Clarification of Two Poorly Known Vittarioid Ferns (Pteridaceae): Haplopteris angustissima and H. capillaris

Authors: Chen, Cheng-Wei, Lindsay, Stuart, Yong, Kien Thai, Mustapeng, Andi Maryani A., Amoroso, Victor B., et al.

Source: Systematic Botany, 44(3) : 483-493

Published By: The American Society of Plant Taxonomists

URL: https://doi.org/10.1600/036364419X15620113920545

Systematic Botany (2019), 44(3): pp. 483–493

DOI 10.1600/036364419X15620113920545
Date of publication August 6, 2019

Clarification of Two Poorly Known Vittarioid Ferns (Pteridaceae): *Haplopteris angustissima* and *H. capillaris*

Cheng-Wei Chen,[1] Stuart Lindsay,[2] Kien Thai Yong,[3] Andi Maryani A. Mustapeng,[4] Victor B. Amoroso,[5] Viet Dai Dang,[6] and Yao-Moan Huang[7,8]

[1]Division of Silviculture, Taiwan Forestry Research Institute, 53 Nan-Hai Road, Taipei 100, Taiwan; wade0504@gmail.com
[2]Native Plant Centre, Horticulture and Community Gardening Division, National Parks Board, 1 Cluny Road, Singapore 259569; stuart_lindsay@nparks.gov.sg
[3]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; yongkt@um.edu.my
[4]Forest Research Centre, Sabah Forestry Department, PO Box 1407, 90715 Sandakan, Sabah, Malaysia; andimaryani.mustapeng@gmail.com
[5]Central Mindanao University, Musuan, Bukidnon, the Philippines; victorbamoroso@gmail.com
[6]Southern Institute of Ecology, Ho Chi Minh city, Vietnam; Biodiversity Taiwan International Graduate Program, Biodiversity Research Center, Taipei, Taiwan; Department of Life Science, National Taiwan Normal University, Taipei, Taiwan; vietdaidang@gmail.com
[7]Division of Silviculture, Taiwan Forestry Research Institute, 53 Nan-Hai Road, Taipei 100, Taiwan; huangym@tfri.gov.tw
[8]Author for correspondence (huangym@tfri.gov.tw)

Communicating Editor: Alejandra Vasco

Abstract—Two poorly known Malesian vittarioid ferns, *Haplopteris angustissima* and *Monogramma capillaris*, are noteworthy for their similar gross morphology. In this study, their systematic relationship was clarified by using molecular phylogenetic analysis which resolved the species in two different clades. Diagnostic morphological characters were further revealed by detailed comparison of almost all the known collections. Although overlaps were found in most of the investigated morphological characters, the venation and silica bodies were found to be useful for species delimitation. Type information, field images, illustrations, and taxonomic notes are provided for both species. A new combination, ***Haplopteris capillaris***, is made and its occurrence in Taiwan as independent gametophytes is confirmed.

Keywords—Borneo, *Monogramma*, new combination, Peninsular Malaysia, Philippines.

This paper concerns two poorly known Malesian vittarioid ferns usually called *Haplopteris angustissima* (Holttum) S.Linds. and *Monogramma capillaris* Copel. The generic delimitation of vittarioids has changed substantially in recent years due to insights from molecular phylogenetic research (e.g. Crane et al. 1995; Ruhfel et al. 2008; Schuettpelz et al. 2016). Here we follow Schuettpelz et al. (2016) and Chen et al. (2016), and therefore treat the above two species under *Haplopteris*.

Holttum (1947) described *Vittaria angustissima* based on Carr's collection from Fraser's Hill in Pahang, Peninsular Malaysia. He mentioned its similarity to *Vittaria angustifolia* Blume [now *Haplopteris angustifolia* (Blume) E.H.Crane] and *Vittaria parvula* Bory, but distinguished it by the smaller scales and fronds, and the fact that many fronds have sori only on one edge of the lamina. After its establishment, subsequent authors have maintained Holttum's species (e.g. Parris and Latiff 1997), but no new argument has been made for its systematic relationship. Lindsay (2010) transferred it to *Haplopteris* following Crane (1997).

Copeland (1911) described *Monogramma capillaris* [*Haplopteris capillaris* (Copel.) C.W.Chen, S.Linds. & K.T.Yong comb. nov., see below] based on Merrill's collections from the island of Negros in the Philippines. He compared it with the congeneric species, *Monogramma dareicarpa* Hook. [now *Haplopteris dareicarpa* (Hook.) S.Linds. & C.W.Chen] and distinguished it from that by the more closely spaced and narrower fronds. *Monogramma capillaris* was later transferred to *Vaginularia* Fée by Christensen (1934) and *Pleurofossa* Nakai ex H.Ito by Ito (1936). Since then, this species has seldom been mentioned in the literature and was even omitted by Copeland himself in his *Fern Flora of the Philippines* (Copeland 1960). Price (1990) synonymized this species under *M. dareicarpa*, and his treatment was followed by Pelser et al. (2011 [onwards]). Recently, Kuo et al. (2017) included a specimen of *H. capillaris* (as *Monogramma capillaris*) from Sabah in their molecular phylogenetic analysis and discovered that it had a close affinity to an independent gametophyte population from Taiwan.

Morphologically, *H. angustissima* and *H. capillaris* are extremely simplified and very similar (Fig. 1); they are diminutive plants with hair-like fronds, ca. 1 mm wide and less than 15 cm long. These two species have not been studied systematically, probably due to the scarcity of materials, and so their relationship is unknown. Excluding the type collections, *H. angustissima* is only known by nine other collections from Peninsular Malaysia, and *H. capillaris* is only known by seven other collections from Borneo. Despite their apparent rarity, we recently found both species during collecting expeditions in Peninsular Malaysia, Borneo, and the Philippines. These new materials covered most of the known localities of both species (including the type locality of *H. capillaris*) and proved critical to deciphering both the relationship of *H. angustissima* and *H. capillaris*, and their systematic position within the vittarioid clade.

Materials and Methods

Plant Materials—Four populations of *H. angustissima* and *H. capillaris* were sampled between 2015–2017 in Borneo (Sabah & Sarawak), Peninsular Malaysia, and the Philippines (*Chen Wade4360*, *YKT 813*, *Chen Wade4825*, and *Chen Wade4959*; Table 1). Due to their morphological similarity, we first identified our specimens based on the type locality, i.e. *Chen Wade4825* from Peninsular Malaysia as *H. angustissima* and *Chen Wade4959* from the Philippines as *H. capillaris*. The two specimens from Borneo were identified later (as *H. capillaris*) from the results of the molecular phylogenetic analysis (see below). As well as our new collections, specimens of *H. angustissima* and *H. capillaris* deposited at K, KLU, KYO, L, MICH, SING, TAIF, and US (including the lectotype of *Monogramma capillaris* at MICH and the holotype of *Vittaria angustissima* at SING) were used for morphological analyses (Table 1) and/or the preparation of taxonomic descriptions.

