American Journal of Botany

American Journal of Botany 98(11): 1782–1800. 2011.

Reticulate evolution in the *Crepidomanes minutum* species complex (Hymenophyllaceae)[1]

Joel H. Nitta[2,4], Atsushi Ebihara[3], and Motomi Ito[2]

[2]Department of System Sciences, Graduate School of Arts and Sciences, University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan; and [3]Department of Botany, National Museum of Nature and Science, 4-1-1 Amakubo, Tsukuba 305-0005, Japan

- *Premise of the Study:* Hybridization is an important mechanism of speciation in plants and often results in complexes that comprise multiple sexual diploids and their hybrid offspring. However, the intricacy of these systems has prevented a thorough understanding of many groups. The *Crepidomanes minutum* species complex (Hymenophyllaceae) is a widely distributed, morphologically variable fern species complex. Although prior reports of apogamy and polyploidy suggest hybridization, it has never been the focus of a phylogenetic study.
- *Methods:* Morphology, nuclear (*gapCp*), and chloroplast (*rbcL*) DNA sequences, cytology, field observation, and spore counts were used to infer phylogeny and trace hybrid origins.
- *Key Results:* The *C. minutum* species complex is composed of at least three major clades: the African clade, clade 1 (East Asia and the Pacific), and clade 2 (Southeast Asia and the South Pacific). Clades 1 and 2 differ strikingly in morphological variation (uniform in clade 1 vs. highly variable in clade 2) and occurrence of hybrids (rare in clade 1 vs. frequent in clade 2). Apogamy and polyploidy were confirmed as likely mechanisms of hybrid stabilization in clade 2. Despite the large genetic distance between clades 1 and 2, several specimens were observed with *gapCp* sequences from both; diploid genome size and sexual reproduction indicate maintenance of genetic diversity via introgression or incomplete lineage sorting, rather than ongoing hybridization, in these specimens.
- *Conclusions:* The *C. minutum* species complex is a reticulate network including multiple diploid lineages and their stabilized hybrid crosses. Additional sampling focused on reproductive mode and ploidy level is needed to delimit diploid species and hybrids.

Key words: *Crepidomanes minutum*; ferns; *gapCp*; *Gonocormus*; Hymenophyllaceae; hybrid; *rbcL*; reticulate evolution; species complex.

Evolution at the species level and above is generally depicted in the form of a bifurcating tree based on the assumption that new species arise by divergence from a common ancestor (Darwin, 1859). However, there are also evolutionary processes that cannot be represented by bifurcation, collectively known as reticulate evolution (Legendre, 2000; Linder et al., 2004). In reticulate evolution at the species level, new species arise by hybridization events between already diverged species; this leads to a network rather than a tree (Otto and Whitton, 2000; Rieseberg, 2001; Linder and Rieseberg, 2004; Linder et al., 2004; Makarenkov and Legendre 2004). Hybridization is particularly likely to occur in plant species complexes in which species are recently derived and the barriers between them relatively weak (Stebbins, 1950; Grant, 1981; Arnold, 1992). In plants F_1 hybrids are capable of overcoming postzygotic barriers through several stabilizing mechanisms, including polyploidy and apogamy (Grant, 1981). Additionally, autopolyploids (polyploids containing multiple copies of chromosomes derived from a single parent species), although not themselves hybrids, may also be present and involved as parents in hybridization events (Ramsey and Schemske, 1998).

Hybridization, polyploidy, and apogamy are all known to occur frequently in ferns (Barrington et al., 1989). Ferns may be able to form hybrids more easily than seed plants because they lack the sophisticated breeding systems (e.g., pollen–stigma interactions) present in seed plants that help prevent interspecific crossing (Haufler, 2002). High incidences of hybrid species have been recorded in North America (20%; Flora of North America Editorial Committee, 1993) and Japan (ca. 300 of 730 species total; Nakaike, 2004). Although apogamous fern species cannot interbreed, they are capable of producing functioning sperm and can thus be involved in reticulate networks within species complexes (e.g., *Pellaea*, *Notholaena*, and *Cheilanthes*; Gastony and Windham, 1989; Grusz et al., 2009).

The *Crepidomanes minutum* (Blume) K. Iwats. species complex (Hymenophyllaceae) was chosen as a study system to investigate reticulate evolution in ferns based on reports of apogamous reproduction, polyploidy, and morphological complexity suggestive of hybridization (see references below). These epiphytic (sometimes epipetric) ferns are similar to other hymenophylloid epiphytes in their extremely reduced morphology, with filamentous rhizomes lacking true roots, leaf

[1]Manuscript received 30 November 2010; revision accepted 29 August 2011.

The authors thank D. Darnaedi, G. Kokubugata, U. Hapid, L.-Y. Kuo, B. Mishler, and C.-I. Peng for assistance with field collections and J.-Y. Dubuisson, H. Kato, L.-Y. Kuo, M. Uzawa, and M. Yokota for providing additional plant material. J. Beck, D. Farrar, B. Mishler, K. Pryer, E. Schuettpelz, M. Windham, and two anonymous reviewers provided helpful comments on drafts. N. Nakato performed chromosome counts and K. Yamamoto assisted with flow cytometry. Research supported in part by the Japanese Government (Monbukagakusho: MEXT) Scholarship and University of Tokyo Academic Research Grant Program (International).

[4]Author for correspondence (e-mail: jnitta@oeb.harvard.edu). Present address: Department of Organismic and Evolutionary Biology, Harvard University Herbaria, 22 Divinity Avenue, Cambridge, Massachusetts 02138-2020, USA

doi:10.3732/ajb.1000484

TABLE 1. Examples of taxonomic treatments recognizing two taxa in the *Crepidomanes minutum* species complex.

Reference	Locality	Taxon 1 [a]	Taxon 2 [b]
Tindale 1963	Australia	*Gonocormus saxifragoides* (C. Presl) Bosch	*Gonocormus minutus* (Blume) Bosch
Holttum 1966	Malaysia	*Trichomanes minutum* Blume	*Trichomanes proliferum* Blume
Sledge 1968	Sri Lanka	*Trichomanes saxifragoides* C. Presl	*Trichomanes proliferum* Blume
Croxall 1975	Australia	*Gonocormus saxifragoides* (C. Presl) Bosch	*Gonocormus prolifer* (Blume) Prantl
Brownlie 1977	Fiji [c]	*Trichomanes saxifragoides* C. Presl	—
Tsai and Shieh 1994	Taiwan [c]	*Gonocormus minutus* (Blume) Bosch	—
Hameed et al. 2003 [d]	India	*Crepidomanes saxifragoides* (C. Presl) P. S. Green	*Crepidomanes proliferum* (Blume) Bostock

[a]Taxon 1 is flabellate and nonproliferous.
[b]Taxon 2 is pinnatifid with proliferations.
[c]Specimens matching in morphology with taxon 2 not reported from Taiwan or Fiji.
[d]Hameed et al. (2003) recognize two varieties of taxon 2.

laminae only a single cell layer thick, lack of cuticle or stomata, and small overall frond size (many taxa ≤1 cm, the largest no greater than 5–6 cm). Gametophytes are capable of asexual reproduction via gemmae (Yoroi, 1972). Proliferous growth of new leaves from petioles or other leaf laminae is often observed (Bell, 1960) and has been used as a character in species delimitation (Table 1). Plants are found growing in mats of individuals with interwoven rhizomes on trees or rocks in the tropics to humid temperate zones and are widely distributed from Africa to the Pacific (Yoroi and Iwatsuki, 1977).

Taxonomy of the *C. minutum* species complex has been characterized by nomenclatural confusion. In one of the earliest treatments, Blume (1828) distinguished three species from Java based on leaf shape and stipe length: *Trichomanes parvulum* Poir., *T. minutum* Blume, and *T. proliferum* Blume; of these, *T. parvulum* was later confused with flabellate *Hymenophyllum* and is now considered to be a synonym of *Hymenophyllum sibthorpioides* (Bory ex Willd.) Mett. Van den Bosch (1861) described the complex as comprising four species, which he differentiated on the basis of frond shape, presence or absence of proliferations, cell walls, and involution of segments. Copeland (1933) initially followed the taxonomy of van den Bosch, later expressed doubt as to these species delimitations (Copeland, 1938), and finally came to the conclusion that no species could be differentiated in the complex because of the continuous nature of morphological characters (Copeland, 1958). Later authors have tended to split the group into two species on the basis of two apparently related characters: leaf shape and presence or absence of proliferations (flabellate and absent vs. pinnatifid and present). However, names for these two taxa vary by author, and further taxonomic confusion has been caused by occasional misapplication of Blume's names (Table 1). In addition to these two widely distributed taxa, several endemic species have been described, including *T. novoguineense* Brause (New Guinea), *Gonocormus bonincola* (Nakai) Tagawa (Ogasawara Islands, Japan), *G. samoensis* Copel. (Samoa), and *G. siamensis* Tagawa & K. Iwats. (Thailand). The most recent family-wide revision of Hymenophyllaceae based on molecular data places all these and several other more obscure names into one polymorphic species complex comprising a monotypic section (*Crepidomanes* subgen. *Crepidomanes* sect. *Gonocormus*; Ebihara et al., 2006).

One possible explanation for the morphological complexity that has hampered species delimitation in the complex is the occurrence of hybrid taxa. These would display intermediate morphologies that could not be easily assigned to one species or another. Copeland (1958) found that specimens of the *C. minutum* complex from Mt. Makiling, Philippines, displayed a great diversity of morphologies with many intermediate forms and was unable to distinguish any separate species (although he attributed this to phenotypic plasticity rather than genetic variation). In addition to morphology, the existence of hybrid taxa in the complex has also been indicated by cytology. At least two sexual cytotypes have been identified: $n = 36$ (most likely the base number; Braithwaite, 1975) and $n = 72$, a sexual tetraploid (Braithwaite, 1975). Evidence for apogamous reproduction following the Braithwaite system (Braithwaite, 1964; Walker, 1985) in the complex has been reported in specimens from multiple localities representing at least two cytotypes, including India ($n = 108$; Mehra and Singh, 1957), Malaysia ($n = 108$; Bell, 1960), the Solomon Islands ($n = 72$; Braithwaite, 1969), and New Hebrides ($n = 108$; Braithwaite, 1975). Apogamy is uncommon in Hymenophyllaceae (Stokey, 1948; Yoroi, 1976; Tilquin, 1978), and no apogamous species complex in Hymenophyllaceae has yet been the subject of a phylogenetic study to our knowledge.

Hybrids are often cited as a source of morphological variation in taxonomic studies; however, this assumption is difficult to defend without genetic data (Rieseberg, 1997). On the other hand, incongruence between molecular phylogenies may be

TABLE 2. Outgroup taxa.

Outgroup taxon	Specimen ID	Subgenus	Section
Crepidomanes humile (G. Forst.) Bosch	V2	*Crepidomanes*	*Crepidium*
Crepidomanes bipunctatum (Poir.) Copel.	N24	*Crepidomanes*	*Crepidomanes*
Crepidomanes christii (Copel.) Copel.	K27	*Crepidomanes*	*Crepidomanes*
Crepidomanes fallax (Christ) Ebihara & Dubuisson	M6	*Crepidomanes*	*Crepidomanes*
Crepidomanes kurzii (Bedd.) Tagawa & K. Iwats.	A16	*Crepidomanes*	*Crepidomanes*
Crepidomanes vitiense (Baker) Bostock	A11	*Crepidomanes*	*Crepidomanes*
Crepidomanes latealatum (Bosch) Copel.	A14	*Crepidomanes*	*Crepidomanes*

Note: Taxonomy follows Ebihara et al. (2006).

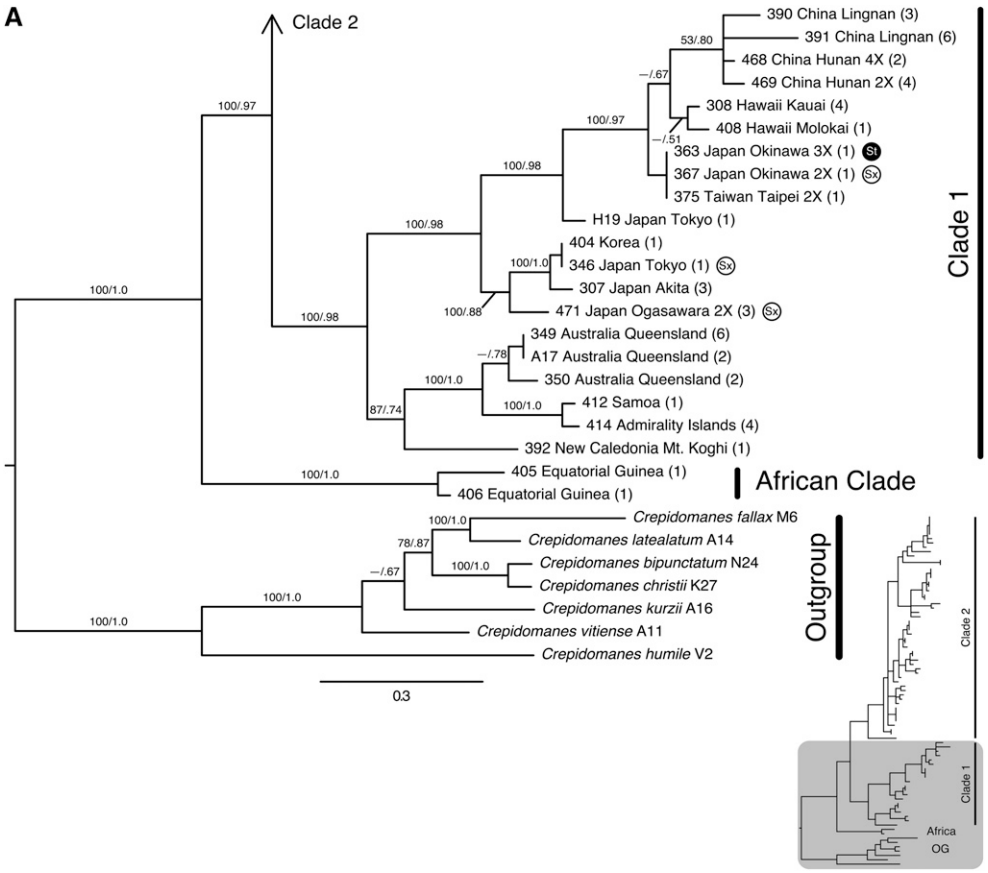

Fig. 1. Phylogeny of the *Crepidomanes minutum* species complex resulting from Bayesian analysis of chloroplast *rbcL*. Specimen ID number and locality plus the following data are indicated for each ingroup taxon when available: ploidy level (>2× indicates polyploid but exactly ploidy unknown), reproductive mode (indicated as follows: white circle with "Sx," sexual; gray circle with "Ap," apogamous; black circle with "St," sterile; white circle with "P," precocious), and number of *gapCp* alleles (in parentheses). Bayesian posterior probabilities (PP) and maximum parsimony bootstrap values (BS) are indicated above each branch (BS/PP); support values are given only for nodes with PP or BS >50%. Branch lengths proportionate to expected amount of change per site as inferred by Bayesian analysis are indicated by scale bar. Inset depicts structure of overall tree; location of the current figure part is indicated by shaded gray box.

due to factors other than hybridization, such as concerted evolution, gene conversion, and incomplete lineage sorting (Wendel and Doyle, 1998). To properly elucidate hybridization events, an integrative approach is needed (Grusz et al., 2009). The present study utilizes multiple data types including DNA (chloroplast *rbcL* and nuclear *gapCp*), cytology, morphology, and observation of spores to investigate: (1) the phylogenetic structure of the *C. minutum* species complex, (2) the occurrence of hybrid taxa within the complex, and (3) the mechanisms by which hybrid taxa are stabilized.

