Abstract

The monotypic fern genus *Dryopolystichum* Copel. combines a unique assortment of characters that obscures its relationship to other ferns. Its thin-walled sporangium with a vertical and interrupted annulus, round sorus with peltate indusium, and petiole with several vascular bundles place it in suborder Polypodiinae, but more precise placement has eluded previous authors. Here we investigate its phylogenetic position using three plastid DNA markers, *rbcL*, *rps4-trnS*, and *trnL-F*, and a broad sampling of Polypodiinae. We also provide new data on *Dryopolystichum* including spore number counts, reproductive mode, spore SEM images, and chromosome counts. Our maximum-likelihood and Bayesian-inference phylogenetic analyses unambiguously place *Dryopolystichum* within Lomariopsidaceae, a position not previously suggested. *Dryopolystichum* was resolved as sister to a clade comprising *Dracoglossum* and *Lomariopsis*, with *Cyclopeltis* as sister to these, but clade support is not robust. All examined sporangia of *Dryopolystichum* produced 32 spores, and the chromosome number of sporophyte somatic cells is ca. 164. Flow cytometric results indicated that the genome size in the spore nuclei is approximately half the size of those from sporophyte leaf tissues, suggesting that *Dryopolystichum* reproduces sexually. Our findings render Lomariopsidaceae as one of the most morphologically heterogeneous fern families. A recircumscription is provided for both Lomariopsidaceae and *Dryopolystichum*, and selected characters are briefly discussed considering the newly generated data.

* Equal contribution
Keywords
Fern, morphology, Papua New Guinea, phylogeny, recircumscription, taxonomy, the Solomon Islands

Introduction

Dryopolystichum Copel., with its single species *D. phaeostigma* (Ces.) Copel., is distributed along streams in lowland forests in New Guinea, the Bismarck Archipelago, and the Solomon Islands (Copeland 1947; Fig. 1A). Christensen (1937) was the first to point out that *D. phaeostigma* had been independently described under three different genera or subgenera. All told, generic placements has included *Aspidium* (≡ *Tectaria*) (Cesati 1877, Baker 1891), *Dryopteris* (Christensen 1906, Alderwerelt van Rosenburgh 1908, Copeland 1911, Brause 1920, Alderwerelt van Rosenburgh 1924), and *Polystichum* (Rosenstock 1911). Copeland (1947) inaugurated the new monotypic genus *Dryopolystichum* in his *Genera Filicum*, and argued that it was closest to *Ctenitis*. Pichi Sermolli (1977) agreed, citing the ctenitoid rachis, free venation, and peltate indusium as critical characters. Holttum included the genus in his “Tectarioideae” in his list of Malaysian pteridophytes (Holttum 1959), but then omitted it in his 1991 treatment of that group.

Although Copeland did not provide an etymological explanation, the name *Dryopolystichum* presumably reflects the combination of peltate indusium (which is similar to those of polystichoid ferns) and pinnate-pinnatifid lamina division (which is similar to that of most *Dryopteris*). Such a combination of characters resulted in taxonomic confusion giving that peltate indusia are never found in *Dryopteris*, and the laminae of *Dryopolystichum* do not include prominulous segment apices, the hallmark of polystichoid ferns (Little and Barrington 2003). A peltate indusium is diagnostic of polystichoid ferns, including *Phanerophlebia* and *Polystichum*, but also found in a few distantly related genera in Polypodiineae such as *Cyclodium*, *Cyclopeltis*, *Rumohra*, *Megastrum*, and *Tectaria* (Kramer and Green 1990).

Despite recent advances in fern phylogenetics and classification, the position of *Dryopolystichum* remains unclear. The thin-walled sporangium with a vertical and interrupted annulus, round sori, and petiole with several vascular bundles suggest that this genus belongs to suborder Polypodiinae (= eupolypods I) (Sundue and Rothfels 2014, PPG I 2016). However, the remaining prominent features including pinnate-pinnatifid leaf dissection (Fig. 1B), peltate indusium (Fig. 1C), catadromous free veins (Fig. 1D), and sulcate rachis-costa architecture (Fig. 1E), do not clearly place it within any Polypodiinae family (Christensen 1937, Copeland 1947).