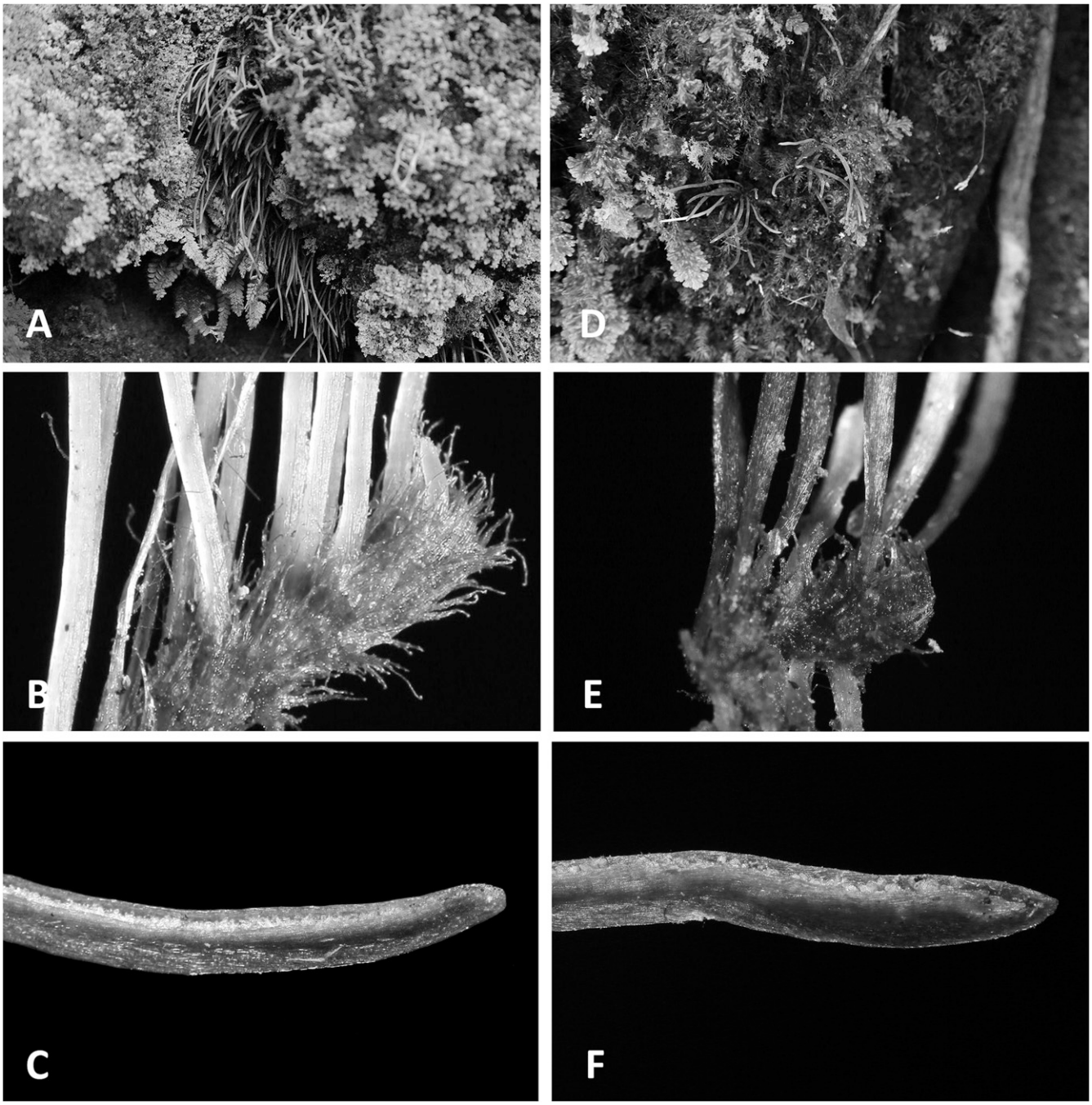

FIG. 1. Habit, rhizome, and sorus of the two *Haplopteris* species studied here. A–C. *Haplopteris angustissima* (based on *Chen Wade4825*). D–F. *Haplopteris capillaris* (based on *Chen Wade4959*).

Molecular Phylogenetic Analysis—To infer the phylogenetic relationship of *H. angustissima* and *H. capillaris*, a phylogeny of 22 *Haplopteris* taxa, including our four recent collections was reconstructed based on seven chloroplast DNA regions (*atpA*, *chlL*, *matK*, *ndhF*, *rbcL*, *rpoA*, and *trnL-F*) with *Radiovittaria* [*Radiovittaria gardneriana* (Fée) E.H. Crane] as outgroup. Voucher information and GenBank accession numbers are listed in Appendix 1. Extraction of genomic DNA was performed using a modified CTAB-Qiagen column (Valencia, CA, USA) protocol from fresh, silica-dried, or herbarium specimens following Kuo (2015). The PCR primer sets used in this study followed Schuettpelz et al. (2016) and Chen et al. (2017). The PCR amplifications and sequencing reactions were performed following the protocol of Chen et al. (2013). Sequences were aligned using default options in Muscle (Edgar 2004). This alignment is available through Dryad (Chen et al. 2019) and TreeBASE (http://www.treebase.org, TreeBASE study # 23706). A maximum likelihood (ML) analysis was conducted using GARLI v. 2.0 (Zwickl 2006) with the substitution models derived in PartitionFinder v. 2.1.1 (Guindon et al. 2010; Lanfear et al. 2012, 2017), and the genthreshfortopoterm option set to 20,000. Branch support was assessed with 1000 bootstrap replicates under the same criteria in the CIPRES Science Gateway (Miller et al. 2010). Branch support was also assessed in a Bayesian framework following Chen et al. (2017) with the partitioning scheme and substitution models determined by PartitionFinder (as above).

Morphological Analyses—In total, 15 specimens (eight of *Haplopteris angustissima* and seven of *H. capillaris*) were used for morphological analyses (Table 1). Morphological characters including rhizomes scales, terminal cells of soral paraphyses (simply referred to as paraphyses hereafter), frond venation, epidermal silica bodies, and spores were examined and measured with a light microscope (DMR, Leica, Wetzlar, Germany) and/or a tabletop scanning electron microscope (TM-3000 Hitachi, Ibaraki, Japan), following the methods of Kao et al. (2008), Vasco et al. (2014), and Chen et al. (2017). The size (length and width) of 30 paraphyses, the thickness of 30 cell walls of rhizome scales, the length of 30 epidermal silica bodies from adaxial laminae (more abundant than

TABLE 1. Morphological traits of *Haplopteris angustissima* and *H. capillaris*.

Taxon	Voucher (herbarium)	Location	Soral line position	Apical cell of soral paraphysis (length × width) (μm)	Rhizome scale cell wall thickness (μm)	Epidermal silica bodies (length)(mm)	Spore size (μm)
Haplopteris angustissima (Holttum) S.Linds.	Carr *s.n.* (SING)	Pahang, Malaysia	Mostly single sorus, rarely double sori	60.9 ± 7.6 × 65.1 ± 8.0	12.7 ± 1.8	0.36 ± 0.11	47.9 ± 2.6
Haplopteris angustissima (Holttum) S.Linds.	*Chen Wade4825* (TAIF)	Pahang, Malaysia	Single sorus	80.5 ± 14.4 × 61.9 ± 8.6	13.3 ± 1.4	0.38 ± 0.10	52.3 ± 3.2
Haplopteris angustissima (Holttum) S.Linds.	*Burkill & Holttum 8835* (SING)	Pahang, Malaysia	Single sorus	63.3 ± 11.4 × 54.2 ± 8.1	NA	0.46 ± 0.08	47.2 ± 3.1
Haplopteris angustissima (Holttum) S.Linds.	*Evans 118* (KLU2422)	Pahang, Malaysia	Single sorus	48.2 ± 6.6 × 48.0 ± 6.1	NA	NA	NA
Haplopteris angustissima (Holttum) S.Linds.	*Haniff 3938* (SING)	Perak, Malaysia	Single sorus	54.7 ± 7.8 × 56.2 ± 4.3	14.5 ± 1.3	NA	50.2 ± 3.7
Haplopteris angustissima (Holttum) S.Linds.	*Molesworth Allen 3941* (SING)	Pahang, Malaysia	Single sorus	53.3 ± 7.9 × 51.5 ± 6.2	NA	0.37 ± 0.09	43.6 ± 2.9
Haplopteris angustissima (Holttum) S.Linds.	*Molesworth Allen 4135* (US)	Pahang, Malaysia	Single sorus	63.5 ± 8.2 × 54.0 ± 6.6	14.5 ± 1.8	NA	46.9 ± 4.2
Haplopteris angustissima (Holttum) S.Linds.	*Smith 899* (SING)	Pahang, Malaysia	Single sorus	66.7 ± 8.8 × 60.0 ± 5.4	NA	0.41 ± 0.16	50.1 ± 3.0
Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong	*Clemens & Clemens 29574* (US)	Sabah, Malaysia	Single sorus	92.3 ± 16.8 × 61.3 ± 8.0	10.7 ± 1.5	NA	42.2 ± 2.2
Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong	*Chen Wade4360* (TAIF)	Sabah, Malaysia	Single sorus	107.6 ± 9.6 × 66.1 ± 7.8	10.8 ± 0.9	0.43 ± 0.09	56.4 ± 3.9
Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong	*Chen Wade4959* (TAIF)	Negros, Philippines	Single sorus	109.7 ± 13.7 × 57.0 ± 8.3	14.8 ± 1.5	0.42 ± 0.08	37.5 ± 2.0
Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong	*Holttum 25715* (SING)	Sabah, Malaysia	Single sorus	82.7 ± 8.5 × 50.4 ± 5.6	12.7 ± 1.1	NA	38.2 ± 2.9
Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong	*Kato et al. B-10358* (L)	East Kalimantan, Indonesia	Single sorus	100.8 ± 14.5 × 55.9 ± 7.9	10.8 ± 1.3	0.34 ± 0.11	48.2 ± 4.7
Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong	*Merrill 6961* (MICH)	Negros, Philippines	Mostly single sorus, rarely double sori	104.8 ± 20.8 × 55.3 ± 8.3	12.0 ± 1.5	NA	39.1 ± 3.0
Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong	*Yong 813* (TAIF)	Sarawak, Malaysia	Single sorus	97.3 ± 13.5 × 54.9 ± 8.0	12.8 ± 1.2	0.42 ± 0.09	44.9 ± 2.9

abaxial surface), and the length of 30 spores were measured for each specimen. The ANOVA with p adjusted by TukeyHSD was used to compare the differences in cell wall thickness in the rhizome scales and the differences in spore size among the specimens. Clustering of the width and length of paraphyses of both taxa was tested using k-means clustering analysis. All statistical analyses were performed using R v. 3.4.0 (R Core Team 2017) with ggplot2 package (Wickham 2009) to visualize the results.