MATERIALS AND METHODS

Materials—Given the wide distribution (Old World tropics) of the *C. minutum* species complex, thorough field collection across the range was not feasible. Rather, field collection was conducted for the current study at selected representative localities (Java, Moorea, Taiwan, and Okinawa) from April 2008 to May 2009; small amounts of fresh material were obtained from collaborators for a few additional localities (Ogasawara Islands, China, Philippines). Voucher specimens were deposited at TI, TNS, and UC. Herbarium specimens representing additional localities from TI, TNS, UC, and KYO were examined for morphological characters and sampled for DNA extraction (recent collections only). Given the confusion over species delimitation in the complex, no attempt was made to sample according to previous species concepts; rather, specimens were chosen to encompass as much morphological and geographic variation as possible. Several con-subgeneric species (sensu Ebihara et al., 2006) were selected as outgroup taxa (see Table 2). *Crepidomanes* has been supported as monophyletic by several recent phylogenetic studies (Pryer et al., 2001; Dubuisson et al., 2003; Ebihara et al., 2007).

Molecular analyses—Total genomic DNA was extracted from material dried on silica gel (field collected specimens) or dried leaves (herbarium specimens) using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's instructions or by modified CTAB method (Hasebe and Iwatsuki, 1990). Approximately 1.4 kb of chloroplast *rbcL* was amplified by polymerase chain reaction (PCR) using primers af and 1379R (Pryer et al., 2001) in 20-μL reaction mixtures containing 1 μL total genomic DNA, 2 μL of 10× Takara Ex Taq buffer (Takara, Shiga, Japan), 1.6 μL of 2.5 mmol/L dNTP mix, 1 μL each primer (10 pmol/μL), and 0.1 μL of Takara Ex Taq DNA polymerase (5 U/μL; Takara) under the following temperature and cycle conditions: initial denature step at 94°C for 30 s, 30 cycles of 94°C for 30 s, 52°C for 90 s, 72°C for 90 s, and a final extension at 72°C for 10 min. The ca. 270 base pair (bp) region containing intron 10 and portions of flanking exons of nuclear *gapCp* was amplified using a nested PCR approach (exon and intron numbering follows that of the *Pinus gapCp* gene; GenBank accession AJ001706; Meyer-Gauen et al., 1994). Primers ESGAPCP8F1 and ESGAPCP11R1 (Schuettpelz et al., 2008) were first used to amplify the ca. 900-bp region between exons 8 and 11 in 25-μL reaction mixtures containing 1 μL total genomic DNA, 2.5 μL of 10× KOD -Plus- version 2 buffer (Toyobo, Osaka, Japan), 1.5 μL of 25 mmol/L $MgSO_4$, 2.5 μL of 2 mM dNTP mix, 0.75 μL each primer (10 pmol/μL), and 0.5 μL of KOD -Plus- DNA polymerase (1 U/μL; Toyobo)

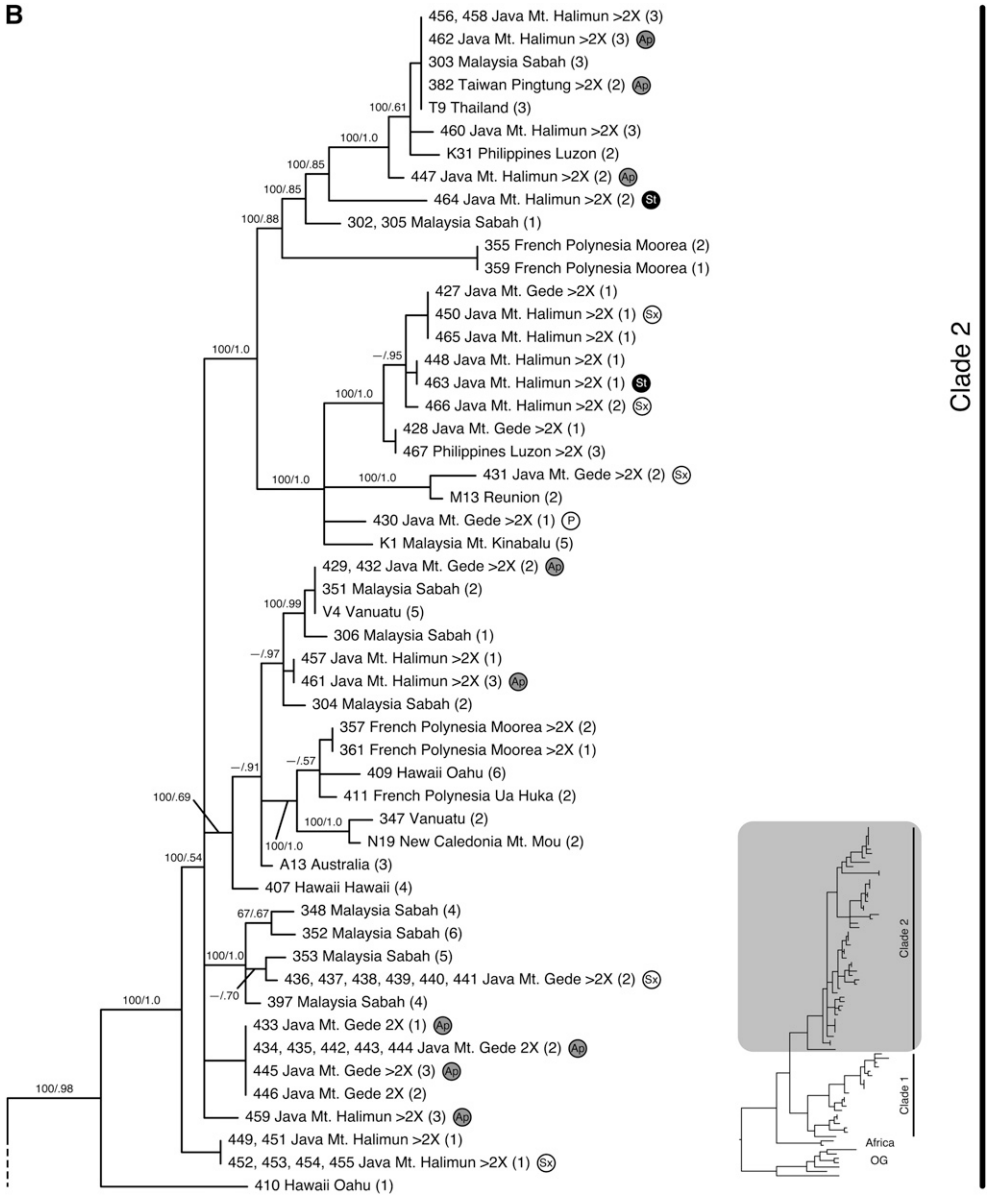

Fig. 1. Continued.

under the following temperature and cycle conditions: initial denature step at 94°C for 2 min, 30 cycles of 98°C for 10 s, 59°C for 30 s, 68°C for 20 s, and a final extension at 68°C for 10 min. Polymerase chain reaction products obtained from the first PCR amplification were used as template in a second PCR amplification. The second PCR amplified intron 10 and portions of flanking exons using primers JNGAPCP10F1 (newly designed for this study; 5′–GAAGGCTCCATGAAAGGGATTATGGG–3′) and ESGAPCP11R1 following the same protocol as the first PCR amplification. Polymerase chain reaction products were checked for successful amplification by gel electrophoresis run at 100 V for 23–30 min on a 1% agarose gel in TBE buffer together with the GeneRuler 100 bp+ ladder (Fermentas AB, Vilnius, Lithuania).

Single strand conformation polymorphism (SSCP) analysis was used to genotype and detect polymorphism in *gapCp* sequences. The *gapCp* PCR products were denatured at 94°C for 3 min, then mixed with loading dye and electrophoresed on a 0.5× mutation detection enhancement (MDE) gel (Cambrex, East Rutherford, New Jersey, USA) containing 2% glycerol in 0.5% TAE buffer at 18°C for 6 h, then visualized with silver nitrate. In the case that the exact same banding pattern was observed in multiple specimens, a single specimen was selected to be sequenced by cloning. Cloning of *gapCp* sequences was performed using the pGEM-T Easy Vector System (Promega, Madison, Wisconsin, USA) following the manufacturer's instructions. To correct for PCR errors, PCR was performed twice for each sample and multiple colonies were sequenced from each PCR product (typically 8–16 colonies total per specimen). Only sequences recovered from multiple colonies were retained for analysis.

Polymerase chain reaction products were purified to remove excess DNA fragments using Montage PCR centrifugal filter devices (Millipore, Billerica, Massachusetts, USA) or ExoSap-IT (USB, Cleveland, Ohio, USA) according to the manufacturer's protocol. Automated cycle sequencing of purified PCR products was carried out on a CEQ2000 genetic analysis system (Beckman Coulter, Fullerton, California, USA) following the manufacturer's protocol. In addition to PCR primers aF and 1379r, *rbcL* was sequenced using the following internal primers: H1F1, H1R1, TKT-F2N-2–TKT-R3N-2, and TKT-F1–TKT-2PRN (Ebihara et al., 2003; Ebihara et al., 2007). We sequenced *gapCp* using only the SP6 Promoter Primer (Promega); because the orientation of the insert in the vector is random and many colonies were sequenced per sample, only a single primer was necessary to obtain both forward and reverse sequence reads.

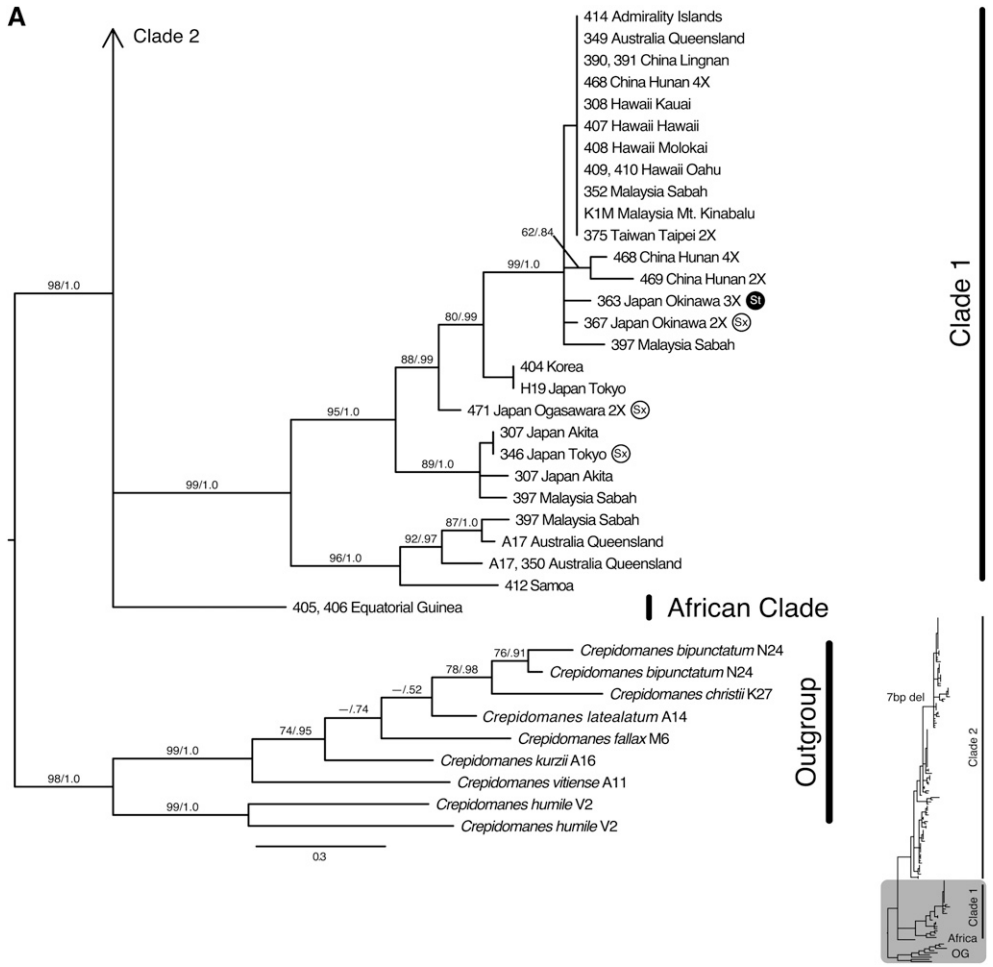

Fig. 2. Phylogeny of the *Crepidomanes minutum* species complex resulting from Bayesian analysis of nuclear *gapCp*. Specimen ID number and locality plus the following data are indicated for each ingroup taxon when available: ploidy level (>2× indicates polyploid but exact ploidy unknown) and reproductive mode (indicated as follows: white circle with "Sx," sexual; gray circle with "Ap," apogamous; black circle with "St," sterile; white circle with "P," precocious). Bayesian posterior probabilities (PP) and maximum parsimony bootstrap values (BS) are indicated above each branch (BS/PP); support values are given only for nodes with PP or BS >50%. Branch lengths proportionate to expected amount of change per site as inferred by Bayesian analysis are indicated by scale bar. Phylogenetic position of 7-bp deletion is indicated with arrow. Inset depicts structure of overall tree; location of the current figure part is indicated by shaded gray box.

All sequences newly obtained in this study (86 *rbcL* and 182 *gapCp* sequences) were deposited in the online database GenBank (http://www.ncbi.nlm.nih.gov/genbank/; Appendix 1).

