JSE Journal of Systematics and Evolution

Journal of Systematics and Evolution 51 (1): 61–70 (2013) doi: 10.1111/j.1759-6831.2012.00234.x

Research Article

Exploring the origin of the latitudinal diversity gradient: Contrasting the sister fern genera *Phegopteris* and *Pseudophegopteris*

[1,2]Harald SCHNEIDER* [1,3]Li-Juan HE [2]Jeannine MARQUARDT [1,2,3]Li WANG
[4]Jochen HEINRICHS [5]Sabine HENNEQUIN [1]Xian-Chun ZHANG

[1](*State Key Laboratory of Systematic and Evolutionary Biology*, *Institute of Botany, Chinese Academy of Sciences*, Beijing 100093, China)
[2](*Department of Life Sciences*, *Natural History Museum*, London SW7 5BD, UK)
[3](*Graduate University of Chinese Academy of Sciences*, Beijing 100049, China)
[4](*Department of Systematic Botany*, *Albrecht-von-Haller Institute of Plant Sciences, Georg-August-University*, 37073 Göttingen, Germany)
[5](*UMR 7207 UPMC CNRS MNHN 'Centre de recherche sur la Paléobiodiversité et les Paléoenvironnements'*, 43 rue de Buffon, 75005 Paris, France)

Abstract The origin of the latitudinal biodiversity gradient has been studied using various approaches. Here, we employ a comparative phylogenetic approach to infer evidence for the hypothesis that differences in diversification rates are one of the main factors contributing to the assembly of this gradient. We infer the phylogeny of the two sister genera *Phegopteris* and *Pseudophegopteris*. The two genera are distinct in their species richness (4 vs. 20 spp.) and their preferences to temperate to subtropical (*Phegopteris*) or tropical climates (*Pseudophegopteris*). Using sequences of three plastid DNA regions, we confirm the monophyly of each genus and infer the inter- and intra-generic phylogenetic differentiation of the sister clades. We recover evidence for distinct net-diversification rate between the two genera, which may be caused either by a higher extinction risk of temperate *Phegopteris* or a higher speciation rate of tropical *Pseudophegopteris*. We discuss our results in the context of our current knowledge on the speciation processes of ferns. We conclude on the crucial influence of other factors such as the rise of the Himalaya on the diversification of these ferns.

Key words BiSSE, climatic preferences, diversification rates, extinction, Himalaya, macroecology, macroevolution, phylogeny, Pleistocene glaciations, speciation.

In the recent years, ecologists and evolutionary biologists have given increasing attention to historical processes that shaped the macroscale distribution of biodiversity (e.g., Wiens & Donoghue, 2004; Ricklefs, 2009; Schemske, 2009; Buckley et al., 2010). In particular, the origin of the latitudinal diversity gradient (LDG) has been subject to controversial discussion (e.g., Mittelbach et al., 2007; Schemske, 2009). Several hypotheses have been considered to explain the distinctly lower number of species found in temperate climatic zones compared with tropical regions. Mittelbach et al. (2007) categorized these concerning the origin of the LDG in three major groups: (1) time dependent historical hypotheses, (2) productivity dependent ecological hypotheses, and (3) diversification rate dependent evolutionary hypotheses.

Inferences of evolutionary hypotheses are currently underrepresented in studies exploring the origin of LDG, although they can be easily carried out via comparison of sister lineages or lineage assemblages that show evidence for the LDG (e.g., Cardillo, 1999; Davis et al., 2004; Condamine et al., 2012). Such sister-group comparisons are commonly used in metadata analyses, as they are simple to implement and powerful (Barraclough et al., 1998). Nevertheless, they show some limits in the context of the LDG that need to be taken into account (Ricklefs, 2006).

In this study, we investigate a group of derived ferns to explore evidence for a diversification rate difference as cause of LDG observed. The chosen genus pair, *Phegopteris* (C. Presl) Fée and *Pseudophegopteris* Ching, is well suited to study the origin of LDG. The two genera demonstrate distinct differences in species number, 3–4 spp. versus ca. 20 spp., and in their climatic preferences: *Phegopteris* occurs in mainly temperate to subtropical zones, whereas *Pseudophegopteris* tends to occur in the tropics of South East Asia and Afromadagascar. However, some species of *Pseudophegopteris* (e.g., *Pseudophegopteris bukoensis*) occur also in cool-temperate climatic conditions. The two genera show some overlap in their distribution range in subtropical SE Asia but in this region

Received: 12 July 2012 Accepted: 14 October 2012
* Author for correspondence. E-mail: h.schneider@nhm.ac.uk. Tel.: 44-20-79426058. Fax: 44-20-79425501.

Phegopteris inhabits usually higher altitudes than *Pseudophegopteris*. The monophyly of the *Phegopteris–Pseudophegopteris* lineage is well supported (Smith et al., 2006; Schuettpelz & Pryer, 2007) and estimates of lineage ages of derived ferns indicate an Eocene origin (Schneider et al., 2004; Schuettpelz & Pryer, 2009). Furthermore, the distribution range of the northern-temperate genus *Phegopteris* is not substantially smaller than the range of the paleotropical genus *Pseudophegopteris*.

To address the hypothesis of whether the observed difference in the species number between the two genera is the result of distinct diversification rates, we generated a dataset in which we sampled sequences of up to three plastid genome regions for all species of *Phegopteris* and for 10 species out of the ca. 20 species estimated for *Pseudophegopteris*. This taxon sampling allowed us to confirm the monophyly of each sister genus which is a critical assumption of this study. In addition, the dataset provided us with reliable estimates for the divergences of the two genera as required to calculate diversification rates. To avoid misleading results caused by erroneous species concepts, we sampled several specimens for most of the studied species. This procedure enabled us to recover DNA-based evidence for the monophyly of these species and to explore evidence for cryptic diversity.

In addition, we considered other factors that have a profound impact on the speciation processes in ferns such as establishment of diploid sister species (cladogenetsis), speciation via autoploidization, and formation of allopolyploid taxa. In this context, we were particularly interested in recovering evidence consolidating the hypothesis of Haufler et al. (2000; see also Haufler, 2002) that suggests different speciation processes in tropical and temperate fern lineages. This hypothesis assumes sympatric speciation as a common process in tropical ferns but allopatric speciation is the dominant process in temperate ferns. The later also enhanced the probability of speciation via auto- or allopolyploidization. Differences in speciation processes in temperate versus tropical lineages were recently also reported for some animal groups (Kozak & Wiens, 2007; Marin & Tewksburry, 2008; Hua & Wiens, 2010; Marin et al., 2010).