Results

Molecular Phylogeny—The final concatenated dataset comprised 7586 aligned sites (1733, 879, 1035, 1208, 1195, 576, and 960 from *atpA*, *chlL*, *matK*, *ndhF*, *rbcL*, *rpoA*, and *trnL-F*, respectively) and included 12.9% (25,479 characters) missing data and 15.1% (1151 sites) parsimony-informative sites. The optimal partitioning scheme based on the corrected Akaike information criterion (AICc), as inferred by PartitionFinder, included 15 data subsets (their corresponding models are listed in parentheses): *atpA* codon position 1 + *chlL* codon position 1 (GTR + I + G); *chlL* codon position 2 (K81uf + I); *chlL* codon position 3 (TVM + G); *matK* codon position 1 + *ndhF* codon position 3 (TVM + G); *matK* codon position 2 + *ndhF* codon position 1 (TVM + G); *matK* codon position 3 (TVM + G); *ndhF* codon position 2 (TVM + I + G); *trnL-F* (TVM + G); *rpoA* codon position 1 (GTR + G); *rpoA* codon position 2 (TVM + G); *rbcL* codon position 3 + *rpoA* codon position 3 (TVM + G); *atpA* codon position 2 (GTR + I + G); *atpA* codon position 3 (GTR + I); *rbcL* codon position 1 (TIM + I); *rbcL* codon position 2 (TIMef + I + G).

The reconstructed phylogeny of the 22 *Haplopteris* taxa is shown in Fig. 2. Overall the relationships within the genus are well supported and congruent with previous studies (Schuettpelz et al. 2016; Chen et al. 2017). Of the 22 resolved nodes, 19 received good support (i.e. BS ≥ 70). Three samples of *H. capillaris* were resolved as monophyletic. The independent gametophyte from Taiwan was resolved within the *H. capillaris* clade and differed by only five bp from the sporophytic sample from the Philippines. Despite their morphological similarity, *H. angustissima* and *H. capillaris* were resolved in different clades.

Morphological Analyses—The results of the morphological analyses are presented in Tables 1–2 and Figs. 3–7. Almost all the fronds of each species have single sorus, but the type specimen of *H. angustissima* has ca. 10 fronds with double sori, and the type specimen of *H. capillaris* has two very small fragments with double sori (Table 1). Considerable variation was observed in the size of paraphyses, rhizome scales, and spores such that there was no clear distinction between the two species (Figs. 3, 4, 5). For example, clustering analysis on the size of paraphyses showed that more than 23% of the paraphyses of *H. capillaris* were clustered with those of *H. angustissima* (Table 2). However, the shape of the silica bodies and the venation patterns were found to be useful for species delimitation. Specifically, the ends of the lobes of the silica bodies in *H. angustissima* are pointed whereas those in *H. capillaris* are blunt (Fig. 6); and fronds (with single sorus) of

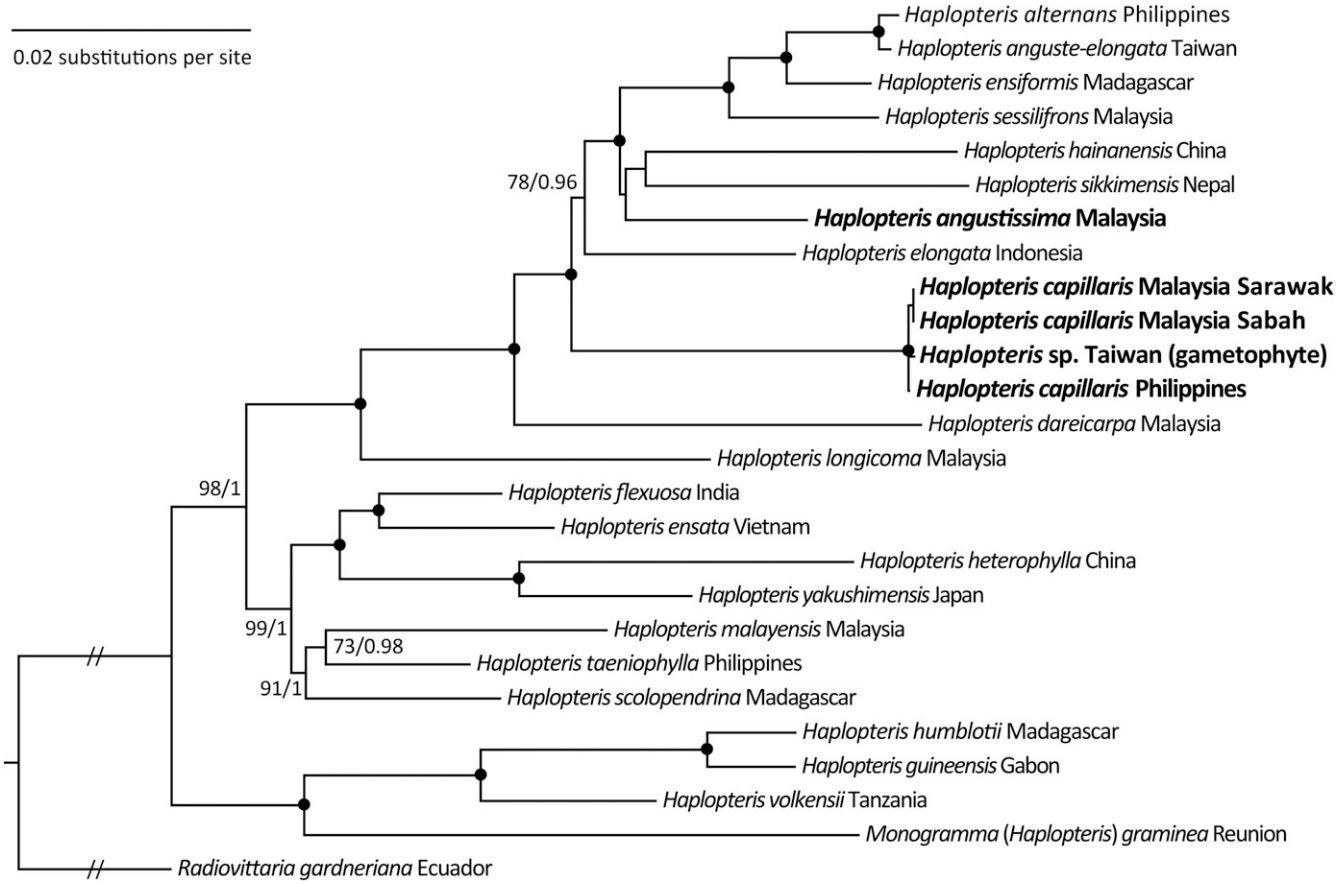

Fig. 2. Phylogram of 22 *Haplopteris* taxa from maximum likelihood analysis of the concatenated plastid dataset (seven loci; 15 data partitions). Maximum likelihood bootstrap support values and Bayesian posterior probabilities are shown for nodes with high support (i.e. BS ≥ 70) and solid circles at nodes indicate bootstrap = 100%, posterior probability = 1. *Haplopteris angustissima* and *Haplopteris capillaris* are in bold-face type. Branch length of the outgroups is not to scale for better visualization of ingroup relationships. The sequence of independent gametophytes of *Haplopteris capillaris* from Taiwan is derived from two samples (*A* and *E30*, Appendix 1).

TABLE 2. K-means clustering analysis on the length and width of paraphyses of *Haplopteris angustissima* and *H. capillaris*. Eleven and 49 data points of *H. angustissima* and *H. capillaris* were misclassified, respectively.

	Species	
Cluster	*Haplopteris angustissima*	*Haplopteris capillaris*
1	11 (5.4%)	161 (76.6%)
2	191 (94.5%)	49 (23.3%)
Total	202	210

H. capillaris contain only a midvein (Fig. 7C) whereas those (with single sorus) of *H. angustissima* have two very close parallel veins connected by a few oblique cross-veins (thereby creating a few very long narrow areoles) (Fig. 7A).

Discussion

Species Delimitation—The phylogenetic analysis resolved *H. angustissima* and *H. capillaris* in different clades that are well-separated from each other (Fig. 2), providing support for their distinctiveness. *Haplopteris angustissima* was clustered with *H. hainanensis* (C.Chr.) E.H.Crane and *H. sikkimensis* (Kuhn) E.H.Crane despite their morphological and geographical dissimilarity. Morphologically, *H. hainanensis* and *H. sikkimensis* can be distinguished from *H. angustissima* by always having fronds with double sori. Additionally, *H. hainanensis* is a much larger species with fronds ca. 10–30 cm long (Zhang et al. 2013) compared to ca. 2–13 cm long in *H. angustissima*. Geographically, *H. angustissima* is known only from Peninsular Malaysia, *H. hainanensis* is known only from southern China and northern Vietnam, and *H. sikkimensis* is known only from the Himalayan area and northern Indochina (Zhang et al. 2013).

