Antrophyum solomonense (Pteridaceae), a New Species from the Solomon Islands, and Its Systematic Position Based on Phylogenetic Analysis

Author(s): Cheng Wei Chen, Joel Hamilton Nitta, Moffat Fanerii, Tsung Yu Aleck Yang, Fred Pitisopa, Chia Wei Li, and Wen Liang Chiou

Source: Systematic Botany, 40(3):645-651.
Published By: The American Society of Plant Taxonomists
URL: http://www.bioone.org/doi/full/10.1600/036364415X689357

Systematic Botany (2015), 40(3): pp. 645–651

DOI 10.1600/036364415X689357
Date of publication September 22, 2015

Antrophyum solomonense (Pteridaceae), a New Species from the Solomon Islands, and its Systematic Position Based on Phylogenetic Analysis

Cheng Wei Chen,[1] Joel Hamilton Nitta,[2] Moffat Fanerii,[3] Tsung Yu Aleck Yang,[4,5] Fred Pitisopa,[6] Chia Wei Li,[1,8] and Wen Liang Chiou[7,8]

[1]Department of Molecular and Cellular Biology, National Tsing Hua University, Hsinchu 30013, Taiwan.
[2]Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts 02138, U. S. A.
[3]Kolombangara Island Biodiversity Conservation Association, Ringii, the Solomon Islands.
[4]Department of Biology, National Museum of Natural Science, Taichung 40453, Taiwan.
[5]Department of Life Science, National Chung Hsing University, Taichung 40227, Taiwan.
[6]Ministry of Forestry and Research, Honiara, the Solomon Islands.
[7]Division of Botanical Garden, Taiwan Forestry Research Institute, Taipei 10066, Taiwan.
[8]Authors for correspondence: CWL: cwli@life.nthu.edu.tw, WLC: chiou@tfri.gov.tw

Communicating Editor: David Morgan

Abstract—A vittarioid fern from the Solomon Islands, which has long been treated as *Antrophyum semicostatum*, is described here as a new species, ***Antrophyum solomonense***. A description, illustration, and distribution of the new species are presented. Boot-shaped paraphysal apical cells, rounded stipe in cross section, and thin rhizome scale cell walls are the diagnostic characteristics of *A. solomonense*. A molecular phylogeny inferred from four chloroplast markers further supports its systematic uniqueness.

Keywords—Chloroplast DNA, fern, flora, morphology, South Pacific.

The "Census and Classification of Plant Resources in the Solomon Islands" project (http://siflora.nmns.edu.tw/) was conducted in collaboration between the Solomon Islands and Taiwan from 2012–2014. During our expeditions, over 4,000 sheets of specimens representing about 300 species of ferns and lycophytes were collected, including several unknown *Antrophyum* Kaulf. specimens. The fern genus *Antrophyum* includes ca. 40 species widely distributed throughout the Old World tropics (Crane 1997), and 15 of these are known from the Pacific (Jones 1998; Matsumoto et al. 2008). In the Solomon Islands, six species of *Antrophyum* are usually recognized (Whitmore 1966; Henderson and Hancock 1988): *A. callifolium* Blume, *A. megistophyllum* Copel., *A. plantagineum* (Cav.) Kaulf., *A. reticulatum* (G. Forst.) Kaulf., *A. semicostatum* Blume, and *A. subfalcatum* Brack. Some of our specimens collected in the Solomon Islands were originally identified as *A. semicostatum* based on previous descriptions (Brownlie 1977; Henderson and Hancock 1988). However, after examination of both living plants and herbarium specimens of *A. semicostatum* from Java, the type locality, we realized our specimens from the Solomon Islands differed from *A. semicostatum* in gross morphology and possibly represented a new species. To further examine the uniqueness of our collections, we conducted detailed morphological comparisons and molecular phylogenetic analyses, and present the results here.

Materials and Methods

The morphology of all 15 Pacific *Antrophyum* species was observed from living plants and/or specimens. Furthermore, 22 *Antrophyum* specimens including two specimens of the putative new species (*Wade 3086* and *Wade 4072*) from the Solomon Islands, two specimens of *A. semicostatum* (*Wade 1072* and *Wade 1810*) from Java, and specimens of seven other species from the Pacific region were compared on the basis of both morphology and DNA sequences (nine species in total). We sought to include all Pacific species with similar morphology to the putative new species from the Solomon Islands in the molecular data set. Voucher specimens were deposited in the herbarium of the Honiara Botanical Gardens of the Solomon Islands (BSIP), Taiwan Forestry Research Institute (TAIF), Harvard University (GH), or the Japanese National Museum of Nature and Science (TNS; Appendix 1).

The morphology of spores, rhizome scales, and paraphyses was examined by both light microscope (LEICA DMR) and a tabletop scanning electron microscope (TM-3000 Hitachi) following the method of Chen et al. (2014). Thirty spores from one specimen (*Wade 4072*) of the putative new species from the Solomon Islands were sampled for measurement and morphological observation. The thickness of cell walls of rhizome scales was measured for all of the *Antrophyum* specimens except for *Wade 1289* and *Wade 1422*, for which the rhizomes are missing. For each specimen, 30 cells were selected from the middle section of three different scales, photographed with a digital camera (EOS 7D, Canon) under a light microscope (LEICA DMR), and measured with Image-Pro Plus 5.0 (IPP; Version 5.0; Media Cybernetics, Silver Spring, Missouri). The median, 25th and 75th percentiles, and variance were calculated using SPSS v.14.0 (IBM Inc., Chicago, Illinois) for each species.

