsule within involucre (left). **29.** Cross section of fertile female plant. The innermost circular envelope is the shoot calyptra; note its thick, multistratose base that tapers to a single stratum above. The outer enclosing tissue on either side is the involucre. **30.** The capsule (excised from within the calyptra in Fig. 29) is spherical, with a 2–3 layered wall. The bottom denser portion is immature seta tissue. **31.** Detail of capsule with immature spores within. **32.** Detail of 2–3 layered capsule wall. **33.** The immature spores appear to push against each other to separate from the tetrad with long protrusions that extend from their proximal face. Photographs: 28 taken "*in situ*" by Z. Pezzillo; 29–33 by A.V. Freire of *Pezzillo 342* (BISH).

TABLE 2. The top three genus hits for DNA sequence reads from the Hawaiian plant to sequences deposited on the NCBI database, GenBank, based on BLASTn searches on 6th July 2023. ¥ The next hit in this series is *Megaceros flagellaris,* also at 91%. § This hit is to five separate accessions of *Ptilidium*.

Locus	BLASTn Hit 1	BLASTn Hit 2	BLASTn Hit 3
26S	*Allisonia* (996/1023 bases, 97%)	*Fossombronia* (968/995 bases, 97%)	*Makinoa* (996/1025 bases, 97%)
***atp*β**	*Allisonia* (919/1044 bases, 88%)	*Petalophyllum* (911/1044 bases, 87%)	*Calycularia* (911/1044 bases, 87%)
ITS2	*Allisonia* (349/420 bases; 83%)	*Fossombronia* (350/426 bases, 82%)	*Petalophyllum* (349/430 bases, 81%)
***psb*A-*trn*H**	*Petalophyllum* (483/515 bases, 94%)	*Fossombronia* (475/516 bases, 92%)	*Nothoceros* (468/512 bases, 91%) ¥
***rbc*L**	*Ptilidium* (937/1041 bases, 90%) §	*Allisonia* (1014/1131 bases, 90%)	*Calycularia* (1008/1127 bases, 89%)
***rpo*C1**	*Pellia* (674/785 bases, 86%)	*Moerckia* (672/786 bases, 85%)	*Porella* (660/785 bases, 84%)
***rps*4**	*Moerckia* (428/517 bases, 83%)	*Calycularia* (456/556 bases, 82%)	*Allisonia* (451/551 bases, 82%)
***trn*L-*trn*F**	*Allisonia* (317/430 bases; 74%)	*Calycularia* (310/435 bases, 71%)	*Fossombronia* (312/438 bases, 71%)

Although there are some similarities, as discussed above, between the Hawaiian plant and each of the genera in the Fossombroniales, there are also significant differences. The BLASTn analyses of the DNA sequences revealed no close matches to any taxa currently on GenBank. The top three genus matches for each marker are reported in Table 2 (i.e., only the first hit to each genus is reported). Where the genus match was only represented by a single GenBank accession, a reciprocal search was undertaken to rule out the possibility that the GenBank accession had been misidentified.

The fact that these closest matches based on genetic similarity are not all in the Fossombroniales, or indeed, not always simple thalloid liverworts (i.e. *psb*A-*trn*H, *rbc*L, *rpo*C1), suggests that the Hawaiian taxon is genetically distinctive from any other taxa that have been sequenced for those markers. None of the genera in Table 2 were present as community associates with the Hawaiian plant.

We conclude that both molecular and morphological evidence are sufficient to support the placement of the Hawaiian plant in its own genus and family.