1 Material and methods

We obtained sequence data for all 4 currently accepted species of *Phegopteris* and for 10 out of presumably 20 species of *Pseudophegopteris*. For six species of *Pseudophegopteris* we included more than one specimen, whereas the rest is represented by only one accession. For *Phegopteris*, two to five specimens were sampled for each species. As outgroup taxa, we sampled *Dictyocline griffithii* and *Leptogramma tottoides* to represent another lineage of Thelypteridaceae and two species of *Macrothelypteris*, which is the sister genus to the clade composed by *Phegopteris* and *Pseudophegopteris* (Smith & Cranfill, 2002). These taxa were included to polarize the clade and establish the divergence time framework. The sampling of *Phegopteris* and *Pseudophegopteris* is taxonomically and geographically representative. The sampled species cover the whole distribution range of the two genera. In particular, the five samples of *Phegopteris connectilis* represent the distribution of this species in the northern temperate climate zone including Asia, Europe, and North America. Our samples were obtained either (1) during fieldwork carried out by one or several of the authors, (2) from silica samples shared by other botanists, or (3) from herbarium specimens deposited at BM or PE. Table 1 summarizes information on voucher specimens and GenBank Accession numbers.

Whole genomic DNA was extracted using a modified CTAB approach (Doyle & Doyle, 1987). Sequences of up to three plastid DNA regions were generated for each specimen included. All primers and protocols were obtained from previous studies. We sampled two intergenic spacer (IGS) regions *psbA-trnH*$_{GUG}$ (Tate & Simpson, 2003) and *rps4-trnS*$_{GGA}$ (Smith & Cranfill, 2002), plus the *trnL*$_{UAA}$*-trnF*$_{GAA}$ (Trewick et al., 2002) region including the *trnL* intron and *trnL-trnF* IGS. All sequences were generated using an ABI capillary sequencer at the NHM and were edited and assembled using standard software such as Sequencher (Gene Codes, Ann Arbor, MI, USA). Alignments were generated manually using MacClade 4v. 4.08 (Maddison & Maddison, 2005). Ambiguously aligned regions were detected visually and excluded from all analyses. We were not able to obtain sequence data of all three regions for all specimens. Thus, we generated two datasets. The first dataset (25 operational taxonomic units, or OTUs) included specimens with all three plastid DNA regions generated with the exception of *Pseudophegopteris cruciata* that was included despite some gaps in the dataset. This dataset was designed to limit the effect of incompletely sampled taxa without reduction of the taxonomic coverage. This dataset was used for analyses that are likely sensitive to incomplete sequence data. The second dataset (33 OTUs) included all specimens irrespective of missing data. This dataset was designed to infer the impact of a denser sampling of specimens on the monophyly of some species.

Table 1 Information on species included, with vouchers and GenBank accession numbers

Taxon	Voucher	*psbA-trnH*	*rps4* + *rps4-trnS*	*trnL-trnF*
Macrothelypteris (H. Ito) Ching				
M. ornata (Wall. ex Bedd.) Ching	Nepal: Fraser-Jenkins 205 (BM)	–	JX874892	HQ890406
M. polypodioides (Hook.) Holttum	Philippines: Price 1552 (BM)	HQ890378	JX874891	HQ890407
Phegopteris Fée				
Ph. connectilis (Michx.) Watt.	Czech Republic: Cult. Prague Bot. Gard.: Zhang 5614 (PI)	HQ890379	JX874896	HQ890408
	Denmark, Greenland, Skjolungen: Bay 92-5 (BM)	HQ890380	–	HQ890409
	Poland: Dzwouko 39 (BM)	–	–	HQ890410
	China, Taiwan: Schuettpelz 1115 (DUKE)	HQ890381	JX874895	HQ890411
	USA, Maryland: Zhang 3795 (PI)	HQ890382	JX874894x	HQ890412
Ph. decursivepinnata Fée	China, Chongqing: Zhang 2619 (PI)	HQ890383	JX874897	HQ890413
	China, Chongqing: Sanxia Exp. 1998 (PI)	HQ890384	JX874898	HQ890414
	Korea: Zhang 3559 (PI)	HQ890385	JX874899	HQ890415
	China, Taiwan: Schuettpelz 1221A (DUKE)	HQ890386	JX874900	HQ890416
Ph. hexagonoptera Fée	USA, South Carolina: Christenhusz 3844 (DUKE)	HQ890387	JX874893	–
	USA, Virginia: Taylor 7665 (BM)	JX874918	–	–
Ph. koreana B. Y. Sun & C. H. Kim	Korea: Zhang 3558 (PI)	HQ890388	JX874901	HQ890417
Pseudophegopteris Ching				
Ps. aurita (Hook.) Ching	China, Tibet: Zhang & Wang 4664 (PI)	HQ890389	–	HQ890418
	New Guinea: Walker T8427 (BM)	–	JX874902	HQ890419
Ps. brevipes Ching & S. K. Wu	China, Tibet: Zhang & Wang 5084 (PI)	HQ890390	JX874912	HQ890420
Ps. cruciata (Willd.) Holttum	France, La Réunion: Janssen 2724 (P)	JX874917	–	–
Ps. levingei (C. B. Clarke) Ching	China, Hubei: Zhang 3390 (PI)	HQ890391	JX874913	HQ890421
	China, Hubei: Zhang 3410 (PI)	HQ890392	JX874914	HQ890422
	China, Tibet: Zhang & Wang 4723 (PI)	HQ890393	JX874915	HQ890423
Ps. microstegia (Hook.) Ching	China, Sichuan: Zhang 4250 (PI)	HQ890391	JX874907	HQ890421
	China, Sichuan: Zhang & Wang 5150 (PI)	HQ890392	JX874908	HQ890422
Ps. paludosa (T. Moore) Ching	China, Taiwan: Schuettpelz 1152A (DUKE)	HQ890396	JX874916	HQ890426
Ps. pyrrhorhachis (Kunze) Ching	China, Chongqing: Zhang 2580 (PI)	HQ890397	JX874905	HQ890427
	China, Guangxi: Zhang 4157 (PI)	HQ890398	JX874906	HQ890428
	China, Yunnan: Shui et al., 81180 (PI)	HQ890399	JX874904	HQ890429
	China, Yunnan: Zhang 2403 (PI)	HQ890400	JX874903	HQ890430
Ps. retangularis (Zoll.) Holttum	Nepal: Fraser-Jenkins 216 (BM)	–	–	HQ890431
	Nepal: Fraser-Jenkins 1068 (BM)	HQ890401	–	HQ890432
Ps. yunkweiensis (Ching) Ching	China, Guizhou: Zhang 4228 (PI)	HQ890402	JX874909	HQ890433
	China, Yunnan: Zhang 3167 (PI)	HQ890403	JX874910	HQ890434
Ps. zayuensis Ching & S. K. Wu	China, Tibet: Zhang & Wang 5058 (PI)	HQ890404	JX874911	HQ890435
Outgroup taxa				
Dictyocline griffithii T. Moore	China, Taiwan: Schuettpelz 1190 (DUKE)	HQ890405	–	–
Leptogramma tottoides H. Ito	China, Taiwan: Schuettpelz 1135 (DUKE)	HQ890406	JX874890	HQ890436