Four chloroplast regions (*chlL*, *ndhF*, *matK*, and *trnL-F*) were sequenced to infer a molecular phylogeny of the nine selected Pacific *Antrophyum* species, with *Haplopteris ensiformis* (Sw.) E. H. Crane as the outgroup. Laboratory protocols for DNA extraction, amplification, and sequencing follow Chen et al. (2013, 2014). Sequences were manually edited and aligned using default options in Muscle (Edgar 2004). There were no strongly supported conflicting relationships (i.e. bootstrap support $\geq$ 70%) between the four DNA regions when analyzed individually (results not shown), so we concatenated the sequences into a single alignment for subsequent analysis. All newly generated DNA sequences were deposited in GenBank (Appendix 1), and the combined dataset was deposited in TreeBASE (study number 17130). Maximum likelihood (ML) analysis was performed with GARLI v.2.0 (Zwickl 2006) using the GTR + I + Γ model of sequence evolution, and the genthreshfortopoterm option set to 20,000. Branch support was assessed with 3,000 bootstrap replicates under the same settings.

Results

Morphological Characteristics—Rhizome scales and soral paraphyses of the nine investigated species are presented in Fig. 1. Rhizome scales of all species are linear-lanceolate and clathrate, with thickened cell walls. The degree of cell wall thickening of the rhizome scales varies among species (Fig. 2). Apical cells of the paraphyses can be classified into five types: filiform, funnel-shaped, clavate, globose, and

The first and second authors contributed equally

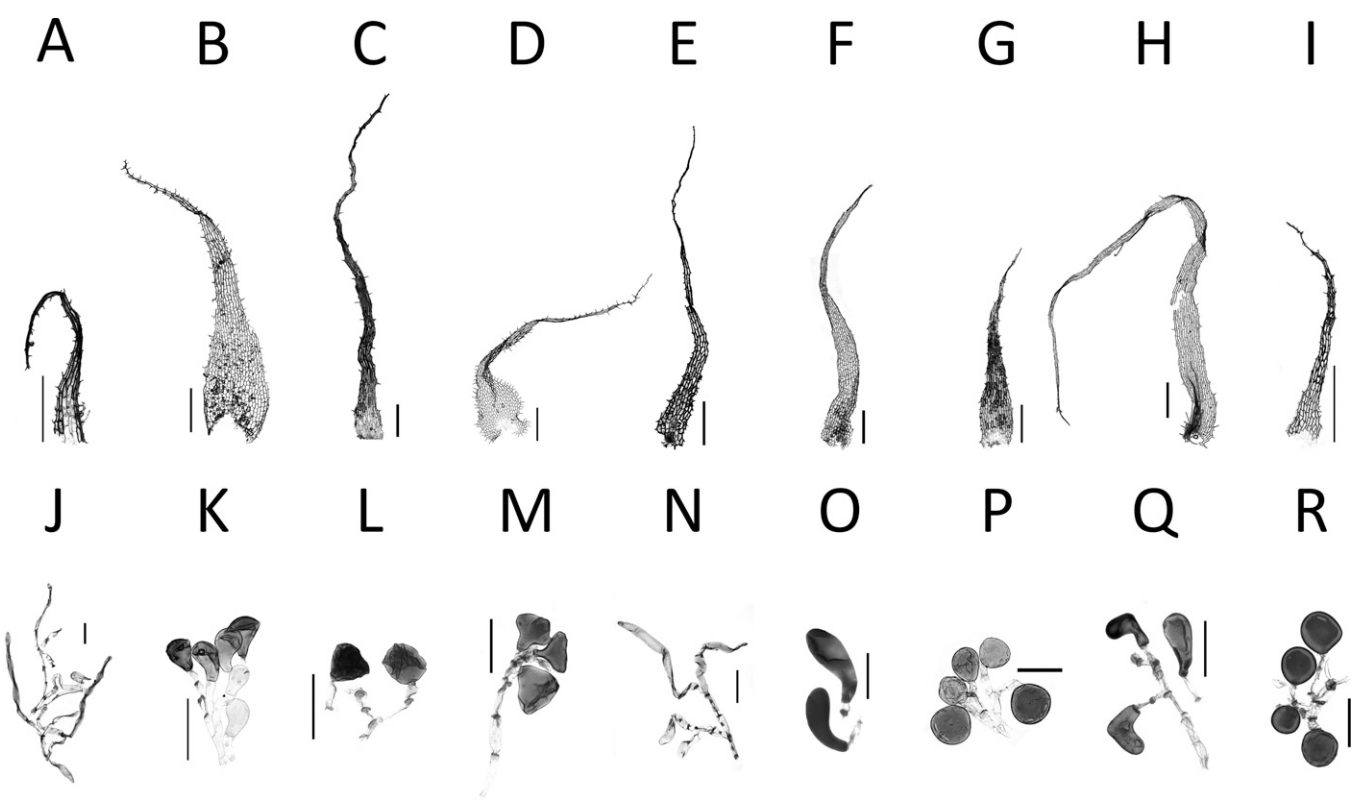

Fig. 1. Rhizome scales (A to I) and paraphyses (J to R) of nine Pacific *Antrophyum* species. A, J. *A. callifolium*. B, K. *A. ledermannii*. C, L. *A. megistophyllum*. D, M. *A. plantagineum*. E, N. *A. reticulatum*. F, O. *A. semicostatum*. G, P. *A. smithii*. H, Q. *A. solomonense*. I, R. *A. subfalcatum*. Bar = 1 mm (A to I) or 100 μm (J to R).

