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RESEARCH ARTICLE

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When it only takes one to tango: assessing the impact of apomixis in the fern genus *Pteris*

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Abstract

Premise: Apomixis (asexual reproduction by seed, spore, or egg) has evolved repeatedly across the tree of life. Studies of animals and angiosperms show that apomictic lineages are often evolutionarily short-lived and frequently exhibit different distributions than their sexual relatives. However, apomixis is rare in these groups. Less is known about the role of apomixis in the evolution and biogeography of ferns, in which ~10% of species are apomictic. Apomixis is especially common in the fern genus *Pteris* (34–39% of species); however, because of the limited taxonomic and geographic sampling of previous studies, the true frequency of apomixis and its associations with geography and phylogeny in this lineage remain unclear.

Methods: We used spore analyses of herbarium specimens to determine reproductive mode for 127 previously unsampled *Pteris* species. Then we leveraged biogeographic and phylogenetic analyses to estimate the global distribution and evolution of apomixis in *Pteris*.

Results: Among all *Pteris* species examined, we found that 21% are exclusively apomictic, 71% are exclusively sexual, and 8% have conflicting reports. Apomixis is unevenly distributed across the range of the genus, with the Paleotropics exhibiting the highest frequency, and has evolved numerous times across the *Pteris* phylogeny, with predominantly East Asian and South Asian clades containing the most apomictic species.

Conclusions: Apomixis arises frequently in *Pteris*, but apomictic species do not appear to diversify. Species that encompass both apomictic and sexual populations have wider ranges than exclusively sexual or apomictic species, which suggests that sexual and apomictic ferns could occupy separate ecological niches.

KEYWORDS

apomixis, biogeography, geographic parthenogenesis, Pteridaceae, *Pteris*

Apomixis is a widespread form of asexual reproduction in which new organisms are formed without the fusion of sperm and egg, often by way of a nonreductive meiosis (Van Dijk, 2009). Like other forms of asexual reproduction, this phenomenon is often considered an evolutionary dead end. Although there are short-term benefits to apomixis, such as maximizing reproductive capacity and skirting the cost of producing males (i.e., the twofold cost of sex; Maynard Smith, 1978), it is generally thought to be an unsuccessful long-term strategy. The primary hypothetical drawbacks to asexuality are (1) the lack of meiotic recombination, and thus the inability to purge deleterious mutations from the genome; and (2) a cap on overall genetic diversity, which in sexual species can buffer against ecological challenges such as parasites, competition among close relatives, or rapid environmental change (Hurst and Peck, 1996).

Despite these drawbacks, apomixis has evolved in numerous disparate lineages, including loaches (Itono et al., 2006), rotifers (Birky and Gilbert, 1971), ostracods (Havel and Hebert, 1989; Bode et al., 2010), weevils (Saura et al., 1993), ferns (Grusz, 2016), and flowering plants (Whitton et al., 2008). Within plant and animal clades that have high proportions of asexual taxa, apomixis is often localized to the tips of the evolutionary tree. In animals, there are a few instances of ancient clades of exclusively parthenogenetic species, most notably the diverse bdelloid rotifers; but among plants, apomicts appear to be evolutionarily young and generally do not diversify (Hörandl, 2006; Whitton et al., 2008), constrained by the dual challenges of low genotypic diversity and the accumulation of deleterious mutations.

A frequent correlate of apomixis is an expanded and distinct geographic distribution compared to close sexual relatives, a pattern described as "geographical parthenogenesis" (Vandel, 1928). Studies of animals and flowering plants have shown that asexual lineages are more likely to inhabit northerly latitudes, higher altitudes, disturbed or marginal habitats, and islands (Stebbins, 1950; Haufler et al., 1985; Suomalainen et al., 1987; Vrijenhoek and Parker, 2009). By contrast, sexual species often have ranges that are smaller or limited to select habitats within an asexual relative's expanded range (Hörandl, 2006). However, the mechanisms underlying both the frequent formation of apomictic lineages and their subsequent proliferation remain poorly understood, as most studies of apomixis come from the angiosperms, in which <1% of species exhibit this form of asexuality (Bicknell and Koltunow, 2004; Becks and Alavi, 2015).

Comparatively little is known about the origins and consequences of apomixis in the ferns, in which apomicts account for ~10% of species examined (Walker, 1985; Liu et al., 2012). The frequency of apomixis varies widely among fern lineages; although entirely absent from some groups (e.g., Cystopteridaceae), it is well established in others (e.g., Dryopteridaceae and Pteridaceae) (Liu et al., 2012). Many studies of apomixis in ferns focus on the Pteridaceae, a large and ecologically diverse family that, with >1200 species (PPG I, 2016), accounts for ~10% of total fern diversity. Across the family, apomixis has been observed in ≥6% of species and ≥28% of genera (Liu et al., 2012; Grusz, 2016), with some of the highest concentrations of known apomicts belonging to the desert-adapted subfamily Cheilanthoideae. The unusually high occurrence of apomixis within ferns makes them ideal for exploring the long-term evolution and biogeography of apomictic lineages.

The available data suggest that apomictic ferns, like apomictic angiosperms, are generally young—for example, ≤0.4 Ma for *Astrolepis* (Beck et al., 2011), 2.5 Ma for *Myriopteris* (Wickell et al., 2017), <8 Ma for the polystichoid ferns (Liu et al., 2012), and <15 Ma for apomictic ferns in Japan (Tanaka et al., 2014). Most species with adequate sampling show evidence of recurrent formation (Beck et al., 2011; Wickell et al., 2017; Grusz et al., 2021), and they frequently exhibit expanded ranges compared to their sexual progenitors (Tryon, 1968; Grusz et al., 2021). Given the elevated frequency of apomixis in ferns occurring in persistently or seasonally dry habitats, most studies characterizing the prevalence of this reproductive mode have focused on xeric-adapted genera (e.g., *Myriopteris* and *Pellaea*) and related lineages concentrated in North, Central, and South America. Morphological, cytogenetic, and phylogenetic studies of the pellaeids provided the foundation for a drought-driven model of the origin, establishment, and spread of obligate apomixis in ferns, with water limitation proposed as a primary driver of the transition to apomixis (Grusz et al., 2021). However, the pellaeids are restricted to xeric habitats and it remains to be shown whether the proposed model applies to apomixis in ferns generally (e.g., in persistently or seasonally wet regions).

Apomixis is not restricted to desert-adapted ferns and is widespread in *Pteris*, the largest genus in the Pteridaceae, which comprises >300 species found in diverse tropical and subtropical habitats across the globe. Previous studies (Walker, 1962; Chao et al., 2012a) indicate that about one-third of *Pteris* species exhibit apomixis. However, these estimates are based on a taxonomically and geographically limited sampling. Thus, the true extent of apomixis in *Pteris*, and whether those apomictic taxa exhibit the phylogenetic or biogeographic patterns observed in other apomictic lineages, has yet to be determined. Here, we build on previous work by greatly expanding the global sampling of reproductive mode in *Pteris*. We used a combination of morphological, biogeographic, and phylogenetic data to explore the following questions: (1) How common is apomixis across *Pteris*? (2) How are apomictic species distributed across the global range of *Pteris*? (3) Is apomixis phylogenetically structured within *Pteris*?

MATERIALS AND METHODS

Taxon sampling

The online database World Ferns (Hassler, 2004–2021) included a total of 331 *Pteris* species when accessed on 27 June 2018. Published data on reproductive mode were available for ~25% of those species (Walker, 1962; Chao et al., 2012a). Our study establishes reproductive mode for 127 previously unsampled species and provides additional data for 31 other taxa (Appendix S1). This sampling is truly global in scope, representing over half of all accepted species as well as spanning all regions of the world and all major clades. Molecular data were obtained for 191 *Pteris* species: 157 from GenBank and 34 newly sequenced here (Appendix 1). Two outgroup taxa, *Onychium japonicum* and *Actiniopteris dimorpha*, which have been shown to form a clade that is sister to *Pteris* (Zhang and Zhang, 2018), were also included in our molecular data set. Overlapping reproductive mode and molecular sequence data were available for 154 *Pteris* species.

Reproductive mode

Reproductive mode was newly determined for 158 species, represented by 158 *Pteris* specimens housed in the United

States National Herbarium (US), based on spore counts from mature sporangia. Spore number per sporangium is considered a reliable indicator of reproductive mode, with most sexually reproducing leptosporangiate ferns producing 64 spores and apomictic individuals producing 32 (Manton, 1950). This reduction in spore number per sporangium is due to a suppressed mitotic division in spore mother cells prior to entering meiosis (i.e., premeiotic endomitosis), resulting in spores of the same ploidy level as the parental sporophyte (diplospores) (Manton, 1950; Lovis, 1978; Walker, 1979; Gastony and Windham, 1989). Previous studies of *Pteris* have confirmed the correlation between spore number per sporangium and reproductive mode in this genus (Walker, 1962; Chao et al., 2012a). To establish a spore count per sporangium for each specimen, an intact, mature sporangium was located, removed from the specimen, and transferred to a drop of glycerin on a glass microscope slide using 0.2 mm insect pins (Austerlitz, Czech Republic). The sporangium was then dissected under a Leica M165C stereo microscope; spores were imaged and counted using a Leica DM4B compound microscope (Leica Microsystems, Wetzlar, Germany).

For each specimen directly examined in this study, a single sporangium was isolated and the spores within were counted. Only sporangia containing spores that were uniformly shaped and approximately equally sized (i.e., likely viable) were used to assess reproductive mode. When we encountered sporangia containing only malformed spores, we first evaluated additional sporangia on the same specimen; if all sporangia isolated housed similarly malformed spores, we selected a different specimen to represent that species. We supplemented our direct examinations with observations from previous studies of apomixis in *Pteris* (Appendix S1). When assigning reproductive mode to a species, we combined these data, as applicable, to determine a consensus reproductive mode: *sexual* (only sexual specimens observed or recorded), *apomictic* (only apomictic specimens observed or recorded), or *conflicting* (both sexual and apomictic specimens observed and/or recorded).