Phylogenetic analyses—Chromatograms were assembled into sequences using ATGC multialignment software (Genetyx, Tokyo, Japan). We aligned *rbcL* sequences manually in MacClade version 4.0 (Maddison and Maddison, 2000). The *gapCp* sequences varied in length and were aligned using MUSCLE (Edgar, 2004) on default settings. Alignments were deposited in the treeBASE online database (http://purl.org/phylo/treebase/phylows/study/TB2:S11059). In some cases, *gapCp* chimeric sequences resulting from recombination during PCR were identified and removed from analysis or corrected manually. Simple gap coding (Simmons and Ochoterena, 2000) of *gapCp* sequences was performed using GapCoder (Young and Healy, 2003). Aligned sequence data matrices were analyzed using maximum parsimony (MP) and Bayesian analysis methods. Aligned sequences were stored as nexus files in MacClade and imported into PAUP* version 4.0b10 (Swofford, 2002); identical sequences were removed at this point and manually added to each tree after analysis. A heuristic search under the maximum parsimony criterion was performed in PAUP* with 1000 random addition sequences, maxtrees set to 100, and tree-bisection-reconnection (TBR) branch swapping. Support values for the MP trees were estimated using 1000 bootstrap (BS) replicates with 10 random addition sequences per replicate under the same settings as the parsimony analysis. For Bayesian analysis of the *rbcL* data set, all data were included in a single partition. The *gapCp* data set was split into two partitions: one for DNA data and another for gap characters treated as binary (restriction) data, using the "lset coding = variable" command. The most appropriate model of evolution for each DNA data set was determined using Akaike's information criterion in ModelTest version 3.7 (Posada and Crandall, 1998). The chosen model of sequence evolution was then implemented in MrBayes 3 (Ronquist and Huelsenbeck, 2003), which searched treespace with two independent runs each comprising four chains (three hot, one cold) starting from flat (i.e., default) priors. Chains were run for 2 500 000 generations, with the cold chains sampled once every 100 generations. Convergence was confirmed when average standard deviation of split frequencies reached 0.01 or less. We discarded 25% of trees as burn-in and summed the remaining trees (using "sumt" command) to construct a 50% majority-rule consensus tree.

Ploidy analysis—Fresh leaf tissue (field collected samples only) was used for flow cytometry following the method of Ebihara et al. (2005). Genome sizes were analyzed using the Epics XL system (Beckman Coulter) according to the manufacturer's protocol with *Nicotiana tabacum* L. cv. Xanthi (diploid genome size 2C = 23.4 pg; Narayan, 1987) as the internal size standard (i.e., control). Genome sizes of specimens were determined by comparing the positions

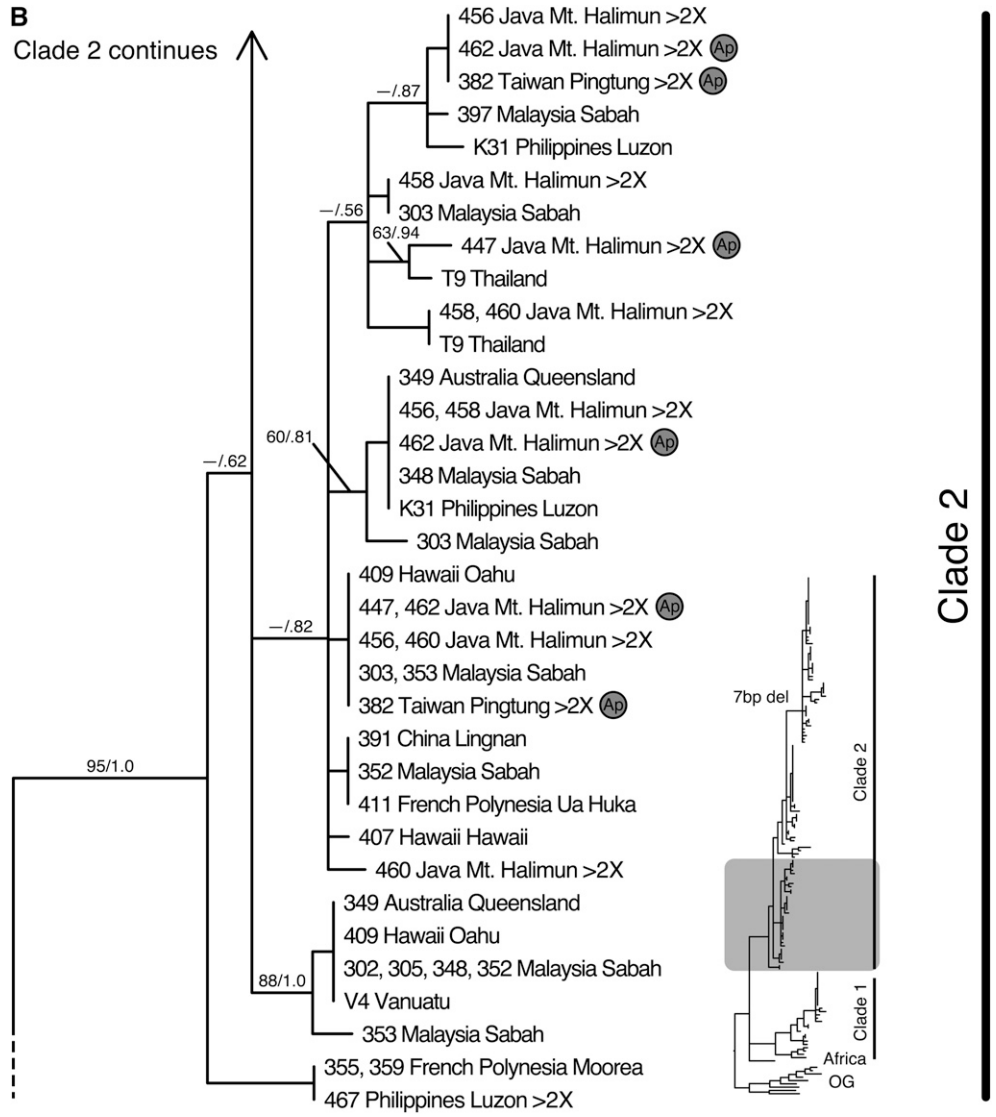

Fig. 2. Continued.

of peaks in fluorescence between sample and control (e.g., if the sample peak was 1.95× greater than the control peak, the sample genome size was calculated to be 2C = 23.4 pg × 1.95 = 45.6 pg). Chromosomes were counted on an exemplar specimen (*Nakato s.n.*; Tokura, Akiruno-shi, Tokyo, Japan [TNS]) using the squash method (Takamiya, 1993) to correlate genome size with ploidy level.

Spore observations—Apogamy in the *C. minutum* species complex follows the Braithwaite system, which results in 32 spores per sporangium instead of the usual 64 observed in sexually reproducing taxa (Braithwaite, 1964). Thus, spore counts can be used as an indirect check for apogamy; however, low spore/sporangium counts occasionally occur in sexually reproducing taxa (Gastony and Windham, 1989) and observations of apogamy presented here must be considered preliminary until they can be confirmed with observation of chromosomes or gametophytes.

Sporangia were isolated from fertile fresh material (field collected samples only) and used for spore counts. Spores per sporangium were counted for 1–3 sporangia per specimen under a Leica DMLB light microscope (Leica, Wetzlar, Germany). Spore size was measured for 1–3 spores per specimen using a BZ-8000 microscope (Keyence, Osaka, Japan). Because some spores are often lost during slide preparation, specimens with sporangia containing 32 or fewer spores were considered apogamous and specimens with sporangia containing 33–64 spores were considered sexual.

Morphological analysis—All specimens were checked for presence or absence of proliferations, leaf shape (flabellate or pinnatifid), and leaf size (length and width). Leaf size was measured for 2–3 leaves per specimen; measurements for proliferous specimens were based on the smallest unit recognizable as a leaf (i.e., not including multiple proliferations). Length was measured from base to tip of the lamina, not including the petiole. Yoroi and Iwatsuki (1977) reported that buds of proliferation occur on all taxa within the *C. minutum* complex, but some remain dormant while others produce new growth; only the presence or absence of proliferous growth and not dormant buds were investigated for the present study.

RESULTS

***Analysis of chloroplast* rbcL**—The aligned *rbcL* data set (1206 bp) included 97 sequences (one sequence per specimen; 90 ingroup sequences + 7 outgroup sequences) with 200 (16.6%) variable characters and 140 (11.6%) parsimony-informative characters. Portions of *rbcL* were missing for some herbarium specimens due to poor recovery of genomic DNA (specimens 390, 391, 392, 397, 407, 411, 412, and 466). The MP analysis recovered 94 969 most parsimonious trees (335 steps each),

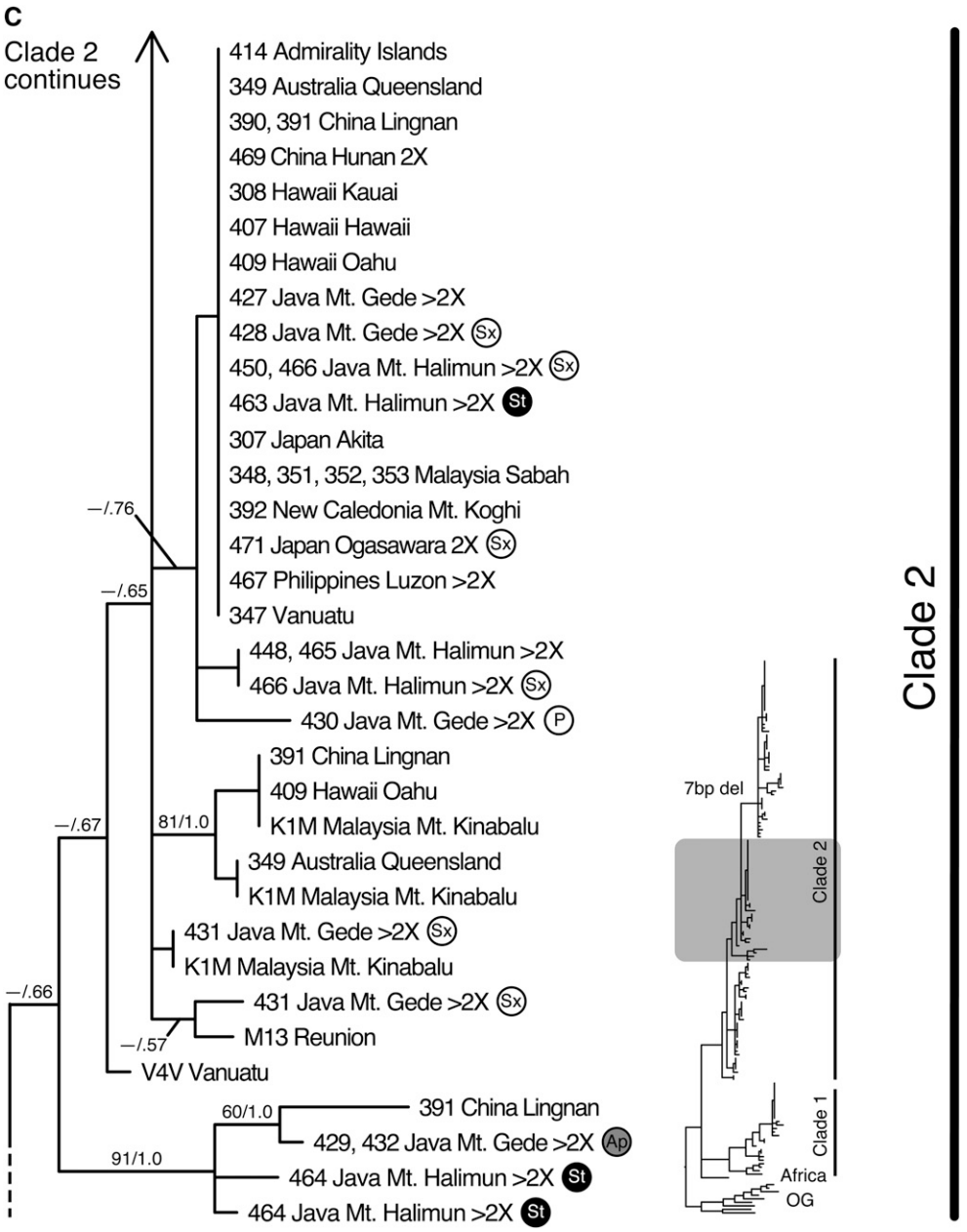

Fig. 2. Continued.

which were summed in a 50% majority-rule consensus tree with a consistency index (CI) of 0.66 and a retention index (RI) of 0.89. TrN+I+Γ was identified by Akaike's information criterion in ModelTest as the most appropriate model of evolution for the *rbcL* data set. However, MrBayes cannot implement the TrN model; because Bayesian inference is relatively robust to slight over-parameterization, the next most complex model, general time reversible (GTR), was used instead (Ronquist et al., 2005). The 50% majority-rule consensus tree resulting from Bayesian analysis of the *rbcL* data set is shown in Figure 1. Maximum parsimony and Bayesian analyses of the *rbcL* data set resulted in nearly identical topologies; only a few unsupported nodes differed between the two analyses and do not affect the findings of the present study (MP phylogeny not shown; MP bootstrap values indicated in Fig. 1). Both MP bootstrap (BS) and Bayesian posterior probability (PP) values strongly support the *C. minutum* species complex as monophyletic (100% BS, 1.0 PP). The first split within the complex resolves African specimens as sister to all other members of the complex (100% BS, 0.97 PP). The remaining specimens are divided into two large, well-supported clades, designated "clade 1" (100% BS, 0.98 PP) and "clade 2" (100% BS, 0.98 PP; Fig. 1).