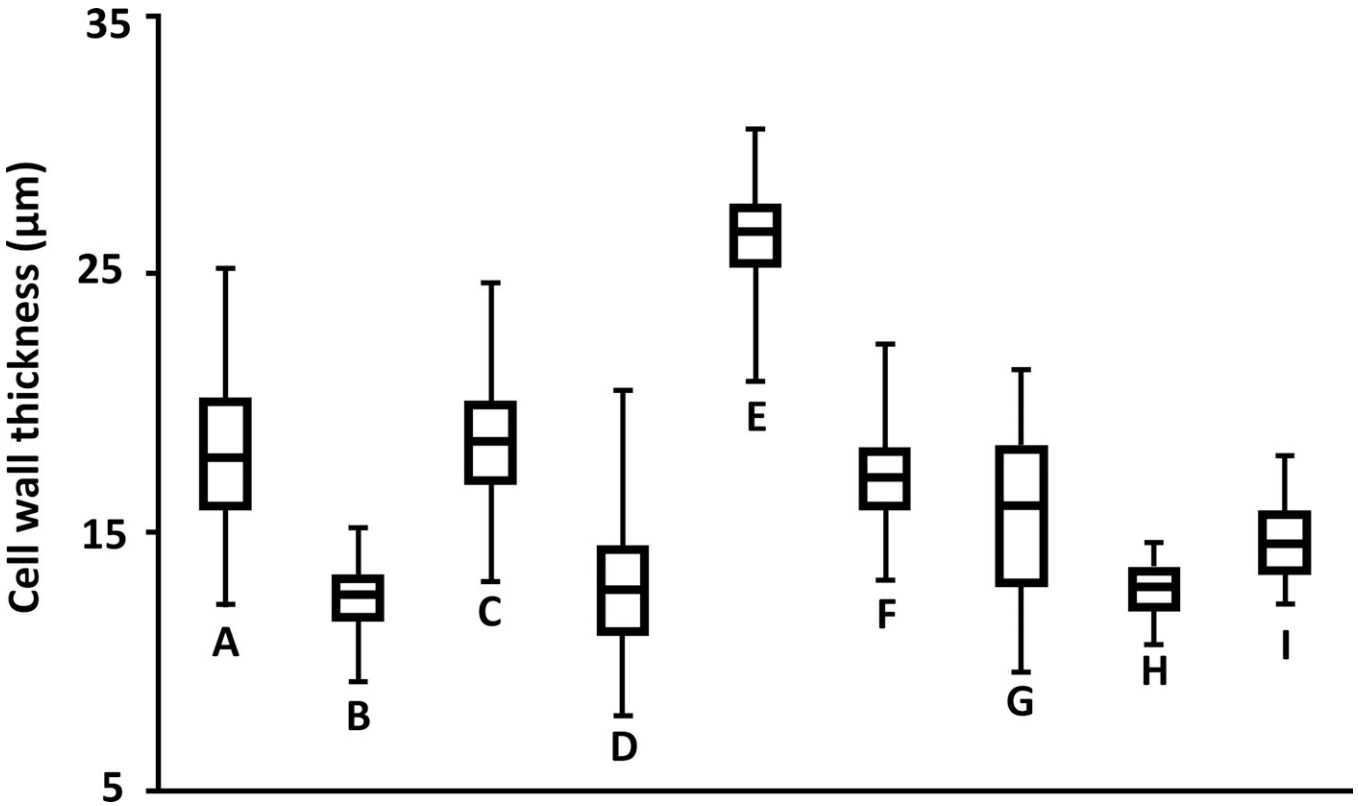

Fig. 2. Cell wall thickness of the rhizome scales of nine Pacific *Antrophyum* species. A. *A. callifolium*. B. *A. ledermannii*. C. *A. megistophyllum*. D. *A. plantagineum*. E. *A. reticulatum*. F. *A. semicostatum*. G. *A. smithii*. H. *A. solomonense*. I. *A. subfalcatum*. The thick horizontal line is the median, the box indicates the variation observed between the 25th and 75th percentiles, and the whiskers show the variance range.

Table 1. Comparison of selected morphological characteristics of nine Pacific *Antrophyum* species.

Taxon	Apical cell of paraphyses	Cross section of stipe	Position of sori	Frond shape
Antrophyum callifolium Blume	Filiform	Flat	Shallowly grooved	Spathulate
Antrophyum ledermannii Hieron.	Funnel-shaped	Flat	Superficial	Spathulate
Antrophyum megistophyllum Copel.	Funnel-shaped	Flat	Shallowly grooved	Spathulate
Antrophyum plantagineum (Cav.) Kaulf.	Funnel-shaped	Rounded	Deeply grooved	Spathulate
Antrophyum reticulatum (Forst.) Kaulf.	Filiform	Flat	Shallowly grooved	Spathulate
Antrophyum semicostatum Blume	Clavate	Flat	Deeply grooved	Spathulate
Antrophyum smithii C. Chr.	Globose	Flat	Shallowly grooved	Linear
Antrophyum solomonense C. W. Chen & J. H. Nitta	Clavate and boot-shaped	Rounded	Shallowly grooved	Spathulate
Antrophyum subfalcatum Brack.	Globose	Flat	Shallowly grooved	Linear

boot-shaped. Boot-shaped apical cells are only found in the putative new species from the Solomon Islands, which also has clavate apical cells. A comparison of other selected morphological characteristics among the nine species is shown in Table 1.

Molecular Phylogeny—The concatenated data set contained 3,957 bp including 873 variable (22%), 369 gap (10%), and 361 missing (9%) characters. The phylogeny inferred from the concatenated data set by ML analysis is presented in Fig. 3. The nine sampled species of *Antrophyum* form two major clades. The first clade (maximum likelihood bootstrap support; MLBS = 100) includes *A. callifolium*, *A. reticulatum*, and *A. semicostatum*. The second clade (MLBS = 97) can be further divided into two subclades: subclade 1 (MLBS = 100) includes only *A. plantagineum*, and subclade 2 (MLBS = 99) includes the rest of the species (*A. ledermannii*, *A. megistophyllum*, *A. smithii*, *A. subfalcatum*, and the putative new species from the Solomon Islands).