One other conspicuous character of *Dryopolystichum* not emphasized by previous authors is that the distal pinnae are decurrent onto the rachis, and the basal pinnules of its distal pinnae are served by veins that emerge from the rachis, rather than the pinna costa (Fig. 1D). This character is relatively uncommon in the Polypodiinae. It can be found in Dryopteridaceae, mostly in *Megastrum*, and less commonly in *Stigmatopteris*, *Ctenitis*, and *Pleocnemia* (Moran et al. 2014, Moran and Labiak 2016).
Phylogenetic analyses place the monotypic *Dryopolystichum* within Lomariopsidaceae. It can also be found in some Tectariaceae such as *Pteridrys* and *Tectaria* (Ding et al. 2014). Among these genera, *Pleocnemia* seems morphologically the most similar to *Dryopolystichum* because its rachises are adaxially sulcate and narrowly winged laterally. *Pleocnemia*, however, lacks a peltate indusium (Holttum 1974).

Subsequent to its establishment as a new genus in *Genera Filicum* (Copeland 1947), and Sermolli’s (1977) contribution, no other substantial argument was made for generic placement of *Dryopolystichum*. More recent studies maintained *Dryopolystichum* as a distinct genus, placing it under Dryopteridaceae (Kramer and Green 1990, Smith et al. 2006, Christenhusz et al. 2011). The recently published community-derived classification for extant lycophytes and ferns also places *Dryopolystichum* in the Dryopteridaceae but without assigning it to subfamily (PPG I 2016).
To resolve the phylogenetic placement of *Dryopolystichum*, we employ a molecular phylogenetic approach using three chloroplast DNA regions, *rbcL*, *rps4-trnS*, and *trnL-F*. Based on our observations, we further provide new data on *Dryopolystichum* including spore counts, reproductive mode, spore SEM images, and a chromosome count. Finally, we discuss its diagnostic characters in the light of the inferred phylogeny.

**Materials and methods**

We examined the morphology of *Dryopolystichum phaeostigma* using material collected from the Solomon Islands (*Braithwaite R.S.S.4557, SING; SITW10443, BSIP, TAIF, TNM*) and Papua New Guinea (*James & Sundue 1688, BISH, LAE, VT*).

Living plants of *SITW10443* were transplanted to the Dr. Cecilia Koo Botanic Conservation Center in Taiwan (KBCC). The collection of *SITW10443* was made under the “Census and Classification of Plant Resources in the Solomon Islands” project ([http://siflora.nmns.edu.tw/](http://siflora.nmns.edu.tw/)). Mitotic chromosomes were counted from these cultivated plants following the protocol of Chen et al. (2014).

Fertile pinnae of *SITW10443* were air-dried in an envelope for one day to release the spores. The spores were observed and measured by a tabletop scanning electron microscope (TM-3000 Hitachi, Ibaraki, Japan). The sizes (the length of equatorial axes including the perine ornamentation) of 35 randomly selected spores were measured. Five intact sporangia were observed under a stereo microscope (Leica MZ6, Wetzlar, Germany) to count the number of spores per sporangium.

The genome sizes of spore and leaf nuclei of *SITW10443* were examined by flow cytometry in order to infer the reproductive mode (Kuo et al. 2017). The genome size of spore nuclei should be half the genome size of leaf nuclei in the case of sexual and the same size in the case of apomictic reproduction (Kuo et al. 2017). We followed Kuo et al. (2017) for the extraction of leaf nuclei. For extraction of spore nuclei, we used an optimized bead-vortexing treatment with vertex duration of 1 minute and vertex speed of 1,900 rpm, as described by Kuo et al. (2017). An external standard was not necessary since we only need to compare the two phases of the life-cycle to each other.

**DNA extraction, amplification and sequencing**

Total DNA was extracted using a modified CTAB-Qiagen column protocol (Kuo 2015). Three plastid DNA regions, *rbcL*, *rps4-trnS* (*rps4* gene + *rps4-trnS* intergenic spacer), and *trnL-F* (*trnL* gene + *trnL-trnF* intergenic spacer), were amplified and sequenced using the primers “ESRBCL1F” and “1379R” for *rbcL* (Pryer et al. 2001, Schuettpelz and Pryer 2007), “RPS5F” and “TRNSR” for *rps4-trnS* (Nadot et al. 1995, Smith and Cranfill 2002), and “FernL 1lr1” and “f” for *trnL-F* (Taberlet et al. 1991, Li et al. 2010).