***Analysis of nuclear* gapCp**—The aligned *gapCp* data set (231 bp) included 210 sequences (200 ingroup + 10 outgroup sequences) recovered from 97 specimens (90 ingroup + 7 outgroup specimens). The final data matrix contained 139 (55.8%) variable characters and 103 (41.4%) parsimony-informative characters, including 18 insertion/deletions (indels) coded as binary data. The MP analysis recovered 99 600 most parsimonious

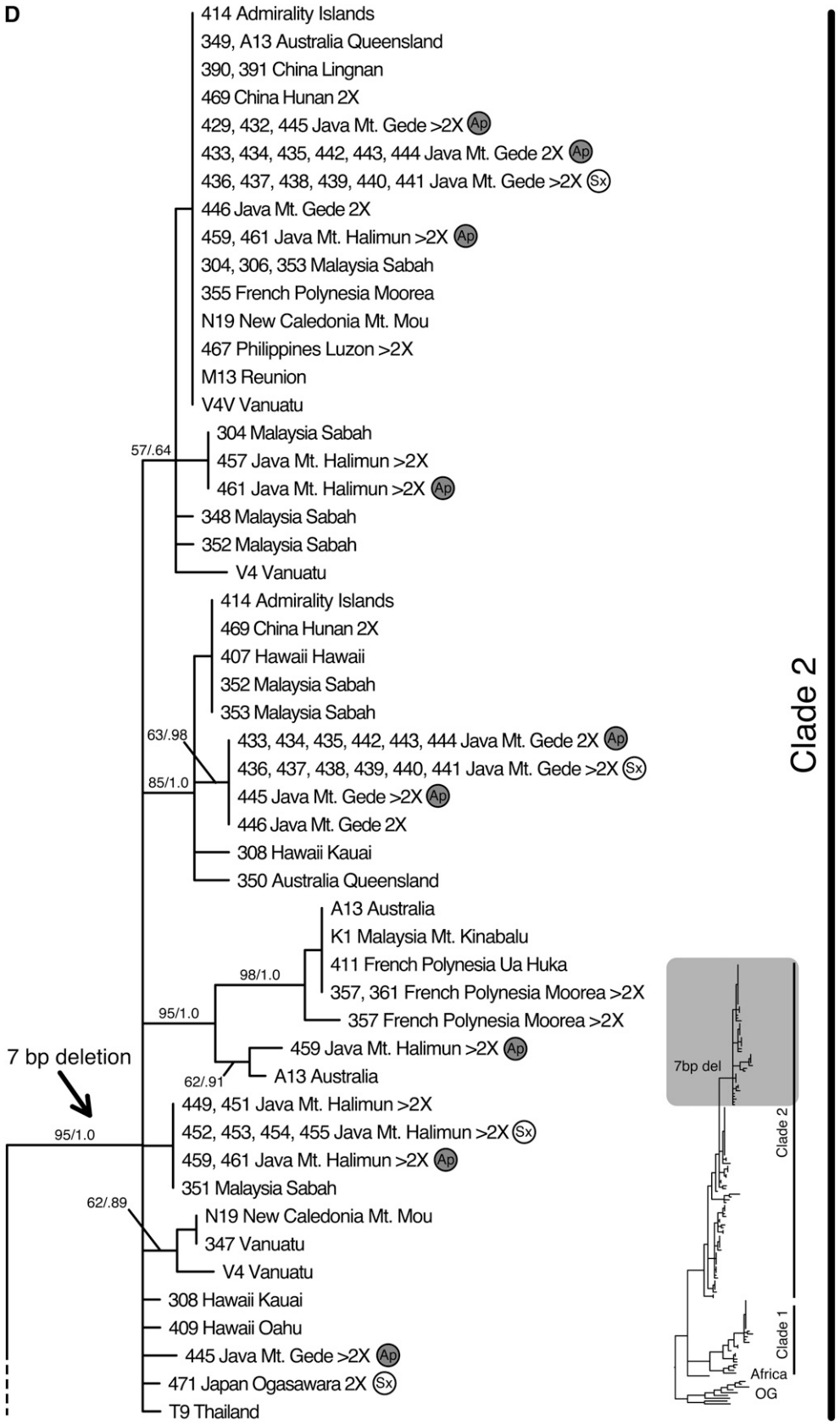

Fig. 2. Continued.

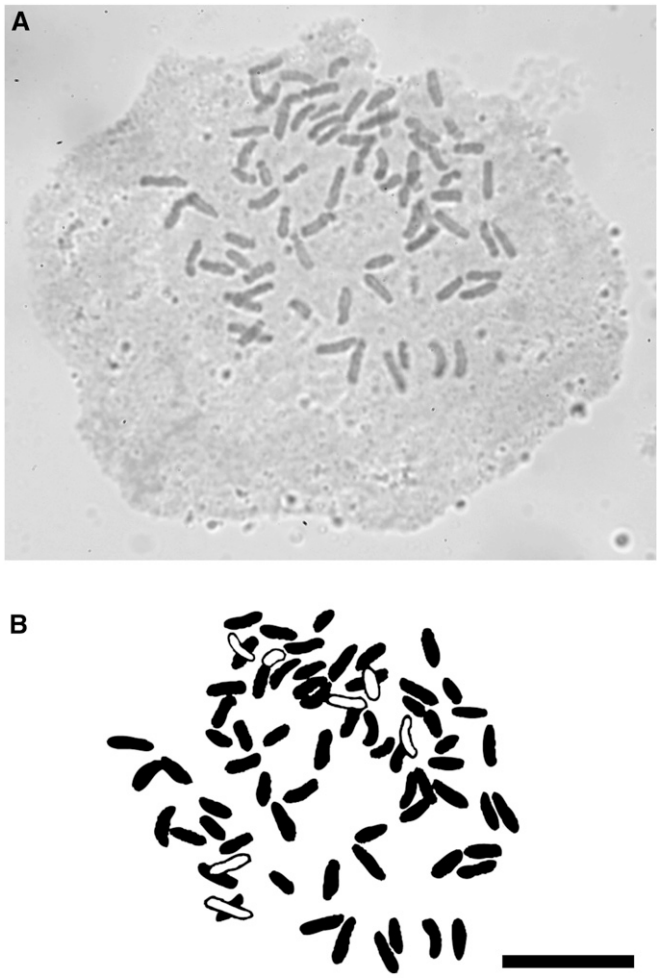

Fig. 3. Chromosomes during mitosis in growing rhizome tips of Japanese *Crepidomanes minutum*. (A) Light micrograph. (B) Diagram of chromosomes in A. Scale bar = 10 μm.

trees (259 steps each), which were summed in a 50% majority-rule consensus tree (CI = 0.70, RI = 0.91). Akaike's information criterion as implemented in ModelTest selected TIM+Γ as the most appropriate model of evolution for the *gapCp* data set; however, because TIM is not available in MrBayes, GTR+Γ was used instead (Ronquist et al., 2005). Maximum parsimony and Bayesian analyses of the *gapCp* data set resulted in nearly identical topologies; only a few differences in internal nodes differed between trees and received little or no support (MP phylogeny not shown; MP bootstrap values indicated in Fig. 2). The *C. minutum* species complex was strongly supported as monophyletic by *gapCp* data (98% BS, 1.0 PP). The first split within the ingroup is a polytomy between the African clade, clade 1, and clade 2. Monophyly of clades 1 (99% BS, 1.0 PP) and 2 (95% BS, 1.0 PP) is strongly supported. Clade 2 includes a strongly supported subclade with a 7-bp deletion (95% BS, 1.0 PP). The African clade (specimens 405 and 406 from Equatorial Guinea) corresponds exactly between the *rbcL* and *gapCp* trees. Clades 1 and 2 correspond between the *rbcL* and *gapCp* tree, with the exception of some specimens with multiple *gapCp* alleles (i.e., different sequences). For specimens with multiple *gapCp* alleles, those in *rbcL* clade 1 possess only *gapCp* clade 1 alleles or a combination of *gapCp* clade 1 and 2 alleles; those in *rbcL* clade 2 possess only *gapCp* clade 2 alleles or a combination of *gapCp* clade 1 and 2 alleles. One specimen (410 from Hawaii) was observed with a genomic constitution of *rbcL* clade 2 + *gapCp* clade 1, and one specimen (N19 from New Caledonia) with *rbcL* clade 1 + *gapCp* clade 2.

Ploidy analysis—We counted 72 chromosomes at mitosis in an exemplar specimen (*Nakato s.n.*; Tokura [TNS]) belonging to clade 1 (Fig. 3; molecular data not shown). Braithwaite (1975) reported that the base number in the *Crepidomanes minutum* complex is $n = 36$; thus, this plant is considered diploid. Genome sizes determined by flow cytometry are shown in Figure 4 by major *rbcL* clade and size (see Appendix 1 for genome sizes by specimen). Sizes within clade 1 fall into three distinct groups: 2C = ca. 48.6, 67.3, or 90.8 pg. The diploid exemplar specimen had an observed genome size of 51.2 pg; thus, we infer that the haploid genome size is C = ca. 24 pg, and these genome sizes correspond to diploids, triploids, and tetraploids, respectively. In *rbcL* clade 2 specimens, a group of small-genome-size Indonesian specimens (2C = ca. 45.9 pg) possibly represent diploids. The sizes of the remaining *rbcL* clade 2 specimens gradually increase from 86.7 to 128.2 pg, followed by a small group of specimens from 161.3 to 199.9 pg. The lack of distinct multiples of genome sizes in clade 2 precludes the assignment of ploidy levels for these specimens, although they are thought to be polyploid. Genome sizes were obtained for only two "clade 1 / 2 hybrid" specimens containing *gapCp* alleles from both clade 1 and clade 2 (specimens 469 and 471); these each had clade 1 *rbcL* sequences and appear to be diploid.

Spore observations—Four types of spores were observed: sexual, apogamous, sterile, and precocious (Table 3). In sexual specimens, spore counts ranged from 48 to 64 spores per sporangium and spores were uniformly round, green, and averaged ca. 61 μm in diameter. In apogamous specimens, spore counts ranged from 22 to 32 spores per sporangium; spores were uniformly round, green, and averaged ca. 76 μm in diameter. In sterile specimens, 40–50 spores were counted per sporangium; spores were irregularly shaped and clear or brown in color. A single precocious specimen (430 from Mt. Gede, Java) was observed with ca. 45 spores per sporangium; spores were green but had already divided to produce gametophytic cells while within the sporangium. Rhizoids were observed but no sexual organs were present. Intrasporangial germination has been reported in *Hymenophyllum* but is less common in trichomanoid species (Stokey, 1940).

Morphology—Results of morphological observations are summarized in Table 4 and Figure 5 by major *rbcL* clade. Nearly all clade 1 specimens were small (ca. 1.0 × 1.0 cm or less), nonproliferating, and flabellate with deeply incised margins; a single clade 1 specimen (471 from the Ogasawara Islands, Japan; previously recognized as the endemic *Gonocormus bonincola*) had large (ca. 1.1 × 1.4 cm), flabellate leaves with shallowly lobed margins and occasional proliferations. African specimens generally matched in morphology with clade 1 specimens. Clade 2 specimens displayed a greater range of morphological variation than clade 1 specimens: leaf size varied from ca. 0.3 × 0.3 cm to 4.0 × 2.4 cm. Leaf shape was generally flabellate in smaller specimens to pinnatifid in larger specimens, although shape and size of segments varied greatly between specimens. Proliferations were observed on most but

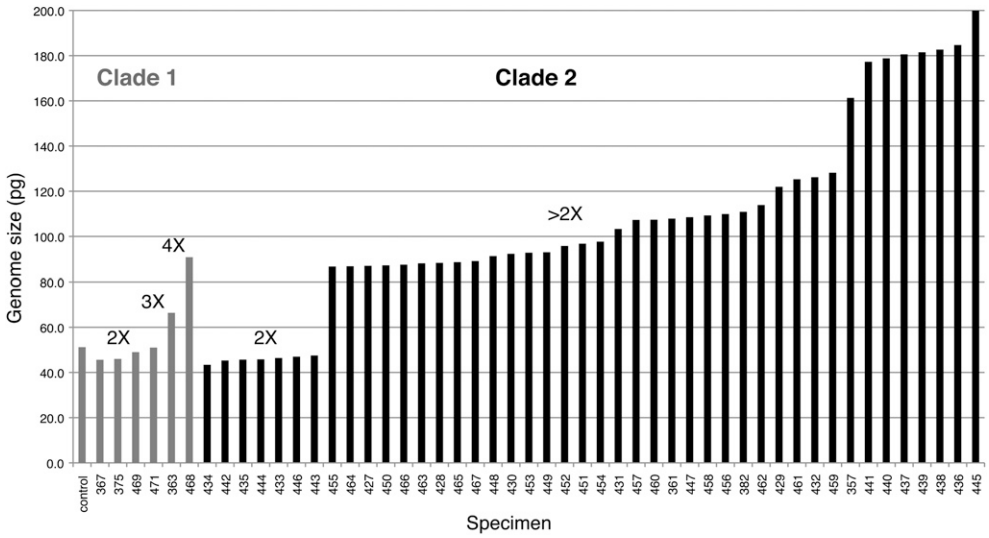

Fig. 4. Results of flow cytometry analysis with putative ploidy levels (>2× indicates polyploid but exact ploidy unknown). Genome sizes (pg) determined by flow cytometry arranged by *rbcL* clade and size. Clade 1 specimens are in gray, clade 2 specimens in black. Sample IDs are indicated along horizontal axis. Diploid exemplar specimen (*Nakato s.n.*; Tokura, Akiruno-shi, Tokyo, Japan) indicated by "control" at far left.

not all clade 2 specimens (74%). A few specimens in clade 2 were observed with highly reduced lamina (specimens 306 from Mt. Alabu, Malaysia; 351 from Mt. Kinabalu, Malaysia; and 459 from Mt. Halimun, Java); these match the description of *G. alagensis* (Christ) Copel. but did not form a clade in either *rbcL* or *gapCp* phylogenies. "Clade 1 / 2 hybrids" containing *gapCp* alleles from both clade 1 and clade 2 included specimens that for the most part displayed otherwise typical clade 1 or clade 2 forms and did not appear morphologically intermediate.

DISCUSSION

Phylogenetic structure of the* Crepidomanes minutum *species complex—Results of phylogenetic analysis of nuclear and chloroplast data reveal that the *C. minutum* species complex is a monophyletic group composed of at least three major clades: the African clade, clade 1, and clade 2 (Figs. 1 and 2). The African clade may be sister to the rest of the complex (supported by *rbcL* but not by *gapCp*), but this is unclear with the present data set. Although topologies were not exactly congruent because of the prevalence of specimens with multiple *gapCp* sequences, clades 1 and 2 largely correspond between *rbcL* and *gapCp* trees (see Results). Clades 1 and 2 differ markedly in several respects, including geographic distribution, morphology, cytology, and occurrence of hybrids, as discussed below (clade membership refers to *rbcL* clade unless otherwise specified).