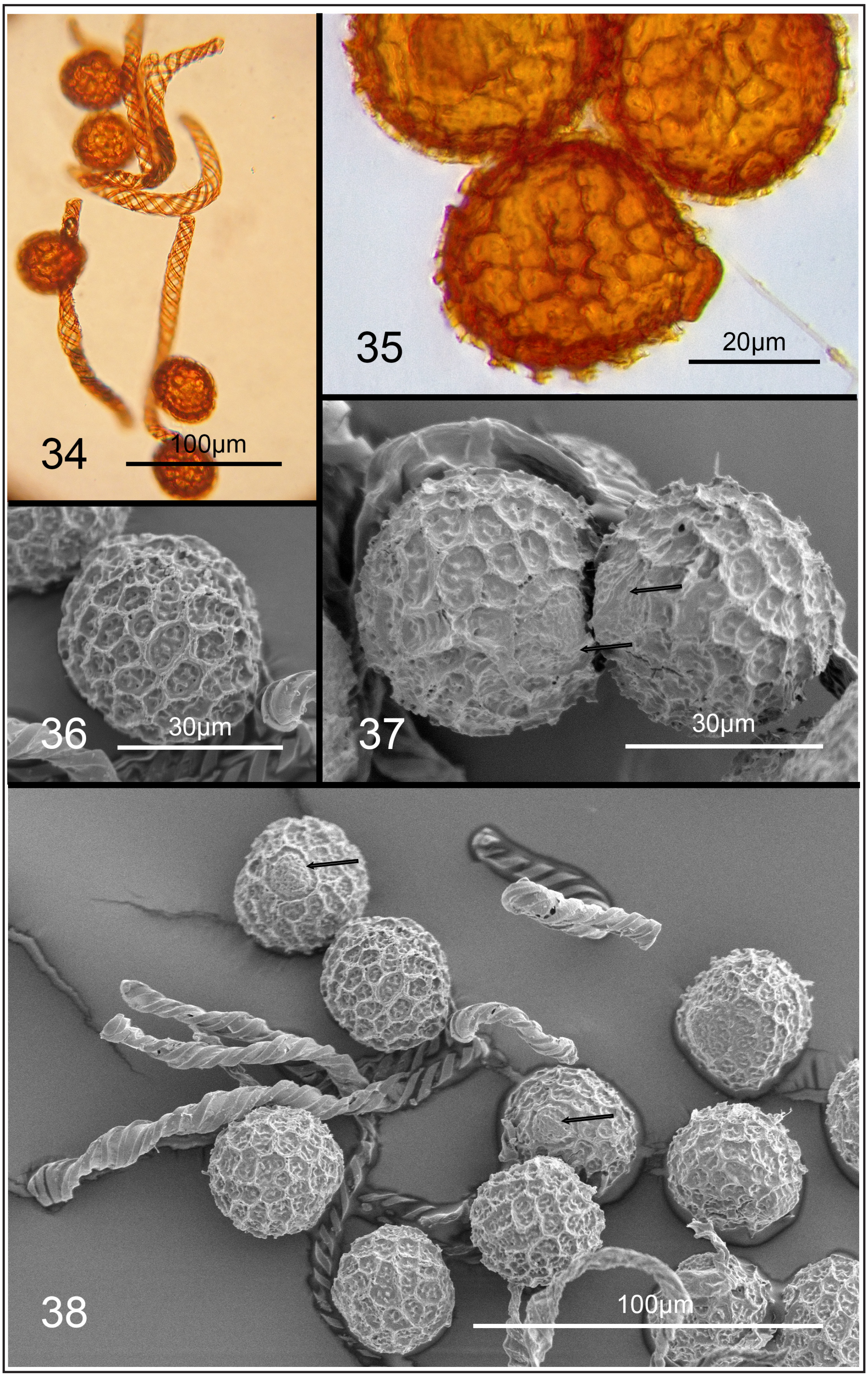
Taxonomic Treatment

We here describe a new endemic simple thalloid liverwort, known from only two bogs on Mauna ʻEʻeka in the Kahakuloa Stream watershed in the Kahakuloa Section of the West Maui Natural Area Reserve. Based on morphological and molecular evidence, we describe it as a new genus and publish the name *Kahakuloa operculispora*. The generic name derives from the region where it grows; it means "the tall lord" (Pukui & Elbert 1986). The species epithet alludes to its unusual spores that are unique in the Marchantiophyta and, as far as we can ascertain, within all bryophytes. We propose its phylogenetic placement in Jungermanniopsida, suborder Fossombroniineae, based on molecular evidence and morphological similarities.

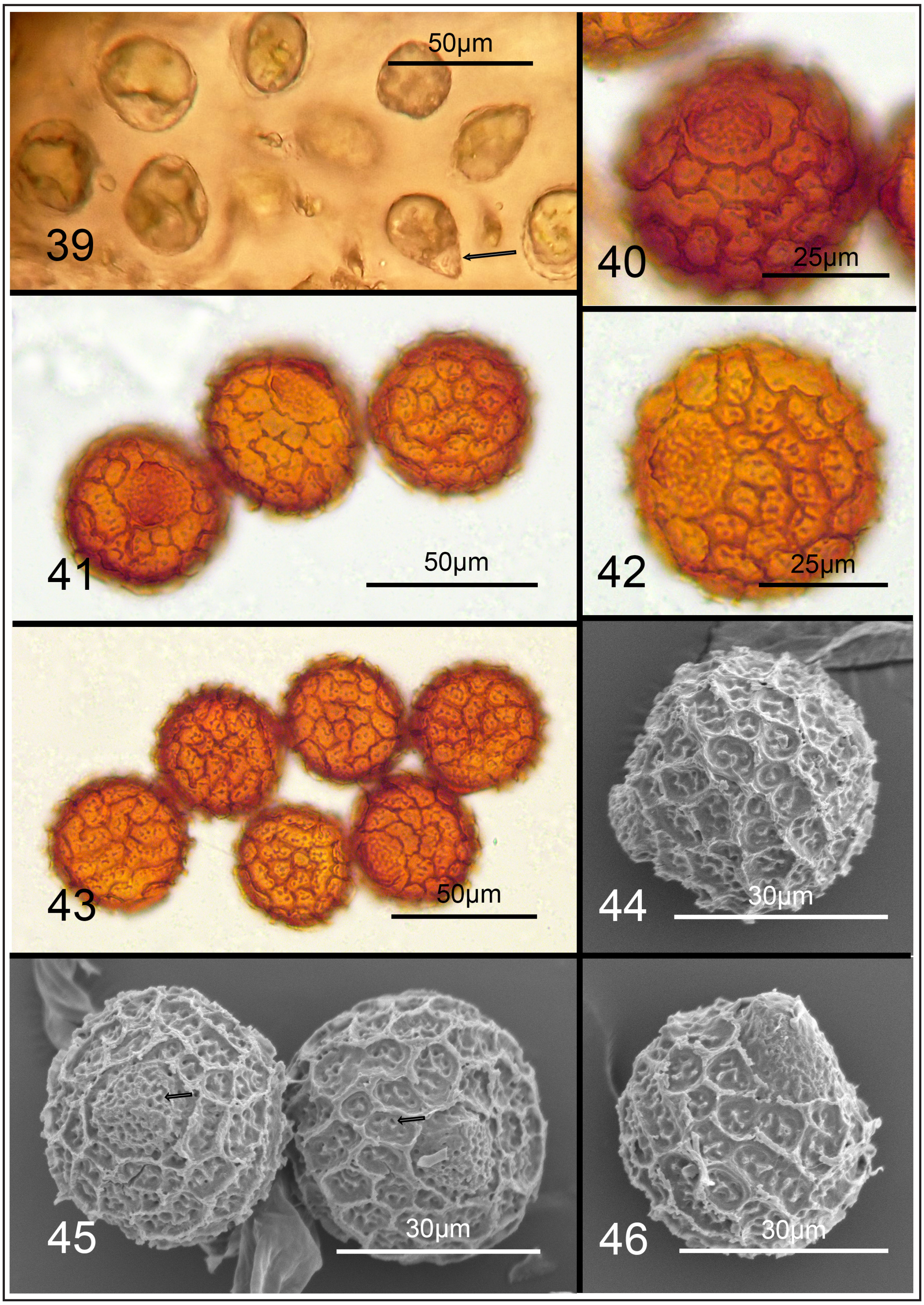
As the genus is monotypic, we provide a single species diagnosis and description here. This is sufficient to validly publish the genus and species names simultaneously. (Art. 38.5: Turland *et al.* 2018).