'–', no sequences were generated for this region.

To explore the monophyly of each species as well as the interspecific relationships we performed both maximum parsimony (MP) and maximum likelihood (ML) analyses. MP analyses were carried out using PAUP* 4.0a109 (Swofford, 2002) with the following parameters: heuristic search until completion, 100 randomly assembled starting trees, and TBR branch swapping. Bootstrap values for MP were calculated with 1 000 replicates and the same conditions as the searches for the optimal tree of the unaltered dataset. Gaps were treated as missing characters. ML analyses were carried out with PhyML 3.0 (Guindon & Gascuel, 2003) with the model implemented and the parameters estimated. The model of sequence evolution was identified using JModelTest (Posada, 2008), which recovered the GTR + invgamma model as the best fit. Bootstrap values for ML were generated by 200 replicates in PHYML with the same model implemented but parameters estimated.

Divergence time estimates were obtained using a Bayesian approach (Drummond & Rambaut, 2007) as implemented in BEAST 1.5.6 (http://beast.bio.ed.ac.uk). These analyses were carried out with a reduced dataset including a single specimen per species. We also reduced the clade including *Pseudophegopteris microstegia* to *Pseudophegopteris paludosa* to two representing species (*Ps. paludosa* and *Pseudophegopteris yunkweiensis*) because species of this clade have nearly identical sequences. *Phegopteris koreana* was excluded to reflect phylogenetic results that recovered this species as nested within *Phegopteris decursivepinnata*. For *Ph. connectilis*, a single specimen was maintained. In total, the dated chronogram included three species of *Phegopteris* and six species of *Pseudophegopteris*. The dataset for divergence analyses was generated with the BEAUTY tool of the BEAST package. The tree was calibrated at its base by using the age estimate of 45.9 ma (Schuettpelz & Pryer, 2009) for the split of

Macrothelypteris and core phegopteroids, with a lognormal distribution as no estimates of the confidence intervals was provided in the former study. The molecular clock was estimated and treated as relaxed using the uncorrelated lognormal model. The analyses were pursued with the GTR model plus GAMMA and INVARIABLE sites with all parameters estimated. The accumulation of species through time was assumed to follow a Yule Process. The results of the BEAST runs were analyzed using TRACER 1.5. (http://wwwbeast.bio.ed.ac.uk) and summarized using TreeANNOTATOR (part of BEAST package). FigTree 1.3.1. (http://tree.bio.ed.ac.uk) was used to visualize the results. Age estimates were reported using the geological standard abbreviation ma for million of years in the past.

The formula for absolute diversification rates given by Magallon and Sanderson (2001) was used to estimate these rates for selected crown or stem-group nodes. The age estimates are based on the described BEAST analyses. The number of species per genus was based on current estimates given in Smith (1990): *Macrothelypteris* (10 spp.), *Phegopteris* (3 spp.), and *Pseudophegopteris* (20 spp.).

We employed binary-state speciation and extinction "BiSSE" model analyses (Maddison et al., 2007) as implemented in Mesquite ver. 2.74 (http://mesquiteproject.org.) to test for hypothetical correlation of the climate preference and diversification rates. All statistics including significance values were calculated using the implementation of the BiSSE approach in Mesquite. The genus *Phegopteris* was scored as temperate, whereas *Macrothelypteris* and *Pseudophegopteris* were scored as subtropical to tropical. Our sampling covers 100% of the extant species diversity of *Phegopteris* but only 45% of *Pseudophegopteris* and 20% of *Macrothelypteris*. We therefore generated a dataset with 3 OTUs for *Phegopteris* and 20 OTUs for *Pseudophegopteris* by duplicating terminal nodes of *Pseudophegopteris*. By doing so, the effect of imbalanced taxon sampling was addressed. We assumed that all missing species of *Pseudophegopteris* are nested within the crown-group inferred from our analyses of the sampled dataset. *Macrothelypteris* was included to enable the estimation the root length but pruned from the chronogram to avoid influencing the sister pair analyses in BiSSE. The required chronogram was generated for the dataset with the corrected number of taxa using the procedure described above for the observed dataset. BiSSE analyses were performed for the dataset with the taxon sampling corrections.

We carried out (1) character independent diversification analyses and (2) character associated analyses calculating Speciation/Extinction rates, ln Likelihood Difference, Net Diversification Likelihood, and Sister Diversification test. This analytical procedure was used to explore evidence supporting the hypothesis of a correlation of diversification rates and occurrence in tropical (scored as 0) or temperate (scored as 1) climate zones. We compared the ln L of the diversification rate independent and dependent on the climate references including estimates of the speciation rate (lamda), extinction rate (mu), and rate of change in climate frequency (q01 and 10). To enable a statistical evaluation of the results, we simulated 1 000 trees with two constraint character sets (tropical versus temperate), including 23 taxa as required to compare the observed data with an expected distribution of trees under the assumption of constant speciation and constant extinction rate.