To reveal the discriminative characters of *H. angustissima* and *H. capillaris*, we conducted a detailed morphological comparison and included not only those characters that are commonly used in *Haplopteris* (i.e. paraphyses, rhizome scales, and spores) (e.g. Chen et al. 2013, 2014, 2017), but also two other less often used characters (i.e. silica bodies and venation). Our results show that only silica bodies and venation can be used to unambiguously delimit the two species.

Our discovery of different venation patterns in *H. angustissima* and *H. capillaris* is based only on fronds with single sori. We were able to determine the venation pattern in one frond of *H. angustissima* with double sori (Fig. 7B), but could not compare this with *H. capillaris* due to the size and condition of the relevant frond fragments. However, we expect those fragments to contain at least two veins because, as far as we know, two sori never arise from the same vein in *Haplopteris*.

Silica bodies appear to be a synapomorphy for all vittarioid ferns, and high variation has been found at both the generic and the species level (Ito 1936; Bhandari and Mukhopadhyay 2009; Sundue 2009). Here we have demonstrated that silica bodies can be used for species delimitation. Future studies with broader sampling are needed to further explore their systematic value.

Convergent Evolution—Biseriate areolate venation, supporting two linear sori per frond, is found in most *Haplopteris* species (Schuettpelz et al. 2016); however, some of the smallest species, which have fronds only a few centimeters long, have only a single vein (midvein) supporting a single sorus. These include *H. capillaris*, *H. dareicarpa* (Hook.) S.Linds. & C.W.Chen, and *H. graminea* (Poir.) comb. ined. Despite the similarity of these three species, the phylogenetic data shows that they are only distantly related (Fig. 2). This indicates that extreme morphological simplification has likely evolved independently multiple times in the history of the genus.

Independent Gametophyte—Kuo et al. (2017) included a specimen of *H. capillaris* from Sabah in a molecular phylogenetic analysis and found that it had a close affinity with an independent gametophyte population in Taiwan. They suspected the independent gametophyte was conspecific with *H. capillaris* but refrained from publishing this conclusion due to the systematic uncertainty of *H. capillaris*. In this study, we were able to confirm the distinctiveness of *H. capillaris* by morphological observations (including examination of the type specimens) and molecular phylogenetic analysis. In addition to the two specimens sequenced by Kuo et al. (2017), we

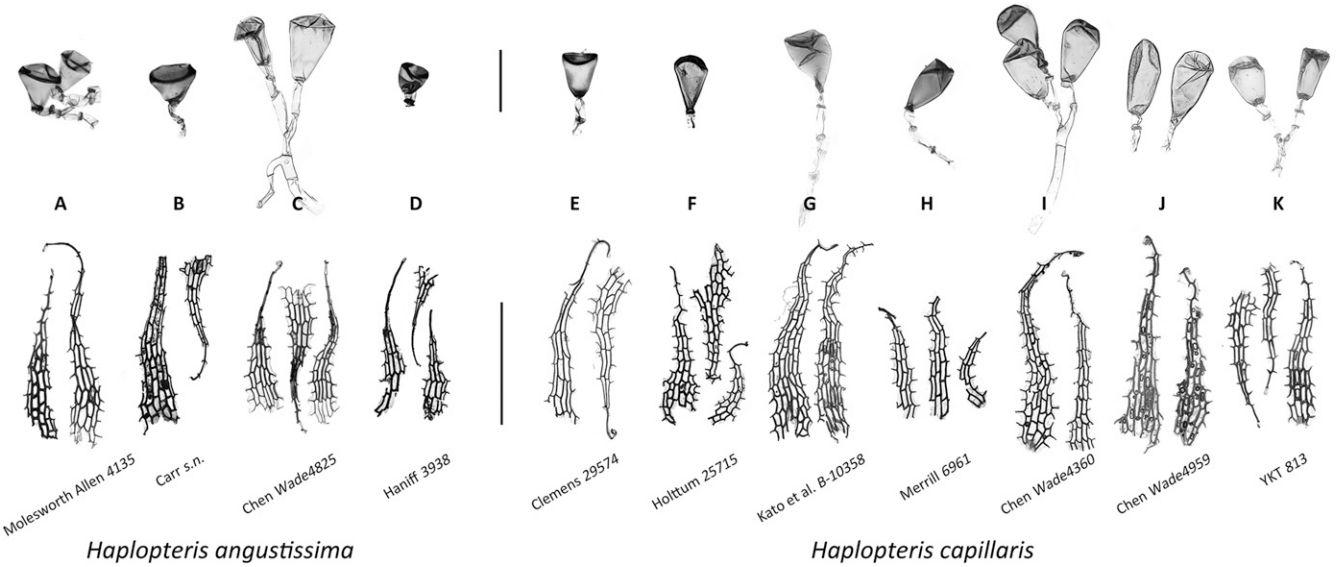

FIG. 3. Paraphyses (upper panel) and rhizome scales (lower panel) of the two *Haplopteris* species studied here. A–D. *Haplopteris angustissima*. E–K. *Haplopteris capillaris*. Scale bars = 100 μm for the paraphyses and 1 mm for the scales.

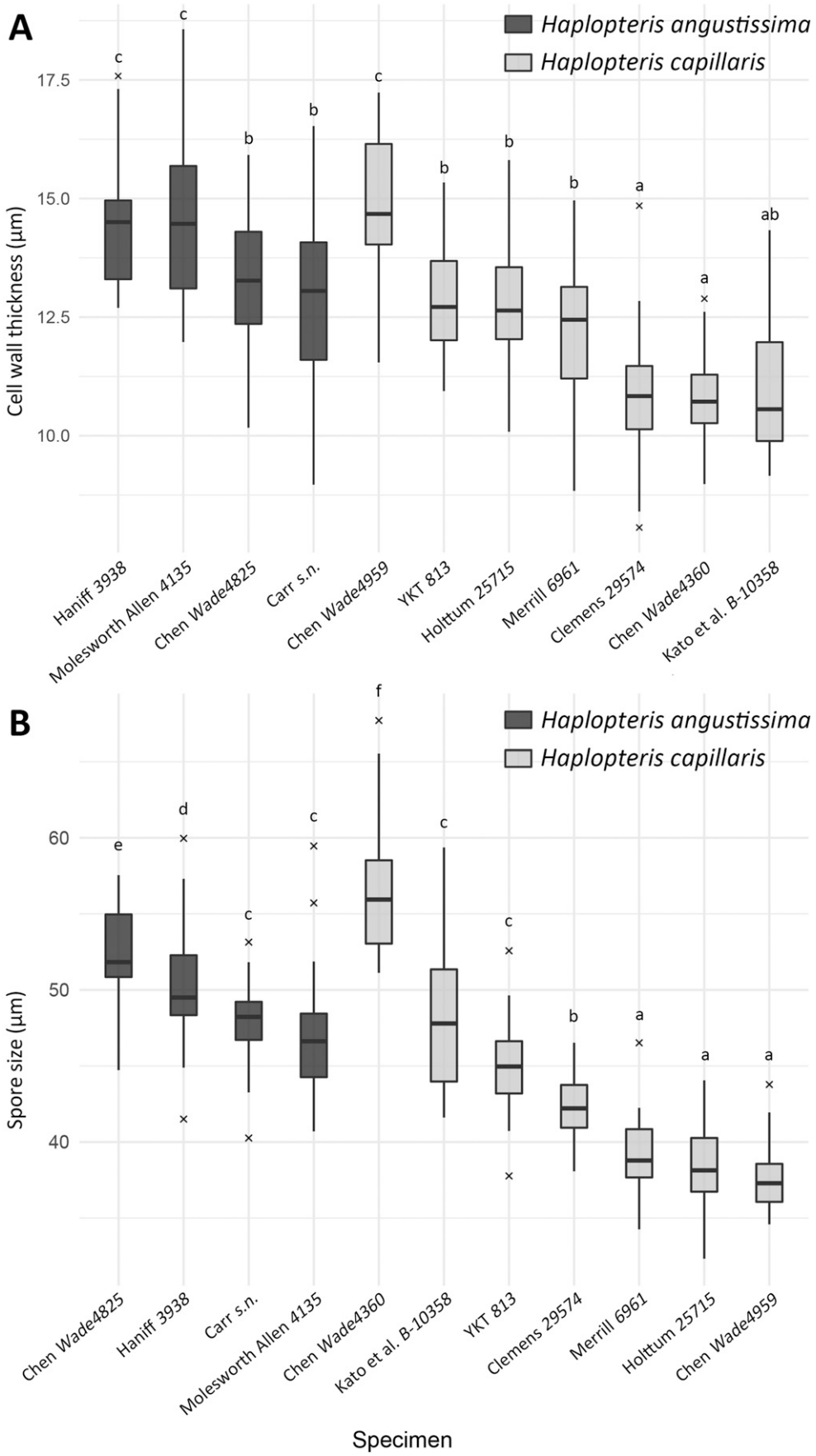

FIG. 4. Rhizome cell wall thickness (A) and spore size (B) in specimens of *Haplopteris angustissima* (dark grey) and *Haplopteris capillaris* (light grey). Median, 25%, and 75% quartiles are shown; whiskers indicate 5% and 95% percentiles. Specimens sharing the same letter are not significantly different ($p < 0.05$, Tukey HSD post-hoc test for significant differences among means).

