

Into Africa: Molecular phylogenetics and historical biogeography of sub-Saharan African woodferns (*Dryopteris*)¹

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PREMISE OF THE STUDY: Our goal was to infer the phylogenetic relationships and historical biogeography of the genus *Dryopteris* with a focus on taxa in sub-Saharan Africa and neighboring islands. In general, little is known about the relationships between African fern species and their congeners in other geographic regions, and our aim was to determine whether the sub-Saharan African species of *Dryopteris* are monophyletic and evolved within Africa or arrived there via repeated dispersals into Africa from other regions.

METHODS: We obtained sequence data for five chloroplast markers from 214 species of *Dryopteris* and 18 outgroups. We performed phylogenetic and molecular dating analyses using a Bayesian relaxed clock method in BEAST with fossil and secondary calibration points and estimated ancestral ranges for the genus globally by comparing multiple models in BioGeoBEARS.

KEY RESULTS: We found that 22 of 27 accessions of sub-Saharan African *Dryopteris* belong to a large clade of 31 accessions that also includes taxa from Indian and Atlantic Ocean islands. Additional accessions of taxa from our regions of interest have Asian, Hawaiian, European, or North American species as their closest relatives.

CONCLUSIONS: The majority of sub-Saharan African *Dryopteris* species are descended from a shared common ancestor that dispersed to Africa from Asia approximately 10 Ma. There have been subsequent dispersal events from the African mainland to islands in the Atlantic and Indian Oceans, including Madagascar. Several additional species are estimated to have descended from ancestors that reached Africa via separate events over the last roughly 20 million years.

KEY WORDS Africa; ancestral range estimation; Dryopteridaceae; ferns; historical biogeography; long-distance dispersal; molecular dating

Understanding how floras assemble at continental scales is of great interest to plant systematists. Botanists have long recognized that vascular plant diversity in Africa is remarkably low compared with areas at similar latitudes in neighboring regions (the “odd man out” hypothesis; Richards, 1973; Aldasoro et al., 2004; Couvreur, 2014). For example, while the American and Australasian tropics have ca. 3000 and ca. 4500 species of ferns and lycophytes, respectively (Tryon, 1986), Africa has only 1400 (Roux, 2009). For ferns and lycophytes, this paucity of taxa in Africa has been attributed to the relatively low rainforest cover and high degree of aridity across

much of the continent as a result of extensive climate change during the late Tertiary and Pleistocene (Tryon, 1986; Maley, 1996; Aldasoro et al., 2004; Pokorny et al., 2015). Despite the generally low number of ferns in Africa, there are several areas of high fern diversity, including the mountainous regions of eastern Africa (Schelpe, 1983; Roux, 2009), the Cape Floristic Region of South Africa, which is a global biodiversity hotspot (Eiserhardt et al., 2011), and nearby Madagascar (Aldasoro et al., 2004).

Three major latitudinal divisions are typically used to describe fern distributions in Africa (Kornaś, 1993): Mediterranean (or North African), Tropical, and Cape. The Mediterranean fern flora is shared almost entirely with southern Europe, and there is almost no overlap in species between this region and sub-Saharan Africa, which includes the Tropical and Cape zones (Dobignard and Chatelain, 2010; Roux, 2012). Within sub-Saharan Africa, Aldasoro et al. (2004) identified three major ecological categories of ferns that roughly correspond to bioclimatic regions: thermophilous (warmth-loving),

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cold-tolerant, and drought-tolerant species. Kornaš' (1993) Tropical zone of sub-Saharan Africa corresponds to two of the bioclimatic regions of Aldasoro et al. (2004): the Gulf of Guinea area, which has a large number of fern species from the thermophilous group, and the tropical Eastern African mountain arc region, which has roughly equal proportions of species from the three ecological components (Aldasoro et al., 2004). The southern Cape area has a high proportion of drought-tolerant species (Aldasoro et al., 2004).

While adhering generally to the ecological groupings and regions described above, individual fern species present in sub-Saharan Africa occupy a diverse set of distribution patterns. Many African ferns have wide pantropical ranges (Kornaš, 1993), while others are disjunct between Africa and Australasia (Parris, 2001) or Africa and the Americas (Moran and Smith, 2001). However, the majority of African species are endemic to the continent (including Madagascar), and many of these species have their closest relatives in Australasia or the Americas (Kornaš, 1993; Moran and Smith, 2001). Kornaš (1993) attributed this pattern to common descent from shared ancestors with wide, pantropical ranges. Given that most extant fern families diversified long after the breakup of the southern continents (Moran and Smith, 2001; Parris, 2001; Schuettpelz and Pryer, 2009), vicariance as an explanation for this pattern can be ruled out, and it seems certain that these transcontinental disjunctions between close relatives were established via long-distance dispersal.

Within Africa, there is relatively little overlap in species' occurrence between the three diversity centers (Mediterranean/Northern, Tropical, and Cape), likely due to the divergent climatic and ecological tolerances of the species found there (Aldasoro et al., 2004). These differences in species assembly across the continent lead to a key question about the affinities of African ferns: Within genera, are African species monophyletic and the result of radiations in situ, or do some or all African taxa have their closest relatives in other regions and thus represent repeated dispersals to the African continent? Moran and Smith (2001) identified 87 sister-species pairs consisting of an African and an American species, supporting a key role for dispersals to the African continent from the neotropics. However, most fern genera with representatives in Africa have only a few species there. Of the 130 fern genera Roux (2009) listed as occurring in Africa, 85 include five or fewer African species. Of the 12 fern genera with 20 or more African members, only one to date (*Cheilanthes* Sw.) has been the subject of comprehensive phylogenetic and biogeographic analyses focused on elucidating the history of the African species; they were found primarily to have radiated within the Cape region, after their ancestors arrived in Africa (Eiserhardt et al., 2011). The genus *Marsilea* L. is also diverse in Africa and is thought to have radiated in situ in southern Africa (Nagalingum et al., 2007). For the remaining fern genera with high representation in Africa, it remains unknown whether these species represent the results of diversification on the continent or of repeated dispersals to Africa.

