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Global phylogeny and biogeography of the fern genus *Ctenitis* (Dryopteridaceae), with a focus on the Indian Ocean region

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Abstract

The diverse and pantropical genus *Ctenitis*, in the Dryopteridaceae, has been largely ignored in phylogenetic studies until now. In this study, we fill in this gap by reconstructing the first comprehensive phylogeny of the genus including 53 species currently recognized in the genus *Ctenitis*, among which seven species formerly were assigned to the genus *Pseudotectaria* and one to *Heterogonium*. Special emphasis was given to the sampling of species occurring in the African-Indian Ocean region. The presented results include reconstruction of a biogeographic scenario based on estimated divergence times and ancestral area reconstruction. Our findings confirm the inclusion, within *Ctenitis*, of the Indian Ocean species formerly placed in *Pseudotectaria* and *Heterogonium*. The crown group divergence was estimated to date back to the Oligocene or Early Miocene. The biogeographical scenario indicates an initial divergence of the Asian-Pacific ranges and the neotropical ranges, and a subsequent colonization of the Afro-Madagascan region by a lineage with neotropical ancestors. The Afro-Madagascan lineage splits into a lineage endemic to the Mascarene islands and a lineage occurring in Madagascar, the Comoros and Africa. The range expansion towards Africa and Madagascar was estimated to date back to the late Miocene, whereas the estimated ages for the onset of the diversification of the Mascarene diversity is consistent with the ages of these young, volcanic islands. The absence of any extant species of *Ctenitis* with a multi-continental distribution range and the rarity of inter-island dispersal and speciation in the Indian Ocean region suggest a limited contribution of long distance dispersal to the biogeographical history of this fern genus, versus a high contribution of local speciation.

Keywords: Biogeography; dispersal; diversification; ferns; Madagascar; Mascarenes

Introduction

According to the most recent fern classifications, *Ctenitis* (C.Chr.) C.Chr. is the fourth genera of the Dryopteridaceae in terms of species richness, with an estimated 125 species (PPG I, 2016). Species of *Ctenitis* are terrestrial, with fronds tufted on an erect or short-creeping rhizome. They occur throughout the tropics, with approximately 50 species in the Neotropics (Viveros and Salino, 2015), 30 in the Asian-Pacific region excluding Southern Pacific such as Australia and New Zealand (Holttum, 1984, 1985), one species in mainland Africa and 40 in the Western Indian Ocean islands (Duan et al., 2017).

The taxonomy of *Ctenitis* has long confounded systematists. *Ctenitis* was first described by Christensen (1911) as a subgenus of *Dryopteris* Adans., based on the occurrence of distinctive “ctenitoid” hairs on the axes of the upper side of fronds, before being erected to genus rank (Christensen, 1938). Several taxa were first included in *Ctenitis* as informal groups by Christensen (1920) because they shared this type of hairs. Although they were later treated as separated genera, some have long been considered close to *Ctenitis*, in particular *Megalastrum* Holttum in the Dryopteridaceae (Holttum, 1986a), and *Triplophyllum* Holttum, *Pseudotectaria* Tardieu, and *Heterogonium* C.Presl in the Tectariaceae (Holttum, 1983, 1986a, 1986b; Holttum and Lin, 1990). *Ctenitis* itself was moved several times between the two families, before being durably placed in the Dryopteridaceae (Ching, 1965; Li and Lu, 2006; Liu et al., 2007, 2016a; Smith et al., 2006). Furthermore, a comprehensive taxonomic treatment of the genus as a whole is lacking, as to date taxonomic treatments or even detailed checklists were focused on geographical regions: the Neotropics (Brade 1972; Christensen, 1913, 1920; Cremers et al., 1993; Mickel and Smith, 2004; Moran, 1995; Proctor, 1977, 1985, 1989; Sehnem, 1979; Smith, 1995; Stolze, 1981, 1990; Tryon and Stolze, 1991; Tryon and Tryon, 1982; Viveros and Salino, 2015), the Asian region (Ching, 1938; Dong and Christenhusz, 2013; Holttum, 1985; Itô, 1939; Wang, 1965, 1999), Africa and Indian Ocean (Badré, 2008; Duan et al., 2017; Holttum, 1983; Roux, 2009).

Surprisingly, the genus has also been largely ignored in phylogenetic studies of ferns. A maximum of five species of *Ctenitis*, more recently 13 (Hennequin et al., 2014), were included in only a few phylogenetic studies, either at the broad scale (Schuettpelez and Pryer, 2007; Testo and Sundue, 2016), or focusing on the Dryopteridaceae (Liu et al., 2007, 2016a) or Tectariaceae (Ding et al., 2014; Moran et al., 2014; Wang et al., 2014; Zhang et al., 2016, 2017). All previous studies confirmed the inclusion of *Ctenitis* in Dryopteridaceae, but therein, its position was either not resolved (Liu et al., 2007) or resolved as sister to a clade mainly composed of *Dryopteris* and *Polystichum* Roth/*Cyrtomium* C. Presl/*Phanerophlebia* C. Presl (clade corresponding to subfam. Dryopteridoideae

according to Ding et al., 2014; Liu et al., 2016a; Schuettpelz and Pryer, 2007; Testo and Sundue, 2016; Wang et al., 2014). Notwithstanding the absence of a consensus on the accurate position of *Ctenitis* in Dryopteridaceae, these studies further resolved the placement of the taxa previously considered allied to *Ctenitis*. *Megalastrum* was confirmed a distinct genus in the Dryopteridaceae (Labiak et al., 2014; Liu et al., 2016a; Schuettpelz and Pryer, 2007). *Triplophyllum* was confirmed in the Tectariaceae (Ding et al., 2014; Moran et al., 2014; Wang et al., 2014; Zhang et al., 2016, 2017), and *Heterogonium* as part of *Tectaria* (Ding et al., 2014; Zhang et al., 2017). However, the single non-Asian species of *Heterogonium*, *H. sieberianum* (Kaulf.) Holttum, was shown to be included in *Ctenitis* by Hennequin et al. (2014). In addition, Wang et al. (2014) sampled three species of *Pseudotectaria* (Tardieu-Blot, 1955; revised by Holttum and Lin, 1990), a genus of eight species endemics to Madagascar and the Comoros (Rakotondrainibe, 2010). This genus was previously included in the Tectariaceae based on the venation pattern (Christenhusz et al., 2011; Smith et al., 2006), but it was suspected to have affinities to *Ctenitis* (Rakotondrainibe, 2010). The latter assumption was confirmed by Wang et al. (2014) retrieving three species of *Pseudotectaria* sister to *Ctenitis*. In their revision of *Ctenitis* in the African-Indian Ocean region, which was conducted in parallel to this study, Duan et al. (2017) proposed the combination of all species formerly placed in *Pseudotectaria*, as well as *Heterogonium sieberianum*, under *Ctenitis*. Despite this progress in delimiting the natural boundaries of *Ctenitis*, a phylogenetic study of the genus is still highly needed to address the affinities among its species as well as its biogeographic history.

Madagascar and the Indian Ocean Islands are well known for their high levels of diversity and endemism and the ferns are no exception, with an estimated 590 species (45% endemics) for Madagascar only (Rakotondrainibe, 2003). The diversity of *Ctenitis* in the African-Indian Ocean (AIO) region was recently re-evaluated, with a previously estimated 14 species (Roux, 2009) rising upward to as many as 40 (Duan et al., 2017). According to Duan et al. (2017), *Ctenitis* is distributed in the region in mainland Africa (supposedly one species, endemic), Madagascar (24 species, 23 endemics), the Mascarenes (12 species, all endemic, 11 single-island endemics), the Comoros (four species, two single-island endemics) but not in the Seychelles. Endemism levels are thus particularly high in *Ctenitis* compared to the mean of 45% fern endemism in Madagascar. Seven *Ctenitis* species occurring in the Mascarene Islands (out of ten species then recognized; Badré, 2008) were included in a study on the assembly of the fern flora on these islands (Hennequin et al., 2014). These authors proposed insular cladogenetic speciation for the genus on the Mascarene Islands, pending further analyses using a broader taxon sampling in the region. This cladogenetic speciation included the Mauritian endemic *Heterogonium sieberianum*. The Western Indian Ocean region thus appears as a dominant center of diversity for the genus, where its complex systematics needs molecular studies in

parallel to the taxonomical treatment by Duan et al. (2017), and whose geographical origin remains to be assessed.