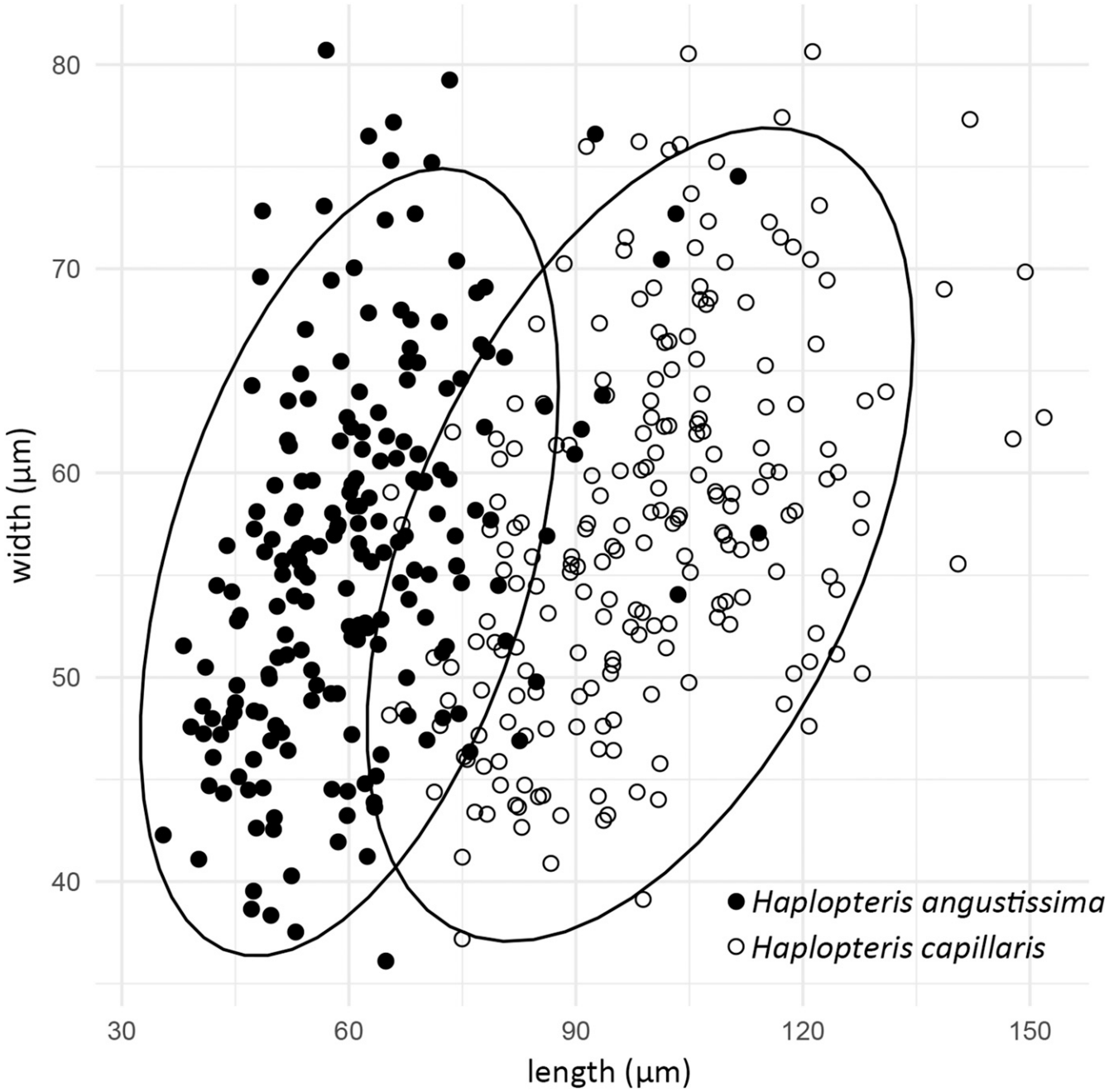

FIG. 5. Scatter plot of length and width of the end cells of the soral paraphyses of *Haplopteris angustissima* (solid circles) and *Haplopteris capillaris* (open circles). The 95% confidence ellipses are shown.

were able to include two other specimens of *H. capillaris* in the molecular phylogenetic analysis, one from Mt. Kanlaon, the type locality in the Philippines, and one from Sarawak. We found that all four samples form a well-supported clade and there is only a five bp difference between the sporophytic specimen from the Philippines and the independent gametophyte from Taiwan. Our results confirm the suspicions of Kuo et al. (2017) that independent gametophytes of *H. capillaris* occur in Taiwan.

Taxonomic Treatment

Haplopteris angustissima (Holttum) S.Linds., Gard. Bull. Singapore 62: 119. 2010. *Vittaria angustissima* Holttum, Gard. Bull. Singapore 11: 274. 1947. TYPE: MALAYSIA. Pahang: Fraser's Hill, 1219 m [4000 ft], Mar 1929, *C.E. Carr s.n.* (holotype: SING! [SING0144295]).

Epiphytic or lithophytic. **Rhizome** short-creeping, densely scaly, ca. 1 mm diam including scales, bearing fronds close together. **Rhizome scales** narrowly lanceolate, gradually narrowing from the base towards a long-tailed and ultimately filiform apex, 0.8–1.8 mm long and 0.2–0.3 mm wide (at base), clathrate, toothed at margin, the walls thickened and dentate. **Fronds** sessile, simple, linear, 2.0–13.2 cm long, 0.5–0.8 mm wide, widest above the middle, gradually narrowing towards both ends, apex acute or (occasionally) truncate, laminae coriaceous, glabrous, margins entire, flat on both surfaces. **Venation** difficult to see even with transmitted light, in fronds