In the current study, we address this question by inferring the phylogenetic relationships and historical biogeography of a large, globally distributed genus of ferns, *Dryopteris* Adans. *Dryopteris* is one of the largest genera in the taxonomically and phylogenetically complex family Dryopteridaceae (Sessa et al., 2015). The genus consists of 250–350 (Fraser-Jenkins, 1986; Zhang et al., 2012) or even 400 (Wu et al., 2013) species, with a primary center of diversity in Eastern Asia (Wu and Lu, 2000; Li and Lu, 2006) and secondary centers in the Americas (Sessa et al., 2012a, 2012c, 2015), Europe (Juslén et al., 2011), Africa (Roux, 2011, 2012), and the Pacific

islands (Geiger and Ranker, 2005). The African *Dryopteris* species composition is relatively well known thanks to a revision of the sub-Saharan species by Roux (2012) and the fact that the northern African flora is very similar to that of the Mediterranean area (Fraser-Jenkins, 1982; Dobignard and Chatelain, 2010).

Roux (2012) described African *Dryopteris* as having three main centers of diversity, in North, West, and Southeast Africa (Fig. 1). The North-African center corresponds to the Mediterranean zone of Kornaš (1993), and no species of *Dryopteris* occur in both the North-African and the West or Southeast centers. The West-African center corresponds to the Gulf of Guinea region of Aldasoro et al. (2004) and extends to include Cape Verde, while the Southeast-African center reaches from Eritrea through the East African mountains to South Africa, with a westward projection encompassing the highlands of Angola (Fig. 1). No *Dryopteris* are known from the extreme deserts of the Sahara/Sahel or Kalahari/Namib regions or from the lowland rainforests of the Congo basin (*Dryopteris* is primarily a genus of temperate forest habitats; Sessa et al., 2012a). Roux (2012) listed 26 *Dryopteris* species from sub-Saharan Africa, including the islands of Cape Verde, São Tomé, Príncipe, and Annobón (Fig. 1), plus an additional seven species endemic to Madagascar and neighboring Indian Ocean Islands (Roux, 2011), and three species endemic to the Atlantic Islands Ascension and St. Helena (Roux, 2013). Eight species occur in the Azores and Canary Islands (Gibby et al., 1977; Roux, 2012).

Previous molecular phylogenetic studies of *Dryopteris* have focused on taxa in the Americas (Sessa et al., 2012a–c), Asia (Li and Lu, 2006; Zhang et al., 2012), and Europe (Juslén et al., 2011), while the relationships of African *Dryopteris* have been almost totally unexplored thus far (Sessa et al., 2015). Very few African species had been sampled before the current study, and Roux (2012) did not assign the African taxa to any subgenera because he felt a more robust phylogeny was needed before making classification decisions. Our goal is to place the sub-Saharan African *Dryopteris* species in a global phylogenetic context to identify their closest relatives and to reconstruct the historical biogeography of the genus globally to determine whether African *Dryopteris* are monophyletic and radiated within Africa or represent a number of separate introductions into Africa from other regions.

MATERIALS AND METHODS

Taxon sampling, DNA extraction, amplification, and sequencing—

We included *Dryopteris* taxa from all major geographic regions of the world. Most species were represented by a single accession but when possible, African species were represented by multiple accessions (Table 1; Appendix S1 [see the Supplemental Data with this article]). We also included 18 outgroup species from eupolypods I and II and Pteridaceae (Smith et al., 2006) to enable the use of fossils in the molecular dating analysis. Sequence data for most species were obtained from GenBank, but we generated new sequences for African taxa, many of which were sequenced here for the first time, from herbarium material. We followed the most recent annotations on each label when determining taxonomy. Voucher information and GenBank accessions numbers for all accessions are listed in Appendix S1.

We extracted total genomic DNA using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA), following the manufacturer's protocol. We amplified five chloroplast regions using primers from