The objectives of this study are therefore three-fold: 1) to reconstruct the first comprehensively sampled, global phylogeny of the genus *Ctenitis*, including especially species formerly placed in the distinct *Pseudotectaria*; 2) to investigate the global biogeographical history of *Ctenitis* in the light of divergence time estimates; and 3) to identify the origin of diversity in the Indian Ocean region.

1. Material and methods

1.1. Taxon sampling

We assembled two different taxon samplings. The first one (dataset 1) was designed to assess the monophyly of *Ctenitis* in its new circumscription (Duan et al., 2017), and to conduct the dating and biogeographical analyses. This dataset is based on *rbcl* and *trnL-F* sequences. We sampled only one specimen per species, and used only specimens for which both markers were available. The sampling includes 45 *Ctenitis* species, of which *C. sieberiana* (Kaulf.) Hennequin & Rouhan which was formerly placed in *Heterogonium* and five species of the former genus *Pseudotectaria* (indicated in the following text and figures with “*Pseudotectaria*” in parenthesis). In addition, we sampled 22 other Dryopteridaceae genera, represented each by one species. Species of the Elaphoglossoideae were used to root the tree.

The second sampling (dataset 2) was designed to focus on the relationships within *Ctenitis*, with a focus on the AIO region. It includes 45 *Ctenitis* species (63 specimens), seven species of the former *Pseudotectaria*, two specimens of *C. sieberiana*, and two species from *Dryopteris* and *Polystichum* as outgroup. Within *Ctenitis*, we sampled 11 species (17 specimens) from the Asian-Pacific region, 11 (12) from the Neotropics, and 22 (35) from the African-Indian Ocean region. Referring to Duan et al. (2017) for the latter region, our sampling includes the single species reported from Africa (*C. cirrhosa* (Schumach.) Ching), 13 out of 24 species for Madagascar (previously to Duan et al., 2017, all but two species reported by Rakotondrainibe, 2003 and Roux, 2009), all 12 species from the Mascarenes, and all four species from the Comoros. We newly generated 152 sequences, and included all published *Ctenitis* sequences provided that at least two of the three markers used in this study were available. The *rbcl* and *psbA-trnH* sequences obtained by Ebihara et al. (2010) for *C. sinii* (Ching) Ohwi were concatenated with the *trnL-F* sequence obtained by Ding et al. (2014; Genbank number KJ196643). All included species are listed in Appendix 1, together with information on their distribution and Genbank accession numbers.

1.2. DNA sequencing, alignment and phylogenetic analyses

DNA was extracted from silica-dried material or herbarium material using either a modified CTAB approach (Doyle and Doyle, 1987) or the DNeasy Plant Mini kit (Qiagen, Valencia, CA, USA). DNA sequences of the chloroplast genome were amplified for up to three regions per specimen using established primers and protocols: *rbcl* (Hennequin et al., 2010), *trnL-F* region (Taberlet et al., 1991), and *psbA-trnH* intergenic spacer (Ebihara et al., 2010). For the *trnL-F* region, we either sequenced the whole region (including *trnL* intron and *trnL-trnF* intergenic spacer) or the *trnL-trnF* intergenic spacer when the whole region could not be amplified. PCR products were cleaned using standard procedures. Sequences were generated using big dye chemistry either on an ABI capillary sequencer (Natural History Museum, London, UK) or by the Eurofins Company (France). Sequences were manually aligned using MacClade 4.03 (Maddison and Maddison, 2000). In the alignment of dataset 1, we excluded all indels in order to minimize the amount of missing data in our dating analyses. In dataset 2, indels were maintained and treated as missing data in subsequent analyses.

We first explored potential conflict among markers by comparing the trees obtained in maximum parsimony analyses of each marker, using the bootstrap 70% rule (Mason-Gamer and Kellogg, 1996). These analyses were performed with PAUP*4.0b10 (Swofford, 2002), using a heuristic approach with TBR branch-swapping, 1,000 replicates of random sequence addition, MulTrees option on, and the robustness of each branch was assessed by bootstrap analysis with 1,000 replicates (Felsenstein, 1985).

We used jModeltest 2.1.1 (Darriba et al., 2012) to identify the optimal model of sequence evolution for each marker, using the Bayesian information criterion (BIC – Schwarz, 1978). All two (dataset 1) or three (dataset 2) markers were then concatenated but each was assigned its own partition.

We carried out ML and BI analyses on the CIPRES science gateway (Miller et al., 2010). For ML, we used the RAxML-HPC BlackBox with the GTRGAMMA+I model of nucleotide substitution for each partition and performed 1,000 rapid bootstrap (BS) replicates. For BI analyses, we used MrBayes 3.2.6 (Ronquist et al., 2012) in two independent but parallel analyses using flat priors and four chains of 5 million generations each, sampling trees every 1,000 generations. We used Tracer 1.5 (Rambaut and Drummond, 2013) to explore the output parameter estimates through time and to recognize the point of convergence to the stationary distribution. This was reached after approximately 500,000 generations, and the first 1,000,000 generations (1,000 trees) of one of the runs were very conservatively excluded as the “burn-in”. We pooled the post burn-in trees (4,000 total trees) and computed a majority consensus with average branch lengths and posterior probability estimated for all nodes.

1.3. Divergence time estimations

Divergence times were estimated using dataset 1 with BEAST 1.8.2 (Drummond et al., 2012) and the CIPRES science gateway (Miller et al., 2010). In the absence of reliable fossil data for the genus *Ctenitis*, we relied on divergence time estimations from previous studies on the diversification of leptosporangiate ferns (Schuettpelez and Pryer, 2009). We used one calibration for the most recent common ancestor of the Dryopteridaceae. The age of the node was set to 81.8 (SD = 5) and assigned a normal distribution. The data was partitioned with each marker assigned a GTR + I + G model of substitution. We used an uncorrelated log-normal clock, a Yule tree prior, and a randomly generated starting tree. Runs were conducted for 100 million generations with parameters sampled every 1000 steps. The results of the BEAST runs were analysed using Tracer 1.5 (Rambaut and Drummond, 2009). Trees were summarized and annotated with median ages estimates and 95% highest posterior density (HPD) intervals using TreeANNOTATOR in BEAST, with burn-in set to 10 million generations.

1.4. Ancestral areas reconstruction (AAR)

We defined six geographical areas encompassing the distribution of the genus: Asia-Pacific; tropical America; Africa; Madagascar; Mascarenes; Comoros. We performed biogeographical analyses using the statistical dispersal–vicariance analysis (S-DIVA; Yu et al., 2010) and the maximum likelihood dispersal–extinction–cladogenesis (DEC) method (Ree et al., 2005; Ree and Smith, 2008), both implemented in the software RASP (Reconstruct Ancestral State in Phylogenies; Yu et al., 2010, 2015). We used time-calibrated trees resulting from the BEAST analyses. To account for both phylogenetic uncertainty and AAR ambiguity (Nylander et al., 2008), we conducted S-DIVA analyses on 1000 randomly sampled trees resulting from the BEAST analyses, with maximum areas set as four. Biogeographical results were summarized on the majority-rule consensus of the BEAST analysis (chronogram). DEC analysis was conducted on the same tree, with an unconstrained model where all areas are treated as equiprobable ancestral range, and maximum areas set as four, then as three to reduce the number of reconstructed ancestral areas. For these analyses, we did not take into account the distribution of the non-*Ctenitis* genera analyzed as outgroup, because most of those can occur in virtually all areas here coded.

2. Results

2.1. Phylogenetic relationships

The final length of the alignment of dataset 1 was 2275 sites, composed of 1227 bp *rbcl* and 1068 bp *trnL-F*, of which 220 sites corresponding to indels were excluded (one site in *rbcl*, 219 in *trnL-F*). Phylogenetic hypotheses with highly similar topologies were reconstructed using ML and BI.