Discussion

The systematic uniqueness of the putative new species from the Solomon Islands is supported by both morphological and molecular evidence. Morphologically, the new species can be clearly distinguished from *A. semicostatum* by its thinner rhizome scale cell walls (Fig. 2) and paraphyses with boot-shaped apical cells (Fig. 1). Boot-shaped paraphysal apical cells have not been reported from any other *Antrophyum* species, or any other fern, to our knowledge. Furthermore, using the combined characteristics of fronds and rhizome scales, the putative new species from the Solomon Islands can be distinguished clearly from other species in the Pacific region (Table 1). The results of the phylogenetic analysis also indicate the distinctiveness of the putative new species: the two specimens of this species share the same sequences in four cpDNA regions and occupy a different clade from *A. semicostatum*, which was previously regarded to occur in the Solomon Islands (Whitmore 1966; Henderson and Hancock 1988). As a result, we suggest that our specimens from the Solomon Islands belong to a new species, *A. solomonense* (see Taxonomic Treatment). A specimen collected in 1965 by Braithwaite (*4717*, deposited in K as *K000706771*) might be the first collection of *A. solomonense* (Glenny, pers. comm.). Although this specimen was identified at that time as *A. semicostatum*, after examination of the specimen image we believe it is actually *A. solomonense*. However, a more confident identification can only be made after examination of distinguishing characters (e.g. rhizome scales and paraphyses).

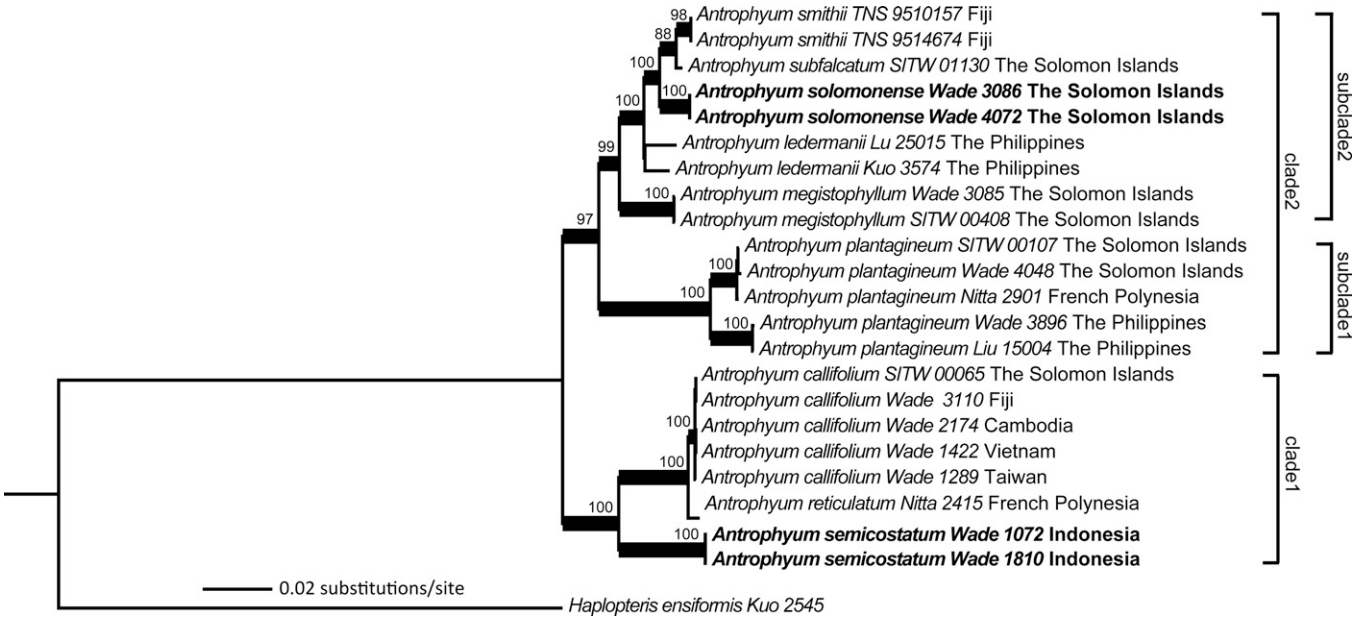

Fig. 3. Maximum likelihood phylogram of Pacific *Antrophyum* obtained from the combined *chlL*, *matK*, *ndhF*, and *trnL-F* data set. Voucher information and GenBank accession numbers are shown in Appendix 1. Maximum likelihood bootstrap support indicated above nodes; thickened lines indicate bootstrap support ≥ 80%. *A. semicostatum* and *A. solomonense* are highlighted in bold.

The taxonomy of vittarioid ferns is not an easy task, due to their high degree of morphological simplification that provides few characteristics for species identification (Crane et al. 1995). Although names for more than 500 vittarioid ferns have been published, the actual estimated species number is no more than 150 (Lindsay 2003). Recently, an integrated methodology utilizing both molecules and morphology has gradually provided better insight into vittarioid species discrimination (Chen et al. 2013, 2014). Additional evidence, e.g. cytology and nuclear markers, should be included in future systematic work to gain a better understanding of species diversity in this fascinating group of ferns.

Taxonomic Treatment

Antrophyum solomonense C. W. Chen & J. H. Nitta, sp. nov. —TYPE: THE SOLOMON ISLANDS. Isabel Province: Santa Isabel Island, Mt. Kobinitu, 800 m, 16 July 2014, *Wade 4072 = SITW 5633* (holotype: BSIP!; isotypes: GH!, K!, P!, TAIF!, TNM!, US!).