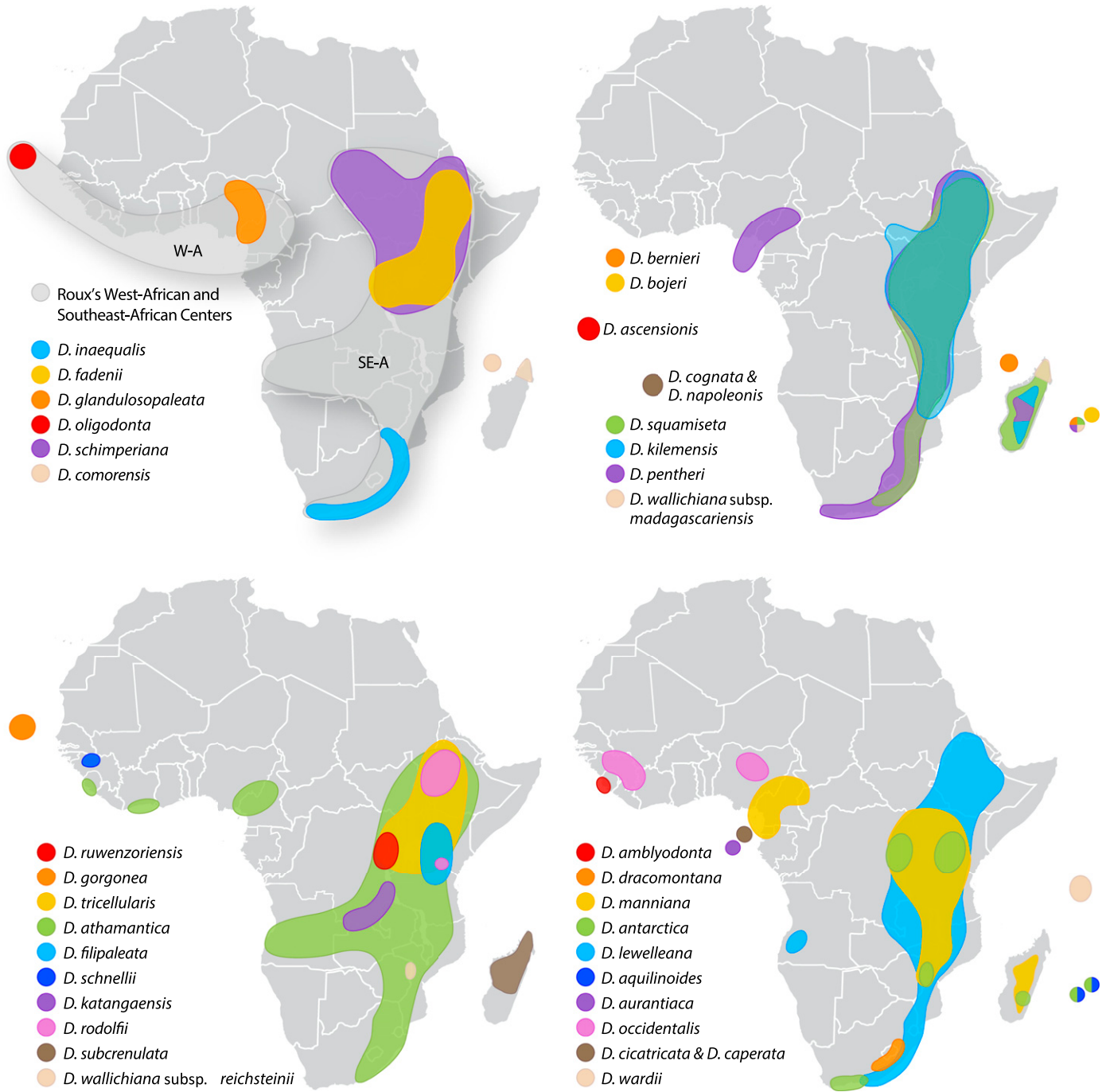


FIGURE 1 Range maps of African *Dryopteris* species, redrawn from Roux (2011, 2012, 2013). Roux’s West-African and Southeast-African centers of sub-Saharan African *Dryopteris* diversity are also shown (from Roux, 2012).

previous studies: intergenic spacers *trnL-F* (Taberlet et al., 1991; Trewick et al., 2002), *trnP-petG* (Small et al., 2005), *trnG-trnR* (Korall et al., 2007), and *rps4-trnS* (Rouhan et al., 2004), and the gene *rbcL* (Korall et al., 2006). PCR protocols followed those of Sessa et al. (2012a, c). PCR products were purified and sequenced by Genewiz (South Plainfield, New Jersey, USA) or at the Interdisciplinary Center for Biotechnology at the University of Florida. Sequences were edited and contigs assembled in the program Geneious R9 (Biomatters, Auckland, New Zealand). Alignments were performed

using the MAFFT 1.3.5 plugin in Geneious and then checked manually. We used the program PartitionFinder (Lanfear et al., 2012) to identify the best model of evolution for each locus, and the best partitioning scheme for the data set as a whole, using the Bayesian information criterion (BIC).

Molecular dating analysis—We estimated divergence times using an uncorrelated, lognormal relaxed clock (UCLN) model in BEAST 2.3.2 (Drummond et al., 2006; Drummond and Rambaut, 2007;

TABLE 1. Presence of *Dryopteris* species in Sub-Saharan Africa, North Africa, Madagascar, Indian Ocean Islands (Seychelles, Réunion, Mauritius, and/or Comoros), and Atlantic Ocean Islands (Azores and/or Canaries, Cape Verde, Ascension and/or St. Helena). Species' inclusion in the current study and number of accessions included are indicated. An asterisk (*) denotes taxa sequenced for the first time in this study. References upon which distribution determinations are based are given (Euro+Med refers to the online Euro+Med PlantBase; Christenhusz and Raab-Straube, 2013).