We here show only the ML tree (Fig. 1), but with both BS support and posterior probabilities (PP) from the BI analysis. All sampled species of *Ctenitis*, including *C. sieberiana* and those previously placed in *Pseudotectaria*, are retrieved as a clade (BS = 100; PP = 1), which is sister to all other five Dryopteridoideae but with no support. Within *Ctenitis*, the topology is well supported with an initial divergence between a clade comprising species occurring in the Asian-Pacific region (BS = 90; PP = 1) and a clade including species occurring outside of this region (BS = 100; PP = 1). Within this latter clade, the neotropical species form a paraphyletic grade, whereas species from Africa and the Indian Ocean region are nested in a monophylum (BS = 94; PP = 1). Species from the Mascarenes form a clade (BS = 97; PP = 0.74) which is in turn sister to a clade comprising species from Madagascar, the Comoros, and Africa (BS = 65; PP = 0.98). The latter includes species previously treated as *Pseudotectaria*, of which three form a clade (BS = 98; PP = 1). *Ctenitis sieberiana* was found to be the putative sister to the remaining Mascarene island species (BS = 75; PP = 1). Species from Reunion and Mauritius did not form distinct clades.

The final length of the alignment of dataset 2 was 2687 sites, composed of 1226 bp *rbcl*, 919 bp *trnL-F*, and 542 bp *psbA-trnH*. The ML and BI trees inferred with this dataset show a similar topology (Fig. 2) but allow further insights into species relationships. Within the Asian-Pacific clade, two main clades are retrieved: one composed of the specimens of *C. sinii*, *C. aciculata*, and *C. pallens* (BS = 100; PP = 1), and one composed of other Asiatic species sampled (*C. eatonii* - *C. yunnanensis*; BS = 98; PP = 1). *Ctenitis sinii* and *C. eatonii* are each retrieved as monophyletic. *C. rhodolepis* is nested with *C. subglandulosa* specimens. In the neotropical grade of *Ctenitis*, three clades diverge subsequently: one consisting of *C. ampla*, *C. equestris* and *C. excelsa* (BS = 99; PP = 1), a second composed of *C. melanosticta* and *C. lanceolata* (BS = 77; PP = 1), and a third composed of *C. submarginalis*, *C. glandulosa*, *C. eriocaulis*, *C. sp.*, and *C. refulgens* (BS = 80; PP = 0.85), which is sister to the AIO clade (BS = 91; PP = 1). *Ctenitis hemsleyana* is in trichotomy including the latter two clades.

The AIO clade (BS = 92; PP = 1) is here again divided in two subclades corresponding to the Mascarene species (BS = 85; PP = 0.94), and species from Madagascar, the Comoros, and Africa (BS = 59). Relationships within the Mascarene clade are not well resolved except for the basal position of *C. sieberiana* (BS = 68; PP = 0.77) and close relationships between: *C. reunionensis* and *C. humida* (BS = 88; PP = 0.99), *C. cycloclamys* and *C. borbonica* (BS = 63; PP = 0.63), and *C. lorencei* and *C. maritima* (BS = 68; not retrieved in BI). Within the Afro-Madagascan-Comorian clade (BS = 59; PP = 0.67), the initial divergence segregates a clade composed of *C. bullescens*, *C. dentata* and *C. exaggerata* (BS = 65; PP = 0.96). A second clade is composed of most species of former *Pseudotectaria* including *C. blepharochlamys* (not supported with this sampling). The last clade shows

C. cirrhosa from Africa in a basal position, then resolves into two subclades composed of 1) species occurring in the Comoros including *C. (Pseudotectaria) biformis* (Madagascar and the Comoros), and 2) Madagascan species *C. subcrenulata*, *C. tardieu-blotae*, *C. brevipetiolulata*, *C. cerea*, and *C. (Pseudotectaria) warburii*. Bayesian results differ by the position of *C. cirrhosa* among the Comorian species. These two clades are not supported using this dataset, but *C. subcrenulata* and *C. brevipetiolulata* showed BS = 98 and PP = 1 with dataset 1.

2.2. Divergence times analyses

BEAST analyses estimated a putative divergence of *Ctenitis* from other Dryopteridaceae around 60.19 Ma (95% HPD: 73.70 - 47.56 Ma), and the divergence of the *Ctenitis* crown group around 25.96 [34.74 - 18.90] Ma (Fig. 3). The Asian-Pacific clade and the neotropical + Afro-Indian Ocean clade were estimated to originate at 19.76 [26.94 - 13.47] and 15.62 [21.36 - 10.80] Ma, respectively. The stem age of the Afro-Indian Ocean clade was estimated at 8.48 [11.63 - 5.98] Ma, followed by divergence between the Mascarene clade and the Afro-Madagascan-Comorian clade at 6.06 [8.38 - 4.10] Ma. The diversification of the Afro-Madagascan-Comorian clade is estimated at 4.94 [6.94 - 3.28] Ma and that of the Mascarene clade at 3.33 [5.34 - 1.71] Ma.

2.3. Ancestral area reconstruction (AAR)

Both S-DIVA and DEC reconstructed for *Ctenitis* an ancestral area spanning the Neotropics and Asia-Pacific, and a subsequent split between the two areas, leading to the Asian-Pacific clade and an ancestrally tropical American clade. DEC reconstructions differ by the inclusion of either Madagascar and/or the Mascarenes as potential ancestral areas at several nodes after the divergence between Asia-Pacific and tropical America. From the latter area, a range extension to either Madagascar or the Mascarenes, or both, is inferred. An ancestral distribution spanning Madagascar and the Mascarenes is proposed for the most recent common ancestor of the Afro-Indian Ocean clade, followed by a split between the two areas. A dispersal to either Africa and/or the Comoros is then inferred, leading to *C. cirrhosa* in Africa and *C. comorensis* in the Comoros. However with ML analyses of dataset 2, *C. cirrhosa* is retrieved as a more basal position, sister to a clade composed of species from both the Comoros and Madagascar. This topology results in an alternative biogeographical scenario for the origin of the Comorian species, which would have originated by dispersal from Madagascar, and not involving mainland Africa (results not shown).

3. Discussion

3.1 Taxonomical implications

Our study confirms the monophyly of *Ctenitis*, with two important results: 1) the species previously attributed to the genus *Pseudotectaria* are not only closely related to *Ctenitis* (as in Wang et al., 2014), but actually included in the genus, and 2) one species formerly placed in *Heterogonium*, *C. sieberiana*, is confirmed as part of *Ctenitis* in agreement with Hennequin et al. (2014). These results are further discussed hereafter. The question of the accurate placement of *Ctenitis* in the Dryopteridaceae was not a goal of the present study, but our results are in line with previous studies (Ding et al., 2014; Liu et al., 2016a; Schuettpelz and Pryer, 2007; Wang et al., 2014), i.e., that *Ctenitis* is sister to a clade including all other genera of the subfamily Dryopteridoideae as defined in Liu et al. (2016a). Given the current topology, we suggest to place *Ctenitis* tentatively into this subfamily until more comprehensive studies are carried out.

The monophyly of the *Ctenitis* species in the Asian-Pacific region is well supported in our analyses, using a sampling that comprises about one third of the estimated species diversity in the region. However, we are aware that the total number of species in the region is likely to rise in the future as new revisions are conducted. Therefore, our results on the Asian-Pacific species are only preliminary. The more recent survey covering the region was produced by Holttum in 1985. He proposed two informal groups for *Ctenitis* species in the region, based on the study of scales of secondary rachis: the group of “*C. subglandulosa*” (including in our sampling *C. lepidogera* (Bak.in Hook. & Baker) Tagawa) and the group of “*C. eatonii*” (including *C. vilis* (Kunze) Ching and *C. iriomotensis* (H.Ito) Nakaike but also *C. aciculata* (Baker) Ching and *C. pallens* (Brack.) Price). These groups, with the species circumscription proposed then, are well retrieved in our molecular study although they will need to be confirmed using an extended sampling, except for the position of *C. aciculata* and *C. pallens*. Our study shows that *C. aciculata* and *C. pallens* are instead closely related to *C. sinii*, early diverging among Asian-Pacific species. Holttum (1984) placed *C. sinii* in its own genus, *Ataxipteris* Holttum, because of frond shape and venation similar to that of *Tectaria* Cav. species, but scales similar to those seen in *Ctenitis*. *Ataxipteris* was later merged into *Ctenitis* by Kramer (1990). The group of species allied to *C. sinii* likely includes at least a further species, *C. dianguiensis* (W.M.Chu & H.G.Zhou) S.Y.Dong, which was previously attributed to *Ataxipteris* but recently combined into *Ctenitis* (Dong, 2008).