Geographic distribution of apomixis in *Pteris*

To examine the relationships between geography and reproductive mode, distribution data for each of the 331 *Pteris* species sampled were determined. The overall range of *Pteris* was divided into 12 distinct ecoregions, modified from Chao et al. (2014) to include three additional regions (West Indies; Mexico and Central America; and New Zealand and Pacific Islands). The 12 ecoregions considered in this study are as follows: North America (N); Mexico and Central America (M); West Indies (W); South America (S); Europe and Near East (E); Africa (F); Madagascar and Western Indian Ocean Islands (G); India, Bangladesh, and Sri Lanka (I); Malesia and Indochina (D); Australia (C); and New Zealand and Pacific Islands (P) (Figure 1). Species were coded according to their known ranges as described by World Ferns (Hassler, 2004–2021), with regional floras used to resolve conflicts for two species (Múlgura et al., 2012; Zhang et al., 2013). Naturalized species were coded according to their native ranges only.

DNA extraction, amplification, and sequencing

Plastid sequences were newly generated for 34 *Pteris* species (Appendix 1). Total genomic DNA was extracted from either silica-dried or herbarium-preserved leaf material following Schuettpelz and Pryer (2007). The plastid gene

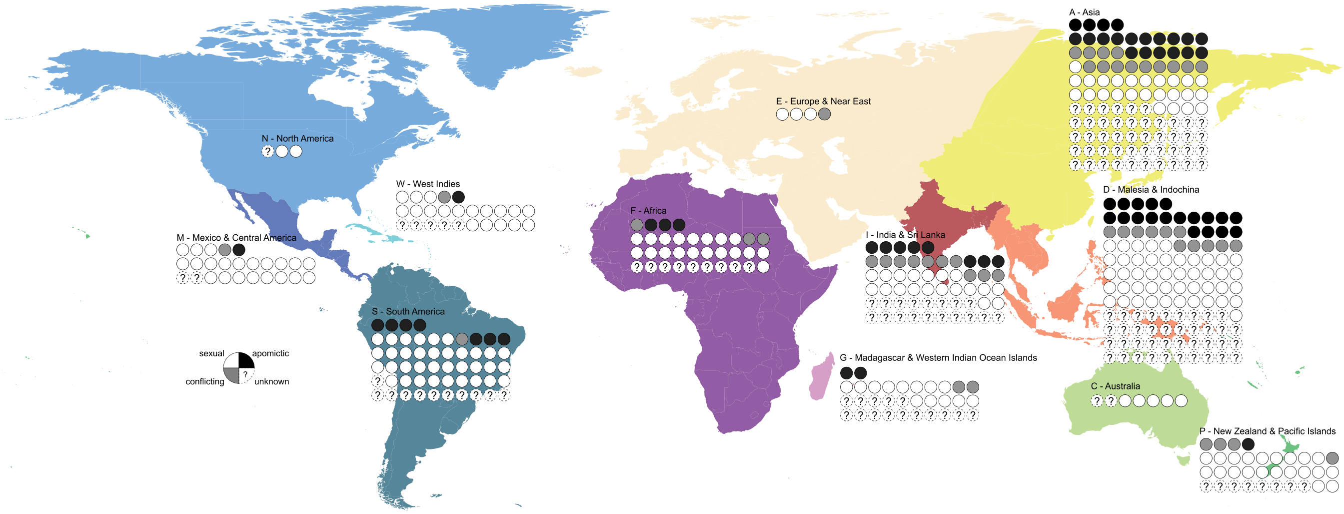

FIGURE 1 Geographic distribution of reproductive mode in *Pteris* in 12 ecoregions (modified from Chao et al., 2014). For each region, circles represent one *Pteris* species. Some species are represented in multiple ecoregions. Color of circles represents reproductive mode: unknown (dotted line, question mark), sexual (white), conflicting (gray), apomictic (black)

rbcL was amplified using primers and PCR conditions from Schuettpelz et al. (2007); sequences were generated on an ABI 3730xl capillary sequencer (Applied Biosystems, Waltham, Massachusetts, USA) at the National Museum of Natural History Laboratories of Analytical Biology (LAB). Newly generated *rbcL* sequences were assembled and edited using Sequencher version 5.1 (Gene Codes, Ann Arbor, Michigan, USA).

Sequence alignment

Published *rbcL* and *matK* sequences from *Pteris* species were retrieved from GenBank (Appendix 1). For each plastid region, these existing sequences and those newly generated here were aligned with MUSCLE version 3.8 (Edgar, 2004) and then manually refined using AliView version 1.26 (Larsson, 2014). Insertions and deletions were coded as missing data.

Phylogenetic analyses

Three data sets were analyzed: two single-locus data sets and one combined data set. For each plastid locus, PartitionFinder2 version 2.1.1 (Lanfear et al., 2017) was used to determine the best model of nucleotide substitution according to the corrected Akaike information criterion (AIC_c): the *rbcL* data set was partitioned by codon position and the *matK* data set was treated as a single partition. For each single-locus data set, maximum likelihood (ML) inferences were performed in IQTree version 1.6.10 (Nguyen et al., 2015) on the CIPRES computing cluster (Miller et al., 2010), with 1000 bootstrap replicates. ML trees for individual loci were compared for significant topological conflicts (≥70% bootstrap support) prior to concatenation. The concatenated, partitioned data set was then analyzed with IQTree using the same per locus parameters as the single-locus data sets.

Bayesian inference (BI) was performed on the partitioned, concatenated data set using MrBayes version 3.2.6 (Ronquist et al., 2012) on CIPRES. Four independent runs, each comprising four chains (one cold, three heated), were run for 10 million generations, with trees sampled every 1000 generations. Sample parameter traces were visualized in Tracer version 1.7.1 (Rambaut et al., 2018) to examine convergence among chains, with the first 25% of samples discarded as burn-in. The remaining trees were used to calculate a majority-rule consensus tree and posterior probabilities (PP).

Phylogeography of reproductive mode in *Pteris*

To investigate the relationships among biogeography, phylogeny, and reproductive mode, geographic profiles were generated for each of the major clades within *Pteris*, as defined by Zhang and Zhang (2018). To calculate clade geographic profiles, each species within a clade was assigned a value of "1" to be divided across the number of ecoregions in which that taxon was present (e.g., a species endemic to one ecoregion contributed 1 to that ecoregion, whereas a species found in three ecoregions contributed 0.33 to each ecoregion). These weighted distribution counts were then summed across the taxa within a clade and used to calculate the proportional geographic distribution of the group. This weighting strategy ensured that widespread species did not contribute disproportionately to the geographic profile of a clade as a whole.

Ancestral character reconstruction and phylogenetic signal of reproductive mode

Reproductive mode in ferns can be a complex trait to reconstruct in evolutionary analyses, as some species are exclusively sexual or apomictic, while others contain both sexual and apomictic populations (Tryon, 1968). Thus, it is possible to overestimate or underestimate the gains/losses of apomixis within a clade based on how reproductive mode is coded. To determine the rate of apomixis gain across the *Pteris* phylogeny while also accounting for these conflicting trait values (i.e., species encompassing sexual and apomictic populations), two character matrices were assembled. In the first data set, reproductive mode was coded as sexual (0) or apomictic (1), with conflicting taxa coded as exclusively sexual species, under the premise that the persistence of sexual reproduction could be indicative of an incomplete transition to apomixis. In the second data set, taxa with conflicting reproductive mode were coded as apomictic, under the premise that the presence of asexual individuals represented a trait gain, irrespective of whether apomixis has gone to fixation in the species. Maximum likelihood reconstructions of ancestral reproductive mode were performed using the Bayesian majority-rule consensus tree inferred from the concatenated data set using the R package ape (Paradis and Schliep, 2019). Tips lacking reproductive mode data were pruned from the topology prior to analysis. For each data set, three transition models were compared: ER (equal rates), SYM (equal forward and reverse rates), and ARD (all rates different).

We also reconstructed ancestral states while accounting for taxa with conflicting reports of reproductive mode using the threshold model from quantitative genetics (Felsenstein, 2012; Revell, 2014), using a Bayesian Markov chain Monte Carlo (MCMC) approach as implemented in the phytools function ancThresh (Revell, 2012). The threshold model estimates an unobserved continuous trait (the "liability"), which governs transitions between states in a discrete character, and is conditioned on the tip states for a given character (in this case, reproductive mode). When the liability crosses an arbitrary threshold, the discrete character trait changes states. Tips for which there are conflicting reports of reproductive mode were assigned equal prior probabilities for each state (sexual and apomictic). Species lacking reproductive mode data were discarded. We used Brownian motion to model the evolution of the liability, with 2 million generations of MCMC sampled every 2000 generations, with the first 20% of generations discarded as burn-in.

We evaluated the phylogenetic signal of reproductive mode across the *Pteris* phylogeny by calculating Fritz and Purvis's *D* statistic for discrete traits (Fritz and Purvis, 2010), as implemented in the phylo.d function in the R package caper (Orme et al., 2013). *D* measures the number of sister-clade differences in a discrete character state for a given phylogeny, with a value of 0 indicating evolution under a Brownian motion model (i.e., strong phylogenetic signal), a value of 1 indicating random trait evolution (i.e., no phylogenetic signal), and negative values or values >1 indicating highly conserved traits and overdispersed traits, respectively. As *D* can be calculated for binary traits only, we carried out simulations for two data sets: one in which taxa with conflicting reports of reproductive mode were coded as sexual and another in which conflicting taxa were coded as apomictic. Each simulation was run for 10,000 permutations using the majority-rule consensus tree estimated from MrBayes. Finally, because reproductive mode is unknown for a subset of taxa included in the phylogenetic analyses, we also calculated the phylogenetic signal of "missingness" using the miss.phylo.d function in the R package sensiPhy (Paterno et al., 2018).