Taxon	Included? (no. of accessions)	Reference	Sub-Saharan Africa	North Africa	Madagascar	Indian Ocean	Ascension/ St. Helena	Azores/ Canaries	Cape Verde
1. <i>D. aemula</i>	✓ (1)	Euro+Med						✓	
2. <i>D. affinis</i> subsp. <i>affinis</i>	✓ (1)	Roux, 2012; Euro+Med		✓				✓	
3. <i>D. aitoniana</i>	✓* (1)	Roux, 2012						✓	
4. <i>D. amblyodonta</i>	—	Roux, 2012	✓						
5. <i>D. antarctica</i>	✓ (2)	Roux, 2011, 2012	✓		✓	✓			
6. <i>D. aquilinoides</i>	✓ (2)	Roux, 2011							
7. <i>D. ascensionis</i>	—	Roux, 2013					✓		
8. <i>D. athamantica</i>	✓ (3)	Roux, 2012	✓						
9. <i>D. aurantiaca</i>	—	Roux, 2012	✓						
10. <i>D. azorica</i>	✓ (1)	Euro+Med						✓ (2)	
11. <i>D. bernieri</i>	✓ (2)	Roux, 2011				✓			
12. <i>D. bojeri</i>	✓* (1)	Roux, 2011				✓			
13. <i>D. caperata</i>	—	Roux, 2012	✓						
14. <i>D. cicatricata</i>	—	Roux, 2012	✓						
15. <i>D. cognata</i>	✓* (1)	Roux, 2013					✓		
16. <i>D. comorensis</i>	✓* (1)	Roux, 2011			✓	✓			
17. <i>D. crispifolia</i>	✓ (1)	Gibby et al., 1977; Euro+Med						✓	
18. <i>D. dracomontana</i>	✓* (1)	Roux, 2012	✓						
19. <i>D. fadenii</i>	✓* (1)	Roux, 2012	✓						
20. <i>D. filipaleata</i>	—	Roux, 2012	✓						
21. <i>D. filix-mas</i>	✓ (2)	Euro+Med		✓					
22. <i>D. glandulosopaleata</i>	✓* (1)	Roux, 2012	✓						
23. <i>D. gorgonea</i>	—	Roux, 2012							✓
24. <i>D. guanchica</i>	✓ (1)	Euro+Med						✓	
25. <i>D. inaequalis</i>	✓ (2)	Roux, 2012	✓						
26. <i>D. katangaensis</i>	✓* (1)	Roux, 2012	✓						
27. <i>D. kilimensis</i>	✓* (2)	Roux, 2012	✓		✓				
28. <i>D. lewalleana</i>	✓* (3)	Roux, 2012	✓						
29. <i>D. maderensis</i>	✓ (1)	Euro+Med						✓	
30. <i>D. manniana</i>	✓* (1)	Roux, 2012	✓		✓				
31. <i>D. napoleonis</i>	✓* (1)	Roux, 2013					✓		
32. <i>D. occidentalis</i>	✓* (1)	Roux, 2012	✓						
33. <i>D. oligodonta</i>	✓ (2)	Roux, 2012						✓	✓
34. <i>D. pallida</i>	✓ (2)	Euro+Med		✓					
35. <i>D. pentheri</i>	✓ (3)	Roux, 2011; 2012	✓		✓	✓			
36. <i>D. rodolfii</i>	—	Roux, 2012	✓						
37. <i>D. ruwenzoriensis</i>	—	Roux, 2012	✓						
38. <i>D. schimperiana</i>	✓* (3)	Roux, 2012	✓						
39. <i>D. schnellii</i>	✓* (1)	Roux, 2012	✓						
40. <i>D. squamiseta</i> (= <i>Nothoperanema</i> <i>squamiseta</i>)	✓ (1)	Roux, 2011, 2012	✓		✓	✓			
41. <i>D. subcrenulata</i>	✓* (1)	Roux, 2011			✓				
42. <i>D. tricellularis</i>	✓* (1)	Roux, 2012	✓						
43. <i>D. wallichiana</i> subsp. <i>madagascariensis</i>	—	Roux, 2011			✓	✓			
44. <i>D. wallichiana</i> subsp. <i>reichsteinii</i>	—	Roux, 2012	✓						
45. <i>D. wardii</i>	—	Roux, 2011				✓			

Bouckaert et al., 2014). We implemented a birth–death process speciation tree prior with unlinked clock models between partitions and employed the best partitioning and model scheme identified by PartitionFinder. Relationships among the outgroups were constrained based on previous phylogenetic analyses of ferns (Smith et al., 2006; Schuettpelz and Pryer, 2009). Three analyses were run for 50,000,000 generations each, with parameters sampled every 1000 generations. Log files were combined using the application

LogCombiner 2.3.2 (Bouckaert et al., 2014), and the posterior distribution and estimated sample size (ESS) of all parameters were examined using the program Tracer 1.6 (Drummond and Rambaut, 2007). We then used TreeAnnotator 2.3.2 (Bouckaert et al., 2014) to combine and summarize a post burn-in set of trees and generate a maximum clade credibility chronogram showing mean divergence time estimates with 95% highest posterior density (HPD) intervals.

Because the fossil record of Dryopteridaceae is sparse (Sessa et al., 2012a; Le Péchon et al., 2016), we employed one reliable fossil from within the family and two additional fossils from Polypodiaceae (eupolypods I) and Blechnaceae (eupolypods II). We also constrained the crown node of the eupolypods following Le Péchon et al. (2016), using a secondarily derived age estimate based on two previous studies of divergence times in leptosporangiate ferns (Schneider et al., 2004; Schuettpelz and Pryer, 2009). All calibration points used lognormal priors because a previous analysis of divergence times in *Dryopteris* found no significant differences in dates between analyses that used uniform vs. lognormal and normal priors (Sessa et al., 2012a). The three fossil calibration points were modeled as follows: (1) the crown node of *Elaphoglossum* Schott ex J.Sm. was constrained to a minimum age of 15 Myr based on the fossil *Elaphoglossum miocenicum* Lóriga, A.R.Schmidt, R.C.Moran, K.Feldberg, H.Schneid. & Heinrichs (Lóriga et al., 2014); (2) the divergence between Polypodiaceae and Davalliaceae was constrained to a minimum age of 33.9 Myr based on the fossil *Protodrynaria takhtajanii* Vikulin & Bobrov (Vikulin and Bobrov, 1987; Van Uffelen, 1991); and (3) the divergence between *Woodwardia* Sm. and *Blechnum* L. was constrained to a minimum age of 55.8 Myr, based on a fossil *Woodwardia* (Wilf et al., 1998). The crown node of eupolypods was constrained to a minimum age of 117.1 Myr following Le Péchon et al. (2016) and based on the age of the clade as estimated by Schneider et al. (2004) and Schuettpelz and Pryer (2009).