Kahakuloa operculispora A.V.Freire, Judz., Cargill, L.L.Forrest & Gradst. new genus and species **(Figures 2, 4–54)**

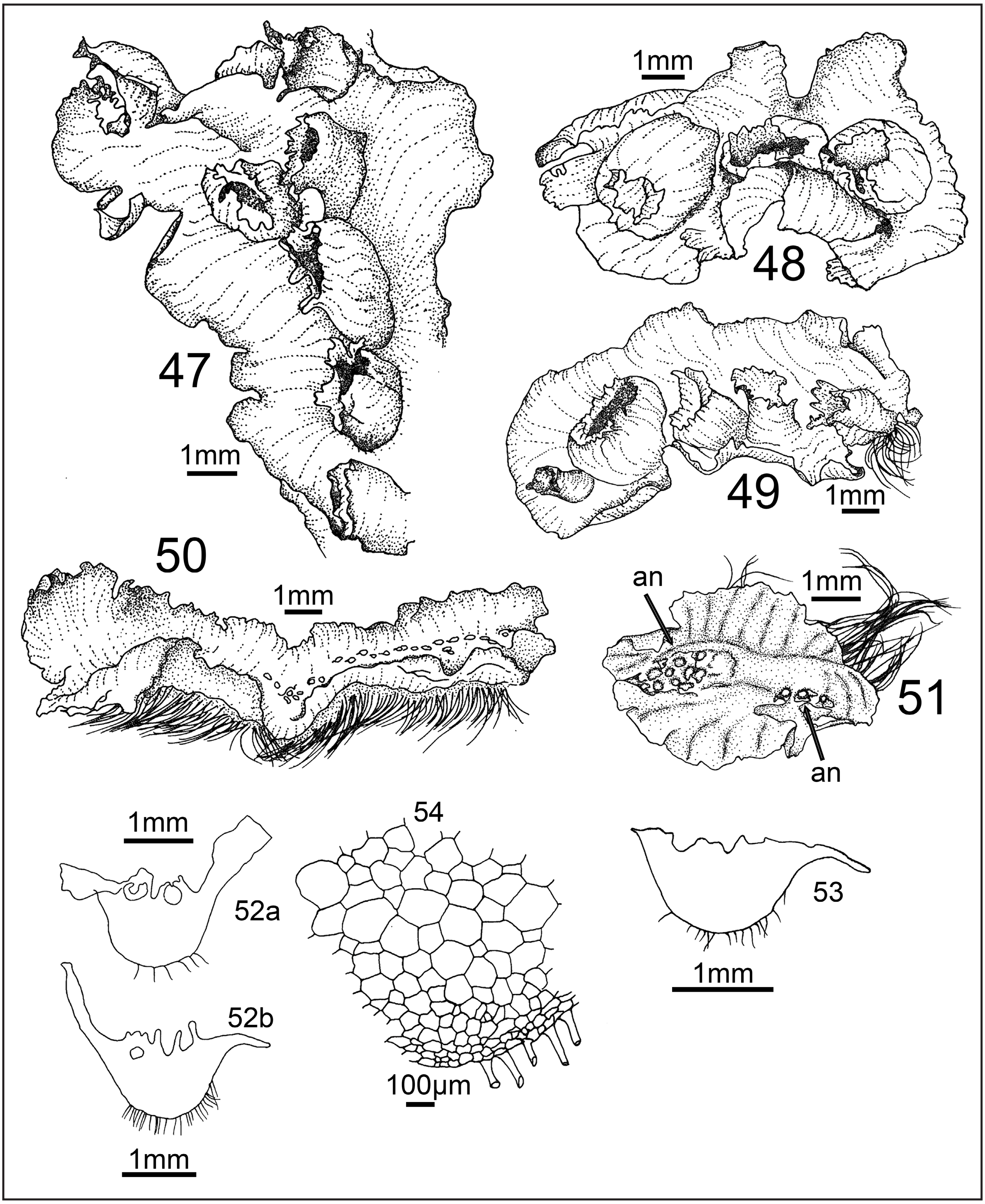
Diagnosis:—*Kahakuloa operculispora* differs from all other members of Fossombroniales in having numerous, apically developing (pre-fertilization), inflated, barrel-shaped involucres, each protecting a small cluster of archegonia; in having sunken antheridia in several rows of dorsal chambers protected by scales; and in having spores with a proximal operculum-like disk. The genus is morphologically most similar to *Allisonia* in its lack of ventral scales, presence of long, reddish-purple rhizoids, and ventral hairs tipped with slime papillae; however, *Allisonia* differs in having archegonia in a single cluster enclosed by partially connate scales, by having superficial antheridia, by having a true calyptra rather than a shoot calyptra; and by having cristate rather than areolate spores that lack an operculum-like disk.



FIGURES 34–38. 34. The spores are circular to slightly elliptical in top view; they are orange-brown and have a reticulate pattern of areolae; some areolae are incomplete. Elaters are robust and usually have three spiral thickenings. **35.** The lamellae (ridges) surrounding the areolae form a low wing on the spore periphery. The proximal disk protrudes in some spores (bottom). **36.** Distal view of a spore showing areolae and sub-lamellar ornamentation. **37.** Proximal face of spores, arrows point at ridges across the operculum-like disks. **38.** Spores and elaters; note the two spores in proximal view (indicated by arrows), showing the unique operculum-like disk, the bottom one with ridge across disk. Photographs: 34 by A.V. Freire of *Pezzillo 342* (BISH); 35 and 36–38 SEMs by S. Sepsenwol of *Pezzillo 342* (BISH).



FIGURES 39–46. 39. Immature spores separate early and asynchronously from their tetrad. Note the proximal protrusions of different lengths that emerge from the proximal disk, one of them indicated by the arrow at the bottom right. **40-42.** LM of spores showing the reticulate pattern of ornamentation and operculum-like disk on proximal face (visible in figures 40, bottom left; middle spores in 41; and figure 42). **43.** LM of distal view of spores, showing areolate reticulation with papillae and short crests on the areolar surface. Note sideway spore at bottom right, showing proximal disk. **44–46**. SEM images of spores showing the operculum-like disk at different stages of protrusion. **45.** Note the small “pinholes” on distal and proximal surfaces (indicated by arrows). On the spore to the right, the arrow points at one of three pinholes within one of the areolae. Photographs: 39 by A.V. Freire of *Pezzillo 342* (BISH); 40–43 by D.C. Cargill from *Pezzillo 342* (CANB); 44–46. SEMs by S. Sepsenwol of *Pezzillo 342* (BISH).