Finally, we also tested the hypothesis that apomixis is more prevalent in species-rich clades using nonparametric Spearman's rank correlation (r_s). We performed two tests of this hypothesis, one in which we compared the number of exclusively apomictic taxa in each recognized section of *Pteris* to total clade richness, and a second in which we compared the sum total of exclusively apomictic taxa and taxa with conflicting reports of reproductive mode to overall clade richness.

RESULTS

Frequency of apomixis in *Pteris*

Through direct examination of herbarium specimens and a review of published data sets, reproductive mode data were gathered for a total of 204 *Pteris* species (62% coverage based on the World Ferns species count; Hassler, 2004–2021). Data were available in the literature for 77 species and we reexamined 31 of these, confirming the published reproductive mode for 26 species and documenting conflicts for 5 species (Table 1; Appendix S1). An additional 127 species were newly examined here. Of the species examined, 145 (71.1%) were found to reproduce only sexually, 42 (20.6%) were found to be exclusively apomictic, and 17 (8.3%) had conflicting reports of reproductive mode (Table 1; Appendix S1). In total, 59 of the species sampled (28.9%) had the ability to reproduce via apomixis.

TABLE 1 Summary of reproductive mode data (and their sources) available for species in the genus *Pteris*

Reproductive mode	Published reports only	New reports	Reexamined	Total species
Apomictic	13	23	6	42
Conflicting	5	–	12[a]	17
Sexual	28	104	13	145
Unknown	–	–	–	127

[a] Seven of these represent conflicts with previously published reports, and the other five are newly uncovered conflicts (between published reports and our findings).

Biogeography and reproductive mode in *Pteris*

Major geographic differences in the relative proportions of sexual and apomictic species were observed. The highest frequencies of apomixis among sampled species were found in Asia (56.9% of taxa examined), the Indian subcontinent (48.6%), and Malesia and Indochina (39.5%) (Figure 1). It should be noted, however, that these regions also had the highest proportions of taxa for which reproductive mode remains unknown (44.2%, 32.7%, and 33.9%, respectively). The lowest frequencies of apomixis were found in the New World, which was better represented in our sampling (Figure 1). Among the New World ecoregions, Mexico and Central America were dominated by sexual species (91.3% of taxa examined), as were the West Indies (90.0%) and South America (81.4%). Missing data comprised just 8.0%, 20.0%, and 20.4% for these regions, respectively. Regions with moderate frequencies of apomictic species include New Zealand and the Pacific Islands (19.2% of taxa examined), Africa (24.0%), and Madagascar and Indian Ocean Islands (23.5%).

Phylogenetic distribution of reproductive mode in *Pteris*

ML and BI analyses both recovered all clades and sections described by Zhang and Zhang (2018), with most of these receiving high support (Appendix S2). Three primary patterns emerged when mapping reproductive mode across the inferred phylogeny: (1) apomixis was found throughout *Pteris*, but was unequally distributed among the clades (Figure 2); (2) clades with the highest proportion of apomictic taxa or taxa with conflicting reports were also generally the most species-rich (Figure 2; Appendix S1); and (3) clades rich in apomixis were dominated by species in the Paleotropics, chiefly Asia, Malesia and Indochina, and the Indian subcontinent (Figure 2). The lone exception to the third pattern was section *Denticulatae*, a small group of

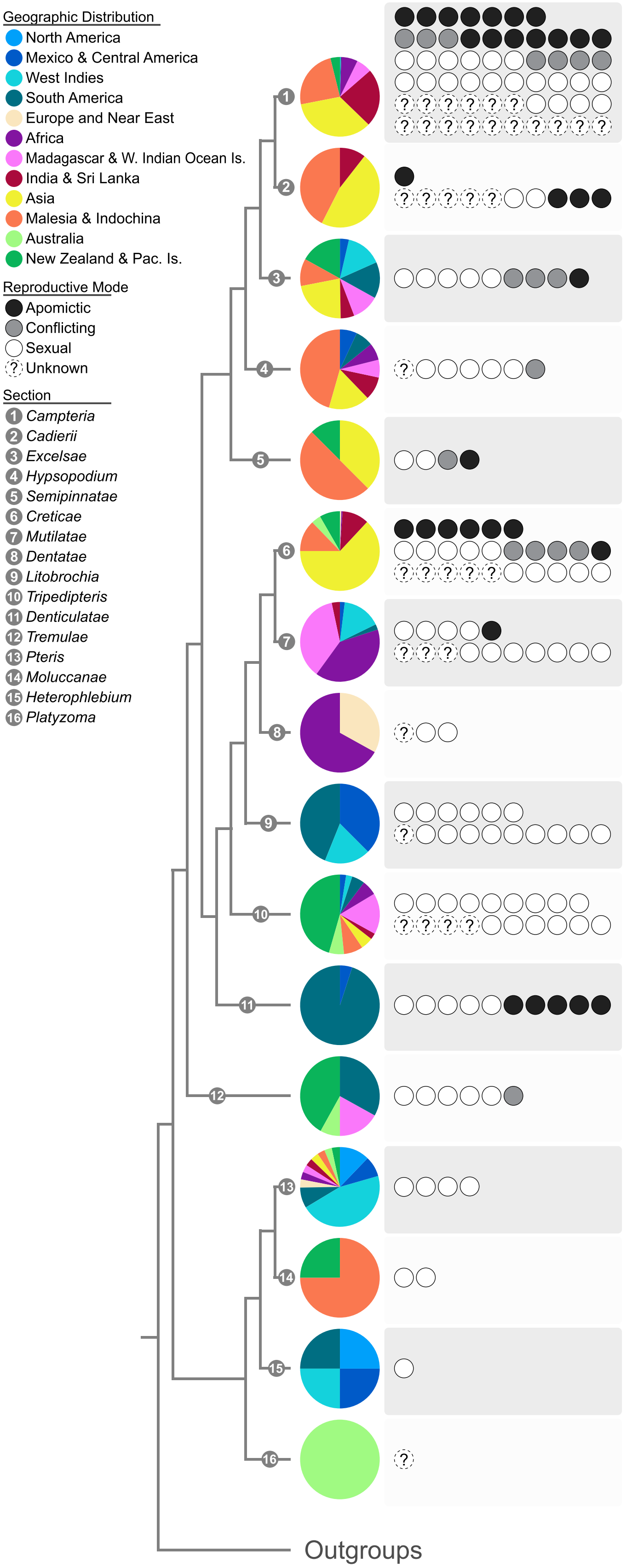

FIGURE 2 Phylogenetic and geographic distribution of reproductive mode in *Pteris*: summary cladogram of *Pteris* relationships derived from Bayesian inference and maximum likelihood analysis of concatenated *rbcL* and *matK* data set. Numbered branches correspond to sections within *Pteris* as delineated by Zhang and Zhang (2018). Pie charts represent weighted geographic profiles for each section. Colored circles for each clade indicate the reproductive modes of the taxa sampled within the clade; each circle corresponds to one taxon within a respective clade. See Appendices S1 and S2 for reproductive modes and geographic distribution of individual taxa

TABLE 2 Comparison of maximum likelihood models tested for the evolution of apomixis in *Pteris*

	Conflicting taxa coded as sexual		Conflicting taxa coded as apomictic	
Model	**lnLik**	**Transition rates**	**lnLik**	**Transition rates**
Equal rates (ER)	−96.785	34.587	−103.477	133.431
Symmetrical (SYM)	−96.785	34.587	−103.477	133.431
All rates different (ARD)	−79.322	115375.14, 31465.95 (forward, reverse)	−95.145	289.642, 131.295 (forward, reverse)

exclusively New World species in which 50% of the sampled taxa were found to be apomictic. For clades within *Pteris*, the numbers of apomictic species were positively correlated with total species richness. This correlation was observed when we included only exclusively apomictic species ($r_s = 0.61$, $P < 0.05$), as well as when we included both apomicts and taxa with conflicting reports of reproductive mode ($r_s = 0.58$, $P < 0.05$).

Ancestral state reconstruction and phylogenetic signal

Our ancestral state reconstructions of reproductive mode in *Pteris* showed that transitions between sexuality and apomixis were best explained by an ARD model, with gains of apomixis two to three times more likely than reversions to sex (Table 2). This pattern was observed whether conflicting taxa were coded as sexual or as apomictic. The results of the threshold model show that independent transitions to apomixis were common, but unevenly distributed across the phylogeny (Appendix S3). The number of transitions to apomixis presented is likely conservative, given that the ancestral state reconstruction included just 75% of the species from our larger phylogeny (Appendix S2). In our analyses of phylogenetic signal, apomixis was found to be weakly phylogenetically structured (Table 3).