Information on the distribution and on the chromosome numbers of species belonging to the studied genera was obtained from various sources including general resources such as Smith (1990), Index of Plant Chromosome Numbers (http://mobot.mobot.org/W3T/Search/ipcn.html), Flora of North America (http://www.efloras.org), as well as species publications (Manton, 1950; Mulligan et al., 1972; Mulligan & Cody, 1979; Masuyama, 1986; Matsumoto & Yano, 1989; Matsumoto & Nakaike, 1990; Ivanova & Piekos-Mirkowa, 2003; Nakato et al., 2012).

2 Results

All phylogenetic analyses recovered the same overall topology with all critical clades having bootstrap support values over 95%. The statistics for the phylogenetic analyses, for each dataset, are given as: ML complete dataset (33 OTUs) ln L = −5 186.825 GTR, gamma = 0.404, Invar = 0.0, f(A) = 0.2982, f(C) = 0.1985, f(G) = 0.2107, f(T) = 0.2926, A–C = 0.8486, A–G = 5.0510, A–T = 0.4364, C–G = 1.3370, C–T = 5.8922, G–T = 1.0 (fixed); ML-reduced dataset (25 OTUs) ln L = −4995.346, GTR model, gamma = 0.472, invar = 0.0, f(A) = 0.2987, f(C) = 0.1986, f(G) = 0.2101, f(T) = 0.2920, A–C = 0.9638, A–G = 5.5999, A–T = 0.4880, C–G = 1.4574, C–T = 5.9612, G–T = 1.0 (fixed); MP complete dataset (33 OTUs), 2 278 characters included, 1 982 constant characters (87.01%), 80 variable, parsimony-uninformative characters (3.51%), 216 parsimony-informative characters (9.48%), 971 MP trees, Length 349 steps, CI* = 0.8358, RI = 0.9449, RC = 0.8258; MP-reduced dataset (25 OUTs), 2 278 characters included, 1 991 constant characters

(87.4%), 140 variable, parsimony-uninformative characters (6.15%), 147 parsimony-informative characters (6.45%), 18 MP trees, Length 329 steps, CI* = 0.8235, RI = 0.9422, RC = 0.8477. (* indicates values calculated after the exclusion of uninformative characters.)

Both genera, *Phegopteris* and *Pseudophegopteris*, were recovered as monophyletic and sister clades to each other (Fig. 1). *Phegopteris* included three clearly separated lineages with the single specimen of *Phegopteris koreana* nested within *Phegopteris decursivepinnata*. The five sampled specimens of *Phegopteris connectilis* fell into two lineages. One clade included two specimens of *Phegopteris hexagonoptera* besides two specimens of *Ph. connectilis*, while the other included three specimens of *Ph. connectilis*.

Pseudophegopteris showed four distinctive clades. The first clade, sister to all other *Pseudophegopteris* clades, comprised two monophyletic and well-separated species, *Pseudophegopteris aurita* (two specimens) and *Pseudophegopteris levingei* (three specimens). *Pseudophegopteris brevipes* (one specimen) was found to be sister to the remaining two clades. *Pseudophegopteris rectangularis* (two specimens) was found to be sister to *Pseudophegopteris pyrrorachis* (four specimens). Together they formed the sister clade to a poorly resolved clade that included five species: *Pseudophegopteris cruciata* (one specimen), *Pseudophegopteris microstegia* (two specimens), *Pseudophegopteris paludosa* (one specimen), *Pseudophegopteris yunkweiensis* (two specimens), and *Pseudophegopteris zayuensis* (one specimen). Analyses of the smaller dataset recovered the same species relationships as described for the larger dataset (not shown, topology as in Fig. 2).

Diversification age estimates supported a separation of the two genera around 33 ma (21.99–45.89) with initial diversification of *Phegopteris* around 26 ma (13.09–42.93) and *Pseudophegopteris* around 17 ma (7.92–29.33). Estimations of absolute diversification rates of the two genera provided distinct values for the sister genera (Table 2). The crown clade of *Phegopteris* showed a rate between $r(\varepsilon = 0) = 0.0177$ to $r(\varepsilon = 0.9) = 0.0020$, whereas the crown clade of *Pseudophegopteris* showed values of $r(\varepsilon = 0) = 0.0581$ to $r(\varepsilon = 0.9) = 0.0256$. Compared with all absolute diversification rates estimates, the two sister genera were at the opposite end of the spectrum (Table 2).

BiSSE analyses with character independent analyses estimated a speciation/extinction $-\ln L = 68.078$ for the *Phegopteris*/*Pseudophegopteris* clade with lamba = 0.218 and mu = 0.181. Character dependent analysis, *Pseudophegopteris* as tropical (0) and *Phegopteris* as temperate (1), found a BiSSE $-\ln L = 63.898$ with lamba(0) = 0.407, lamda(1) = 0.0260, mu(0) = 0.405, mu(1) = 6.361e-5, q01 = 2.127e-5, q10 = 0.009 and BiSSE (NET Div) speciation/extinction (0) = 0.004, speciation/extinction (1) = 0.026, speciation/extinction (0) = 0.99, speciation/extinction (1) = 0.003. The character dependent analysis provided a better explanation of diversification of the two genera than the character independent one (ln independent = −68.078, ln L = character dependent = −63.898, $p < 0.01$). The observed BiSSE Net Diversification Likelihood of ln L = −68.078 was significantly closer to zero than the likelihood expected based on the 1000 simulated trees with a median BiSSE Net Diversification of ln L = −105.4. Thus, the results of these analyses supported the hypothesis of a dependence of the diversification rates and preferred occurrences in temperate versus tropical climatic regimes.