The PCR amplifications were performed in 16 μl reactions containing ca. 10 ng template DNA, 1×Taq DNA Polymerase Master Mix RED solution (Ampliqon, Den-
Phylogenetic analyses place the monotypic *Dryopolystichum* within Lomariopsidaceae.

DNA alignment and phylogenetic analyses

Initial BLAST against the NCBI nucleotide database (Altschul et al. 1990) based on *rbcL* sequences indicated that *Dryopolystichum phaeostigma* is closely related to the species of Polypodiineae families, including Lomariopsidaceae, Nephrolepidaceae, Tectar-iaceae, and Dryopteridaceae. Accordingly, we assembled a data matrix including 250 species representing 36 genera from these families (Appendix). Sampling included all the four genera in which *D. phaeostigma* has been placed (i.e., *Dryopteris*, *Polystichum*, and *Tectaria*).

Sequences were aligned using Geneious v6.1.8 (Drummond et al. 2011) and then manually checked for errors. The three single-region (*rbcL*, *rps4-trnS*, and *trnL-F*) and dataset combining all three were independently subjected to both maximum likelihood (ML) and Bayesian inference (BI) phylogenetic analyses. Data matrices are available in TreeBASE, study number 20506, at https://treebase.org/. ML tree searches were conducted using RAxML (Stamatakis 2006) employing the GTRGAMMA substitution model through the CIPRES portal (Miller et al. 2010). Five independent searches for the ‘best tree’ and 1,000 bootstrap replicates were performed using a region-partitioned dataset. BI analyses were conducted using MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003) employing the same substitution model as in ML analysis. Each analysis consisted of two independent runs with four chains for $10^6$ generations, sampling one tree every 1000 generations. Burn-in was set to 10000 based on our preliminary analysis. The convergences of MCMC runs were checked using Tracer v.1.6 (Rambaut et al. 2014).

We addressed the possibility of phylogenetic bias due to long branches following the recommendation of Siddal and Whiting (1999). Since *Dracoglossum* and *Lomariopsis* were resolved on long branches in preliminary analyses (not shown), we conducted two additional analyses in which each one of the two long-branched genera, *Dracoglossum* and *Lomariopsis*, was excluded to examine whether phylogenetic placement and branch support for *Dryopolystichum*’s placement changed. Since maximum parsimony (MP) phylogeny is considered to be more susceptible to long-branch attraction (Philippe et al. 2005), we analyzed the concatenated dataset under MP in order to compare those results with our ML phylogeny. The MP analyses were conducted using TNT (Goloboff et al. 2008) following the search strategy detailed in Sundue et al. (2014).
Results

Phylogenetic analyses

All single-region phylogenies resolved *Dryopolystichum phaeostigma* in Lomariopsidaceae, but with two slightly different topologies. The *rbcL* and *rps4-trnS* phylogenies placed *D. phaeostigma* sister to a clade of *Dracoglossum + Lomariopsis* with 93% and 72% maximum likelihood bootstrap percentages (BS), respectively (Suppl. materials 2, 3). In comparison, the *trnL-F* phylogeny placed *D. phaeostigma* sister to *Cyclopeltis* (BS = 74%), and *Dryopolystichum + Cyclopeltis* was sister to *Dracoglossum + Lomariopsis* (Suppl. material 4). There was no strongly supported conflict between the ML and BI phylogenies (Suppl. materials 1–4). Both the ML and BI phylogenies based on the combined dataset (Fig. 2, Suppl. material 1) reveal the same topology as those based on the *rbcL* and *rps4-trnS* regions. Bootstrap support and posteriori probability (PP) for the above relationships were generally very high except for the branches placing *D. phaeostigma*, where BS was ≤ 70% and PP were ≤ 0.9 in all the phylogenies.