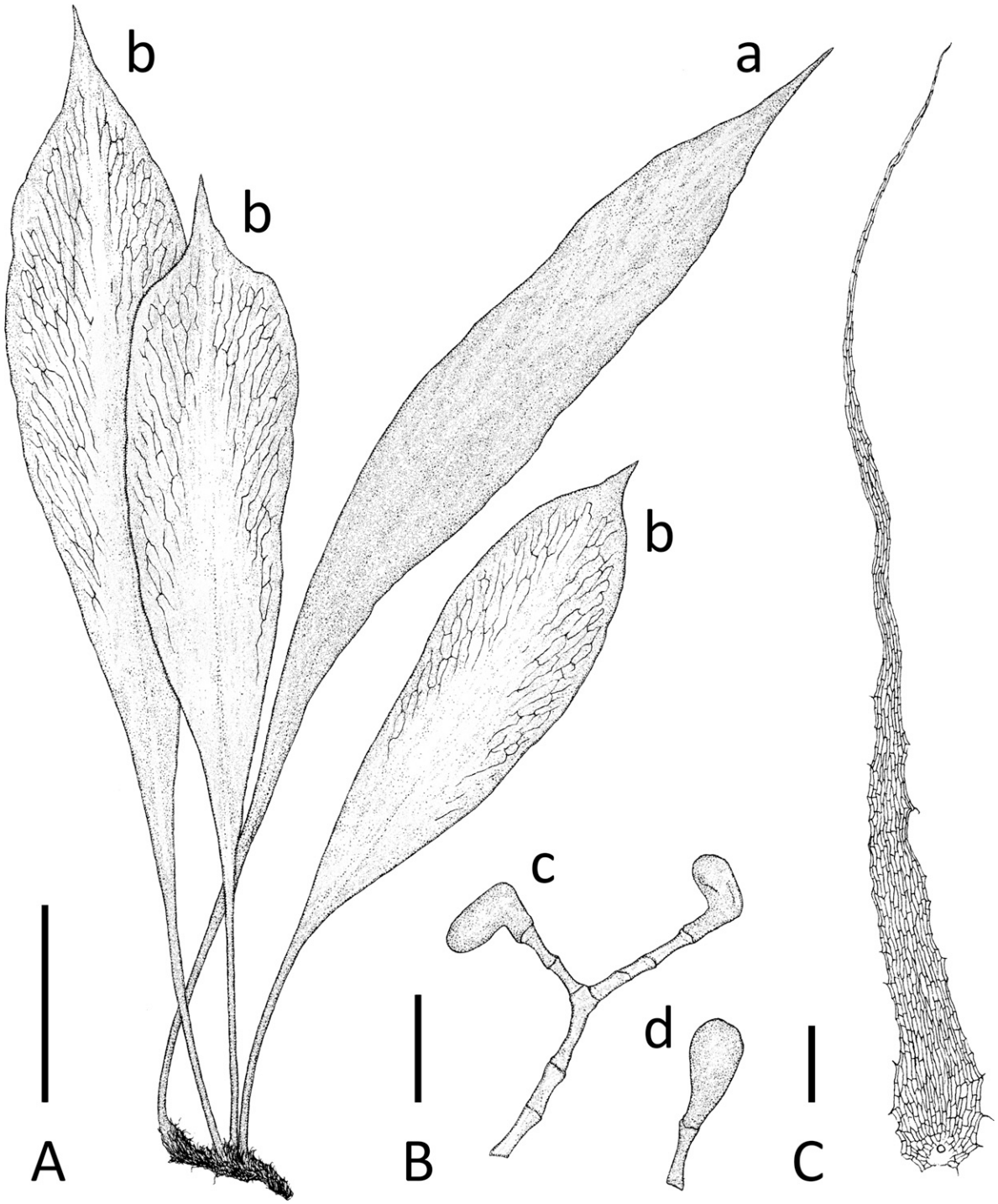

Fig. 4. *Antrophyum solomonense* (from the type). A. Habit; a and b indicate adaxial and abaxial surface, respectively. B. Paraphyses; c and d indicate boot-shaped and clavate apical cells, respectively. C. Rhizome scale. Scale bars: A = 5 cm, B = 100 μm, C = 1 mm.