Ancestral range estimation—We estimated ancestral ranges using the R package BioGeoBEARS (Matzke, 2014). Contemporary ranges of taxa were determined by consulting existing literature, primarily Roux (2011, 2012, 2013), Sessa et al. (2012a), the Flora of China entry on *Dryopteris* (Wu et al., 2013), and the web-based Catalogue of Life (Roskov et al., 2017) and Euro+Med PlantBase (Christenhusz and Raab-Straube, 2013). We compared three models: DEC (dispersal–extinction–cladogenesis; Ree and Smith, 2008), DIVA-like (dispersal–vicariance analysis; Yu et al., 2010), and BayArea-like (Landis et al., 2013), each with and without the “jump dispersal” (*j*) parameter available in BioGeoBEARS, for a total of six models. The models were constructed using the maximum clade credibility tree from BEAST after pruning outgroups outside Dryopteridoideae Link (Liu et al., 2016). We also collapsed accessions of African *Dryopteris* species if multiple accessions were included for a taxon and they formed a clade in the BEAST analysis, so that only a single tip in the BioGeoBEARS analysis represented each such taxon. We performed a time-stratified analysis to account for changes in proximity of landmasses over the last 60+ Myr; for example, the Hawaiian islands are volcanic in origin and would not have been available as a target for dispersal until within the last 10–15 Myr. The four time slices in the analysis corresponded to 0–10 Ma, 10–30 Ma, 30–60 Ma, and more than 60 Ma. Dispersal multipliers, which set the rate of dispersal between geographic areas, were chosen for each time period based on recent studies that have performed similar analyses over similar temporal and spatial scales (e.g., Sessa et al., 2012a; Spalink et al., 2016).

RESULTS

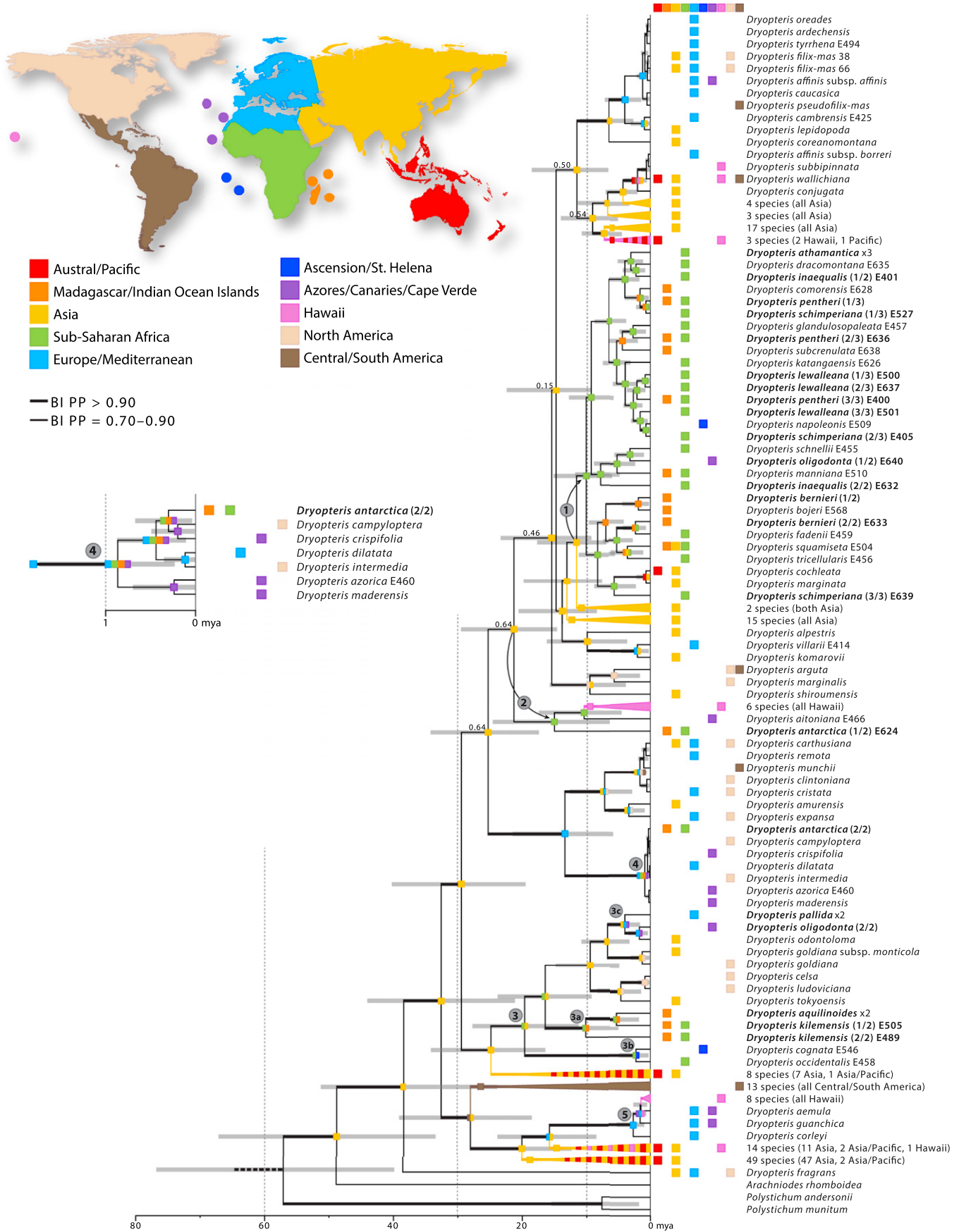
The final data matrix includes 247 accessions (18 outgroups and 229 *Dryopteris* from 214 species and subspecies) and is 5055 bp