Removing *Dracoglossum* from the analysis had little effect on the topology within Lomariopsidaceae, and BS supports for the generic placement of *Dryopolystichum* remained low (≤ 70%, data not shown). In contrast, the removal of *Lomariopsis* resulted in higher BS values for all clades within Lomariopsidaceae (≥ 99%, data not shown). MP analyses also resulted in a clade comprising all the Lomariopsidaceae genera and *Dryopolystichum*, but *Dryopolystichum* was resolved as sister to *Cyclopeltis* (data not shown).

Karyology, reproductive mode, and spore measurements

All examined sporangia (*SITW10443*) produced 32 normal spores, and the mean spore length was 64.1 ± 4.5 μm (Fig. 3). The chromosome number of the three sporophyte somatic cells observed was ca. 164 (Fig. 4). Results of flow cytometry revealed that the genome size of spore nuclei is approximately half of those of leaf nuclei (Fig. 5).

Discussion

Phylogenetic placement of *Dryopolystichum*

The reconstructed maximum likelihood and Bayesian inference phylogenies unambiguously resolved *Dryopolystichum* within Lomariopsidaceae (Fig. 2), a position not previously suggested (Kramer and Green 1990, Smith et al. 2006, Christenhusz et al. 2011, PPG I 2016). This placement is consistent in all our analyses. Nonetheless, the generic position of *Dryopolystichum* within Lomariopsidaceae remains poorly resolved.
Phylogenetic analyses place the monotypic *Dryopolystichum* within Lomariopsidaceae.

Figure 2. Simplified maximum likelihood phylogram of Polypodiinae obtained from the $rbcL + rps4-trnS + trnL-F$ combined dataset. Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) ≥ 0.9. Original phylogram with support values for all the nodes is available in Suppl. materials 1. Voucher information and GenBank accession numbers are shown in Appendix.

This uncertainty may be partially explained by the incongruence between $trnL-F$ and the other analyzed regions, but our process of removing the long-branched genera showed that low BS was retrieved only when *Dryopolystichum* and *Lomariopsis* were
both included in the analysis. These results may also be explained by the large amounts of missing data in *Lomariopsis*; 19 of the 25 species included were represented by *trnL*-*F* data alone. We recommend further phylogenetic study using an expanded dataset to resolve the intergeneric relationships within Lomariopsidaceae.

**Figure 3.** Spores SEM of *Dryopolystichum phaeostigma*. A Lateral view of the spore B Detail of surface. Scale bars: A = 50 μm, B = 10 μm.

**Figure 4.** Chromosome number of *Dryopolystichum phaeostigma*. A Chromosomes at mitosis metaphase, 2n = ca. 164 (SITW10443) B explanatory illustration of A. Scale bars = 10 μm.
Phylogenetic analyses place the monotypic Dryopolystichum within Lomariopsidaceae

Figure 5. Relative DNA contents of Dryopolystichum phaeostigma spore and leaf nuclei inferred by flow cytometry.

Recircumscription of Lomariopsidaceae

Phylogenetic analyses using DNA sequences have served as the basis for redrawing fern classifications in the 21st century (Smith et al. 2006, Christenhusz et al. 2011, PPG I 2016). With respect to family circumscription, one of the most dramatically changed families is Lomariopsidaceae (Tsutsumi and Kato 2006, Schuettpelz and Pryer 2007, Christenhusz et al. 2013). Just prior to the molecular era, Lomariopsidaceae was treated as one of the largest fern families with six genera and over 500 species (e.g., Kramer and Green 1990) and was strongly supported by the following combination of characters: rhizomes with ventral root insertion, dictyosteles with elongate ventral meristeles, and dimorphic leaves where the fertile leaves had acrostichoid sori (Holttum and Hennipman 1959, Kramer and Green 1990).

Subsequent molecular phylogenetic analyses demonstrated that most genera previously treated in Lomariopsidaceae should be transferred to Dryopteridaceae (Tsutsumi and Kato 2006, Schuettpelz and Pryer 2007). The combination of characters uniting the former Lomariopsidaceae are now interpreted to have evolved multiple times, and to be correlated with dorsiventrality of the rhizome (Moran et al. 2010, McKeown et al. 2012). Meanwhile, Cyclopeltis was transferred from Dryopteridaceae to Lomariopsidaceae as suggested by molecular phylogeny (Schuettpelz and Pryer 2007), although it has none of the characters formerly used to circumscribe Lomariopsidaceae (Holttum and Hennipman 1959, Kramer and Green 1990).