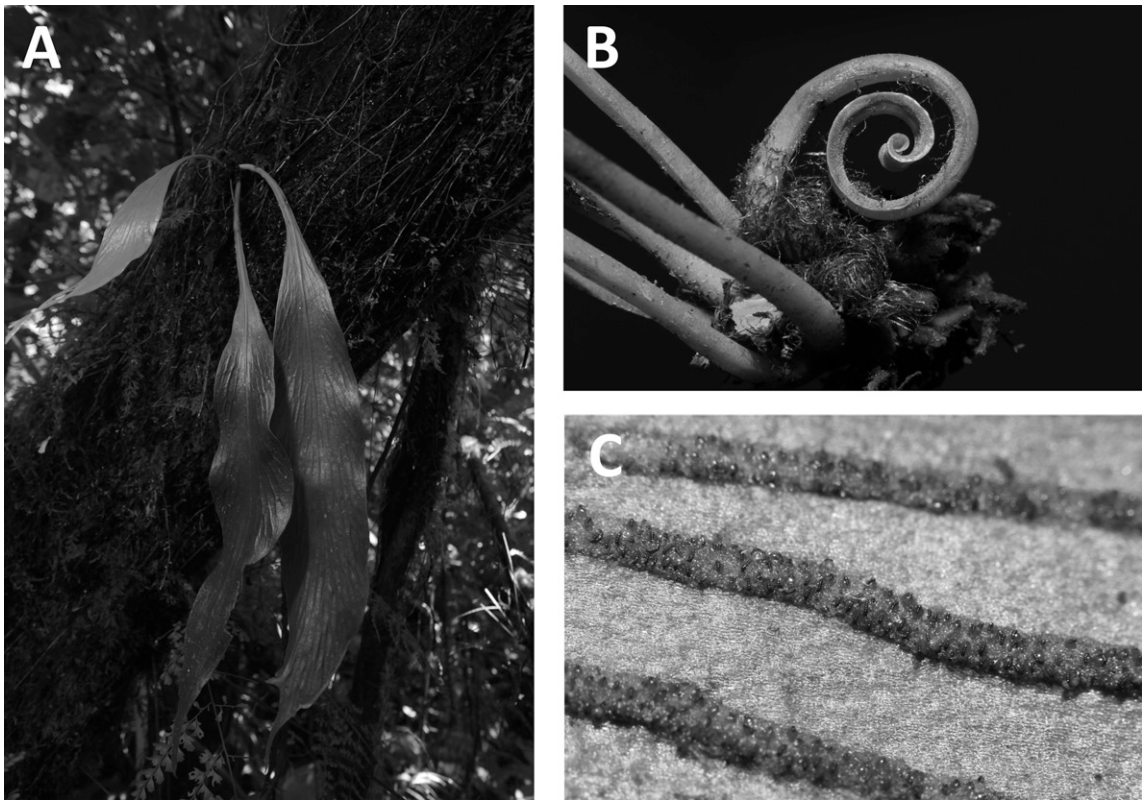

FIG. 5. *Antrophyum solomonense*. A. Habit. B. Basal part of the plant showing the rhizome scales, stipes, and young frond. C. Sori.

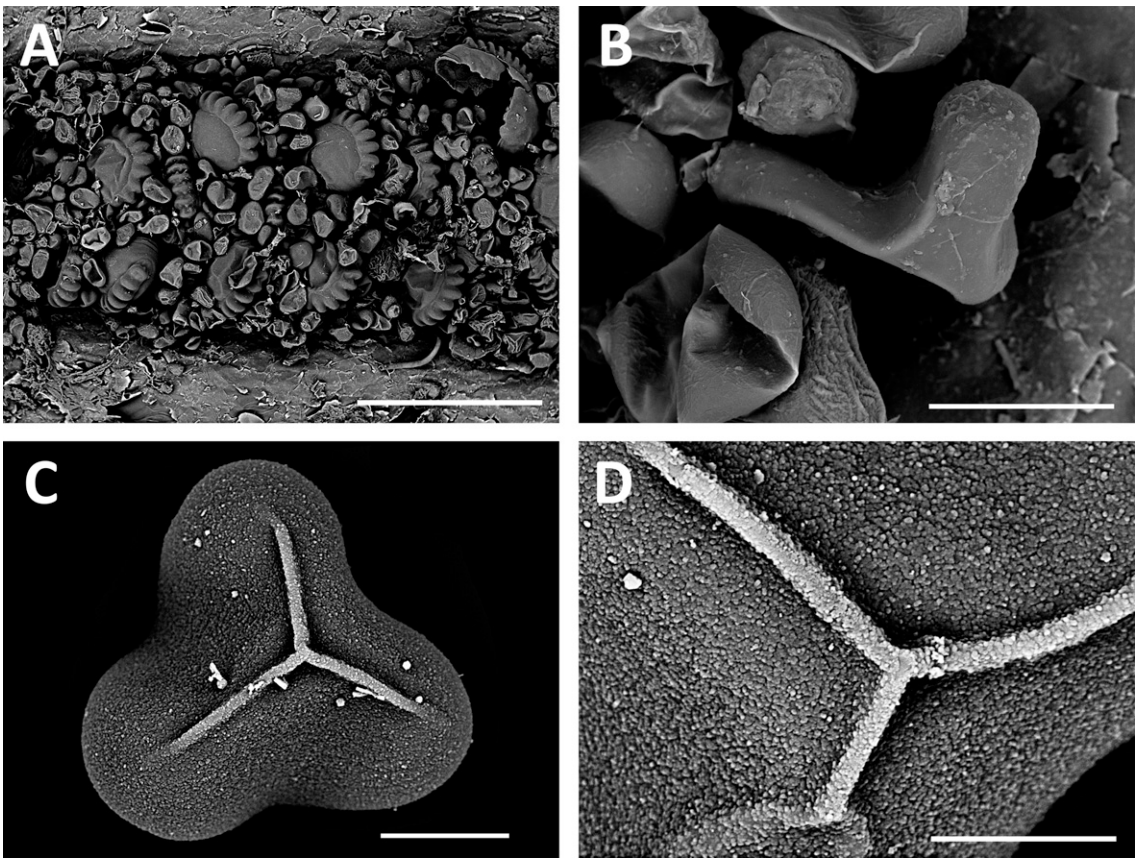

FIG. 6. SEM images of the paraphyses and spores of *Antrophyum solomonense*. A. Sorus showing the distribution of sporangia and paraphyses. B. Paraphyses with boot-shaped apical cells. C. Proximal face of the spore. D. Detail of surface and aperture arm. Scale bars: A = 500 μm, B = 50 μm, C = 15 μm, D = 10 μm.