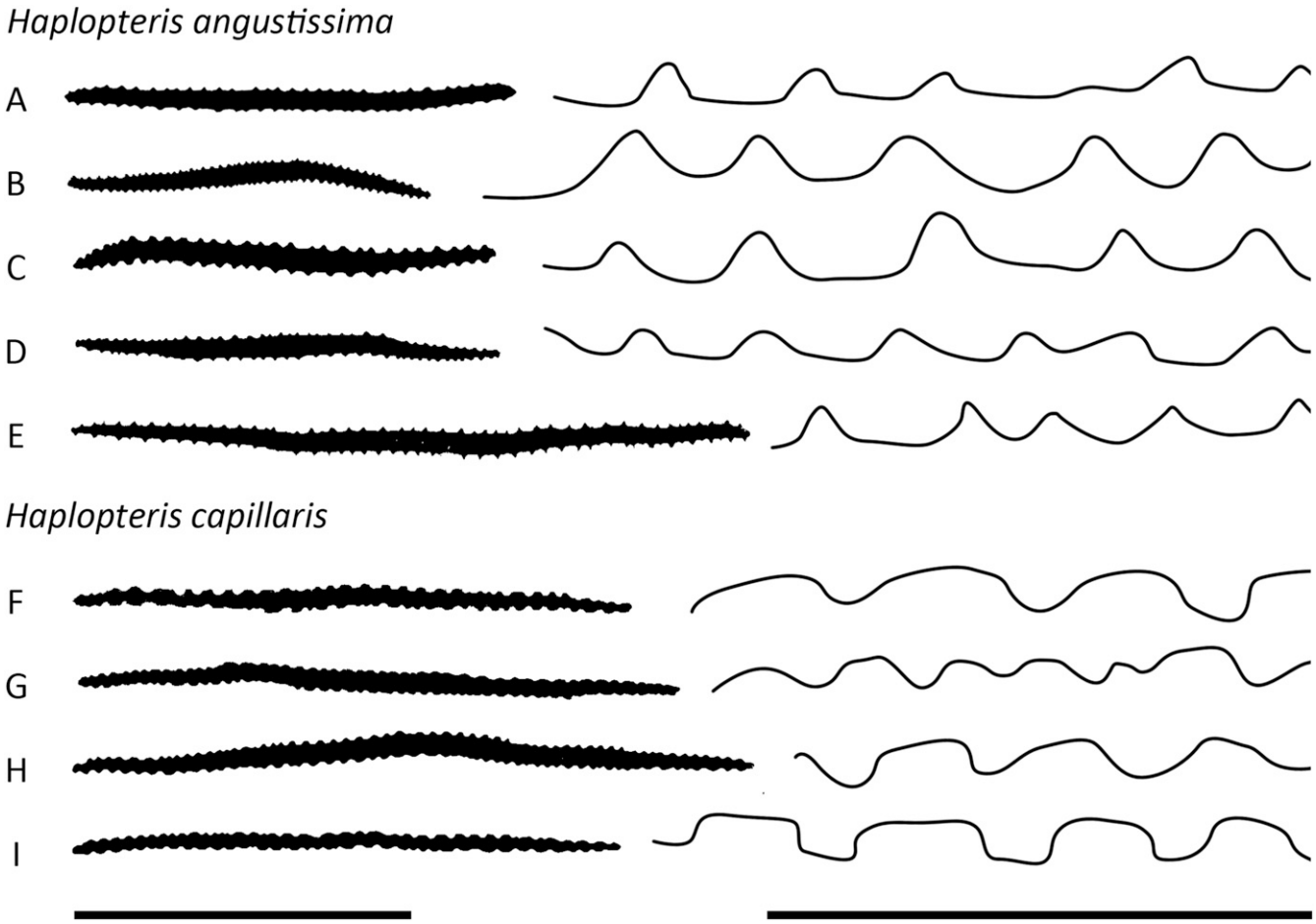

FIG. 6. Illustrations (left panel) and enlarged margins (right panel) of silica bodies on the adaxial surfaces of *Haplopteris angustissima* (A–E) and *Haplopteris capillaris* (F–I). A. *Carr s.n.* (holotype). B. *Chen Wade4825*. C. *Burkill & Holttum 8835*. D. *Molesworth Allen 3941*. E. *Smith 899*. F. *Chen Wade4360*. G. *Chen Wade4959*. H. *Kato et al. B-10358*. I. *Yong 813*. Scale bars = 300 μm (left panel) and 50 μm (right panel).

with single sori, midvein branching near frond base into two very close parallel main veins and reuniting near the frond apex, a few oblique lateral veins between the two main veins, forming a few long, narrow areoles; in fronds with double sori (our observation is based on the examination of one frond), midvein branching into three almost parallel main veins (midvein and two lateral veins) near frond base, very sparse oblique lateral veins present only on one side of midvein. **Epidermal silica bodies** linear, 0.1–0.7 mm long, present on both sides of the lamina, margin lobed, ends of lobes pointed. **Sori** linear, uninterrupted, following one main vein, slightly covered by a soral flap and open to one side, or rarely, fertile on both sides, each following a lateral main vein. **Soral paraphyses** copious, stalks uniseriate, filiform, colourless; apical cells large, funnel-shaped, length-to-width ratio of 1.1 ± 0.23, yellowish. **Spores** bilateral, bean-shaped, monolete, yellowish, surface smooth.

Distribution—Peninsular Malaysia. Known from only three areas on the Main Range (Fraser's Hill, Gunung Kerbau, and Cameron Highlands).

Ecology—Epiphytic on mossy tree trunks in damp forests or lithophytic on quartz outcrops near the ridge of mossy forests. The lowest recorded elevation is 1200 m and the highest is 1730 m.

Additional Specimens Examined—**Malaysia.**—PENINSULAR MALAYSIA: Pahang, Cameron Highlands, 1524 m [5000 ft], 4 Mar 1948, *Molesworth Allen, B.E.G. 1061* (K! [K000637847]); Pahang, Cameron Highlands, slope of G. Jasar, 1615 m [5300 ft], 20 Apr 1958, *Molesworh Allen, B.E.G. 3928* (K! [K000637844]); Pahang, Cameron Highlands, [See note below concerning discrepancies in locality data], underside of sloping trunk hanging over stream in tall dark forest. A mossy place. Only one patch (but found in other localities), rare, 1524 m [5000 ft], 22 Apr 1958, *Molesworth Allen, B.E.G. 3941* (K!, SING! [SING0085700]); Pahang, Cameron Highlands, Many fronds now dead, others medium green. In small clumps, very dwarfed, looking like *Monogramma trichoidea*. Abundant locally on vertical sites, in tiny crevices on the actual rock. Quartz outcrop [sometimes called "Castle Rock"] beyond Brinchang Village., 1615 m [5300 ft], 9 Feb 1959, *Molesworth Allen, B.E.G. 4135* (K!, 2 sheets [one with barcode K000637843], the other without barcode, US! [US01483064]); Pahang, Cameron Highlands, Brinchang Village, trail to Coral hill, lithophyte on quartz outcrop, rare, 4.495282°, 101.3955°, 1730 m, 5 Jul 2017, *Chen, C.-W. Wade4825* (KLU!), TAIF! [TAIF513482]); Pahang, Cameron Highlands, on Jacob's ladder, on underside of root base tangle, in very damp and dark position, 1524 m [5000 ft], 25 Oct 1965, *Evans 118* (KLU!); Pahang, Fraser's Hill ["Bukit Fraser"], 1219 m [4000 ft], Jun 1922, *Smith, E. 899* (K! [K000637846], SING! [SING0085650]); Pahang, Frasers Hill, upon the Selangor border, epiphyte on tree stump among moss. 1219–1331 m [4000–4370 ft], 26 Sep 1922, *Burkill, I.H, & Holttum, R.E. 8835* (SING! [SING0085634, SING0085635]); Pahang, Fraser's Hill, 1280 m [4200 ft], 20 Apr 1957, *Molesworth Allen, B.E.G. 3347* (US! [US1485955]); Perak, Gunung Inas, 1158 m, 7 Dec 1899, *Yapp, R.H. 417* (K! [K000637845]); Perak, Gunong Kerbau, 1371 m [4500 ft], May 1909, *Haniff, M. 3938* (SING! [SING0085673]).

Notes—1) The two sheets of *Molesworth Allen 3941*, from the Cameron Highlands show the same elevation data, collection date, and ecological information, but the one at K is labelled "slopes of G. Kemmiting" whereas the one at SING is labelled

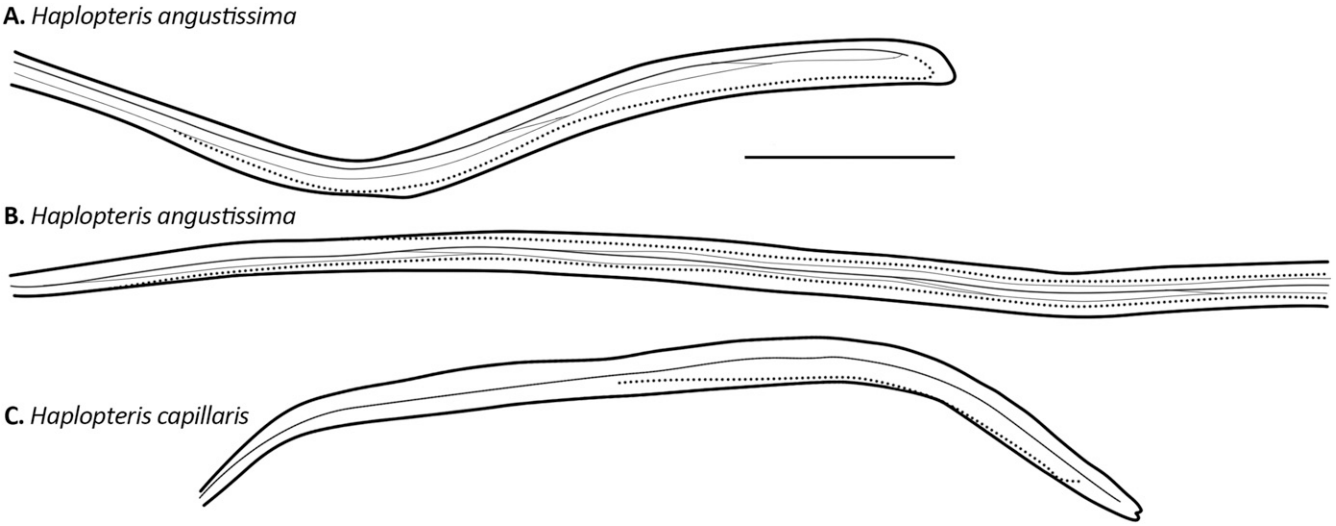

FIG. 7. Venation of *Haplopteris angustissima* (A from *Chen Wade4825*, B from *Carr s.n.* (holotype)) and *Haplopteris capillaris* (C from *Chen Wade4959*). The open margins of the soral flaps are indicated by dotted lines. Scale bar = 5 mm.

"Forest above Walkerburn Cottage." 2) The locality of *Chen Wade4825* is possibly the same locality as *Molesworth Allen 4135*.