long (Appendices S2 [NEXUS file] and S3 [XML file]). The chronogram provides moderate to strong support for most backbone nodes and within many clades, but support is weak at several points along the backbone (Fig. 2). The split between *Dryopteris* and its closest relative, *Arachniodes*, occurs at 48.9 Ma (95% highest posterior density interval [HPD] = 67.1–33.4 Ma). Within *Dryopteris*, *D. fragrans* L.Schott is sister to the rest of the genus, with a divergence date of 38.5 Ma (95% HPD = 51.3–23.4 Ma). Subsequent diversifications within the genus have occurred over the last ca. 32.5 Myr, with many radiations in the last ca. 10 Myr (Fig. 2). The DEC model produced the most likely ancestral range estimates of the models tested in BioGeoBEARS, and likelihood ratio tests support the inclusion of the *j* parameter as producing significantly more likely reconstructions than DEC alone (DEC+*j* LnL = –387; DEC LnL = –397; df = 1, $D_{\text{stat}} = 21.84$, $P = 3.0 \times 10^{-6}$).

Our sampling includes 49 accessions representing 33 of the 45 species of *Dryopteris* that occur in our regions of interest (sub-Saharan and North African, Madagascar and Indian Ocean Islands, Ascension/St. Helena, and Canaries/Azores/Cape Verde; Table 1). Many species occur in more than one of these areas (Figs. 1, 2). Of the 33 included species, we had multiple accessions for 12 of them (bolded taxon names in Fig. 2); of these, the accessions for eight species are placed in different parts of the tree (i.e., accessions for a single taxon are not monophyletic).

Of 27 accessions for sub-Saharan African species, 22 belong to a large clade that also includes taxa from the Indian Ocean region (Madagascar and neighboring islands), St. Helena (*D. napoleonis* Kuntze), the Canary Islands [*D. oligodonta* (Desv.) Pic.Serm.], and two species from Asia/Austral-Pacific. This clade descended from an ancestor that the model reconstructs as having arrived in sub-Saharan Africa via dispersal from Asia by approximately 10.2 Ma (95% HPD = 15.2–5.9 Ma) (Fig. 2, label 1). In addition to this large clade, other accessions of sub-Saharan African species are placed in three smaller clades (Fig. 2, labels 2–4). The first of these is the result of another ancestral long-distance dispersal event from Asia to Africa that had occurred by 15.1 Ma (95% HPD = 24.6–6.5 Ma). A subclade of six Hawaiian species is nested within this group, sister to African *D. aitoniana* Pic.Serm. (Fig. 2, label 2). Another clade that includes African taxa descends from a node with a broad ancestral range estimation that includes both Asia and Africa around 19.7 Ma (95% HPD = 27.7–11.3 Ma) (Fig. 2, label 3). Two accessions each of *D. kilemensis* (Kuhn) Kuntze and *D. aquilinoidea* C.Chr. are nested in this clade, with their immediate ancestors reconstructed as occurring in sub-Saharan Africa and Madagascar (Fig. 2, label 3a). A species from St. Helena (*D. cognata* Kuntze) also belongs to this clade and is descended from an ancestor reconstructed as having a range in Africa as well as Ascension/St. Helena, which were coded together (Fig. 2, label 3b). One accession of another island endemic, *D. oligodonta*, also falls in this clade, sister to a species from Asia [*D. odontoloma* (Moore) C. Chr.], with these two together sister to two accessions of European/North African *D. pallida* Fomin. The ancestor of all four is estimated to have been widespread in Asia, Europe/North Africa, and the Azores/Canaries/Cape Verde island groups (Fig. 2, label 3c).

Two additional clades include species from the Azores and Canaries: three endemics to these islands, *D. crispifolia* Rasbach, Reichst. & G.Vida, *D. azorica* (Christ) Alston, and *D. maderensis* Alston, belong to a clade that also includes one accession of African *D. antarctica* (Baker) C.Chr., as well as North American *D. intermedia* (Muhl. ex Willd.) A.Gray and *D. campyloptera* (Kunze) Clarkson,



and European *D. dilatata* (Hoffm.) A.Gray. This group diversified in the last 1 Myr from ancestors that were widespread in Europe, Africa, Madagascar, and the Atlantic islands (Fig. 2, label 4). *Dryopteris aemula* Aiton (Kuntze) from western Europe and Macaronesia, and *D. guanchica* Gibby & Jermy from the Canaries and Atlantic coast of the Iberian Peninsula, are sister to northern Spanish narrow endemic *D. corleyi* Fraser-Jenk. and descended from an ancestor in Europe in the last 2.6 Myr (95% HPD = 5.2–0.95 Ma) (Fig. 2, label 5). A clade of eight Hawaiian species is embedded in this group.

DISCUSSION

Overall, the phylogenetic results produced by our analyses agree well with those from previous studies of *Dryopteris*. Despite the low support at several backbone nodes, the topology is generally congruent with those recovered by Zhang et al. (2012) and Sessa et al. (2012a). The majority of new accessions included here, for sub-Saharan African species, fall into a clade that is itself embedded in clade I of Sessa et al. (2012a) and the “*Dryopteris* clade” of Zhang et al. (2012), which roughly correspond to *Dryopteris* subgen. *Dryopteris* sensu Fraser-Jenkins (1986) and Wu and Lu (2000). The dates for the initial divergences within *Dryopteris* (e.g., at the split of *D. fragrans* from the ancestor of the rest of the genus, and for the first divergence within the remaining species) are very similar between this study and Sessa et al. (2012a): 42.4 Ma for the split between *D. fragrans* and all other *Dryopteris* species (Sessa et al., 2012a) vs. 38.5 Ma (this study), and 35.9 Ma for the *Dryopteris* crown group (Sessa et al., 2012a) vs. 32.5 Ma (this study).