3 Discussion

3.1 Species diversity

Correct assessment of the species number per lineage is a critical issue in studies exploring the processes involved in macroecological pattern formation. Incorrect species concepts or undiscovered species diversity may create misleading results. Sister pair comparisons are expected to be especially sensitive to errors that increase or decrease the number of only one of the two sister pairs (Ricklefs, 2006). Thus, we took particular care of this issue by including several specimens to confirm species concepts wherever possible. Our study found support for three distinct species within *Phegopteris* under the assumption that cpDNA sequence differences are sufficient to define species identity. *Phegopteris koreana* cannot be distinguished from *Phegopteris decursivepinnata* in its cpDNA. This result is congruent with the high morphological similarity between the sporophytes of these two species (Kim et al., 2004). Additional molecular and cytological evidence will be required to confirm the status of this taxon, in the meantime we do not accept it as an independent species. *Phegopteris tibetica* was treated as a synonym of *Phegopteris connectilis* in the present study, although the status of this taxon needs further attention. We found two different plastid DNA types in specimens identified as *Ph. connectilis*. Some shared the cpDNA with *Phegopteris hexanoptera* from North America, whereas specimens from Taiwan and Greenland showed a type unique to *Ph. connectilis*. This species was known to include diploids, triploids, and tetraploids (Ivanova & Piekos-Mirkowa, 2003). Sexual diploids were reported

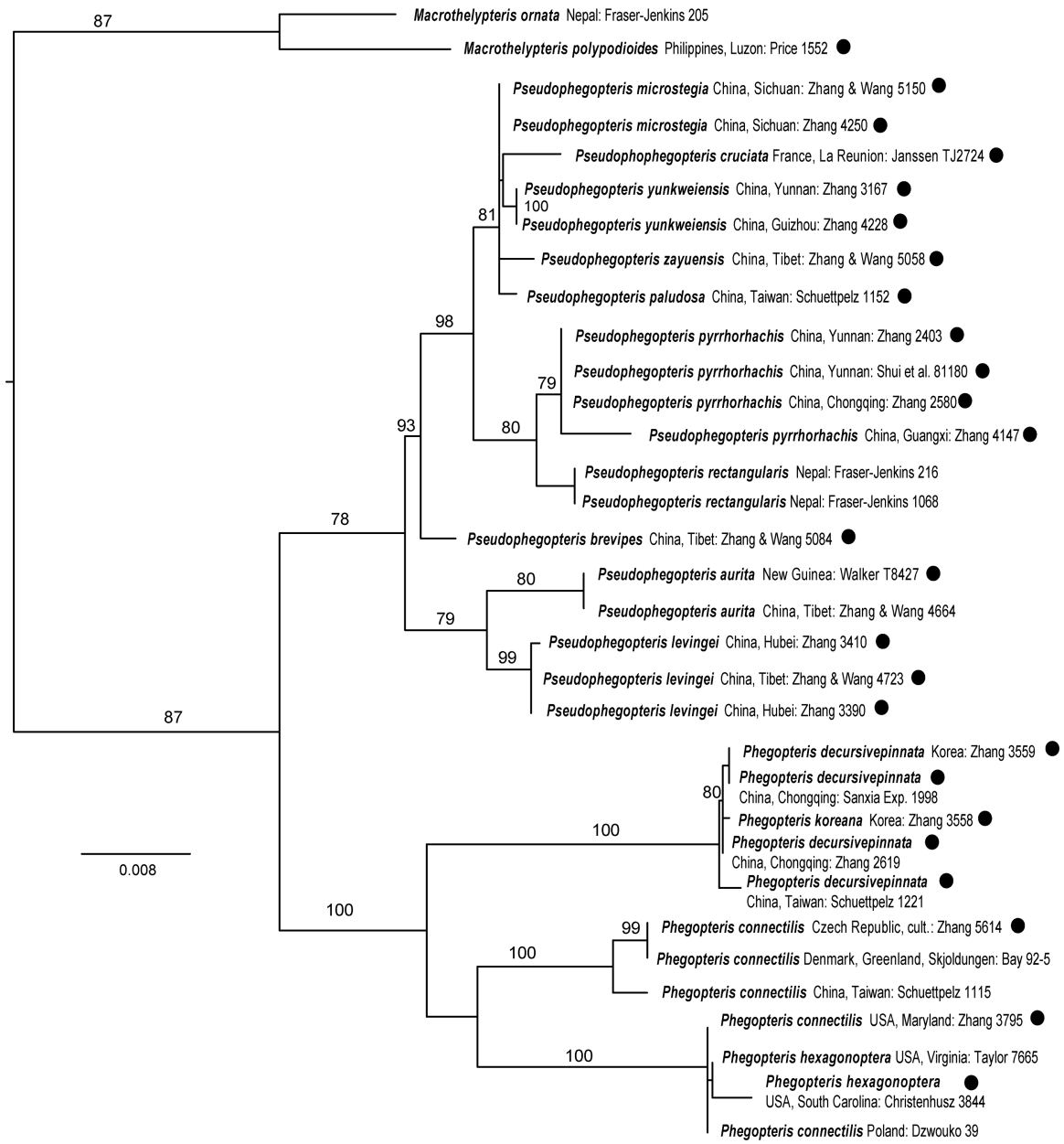

Fig. 1. Phylogeny as reconstructed in maximum likelihood (ML) analysis of the large dataset based on three plastid DNA regions. The lengths of branches correspond to the reconstructed likelihood of character state changes. Black dots indicate terminal taxa for which we obtained all sequenced regions. The analyses of the complete and reduced dataset resulted in the same relationships of the taxa, for both ML and maximum parsimony (MP) analyses. The large dataset was rooted with *Dictycline griffithii* and *Leptogramma tottoides*, whereas the small dataset was rooted with the two specimens of *Macrothelypteris*. Bootstrap values of the ML analyses of the large dataset are given above branches or behind nodes.

to be restricted to Japan whereas all European records were apomictic triploids, and North American records were mainly apomictic triploids with the exception of some tetraploids (Manton, 1950; Mulligan et al., 1972; Mulligan & Cody, 1979; Matsumoto & Yano, 1989; Matsumoto & Nakaike, 1990; Ivanova & Piekos-Mirkowa, 2003). Thus, our results may be explained by hybridization between *Ph. connectilis* and *Ph. hexanoptera*. However, a study using isozymes (Driscoll et al., 2003) did not recover evidence supporting the hypothesis of the diploid *Ph. hexanoptera* as one of the parents of these tetraploid apogamous forms of *Phegopteris*. Thus, we cannot rule out alternative hypothesis such as incomplete lineage sorting. The taxonomy of *Ph. connectilis* requires attention in the context of our results. Despite these issues, we