Haplopteris capillaris (Copel.) C.W.Chen, S.Linds. & K.T.Yong, comb. nov. *Monogramma capillaris* Copel., Philipp. J. Sci. C. 6: 147. 1911. TYPE: PHILIPPINES. Negros. Mt. Kanlaon ["Canlaon"], Apr 1910, *E.D. Merrill 6961* (holotype PNH (lost); lectotype MICH! [MICH1309835], designated by Price (1990); isolectotypes P [P00608464], NY [NY00127501]).

Vaginularia capillaris (Copel.) C.Chr., Index Filic., Suppl. Tert. 193. 1934.

Pleurofossa capillaris (Copel.) Nakai ex H.Ito, J. Jap. Bot. 12: 408. 1936.

Epiphytic. **Rhizome** short-creeping, densely scaly, ca. 1 mm diam including scales, bearing fronds close together. **Rhizome scales** narrowly lanceolate, gradually narrowing from the base towards a long-tailed and ultimately filiform apex, 0.7–2 mm long and 0.2–0.3 mm wide (at base), clathrate, toothed at margin, the walls thickened and dentate, sometimes reddish. **Fronds** sessile, simple, linear, 2.7–4.5 cm long, 0.6–0.8 mm wide, widest above the middle, gradually narrowing towards both ends, apex acute or (occasionally) retuse, laminae chartaceous, glabrous, margins entire, flat on both surfaces. **Venation** difficult to see even with transmitted light, simple, with only a midvein (in fronds with single sori). **Epidermal silica bodies** linear, 0.1–0.64 mm long, present on both sides of lamina, margin lobed, ends of lobes blunt. **Sori** linear, uninterrupted, following the midvein, covered by a soral flap and open to one side, very rarely fertile on both sides. **Soral paraphyses** copious, stalks uniseriate, filiform, colourless; apical cells large, funnel-shaped, length-to-width ratio of 1.7 ± 0.23, yellowish. **Spores** bilateral, bean-shaped, monolete, yellowish, surface smooth.

Distribution—Only known from 10 collections. The type and *Chen Wade4959* are from Negros, the Philippines, seven collections are from Borneo (Sabah, Sarawak, Kalimantan, Brunei), and one collection is from an independent gametophyte population in Northern Taiwan.

Ecology—A low epiphyte on mossy tree trunks in damp forests. The lowest recorded elevation is 1100 m and the highest is 1600 m.

Additional Specimens Examined—**Brunei.**—TEMBURONG: Ulu Belalong, 540 m, 17 Jan 1994, *Dransfield, J. 7350* (K! [K000507730]); NE of G. Retak, 1125 m, 10 Mar 1991, *Johns, R.J. 6637* (K! [K000705731]). **Indonesia.**—EAST KALIMANTAN: Gunung Batu Harun, 1150–1650 m, 25 Jul 1981, *Kato, M., Okamoto, M., & Walujo, E.B. B-9856* (KYO!); Gunung Batu Linanit, 1100 m, 31 Jul 1981, *Kato, M., Okamoto, M., & Walujo, E.B. B-10358* (KYO! & L! [L3641722]). **Malaysia.**—SABAH: Mt. Kinabalu, ca. 1676 m [5500 ft], 15 Feb 1957, *Molesworth Allen, B.E.G. 3217* (US! [US01485956], K! [K000699438]); Tenompok, 1432 m [4700 ft], 10 Nov 1931, *Holttum, R.E. 25715* (SING! [SING0040889]); Tenompok, 1676 m [5500 ft], 5 May 1932, *Clemens, J. & M.S. 29574* (G! US! [US01503549]); Mt. Trus Madi, Jaafar Nyiro Cabin, epiphyte on tree fern trunk, 1400 m, 10 May 2015, *Chen, C.-W. Wade4360* (SAN! [SAN130433], TAIF! [TAIF468793]).—SARAWAK: Miri Division, Upper Baram, Tama Abu Protected Forest, patches of virgin and > 20 yr old logged over forest in adjacent to Sungai (River) Baleh, between Long Beruang and Pa Dali. Transect no. 7. Kerangas forest near the transect end, 980 m, 22 Aug 2017, *Yong, K.T. 813* (TAIF! [TAIF513503]). **Philippines.**—NEGROS: Murcia, trail from Wasay DENR Ranger Station to Mt. Canlaon, 10.47358°, 123.1242°, 1300 m, 26 Oct 2017, *Chen, C.-W. Wade4959* (CMUH! [CMUH00010961], TAIF! [TAIF512395]).

ACKNOWLEDGMENTS

We thank the Department of Environment & Natural Resources (DENR), Sabah Biodiversity Centre, and Sabah Forestry Department for granting us collecting permits in the Philippines and Sabah, respectively. We also thank Germinal Rouhan for sharing samples; Lisa Paguntalan for logistics support while exploring Mt. Kanlaon; the curators of K, KLU, KYO, L, MICH, SING, TAIF, and US for access to and loans of specimens; and two anonymous reviewers for their valuable suggestions. Funding for this study was provided by the Taiwan Ministry of Science and Technology (MOST 106-2621-B-054-002).

AUTHOR CONTRIBUTIONS

CWC designed the study and analyzed the data. CWC and SL collected the data and drafted the paper. CWC, KTY, AMAM, and VBA collected the samples. VDD analyzed the data and did statistics. All the authors edited, contributed comments, and approved the final manuscript.

LITERATURE CITED

Bhandari, J. B. and R. Mukhopadhyay. 2009. *The Vittarioid Ferns of India*. Dehre Dun: Bishen Singh Mahendra Pal Singh.

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.

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., E. Schuettpelz, S. Lindsay, and D. J. Middleton. 2016. (2454) Proposal to conserve the name *Haplopteris* against *Monogramma* (Pteridaceae). *Taxon* 65: 884–885.

Chen, C.-W., S. Lindsay, L.-Y. Kuo, C. R. Fraser-Jenkins, A. Ebihara, H. T. Luu, C. W. Park, Y.-S. Chao, Y.-M. Huang, and W.-L. Chiou. 2017. A systematic study of East Asian vittarioid ferns (Pteridaceae: Vittarioideae). *Botanical Journal of the Linnean Society* 183: 545–560.

Chen, C.-W., S. Lindsay, K. T. Yong, A. M. A. Mustapeng, V. B. Amoroso, V. D. Dang, and Y.-M. Huang. 2019. Data from: Clarification of two poorly known vittarioid ferns (Pteridaceae): *Haplopteris angustissima* and *H. capillaris*. Dryad Digital Repository. https://doi.org/10.5061/dryad.k1466kq.

Christensen, C. 1934. Index Filicum, Supplementum Tertium pro Annis 1917–1933. Hafniae: H. Hagerup.

Copeland, E. B. 1911. New or interesting Philippine ferns, V. *The Philippine Journal of Science, C* 6: 145–148.

Copeland, E. B. 1960. *Fern Flora of the Philippines,* vol. 3. Manila: Bureau of Printing.

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.

Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321.

Holttum, R. E. 1947. New species of vascular plants from the Malay Peninsula. *Gardens' Bulletin (Singapore)* 11: 267–298.

Ito, H. 1936. Vittarieae Japonenses I. *Journal of Japanese Botany* 12: 401–410.

Kao, T.-T., S.-J. Chen, W.-L. Chiou, Y.-C. Chuang, and L.-L. Kuo-Huang. 2008. Various microscopic methods for investigating the venuloid idioblasts of *Pteris grevilleana* Wall. *Taiwania* 53: 394–400.

Kuo, L.-Y. 2015. Polyploidy and Biogeography in Genus Deparia and Phylogeography in Deparia lancea. Ph.D. thesis. Taipei: National Taiwan University.

Kuo, L.-Y., C.-W. Chen, W. Shinohara, A. Ebihara, H. Kudoh, H. Sato, Y.-M. Huang, and W.-L. Chiou. 2017. Not only in the temperate zone: Independent gametophytes of two vittarioid ferns (Pteridaceae, Polypodiales) in East Asian subtropics. *Journal of Plant Research* 130: 255–262.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.

Lanfear, R., P. Frandsen, A. Wright, and T. Senfeld. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. *Molecular Biology and Evolution* 34: 772–773.

Lindsay, S. 2010. New combinations in *Haplopteris* (Adiantaceae) for the Flora of Peninsular Malaysia. *Gardens' Bulletin (Singapore)* 62: 119–120.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. Pp. 1–8 in *Proceedings of the Gateway Computing Environments Workshop (GCE)*. New Orleans: Gateway Computing.

Parris, B. S. and A. Latiff. 1997. Towards a pteridophyte flora of Malaysia: a provisional checklist of taxa. *Malayan Nature Journal* 50: 235–280.