About 40 species of *Ctenitis* were reported in the Neotropics by Tryon and Stolze (1991), a figure recently revised upward to 50 (Viveros and Salino, 2015). Christensen (1920) proposed five groups for the neotropical species, revised to four groups by Tryon and Tryon (1982). Two of

Christensen's groups were since assigned to *Triplophyllum* and *Megalastrum* (Holttum, 1986a, 1986b). The three remaining groups, "*C. ampla*", "*C. hirta*", and "*C. submarginalis*", were reviewed by Stolze (1990) whose study focused on relevant morphological characters. Stolze (1990) concluded on the importance of scales, in accordance with taxonomic treatments by Christensen (1920, 1938) and Holttum (1985). Our sampling for neotropical species is limited; however the species we sampled in the "*C. submarginalis*" group are indeed retrieved in the same clade (*C. submarginalis* (Langsd. & Fisch.) Ching, *C. eriocaulis* (Fée) Alston - considered as *Dryopteris cirrhosa* by Christensen, 1920 -, and *C. refulgens* (Kl. ex Mett.) Vareschi). These species are pinnate-pinnatisect. In the "*C. hirta*" group, we sampled *C. melanostica* (Kunze) Copel. and *C. lanceolata* (Kunze) Copel., which are retrieved as sisters, and *C. hemsleyana* (Baker) Copel. which is retrieved in an unresolved position. In the "*C. ampla*" group, the two species sampled (*C. excelsa* (Desv.) Proctor and *C. equestris* (Kunze) Ching) also form a clade together with *C. ampla* (Humb. & Bonpl. ex Willd.) Ching which was not addressed by Christensen (1920). The groups proposed by Christensen (1920) are therefore rather well supported by our phylogenetic study, although an extended sampling is required to confirm these findings. Globally, there seems to be a pattern in which the first two diverging neotropical clades have bipinnate-pinnatifid to more divided laminae, while the last diverging clade and *C. hemsleyana* are characterized by pinnate-pinnatifid laminae. In addition, species from South America seem to group together and to be in a more derived position than species of Central America and West Indies (*C. submarginalis* from Costa Rica belong here, but is the most widely distributed species from South to Central and North Americas). However, our sampling is currently too reduced to allow any further conclusion regarding the systematics of the neotropical species. Interestingly, Christensen (1913) considered *C. eriocaulis* a variety of the African *Dryopteris* [*Ctenitis*] *cirrhosa*, and treated it as *Dryopteris cirrhosa* var. *eriocaulis* (Fée) C.Chr. The morphological similarities between the two taxa were then considered evidence of neotropical-African disjunctions in pteridophytes (Moran and Smith, 2001). Based on our results, we cannot uphold the affinity between these two species.

Regarding the African-Indian Ocean region, a main result of the study is the position of the species of the former genus *Pseudotectaria*, which all fall into the Afro-Indian Ocean clade of *Ctenitis*, but are not always closely related. The combination of these species into *Ctenitis* was proposed by Duan et al. (2017). Holttum and Lin (1990) proposed two sections within the genus *Pseudotectaria*: sect. *Pseudotectaria* Tardieu with two species, *P. crinigera* (C.Chr.) Tardieu and *P. decaryana* (C.Chr.) Tardieu, and sect. *Isopinnae* Holttum with four species, *P. arthrothrix* (Hook.) Holttum, *P. biformis* (Mett.) Holttum, *P. fibrillosa* (Baker) Holttum, and *P. warburii* (C.Chr.) Holttum. The two sections were distinguished based on the shape of the basal pinnae, being longer than the suprabasal ones and more developed on the basiscopic side in sect. *Pseudotectaria*, while identical to the supra-basal

ones in sect. *Isopinnatae*. Rakotondrainibe (2010) later described two additional species for sect. *Pseudotectaria*: *P. analamazaotrensis* Rakotondr. and *P. jouyana* Rakotondr. She further recognized two additional characters to distinguish between the two sections, namely the absolute length of the median sterile pinnae (fertile and sterile fronds being more or less dimorph), and a different number of vascular bundles at the base of the petioles: three in sect. *Pseudotectaria* and four in sect. *Isopinnatae*. Rakotondrainibe (2010) also noted that sect. *Isopinnatae* was more heterogeneous than sect. *Pseudotectaria*, and probably closer to *Ctenitis* than *Tectaria*. Our results clearly support this latter view, with the three species of sect. *Isopinnatae* sampled here retrieved in different positions. Species of sect. *Pseudotectaria* do form a clade, but this includes *C. (Pseudotectaria) arthrotrix* (Hook.) Tardieu from sect. *Isopinnatae* and *C. blepharochlamys* (C.Chr.) Tardieu, never treated as *Pseudotectaria*. We conducted investigations on the number of vascular bundles at the base of the petioles for several species of *Ctenitis* and *Pseudotectaria*. We usually observed four or five vascular bundles, more rarely three (in a few Mascarene species in addition to sect. *Pseudotectaria*) or six, but this character does not seem to be pertinent to support the main clades retrieved here for *Ctenitis*.

Another interesting result is the inclusion, in *Ctenitis*, of *C. sieberiana*, previously placed in the genus *Heterogonium* but combined into *Ctenitis* by Duan et al. (2017). This species is endemic to Mauritius, while *Heterogonium* is otherwise distributed in South-Eastern Asia and Malesia. *Heterogonium* includes 14 species with either free or anastomosing veins, and *C. sieberiana* displays free veins. To Holttum (1983), *C. sieberiana* “differs from the free-veined species of Malesia in its broader pinnae which are thick and opaque”, a feature also found in some Mascarene species of *Ctenitis* such as *C. maritima*. Based on this feature and its isolated area of distribution, we assume that *C. sieberiana* is the only species of the former *Heterogonium* that required combination into *Ctenitis*, while the remainder of *Heterogonium* is to be merged in *Tectaria* (Ding et al., 2014). Interestingly, *C. sieberiana* is the first species diverging at the base of the Mascarene clade (Figs. 1–3).

Finally, our results confirm the suspected polyphyly of two species as defined by Holttum (1983), Badré (2008) and Roux (2009): *C. crinita* (Poir.) Ching, a species formerly considered as distributed in Mauritius and Madagascar, and *C. cirrhosa* which was formerly described as distributed in the whole Indian Ocean region and Africa. In their revision, Duan et al. (2017) proposed to distinguish the Madagascar specimens formerly identified as *C. crinita* as either *C. dentata* Li Bing Zhang & Yi F. Duan or *C. tardieu-blotae* Li Bing Zhang & Yi F. Duan. Regarding the Mauritian species, three varieties were described by Holttum (1983), mainly based on scales differences. The variety *setacea*, characterized by long, conspicuous spreading bristle-like scales on rachises, is clearly distinct molecularly from the other two varieties *atrata* and *crinita*, whereas no molecular difference was

observed between the DNA sequences of the varieties *crinita* and *atrata* for each three markers. *Ctenitis crinita* var. *setacea* was thus treated as a separate species (*C. hispida* (Kuhn) Li Bing Zhang & Yi F. Duan, while the other two varieties were treated as a single species *C. crinita* by Duan et al. (2017). As to *C. cirrhosa*, the polyphyly of the specimens sampled from different areas (Africa, Madagascar, Comoros, Mascarenes) support their attribution to separate species by Duan et al. (2017). The type of *C. cirrhosa* being from Ghana, this name was restricted to specimens from mainland Africa. Recent studies on African ferns showed evidence for a west-central African fern flora distinct from the east African flora (Liu et al., 2016b). Further studies should thus investigate if only one, or more species of *Ctenitis*, are present on the continent. In agreement with our study, the other specimens formerly attributed to *C. cirrhosa* are described by Duan et al. (2017) as: *Ctenitis cerea* Li Bing Zhang & Yi F. Duan and *C. pulvinata* (C.Chr.) Ching (not present in our sampling) for Madagascar, *C. mayottensis* Li Bing Zhang & Yi F. Duan for Mayotte, *C. comorensis* Li Bing Zhang & Yi F. Duan for the Comoros, and *C. reunionensis* Hennequin, Li Bing Zhang & Yi F. Duan for Reunion.