FIGURES 47–54. 47–49. Female plants with serial involucres of varied sizes. **47.** Female plant with bifurcate branching. Note the involucres at early stage of development at the apex of both branches and the paired involucres (side by side) at the point of bifurcation. **50, 51.** Male plants with dorsal, sunken antheridia protected by scales. **51.** Young male plant, note the gap between clusters of antheridia sometimes observed. **52–54.** Transverse sections through thalli. **52, 53.** Thick thallus midrib tapers towards wings. **52a, 52b.** Sections through male thalli showing antheridial chambers and scales. **53.** Early stages of antheridial chamber development. **54.** Detail of transverse section showing cells of reduced size on dorsal region and lack of central strand. Illustrations by D.C. Cargill. Final preparation of **51** for publication by Charlotte Nuessler.

FIGURES 55−60. Genera of Fossombroniales (excepting *Kahakuloa operculispora*). **55.** *Sewardiella tuberifera* Kashyap. **56.** *Allisonia cockaynei* female plants. **57.** *Petalophyllum preissii* Gottsche ex Lehm. **58.** *Fossombronia sp.* **59.** *Makinoa crispata.* **60.** *Calycularia crispula.* Photographs courtesy of: Sapana Pant (55); D.C. Cargill (56–58); Yu-Feng Yang (59); and Matt Goff (60).

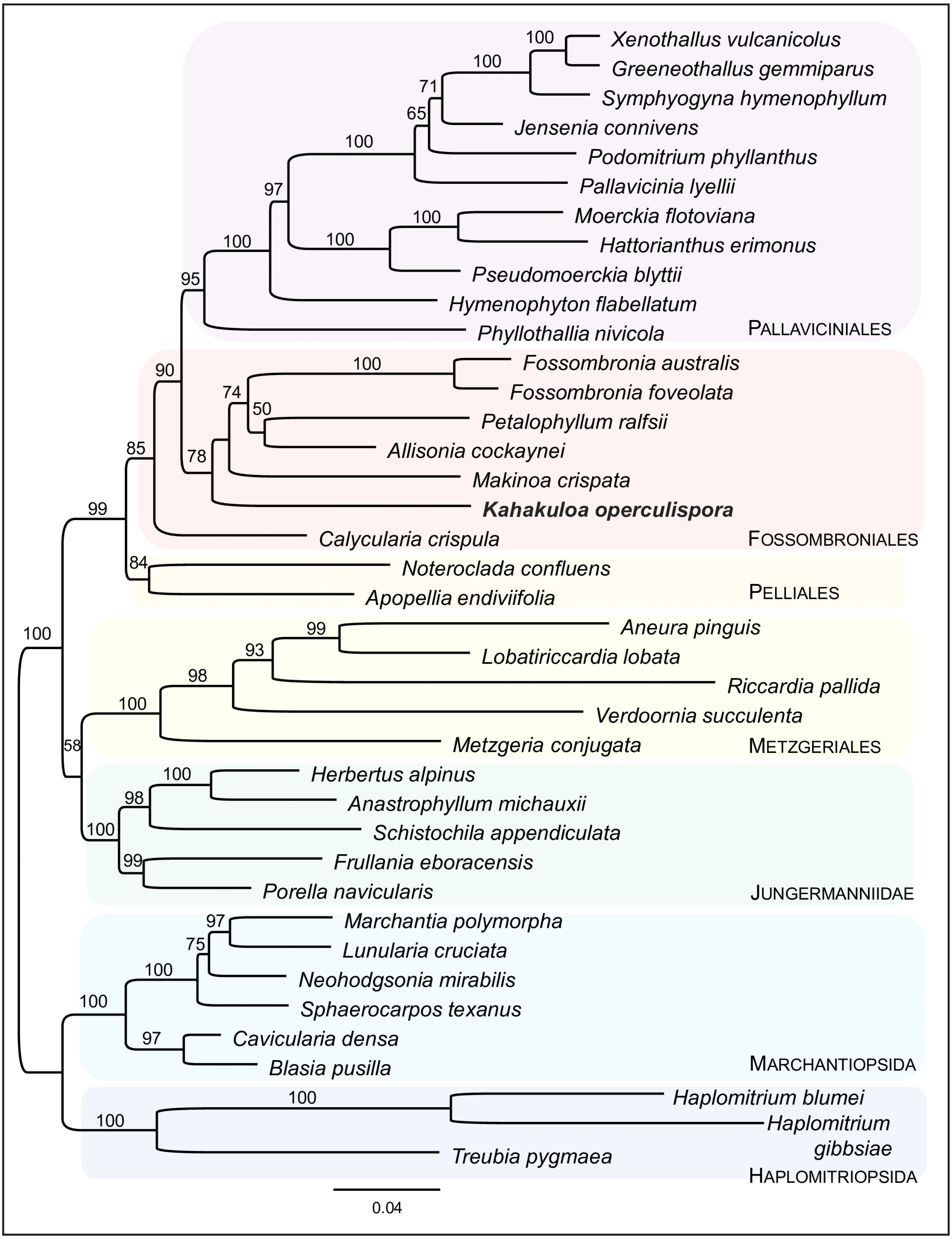


FIGURE 61. Maximum likelihood phylogram generated using RAxML from sequence data of *rbc*L, *rps*4, *atp*β, *psb*A-*trn*H, *trn*L-*trn*F and 26S, with likelihood bootstrap values above the branches. Sample voucher information and GenBank accession numbers as in Crandall-Stotler *et al.* (2005) and Forrest *et al.* (2006), with the exception of sequence data for the Hawaiian plant, which were newly generated for this study: 26S OR842236; *atp*β OR828561; *rbc*L OR828559; *rps*4 OR828562; *psb*A-*trn*H OR828563; *trn*L-*trn*F OR828564. Analysis by L.L. Forrest.