The primary purpose of this study was to determine whether the sub-Saharan African species of *Dryopteris* are monophyletic and radiated following a single ancestral colonization of Africa or instead represent the result of multiple introductions to the continent from other regions. Our results clearly show that while most accessions of African *Dryopteris* species do form one large clade (descended from an ancestor that dispersed to Africa from Asia in a single event by roughly 10 Ma), several movements into sub-Saharan Africa over the last 20 Myr have contributed to the modern *Dryopteris* flora there and in neighboring island groups (Fig. 2). The history of *Dryopteris* in Madagascar, in particular, is dominated by connections to sub-Saharan Africa. Of the 12 species of *Dryopteris* in the Indian Ocean region, seven are endemic to that area (Roux, 2011), and four of the five of these that we were able to include are nested within the large sub-Saharan African clade, as are most of the non-endemic species that also occur in sub-Saharan Africa. Several species present in the Atlantic island groups (e.g., *D. aitoniana* (Fig. 2, label 2), *D. cognata* (Fig. 2, label 3b), and *D. maderensis*, *D. azorica*, and *D. crispifolia* (Fig. 2, label 4) have also descended from ancestors whose ranges included Africa. Africa has

therefore acted as both a source for island colonists relatively recently, and as a destination for dispersal events in the more distant past. At least one of these events involved the ancestor of a subsequent radiation that makes up the bulk of the sub-Saharan African *Dryopteris* flora today (Fig. 2, label 1).

After the placement of most of the African accessions into one large clade, the next most striking aspect of our results is that, of the species for which we had multiple accessions (12 taxa), in most cases (eight taxa), these accessions were not monophyletic. This result suggests that considerable confusion exists in the identification and labeling of sub-Saharan African *Dryopteris* species in herbaria since all of our accessions were sampled from herbarium sheets rather than being newly collected in the field. That such extensive confusion might exist is not particularly surprising, given that a thorough and comprehensive monographic treatment of these taxa was only produced in 2012 (Roux, 2012). Many of the herbarium collections we sampled are much older than that, so collectors were likely using concepts when labeling their specimens that were not based on rigorous comparative analyses such as those carried out by Roux (2012). Substantial specimen misidentification for tropical plant taxa is not unprecedented; Goodwin et al. (2015) reported that a high percentage of specimens of African gingers (genus *Aframomum* K.Schum.), Dipterocarpaceae, and *Ipomoea* species were incorrectly identified before focused taxonomic studies. We also note that support was weak for most branches in this section of the tree, so the nonmonophyly of taxa may also reflect lack of resolution, with accessions for these taxa becoming monophyletic if additional data are added. However, it seems to us most likely that this pattern does mostly reflect confusion in identification of specimens, driven by the fact that several of the sub-Saharan African *Dryopteris* species are morphologically complex, and many have wide and/or disjunct distributions over which morphology may vary. Roux (2012) discussed the ranges and morphological complexities of these species at length. For example, *Dryopteris antarctica* (Baker) C.Chr. has a remarkably wide and disjunct distribution, occurring in the Indian Ocean, South Africa, and several noncontiguous locations in eastern Africa (Fig. 1). Our sequences are from Réunion and South Africa (Appendix S1) and fall into quite different locations in our tree (Fig. 2). There has been debate about the identity of this taxon in South Africa, and while Roux (2012) noted that there is “little variation in overall morphology” between the widespread populations of this species, it may be that they in fact represent cryptic diversity and do not belong to the same evolutionary entity. In contrast, Roux (2012) remarked on the high variability in *Dryopteris lewalleana* Pic.Serm. and noted that this species is likely part of a complex with *D. pentheri* (Krasser) C.Chr. and *D. inaequalis* (Schltdl.) Kuntze; the three are morphologically similar and specimens of each have at various times been ascribed to the other two. In our topology, accessions of these three are present in

FIGURE 2 Maximum clade credibility (MCC) chronogram from molecular dating analysis of *Dryopteris*, overlaid with results of ancestral range estimation. The map at top left is colored according to the areas used in the BioGeoBEARS analysis, and species’ contemporary ranges are indicated in the columns beside their names. Names are bolded for species for which we had multiple accessions, and numbers in parentheses indicate numbers of accessions. Identification numbers are also given following taxon names for samples that were newly sequenced in this study. Clades that contain only taxa not from the regions of interest in this study are collapsed and shown as triangles. Vertical dotted lines correspond to time slices in the BioGeoBEARS analysis. The dotted line at the root of the tree leads to additional outgroups that were included in the analyses but are not shown in the figure. Horizontal gray bars give the 95% highest posterior density (HPD) intervals around mean node ages from the BEAST analysis. Numbers 1–5 indicate nodes discussed in the text; arrows accompanying numbers 1 and 2 denote major long-distance dispersal events inferred in the history of African *Dryopteris*. Detail is provided at left for node 4.

the same subclades, along with accessions of several other species (Fig. 2), including *D. schimperiana* (Hochst.) C.Chr., which Roux (2012) notes is also easily confused with *D. pentheri*. We stress that support in this section of the tree is very low, so the relationships of all these accessions should be considered unresolved at present. A contrast to the pattern noted above is *D. athamantica* (Kunze) Kuntze, a widespread taxon for which we included three accessions that were monophyletic in the tree. Overall, our results suggest that studies of African ferns should begin with careful consideration of the specimens to be included and that many specimens in collections are likely in need of re-annotation using uniform taxonomic treatments. We doubt that this problem is limited to *Dryopteris*, and our results suggest that the issue of “mistaken identity” described above for several groups of flowering plants (Goodwin et al., 2015) may plague ferns as well.