Clade 1 comprises taxa from East Asia (China, Japan, Korea, Taiwan) and the Pacific (the Admiralty Islands, Australia, Hawaii, New Caledonia, and Samoa). In the *rbcL* tree, these two geographic areas form nearly reciprocally monophyletic groups (Hawaii is the exception, included with East Asian specimens); within the East Asian subclade, specimens from mainland Japan and Korea form one subclade (except for specimen H19 from Tokyo) and specimens from China, Hawaii, Okinawa, and Taiwan form another. This indicates that Okinawa, where *C. minutum* is rare, is the northern limit of the China/Hawaii/Okinawa/Taiwan group, rather than the southern limit of mainland Japanese taxa as proposed by Iwatsuki (1975). The unusual phylogenetic position of specimen H19, sister to the China/Hawaii/Okinawa/Taiwan group, suggests possible gene flow between these two subclades. Clade 1 taxa are nearly uniform in morphology: almost all are small (ca. 1 × 1 cm or less), flabellate, and nonproliferating. Of the clade 1 specimens with fresh material available for flow cytometry and spore observation, most were simple in terms of cytology and reproductive mode (i.e., diploid and sexually reproducing). The single clade 1 triploid observed (specimen 363 from Okinawa) appears to be autopolyploid with aborted spores. Frequency of hybridization seems to be low within clade 1; nearly half of clade 1 specimens had only a single *gapCp* clade 1 allele, and only two clade 1 specimens, 468 from Hunan, China (tetraploid), and A17 from Australia (herbarium specimen, ploidy unknown), were found to have multiple *gapCp* clade 1 alleles. The single specimen with a *gapCp* clade 1 / *rbcL* clade 2 genomic composition (specimen 410 from Hawaii) matched exactly in morphology

TABLE 3. Minimum, maximum, and mean number of spores per sporangium (± SE) and minimum, maximum, and mean diameter (μm) of spores (± SE) by reproductive mode for fertile, field collected specimens of the *Crepidomanes minutum* species complex.

Reproductive mode	Minimum count	Maximum count	Mean count	Minimum diameter	Maximum diameter	Mean diameter
Sexual	48	64	56.3 ± 5.5 (n = 17)	50.0	75.0	61.1 ± 6.4 (n = 17)
Apogamous	22	32	29.7 ± 2.6 (n = 13)	65.0	82.5	75.8 ± 5.2 (n = 13)
Sterile	40	50	45 ± 7.1 (n = 3)	51.0	60.5	57.2 ± 5.3 (n = 3)

TABLE 4. Minimum, maximum, and mean (± SE) length (cm) and width (cm) of leaves, percentage of specimens observed with proliferations, and percentage of specimens observed with pinnatifid leaves for all specimens of the *Crepidomanes minutum* species complex in the present study.

Specimen group	Minimum length	Maximum length	Mean length	Minimum width	Maximum width	Mean width	Percent proliferous	Percent pinnatifid
rbcL African clade	0.95	0.97	0.96 ± 0.02 (n = 2)	0.59	0.80	0.70 ± 0.15 (n = 2)	100% (2/2)	0% (0/2)
rbcL clade 1	0.37	1.14	0.57 ± 0.18 (n = 20)	0.41	1.39	0.72 ± 0.21 (n = 20)	15% (3/20)	5% (1/20)
rbcL clade 2	0.28	4.12	1.51 ± 0.87 (n = 68)	0.31	2.43	0.95 ± 0.38 (n = 68)	74% (50/68)	62% (42/68)
gapCp clade 1 / 2 hybrids	0.42	2.09	0.90 ± 0.52 (n = 14)	0.54	1.54	0.82 ± 0.3 (n = 14)	43% (6/14)	21% (3/14)
rbcL clade 1 (not including *gapCp* clade 1 / 2 hybrids)	0.37	0.59	0.48 ± 0.08 (n = 11)	0.41	0.90	0.67 ± 0.15 (n = 11)	0% (0/11)	0% (0/11)
rbcL clade 2 (not including *gapCp* clade 1 / 2 hybrids)	0.28	4.12	1.52 ± 0.89 (n = 63)	0.31	2.43	0.96 ± 0.38 (n = 63)	75% (47/63)	63% (40/63)

with other nonhybrid Hawaiian clade 1 specimens, and is thought to represent an episode of chloroplast capture (Rieseberg and Soltis, 1991). Despite the low rate of hybrids with multiple *gapCp* clade 1 alleles, several specimens were observed with both *gapCp* clade 1 and 2 alleles, and are discussed further below.

In contrast with clade 1, clade 2 shows much more complicated patterns of geographic affinities, morphology, and frequency of polyploidization and hybridization. Clade 2 taxa are primarily from Southeast Asia (Indonesia, Malaysia, Philippines, Thailand) and the South Pacific (French Polynesia, Vanuatu), but extend as far west as La Reunion and also overlap in some localities with clade 1 (Australia, Hawaii, New Caledonia, Taiwan; Fig. 6). Specimens from the same locality do not form reciprocally monophyletic groups in either the *rbcL* or *gapCp* tree. Clade 2 taxa are morphologically complex, varying greatly in frond shape and size, although most (74%) produce proliferations. Although clusters of morphologically similar specimens with identical (or nearly identical) *rbcL* and *gapCp* sequences were occasionally found (e.g., specimens 305 from Mt. Alabu, Malaysia and 302 from Mt. Kinabalu, Malaysia), sister specimens often did not match in morphology. The majority of clade 2 specimens had multiple *gapCp* alleles per specimen (48 out of 68 specimens), many of which failed to form monophyletic groups (i.e., alleles from different specimens were found to be more closely related than alleles from the same specimen). A large number of putative hybrid taxa with multiple *gapCp* alleles from within clade 2 only and from both clades 1 and 2 were observed. This allelic diversity, plus the fact that many clade 2 specimens appear to be polyploid (37 of 44 specimens analyzed by flow cytometry), suggests hybrid origins, introgression, or incomplete lineage sorting. Thus, it appears that the evolutionary history of clade 2 is highly complicated,

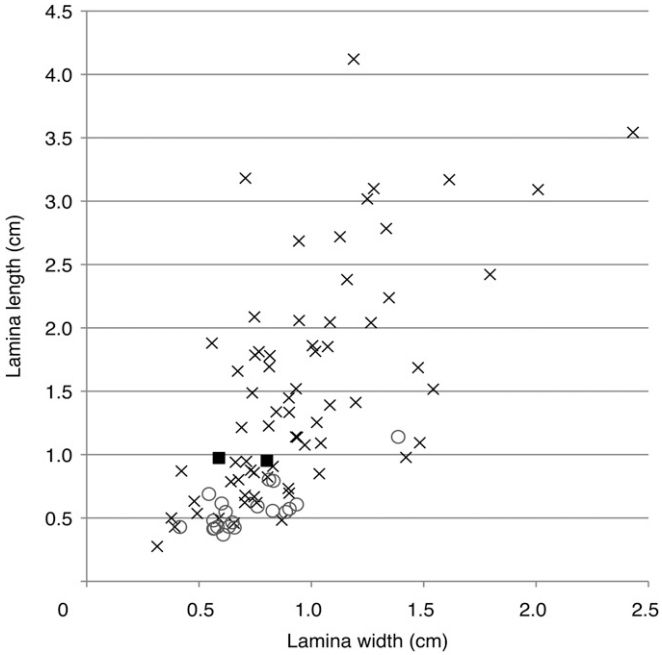

Fig. 5. Distribution of leaf sizes by *rbcL* clade. Circle indicates *rbcL* clade 1 specimens; "X" indicates *rbcL* clade 2 specimens; square indicates *rbcL* African clade specimens.

Fig. 6. Geographic distribution of specimens in the present study. Circle indicates *rbcL* clade 1 specimens; "X" indicates *rbcL* clade 2 specimens; square indicates *rbcL* African clade specimens. Arrows indicate *gapCp* "clade 1 / 2 hybrid" specimens.

involving repeated contact and genetic exchange between different lineages in Southeast Asia and the South Pacific.

The genetic and morphological diversity uncovered in this study has implications for species delimitation in the *C. minutum* complex. The complex evolutionary history of clade 2 suggests multiple biological species and their interspecific crosses; specific cases of hybridization and issues relating to circumscription of species within clade 2 are discussed in the next section. Clade 2 aside, the strongly contrasting evolutionary histories between clade 1 and clade 2 indicate that these two groups may represent distinct taxonomic entities at the species level or higher. Genetic distances between sequences from clades 1 and 2 are comparable to or greater than interspecific genetic distances between outgroup taxa (Figs. 1, 2), and are thus not likely due to population-level allelic diversity. Furthermore, taxa traditionally assigned to these two groups on the basis of morphology have been considered to be separate species by many authors (Table 1): most specimens in clade 1 match in morphology with taxa called "*saxifragoides*," (flabellate and nonproliferating) whereas most of those in clade 2 match morphologically with taxa called "*proliferum*," (pinnatifid and proliferating). A caveat to this seemingly clear difference in morphology is that, when taken together, the morphological diversity of clade 2 encompasses several small forms that overlap with those of clade 1 (Table 4, Fig. 5); however, there is usually enough of a gap between forms present at any given locality such that two taxa can be distinguished. The two clades may differ in ecology as well: Braithwaite (1969) describes observing "*saxifragoides*" (clade 1) taxa at "at low altitudes sometimes on trees overhanging the beach" and "*proliferum*" (clade 2) taxa at "approximately 6,000 ft…in the mist forest" on the Solomon Islands. During the field survey conducted for the current study in Taiwan, a similar pattern was also observed on Mt. Li Long, where clade 1 taxa were present in low altitude mesic forests and clade 2 taxa at high altitudes in the cloud forest, possibly reflecting different moisture requirements.

One question that must be resolved to determine if these two clades should be recognized as separate species is the origin of taxa with *gapCp* sequences from both clades 1 and 2. These "clade 1 / 2 hybrids" varied in their morphology, including both typical clade 1 and clade 2 forms. However, data on reproductive mode and ploidy level is lacking for most specimens, and it is currently unclear whether "clade 1 / 2 hybrids" represent infertile F_1 crosses, stabilized true hybrids, allohomoploids, or are the results of introgression or incomplete lineage sorting (more data are available for field-collected specimens 469 and 471; see next section). Further studies of "clade 1 / 2 hybrids" are necessary before any attempt is made at species delimitation.

Occurrence of hybrids and mechanisms of stabilization—The strongest evidence for existence of hybrids within the *C. minutum* species complex is the incongruence between nuclear and chloroplast trees caused by multiple *gapCp* alleles recovered from single specimens. It is unlikely that such alleles are due to paralogous genes: all *C. minutum* species complex *gapCp* sequences were found to be monophyletic, and were easily aligned to each other and the outgroup (neither of which would be expected in the case of paralogous sequences). Schuettpelz et al. (2008) found multiple copies of *gapCp* in some ferns, but hypothesized that this duplication occurred after the split giving rise to Hymenophyllaceae. It is possible that some alleles simply represent population-level variation within nonhybrid species (or PCR artifacts); however, the majority of alleles recovered were found to be more closely related between rather than within specimens. Given that the multiple alleles found in these specimens represent the mixing of distinct lineages, the question arises: do these specimens represent extant, stabilized hybrid taxa, or are they nonhybrid taxa harboring allelic diversity via mechanisms such as introgression or incomplete lineage sorting?

The complex phylogenetic structure of *gapCp* alleles and lack of data on reproductive mode and chromosome number from fresh samples makes this question difficult to answer. However, one case from Java, Indonesia, where the most thorough field collection was conducted (40 specimens total, most with genome size and spore observation data), seems to indicate ongoing hybridization (Fig. 7). Specimens 433–444 all had the same two *gapCp* alleles, but were split into two groups by *rbcL* sequences: specimens 436–441 with one *rbcL* sequence ("taxon A"), and specimens 433–435 plus 442–444 with a different *rbcL* sequence ("taxon B"). Taxa A and B are also well differentiated by their morphology: taxon A is ca. 0.6 × 0.7 cm

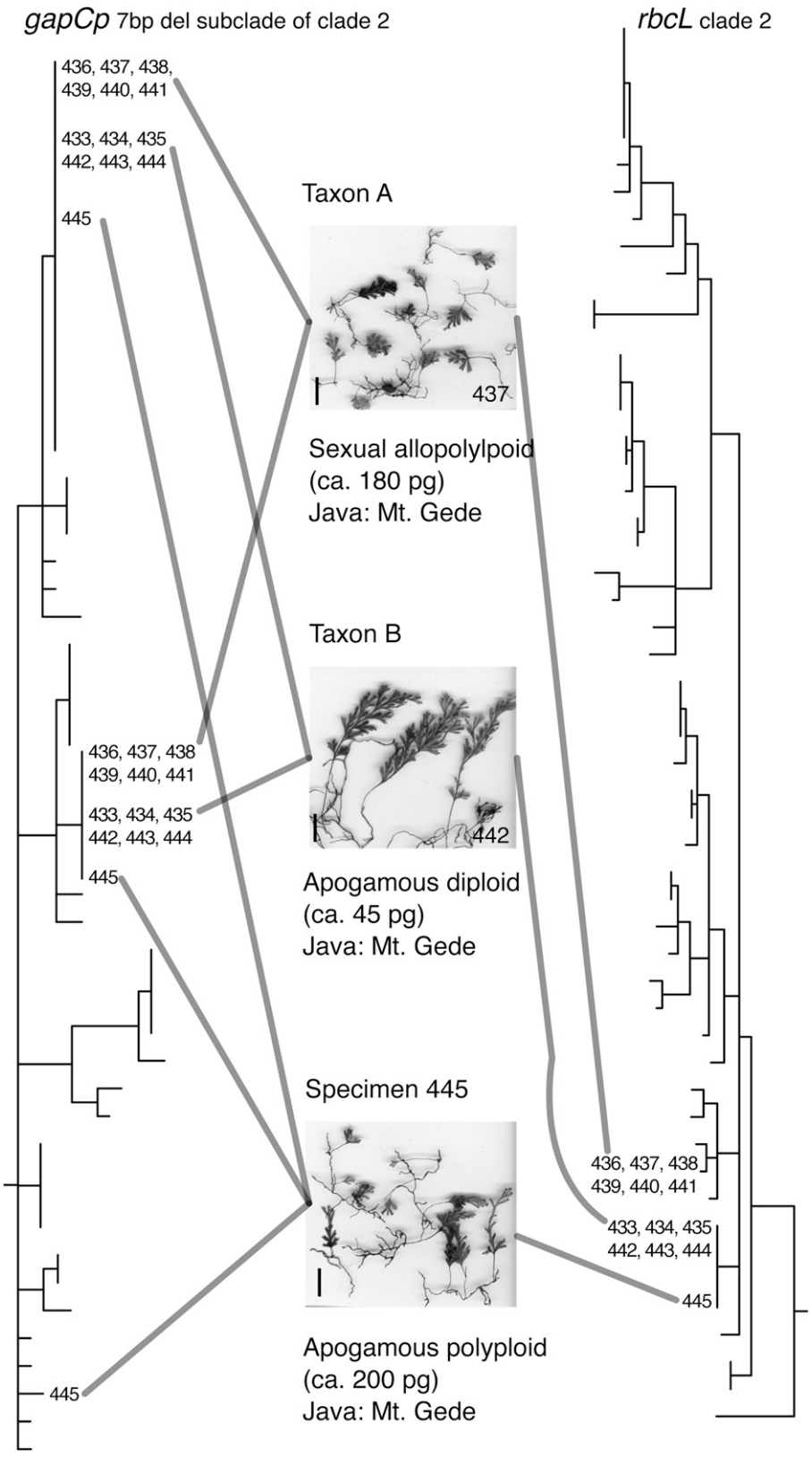

Fig. 7. Putative hybridization in the *Crepidomanes minutum* species complex. Partial phylogenies are reproduced from Figures 1 and 2; tip labels are shown only for taxon A, taxon B, and specimen 445. Bar = 1 cm. Taxa A and B appear to be hybrids that have been stabilized by polyploidy and apogamy, respectively; specimen 445 may represent a hybridization between taxon B and another unknown taxon.