Type:—UNITED STATES: Hawai‘i: Maui, Mauna ‘E‘eka, West Maui Natural Area Reserve-Kahakuloa Section, eastern slopes of ‘E‘eka, NE aspect, bog region surrounding ‘Eke crater, rare in high elevation bog with *Metrosideros* sp., *Machaerina* sp., *Scaevola* sp., *Dicranopteris* sp., *Diplopterygium* sp., *Lobelia gloria-montis* Rock, *Sadleria pallida* Hook. & Arn., *Argyroxiphium caliginis* C.N.Forbes, *A. grayanum* O.Deg., *Dichanthelium cynodon* (Reichardt) C.A.Clark & Gould, *D. koolauense* (H.St. John & Hosaka) C.A.Clark & Gould, *D. isachnoides* (Munro ex Hillebrand) C.A.Clark & Gould, *D. hillebrandianum* (Hitchc.) C.A.Clark & Gould, *Myrsine vaccinioides* W.L.Wagner, D.R.Herbst & Sohmer, *Ilex* sp., *Viola maviensis* H.Mann, *Eurya* sp., *Schizaea robusta* Baker, *Plantago pachyphylla* A.Gray, *Oreobolus furcatus* H.Mann, and the liverworts *Bazzania* cf. *minuta* (Austin) A.Evans, *Cephalozia lucens* (A.Evans) Steph., *Herbertus* sp., *Kurzia mauiensis* (H.A.Mill.) H.A.Mill., *Leptoscyphus baldwinii* (Steph.) Judz. & A.V.Freire, *Riccardia* sp., and *Symphyogyna* sp., and mosses including *Leucobryum* sp., 4,200 ft. [1,280 m], 17 March 2023, *Z. Pezzillo 342* (holotype BISH!, isotypes BISH!, BR!, CANB!, F!, PTBG!). **Additional specimens examined (paratypes):** United States: Hawai‘i: Maui, Mauna ‘E‘eka, West Maui Natural Area Reserve-Kahakuloa Section, eastern slopes of ‘E‘eka, NE aspect, bog region surrounding ‘Eke crater, 4,200 ft. [1,280 m], 17 March 2023, *Z. Pezzillo 351* (BISH); Keahikauō bog, with *Bazzania* sp., *Cephalozia lucens*, and *Kurzia mauiensis*, 975 m, 10 Dec. 1980, *D. R. Herbst 6609* (BISH!)*, 6609.1* (BISH!).

Description:—Plants dioicous, terrestrial, bright green, prostrate, with erect to semi-erect, undulate wings with entire margins; plant apex fleshy and rounded; plants mostly unbranched, occasionally bifurcate; tubers absent; heterothallic, the male plants smaller than the female plants; not aromatic. **Midrib** (seen in both sexes) broad, fleshy, 15–25% of the width of the thallus, slightly concave to flat dorsally and convex ventrally, with scattered, uniseriate slime papillae (3–5)6–8 cells long, forming small clusters immediately below the apical region of the thallus and extending downwards and laterally, along both sides of the midrib, between the base of the thallus wings and above the rhizoid zone; midrib in transverse section up to 13 cells thick, strongly tinged with purple pigment in the ventral layers of cells, which are smaller than the dorsal layers of cells above; central strand absent; wing cells thin-walled, lacking trigones; wings multistratose but gradually tapering into a unistratose marginal region, the multistratose portion occupying about 80% of the wing, its cells rectangular-hexagonal, 135–175 µm long by 55–70 µm wide, the wing margin hyaline, unistratose, 4–6 cells wide, cells isodiametric, 55–65 µm in diameter, the border cells more globose and slightly inflated, tinged with yellow-orange with age. **Ventral scales** absent. **Oil bodies** 7–12 (in most epidermal and apparently in some subepidermal cells), heterogeneous, 7–13 µm in diameter, varying from granular, made up of many small droplets, to homogeneous, consisting of a single large spherical droplet, some showing an intermediate morphology of small droplets combined with one to few large droplets. **Rhizoids** abundant, often uncinate, often twisted around substrate particles, and slightly clavate at their apices, reddish-purple, restricted to the ventral side of the midrib, up to 15 mm long, ca. 50 µm wide at base, 20–25 µm wide along most of their length, often with evident endophytic fungal hyphae, forming part of a complex substrate that sustains a community of several liverworts, fungi, and algae. **Male plants** as small as 6 mm long at early stages of sexual expression, but 9–16 mm long when mature, (3.2–)4–5 mm wide (flattened), the midribs 1–1.5 mm wide. **Female plants** (3–)6–12 mm long at early stages of sexual expression but 18–26 mm long when mature, 5–9 mm wide (flattened), with midribs 1.5–2.5 mm wide. **Antheridia** dorsal, with a single layer of jacket cells, sunken in thallus chambers with raised ostioles, the chambers in several rows along the midrib and subtended by variously shaped scales; apical (immature) antheridia single or in groups of 2 or 3, subtended by triangular to irregularly shaped conspicuous scales, shallowly sunken in underdeveloped chambers; mature, sunken chambers (farther from the apex), each with just a single antheridium, often subtended by a very reduced yellow scale a few cells high, some with fingerlike lobes. **Archegonia** in small clusters along the dorsal surface of the midrib, naked, enclosed by an involucre that develops apically and matures before fertilization. **Involucres** in a single row of (1)2–16 per plant along the midrib, or occasionally extending to the dorsal lower portion of the wings on either side of the midrib, a few in small clusters at the apex when plants begin to bifurcate, 2.5–3.2 mm high, (0.8–1)1.5–2.2 mm wide at the mouth, 2–3.5 mm wide at the base, green, cylindrical to barrel-shaped, sometimes slightly flattened, thick, inflated, not subtended by scales, 4–5 cells thick at the base, for (5)9–10 tiers of cells, transitioning to only 3 cells thick for the next 3–7(8) tiers of cells, 2 cells thick for the next (5)8–9(10) tiers of cells, and finally a single cell layer thick in the apical 1–2 tiers of cells, the mouth laciniate-lobed, often hyaline; slime papillae sometimes present on the inner involucral surface. **Pseudoperianth** (defined here as a post-fertilization structure of thallus origin) absent. **Shoot calyptra** one (rarely two) per involucre, with 7–8 layers of cells at the base and gradually tapering to a bistratose and then a unistratose apical portion. **Sporophyte** (when present) one per plant. **Capsules** ca. 2 mm in diameter, spherical to slightly elliptical, dehiscing irregularly into large rectangular plates; capsule walls 2–3 layered, with semi-annular thickenings. **Seta** short, to ca. 5 mm long. **Spores** brown to orange-brown, spherical to slightly ellipsoidal, (33.5–)37.5–42.2 µm in diameter, the distal face reticulate, with (6–7)8–9 areolae across the spore diameter, 7–12 µm wide, isodiametric or elongate or irregularly-shaped, delimited by short, blunt lamellae 1−1.4 µm wide and 1–2 µm high, sinuous in profile; spore surface with low, irregular vermiculate ridges with occasional bulging projections or small crests, with smaller, perpendicular side ridges; small "pinhole" perforations are also present; proximal face with an operculum-like disk 10.5–12.5(–14) µm in diameter, surrounded by a groove 2–3 µm wide, sometimes with an "aperture-like" ridged slit across the disk; triradiate ridge and thumbprints absent; spores often with a protuberant bulge that elevates the proximal disk; immature spores with armlike proximal protuberances, these seemingly separating them from their sister tetrad spores. **Elaterophore** absent. **Elaters** free, 225–275 µm long, 10–15 µm wide, golden-brown, smooth, with three spiral thickening bands, often tapering to two towards the elater tips, or infrequently bispiraled throughout.