Another phenomenon that may have contributed to the non-monophyly of accessions, and which has been almost totally unexplored in sub-Saharan African *Dryopteris*, is polyploidy. Ferns in general (Wood et al., 2009), and this genus in particular (Sessa et al., 2012b, c), are known to be highly prone to polyploidy. Based on estimates of guard cell sizes, Roux (2012) suggested that several of the African *Dryopteris* species might be polyploids [e.g., *D. antarctica*, *D. manniana* (Hook.) C.Chr., and *D. pentheri*]. No nuclear sequence data have yet been produced to test these suggestions, but the potential for allopolyploid hybridization to have occurred in the history of this group is high, given how frequent this phenomenon is in other groups of *Dryopteris* (Juslén et al., 2011; Sessa et al., 2012b, 2012c, 2015). If any of these species are allopolyploids, there is also the potential for multiple origins of those entities (Werth et al., 1985; Soltis et al., 2014), which may or may not have had reciprocal parentage (Sigel et al., 2014). All of these phenomena—allopolyploidy, multiple origins, and reciprocal parentage—alone or in combination, may have complicated the history of sub-Saharan African *Dryopteris* substantially and in ways that plastid sequence data alone are insufficient to untangle. Clearly, all of these species are in need of further study, and fresh collections, identified using a unified treatment of the group (Roux’s being the obvious choice), will be essential for further clarifying the relationships and histories of individual *Dryopteris* taxa in Africa.

Before this study, phylogenetic analyses of African ferns had been carried out for the genera *Cheilanthes* (Eiserhardt et al., 2011) and *Marsilea* (Nagalingum et al., 2007). Only the first of these studies included molecular dating and analysis of historical biogeographic movements, and Eiserhardt and colleagues concluded that several clades of *Cheilanthes* diversified within the Cape region of South Africa, subsequent to their ancestors’ introductions to the region between 20 and 10 Ma. This is the same time frame that we infer for the arrival of *Dryopteris* to the continent. While Nagalingum et al. (2007) did not include molecular dating or biogeographic analyses, they did infer that *Marsilea*, which includes several small African clades whose relationships to one another are unresolved, likely diversified in Africa in the last 20 Myr, which they note corresponds to a period of increasing aridification in the Cape region (Linder and Hardy, 2004). Diversification of both of these groups in situ in Africa within the last 20 Myr concurs with our findings from *Dryopteris*, where the bulk of accessions of African species belong to one major clade whose ancestor arrived in Africa by 10.2 Ma (Fig. 2, label 1). None of our estimates of ancestral ranges included Africa before ca. 20 Ma (Fig. 2).

In addition to the above studies, Janssen et al. (2007) evaluated the relationships of African members of Polypodiaceae using data for six of the eight lineages in the family that include African species. These authors concluded that the African taxa represent multiple dispersals to the continent, based on sister-group relationships with species in the neotropics and/or Asia, where the lineages were inferred to have originated. The African representatives of each lineage generally consisted of several single species that were not each other’s closest relatives, with the exceptions of the drynarioid lineage, which includes a clade of three African species, and the platyceroid lineage, which includes a clade of seven African plus one neotropical species. These clades were inferred to have evolved in Africa (with one dispersal to the neotropics) (Janssen et al., 2007). Similarly, in studies of two groups in *Asplenium* L., Van den Heede et al. (2003) and Bellefroid et al. (2010) concluded that *Asplenium* subgenus *Ceterach* (Willd.) Bir et al. (Van den Heede et al., 2003) and the “loxoscapoid” group of *Asplenium* (Bellefroid et al., 2010), each of which contain only three to four species, both diversified after arriving in Africa. These conclusions were also based on sister-group relationships, as dating and historical biogeographic analyses were not done. It may be that the inferred dispersals of *Asplenium* and Polypodiaceae species or their ancestors to Africa have occurred more recently than in *Dryopteris*, *Cheilanthes*, and *Marsilea*, that they thus have not had time to radiate on the continent, and they will do so in the future. Without dated phylogenies for each group, however, it is impossible to infer the times at which the introductions occurred and whether the lack of radiation in Africa in some genera reflects their presence in Africa for relatively less time than for other genera. Taken together, the groups for which we do have dated phylogenies (*Dryopteris*, *Cheilanthes*) or a reasonable estimation of dates (*Marsilea*) suggest that fern diversity in Africa may be lower than in other regions (Tryon, 1986; Aldasoro et al., 2004) because ferns have only been diversifying there during the last 20 Myr. These conclusions based on only three genera are not enough to establish a definite pattern, but they are suggestive.

Numerous phylogenetic studies of other fern groups have included one or more African taxa (e.g., Schneider et al., 2008; Korall and Pryer, 2013; Ohlsen et al., 2014; Le Péchon et al., 2016, among many others), but not with the direct goal of determining when or from whence those species arrived on the continent and whether they underwent local diversification in Africa or not. Those were the goals of the present paper, and the new data we report for *Dryopteris*, together with existing data from several other genera, suggest that Africa has been a destination for dispersal as well as the source of subsequent diversification, the latter largely within the last 20 Myr. Dated molecular phylogenies coupled with historical biogeographic and diversification analyses for additional groups may help to strengthen (or weaken) this conclusion and will shed further light on the evolutionary history of ferns in Africa.

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