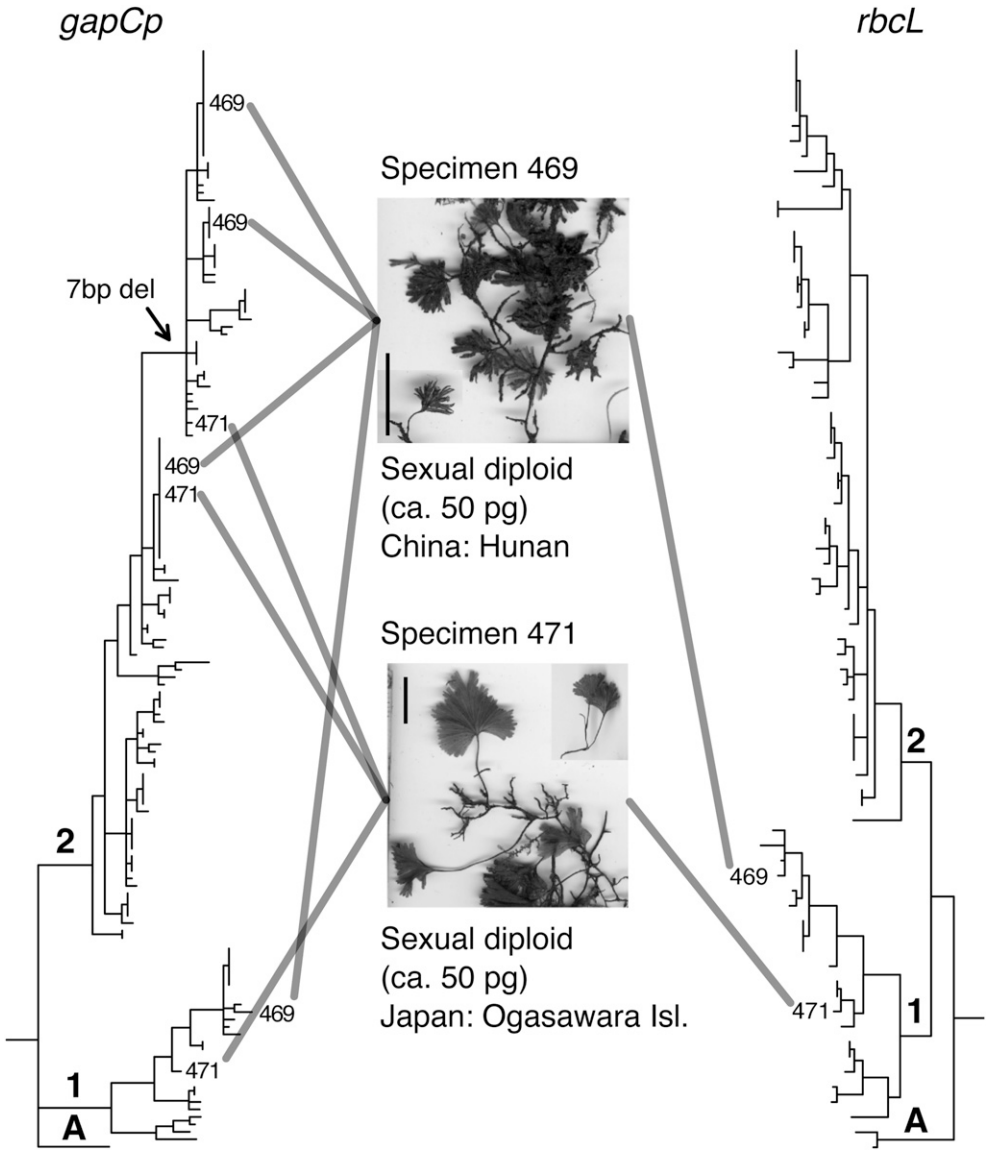

Fig. 8. Putative introgression or incomplete lineage sorting in the *Crepidomanes minutum* species complex. Partial phylogenies are reproduced from Figures 1 and 2; tip labels are shown only for specimens 469 and 471. "1" and "2" indicate clades 1 and 2; "A" indicates African clade; arrow indicates subclade of *gapCp* clade 2 with 7-bp deletion. Bar = 1 cm. Diploidy and sexual reproduction suggest acquisition of genetic diversity via introgression or incomplete lineage sorting rather than hybridization in specimens 469 and 471.

and flabellate-pinnatifid, whereas taxon B is ca. 3.0–4.0 × 1.0 cm and clearly pinnatifid. These two taxa seem to be hybrids that have become stabilized via two different mechanisms. Taxon A specimens all have large genome sizes (ca. 180 pg) and sexual type spores, indicating hybrid stabilization via polyploidy; taxon B specimens all had putatively diploid genome sizes (ca. 45 pg) and 32 spores per sporangium, indicating hybrid stabilization via apogamy. It is unknown whether taxa A and B are capable of interbreeding; however, taxon B may involved in reticulation with other Indonesian taxa, as evidenced by specimen 445 which had a third *gapCp* sequence in addition to typical taxon B *rbcL* and *gapCp* sequences, a large genome size (ca. 200 pg), and apogamous type spores. Apogamy was also observed during the current study in several other distantly related specimens, and prior reports of apogamy in the *C. minutum* species complex include specimens from a variety of localities (Mehra and Singh, 1957; Bell, 1960; Braithwaite, 1969, Braithwaite, 1975); thus it is likely that this mechanism of hybrid stabilization has evolved multiple times within the complex (or has been transferred via sexual intermediaries; Gastony and Windham, 1989).

On the other hand, if allelic diversity is due to rare introgression or incomplete lineage sorting, alleles originating from distinct lineages could be observed in nonhybrid, sexual diploid taxa. This may be the case for specimens 471 from the Ogasawara Islands, Japan and 469 from Hunan, China, which both had *gapCp* alleles from clades 1 and 2 but "sexual" type spores and a putatively diploid genome size (Fig. 8). The great genetic difference between these two clades cannot be attributed to population-level diversity. Polymerase chain reaction error or possible isolated duplication of the *gapCp* gene does seem to be an issue, given that specimens 469 and 471 each had greater

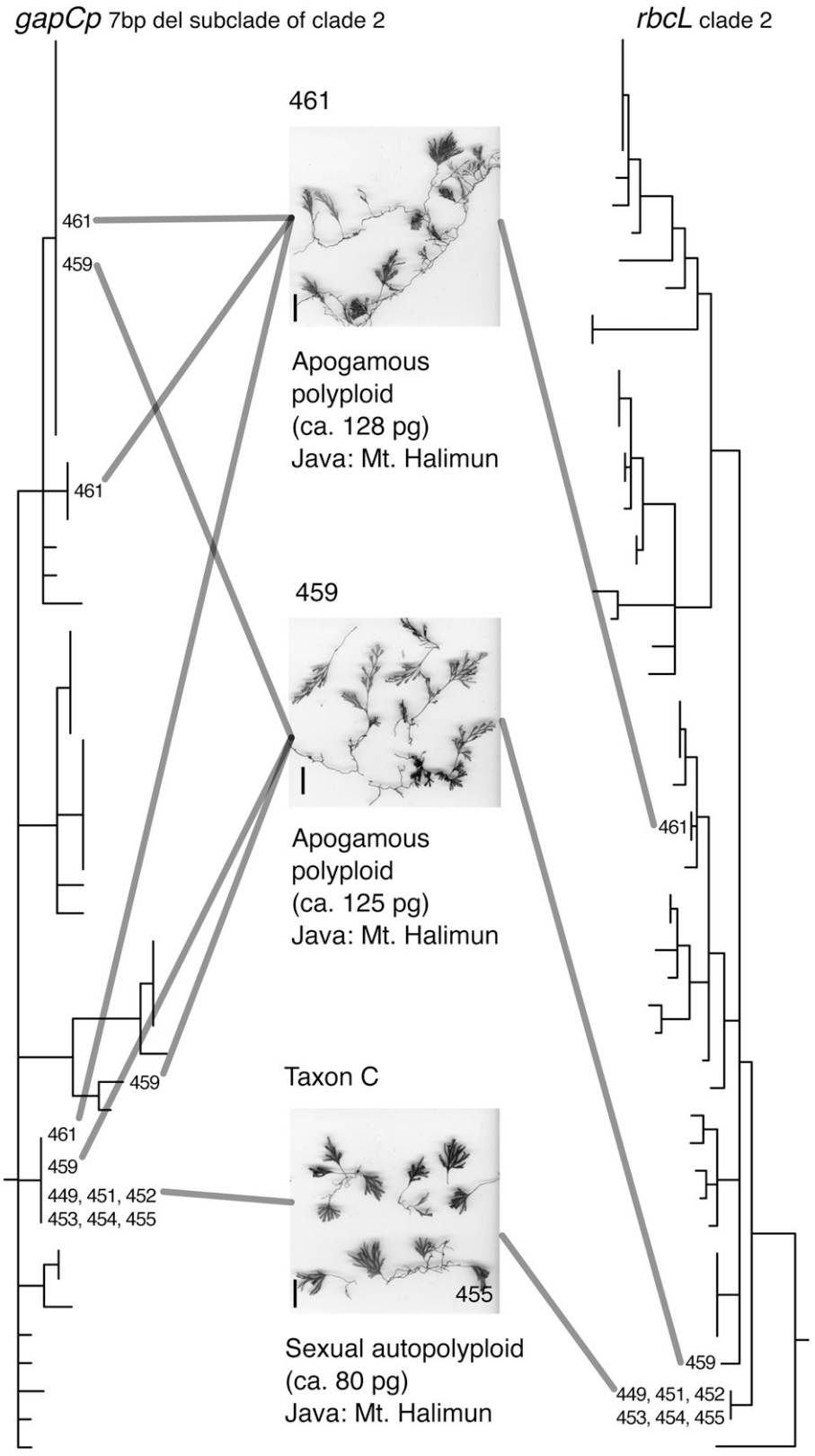

Fig. 9. Putative parent taxon of hybrids in the *Crepidomanes minutum* species complex. Partial phylogenies are reproduced from Figures 1 and 2; tip labels are shown only for taxon C and specimens 459 and 461. Bar = 1 cm. Specimens 459 and 461 each appear to be apogamous hybrids that share an allele with putative sexual parent taxon C.

than two alleles despite their putative diploid genome size (4 total in 469, 3 total in 471); however, PCR errors or isolated gene duplication would only result in slightly different sequences that should be recovered as sister or closely related, not in separate deeply diverging clades like clades 1 and 2. Further sequencing including additional nuclear markers and/or a larger portion of the *gapCp* gene is needed to identify any possible PCR errors or gene duplications.

Given the presence of hybrids such as taxa A and B from Java, one of the most pressing issues is to find their diploid, sexual progenitors (Beck et al., 2010). However, multiple alleles per specimen seems to be the rule rather than the exception in clade 2, and individuals possessing only one of the two *gapCp* alleles present in taxa A and B have not yet been found. One taxon was identified from Java that is likely nonhybrid: specimens 449 and 451–455 (identical *rbcL* sequences) were observed to have "sexual" type spores and a single *gapCp* allele but putatively polyploid genome sizes (ca. 80 pg). It is possible that these specimens represent an autopolyploid entity, designated "taxon C" (Fig. 9). Putative hybrid specimens 459 and 461 (Mt. Halimun, Java) with multiple *gapCp* alleles each shared at least one allele with taxon C, thus implicating it as a possible "parent" species. Another difficulty in identifying diploid progenitor taxa is the apparent variety of genome sizes in clade 2 (Fig. 4). Clusters of specimens likely representing distinct biological entities (specimens with identical *gapCp* and *rbcL* sequences and matching morphology) each had very similar genome sizes, which indicates that overall variation of genome size in clade 2 is due to real variation between taxa rather than errors in measurement. Considering that genetic distances between clade 2 specimens are comparable to con-subgeneric interspecific distances in outgroup taxa, it seems reasonable that clade 2 comprises multiple biological species with varying diploid genome sizes. It should be possible to resolve this issue by obtaining chromosome counts of each major lineage within clade 2; additional field work will hopefully result in collection of diploid progenitor taxa and allow circumscription of species.

Conclusion—This study is the first to investigate the evolutionary history of the *C. minutum* species complex using molecular data and has focused on elucidating the overall phylogenic structure and confirming occurrence of hybridization within the group. Phylogenetic analyses of both plastid *rbcL* and nuclear *gapCp* support the existence of two large clades previously identified by morphological taxonomic studies that differ greatly in their evolutionary history. At least two putatively hybrid taxa (taxa A and B) were identified within clade 2 that may be stabilized by polyploidy and apogamy, respectively. Occurrence of apogamy was inferred by spore counts in several other distantly related specimens, which suggests that this mode of hybrid stabilization may have evolved independently within the complex multiple times. The *gapCp* sequences suggest the presence of many more hybrids within clade 2, but data for ploidy level and reproductive mode are lacking to confirm hybrid status. Nonetheless, the high genetic, morphological, and cytological diversity of clade 2 revealed in this study highlight the extreme complexity that can be produced by reticulate evolution. It will be critical during future investigation into this and other fern species complexes to obtain not only molecular data, but material that can be used for chromosome counts and spore observation, to obtain a complete picture of reticulation involving hybridization and polyploidy.

LITERATURE CITED

ARNOLD, M. L. 1992. Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics* 23: 237–261.