Familial placement

The Sanger sequence data generated for this study is sufficient to rule out the placement of *Kahakuloa* in any extant liverwort family. *Kahakuloa* is not only a new genus but also a new family for which we propose the name Kahakuloaceae. We recommend a more robust phylogenetic placement of this family in the liverwort tree of life using the hybrid capture loci selected in the National Science Foundation's GoFlag project "Building a comprehensive evolutionary history of flagellate plants" (Breinholt *et al.,* 2021), as in Bechteler *et al*. (2023).

Kahakuloaceae A.V.Freire, Judz., Cargill, L.L.Forrest & Gradst., new family

Diagnosis:—Differing from all other Fossombroniales in having numerous, inflated female involucres, and from all other Marchantiophyta in having spores with an operculum-like disk.

Description:—Plants thalloid, thallus wings multistratose, tapering to a unistratose margin; ventral uniseriate slime papillae present; ventral scales absent; rhizoids reddish-purple; antheridia sunken in dorsal thallus chambers in several rows, subtended by scales; archegonia in small clusters along the dorsal side of the midrib, lacking perichaetial scales, enclosed by a thick, globose or barrel-shaped involucre that develops apically, before fertilization; post-fertilization pseudoperianth absent; shoot calyptra present; capsule globose, dehiscing irregularly in long plates, walls 2–3 layered; spores areolate with a proximal operculum-like disk.

Type genus:—*Kahakuloa* A.V.Freire, Judz., Cargill, L.L.Forrest & Gradst.

Based on our observations of *Kahakuloa* and published descriptions of other genera of simple thalloid liverworts (e.g., Crandall-Stotler *et al.* 2009), we present alternate keys to the families and genera of the Fossombroniales, including the new Kahakuloaceae:

Key to the families and genera of Fossombroniales based on reproductive and vegetative characters

1. Spores with a proximal operculum-like disk; archegonia enclosed by large, barrel-shaped involucres (uniseriate, numerous, up to 16 per plant); antheridia sunken into thallus chambers; rhizoids reddish-purple, Hawai'i endemic ..... Kahakuloaceae (*Kahakuloa*)

\- Spores lacking a proximal operculum-like disk; archegonia not enclosed by large, barrel-shaped involucres; antheridia flush with thallus surface or sunken into thallus chambers; rhizoids hyaline, yellowish, reddish-purple, or brownish 2

2. Spores with elongate cylindrical to conical projections; thallus with ventral foliose scales bearing slime papillae on their margins; rhizoids hyaline, yellowish or brownish; elaterophores present; eastern and northern Asia, northwestern North America, Central America and eastern Africa .... Calyculariaceae (*Calycularia*)

\- Spores variously sculptured but not with elongate cylindrical to conical projections; thallus with ventral foliose scales without slime papillae on their margins, or with uniseriate hairs tipped with a slime papilla; rhizoids hyaline, reddish-purple or brownish; elaterophores absent or rudimentary 3

3. Capsules cylindrical, dehiscing along one slit; elaters 1-spiralled; plants monoicous; calyptra thick and fleshy throughout (a coelocaule); rhizoids reddish-brown; elaterophores rudimentary; tropical Asia and Papua New Guinea ... Makinoaceae (*Makinoa*)

\- Capsules spherical, dehiscing irregularly; elaters 2–4-spiralled; plants monoicous or dioicous; calyptra one cell layer thick throughout (a true calyptra) or multicellular at the base, tapering to 1–2 cells thick at the apex (a shoot calyptra); rhizoids hyaline to reddish-purple or brownish; without elaterophores; temperate to tropical 4

4. Thallus with leaf-like segments; rhizoids reddish-purple or hyaline; monoicous or dioicous; nearly cosmopolitan .... Fossombroniaceae (*Fossombronia*)

- Thallus lacking leaf-like segments; rhizoids hyaline to reddish-purple or brownish; dioicous 5

5. Ventral scales absent, uniseriate hairs tipped with a slime papilla present; plants lacking apical tubers; rhizoids reddish-purple; Aoteoroa/New Zealand endemic ....Allisoniaceae (*Allisonia*)

- Ventral scales present, uniseriate hairs tipped with a slime papilla absent; plants usually with prominent apical tubers; rhizoids hyaline to brownish 6

6. Thallus with dorsal lamellae; ventral scales hyaline; Mediterranean and in warm-temperate regions of Europe, southeastern U.S.A and Australasia..Petalophyllaceae (*Petalophyllum*)

- Thallus dorsal lamellae absent; ventral scales reddish; India endemic ....Petalophyllaceae (*Sewardiella*)

Key to the families and genera of Fossombroniales based only on vegetative characters

1. Thallus with leaf-like segments; rhizoids usually reddish-purple.. Fossombroniaceae (*Fossombronia*)

- Thallus lacking leaf-like segments; rhizoids reddish-purple, hyaline, yellowish, or brownish 2

2. Thallus wings with dorsal lamellae; ventral scales present..Petalophyllaceae (*Petalophyllum*)

- Thallus wings lacking dorsal lamellae; ventral scales present or absent...3

3. Rhizoids reddish-purple; ventral scales absent, ventral hairs tipped with a slime papilla present..4

- Rhizoids hyaline, yellowish, or brownish; ventral scales present or absent, ventral hairs tipped with a slime papilla present or absent 5