Further studies are required to investigate the morphology in relation to the phylogeny. Clearly, the scales characters are of major importance for the systematics of the genus in this region too (Duan et al., 2017). All species of the AIO region have pinnate to pinnate-pinnatifid laminae, except for two species with bipinnate to bipinnate-pinnatisect laminae: *C. magna* (Baker) Tardieu which was not available for our study, and *C. exaggerata* (Baker) Ching which falls as sister to *C. dentata* Li Bing Zhang & Yi F. Duan.

3.2. Biogeographical history of the genus: evidence for boreotropical migrations and unique transoceanic dispersal

As most Polypodiales, the Dryopteridaceae lineage underwent an initial diversification in the Late Cretaceous resulting in the establishment of all main lineages including the *Ctenitis* lineage (see Schuettpelz and Pryer, 2009). However, the initial divergence of the *Ctenitis* crown group is estimated to have occurred much later, between the Late Eocene and the Early Miocene. Our estimate (26 Ma) predates that of 17.5 Ma previously published by Schuettpelz and Pryer (2009). This result is likely caused by the denser taxon sampling. Extant *Ctenitis* species thrive under wet tropical climates and we assume that the ancestor of the genus shared its climatic niche preferences with its extant offspring. A major caveat in studying the biogeographical history of *Ctenitis*, however, is the absence of this fern in the paleontological record. Our biogeographical and dating analyses indicate that the genus originated in Asia-Pacific and the Neotropics. In view of its age estimate, a possible interpretation is that the genus originated in the boreotropical forest belt spanning Eurasia and North America from the Paleocene to the Early Oligocene (Morley, 2003; Tiffney, 1985a, 1985b;

Wolfe, 1975). The initial divergence within *Ctenitis* then shows an Asian-neotropical disjunction which may correspond to the fragmentation of the boreotropical forest belt due to climate cooling (Zachos et al., 2001) and tectonic movement during the early Oligocene, followed by the migration of the boreotropical elements southward to tropical refuges, mainly Central America then South America along the Caribbean corridor, or the Indo-Malayan region (Morley, 2003, 2007; Wolfe, 1975). Although our results on the relationships among the American species of *Ctenitis* are only preliminary, the basal position of Central American and West Indies species compared to South American ones is consistent with this scenario. Our age estimates for the split between the Asian and neotropical lineages of *Ctenitis* are rather young to account for boreotropical migration, however the 95% HPD interval [34.74 - 18.90 Ma] does not preclude this scenario. Notably, it has been suggested that boreotropical migration of megathermal taxa through the North Atlantic bridge could have been maintained until the Oligocene (Davis et al., 2004). The boreotropical hypothesis has been invoked to explain the distribution of many angiosperms taxa, such as Lauraceae (Chanderbali et al., 2001), Melastomataceae (Renner et al., 2001), Malpighiaceae (Davis et al., 2004), Chloranthaceae (Antonelli and Sanmartín, 2011), Rubiaceae (Smedmark et al., 2014), Symplocaceae (Fritsch et al., 2015). Conversely this hypothesis has been also considered in studies on derived fern lineages such as *Asplenium* (Schneider et al., 2004), *Nephrolepis* (Hennequin et al., 2010), *Diplazium* (Wei et al., 2015) and in two other Dryopteridaceae genera, *Polystichum* and *Phanerophlebia* (Le Péchon et al., 2016) in accordance with previous studies on *Polystichum* (Driscoll and Barrington, 2007; McHenry and Barrington, 2014). Additional support to this hypothesis may be provided by the well-recorded examples of eastern Asian - North American biogeographic disjunctions in ferns and lycophytes (Xiang et al., 2015). Most recently, Liu et al. (2016a) also reported a disjunction involving the eastern-Asian genus *Trichoneuron* and the Caribbean-Mesoamerican genus *Polystichopsis*. Therefore, boreotropical migrations may have played a more important role in the biogeography of many fern groups than previously considered (especially in scenarios assuming the Gondwana breakup as the core process in shaping the distribution of tropical plant diversity).

Two alternative hypotheses to the boreotropical one still need to be considered to explain the recovered biogeographic scenario. First, the distribution of the clades would also fit with the predictions of vicariance events consistent with the breakup of the Southern Gondwana area in the Late Cretaceous (> 80 Ma; McLoughlin, 2001). However, this scenario predicts substantially older divergence time estimates for the crown group of *Ctenitis* as those recovered in our analyses. In addition, *Ctenitis* is absent from southernmost areas. The other alternative, a pattern created mainly by frequent long distance dispersal (LDD) is arguably still valid. Dispersal is the general null hypothesis in biogeographic studies designed to test vicariance hypothesis. Thus, many statistical

tests have limited power to refute dispersal as the key process. This is especially difficult in ferns because of their general trend to have high dispersal capacities as a result of the dispersal of small meiospores (Wolf et al., 2001). These arguments are especially important in taxa containing species having wide distribution ranges such as some species of *Nephrolepis* (Hennequin et al., 2010). However, the majority of fern species show rather limited and well defined ranges because the long range dispersal capacity is not only a function of the dispersal capacity of meiospores but also shaped by ecological factors and the reproductive biology of fern species. The latter aspect has not been explored in ferns but some evidence was recently reported on European ferns (Bystriakova et al., 2014; de Groot et al., 2012). Given the absence of any species of *Ctenitis* with a multi-continental distribution range, and the phylogenetic pattern inferred, long-distance dispersal likely has a very limited contribution to the biogeographical history of this fern genus.

With the sampling used in this study, no event of long-distance dispersal are inferred outside the Asian-Pacific and the neotropical regions, apart from one transoceanic dispersal from the Neotropics to an area spanning Africa and the Indian Ocean region, dated from middle to late Miocene. By that time the continents had nearly attained their present positions, meaning dispersal over at least 3000 km of ocean. Our AAR for this colonist infers an establishment in an area spanning Madagascar or the Mascarenes or both – instead of a colonization of Africa. However, continental Africa and especially west Africa would have been a much closer area for initial colonization, and with an adequate lowland forest ecosystem (Linder, 2014). Our taxon sampling lacks representatives from the west-central African fern flora described by Liu et al. (2016b). Their inclusion in future studies may alter the AAR for the AIO clade if the species is retrieved as non-monophyletic. Furthermore, the initial African colonizer could have been affected by the major extinctions that took place during the Tertiary and Quaternary (Kornas, 1993; Linder, 2014 and references therein), thus resulting in the pattern currently observed. The alternative hypothesis involving LDD from the Neotropics directly to Madagascar is plausible, although less probable since it involves a higher distance from the source: the island's wet tropical forests were well established by the Miocene (Wells, 2003), providing suitable habitats for the colonization of *Ctenitis*. On the other hand, LDD directly to the Mascarenes seems quite improbable considering the distance between the areas and the reduced area of the islands involved, whatever island of the archipelago was emerged at that time. Long-distance dispersal from the Neotropics to AIO appears to be rather frequent in ferns; it is suspected in Hymenophyllaceae (Bauret et al., 2015; pers. data) and was inferred – but with no age estimate of the event - in *Elaphoglossum* (Rouhan et al., 2004; Vasco et al., 2009), *Lomariopsis* (Rouhan et al., 2007), and some Polypodiaceae (Janssen, 2007; Labiak et al., 2010; Rouhan et al., 2012). It was also inferred in *Parapolystichum* during the Eocene to Oligocene (Labiak et al. 2014), in

the tree fern *Alsophila* likely in the Miocene (Janssen et al., 2008), and at least 12 times within the last 20 Ma in the grammitid ferns (Bauret et al., 2017; Sundue et al., 2014). These more recent ages coincide well with the age estimated in this study, suggestive of a time window for LDD in this direction. Such LDD with similar dates were also observed in angiosperms (for example Melastomataceae; Renner et al., 2001). Long-distance dispersal in the reverse direction (AIO to Neotropics) is also possible, as shown in *Nephrolepis* (three species involved, Hennequin et al., 2010). Finally, further studies will need to address the biogeographical scenarios of expansion within both the Asian-Pacific and the neotropical regions, using adequate sampling. It is noteworthy that the genus is absent from Australia, New Zealand and New Caledonia. Actually, only two species (*C. aciculata* and *C. subglandulosa*) appear to be distributed on both sides of Wallacea *sensu* van Welzen et al. (2011), and less than ten species could be distributed east of Wallacea (including the Pacific islands), while the genus is well diversified both in Wallacea and west of the area. Crayn et al. (2015) have shown that the major exchanges between east and west of Wallacea took place after 12 Ma. How the region acted as a filter for *Ctenitis* is an interesting question to explore. On the other hand, the genus managed to reach isolated islands such as the Society Islands, Tahiti, the Austral Islands or Hawaii, involving several LDD events. Follow-up studies should seek to include these island species and explore their geographic origin.