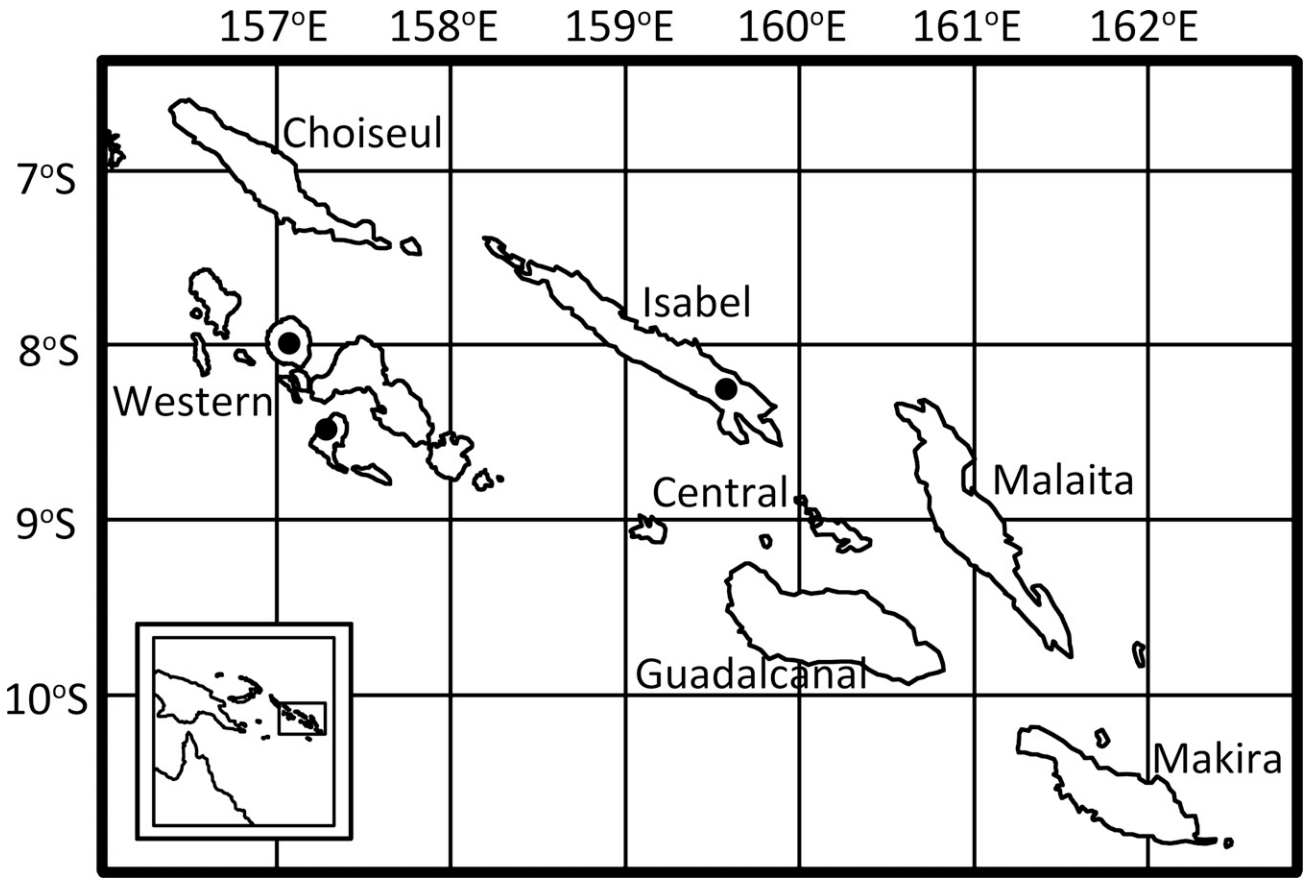

Fig. 7. Distribution map of *Antrophyum solomonense*. Dots indicate known populations.

Plants epiphytic. Rhizomes short-creeping, scaly; rhizome scales clathrate, linear-lanceolate, 5–14 × 0.5–1.2 mm at the base, tapering to one cell wide at the apex, brown, edges slightly denticulate; rhizome cell walls thin, 8–16 μm thick. Leaves approximately clustered; stipes long, over 1/5 the length of the leaf, rounded in cross section; laminae sub-coriaceous, spathulate, 15–30 × 3–4 cm, broadest near the upper 2/3, tapering to base, the midribs visible. Sori linear, in shallow grooves, seldom reticulate; paraphyses 4–6 cells long, branched, apical cell clavate or boot-shaped. Spores tetrahedral, 47.1 ± 4.8 μm long, surface papillate. Figures 4, 5, and 6.

This species differs from other members of the genus by having boot-shaped paraphysal apical cells (Figs. 1, 4, and 6) and from *A. semicostatum* by having a long stipe that is rounded in cross section, with thin-walled rhizome scale cells. This species is phylogenetically closely related to *A. smithii* and *A. subfalcatum* (Fig. 3). However, it can be clearly distinguished from these two species by the morphology of the paraphyses. The paraphyses of *A. solomonense* have clavate and boot-shaped apical cells, whereas those of *A. smithii* and *A. subfalcatum* are globose.

Ecology and Distribution—This species has been collected from several islands of the Solomon Islands (Fig. 7). It is found in primary forests near streams, and grows as an epiphyte on trunks of angiosperms and tree ferns at a height of ca. 1.5 m.

Etymology—The species is named for the type locality.

Additional Specimens Examined—THE SOLOMON ISLANDS. Western Province: Kolombangara Island, Conku Rano crater, 600 m, 5 November 2013, *Wade 3600 = SITW 4045* (BSIP!, TAIF!, TNM!). Western Province: Rendova Island, Ugele, 700 m, 26 August 2013, *Wade 2946 = SITW 3384* (BSIP!, TAIF!, TNM!). Rendova Island, Ugele, 100 m, 29 August 2013, *Wade 3086 = SITW 3598* (BSIP!, TAIF!, TNM!).

Acknowledgments. We thank Tian Chuan Hsu for his assistance during the field work, Atsushi Ebihara, Li Yaung Kuo, Pi Fong Lu, and Yea Chen Liu for providing materials, GH, K, and TNS for loaning specimens, Che Wei Lin for the illustrations, Anna Haigh at K for providing the specimen image (*K000706771*), Stuart Lindsay and two anonymous reviewers for their valuable opinions, and David Glenny for kindly sharing his unpublished book of ferns of the Solomon Islands and informative discussion. This research was supported by the Taiwan Forestry Research Institute (102AS-4.1.1-FI-G1) for WLC, by Taiwan ministry of science and technology (MOST 103-2321-B-007-008) for CWC, and by Taiwan International Cooperation and Development Fund (TH-410-2012-085) for CWL.