4. Hyaline marginal region of thallus wings broad, conspicuous, about 8 –12 cells wide, Aoteoroa/New Zealand ....Allisoniaceae (*Allisonia*)

- Hyaline marginal region of thallus wings narrow, inconspicuous, about 4–6 cells wide; Maui, Hawai‘i ....Kahakuloaceae (*Kahakuloa*)

5. Plants with apical tubers; ventral scales present, reddish...Petalophyllaceae (*Sewardiella*)

- Plants lacking tubers; ventral scales, if present, hyaline, purplish or red..6

6. Plants with foliose ventral scales bearing marginal filamentous hairs tipped with a slime papilla .... Calyculariaceae (*Calycularia*)

- Plants lacking foliose ventral scales (but ventral filamentous hairs tipped with a slime papilla present) .... Makinoaceae (*Makinoa*)

Notes on salient morphological features and phytogeography of *Kahakuloa operculispora*

Interpretation of the spore proximal disk

Based on our observations, we speculate that spores separate from their tetrad sisters early, while still immature, by producing an arm-like protuberance from their proximal disk area. The protuberance appears to push the spores apart from each other, separating them (Figure 33). Once separated, immature single spores either lack a protuberance or have them at different lengths within the same capsule (Figures 32, 39); the same is observed in mature spores from dehisced capsules (Figures 44–46). This seems to suggest that the process of tetrad separation is asynchronous, and perhaps that proximal protuberances may be slowly “retracted back” into the spore wall after spores separate. The “proximal disk” (Figures 35, 38, 44–46) appears to be the site of protuberance development-retraction, and later, we speculate that it is the site of spore germination. We refrain from referring to the proximal disk as a true operculum because we did not observe it opening or separating from the spore as a true operculum would do. We speculate that the disk is a portion of the spore with a thinner wall deposit, similar to the sulcus on a pollen grain. Future developmental studies are recommended to test the function of the spore proximal operculum-like disk. If confirmed, this protuberant growth and retraction pattern would be unique within liverworts (and perhaps bryophytes).

Across the proximal disk of some spores, there is a low ridge with a central slit that resembles an aperture (Figures 37 and 38). The fact that it is present only in some spores does not support its function as an aperture. It could represent the remnant of a tetrad ridge, or just a different ornamentation pattern. The nature of this structure is unclear to us.

Gametangia, involucre development and morphology

The unusual barrel-shaped involucre that protects the archegonia develops before fertilization, and thus differs from the pseudoperianth and caulocalyx of simple thalloid liverworts, which are post-fertilization structures. The development of involucres in *Kahakuloa* occurs at the apex of the plant, and at least partially from wing tissue that is pushed back, towards the dorsal surface of the plant, at intervals, during thallus growth (Figure 10). In this way a cylindrical "tube-like" structure that becomes fused early to form an involucre is produced. Each involucre shelters and protects a small cluster of archegonia (Figures 15, 16). The involucres expand and can become rather large, sometimes extending beyond the midrib to the lower dorsal portion of the thallus wings. Involucre production starts early. We observed small (3–6 mm long), young female plants already bearing one or two involucres, but their production continues throughout the life of the plant. We observed some mature female plants with up to 16 involucres all along their dorsal surface. The involucral mouth is constricted compared to the base, giving the involucre an urn shape. The function of such "energy costly" structures could be to protect the fragile archegonia from desiccation and exposure. The plants occur in open bogs with little shade (Figures 3a, 3b). Additionally, the laciniate, hyaline involucral mouth may reflect solar radiation away from archegonia and developing sporophytes. It may also collect dew or droplets of water in the air (Figures 5, 17 and 18).

There are more female than male plants in the populations studied. Male plants (Figures 21–26, 50–53) appear less robust and produce antheridia initially on the thallus surface, protected by scales. Later in their development (farther away from the apical region), antheridia are sunken into thallus chambers with a small ostiole and a reduced scale. Mature antheridia are thus well protected by the surrounding thallus tissues from desiccation and exposure.

The fertilization rate appears to be low (with the caveat that our observations are limited to the Herbst and Pezzillo collections). We observed only a few (about 5%) involucres enclosing either immature or mature sporophytes in the Pezzillo population. None of the fertile plants studied had more than one involucre with evidence of fertilization (enclosing a sporophyte or empty calyptra). Only in one instance, we saw an involucre with two calyptras, representing two fertilization events. Splashing spermatozoids out of sunken antheridia with a small ostiole (opening) and up above the barrier of a large involucre could be challenging, but the plants grow in a wet climate with periods of heavy rain and high relative humidity.

Phytogeographic significance

Kahakuloa operculispora is known only from bogs at 975–1,280 m elevation on Mauna ʻEʻeka (also known as the West Maui Mountains; summit elevation 1,764 m), an extinct volcano dominating the western end of the island of Maui that is no more than about 1.9 million years old (Sherrod *et al*. 2007). Its last eruption was about 500,000 years ago (Sinton 2012). The area where *Kahakuloa* grows is an open bog with very high rainfall distributed evenly throughout the year. During the period of 30 June 2022 to 30 June 2023, 229 inches (over 5,800 mm) of rain were measured at the summit of Puʻu Kukui (1,764 m) about 3.5 km south of the type locality (U.S. Geological Survey 2023 https://waterdata.usgs.gov/monitoring-location/ 205327156351102/#parameterCode=00045&period=P365D&compare=true).

Maui is rich in endemic vascular plants, but relatively poor in endemic bryophytes. There are at least 90 endemic vascular plant species on Maui, of which 24 are endemic to West Maui; several more taxa are endemic at the level of subspecies (Gustaffson *et al.* 2014, and updates). Several of these endemic species grow with *Kahakuloa* at its type locality including *Argyroxiphium caliginis* and *Myrsine vaccinioides*.

So far, *Kahakuloa operculispora* is one of only two bryophyte species endemic to West Maui. The other is the moss *Glossadelphus mauiensis* Broth. (Hypnaceae). No other liverworts (or hornworts) are endemic to West Maui, although the near-endemic *Protolophozia perssoniana* (H.A.Mill.) Váňa & L.Söderstr. (Scapaniaceae) also occurs in bogs on the neighboring island of Molokaʻi (Staples & Imada 2006) about 40 km away. This species is biogeographically interesting as it is the only tropical member of an otherwise North and South Temperate genus.