The biogeographical scenario inferred in *Ctenitis* is therefore particularly remarkable in the rarity of dispersal events it would involve. As far as we are aware, no other pantropical fern genus whose biogeographical history has been investigated so far shows such a clear pattern, with clades distributed in an area and not dispersing at all, or very scarcely, outside of this area. Variations in the capacities of establishment after LDD are likely to be due notably to gametophytic life form (notably, terrestrial versus epiphytic; Dassler and Farrar, 2001) and reproductive strategies (i.e., outcrossing versus selfing; de Groot et al., 2012; Peck et al., 1990; Ranker and Geiger, 2008). Both polyploidy and apomixis are also known to contribute to a raise in the dispersal capacity of species by overcoming some of the genetic barriers constraining the dispersal biology of ferns. *Ctenitis*, as a terrestrial genus, is already likely to be on the side of the poor fern dispersers. Reproductive strategies in the genus are unknown and no evidence for apomixis has been reported until now for this genus (Liu et al., 2012). Considering polyploidy, only a few species have been investigated for chromosome numbers (Rice et al., 2015; Robert, 2014); all showed $n = 41$ except three species with $n = 82$: *C. eatonii* (Tsai and Shieh, 1985), *C. sloanei* (Poepp. ex Spreng) C.V.Morton (a synonym to *C. amplax*; Walker, 1985), and *C. grisebachii* (Baker) Ching (Rice et al., 2015). We may therefore assume that the low dispersal capacities of *Ctenitis* are due to a combination of its terrestrial growth form, the probable rarity of polyploidy and a predominantly outcrossing mating system, which however

requires investigation. In turn, *Ctenitis* appears to be a well suited model system to investigate the impact of preferably outbreeding strategies on the diversification of ferns colonizing oceanic islands.

3.3. Biogeography and diversification in the African-Indian Ocean region

Pending further analyses using an exhaustive taxon sampling, the entire AIO diversity of *Ctenitis* appears to be of neotropical origin. Such an origin was found for many other ferns as described here-above, but contrasts with an origin from Asia-Pacific for several other groups, e.g., *Nephrolepis* (Hennequin et al., 2010), *Deparia* (Kuo et al., 2016), *Pteris* (Chao et al., 2014) and *Diplazium* (Wei et al., 2015) but with incomplete taxon sampling from the AIO region, and *Dryopteris* (Sessa et al., 2017). Interestingly, arrival and potential diversification of these genera in AIO were here again dated largely within the last 20 Myr. Agnarsson and Kuntner (2012) proposed that for the Indian Ocean region, Africa would have been the predominant source of colonizers for poor dispersers and Asia for the good dispersers. A striking pattern in the distribution of *Ctenitis* in the region is the general absence of inter-island dispersal and speciation, providing further evidence for the low dispersability of the genus. A similar pattern was observed in the fern genus *Alsophila* (Janssen et al., 2008) and the angiosperm genera *Dombeya* (Le Pechon et al., 2010) and *Psiadia* (Strijk et al., 2012).

The most likely oldest age inferred for the colonization of Madagascar falls in the Miocene (8.48 Ma [11.63 - 5.98 Ma]), thus after the onset of the formation of the Madagascar rain forest biome (Wells, 2003). This age is congruent with those estimated for the colonization of Madagascar by other plant lineages, for example the tree fern family Cyatheaceae (Janssen et al., 2008) and the angiosperms genera *Psiadia* (Strijk et al., 2012), *Oncostemum* (Strijk et al., 2013), and *Canarium* (Federman et al., 2015). Interestingly, the diversification of the three Madagascar *Ctenitis* clades is quite recent, starting only in the end of the Pliocene (3.09 Ma [4.97-1.47 Ma] for the *C. bullescens-C. exaggerata* clade). Such a time gap was also observed in tree ferns (Janssen et al., 2008; Schneider et al., 2010) and *Psiadia* (Strijk et al., 2012). These authors proposed a link between the recent speciation bursts observed in these lineages and late Tertiary climatic fluctuations. This could be further explored in follow-up studies of *Ctenitis*.

The three species endemic to the Comoros archipelago (*C. spekei*, *C. comorensis* and *C. mayottensis*) are retrieved as a putative monophylum including *C. (Pseudotectaria) biformis* which is also present in Madagascar (Fig. 2). The appearance of the Comoros in our AAR (most likely, 3.62 Ma [6.03-2.70 Ma]) is consistent with the estimated age of the Comoros archipelago (7.7 - 15 Ma for the oldest island, Mayotte; Emerick and Duncan, 1982; Nougier et al., 1986). However, the topology is

not supported enough to unambiguously infer the directions of biogeographical connections between Africa, Madagascar and the Comoros.

Finally, we confirm the monophyly of the 12 species endemic to the Mascarenes, using a sampling covering 28 out of the 38 species now recognized for the AIO region. The evidence for cladogenesis in the Mascarenes, as already suggested by Hennequin et al. (2014) is therefore supported with even higher confidence, although an exhaustive sampling is required to rule out any alternative hypothesis. Consequently, *Ctenitis* is considered as the fern genus whose cladogenetic diversification (= radiation) is the most important in the Mascarene Islands. Other fern candidates for radiation in the Mascarene islands include the thelypteroid genus *Amauropelta* (Almeida et al., 2016; Hennequin et al., 2014), scaly tree ferns of the genus *Alsophila* (Janssen et al., 2008), the saxicolous to epiphytic genus *Elaphoglossum* (Hennequin et al., 2014; Rouhan et al., 2004), one or two clades of the spleenwort genus *Asplenium* (Hennequin et al., 2014). Even more stunning is the fact that, apart *C. maritima* (Cordem.) Tardieu, all *Ctenitis* species are single-island endemics. Our dataset does not allow us to accurately infer the tempo of colonization and diversification in the three main Mascarene Islands, Reunion, Mauritius and Rodrigues. Nonetheless, the basal position of two species from Mauritius (*C. sieberiana* and *C. bivestita* (Mett.) Tardieu) indicates that, of all islands existing presently, it may have been colonized first, around 3.33 Ma [5.34-1.70 Ma] according to our analyses. This is consistent with the fact that Mauritius is older than Reunion (8 and 2 Ma, respectively; Duncan, 2009; Duncan and Hargraves, 1990; McDougall and Chamalaun, 1969) and, compared to the older Rodrigues (7-11.5 Ma; Duncan, 2009; McDougall et al., 1965), closest to Africa and Madagascar. Interestingly, the Rodrigues endemic, *C. lorencei* Holttum, is not retrieved as basal in the Mascarene clade. The high level of speciation in the Mascarenes is consistent with the assumed low dispersal capacity of these species, which would restrict their distribution range. While *C. cyclochlams* and *C. reunionensis* are rather well distributed on Reunion with broad altitudinal preferences, as well as *C. crinita* on Mauritius, the other species appear rather localized and/or with more restricted altitudinal preferences. Studies are underway to characterize more accurately the distribution and the ecological preferences of the Mascarene species of *Ctenitis*. Low dispersal/colonization capacities are expected to increase chances of speciation on an island (Agnarsson et al., 2014; Hennequin et al., 2014). Still, it is surprising that no further species from the surrounding species-rich areas was able to colonize again the Mascarene Islands. We could speculate that the diversity in surrounding areas is too young to have had time to colonize the Mascarenes, and that the Mascarene and the African/Madagascan/Comorian species would have diversified in parallel. Considering the diversification times inferred, this could be the case although several lineages may have already been present in Africa and Madagascar by the time of the Mascarene colonization. Alternatively, we can

speculate that early arriving species may have inhibited subsequent establishment of related species from the region, a hypothesis advanced by Fukami et al. (2007) and Hennequin et al. (2014), all the more if the initial colonist radiated into several species occupying a variety of niches (i.e., niche preemption; Silvertown, 2004; Silvertown et al., 2005). However, we need to study the population structure and reproductive biology of several species to confirm that some or all species of *Ctenitis* have a reduced ability to expand their ranges as pioneers.