DISCUSSION

The evolutionary and ecological factors contributing to the prevalence of apomixis in ferns and its asymmetric distribution among fern lineages have long remained elusive. One obstacle to understanding the importance of apomixis, as well as its role in fern diversification, is the paucity of comprehensive surveys of reproductive mode. Most studies of apomixis in ferns to date have focused on breeding system dynamics within particular species complexes (Gastony, 1988; Grusz et al., 2009; Dyer et al., 2012; Hori et al., 2014; Patel et al., 2018). In the present study, we build on earlier surveys of reproductive mode in the apomict-rich genus *Pteris* by expanding both the taxonomic and geographic sampling of species within the group. Using a combination of direct examination and literature searches, we compiled the most comprehensive assessment of apomixis for the genus *Pteris* to date. Among the 204 species that have now been examined, ~29% are either exclusively apomictic or include populations of apomictic individuals (Appendix S1). This finding is lower than previous estimates of apomixis in *Pteris*, which found 34–39% of taxa reproducing via apomixis (Walker, 1962; Chao et al., 2012a). Nonetheless, we confirm that apomixis is exceedingly common in this genus, and possibly more common than observed in the well-studied, xeric-adapted genera *Myriopteris* (27% of named species; Grusz et al., 2014) and *Pellaea* (≥15% of named species, but perhaps ≥30% when considering that significant taxonomic revision is needed to make *Pellaea* monophyletic; Grusz et al., 2021). Further, ours may well be an underestimate, given that spore analysis was performed on a single specimen for most of the species we examined directly. There was also substantial variation in the number of specimens used to assign reproductive mode in previous surveys, with determinations based on data from as few as one to as many as a dozen observations. Thus, some taxa that have both sexual and apomictic populations could have been overlooked. Moreover, taxa for which specimens were unavailable and whose reproductive mode(s) remain unknown are concentrated primarily in regions with the highest frequencies of apomixis (Figure 1). Increased sampling from these regions

TABLE 3 Phylogenetic signal of apomixis in *Pteris*. Values for Fritz and Purvis's *D* statistic for measured phylogenetic signal in a discrete binary trait

Trait	**Fritz and Purvis's *D***	***P* (observed vs. random distribution)**	***P* (observed vs. Brownian motion evolution)**
Apomixis (conflicting coded as sexual)	0.564	<0.001	0.002
Apomixis (conflicting coded as apomictic)	0.499	<0.001	0.003
Missing reproductive mode data	0.866	0.112	<0.001

will likely reveal further instances of apomixis, and our estimate of ~29% could thus be considered conservative.

Phylogenetic and ancestral state reconstruction analyses revealed that, in addition to having evolved multiple times, apomixis is also differentially distributed among the major clades of *Pteris*. For example, none of the species sampled in sections *Litobrochia* and *Tripedipteris* showed evidence of apomixis, whereas ≥50% of the sampled species belonging to sections *Creticae* and *Denticulatae* were capable of apomixis (Figure 2 and Appendix S1). Similar discrepancies have been observed elsewhere within the Pteridaceae and more broadly across ferns (Grusz et al., 2009; Beck et al., 2011; Sigel et al., 2011; Dyer et al., 2012; Li et al., 2012; Liu et al., 2012). Given our incomplete taxonomic sampling and low support values for many subterminal nodes, it is difficult to conclude precisely how many times apomixis has arisen. However, we find no evidence that the switch to apomixis leads to a burst of diversification, as no clades of exclusively apomictic taxa were recovered. In our analyses, there were only three examples of sister-species pairs of exclusively apomictic taxa (*P. dataensis* + *P. glaucovirens* in section *Campteria*; *P. esquirolii* + *P. pellucidifolia* in section *Creticae*; and *P. brasiliensis* + *P. denticulata* in section *Denticulatae*), and just four instances of sister-species pairs between an apomictic species and a species with conflicting reproductive mode (*P. normalis* + *P. scabripes* in section *Campteria*; *P. inaequalis* + *P. terminalis* in section *Excelsae*; *P. dispar* + *P. dissitifolia* in section *Semipinnatae*; and *P. actiniopteroides* + *P. gallinopes* in section *Creticae*) (Appendix S2). Thus, our results are consistent with the hypothesis that apomixis is a tip-level phenomenon in ferns, with apomictic taxa representing recurrently formed, but ultimately short-lived, evolutionary entities (Beck et al., 2011; Wickell et al., 2017).

Our analyses also reveal that apomixis is only weakly phylogenetically structured, despite the ostensible overrepresentation of apomixis in select *Pteris* clades. And, given that the absence of reproductive data from taxa within the molecular analysis (i.e., “missingness”) was shown to have no phylogenetic signal (Table 3), it remains unclear whether improved taxonomic and reproductive mode sampling would reveal additional phylogenetic patterns of apomixis in this genus. The lack of a strong phylogenetic signal in the distribution of apomixis across *Pteris*, coupled with the high incidence of apomixis across the Pteridaceae as a whole, suggests that the genetic and epigenetic factors that promote this phenomenon (Wyder et al., 2020) originated early in the evolution of this fern lineage.

Transitions to apomixis are frequently associated with hybridization and polyploidy, though the precise roles these two processes play in the establishment and persistence of apomixis are uncertain (Hörandl, 2006). Although polyploidy and hybridization are recognized as major speciation mechanisms across plants (Mable, 2003; Soltis et al., 2007), they have played particularly prominent roles in the diversification of ferns (Barrington et al., 1989; Dauphin et al., 2018), in which ≥31% of species result from autopolyploid or allopolyploid events (Wood et al., 2009). Cytogenetic studies of apomictic ferns show that most are polyploid (Manton, 1950; Walker, 1962; Windham and Yatskievych, 2003), and apomixis is particularly common among fern lineages that form reticulate complexes (Grusz et al., 2009; Chao et al., 2012b). Grusz et al. (2021) highlight the strong association between whole genome duplication and the origin and establishment of apomixis in ferns, for which apomixis provides a critical mechanism to escape unbalanced chromosome pairing—and thus the formation of abortive spores—during meiosis. *Pteris* is characterized by an especially high proportion of polyploids, with perhaps as many as 60% of species arising from polyploid speciation (Chao et al., 2012a). Moreover, many species within *Pteris* readily hybridize, forming large species complexes (Walker, 1958; Chao et al., 2010, 2012b). Thus, it would appear that these genetic underpinnings of apomixis are present across *Pteris* as a whole. Apomixis is found disproportionately in some clades over others, however, indicating that a predisposition toward polyploidy and/or hybridization, though necessary for the genesis of apomictic lineages, is hardly sufficient.

Grusz et al. (2021) provide a model for the evolution of obligate apomixis that incorporates both a genetic propensity for polyploid formation and strong selection due to water limitation in xeric habitats. In ferns, there is ample reason to believe that apomixis confers some competitive advantage in habitats with low water availability, as evidenced by the prevalence of apomictic taxa in the xeric-adapted cheilanthoid clade. Given the crucial role that water plays in the life cycle of sexual ferns, apomixis could be a viable alternative to maintaining sex in habitats where overall water availability is low, as in deserts or dry forests.

Considering the interplay of genetic and environmental factors proposed in the origin and maintenance of apomixis, could water availability explain the uneven geographic distribution of apomixis in *Pteris*? The highest frequencies of apomictic taxa in our study were observed in the Paleotropics, where annual precipitation is substantial but also highly seasonal due to the Southeast (SE) Asian monsoon. The uplift of the Qinghai–Tibetan Plateau, and the subsequent strengthening of the SE Asian monsoon, has been credited with driving the rapid diversification of many angiosperm lineages (Zhang and Fritsch, 2010; Chen et al., 2012; Sun et al., 2012; Ding et al., 2020) and several groups of ferns, including the polystichoids (Liu et al., 2012; Le Pechon et al., 2016) and lepisoroids (Wang et al., 2012). Overall fern richness is strongly correlated with mean annual precipitation (Kreft et al., 2010), but at least one study has shown that apomictic species richness in ferns is associated with seasonality of precipitation (Tanaka et al., 2014). Divergence time estimates for apomict-rich clades in SE Asia, including the Pteridaceae, support repeated origins of apomixis across the fern phylogeny and show that the crown groups of apomictic ferns diversified <15 mya (Liu et al., 2012; Tanaka et al., 2014), coinciding with a proposed period of intensification of the SE Asian monsoon. Estimated ages of major, apomict-rich clades within *Pteris*, particularly those with origins in SE Asia, Malesia, and the Indian subcontinent, also seem to parallel the strengthening of the monsoon regime (Chao et al., 2014), but further study is required to elucidate what effect, if any, the evolving SE Asian monsoon may have had on the origin and persistence of apomixis in this group and across ferns more broadly.

Although previous studies of angiosperms and ferns have shown that apomicts often exhibit larger geographic ranges than their sexual progenitors (Tryon, 1968; Kearney, 2005; Hörandl and Paun, 2007; Wickell et al., 2017; Grusz et al., 2021), the average number of ecoregions occupied by exclusively sexual and exclusively apomictic *Pteris* species were approximately equal (1.57 and 1.48 ecoregions, respectively). By contrast, *Pteris* species that include both apomictic and sexual individuals are found in 2.76 ecoregions on average, raising the intriguing possibility that these taxa may represent diverging sexual and apomictic lineages that are inhabiting distinct ecological niches (i.e., geographical parthenogenesis). The divergent distributions of apomicts and sexuals have been attributed to differences in competitive ability or ecological tolerance between individuals with different reproductive modes (Hörandl, 2006). Alternatively, apomixis may facilitate long-distance dispersal in ferns (Schneller et al., 1998), and the broader distributions of species with both sexual and apomictic populations could reflect dispersal of apomictic individuals out of a founding sexual population's range, independent of any changes in niche breadth or preference.

Generally, apomictic angiosperms and animals are more likely to populate—and often dominate—higher latitudes and altitudes, and better tolerate xeric or disturbed habitats than their sexual progenitors (Glesener and Tilman, 1978). Studies of the distributions of apomictic ferns from desert-adapted lineages show that apomicts have expanded ranges compared to their sexual progenitors (Tryon, 1968; Wickell et al., 2017; Grusz et al., 2021), but this is not well studied in apomictic ferns outside of xeric habitats. Contrary to the northerly pattern observed in many apomictic angiosperms, we see a clear easterly trend in *Pteris* whereby apomicts are highly concentrated in the Paleotropics, particularly from the Indian subcontinent to Malesia and Indochina. Curiously, limited data have shown that apomictic ferns in monsoonal regions have a smaller range size and are more likely to occur at lower latitudes and altitudes (Tanaka et al., 2014). However, it is outside the scope of this study to determine whether the average range size of apomictic *Pteris* species is significantly different from that of sexual species, and further work is needed to ascertain whether apomixis in tropical ferns leads to ecological and biogeographic shifts comparable to those observed in apomictic angiosperms.