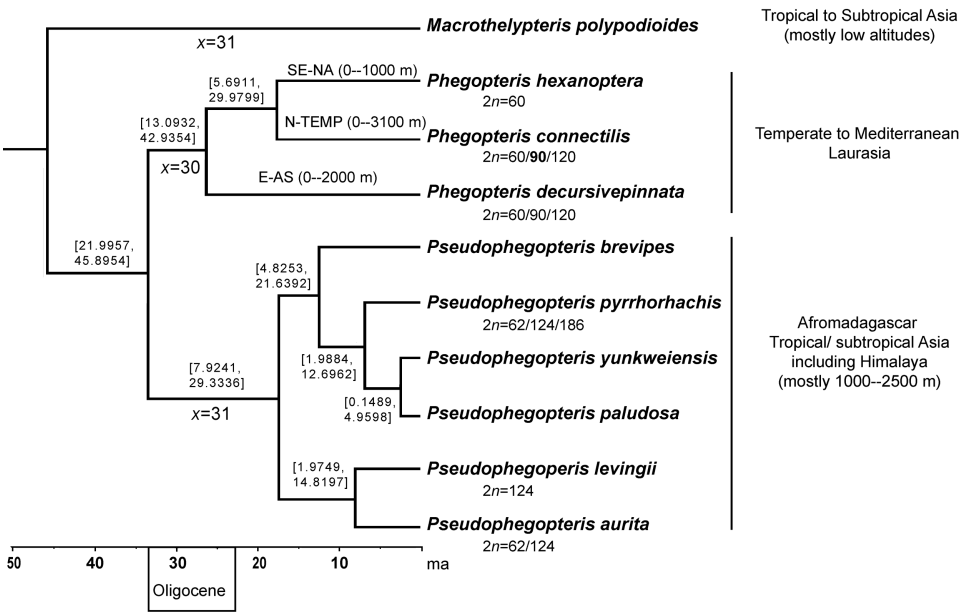

Fig. 2. Diversification time estimates of the core phegopteroid clade generated using the uncorrelated relaxed clock model as implemented in BEAST. The cladogram is calibrated with the divergence of *Macrothelypteris* and core phegopteroids at about 45.92 ma (Schuettpelz & Pryer, 2009). The median divergence time is shown with the 95% Confidence Intervals given in brackets above/below each branch leading to nodes. Reported chromosome numbers are given below each species whereas the estimated basal chromosome number is given below major clades ($x = 30$ or 31). The geographic range of each taxon is given for each species of *Phegopteris* (SE-NA = Southeast North America, N-TEMP = Northern temperate zone, E-AS = East Asia) with the altitudinal range given in brackets. ma = geological standard abbreviation for million years in the past. The geographic range/altitudinal range of each genus is summarized on the right side of the chronogram. The Oligocene period is indicated as a box below the time scale.

considered three as the best approximation of the number of species belonging to *Phegopteris* as long as we follow the cpDNA branching pattern. Intraspecific polyploidy is has also been reported for *Phegopteris decirsivepinnata* (2x, 3x, 4x) which indicates similar speciation processes in the two taxa occurring in SE Asia (Masuyama, 1986).

The number of species belonging to *Pseudophegopteris* appeared at the first sight much less problematic. Several pairs of species, such as *Pseudophegopteris pyrrorhachis* versus *Pseudophegopteris rectangularis* and *Pseudophegopteris aurita* versus *Pseudophegopteris levingei*, were found to form phylogenetically distinct clades. The accumulated phylogenetic distances among these species allowed unambiguous separation. In contrast, the number and separation of species was ambiguous in the clade comprising *Pseudophegopteris microstegia* to *Pseudophegopteris paludosa*. The low resolution of this clade may be the result of rather recent speciation events in this clade.

Our study included a limited number of species belonging to *Pseudophegopteris* which is about half of the currently accepted species (Smith, 1990). We also relied here on estimates based on morphological species descriptions although we lack a comprehensive revision of the genus using biosystematic evidence. Nevertheless, our sampling covers the East Asian diversity center

Table 2 Absolute diversification rates estimated according to Magallon & Sanderson (2001)

	ma	Spp.	r ($\varepsilon = 0$)	r ($\varepsilon = 0.9$)
Stem phegopteroids	68.5*	33	0.0222	0.0091
Crown phegopteroids	45.9*	33	0.0245	0.0131
Stem *Macrothelypteris*	45.9*	10	0.0218	0.0061
Stem *Phegopteris* + *Pseudophegopteris* clade	45.9*	23	0.0297	0.0110
Crown *Phegopteris* + *Pseudophegopteris* clade	33.2	23	0.0319	0.0145
Crown *Phegopteris*	26.9	3	0.0177	0.0020
Crown *Pseudophegopteris*	17.2	20	0.0581	0.0256

Estimates obtained from Schuettpelz & Pryer (2009). Species numbers (n): *Macrothelypteris* $n = 10$, *Phegopteris* $n = 3$, *Pseudophegopteris* $n = 20$. Our limited sampling of *Macrothelypteris* does not allow the estimation of its crown group age. ma, million years until today; Spp., number of species; r ($\varepsilon = 0$), diversification rate under the assumption of an extinction rate of 0; r ($\varepsilon = 0.9$), diversification rate under the assumption of an extinction of 0.9.

of the genus including specimens from New Guinea representing the southern border of the distribution range, and La Réunion representing the Afro-Madagascan component of the genus. Given the much lower knowledge on tropical to subtropical fern diversity in comparison to northern temperate fern diversity, we expect a more likely underestimation of species numbers in *Pseudophegopteris* than in *Phegopteris*. In conclusion, the revealed differences in the speciation rates between the two genera are likely to reflect real differences and are not the result of incorrect estimates of species numbers.

3.2 Chronological pattern and diversification rates

The two sister clades show remarkably distinct patterns of diversification through time (Fig. 2). The *Phegopteris* clade has gained its diversity in the first half of the lineage lifetime. The second half is characterized by zero increase in species diversity. In contrast, *Pseudophegopteris* showed a slower start and has accumulated most of its diversity in the latter part the lineage life span. The low taxonomic coverage of the *Pseudophegopteris* clade has to be considered because some unsampled species may represent the outcome of speciation events within the first half of the lineages' life span. However, we do not have strong evidence for this hypothesis because our sampling covers the distribution range and morphological disparity of the genus very well. A further issue is the restriction of the inferred evidence to the cytoplasmic inherited cpDNA genome. Thus, we can only recognize speciation events that are the results of isolation and do not involve hybridization and/or polyploidization. In *Phegopteris*, polyploid speciation is known to involve at least two out of the three lineages inferred using cpDNA data. Polyploid speciation may also be a common process in *Pseudophegopteris* but chromosome counts exists only for a fraction of the species recognized. Of these six species, two were reported as diploids, one as tetraploid and three include both diploids and tetraploids.