4. Conclusions

Our study provides the first phylogeny of the genus *Ctenitis*, confirming the inclusion in this genus of the species formerly assigned to *Pseudotectaria* and of *C. sieberiana*. It furthermore reveals a surprisingly clear biogeographical pattern suggestive of boreotropical origin and migrations, followed by a unique LDD over the Atlantic Ocean. Follow-up studies should aim to expand the taxon sampling in order to confirm this scenario and to study more precisely the diversification of the genus in Asia-Pacific, in the Neotropics, as well as in the AIO region. Both Madagascar (see Vences et al., 2009) and the Mascarenes are confirmed as important centers of diversification for *Ctenitis*, the mechanisms of which need to be investigated using additional data. The limited number of LDD events, combined with a high level of endemism, indicates low capacities of LDD and colonization for a fern lineage, more consistent to those observed for Angiosperms. The biogeographical history of many fern lineages remains to be studied. Hence, these future studies will indubitably bring some clues as to whether local speciation events (over LDD events) is not, actually, a much more frequent process in the assembly of fern diversity than what is generally expected.

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Acknowledgments

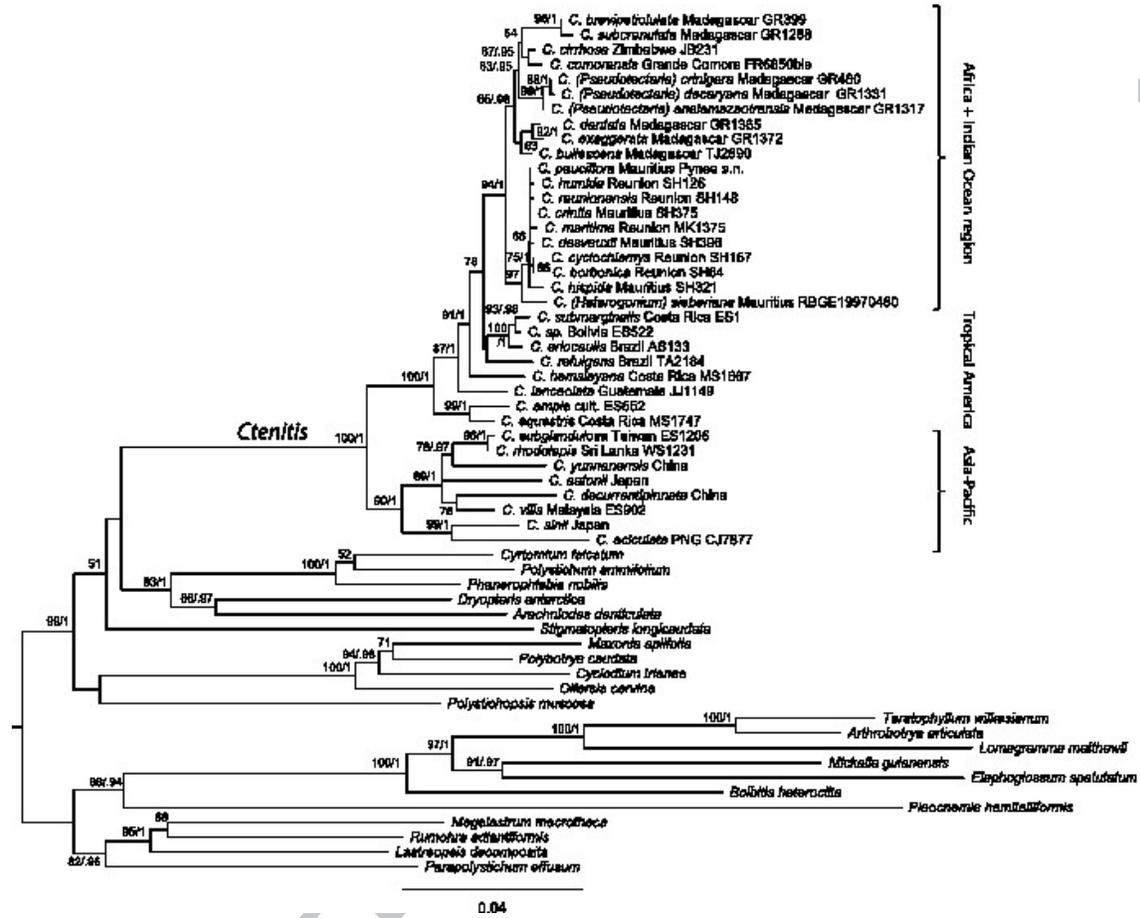
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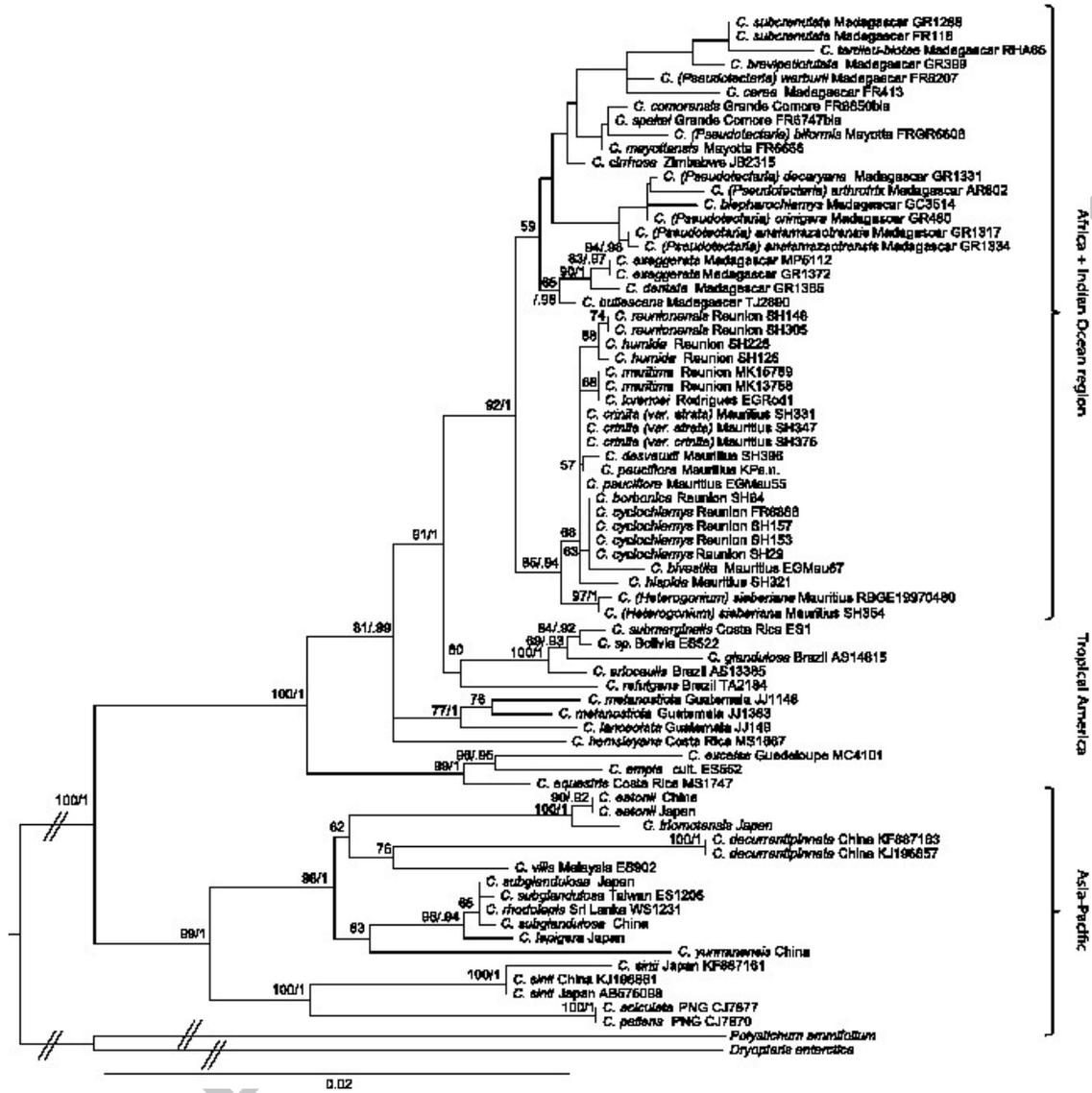
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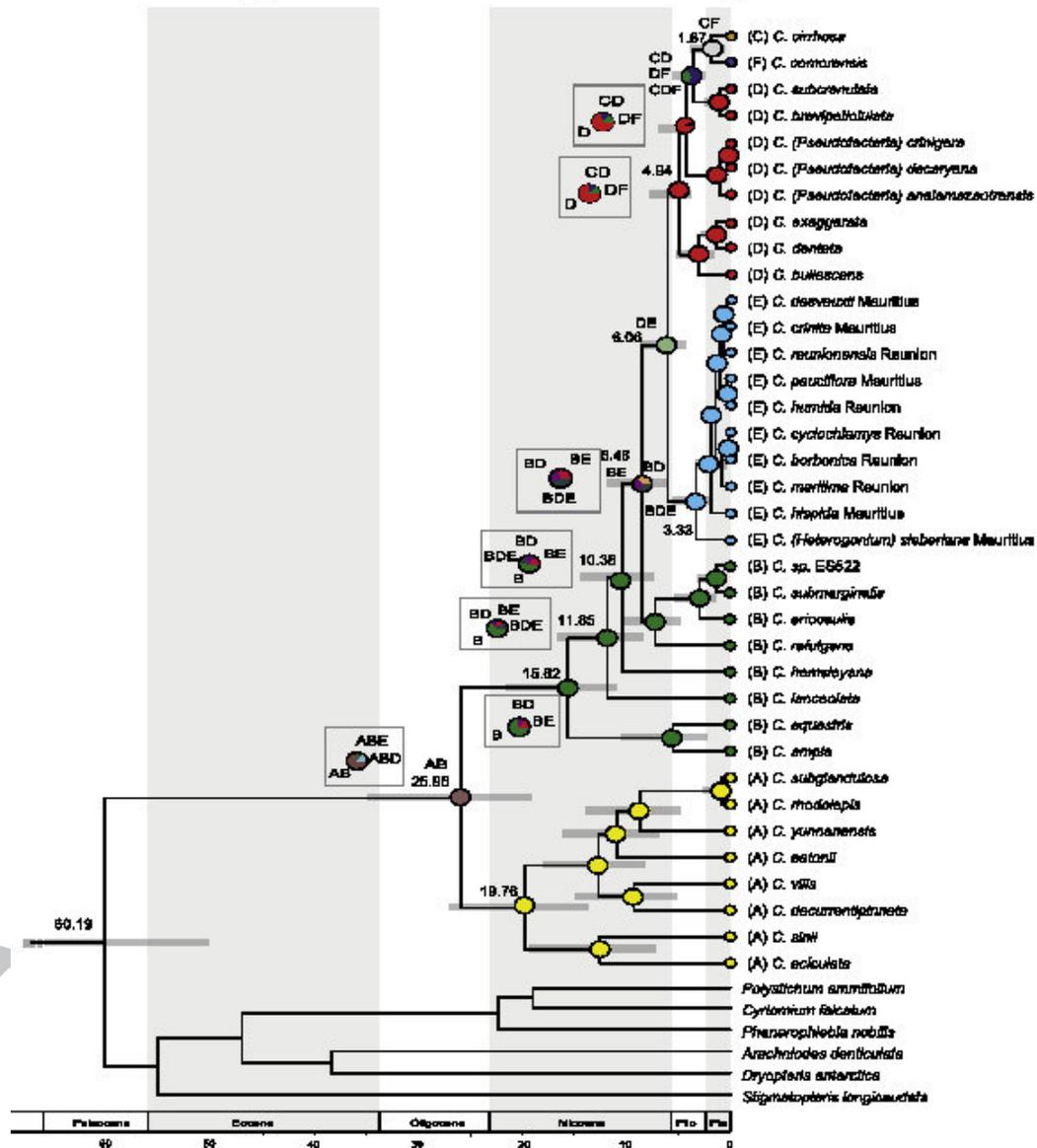
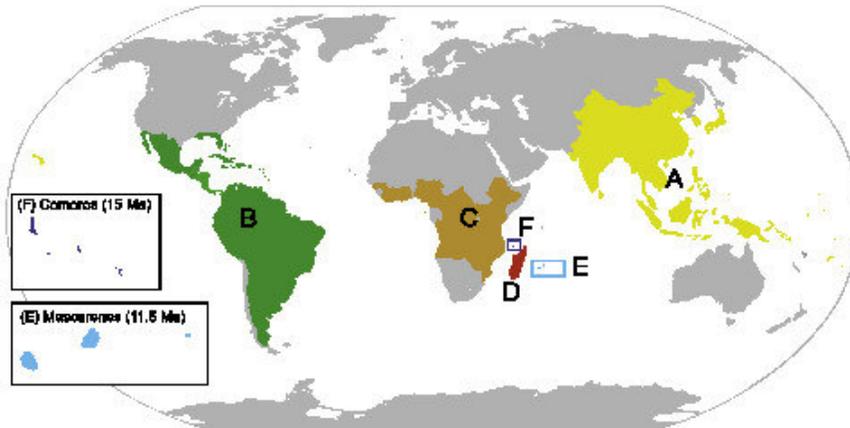