CONCLUSIONS

The remarkable frequency and persistence of apomixis across ferns—despite the theoretical disadvantages of abandoning sex—have perplexed the pteridological community for nearly a century. Our results show that apomixis is a widespread and recurrently evolving reproductive strategy in *Pteris* but also an ultimately short-lived one. Due to gaps in available reproductive mode data, it is difficult to estimate how many independent origins of asexuality have occurred in this genus, and increased sampling will likely reveal additional apomictic species. Preliminary evidence also suggests that apomixis varies across the global range of *Pteris*, with East Asia and South Asia being home to the highest concentrations of apomictic species.

Although our data are insufficiently granular to confirm a direct link between apomixis and specific regional conditions (e.g., seasonal precipitation) or to conclude that apomictic species in *Pteris* occupy different niches or ranges than sexual species, our findings provide potentially intriguing avenues for future research into the biotic and abiotic factors underlying the prevalence of apomixis in tropical ferns. Given the asymmetrical distribution of apomixis across *Pteris*, additional work is needed to investigate clade-specific characteristics—whether cytogenetic or ecological—associated with the adoption of asexuality. Furthermore, studies of species with both documented sexual and apomictic individuals are likely to prove integral for disentangling the respective roles of polyploidy and environmental stressors in the evolution of apomixis. Range-wide surveys of reproductive mode combined with spore-based ploidy estimates and ecological niche modeling could illuminate how breeding system dynamics and environmental factors govern shifts to asexuality, and subsequent range expansions/contractions, in ferns.

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AUTHOR CONTRIBUTIONS

K.T.P., E.S., A.L.G., and M.D.W. designed the study. H.R. and K.T.P. collected reproductive mode data. K.T.P. and H.R. assembled molecular data sets. K.T.P. performed phylogenetic analyses and applied phylogenetic comparative methods. K.T.P. and H.R. wrote the manuscript, with contributions from A.L.G., M.D.W., and E.S.

DATA AVAILABILITY STATEMENT

DNA sequences used in this study are deposited in GenBank (Appendix 1). Species distributions and reproductive mode data (and associated references for previously published reports of reproductive mode) are available in Appendix S1.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Appendix S1. Geographic and reproductive mode data for *Pteris.*

Appendix S2. *Pteris* phylogeny with associated reproductive mode and biogeographic data.

Appendix S3. Ancestral state reconstruction of reproductive mode in *Pteris.*

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APPENDIX 1

List of *Pteris* and outgroup material included in this study. For each collection, the species name, voucher information, and GenBank accession numbers (*rbcL*, *matK*) are provided.

Taxon Authority, *Voucher*, collection locality, *rbcL*: GenBank accession (source), *matK*: GenBank accession (source), herbarium (NA = not available).

Actiniopteris dimorpha Pic. Serm, *Schneider s.n.*, cultivated, *rbcL*: EF452130 (Schuettpelz et al., 2007), *matK*: MF972721 (Zhang and Zhang, 2018), GOET; ***Onychium japonicum*** (Thunb.) Kunze, *Zhang et al. 5997*, China, *rbcL*: KM008138 (Zhang et al., 2015), *matK*: MF972723 (Zhang and Zhang, 2018), CDBI; ***Pteris actiniopteroides*** Christ, *Zhang 1384*, China, *rbcL*: KM008146 (Zhang et al., 2015), *matK*: MF972725 (Zhang et al., 2015), CDBI; ***Pteris altissima*** Poir., *Nitta 863*, Costa Rica, *rbcL*: KM008147 (Zhang et al., 2015), *matK*: MF972726 (Zhang et al., 2015), UC; ***Pteris amoena*** Blume, *Qingzang 74-5078*, China, *rbcL*: KM008148 (Zhang et al., 2015), KUN; ***Pteris angustata*** (Fée) C. V. Morton, *Mynssen 1430*, Brazil, *rbcL*: MW660786 (this study), SP; ***Pteris angustipinna*** Tagawa, *Lu s.n.*, Taiwan, *rbcL*: KF289683 (Chao et al., 2014), *matK*: KF289553 (Chao et al., 2014), TAIF; ***Pteris arborea*** L., *Christenhusz 4050*, Guadeloupe, *rbcL*: KM008149 (Zhang et al., 2015), TUR; ***Pteris argyraea*** T. Moore, *Fraser-Jenkins FN145*, India, *rbcL*: KF289684 (Chao et al., 2014), *matK*: KF289554 (Chao et al., 2014), TAIF; ***Pteris arisanensis*** Tagawa, *Chao 1621*, Vietnam, *rbcL*: KF289677 (Chao et al., 2014), *matK*: KF289547 (Chao et al., 2014), TAIF; ***Pteris aspericaulis*** Wall. ex J. Agardh, *Fraser-Jenkins FN36*, India, *rbcL*: KF289685 (Chao et al., 2014), *matK*: KF289555 (Chao et al., 2014), TAIF; ***Pteris assamica*** Fraser-Jenk., *Fraser-Jenkins FN5*, Nepal, *rbcL*: KF289686 (Chao et al., 2014), *matK*: KF289556 (Chao et al., 2014), TAIF; ***Pteris atrovirens*** Willd., *Fay 1049*, Sierra Leone, *rbcL*: MW660785 (this study), US; ***Pteris bahamensis*** (J. Agardh) Fée, *Rothfels 4024*, United States, *rbcL*: KM008150 (Zhang et al., 2015), DUKE; ***Pteris bella*** Tagawa, *Zhang 1300*, China, *rbcL*: KM008152 (Zhang et al., 2015), *matK*: MF972730 (Zhang et al., 2015), CDBI; ***Pteris berteroana*** J. Agardh, *Solbrig 3838*, Chile, *rbcL*: MW660784 (this study), US; ***Pteris biaurita*** L., *Lu 17285*, China, *rbcL*: KF289676 (Chao et al., 2014), *matK*: KF289546 (Chao et al., 2014), TAIF; ***Pteris blumeana*** J. Agardh, *Barcelona 2320*, Philippines, *rbcL*: MW660783 (this study), US; ***Pteris boliviensis*** J. Prado & A. R. Sm., *Schuettpelz 268*, Brazil, *rbcL*: KM008212 (Zhang et al., 2015), *matK*: MF972781 (Zhang et al., 2015), DUKE; ***Pteris boninensis*** H. Ohba, *Chao 1941*, Japan, *rbcL*: KF289743 (Chao et al., 2014), *matK*: KF289613 (Chao et al., 2014), TAIF; ***Pteris borneensis*** Y. S. Chao, *Chao 2382*, Malaysia, *rbcL*: KY705067 (Chao et al., 2017b), *matK*: KY705069 (Chao et al., 2017b), TAIF; ***Pteris brasiliensis*** Raddi, *Prado 2049*, Brazil, *rbcL*: KM008155 (Zhang et al., 2015), *matK*: MF972733 (Zhang et al., 2015), SP; ***Pteris buchananii*** Baker ex Sim, *Kamau 382*, Kenya, *rbcL*: KM008156 (Zhang et al., 2015), EA; ***Pteris burtonii*** Baker, *Kamau 227*, Uganda, *rbcL*: KM008157 (Zhang et al., 2015), EA; ***Pteris cadieri*** Christ, *Zhang 1240*, Taiwan, *rbcL*: KM008158 (Zhang et al., 2015), *matK*: MF972734 (Zhang et al., 2015), CDBI; ***Pteris catoptera*** Kunze, *Kamau 463*, Kenya, *rbcL*: KM008159 (Zhang et al., 2015), EA; ***Pteris chiapensis*** A. R. Sm., *Pérez-Farrera 2978*, Mexico, *rbcL*: KM008160 (Zhang et al., 2015), MO; ***Pteris chilensis*** Desv., *Zöllner 17416*, Chile, *rbcL*: KM008161 (Zhang et al., 2015), MO; ***Pteris ciliaris*** D. C. Eaton, *Thompson 10709*, Dominican Republic, *rbcL*: MW660782 (this study), US; ***Pteris comans*** G. Forst., *B.D. Duncan 80056*, Australia, *rbcL*: KF289651 (Chao et al., 2014), *matK*: KF289521 (Chao et al., 2014), MEL; ***Pteris commutata*** Kuhn, *Kamau 331*, Uganda, *rbcL*: KM008162 (Zhang et al., 2015), EA; ***Pteris congesta*** J. Prado, *Mynssen 1440*, Brazil, *rbcL*: MW660781 (this study), SP; ***Pteris cretica*** L., *Zhang 5034*, China, *rbcL*: MF972806 (Zhang and Zhang, 2018), *matK*: MF972736 (Zhang and Zhang, 2018), CDBI; ***Pteris croesus*** Bory, *Le Pechon 1215*, Réunion, *rbcL*: MF972807 (Zhang and Zhang, 2018), *matK*: MF972738 (Zhang and Zhang, 2018), CDBI; ***Pteris dactylina*** Hook., *Zhang 1391*, China, *rbcL*: KM008164 (Zhang et al., 2015), *matK*: MF972739 (Zhang et al., 2015), CDBI; ***Pteris dataensis*** Copel., *Liu 9973*, Philippines, *rbcL*: KF289703 (Chao et al., 2014), *matK*: KF289573 (Chao et al., 2014), TAIF; ***Pteris decrescens***