The differences in the speciation rates correlated with the climate preferences as the mainly tropical genus *Pseudophegopteris* shows a higher diversification rate than mainly temperate genus *Phegopteris*. Thus, the hypothesis of higher speciation rates appears to explain the observed LDG-like distribution pattern of the species diversity of these two sister genera. However, a closer look at the diversification pattern suggests the need to consider alternative explanations. First, the lower number of species in *Phegopteris* may be the result of an increased extinction rate for this clade in the Pleistocene, leading to the survival of only a few lineages. This is still consistent with the LDG hypothesis. Secondly, the higher diversification rate of *Pseudophegopteris* may be a response to the dramatic changes in the geography and climate of tropical and subtropical SE Asia in the Miocene that are caused by the rise of the Himalaya and/or by the collision of SE Asia and Australia (Zheng et al., 2004; Zhang & Fritsch, 2010). The slightly later onset of diversity assembly in the *Pseudophegopteris* clade compared with *Phegopteris* is consistent with this hypothesis. These observations are drawn without taking the absolute divergence time estimates into account, thus restricting the arguments to relative time changes. Age estimations for these ferns are challenging as the result of their absence or rarity in the fossil record. The lack of unequivocally assigned fossils prevents us from pursuing the dating using fossil calibration(s). Instead, the tree was calibrated with an age estimate obtained in a global study of the diversification pattern of ferns (Schuettpelz & Pryer, 2009). We employed a lognormal distribution of the calibration, which takes into account uncertainty especially in the context of a potentially older age of the calibrated node.

The putative Oligocene age of the split of the two groups is notable. The global climate was rather cold during the early and mid Oligocene whereas the period of late Oligocene to early Miocene was characterized by a rapid global warming. Consequently, both lineages may have benefited from this rise in temperature and maintained the separation of their climate preferences since more than 20 million years ago.

3.3 Differences in speciation processes

Some authors (Haufler et al., 2000; Haufler, 2002) suggested contrasting modes of speciation in temperate and upland tropical habitats. This hypothesis was based on studies of North American species of *Polypodium* compared with Mexican species of *Pleopeltis*. In this scenario, speciation events are either slow or ancient in temperate regions compared with rapid or recent in the tropics. These differences will result in lower speciation rates in temperate regions. Our study may provide further evidence here. The *Phegopteris* clade shows evidence for ancient speciation events, whereas *Pseudophegopteris* shows more recent events. The poor resolution within one of the *Pseudophegopteris* clades (Fig. 1) is consistent with the expected pattern of a rapid radiation in which the speciation process is too fast to allow for accumulation of neutral variation in the generated sequences of non-coding plastid genome regions.

Haufler (2002) discussed a greater impact of isolation by distance in temperate ferns, whereas

ecological speciation may be the dominant pattern in tropical taxa. However, it is not clear if this argument can be applied to the two genera studied. The North American species *Ph. hexanoptera* does not show a co-occurrence with diploids of its sister species *Ph. connectilis* but has an overlap with triploid *Ph. connectilis* along the northern border of its range. This overlap may result in reticulation as indicated by the similarity of the cpDNA found in *Ph. connectilis* and *Ph. hexanoptera*. However, diploid *Ph. connectilis* and diploid *Ph. decursivepinnata* have overlapping ranges in Japan (Masuyama, 1986; Matsumoto & Yano, 1989; Nakato et al., 2012). In *Pseudophegopteris*, sympatric occurrences are found in some species pairs, e.g. *Ps. pyrrorhachis* and *Ps. rectangularis*. Similarly, the three species of *Phegopteris* are well distinct in their morphology whereas some species of *Pseudophegopteris* show high morphological similarities. In summary, our data provide support only to one element of Haufler's hypothesis: ancient versus more recent speciation events. This finding further raises two questions: are observed differences in diversification rates (1) due to a greater extinction risk of temperate taxa during the Pleistocene glaciations or (2) the result of larger effective population size that restricts isolation by ecological differentiation in temperate taxa. These questions may be addressed via comparative studies aiming to reconstruct the history of the population structure of diploid species of both genera that occur in SE Asia. These studies require dense population sampling and the establishment of genomic markers that are variable enough to resolve the population history.

Acknowledgements We acknowledge the support by the team of the molecular laboratories including sequencing facilities of the Natural History Museum (London, UK). We are also grateful to Fabienne Hennequin who helped with generating sequence data. We acknowledge the Senior Visiting Professorship granted to HS by the Chinese Academy of Sciences and the Marie Curie Fellowship PIEF-GA-2008-220447 granted to SH.

References

Barraclough TG, Nee S, Harvey PA. 1998. Sister-group analysis in identifying correlates of diversification. Evolutionary Ecology 12: 751–754.

Buckley LB, Davis TJ, Ackerly DD, Kraft NJB, Harrison SP, Anacker BL, Cornell HV, Damschen EI, Gryntes JA, Hawkins BA, McCain CM, Stephens PR, Wiens JJ. 2010. Phylogeny, niche conservatism and the latitudinal diversity gradient in mammals. Proceedings of the Royal Society B: Biological Sciences 277: 2131–2138.

Cardillo M. 1999. Latitude and rates of diversification in birds and butterflies. Proceedings of the Royal Society B: Biological Sciences 266: 1220–1225.

Condamine FL, Sperling FAH, Wahlberg N, Rasplus JY, Kergoat GJ. 2012. What causes latitudinal gradients in species diversity? Evolutionary processes and ecological constraints on swallowtail biodiversity. Ecology Letters 15: 267–277.

Davis TJ, Savolainen V, Chase MW, Moat J, Barraclough TG. 2004. Environmental energy and evolutionary rates in flowering plants. Proceedings of the Royal Society B: Biological Sciences 271: 2195–2200.

Doyle JJ, Doyle JL. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19: 11–15.

Driscoll HE, Barrington DS, Gilman AV. 2003. A re-examination of the apogamous tetraploid *Phegopteris* (Thelypteridaceae) from northeastern North America. Rhodora 105: 309–321.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214.

Guindon S, Gascuel O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology 52: 696–704.

Haufler CH. 2002. Homospory 2002: an odyssey of progress in pteridophyte genetics and evolutionary biology. Bioscience 52: 1081–1093.