More recently, the neotropical genus Dracoglossum was established (Christenhusz 2007) and later transferred to Lomariopsidaceae from Tectariaceae based on a molecular phylogeny (Christenhusz et al. 2013). This pattern was also unexpected since there are essentially no shared morphological characters by Dracoglossum and Lomariopsis, except for the ribbon-like gametophyte (R. C. Moran pers. com.). Our finding, that Dryopolystichum belongs to Lomariopsidaceae, comes as a further surprise. With these
Table 1. Comparison of morphological characters of the five Lomariopsidaceae genera [based on Holttum and Hennipman (1959), Holttum (1991), Roubik and Moreno (1991), Moran (2000), Christenhusz (2007), Rouhan et al. (2007), and this study].

<table>
<thead>
<tr>
<th>Genera</th>
<th>Cyclopeltis</th>
<th>Dracoglossum</th>
<th>Dryopolystichum</th>
<th>Lomariopsis</th>
<th>Thysanosoria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habit</td>
<td>terrestrial</td>
<td>terrestrial</td>
<td>terrestrial</td>
<td>hemiepiphyte</td>
<td>hemiepiphyte</td>
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<tr>
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<td>short creeping</td>
<td>erect</td>
<td>climbing</td>
<td>climbing</td>
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<td>Frond division*</td>
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<td>simple</td>
<td>pinnate-pinnatifid</td>
<td>pinnate</td>
<td>pinnate</td>
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<tr>
<td>Pinnae articulation</td>
<td>articulate</td>
<td>-</td>
<td>not articulate</td>
<td>articulate</td>
<td>articulate</td>
</tr>
<tr>
<td>Venation</td>
<td>free</td>
<td>reticulate, with included veinlet</td>
<td>free</td>
<td>free</td>
<td>free</td>
</tr>
<tr>
<td>Rachis-costa architecture</td>
<td>prominent</td>
<td>prominent</td>
<td>grooved</td>
<td>grooved or flat</td>
<td>grooved</td>
</tr>
<tr>
<td>Sporangia</td>
<td>form rounded sori</td>
<td>form rounded sori</td>
<td>form rounded sori</td>
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<td>peltate</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Perine ornamentation</td>
<td>broad folds</td>
<td>narrow crests</td>
<td>narrow crests</td>
<td>various</td>
<td>broad folds</td>
</tr>
</tbody>
</table>

*matured plant, -not applicable

changes, Lomariopsidaceae is a family of five genera (*Cyclopeltis*, *Dracoglossum*, *Dryopolystichum*, *Lomariopsis*, and *Thysanosoria*) and ca. 70 species. As far as we can tell, none of the morphological traits commonly used unify these genera (Table 1). In the following paragraphs, we provide a recircumscription of both Lomariopsidaceae and *Dryopolystichum*, and then discuss selected characters in the light of our phylogenetic placement.

**Taxonomic treatment**


**Description.** Habit erect, creeping, or climbing; rhizomes dictyostelic, the ventral meristele elongate in transverse section or not; scaly at least when young; scales non-clathrate, basally attached or shallowly peltate, margins entire, toothed, or ciliate; fronds monomorphic or dimorphic; petioles with multiple vascular bundles arranged in a U-shape; laminae simple, pinnate, or pinnate-pinnatifid, provided distally with proliferous buds or not; pinnae articulate to the rachis or not; veins free, ± parallel or pinnate; sori acrostichoid or discrete and then round, with peltate indusia or exindusiate; spores brown, olive or green, chlorophyllous or not, bilateral, monolete, perine loosely attached, variously winged or ornamented.

Five genera and an estimated 70 species. *Thysanosoria* is included based on its morphological similarity to *Lomariopsis* (Holttum and Hennipman 1959), but it has not been, to the present, subject to molecular phylogenetic analysis.
Phylogenetic analyses place the monotypic *Dryopolystichum* within Lomariopsidaceae.