Figure 1: Maximum likelihood phylogram derived from molecular dataset 1 (*rbcl*, *trnL-F*), showing the relationships among *Ctenitis* and other Dryopteridaceae genera. Numbers correspond to bootstrap support and posterior probabilities from the BI analysis (only given if more than 50% and 0.9, respectively).

Figure 2: Maximum likelihood phylogram derived from molecular dataset 2 (*rbcl*, *trnL-F*, *psbA-trnH*) with expanded taxon sampling within *Ctenitis*. Numbers correspond to bootstrap support and and posterior probabilities from the BI analysis (only given if more than 50% and 0.9, respectively).

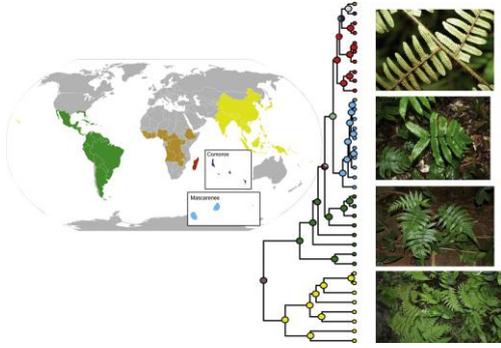
Figure 3: Biogeographical hypothesis for *Ctenitis* inferred by S-DIVA and the DEC analyses and traced on the maximum clade credibility chronogram from the BEAST analysis. Numbers at selected nodes indicate the median divergence time estimates, and grey bars show the 95% HPD intervals of each node age. Pie charts at nodes represent the most likely ancestral areas reconstructed by S-DIVA and DEC analyses. When DEC analyses produced different AARs, they are indicated in a frame next to the node.







Graphical abstract



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Highlights:

Ctenitis forms a monophylum including species formerly placed in *Pseudotectaria*.

An initial disjunction between Asian-Pacific and neotropical lineages is inferred, dated in the Oligocene - Early Miocene.

The Afro-Madagascan diversity seems to result from one transoceanic dispersal from the Neotropics.

Cladogenetic speciation in the Mascarenes is confirmed and consistent with the age of these islands.

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