Endemic liverwort genera are rare in Oceania east of Australia and New Guinea, and, until the discovery of

Kahakuloa, were thought to be absent from Pacific Ocean islands east of New Caledonia and Aoteoroa/New Zealand. New Caledonia has two endemic liverwort genera, *Meinungeria* Frank Müll. (Lepidoziaceae) and *Otoscyphus* J.J.Engel, Bardat & Thouvenot (Lophocoleaceae) (Müller 2007, Thouvenot *et al.* 2011, Engel *et al*. 2012).

In Aoteoroa/New Zealand there are ten endemic liverwort genera: the thalloid genera *Allisonia* (Allisoniaceae) and *Neohodgsonia* Perss. (Neohodgsoniaceae), and the leafy genera *Bragginsella* R.M.Schust. (Lophocoleaceae), *Echinolejeunea* R.M.Schust. (Lejeuneaceae), *Herzogianthus* R.M.Schust. (Herzogianthaceae), *Kymatolejeunea* Grolle (Lejeuneaceae), *Lamellocolea* J.J.Engel (Lophocoleaceae), *Lembidium* Mitt. (Lepidoziaceae), *Megalembidium* R.M.Schust. (Lepidoziaceae), and *Pseudolophocolea* R.M.Schust. & J.J.Engel (Plagiochilaceae). The leafy liverwort genus *Goebeliella* Steph. (Goebeliellaceae) occurs in both New Caledonia and Aoteoroa/New Zealand (https://datastore.landcareresearch.co.nz/dataset/checklist-of-the-new-zealand-flora-hornworts-liverworts-and-mosses-2020, Söderström *et al.* 2016).

Kahakuloa is the first liverwort genus endemic to Hawaiʻi and joins the moss genus *Baldwiniella* M.Fleisch. (Stone 1967, Staples *et al.* 2004) as one of only two endemic genera of bryophytes there. It is also the only genus of land plants endemic to Maui, and the first new endemic embryophyte (land plant) genus discovered in Hawaiʻi since *Kanaloa kahoolawensis* Lorence & K.R.Wood (Fabaceae) was discovered on Kahoʻolawe in 1992 (Lorence & Wood 1994). Although other new Hawaiian endemic vascular plant genera have been proposed since that time such as the monocot *Chrysodracon* P.L.Lui & Morden (Lui *et al.* 2014) and the fern *Hoiokula* S.E.Fawc. & A.R.Sm. (Fawcett & Smith 2021), in both cases the species transferred to these new genera were first described in the 19th century.

The Kahakuloaceae is the first (and so far, only) endemic Hawaiian land plant family. It is also the first new liverwort family based on a "novel" discovery since 1964, when the Grolleaceae R.M.Schust. and Phyllothalliaceae E.A.Hodgs. were first described (data from Söderström *et al.* 2016, with nomenclatural updates from Tropicos through 1 October 2023). Several further liverwort families have been newly described since the 1960s based on novel discoveries, such as Phycolepidoziaceae R.M.Schust., Trabacellulaceae Fulford, Cladomastigaceae Fulford, Perssonelliaceae Grolle, Vandiemeniaceae Hewson and Mizutaniaceae Furuki & Z.Iwats., but these taxa are no longer accepted. Using these data sources, the Kahakuloaceae is the first "novel" liverwort family to be discovered and described from the Northern Hemisphere since *Makinoa* (later placed in the Makinoaceae) was described from Japan in 1899.

How did *Kahakuloa* or its ancestor reach Maui? The species does not appear to exhibit asexual reproduction, and its dioicous sexuality suggests that a cluster of spores must have reached Hawaiʻi via wind or birds for successful colonization to occur. Colonization of Hawaiʻi could have occurred since the formation of Mauna ʻEʻeka 1.9 million years ago, but it is worth noting that the high elevation bog habitat of *Kahakuloa* probably did not develop until the volcano broached the ocean, attained maximum height, eruptions ceased, and then weathered and eroded to its present form – so it seems likely that suitable habitat for *Kahakuloa* has been present there for only a few hundred thousand years.

Alternatively, the liverwort could be much older and could have "island-hopped" its way east down the chain as islands formed, matured, and disappeared, as is apparently the case with the vascular plant *Hillebrandia sandwicensis* Oliv. (Begoniaceae); Stevens (2023) notes that: "The age of *Hillebrandia*, anything from 58 Ma [million years] (Clement *et al*. 2004, errata 2005) to 24.6(-16.8) Ma (Moonlight *et al.* 2018), causes biogeographical problems. The oldest Hawaiian island on which *Hillebrandia* is found is Kaua'i, which is perhaps 5 Ma old. Either *Hillebrandia* arrived in the Hawaiian area from some presumably continental region where it is now extinct, or it has been island hopping in the Pacific for 20-50 Ma or so…"

If the "island-hopping" scenario applies, then it seems likely that *Kahakuloa* could be extant in high elevation bogs on islands older than Maui: Molokaʻi, and especially the summit bogs Kawaikini and Waiʻaleʻale on Kauaʻi, are good candidates. It might also be present on the younger but more extensive and remote bogs of Haleakalā on eastern Maui. Sequence data from further samples of *Kahakuloa,* particularly from geographically distinct populations, could help shed light on the age of the species on the islands.

Kahakuloa operculispora is phylogenetically distinctive, yet occurs on a remote, geologically young island. The geological age of Hawaii is difficult to reconcile with Bechteler *et al.'s* (2023) estimated stem age for the Fossombroniaceae of 277-294 million years, which suggests that the Kahakuloaceae lineage has been around for at least a similar length of time. We speculate that it, or a near relative, may yet be discovered in high elevation bogs in tropical Asia or Australasia.

Dedication

We dedicate this paper to Dr. Barbara Crandall-Stotler and the late Dr. Raymond Stotler, for their rigorous, outstanding contributions to the study of liverworts. Five of the authors either studied with them (Cargill, Forrest and Freire), were mentored by one of their students (Judziewicz by Freire) or collaborated with them (Cargill, Forrest, Gradstein). The Stotlers' influence now extends to the Hawaiian bryoflora.

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