Pelser, P. B., J. F. Barcelona, and D. L. Nickrent (eds). 2011 [onwards]. *Co's Digital Flora of the Philippines*. http://www.philippineplants.org (last accessed May 2018).

Price, M. G. 1990. Philippine fern notes. *Contributions from the University of Michigan Herbarium* 17: 267–278.

R Core Team. 2017. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Ruhfel, B., S. Lindsay, and C. C. Davis. 2008. Phylogenetic placement of *Rheopteris* and the polyphyly of *Monogramma* (Pteridaceae s.l.): Evidence from *rbcL* sequence data. *Systematic Botany* 33: 37–43.

Schuettpelz, E., C.-W. Chen, M. Kessler, J. B. Pinson, G. Johnson, A. Davila, A. T. Cochran, L. Huiet, and K. M. Pryer. 2016. A revised generic classification of vittarioid ferns (Pteridaceae) based on molecular, micromorphological, and geographic data. *Taxon* 65: 708–722.

Sundue, M. 2009. Silica bodies and their systematic implications in Pteridaceae (Pteridophyta). *Botanical Journal of the Linnean Society* 161: 422–435.

Vasco, A., M. Thadeo, M. Conover, and D. C. Daly. 2014. Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Applications in Plant Sciences* 2: 1400038.

Wickham, W. 2009. ggplot2: Elegant graphics for data analysis. New York: Springer-Verlag.

Zhang, G. M., W. B. Liao, M. Y. Ding, Y. X. Lin, Z. H. Wu, X. C. Zhang, S. Y. Dong, J. Prado, M. G. Gilbert, G. Yatskievych, T. A. Ranker, E. A. Hooper, E. R. Alverson, J. S. Metzgar, A. M. Funston, S. Masuyama, and M. Kato. 2013. Pteridaceae. Pp. 169–256 in *Flora of China,* vol. 2–3 *(Pteridophytes)*, eds. Z. Y. Wu, P. H. Raven, D. Y. Hong. Beijing: Science Press; St. Louis: Missouri Botanical Garden Press.

Zwickl, D. J. 2006. GARLI. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion. Ph.D. thesis. Austin: University of Texas.

Appendix 1. Specimens used for molecular phylogenetic analysis. For each specimen, the species name, voucher information (herbarium), place of origin, and GenBank accession numbers (*atpA*, *chlL*, *matK*, *ndhF*, *rbcL*, *rpoA*, *trnL-F*) are provided. An em-dash (—) indicates unavailable information; new sequences are in bold.

Haplopteris alternans (Copel.) S.Linds. & C.W.Chen, *Chen Wade4000* (TAIF), Negros, Philippines, **MH359241**, KX896804, KX896840, KX896882, **MH359275**, **MH359296**, KX896914. *Haplopteris angusta-elongata* (Hayata) E.H.Crane, *Chen Wade1467* (TAIF), Pingdong, Taiwan, **MH359242**, KF815947, KC812873, KC812907, **MH359276**, **MH359297**, JN869336. *Haplopteris angustissima* (Holttum) S.Linds., *Chen Wade4825* (TAIF), Pahang, Malaysia, **MH359243**, **MH359257**, **MH359263**, **MH359269**, **MH359277**, **MH359298**, **MH359315**. *Haplopteris capillaris* (Copel.) C.W.Chen, S.Linds. & K.T.Yong, *Chen Wade4360* (TAIF), Sabah, Malaysia, **MH359244**, KX896825, KX896861, KX896903, **MH359278**, **MH359299**, KX896948. *Haplopteris capillaris* (Copel.) C.W.Chen, S.Linds. & K.T.Yong, *Chen Wade4959* (TAIF), Negros, Philippines, **MH359245**, **MH359258**, **MH359264**, **MH359270**, **MH359279**, **MH359300**, **MH359316**. *Haplopteris capillaris* (Copel.) C.W.Chen, S.Linds. & K.T.Yong, *YKT 813* (TAIF), Sarawak, Malaysia, **MH359246**, **MH359259**, **MH359265**, **MH359271**, **MH359280**, **MH359301**, **MH359317**. *Haplopteris capillaris* (Copel.) C.W.Chen, S.Linds. & K.T.Yong, *A* (TAIF), Ilan, Taiwan, —, KX896820, KX896856, KX896898, —, —, KX896941. *Haplopteris capillaris* (Copel.) C.W.Chen, S.Linds. & K.T.Yong, *E30* (TAIF), Ilan, Taiwan, **MH359247**, —, —, —, **MH359281**, **MH359302**, —. *Haplopteris dareicarpa* (Hook.) S.Linds. & C.W.Chen, *Chen Wade4217* (TAIF), Sabah, Malaysia, **MH359248**, KX896806, KX896842, KX896884, **MH359282**, **MH359303**, KX896915. *Haplopteris elongata* (Sw.) E.H.Crane, *Chen Wade1731* (TAIF), Java, Indonesia, **MH359249**, KF815953, KC812892, KC812926, **MH359283**, **MH359304**, KC812960. *Haplopteris ensata* (Christ) C.W.Chen & S.Linds., *Chen Wade2588* (TAIF), Lam Dong, Vietnam, KX165188, KY101215, KY101285, KY101355, KX164982, KX164880, KY101421. *Haplopteris ensiformis* (Sw.) E.H.Crane, *Bauret 29* (P), Toamasina, Madagascar, **MH359250**, **MH359260**, **MH359266**, **MH359272**, **MH359284**, —, **MH359318**. *Haplopteris flexuosa* (Fée) E.H.Crane, *Fraser-Jenkins 33825* (TAIF), Meghalaya, India, **MH359251**, KF815952, KC812888, KC812922, **MH359285**, **MH359305**, KC812956. *Haplopteris graminea* (Poir.) comb. ined., *Janssen 2692* (P), St. Philippe, La Réunion, KX165202, KX896826, KX896862, KX896904, EF452157, KU147304, KC812964. *Haplopteris guineensis* (Desv.) E.H.Crane, *Nek 223* (MO), Ogooué-Maritime, Gabon, —, MG983934, MG983944, MG983954, **MH359286**, —, KX896921. *Haplopteris hainanensis* (C.Chr.) E.H.Crane, *Wu 959* (TAIF), Hainan, China, KX165205, KF815958, KC812904, KC812938, KX165007, KX164899, KC812972. *Haplopteris heterophylla* C.W.Chen, Y.H.Chang & Yea C.Liu, *Wu 1038* (TAIF), Hainan, China, KX165206, KY101192, KC812903, KC812937, KX165008, KX164900, KC812971. *Haplopteris humblotii* (Hieron.) S.Linds. & C.W.Chen, *Rasolohery 663* (MO), Toamasina, Madagascar, —, KX896811, KX896847, KX896889, MH359287, **MH359306**, KX896926. *Haplopteris longicoma* (Christ) E.H.Crane, *Chen Wade4252* (TAIF), Sabah, Malaysia, **MH359252**, KX896812, KX896848, KX896890, **MH359288**, **MH359307**, KX896927. *Haplopteris malayensis* (Holttum) E.H.Crane, *Chen Wade4412* (TAIF), Pahang, Malaysia, —, **MH359261**, **MH359267**, **MH359273**, **MH359289**, **MH359308**, **MH359319**. *Haplopteris scolopendrina* (Bory) C.Presl, *Rouhan*

363 (P), Toamasina, Madagascar, —, **MH359262**, **MH359268**, **MH359274**, **MH359290**, **MH359309**, **MH359320**. *Haplopteris sessilifrons* (Miyamoto & H.Ohba) S.Linds., *Chen Wade4209* (TAIF), Sabah, Malaysia, **MH359253**, KX896816, KX896852, KX896894, **MH359291**, —, KX896935. *Haplopteris sikkimensis* (Kuhn) E.H.Crane, *C.R.Fraser-Jenkins 34211* (TAIF), Kathmandu, Nepal, **MH359254**, KF815951, KC812889, KC812923, **MH359292**, **MH359310**, KC812955. *Haplopteris taeniophylla* (Copel.) E.H.Crane, *FWL 974* (TAIF), Luzon, Philippines, **MH359255**, KY101190, KY101261, KY101331, **MH359293**, **MH359311**, KC812969. *Haplopteris volkensii* (Hieron.) E.H.Crane, *Mwangoka et al. 7722* (MO), Morogoro, Tanzania, —, KX896822, KX896858, KX896900, —, **MH359312**, KX896944. *Haplopteris yakushimensis* C.W.Chen & Ebihara, *Oka K-090106* (TNS), Yakushima, Japan, **MH359256**, KF815961, KF815967, KF815973, **MH359294**, **MH359313**, KX896946. *Radiovittaria gardneriana* (Fée) E.H.Crane, *Rothfel 3740* (DUKE), Pichincha, Ecuador, —, KX896828, KX896864, KX896906, **MH359295**, **MH359314**, **MH359321**.