BARRINGTON, D. S., C. H. HAUFLER, AND C. R. WERTH. 1989. Hybridization, reticulation, and species concepts in the ferns. *American Fern Journal* 79: 55–64.

BECK, J. B., M. D. WINDHAM, G. YATSKIEVYCH, AND K. M. PRYER. 2010. A diploids-first approach to species delimitation and interpreting polyploid evolution in the fern genus *Astrolepis* (Pteridaceae). *Systematic Botany* 35: 223–234.

BELL, P. R. 1960. The morphology and cytology of sporogenesis of *Trichomanes proliferum* Bl. *New Phytologist* 59: 53–59.

BLUME, C. L. 1828. Enumeratio plantarum Javae et insularum adjacentium: minus cognitarum vel novarum ex herbariis Reinwardtii, Kohlii, Hasseltii et Blumii. Lugduni Batavorum, Leiden, The Netherlands.

BRAITHWAITE, A. F. 1964. A new type of apogamy in ferns. *New Phytologist* 63: 293–305.

BRAITHWAITE, A. F. 1969. The cytology of some Hymenophyllaceae from the Solomon Islands. *British Fern Gazette* 10: 81–91.

BRAITHWAITE, A. F. 1975. Cytotaxonomic observations on some Hymenophyllaceae from the New Hebrides, Fiji and New Caledonia. *Botanical Journal of the Linnean Society* 71: 167–189.

BROWNLIE, G. 1977. The pteridophyte flora of Fiji. *Nova Hedwigia. Beiheft* 55: 1–397.

COPELAND, E. B. 1933. Trichomanes. *Philippine Journal of Science* 51: 19–280.

COPELAND, E. B. 1938. Genera Hymenophyllacearum. *Philippine Journal of Science* 51: 2–110.

COPELAND, E. B. 1958. Fern flora of the Philippines. Bureau of Printing, Manila, Philippines.

CROXALL, J. P. 1975. The Hymenophyllaceae of Queensland. *Australian Journal of Botany* 23: 509–547.

DARWIN, C. 1859. The origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. John Murray, London, UK.

DUBUISSON, J.-Y., S. HENNEQUIN, E. J. P. DOUZERY, R. B. CRANFILL, A. R. SMITH, AND K. M. PRYER. 2003. *rbcL* phylogeny of the fern genus *Trichomanes* (Hymenophyllaceae), with special reference to Neotropical taxa. *International Journal of Plant Sciences* 164: 753–761.

EBIHARA, A., J.-Y. DUBUISSON, K. IWATSUKI, S. HENNEQUIN, AND M. ITO. 2006. A taxonomic revision of Hymenophyllaceae. *Blumea* 51: 221–280.

EBIHARA, A., H. ISHIKAWA, S. MATSUMOTO, S.-J. LIN, K. IWATSUKI, M. TAKAMIYA, Y. WATANO, AND M. ITO. 2005. Nuclear DNA, chloroplast DNA, and ploidy analysis clarified biological complexity of the *Vandenboschia radicans* complex (Hymenophyllaceae) in Japan and adjacent areas. *American Journal of Botany* 92: 1535–1547.

EBIHARA, A., K. IWATSUKI, M. ITO, S. HENNEQUIN, AND J.-Y. DUBUISSON. 2007. A global molecular phylogeny of the fern genus *Trichomanes* (Hymenophyllaceae) with special reference to stem anatomy. *Botanical Journal of the Linnean Society* 155: 1–27.

EBIHARA, A., K. IWATSUKI, T. A. OHSAWA, AND M. ITO. 2003. *Hymenophyllum paniense* (Hymenophyllaceae), a new species of filmy fern from New Caledonia. *Systematic Botany* 28: 228–235.

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

FLORA OF NORTH AMERICA EDITORIAL COMMITTEE. 1993. Flora of North America, vol. 2, Pteridophytes and gymnosperms. Oxford University Press, New York, New York, USA.

GASTONY, G. J., AND M. D. WINDHAM. 1989. Species concepts in pteridophytes: the treatment and definition of agamosporous species. *American Fern Journal* 79: 65–77.

GRANT, V. 1981. Plant speciation. Columbia University Press, New York, New York, USA.

GRUSZ, A. L., M. D. WINDHAM, AND K. M. PRYER. 2009. Deciphering the origins of apomictic polyploids in the *Cheilanthes yavapensis* complex (Pteridaceae). *American Journal of Botany* 96: 1636–1645.

HAMEED, C. A., K. P. RAJESH, AND P. V. MADHUSOODANAN. 2003. Filmy ferns of South India. Penta Book Publishers and Distributors, Calicut, India.

HASEBE, M., AND K. IWATSUKI. 1990. *Adiantum capillus–veneris* chloroplast DNA clone bank: As useful heterologous probes in the systematics of leptosporangiate ferns. *American Fern Journal* 80: 20–25.

HAUFLER, C. H. 2002. Homospory 2002: An odyssey of progress in pteridophyte genetics and evolutionary biology. *BioScience* 52: 1081–1093.

HOLTTUM, R. E. 1966. Flora of Malaya, vol. 2: Ferns of Malaya, 2nd ed. Government Printing Office, Singapore.

IWATSUKI, K. 1975. Contributions to the classification of filmy ferns (5). *Acta Phytotaxonomica et Geobotanica* 26: 173–179.

LEGENDRE, P. 2000. Reticulate evolution: from bacteria to philosopher. *Journal of Classification* 17: 153–195.

LINDER, C. R., B. M. E. MORET, L. NAKHLEH, AND T. WARNOW. 2004. Network (reticulate) evolution: Biology, models, and algorithms. The Ninth Pacific Symposium on Biocomputing (PSB) in Waimea, Hawaii, USA.

LINDER, C. R., AND L. H. RIESEBERG. 2004. Reconstructing patterns of reticulate evolution in plants. *American Journal of Botany* 91: 1700–1708.

MADDISON, D. R., AND W. P. MADDISON. 2000. MacClade 4: Analysis of phylogeny and character evolution. Sinauer, Sunderland, Massachusetts, USA.

MAKARENKOV, V., AND P. LEGENDRE. 2004. From a phylogenetic tree to a reticulated network. *Journal of Computational Biology* 11: 195–212.

MEHRA, P. N., AND G. SINGH. 1957. Cytology of Hymenophyllaceae. *Journal of Genetics* 55: 379–393.

MEYER–GAUEN, G., C. SCHNARRENBERGER, R. CERFF, AND W. MARTIN. 1994. Molecular characterization of a novel, nuclear–encoded, NAD+–dependent glyceraldehyde–3–phosphate dehydrogenase in plastids of the gymnosperm *Pinus sylvestris* L. *Plant Molecular Biology* 26: 1155–1166.

NAKAIKE, T. 2004. Index to scientific names of Japanese pteridophytes. *Journal of the Nippon Fernist Club* 3 (Supplement 2): 1–207.

NARAYAN, R. K. J. 1987. Nuclear DNA changes, genome differentiation and evolution in *Nicotiana* (Solanaceae). *Plant Systematics and Evolution* 157: 161–180.

OTTO, S. P., AND J. WHITTON. 2000. Polyploid incidence and evolution. *Annual Review of Genetics* 34: 401–437.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14: 817–818.

PRYER, K. M., A. R. SMITH, J. S. HUNT, AND J.-Y. DUBUISSON. 2001. *rbcL* data reveal two monophyletic groups of filmy ferns (Filicopsida: Hymenophyllaceae). *American Journal of Botany* 88: 1118–1130.

RAMSEY, J., AND D. W. SCHEMSKE. 1998. Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology and Systematics* 29: 467–501.

RIESEBERG, L. H. 1997. Hybrid origins of plant species. *Annual Review of Ecology and Systematics* 28: 359–389.

RIESEBERG, L. H. 2001. Polyploid evolution: Keeping the peace at genomic reunions. *Current Biology* 11: R925–R928.

RIESEBERG, L. H., AND D. E. SOLTIS. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5: 65–84.

RONQUIST, F., AND J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

RONQUIST, F., J. P. HUELSENBECK, AND P. VAN DER MARK. 2005. MrBayes 3.1 manual, draft 5/17/2005. http://mrbayes.csit.fsu.edu/mb3.1_manual.pdf [Accessed 6 April 2011].

SCHUETTPELZ, E., A. L. GRUSZ, M. D. WINDHAM, AND K. M. PRYER. 2008. The utility of nuclear *gapcp* in resolving polyploid fern origins. *Systematic Botany* 33: 621–629.

SIMMONS, M. P., AND H. OCHOTERENA. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

SLEDGE, W. A. 1968. The Hymenophyllaceae of Ceylon. *Journal of the Linnean Society of London*, *Botany* 60: 289–308.

STEBBINS, G. L. 1950. Variation and evolution in plants. Columbia University Press, New York, New York, USA.

STOKEY, A. G. 1940. Spore germination and vegetative stages of the gametophytes of *Hymenophyllum* and *Trichomanes. Botanical Gazette* 101: 759–790.

STOKEY, A. G. 1948. Reproductive structures of the gametophytes of *Hymenophyllum* and *Trichomanes. Botanical Gazette* 109: 363–380.

SWOFFORD, D. L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4b10. Sinauer, Sunderland, Massachusetts, USA.

TAKAMIYA, M. 1993. Chromosome numbers of *Woodsia kitadakensis* and *W. subcordata* (Woodsiaceae). *Journal of Japanese Botany* 68: 73–76.

TILQUIN, J. P. 1978. Observations cytotaxonomiques sur des Hymenophyllacees Africaines: I. Genres *Didymoglossum* Desv. et *Microgonium* Presl. *Caryologia* 31: 23–42.

TINDALE, M. D. 1963. Hymenophyllaceae. *Contributions of the New South Wales National Herbarium*. *Flora Series* 201: 1–49.

TSAI, J.-L., AND W.-C. SHIEH. 1994. Hymenophyllaceae. *In* T.-C. Huang [ed.], Flora of taiwan, vol. 1: Pteridophyta, gymnospermae, 2nd ed., 99–133. Editorial Committee of the Flora of Taiwan, Taipei, Taiwan.

VAN DEN BOSCH, R. B. 1861. Hymenophyllaceae Javanicae. Akad. van Wetenschappen, Amsterdam, The Netherlands.

WALKER, T. G. 1985. Some aspects of agamospory in ferns—the Braithwaite System. *Proceedings of the Royal Society of Edinburgh* 86: 59–66.

WENDEL, J. F., AND J. J. DOYLE. 1998. Phylogenetic incongruence: Window into genome history and molecular evolution. *In* D. E. Soltis, P. S. Soltis, and J. J. Doyle [eds.], Molecular systematics of plants II: DNA sequencing, 265–296. Kluwer, Boston, Massachusetts, USA.

YOROI, R. 1972. Studies on spore germination and gametophyte of Japanese Hymenophyllaceae. *Science Reports of the Tokyo Kyoiku Daigaku, Section B*, *Zoology and Botany* 15: 81–110.

YOROI, R. 1976. Gametophyte and apogamous embryo of *Crepidomanes latemarginale* from Isl. Ishigaki, Ryukyu. *Journal of Japanese Botany* 51: 257–267.

YOROI, R., AND K. IWATSUKI. 1977. An observation on the variation of *Trichomanes minutum* and allied species. *Acta Phytotaxonomica et Geobotanica* 28: 152–159.

YOUNG, N. D., AND J. HEALY. 2003. GapCoder automates the use of indel characters in phylogenetic analysis. *BMC Bioinformatics* 4: 6.

APPENDIX 1. Specimen Collection Data

Specimens sampled for DNA extraction in this study, including locality, voucher information, genome size (when available), and GenBank accession numbers.

Taxon Authority—**Specimen ID number**: Locality; Voucher (Hebarium); genome size (pg); *gapCp* GenBank accession number(s); *rbcL* GenBank accession number.