Haufler CH, Hooper EA, Therrien JP. 2000. Modes and mechanisms of speciation in pteridophytes: implications of contrasting patterns in ferns representing temperate and tropical habitats. Plant Species Biology 15: 223–236.

Hua X, Wiens JJ. 2010. Latitudinal variation in speciation mechanisms in frogs. Evolution 64: 429–443.

Ivanova D, Piekos-Mirkowa H. 2003. Chromosome numbers of polish ferns. Acta Biologica Cracoviensia Botanica 45: 93–99.

Kim CH, Sun YY, Park SH. 2004. A new species of *Phegopteris* (Thelypteridaceae) from Korea. Novon 14: 440–443.

Kozak KH, Wiens JJ. 2007. Climatic zonation drives latitudinal variation in speciation mechanisms. Proceedings of the Royal Society B: Biological Sciences 274: 2995–3003.

Maddison WP, Maddison DR. 2005. MacClade 4.08. Sunderland: Sinauer Associates.

Maddison WP, Midford PE, Otto S. 2007. Estimating a binary character's effect on speciation and extinction. Systematic Biology 56: 701–710.

Magallon S, Sanderson MJ. 2001. Absolute diversification rates in angiosperm clades. Evolution 55: 1762–1780.

Manton M. 1950. Problems of cytology and evolution in pteridophytes. Cambridge: Cambridge University Press.

Marin PR, Montgomerie R, Lougheed SC. 2010. Rapid sympatry explains greater color pattern divergence in high latitude birds. Evolution 64: 336–337.

Marin PR, Tewksburry JJ. 2008. Latitudinal variation in subspecific diversification of birds. Evolution 62: 2775–2788.

Masuyama S. 1986. Reproductive biology of the fern *Phegopteris decursive-pinnata*. 2. Genetic analyses of self-sterility in diploids. Botanical Magazine Tokyo 99: 107–121.

Matsumoto S, Nakaike T. 1990. Cytological observations of some ferns in Nepal (1). On the related taxa in Japan. In: Watanabe M, Malla SB eds. Cryptogams of the Himalayas. 2: 163–178.

Matsumoto S, Yano Y. 1989. The invasion of the sexual and apogamous types of *Phegopteris connectilis* in an avalanched region on Mt Fuji. Annals of the Tsutsuka Botanical Garden 8: 27–36.

Mittelbach GG, Schemske DW, Cornell HV, Cornell HV, Allen AP, Brown JM, Bush MB, Harrison SP, Hurlbert AHH, Knowlton N, Lessios HA, McCain CM, McCune AR, McDade LA, McPeek MA, Near TJ, Price TD, Ricklefs EE, Roy K, Sax DF, Schluter D, Sobel JM, Turelli M. 2007. Evolution and the latitudinal diversity gradient: speciation, extinction and biogeography. Ecology Letters 10: 315–331.

Mulligan GA, Cinq-Mars L, Cody WJ. 1972. Natural interspecific hybridization between sexual and asexual *Phegopteris*. Canadian Journal of Botany 50: 1295–1300.

Mulligan GA, Cody WJ. 1979. Chromosome numbers in Canadian *Phegopteris*. Canadian Journal of Botany 57: 1813–1819.

Nakato N, Ootsuki R, Murakami N, Masuyama S. 2012. Two types of partial fertility in a diploid population of the fern *Thelypteris decursive-pinnata* (Thelypteridaceae). Journal of Plant Research 125: 465–474.

Posada D. 2008. jModeltest: phylogenetic model averaging. Molecular Biology and Evolution 25: 1253–1256.

Ricklefs RE. 2006. Global variation in the diversification rate of passerine birds. Ecology 87: 2468–2478.

Ricklefs RE. 2009. Speciation, extinction and diversity. In: Butlin RK, Bridle JR, Schluter D eds. Speciation and patterns of diversity. Cambridge: Cambridge University Press. 257–277.

Schemske DW. 2009. Biotic interactions and speciation in the tropics. In: Butlin RK, Bridle JR, Schluter D eds. Speciation and patterns of diversity. Cambridge: Cambridge University Press. 219–239.

Schneider H, Schuettpelz E, Pryer KM, Cranfill R, Magallon S, Lupia R. 2004. Ferns diversified in the shadow of angiosperms. Nature 428: 553–557.

Schuettpelz E, Pryer KM. 2007. Fern phylogeny inferred from 400 leptosporangiate fern species and three plastid genes. Taxon 46: 1037–1050.

Schuettpelz E, Pryer KM. 2009. Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. Proceedings of the National Academy of Sciences USA 106: 11200–11205.

Smith AR. 1990. Thelypteridaceae. In: Kubitzki K ed. The families and genera of vascular plants. Vol. I. Pteridophytes and Gymnosperms (Volume editors: Kramer KU, Green PS). Berlin: Springer. 263–272.

Smith AR, Cranfill R. 2002. Infrafamilial relationships of the thelypteroid ferns (Thelypteridaceae). American Fern Journal 92: 131–149.

Smith AR, Pryer KM, Schuettpelz E, Korall P, Schneider H, Wolf PG. 2006. A classification for extant ferns. Taxon 55: 705–731.

Swofford DL. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4. Sunderland: Sinauer Associates.

Tate JA, Simpson BB. 2003. Paraphyly of *Tarasa* (Malvaceae) and diverse origins of the polyploid species. Systematic Botany 28: 723–737.

Trewick SA, Morgan-Richards M, Russell SJ, Henderson S, Rumsey FJ, Pinter I, Barrett JA, Gibby M, Vogel JC. 2002. Polyploidy, phylogeography ad Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. Molecular Ecology 11: 2003–2012.

Wiens JJ, Donoghue MJ. 2004. Historical biogeography, ecology and species richness. Trends in Ecology and Evolution 19: 639–644.

Zhang ML, Fritsch PW. 2010. Evolutionary response of *Caranga* (Fabaceae) to Quinghai-Tibetan plateau uplift and Asian interior acidification. Plant Systematics and Evolution 299: 191–199.

Zheng HB, McPowell CA, Red DK, Wang JL, Wang PX. 2004. Late Miocene and mid-Pliocene enhancement of the East Asian monsoon as viewed from the land and sea. Global Planetary Change 41: 147–155.