**Dryopolystichum** Copel., Gen. Fil. 125, t. 4. 1947.

**Type.** *Dryopolystichum phaeostigma* (Ces.) Copel., Gen. Fil. 125, t. 4. 1947.

**Description.** Habit terrestrial, on slopes along streams at lowland forests; rhizome short erect, stout and woody, apex densely scaly, blackish sclerenchyma strands visible in sections; scales dark brown, linear-lanceolate, entire, not clathrate; fronds approximate, stipe not articulate, scaly at base, scales similar to those on rhizome; lamina ovate, pinnate-pinnatifid, catadromous, subleathery, nearly glabrous, only very sparse narrow scales on rachis, costa, and costule; rachis and costa grooved adaxially, not connected to each other; veins free, pinnate, veins of basal pinnules on upper pinnae emerge from the rachis rather than costa, all veins terminating in a prominent hydathode, not reaching frond margin; sori round, dorsally on veinlets near hydathode, indusiate; indusia round, persistent, superior, entire, brownish, thick; sporangia long-stalked, annulus with ca. 14 indurated cells, 32 normal spores in each sporangium; spores monolete, 64.1 ± 4.5 μm in lateral view, surface with broadly winged wall; 2n = ca. 164.

Monotypic.

**Dryopolystichum phaeostigma** (Ces.) Copel., Gen. Fil. 125, t. 4. 1947.


Type. Papua New Guinea. Andai, Beccari 12533 (FI [FI013622]).

*Dryopteris phaeostigma* (Ces.) C.Chr., Index Filic. 284. 1905

Type. Based on *Aspidium phaeostigma* Ces.

*Dryopteris tamatana* C.Chr., Index Filic., Suppl. (1906-1912) 40. 1913.


Type. Papua New Guinea. Tamata, C. King 149 (MICH [MICH1287049]).


Type. Papua New Guinea. C. King 194 (MICH [MICH1190927]).


Type. Papua New Guinea. Sepik, Ledermann 9619 (B [B_20_005865], L [L0063060], S [S-P-8581]).


Type. Indonesia. Irian Jaya, H. J. Lam 1086 (BO [BO1529719, BO1529720], K [K000666126], L [L0051583], U [U0007385]).

**Type.** Based on *Aspidium phaeostigma* Ces.

**Description.** Equal to the genus.

**Distribution.** New Guinea, the Bismark archipelago, and the Solomon Islands.
Comparison of selected characters of *Dryopolystichum*

Perine architecture of *Dryopolystichum* is very similar to that of *Dracoglossum plantagineum* (Christenhusz 2007, Fig. 3). They are loosely attached, forming thin crests, and having a spiculate microstructure. Perine of *Cyclopeltis* and *Thysanosoria* are also similar in being loosely attached and having a spiculate microstructure, but they differ by having broader folds (Holttum and Hennipman 1959, Tryon and Lugardon 1991). The perine characters, however, are not shared by all the taxa of Lomariopsidaceae especially considering the variation of ornamentation existing in *Lomariopsis* (Rouhan et al. 2007). Moreover, these perine characters also appear in other Polypodiineae lineages particularly in bolbitidoid ferns (Moran et al. 2010) as well as in various Aspleniineae lineages (Sundue and Rothfels 2014, PPG I 2016).

Blackish sclerenchyma strands are visible in the rhizome sections of *Dryopolystichum* (Fig. 1F). These are also present in *Dracoglossum*, *Cyclopeltis*, and *Lomariopsis*, but similar characters are known from various groups throughout Polypodiineae (Hennipman 1977, Moran 1986, Hovenkamp 1998). Further studies might reveal variation in these strands to be of systematic value.

The rachis-costae architecture of *Dryopolystichum* is characterized by an adaxially sulcate rachis with grooves that do not connect to those of the pinna-costae. The rachis is also narrowly winged laterally. Both characters are seen in *Thysanosoria* and in some species of *Lomariopsis* (Holttum and Hennipman 1959, Moran 2000). In contrast, *Dracoglossum* and *Cyclopeltis* have non-winged and non-sulcate rachises (Holttum 1991, Christenhusz 2007).

The chromosome number in somatic cells of *Dryopolystichum phaeostigma* was ca. 164 (Fig. 4). The base numbers for Lomariopsidaceae genera (*Cyclopeltis, Dracoglossum, and Lomariopsis*) are 40 or 41 (Walker 1985, Kato and Nakato 1999, Moran 2000), suggesting that *D. phaeostigma* is a tetraploid.