Literature Cited

Brownlie, G. 1977. *The pteridophyte flora of Fiji*. Vaduz: Cramer.

Chen, C. W., A. Ebihara, W. L. Chiou, and C. W. Li. 2014. *Haplopteris yakushimensis* (Pteridaceae, Vittarioideae), a new species from Yakushima Island, Japan. *Phytotaxa* 156: 229–234.

Chen, C. W., Y. M. Huang, L. Y. Kuo, Y. H. Chang, Y. C. Liu, and W. L. Chiou. 2013. A new vittarioid fern species, *Haplopteris heterophylla* (Pteridaceae). *Systematic Botany* 38: 901–909.

Crane, E. H., D. R. Farrar, and J. F. Wendel. 1995. Phylogeny of the Vittariaceae: Convergent simplification leads to a polyphyletic *Vittaria*. *American Fern Journal* 85: 283–305.

Crane, E. H. 1997. A revised circumscription of the genera of the fern family Vittariaceae. *Systematic Botany* 22: 509–517.

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.

Henderson, C. P. and I. R. Hancock. 1988. *A guide to the useful plants of Solomon Islands*. Honiara, Solomon Islands: Research Department, Ministry of Agriculture and Lands.

Jones, D. L. 1998. Vittariaceae. Pp. 288–295 in *Flora of Australia*, vol. 48, *Ferns, gymnosperms and allied groups*, ed. P. M. McCarthy. Melbourne: ABRS/CSIRO.

Lindsay, S. 2003. Considerations for a revision of the fern family Vittariaceae for Flora Malesiana. *Telopea* 10: 99–112.

Matsumoto, S., T. Nose, T. Nakamura, Y. Sugisaki, and N. Yoshikawa. 2008. Pteridaceae. Pp. 105–131 in *Illustrated flora of ferns and fern allies of South Pacific islands*, ed. National Museum of Nature and Science. Tokyo: Tokai University Press.

Whitmore, T. C. 1966. *Guide to the forests of the British Solomon Islands*. London: Oxford University Press.

Zwickl, D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph. D. dissertation. Austin: The University of Texas.

Appendix 1. Voucher specimens and GenBank accession numbers for DNA sequences used in this study. Information is presented in the following order: taxon name, locality, collection number, deposited herbarium, *chlL*, *matK*, *ndhF*, and *trnL-F*.

Antrophyum callifolium, Taiwan, *Wade 1289*, TAIF, KM884673, KM884697, KM884721, KM884745. *Antrophyum callifolium*, Vietnam, *Wade 1422*, TAIF, KM884674, KM884698, KM884722, KM884746. *Antrophyum callifolium*, the Solomon Islands, *SITW 00065*, BSIP, KM884675, KM884699, KM884723, KM884747. *Antrophyum callifolium*, Fiji, *Wade 3110*, TAIF, KM884676, KM884700, KM884724, KM884748. *Antrophyum callifolium*, Cambodia, *Wade 2174*, TAIF, KM884677, KM884701, KM884725, KM884749. *Antrophyum ledermannii*, the Philippines, *Kuo 3574*, TAIF, KM884689, KM884713, KM884737, KM884761. *Antrophyum ledermannii*, the Philippines, *Lu 25015*, TAIF, KM884690, KM884714, KM884738, KM884762. *Antrophyum megistophyllum*, the Solomon Islands, *SITW 00408*, BSIP, KM884691, KM884715, KM884739, KM884763. *Antrophyum megistophyllum*, the Solomon Islands, *Wade 3085*, TAIF, KM884692, KM884716, KM884740, KM884764. *Antrophyum plantagineum*, the Solomon Islands, *SITW 00107*, BSIP, KM884678, KM884702, KM884726, KM884750. *Antrophyum plantagineum*, French Polynesia, *Nitta 2901*, GH, KM884679, KM884703, KM884727, KM884751. *Antrophyum plantagineum*, the Philippines, *Liu 15004*, TAIF, KM884680, KM884704, KM884728, KM884752. *Antrophyum plantagineum*, the Philippines, *Wade 3896*, TAIF, KM884681, KM884705, KM884729, KM884753. *Antrophyum plantagineum*, the Solomon Islands, *Wade 4048*, TAIF, KM884682, KM884706, KM884730, KM884754. *Antrophyum reticulatum*, French Polynesia, *Nitta 2415*, GH, KM884684, KM884708, KM884732, KM884756. *Antrophyum semicostatum*, Indonesia, *Wade 1072*, TAIF, KM884685, KM884709, KM884733, KM884757. *Antrophyum semicostatum*, Indonesia, *Wade 1810*, TAIF, KM884686, KM884710, KM884734, KM884758. *Antrophyum smithii*, Vanuatu, *TNS 9510157*, TNS, KM884687, KM884711, KM884735, KM884759. *Antrophyum smithii*, Vanuatu, *TNS 9514674*, TNS, KM884688, KM884712, KM884736, KM884760. *Antrophyum solomonense*, the Solomon Islands, *Wade 3086*, TAIF, KM884693, KM884717, KM884741, KM884765. *Antrophyum solomonense*, the Solomon Islands, *Wade 4072*, TAIF, KM884694, KM884718, KM884742, KM884766. *Antrophyum subfalcatum*, the Solomon Islands, *SITW 01130*, BSIP, KM884695, KM884719, KM884743, KM884767. *Haplopteris ensiformis*, the Philippines, *Kuo 2545*, TAIF, KM884696, KM884720, KM884744, KM884768.