Christ, *Lin s.n. 20120819*, China, *rbcL*: KF289751 (Chao et al., 2014), *matK*: KF289621 (Chao et al., 2014), P; ***Pteris decurrens*** C. Presl, *Prado 1082*, Brazil, *rbcL*: EF473703 (Prado et al., 2007), SP; ***Pteris deflexa*** Link, *Prado 2124*, Brazil, *rbcL*: KM008167 (Zhang et al., 2015), SP; ***Pteris deltodon*** Baker, *Zhang 1356*, China, *rbcL*: KM008168 (Zhang et al., 2015), *matK*: MF972742 (Zhang et al., 2015), CDBI; ***Pteris dentata*** Forssk., *Kamau 563*, Kenya, *rbcL*: MF972808 (Zhang and Zhang, 2018), EA; ***Pteris denticulata*** Sw., *Prado 2159*, Brazil, *rbcL*: KM008170 (Zhang et al., 2015), *matK*: MF972743 (Zhang and Zhang, 2018), SP; ***Pteris dimorpha*** Copel., *Moran 5447*, Taiwan, *rbcL*: MW660780 (this study), US; ***Pteris dispar*** Kunze, *Zhang 1463*, China, *rbcL*: KM008217 (Zhang et al., 2015), *matK*: MF972744 (Zhang et al., 2015), CDBI; ***Pteris dissitifolia*** Baker, *Chao 1550*, Vietnam, *rbcL*: KF289645 (Chao et al., 2014), *matK*: KF289515 (Chao et al., 2014), TAIF; ***Pteris elongatiloba*** Bonap., *Janssen 2958*, Madagascar, *rbcL*: KF289717 (Chao et al., 2014), *matK*: KF289587 (Chao et al., 2014), P; ***Pteris ensiformis*** Burm. f., *Zhang 1312*, China, *rbcL*: KM008174 (Zhang et al., 2015), *matK*: MF972746 (Zhang et al., 2015), CDBI; ***Pteris esquirolii*** Christ, *S.-Y. Dong s.n.*, China, *rbcL*: KF289712 (Chao et al., 2014), *matK*: KF289582 (Chao et al., 2014), IBSC; ***Pteris exigua*** O. G. Martínez & J. Prado, *Martinez 1895*, Argentina, *rbcL*: MW660779 (this study), SP; ***Pteris finotii*** Christ, *Zhang 1323*, China, *rbcL*: KM008176 (Zhang et al., 2015), *matK*: MF972749 (Zhang et al., 2015), CDBI; ***Pteris formosana*** Baker, *Chao 951*, China, *rbcL*: KF289630 (Chao et al., 2014), *matK*: KF289491 (Chao et al., 2014), TAIF; ***Pteris fraseri*** Mett. ex Kuhn, *Rothfels 3712*, Ecuador, *rbcL*: KM008177 (Zhang et al., 2015), *matK*: MF972750 (Zhang et al., 2015), DUKE; ***Pteris friesii*** Hieron., *Rasolohery 897*, Madagascar, *rbcL*: KF289719 (Chao et al., 2014), *matK*: KF289589 (Chao et al., 2014), P; ***Pteris gallinopes*** Ching, *He 1378*, China, *rbcL*: KM008179 (Zhang et al., 2015), *matK*: MF972751 (Zhang et al., 2015), CTC; ***Pteris geminata*** Wall., *Pignal 1836*, Comoros, *rbcL*: KF289745 (Chao et al., 2014), *matK*: KF289615 (Chao et al., 2014), P; ***Pteris giasii*** Fraser-Jenk. & Pasha, *Fraser-Jenkins 30176*, Bangladesh, *rbcL*: KF289660 (Chao et al., 2014), *matK*: KF289530 (Chao et al., 2014), TAIF; ***Pteris glaucovirens*** Goldm., *Fraser-Jenkins FN115*, Philippines, *rbcL*: KF289661 (Chao et al., 2014), *matK*: KF289531 (Chao et al., 2014), TAIF; ***Pteris grandifolia*** L., *Rodas 296*, Guatemala, *rbcL*: KM008180 (Zhang et al., 2015), *matK*: MF972752 (Zhang et al., 2015), MO; ***Pteris grevilleana*** Wall. ex J. Agardh, *Schuettpelz 891*, Malaysia, *rbcL*: MF972811 (Zhang and Zhang, 2018), *matK*: MF972753 (Zhang and Zhang, 2018), DUKE; ***Pteris griffithii*** Hook., *Deng 3818*, Myanmar, *rbcL*: KM008182 (Zhang et al., 2015), *matK*: MF972755 (Zhang et al., 2015), CDBI; ***Pteris griseoviridis*** C. Chr., *Janssen 2799*, Madagascar, *rbcL*: KF289715 (Chao et al., 2014), *matK*: KF289585 (Chao et al., 2014), P; ***Pteris guangdongensis*** Ching, *Chao 1350*, China, *rbcL*: KF289638 (Chao et al., 2014), *matK*: KF289508 (Chao et al., 2014), TAIF; ***Pteris haenkeana*** C. Presl, *van der Werff 17869*, Peru, *rbcL*: KM008183 (Zhang et al., 2015), MO; ***Pteris hainanensis*** Ching, *Chao 1239*, China, *rbcL*: HM582625 (Chao et al., 2012b), *matK*: KF289505 (Chao et al., 2014), TAIF; ***Pteris henryi*** Christ, *Zhang 6038*, China, *rbcL*: KM008184 (Zhang et al., 2015), *matK*: MF972757 (Zhang et al., 2015), CDBI; ***Pteris heteroclita*** Desv., *Rouhan 1296*, Madagascar, *rbcL*: KF289749 (Chao et al., 2014), *matK*: KF289619 (Chao et al., 2014), P; ***Pteris heteromorpha*** Fée, *Wade 1327*, Vietnam, *rbcL*: KF289673 (Chao et al., 2014), *matK*: KF289543 (Chao et al., 2014), TAIF; ***Pteris hexagona*** (L.) Proctor, *Monsegur 1009*, Puerto Rico, *rbcL*: MW660778 (this study), US; ***Pteris hillebrandii*** Copel., *Wood 1069*, United States, *rbcL*: MW660777 (this study), US; ***Pteris hirtula*** (C. Chr.) C. V. Morton, *Fraser-Jenkins FN180*, India, *rbcL*: KF289687 (Chao et al., 2014), *matK*: KF289557 (Chao et al., 2014), TAIF; ***Pteris hui*** Ching, *Zhang 5042*, China, *rbcL*: MF972813 (Zhang and Zhang, 2018), *matK*: MF972758 (Zhang and Zhang, 2018), CDBI; ***Pteris humbertii*** C. Chr., *Rakotondrainibe 5965*, Madagascar, *rbcL*: KF289718 (Chao et al., 2014), *matK*: KF289588 (Chao et al., 2014), P; ***Pteris inaequalis*** (Fée) Jenman, *Iwatsuki 1517*, Japan, *rbcL*: MW660776 (this study), US; ***Pteris incompleta*** Cav., *Rouhan 990*, Portugal, *rbcL*: KF289750 (Chao et al., 2014), *matK*: KF289620 (Chao et al., 2014), P; ***Pteris insignis*** Mett. ex Kuhn, *Zhang 1274*, China, *rbcL*: KM008186 (Zhang et al., 2015), *matK*: MF972759 (Zhang et al., 2015), CDBI; ***Pteris irregularis*** Kaulf., *Bishop 03017006*, United States, *rbcL*: KF289729 (Chao et al., 2014), *matK*: KF289599 (Chao et al., 2014), HAW; ***Pteris kathmanduensis*** Fraser-Jenk. & T. G. Walker, *Fraser-Jenkins FN35*, Nepal, *rbcL*: KF289663 (Chao et al., 2014), *matK*: KF289533 (Chao et al., 2014), TAIF; ***Pteris kawabatae*** Sa. Kurata, *Chao 1637*, Taiwan, *rbcL*: KF289655 (Chao et al., 2014), *matK*: KF289525 (Chao et al., 2014), TAIF; ***Pteris keysseri*** Rosenst., *Chao 1403*, Philippines, *rbcL*: KF289640 (Chao et al., 2014), *matK*: KF289510 (Chao et al., 2014), TAIF; ***Pteris khasiana*** Hieron., *Fraser-Jenkins FN129*, India, *rbcL*: KF289688 (Chao et al., 2014), *matK*: KF289558 (Chao et al., 2014), TAIF; ***Pteris kidoi*** Sa. Kurata, *Dai 1917*, Taiwan, *rbcL*: HM582613 (Chao et al., 2012b), *matK*: KF289500 (Chao et al., 2014), TAIF; ***Pteris kingiana*** Endl., *Duncan 86069*, Norfolk Island, *rbcL*: KF289653 (Chao et al., 2014), *matK*: KF289523 (Chao et al., 2014), MEL; ***Pteris kiuschiuensis*** Hieron., *Chao 1852*, Japan, *rbcL*: KF289739 (Chao et al., 2014), *matK*: KF289609 (Chao et al., 2014), TAIF; ***Pteris laevis*** Mett., *Franc s.n.*, New Caledonia, *rbcL*: MW660775 (this study), US; ***Pteris langsonensis*** Li Bing Zhang, Liang Zhang & N. T. Lu, *Zhang 6593*, Vietnam, *rbcL*: MF972814 (Zhang and Zhang, 2018), CDBI; ***Pteris latipinna*** Y. S. Chao & W. L. Chiou, *Lu 24585*, Taiwan, *rbcL*: MF416317 (Chao et al., 2017a), *matK*: MF416323 (Chao et al., 2017a), TAIF; ***Pteris laurea*** Desv., *Cremers 3291*, Madagascar, *rbcL*: KF289727 (Chao et al., 2014), *matK*: KF289597 (Chao et al., 2014), P; ***Pteris laurisilvicola*** Sa. Kurata, *Chao 1848*, Japan, *rbcL*: KF289738 (Chao et al., 2014), *matK*: KF289608 (Chao et al., 2014), TAIF; ***Pteris lechleri*** Mett., *Prado 2061*, Brazil, *rbcL*:

KM008188 (Zhang et al., 2015), *matK*: MF972760 (Zhang et al., 2015), SP; **Pteris leptophylla** Sw., *Boldrin 160*, Brazil, *rbcL*: EF473707 (Prado et al., 2007), SP; **Pteris liboensis** P. S. Wang, *Zhang 5537*, China, *rbcL*: MF972815 (Zhang and Zhang, 2018), *matK*: MF972762 (Zhang and Zhang, 2018), CDBI; **Pteris ligulata** Gaud., *Rinehart LR17125*, Palau, *rbcL*: MW660774 (this study), US; **Pteris linearis** Poir., *Grangaud s.n.*, France, *rbcL*: KM008190 (Zhang et al., 2015), *matK*: MF972763 (Zhang et al., 2015), MO; **Pteris litoralis** Rechinger, *Iwashina 3271*, Vanuatu, *rbcL*: KF289734 (Chao et al., 2014), *matK*: KF289604 (Chao et al., 2014), TNS; **Pteris livida** Mett., *Schuettpelz 936*, Ecuador, *rbcL*: KM008192 (Zhang et al., 2015), *matK*: MF972765 (Zhang et al., 2015), DUKE; **Pteris longifolia** L., *Sundue 251*, Belize, *rbcL*: KF289656 (Chao et al., 2014), *matK*: KF289526 (Chao et al., 2014), TAIF; **Pteris longipinna** Hayata, *Lu 11383*, Taiwan, *rbcL*: HM582603 (Chao et al., 2012b), *matK*: KF289495 (Chao et al., 2014), TAIF; **Pteris macilenta** A. Rich., *WELT P021006*, New Zealand, *rbcL*: GU136797 (Bouma et al., 2010), WELT; **Pteris madagascarica** J. Agardh, *Janssen 2933*, Madagascar, *rbcL*: KF289748 (Chao et al., 2014), *matK*: KF289618 (Chao et al., 2014), P; **Pteris majestica** Ching, *Zhang 310*, China, *rbcL*: MW660773 (this study), MO; **Pteris marquesensis** Lorence & K. R. Wood, *Schuettpelz 1946*, Marquesas Islands, *rbcL*: MW660772 (this study), US; **Pteris medogensis** Ching & S. K. Wu, *FraserJenkins FN112*, India, *rbcL*: KF289689 (Chao et al., 2014), *matK*: KF289559 (Chao et al., 2014), TAIF; **Pteris melanocaulon** Fée, *Barcelona 1015*, Philippines, *rbcL*: MW660771 (this study), US; **Pteris mertensioides** Willd., *Barcelona 1936*, Philippines, *rbcL*: KF289664 (Chao et al., 2014), *matK*: KF289534 (Chao et al., 2014), TAIF; **Pteris microptera** Mett. ex Kuhn, *Papadopulos AP960*, Australia, *rbcL*: JF950814 (Papadopulos et al., 2011), NA; **Pteris mildbraedii** Hieron., *Carvalho 3630*, Equatorial Guinea, *rbcL*: MW660770 (this study), US; **Pteris mkomaziensis** Verdc., *Hemp 42766*, Tanzania, *rbcL*: MW660769 (this study), US; **Pteris moluccana** Blume, *Demoulin 5790*, New Guinea, *rbcL*: KF289733 (Chao et al., 2014), *matK*: KF289603 (Chao et al., 2014), TNS; **Pteris morii** Masam., *Zhang 1314*, China, *rbcL*: KM008197 (Zhang et al., 2015), *matK*: MF972770 (Zhang et al., 2015), CDBI; **Pteris mucronulata** Copel., *Chao 1410*, Philippines, *rbcL*: KF289641 (Chao et al., 2014), *matK*: KF289511 (Chao et al., 2014), TAIF; **Pteris multiaurita** J. Agardh, *Chiou 15290*, India, *rbcL*: KF289665 (Chao et al., 2014), *matK*: KF289535 (Chao et al., 2014), TAIF; **Pteris multifida** Poir., *Chao 1022*, China, *rbcL*: KF289631 (Chao et al., 2014), *matK*: KF289494 (Chao et al., 2014), TAIF; **Pteris muricata** Hook., *Rothfels 3745*, Ecuador, *rbcL*: KM008201 (Zhang et al., 2015), *matK*: MF972774 (Zhang et al., 2015), DUKE; **Pteris muricatopedata** Arbeláez, *Campos 4643*, Peru, *rbcL*: MW660768 (this study), MO; **Pteris muricella** Fée, *Lellinger 212*, Costa Rica, *rbcL*: MW660767 (this study), US; **Pteris mutilata** L., *Sundue 2096*, United States, *rbcL*: KM008202 (Zhang et al., 2015), MO; **Pteris nanlingensis** R. H. Miau, *Zhang 6003*, China, *rbcL*: KM008203 (Zhang et al., 2015), *matK*: MF972775 (Zhang et al., 2015), CDBI; **Pteris natiensis** Tagawa, *Chao 1835*, Japan, *rbcL*: KF289744 (Chao et al., 2014), *matK*: KF289614 (Chao et al., 2014), TAIF; **Pteris navarrensis** Christ, *Mickel 3303*, Costa Rica, *rbcL*: MW660766 (this study), US; **Pteris nevillei** Baker, *Hennequin R133*, Réunion, *rbcL*: JX144883 (Schneider et al. 2013), REU; **Pteris normalis** D. Don, *Fraser-Jenkins FN11*, India, *rbcL*: KF289690 (Chao et al., 2014), *matK*: KF289560 (Chao et al., 2014), TAIF; **Pteris novae-caledoniae** Hook., *Mackee 19*, New Caledonia, *rbcL*: MW660765 (this study), US; **Pteris opaca** J. Sm. in Hook., *Barcelona 2529*, Philippines, *rbcL*: MW660764 (this study), US; **Pteris oppositipinnata** Fée, *Fraser-Jenkins 4701*, Nepal, *rbcL*: MW660763 (this study), US; **Pteris orizabae** M. Martens & Galeotti, *Reyes-Garcıa 7311*, Mexico, *rbcL*: KM008205 (Zhang et al., 2015), MO; **Pteris oshimensis** Hieron., *Chao 1881*, Japan, *rbcL*: KF289741 (Chao et al., 2014), *matK*: KF289611 (Chao et al., 2014), TAIF; **Pteris otaria** Bedd., *Fraser-Jenkins FN26*, India, *rbcL*: KF289666 (Chao et al., 2014), *matK*: KF289536 (Chao et al., 2014), TAIF; **Pteris pallens** (Sw.) Mett., *Janssen 2677*, France, *rbcL*: KM008207 (Zhang et al., 2015), P; **Pteris papuana** Ces., *Chen & Hsu SITW00424*, Solomon Islands, *rbcL*: KF289755 (Chao et al., 2014), *matK*: KF289625 (Chao et al., 2014), TAIF; **Pteris parkeri** hort. ex J. J. Parker, *Yang s.n.*, Taiwan, *rbcL*: KF289705 (Chao et al., 2014), *matK*: KF289575 (Chao et al., 2014), TAIF; **Pteris pearcei** Baker, *Fay 2711*, Bolivia, *rbcL*: MW660762 (this study), US; **Pteris pellucens** Ag., *Chen Wade4706*, Malaysia, *rbcL*: KY705068 (Chao et al., 2017b), *matK*: KY705070 (Chao et al., 2017b), TAIF; **Pteris pellucida** C. Presl, *Fraser-Jenkins FN220*, Nepal, *rbcL*: KF289691 (Chao et al., 2014), *matK*: KF289561 (Chao et al., 2014), TAIF; **Pteris pellucidifolia** Hayata, *Chao 792*, Taiwan, *rbcL*: HM582654 (Chao et al., 2012b), *matK*: KF289485 (Chao et al., 2014), TAIF; **Pteris perrottetii** Hieron., *Fraser-Jenkins FN215*, Nepal, *rbcL*: KF289736 (Chao et al., 2014), *matK*: KF289606 (Chao et al., 2014), TAIF; **Pteris platyzomopsis** Christenh. & H. Schneid., *Bostock s.n.*, Australia, *rbcL*: AY168721 (Nakazato & Gastony, 2003), *matK*: KF289519 (Nakazato & Gastony, 2003), IND; **Pteris podophylla** Sw., *Rothfels 3746*, Ecuador, *rbcL*: KM008209 (Zhang et al., 2015), *matK*: MF972779 (Zhang et al., 2015), DUKE; **Pteris praestantissima** (Bory ex Fée) Christenh., *Rothfels 2680*, Costa Rica, *rbcL*: KM008210 (Zhang et al., 2015), *matK*: MF972780 (Zhang et al., 2015), DUKE; **Pteris praetermissa** T. G. Walker, *Fraser-Jenkins FN64*, India, *rbcL*: KF289692 (Chao et al., 2014), *matK*: KF289562 (Chao et al., 2014), TAIF; **Pteris preussii** Hieron., *Kamau 212*, Uganda, *rbcL*: KM008211 (Zhang et al., 2015), EA; **Pteris propinqua** J. Agardh, *Fôrther 10161(181)*, Guatemala, *rbcL*: MW660761 (this study), US; **Pteris pseudolonchitis** Bory, *Hennequin R147*, Réunion, *rbcL*: JX144884 (Schneider et al., 2013), REU; **Pteris pseudopellucida** Ching, *Fraser-Jenkins FN26 20090520*, Bhutan, *rbcL*: KF289693 (Chao et al., 2014), *matK*: KF289563 (Chao et al., 2014), TAIF; **Pteris pteridioides** (Hook.) Ballard, *Janssen 2952*, Madagascar, *rbcL*: KF289716 (Chao et al., 2014), *matK*: KF289586 (Chao et al., 2014), P; **Pteris puberula** Ching, *Fraser-Jenkins FN241*, Nepal, *rbcL*: KF289667 (Chao et al., 2014), *matK*: KF289537 (Chao et al., 2014), TAIF; ***Pteris pungens*** Willd., *Rothfels 08-165*, Costa Rica, *rbcL*: KM008215 (Zhang et al., 2015), *matK*: MF972783 (Zhang et al., 2015), DUKE; ***Pteris purdoniana*** Maxon, *Morton 8755*, Cuba, *rbcL*: MW660760 (this study), US; ***Pteris quadriaurita*** Retz., *Fraser-Jenkins 31110*, Sri Lanka, *rbcL*: KF289671 (Chao et al., 2014), *matK*: KF289541 (Chao et al., 2014), TAIF; ***Pteris quadristipitis*** X. Y. Wang & P. S. Wang, *Sun s.n.