Our flow cytometry and spore count results indicate that *Dryopolystichum phaeostigma* is sexually reproducing and has 32 spores per sporangium (Fig. 5). In Polypodiales, sporogenesis leading to the formation of 64 spores in a sporangium is by far the most common pattern of sexually reproducing species, e.g., Aspleniaceae (Gabancho et al. 2010), Arthyriaceae (Kato et al. 1992, Takamiya et al. 1999), Davalliaceae (Chen et al. 2014), Dryopteridaceae (Lu et al. 2006), Polypodiaceae (Wang et al. 2011), Pteridaceae (Huang et al. 2006), and Thelypteridaceae (Ebihara et al. 2014). Cases of sporogenesis resulting in 32 spores per sporangium are known from a few Polypodiales ferns but all belong to the suborders Lindsaeineae and Pteridineae, i.e., Lindsaeaceae (Lin et al. 1990), Cystodiaceae (Gastony 1981), and *Ceratopteris* (Pteridaceae; Lloyd 1973). Our study provides the first confirmed case of a sexual reproduction with 32 spores per sporangium in the suborder Polypodiineae.
Conclusion

We have shown, based on molecular phylogenetic evidence, the placement of *Dryopolystichum* within Lomariopsidaceae. A revised description was provided for both Lomariopsidaceae and *Dryopolystichum* resulting from a review of literature and our own observations. Future studies using an expanded dataset are necessary to resolve intergeneric relationships in Lomariopsidaceae.

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Phylogenetic analyses place the monotypic *Dryopolystichum* within Lomariopsidaceae.


Phylogenetic analyses place the monotypic Dryopolystichum within Lomariopsidaceae.


Appendix

Individuals sampled in this study. For each individual, the species name and GenBank accession numbers (rbcL, rps4-trnS, trnL-F) are provided. A n-dash (–) indicates unavailable information; new sequences are in bold.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Genbank accession numbers</th>
</tr>
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<td></td>
<td>rbcL</td>
</tr>
<tr>
<td>Dryopteridaceae</td>
<td></td>
</tr>
<tr>
<td>Arachniodes aristata (G.Forst.) Tindale</td>
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Phylogenetic analyses place the monotypic *Dryopolystichum* within Lomariopsidaceae.

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Phylogenetic analyses place the monotypic *Dryopolystichum* within Lomariopsidaceae

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Supplementary material 1

Figure S1. Maximum likelihood phylogram of Polypodiineae obtained from the combined \((rbcL + rps4-trnS + trnL-F)\) dataset.
Authors: Cheng-Wei Chen, Michael Sundue, Li-Yaung Kuo, Wei-Chih Teng, Yao-Moan Huang
Data type: statistical data
Explanation note: Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) \(\geq 0.9\).
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Supplementary material 2

Figure S2. Maximum likelihood phylogram of Polypodiineae obtained from the \(rbcL\) dataset.
Authors: Cheng-Wei Chen, Michael Sundue, Li-Yaung Kuo, Wei-Chih Teng, Yao-Moan Huang
Data type: statistical data
Explanation note: Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) \(\geq 0.9\).
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Supplementary material 3

Figure S3. Maximum likelihood phylogram of Polypodiineae obtained from the rps4-trnS dataset.
Authors: Cheng-Wei Chen, Michael Sundue, Li-Yaung Kuo, Wei-Chih Teng, Yao-Moan Huang
Data type: statistical data
Explanation note: Maximum likelihood phylogram of Polypodiineae obtained from the rps4-trnS dataset. Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) ≥ 0.9.
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Supplementary material 4

Figure S4. Maximum likelihood phylogram of Polypodiineae obtained from the trnL-F dataset.
Authors: Cheng-Wei Chen, Michael Sundue, Li-Yaung Kuo, Wei-Chih Teng, Yao-Moan Huang
Data type: statistical data
Explanation note: Maximum likelihood bootstrap percentages (BS) are provided at each node. Thickened lines indicate Bayesian inference posterior probability (PP) ≥ 0.9.
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