Crepidomanes bipunctatum (Poir.) Copel.—**N24**: New Caledonia, Mt. Koghi; *A. Ebihara 001220-01* (TNS); *gapCp* HQ638567, HQ638568; *rbcL* AB479128. ***Crepidomanes christii*** (Copel.) Copel.—**K27**: Malaysia, Sabah, Mt. Kinabalu; *A. Ebihara 000226-038* (TNS); *gapCp* HQ638559; *rbcL* HQ638660. ***Crepidomanes fallax*** (Christ) Ebihara & Dubuisson—**M6**: Madagascar; *F. Rakotondrainibe 6467* (P); *gapCp* HQ638564; *rbcL* AB257459. ***Crepidomanes humile*** (G. Forst.) Bosch—**V2**: Vanuatu, Espiritu Santo Isl., Mt. Tabwemasana; *S. Matsumoto 648* (TNS); *gapCp* HQ638572, HQ638573; *rbcL* AB479139. ***Crepidomanes latealatum*** (Bosch) Copel.—**A14**: Australia; *T. A. Ohsawa 001202-03* (TNS); *gapCp* HQ638549; *rbcL* AB257469. ***Crepidomanes kurzii*** (Bedd.) Tagawa & K. Iwats.—**A16**: Australia, Queensland; *A. Ebihara 010908-04* (TNS); *gapCp* HQ638550; *rbcL* HQ638663. ***Crepidomanes minutum*** (Blume) K. Iwats.—**302**: Malaysia, Sabah, Mt. Kinabalu; *Sugawara SB2007-68* (TNS); *gapCp* HQ638397; *rbcL* HQ638579. **303**: Malaysia, Sabah, Mt. Kinabalu; *Sugawara SB2007-70* (TNS); *gapCp* HQ638398, HQ638399, HQ638400; *rbcL* HQ638580. **304**: Malaysia, Sabah, Mt. Kinabalu; *Sugawara SB2007-75* (TNS); *gapCp* HQ638401, HQ638402; *rbcL* HQ638581. **305**: Malaysia, Sabah, Mt. Alabu; *Sugawara SB2007-90* (TNS); *gapCp* HQ638403; *rbcL* HQ638582. **306**: Malaysia, Sabah, Mt. Alabu; *Sugawara SB2007-98* (TNS); *gapCp* HQ638404; *rbcL* HQ638583. **307**: Japan, Akita Pref.; *A. Ebihara TH2007-494* (TNS); *gapCp* HQ638405, HQ638406, HQ638407; *rbcL* HQ638584. **308**: USA, Hawaii, Kauai; *D. Lorence 9468* (UC); *gapCp* HQ638408, HQ638409, HQ638410, HQ638411; *rbcL* HQ638585. **346**: Japan, Mt. Takako; *J. Nitta 185* (UC); *gapCp* HQ638412; *rbcL* HQ638586. **347**: Vanuatu, Espiritu Santo Isl.; *S. Matsumoto 0811-2* (TNS); *gapCp* HQ638413, HQ638414; *rbcL* HQ638587. **348**: Malaysia, Sabah, Mt. Kinabalu; *T. Arikawa 316B* (TNS); *gapCp* HQ638415, HQ638416, HQ638417, HQ638418; *rbcL* HQ638588. **349**: Australia, Queensland; *T. A. Ohsawa 001202-01* (TNS); *gapCp* HQ638419, HQ638420, HQ638421, HQ638422, HQ638423, HQ638424; *rbcL* HQ638589. **350**: Australia, Queensland; *T. A. Ohsawa 001202-02* (TNS); *gapCp* HQ638425, HQ638426; *rbcL* HQ638590. **351**: Malaysia, Sabah, Mt. Kinabalu; *A. Ebihara 000225-021* (TNS); *gapCp* HQ638427, HQ638428; *rbcL* HQ638591. **352**: Malaysia, Sabah, Mt. Kinabalu; *A. Ebihara 000224-014* (TNS); *gapCp* HQ638429, HQ638430, HQ638431, HQ638432, HQ638433, HQ638434; *rbcL* HQ638592. **353**: Malaysia, Sabah, Mt. Kinabalu; *A. Ebihara 000223-007* (TNS); *gapCp* HQ638435, HQ638436, HQ638437, HQ638438, HQ638439; *rbcL* HQ638593. **355**: French Polynesia, Moorea; *J. Nitta 250A* (UC); *gapCp* HQ638440, HQ638441; *rbcL* HQ638594. **357**: French Polynesia, Moorea; *J. Nitta 251A* (UC); 161.3 pg; *gapCp* HQ638442, HQ638443; *rbcL* HQ638595. **359**: French Polynesia, Moorea; *J. Nitta 253A* (UC); *gapCp* HQ638444; *rbcL* HQ638596. **361**: French Polynesia, Moorea; *J. Nitta 276A* (UC); 107.9 pg; *gapCp* HQ638445; *rbcL* HQ638597. **363**: Japan, Okinawa; *J. Nitta 353* (UC, TNS); 66.4 pg; *gapCp* HQ638446; *rbcL* HQ638598. **367**: Japan, Okinawa; *J. Nitta 369* (UC, TNS); 45.6 pg; *gapCp* HQ638447; *rbcL* HQ638599. **375**: Taiwan, Taipei Co.; *J. Nitta 408* (UC, TNS); 46.0 pg; *gapCp* HQ638448; *rbcL* HQ638600. **382**: Taiwan, Pingtung Co.; *J. Nitta 428* (UC, TNS); 110.9 pg; *gapCp* HQ638449, HQ638450; *rbcL* HQ638601. **390**: China, Lingnan; *K. Yao 11276* (TNS); *gapCp* HQ638451, HQ638452, HQ638453; *rbcL* HQ638602. **391**: China, Lingnan; *K. Yao 11109* (TNS); *gapCp* HQ638454, HQ638455, HQ638456, HQ638457, HQ638458, HQ638459; *rbcL* HQ638603. **392**: New Caledonia, Mt. Koghi; *M. Mitsuhashi 44* (TNS); *gapCp* HQ638460; *rbcL* HQ638604. **397**: Malaysia, Sabah, Mt. Kinabalu; *A. Ebihara 000223-012* (TNS); *gapCp* HQ638461, HQ638462, HQ638463, HQ638464; *rbcL* HQ638605. **404**: South Korea; *Shevok 16266* (UC); *gapCp* HQ638465; *rbcL* HQ638606. **405**: Equatorial Guinea; *D. H. Norris 106343* (UC); *gapCp* HQ638466; *rbcL* HQ638607. **406**: Equatorial Guinea; *D. H. Norris 106043* (UC); *gapCp* HQ638467; *rbcL* AB257474. **407**: USA, Hawaii, Hawaii; *Guerrant s.n.* (UC); *gapCp* HQ638468, HQ638469, HQ638470, HQ638471; *rbcL* HQ638608. **408**: USA, Hawaii, Molokai; *Wood 11105* (UC); *gapCp* HQ638472; *rbcL* HQ638609. **409**: USA, Hawaii, Oahu; *Wood 5619* (UC); *gapCp* HQ638473, HQ638474, HQ638475, HQ638476, HQ638477, HQ638478; *rbcL* HQ638610. **410**: USA, Hawaii, Oahu; *Wood 5061* (UC); *gapCp* HQ638479; *rbcL* HQ638611. **411**: French Polynesia, Ua Huka; *Wood 10752* (UC); *gapCp* HQ638480, HQ638481; *rbcL* HQ638612. **412**: Samoa; *Wall 3242* (UC); *gapCp* HQ638482; *rbcL* HQ638613. **414**: Papua New Guinea; *D. F. Grether 4077* (UC); *gapCp* HQ638483, HQ638484, HQ638485, HQ638486; *rbcL* HQ638614. **427**: Indonesia, Java, Mt. Gede; *U. Hapid 469* (BO, UC, TNS); 87.0 pg; *gapCp* HQ638487; *rbcL* HQ638615. **428**: Indonesia, Java, Mt. Gede; *U. Hapid 470* (BO, UC, TNS); 88.3 pg; *gapCp* HQ638488; *rbcL* HQ638616. **429**: Indonesia, Java, Mt. Gede; *U. Hapid 481* (BO, UC, TNS); 122.0 pg; *gapCp* HQ638489, HQ638490; *rbcL* HQ638617. **430**: Indonesia, Java, Mt. Gede; *U. Hapid 482* (BO, UC, TNS); 92.3 pg; *gapCp* HQ638491; *rbcL* HQ638618. **431**: Indonesia, Java, Mt. Gede; *U. Hapid 483* (BO, UC, TNS); 103.3 pg; *gapCp* HQ638492, HQ638493; *rbcL* HQ638619. **432**: Indonesia, Java, Mt. Gede; *U. Hapid 484* (BO, UC, TNS); 126.2 pg; *gapCp* HQ638494, HQ638495; *rbcL* HQ638620. **433**: Indonesia, Java, Mt. Gede; *U. Hapid 485* (BO, UC, TNS); 46.4 pg; *rbcL* HQ638621. **434**: Indonesia, Java, Mt. Gede; *U. Hapid 486* (BO, UC, TNS); 43.4 pg; *gapCp* HQ638496, HQ638497; *rbcL* HQ638622. **435**: Indonesia, Java, Mt. Gede; *U. Hapid 487* (BO, UC, TNS); 45.7 pg; *rbcL* HQ638623. **436**: Indonesia, Java, Mt. Gede; *U. Hapid 488* (BO, UC, TNS); 184.7 pg; *rbcL* HQ638624. **437**: Indonesia, Java, Mt. Gede; *U. Hapid 489* (BO, UC, TNS); 180.6 pg; *rbcL* HQ638625. **438**: Indonesia, Java, Mt. Gede; *U. Hapid 490* (BO, UC, TNS); 182.7 pg; *rbcL* HQ638626. **439**: Indonesia, Java, Mt. Gede; *U. Hapid 491* (BO, UC, TNS); 181.5 pg; *rbcL* HQ638627. **440**: Indonesia, Java, Mt. Gede; *U. Hapid 492* (BO, UC, TNS); 178.8 pg; *rbcL* HQ638628. **441**: Indonesia, Java, Mt. Gede; *U. Hapid 493* (BO, UC, TNS); 177.3 pg; *rbcL* HQ638629. **442**: Indonesia, Java, Mt. Gede; *U. Hapid 494* (BO, UC, TNS); 45.3 pg; *rbcL* HQ638630. **443**: Indonesia, Java, Mt. Gede; *U. Hapid 495* (BO, UC, TNS); 47.5 pg; *rbcL* HQ638631. **444**: Indonesia, Java, Mt. Gede; *U. Hapid 496* (BO, UC, TNS); 45.8 pg; *rbcL* HQ638632. **445**: Indonesia, Java, Mt. Gede; *U. Hapid 497* (BO, UC, TNS); 199.9 pg; *gapCp* HQ638498, HQ638499, HQ638500; *rbcL* HQ638633. **446**: Indonesia, Java, Mt. Gede; *U. Hapid 498* (BO, UC, TNS); 47.0 pg; *rbcL* HQ638634. **447**: Indonesia, Java, Mt. Halimun; *U. Hapid 510* (BO, UC, TNS); 108.5 pg; *gapCp* HQ638501, HQ638502; *rbcL* HQ638635. **448**: Indonesia, Java, Mt. Halimun; *U. Hapid 511* (BO, UC, TNS); 91.3 pg; *gapCp* HQ638503; *rbcL* HQ638636. **449**: Indonesia, Java, Mt. Halimun; *U. Hapid 517* (BO, UC, TNS); 93.0 pg; *gapCp* HQ638504; *rbcL* HQ638637. **450**: Indonesia, Java, Mt. Halimun; *U. Hapid 519* (BO, UC, TNS); 87.2 pg; *gapCp* HQ638505; *rbcL* HQ638638. **451**: Indonesia, Java, Mt. Halimun; *U. Hapid 520* (BO, UC, TNS); 96.8 pg; *gapCp* HQ638506; *rbcL* HQ638639. **452**: Indonesia, Java, Mt. Halimun; *U. Hapid 521* (BO, UC, TNS); 95.8 pg; *rbcL* HQ638640. **453**: Indonesia, Java, Mt. Halimun; *U. Hapid 522* (BO, UC, TNS); 92.8 pg; *rbcL* HQ638641. **454**: Indonesia, Java, Mt. Halimun; *U. Hapid 525* (BO, UC, TNS); 97.7 pg; *rbcL* HQ638642. **455**: Indonesia, Java, Mt. Halimun; *U. Hapid 527* (BO, UC, TNS); 86.7 pg; *gapCp* HQ638507; *rbcL* HQ638643. **456**: Indonesia, Java, Mt. Halimun; *U. Hapid 529* (BO, UC, TNS); 109.9 pg; *gapCp* HQ638508, HQ638509, HQ638510; *rbcL* HQ638644. **457**: Indonesia, Java, Mt. Halimun; *U. Hapid 530* (BO, UC, TNS); 107.3 pg; *gapCp* HQ638511; *rbcL* HQ638645. **458**: Indonesia, Java, Mt. Halimun; *U. Hapid 533* (BO, UC, TNS); 109.3 pg; *gapCp* HQ638512, HQ638513, HQ638514; *rbcL* HQ638646. **459**: Indonesia, Java, Mt. Halimun; *U. Hapid 534* (BO, UC, TNS); 128.2 pg; *gapCp* HQ638515, HQ638516, HQ638517; *rbcL* HQ638647. **460**: Indonesia, Java, Mt. Halimun; *U. Hapid 536* (BO, UC, TNS); 107.4 pg; *gapCp* HQ638518, HQ638519, HQ638520; *rbcL* HQ638648. **461**: Indonesia, Java, Mt. Halimun; *U. Hapid 538* (BO, UC, TNS); 125.3 pg; *gapCp* HQ638521, HQ638522, HQ638523; *rbcL* HQ638649. **462**: Indonesia, Java, Mt. Halimun; *U. Hapid 539* (BO, UC, TNS); 113.9 pg; *gapCp* HQ638524, HQ638525, HQ638526; *rbcL* HQ638650. **463**: Indonesia, Java, Mt. Halimun; *U. Hapid 540* (BO, UC, TNS); 88.1 pg; *gapCp* HQ638527; *rbcL* HQ638651. **464**: Indonesia, Java, Mt. Halimun; *U. Hapid 545* (BO, UC, TNS); 86.8 pg; *gapCp* HQ638528,

HQ638529; *rbcL* HQ638652. **465**: Indonesia, Java, Mt. Halimun; *U. Hapid 546* (BO, UC, TNS); 88.6 pg; *gapCp* HQ638530; *rbcL* HQ638653. **466**: Indonesia, Java, Mt. Halimun; *U. Hapid 547* (BO, UC, TNS); 87.5 pg; *gapCp* HQ638531, HQ638532; *rbcL* HQ638654. **467**: Philippines, Luzon; *L. Kuo 574* (UC, TNS); 89.1 pg; *gapCp* HQ638533, HQ638534, HQ638535; *rbcL* HQ638655. **468**: China, Hunan; *L. Kuo 740* (UC, TNS); 90.8 pg; *gapCp* HQ638536, HQ638537; *rbcL* HQ638656. **469**: China, Hunan; *L. Kuo 769* (UC, TNS); 49.0 pg; *gapCp* HQ638538, HQ638539, HQ638540, HQ638541; *rbcL* HQ638657. **471**: Japan, Ogasawara Islands; *H. Kato 090002* (UC, TNS); 51.0 pg; *gapCp* HQ638542, HQ638543, HQ638544; *rbcL* HQ638658. **A13**: Australia; *T. A. Ohsawa 001125-04* (TNS); *gapCp* HQ638546, HQ638547, HQ638548; *rbcL* AB479140. **A17**: Australia, Queensland; *T. A. Ohsawa 001201-03* (TNS); *gapCp* HQ638551, HQ638552; *rbcL* HQ638659. **H19**: Japan, Tokyo; *A. Ebihara 001015-01* (TNS); *gapCp* HQ638553; *rbcL* HQ638661. **K1**: Malaysia, Sabah, Mt. Kinabalu; *A. Ebihara 000221-01-1* (TNS); *gapCp* HQ638554, HQ638555, HQ638556, HQ638557, HQ638558; *rbcL* AB479136. **K31**: Philippines, Luzon; *A. Ebihara 040922-06* (TNS); *gapCp* HQ638560, HQ638561; *rbcL* HQ638662. **M13**: Reunion; J.-Y. *Dubuisson HR2003-5* (TNS); *gapCp* HQ638562, HQ638563; *rbcL* AB479134. **N19**: New Caledonia, Mt. Mou; *A. Ebihara 001228-02* (TNS); *gapCp* HQ638565, HQ638566; *rbcL* AB479138. **T9**: Thailand; *Iwatsuki 99H23B-1* (TNS); *gapCp* HQ638569, HQ638570, HQ638571; *rbcL* AB479135. **V4**: Vanuatu, Espiritu Santo Isl., Mt. Tabwemasana; *S. Matsumoto 649* (TNS); *gapCp* HQ638574, HQ638575, HQ638576, HQ638577, HQ638578; *rbcL* HQ638664. *Crepidomanes vitiense* (Baker) Bostock—**A11**: Australia, Queensland, Mt. Melium; *P. Bostock s.n.* (TI); *gapCp* HQ638545; *rbcL* AB162689.