*, China, *rbcL*: KM008216 (Zhang et al., 2015), *matK*: MF972784 (Zhang et al., 2015), CDBI; ***Pteris reptans*** T. G. Walker, *Fraser-Jenkins FN393*, Sri Lanka, *rbcL*: KF289668 (Chao et al., 2014), *matK*: KF289538 (Chao et al., 2014), TAIF; ***Pteris roseolilacina*** Hieron., *Fraser-Jenkins FN3191*, Nepal, *rbcL*: KF289669 (Chao et al., 2014), *matK*: KF289539 (Chao et al., 2014), TAIF; ***Pteris ryukyuensis*** Tagawa, *Lu 11375*, Taiwan, *rbcL*: HM582601 (Chao et al., 2012b), *matK*: KF289492 (Chao et al., 2014), TAIF; ***Pteris satsumana*** Sa. Kurata, *Chao 1853*, Japan, *rbcL*: KF289740 (Chao et al., 2014), *matK*: KF289610 (Chao et al., 2014), TAIF; ***Pteris saxatilis*** Carse, *Welt P022567*, New Zealand, *rbcL*: GU136798 (Bouma et al., 2010), WELT; ***Pteris scabra*** Bory & Willd., *Rakotondrainibe 6851*, Comoros, *rbcL*: KF289720 (Chao et al., 2014), *matK*: KF289590 (Chao et al., 2014), P; ***Pteris scabripes*** Wall. ex J. Agardh, *FraserJenkins FN65*, India, *rbcL*: KF289694 (Chao et al., 2014), *matK*: KF289564 (Chao et al., 2014), TAIF; ***Pteris scabririgens*** Fraser-Jenk., S. C. Verma & T. G. Walker, *FraserJenkins FN138*, India, *rbcL*: KF289698 (Chao et al., 2014), *matK*: KF289568 (Chao et al., 2014), TAIF; ***Pteris schlechteri*** Brause, *Fraser-Jenkins FN58*, Philippines, *rbcL*: KF289670 (Chao et al., 2014), *matK*: KF289540 (Chao et al., 2014), TAIF; ***Pteris schwackeana*** Christ, *Mazziero 861*, Brazil, *rbcL*: MW660759 (this study), SP; ***Pteris semiadnata*** Phil., *Solbrig 3745*, Chile, *rbcL*: MW660758 (this study), US; ***Pteris setulosocostulata*** Hayata, *Zhang 1379*, China, *rbcL*: KM008218 (Zhang et al., 2015), *matK*: MF972787 (Zhang et al., 2015), CDBI; ***Pteris similis*** Kuhn, *Carvalho 5915*, Equatorial Guinea, *rbcL*: MW660757 (this study), US; ***Pteris speciosa*** Mett. ex Kuhn, *Nitta 777*, Costa Rica, *rbcL*: KM008219 (Zhang et al., 2015), *matK*: MF972789 (Zhang et al., 2015), UC; ***Pteris spinescens*** C. Presl, *Fraser-Jenkins FN113*, India, *rbcL*: KF289695 (Chao et al., 2014), *matK*: KF289565 (Chao et al., 2014), TAIF; ***Pteris splendens*** Kaulf., *Prado 1131a*, Brazil, *rbcL*: EF473708 (Prado et al., 2007), SP; ***Pteris splendida*** Ching, *Zhang 5632*, China, *rbcL*: KM008220 (Zhang et al., 2015), *matK*: MF972790 (Zhang et al., 2015), CDBI; ***Pteris stenophylla*** Wall. ex Hook. & Grev., *Fraser-Jenkins 4945*, Nepal, *rbcL*: MW660756 (this study), US; ***Pteris stridens*** J. Agardh, *Holdridge 1991*, Haiti, *rbcL*: MW660755 (this study), US; ***Pteris subindivisa*** C. B. Clarke, *Fraser-Jenkins FN266*, Bhutan, *rbcL*: KF289700 (Chao et al., 2014), *matK*: KF289570 (Chao et al., 2014), TAIF; ***Pteris subquinata*** Wall. ex J. Agardh, *Fraser-Jenkins FN315*, Nepal, *rbcL*: KF289696 (Chao et al., 2014), *matK*: KF289566 (Chao et al., 2014), TAIF; ***Pteris tahuataensis*** Lorence & K. R. Wood, *Wood 10250*, French Polynesia, *rbcL*: MF972821 (Zhang and Zhang, 2018), PTBG; ***Pteris terminalis*** Wall. ex J. Agardh, *P.-F. Lu s.n. 20071202*, Taiwan, *rbcL*: KF289639 (Chao et al., 2014), *matK*: KF289509 (Chao et al., 2014), TAIF; ***Pteris tibetica*** Ching, *Fraser-Jenkins FN10*, Nepal, *rbcL*: KF289697 (Chao et al., 2014), *matK*: KF289567 (Chao et al., 2014), TAIF; ***Pteris togoensis*** Hieron., *Fay 1022*, Sierra Leone, *rbcL*: MW660754 (this study), US; ***Pteris tremula*** R. Br., *Latz 20040*, Australia, *rbcL*: KF289650 (Chao et al., 2014), *matK*: KF289520 (Chao et al., 2014), MEL; ***Pteris tricolor*** Linden, *Fraser-Jenkins FN82*, India, *rbcL*: KF289682 (Chao et al., 2014), *matK*: KF289552 (Chao et al., 2014), TAIF; ***Pteris tripartita*** Sw., *Chao 1199*, China, *rbcL*: KF289636 (Chao et al., 2014), *matK*: KF289504 (Chao et al., 2014), TAIF; ***Pteris umbrosa*** R. Br., *P.I. Forster*, Australia, *rbcL*: KF289648 (Chao et al., 2014), *matK*: KF289518 (Chao et al., 2014), MEL; ***Pteris usambarensis*** Hieron., *Kamau 388*, Kenya, *rbcL*: KM008228 (Zhang et al., 2015), EA; ***Pteris vaupelii*** Hieron., *Whistler 3794*, Samoa, *rbcL*: MW660753 (this study), US; ***Pteris venulosa*** Blume, *Wade 2063*, Indonesia, *rbcL*: KF289680 (Chao et al., 2014), *matK*: KF289550 (Chao et al., 2014), TAIF; ***Pteris venusta*** Kunze, *Chao 873*, Taiwan, *rbcL*: HM582650 (Chao et al., 2012b), *matK*: KF289486 (Chao et al., 2014), TAIF; ***Pteris vieillardii*** Mett., *Chinnock 7609*, New Caledonia, *rbcL*: KF289652 (Chao et al., 2014), *matK*: KF289522 (Chao et al., 2014), MEL; ***Pteris viridissima*** Ching, *Zhang 5944*, China, *rbcL*: KM008230 (Zhang et al., 2015), *matK*: MF972796 (Zhang et al., 2015), CDBI; ***Pteris vitiensis*** Baker, *Chen & Hsu SITW00366*, Solomon Islands, *rbcL*: KF289754 (Chao et al., 2014), *matK*: KF289624 (Chao et al., 2014), TAIF; ***Pteris vittata*** L., *Chao 1427*, Taiwan, *rbcL*: KF289642 (Chao et al., 2014), *matK*: KF289512 (Chao et al., 2014), TAIF; ***Pteris wallichiana*** J. Agardh, *Zhang 1284*, China, *rbcL*: KM008235 (Zhang et al., 2015), *matK*: MF972801 (Zhang et al., 2015), CDBI; ***Pteris warburgii*** Christ, *Chen & Hsu SITW00141*, Solomon Islands, *rbcL*: KF289753 (Chao et al., 2014), *matK*: KF289623 (Chao et al., 2014), TAIF; ***Pteris woodwardioides*** Bory, *Le Péchon 1304*, France, *rbcL*: MF972824 (Zhang and Zhang, 2018), CDBI; ***Pteris wulaiensis*** C. M. Kuo, *Chao 1743*, Taiwan, *rbcL*: KF289678 (Chao et al., 2014), *matK*: KF289548 (Chao et al., 2014), TAIF; ***Pteris xiaoyingiae*** H. He & Li Bing Zhang, *Zhang 5326*, China, *rbcL*: KM008236 (Zhang et al., 2015), *matK*: MF972802 (Zhang et al., 2015), CDBI; ***Pteris yakuinsularis*** Sa. Kurata, *Chao 1906*, Japan, *rbcL*: KF289742 (Chao et al., 2014), *matK*: KF289612 (Chao et al., 2014), TAIF; ***Pteris yamatensis*** Tagawa, *Jaruwattanaphan TJ-TBG054*, Japan, *rbcL*: AB697640 (Jaruwattanaphan et al., 2013), TNS.