TAXON 60 (5) • October 2011: 1284–1294

Relationships and evolutionary origins of polyploid *Dryopteris* (Dryopteridaceae) from Europe inferred using nuclear *pgiC* and plastid *trnL-F* sequence data

Aino Juslén,[1] Henry Väre[2] & Niklas Wikström[3]

1 *Finnish Museum of Natural History, Zoology, P.O. Box 17, 00014 University of Helsinki, Finland*
2 *Finnish Museum of Natural History, Botany, P.O. Box 7, 00014 University of Helsinki, Finland*
3 *Bergius Foundation, Royal Swedish Academy of Sciences, P.O. Box 2263, 10316 Stockholm, Sweden*
Author for correspondence: *Aino Juslén, aino.juslen@helsinki.fi*

Abstract To test earlier hypotheses on the evolutionary origins of European polyploid *Dryopteris*, their phylogenetic relationships are investigated using nuclear *pgiC* and plastid *trnL-F* sequence data. The nuclear *pgiC region* is for the first time used successfully in phylogenetic analyses of ferns, and is shown to provide appropriate variability to address species-level questions in *Dryopteris*. The *trnL-F* dataset is used to infer maternal relationships for the allopolyploid species and provides strong support for some of the smaller clades resolved by *pgiC*. Allopolyploid origins of *Dryopteris carthusiana*, *D. cristata*, and *D. guanchica* are supported and some, but not all, progenitors are indicated by the analyses. The results are in disagreement with the "*D. semicristata*" hypothesis proposed by several authors. The allopolyploid origin of *D. crispifolia* remained unresolved and allopolyploid origins of *D. dilatata* and *D. filix-mas* were unsupported. This study provides the first insight in the molecular phylogeny of the diploid *D. fragrans*, which shows major genetic variation within the only European population of the species.

Keywords allotetraploid; diploid; ferns; phylogeny; polyploids

Supplementary Material The alignment is available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

Hybridization and the formation of polyploids are important evolutionary mechanisms contributing to the development of new species in ferns and other vascular plants (Haufler, 2002; Moran, 2004; Wood & al., 2009). Polyploidy seems to be particularly common in ferns (Walker, 1966, 1984) and as many as 95% of ferns species are supposed to have undergone polyploidization during their evolutionary history (Haufler, 1987). In general, polyploids are classified as allopolyploids or autopolyploids depending on the degree of divergence between the parental genomes. Allopolyploids result from interspecific hybridization followed by genome doubling, whereas autopolyploids are formed as a result of genome doubling within a single species (Thompson & Lumaret, 1992; Soltis & Soltis, 1999; Ramsey & Schemske, 2002). No recombination is expected between the homeologous chromosomes in a polyploid species and single-copy nuclear loci should display haplotypes representing the parental orthologs (Soltis & Soltis, 1993). This "fixed heterozygosity" allows us to infer the evolutionary origins of polyploid species (Soltis & Soltis, 1993). Depending on whether a polyploid is an autopolyploid or allopolyploid, sequences from homeologous loci for a given nuclear gene are expected to resolve with either the same (autopolyploid) or with different (allopolyploid) parental lineages in a phylogenetic analysis (Linder & Rieseberg, 2004).

Dryopteris Adans. is a nearly cosmopolitan genus of approximately 225 species, distributed in primarily mountainous regions with wet, shady forest habitats or open grassy areas. *Dryopteris* is most diverse in south-eastern Asia (Fraser-Jenkins, 1986; Hoshizaki & Wilson, 1999). In Europe there are 22 species of *Dryopteris* (Blockeel, 2006; Table 1); in addition, Blockeel (2006) lists *D. intermedia* subsp. *azorica* (Christ) Jermy and subsp. *maderensis* (Alston) Fraser-Jenk. as subspecies of the North American *D. intermedia* (Muhl. ex Wild.) A. Gray. Both taxa have also been recognized at species level (Jalas & Suominen, 1972; Gibby & al., 1978; Fraser-Jenkins, 1982). A list of European *Dryopteris* and hypothesized diploid progenitors of the polyploids is presented in Table 1. *Dryopteris* diversity in Europe is greatest along the Atlantic Coast and in Macaronesia, where over three quarters of the species are present (Fraser-Jenkins, 1982). Many European species are also found in North America or are known to hybridize with North American species (Montgomery & Paulton, 1981).

There has been much confusion regarding the phylogenetic relationships of *Dryopteris* (Hoshizaki & Wilson, 1999) and the taxonomy of several species complexes, such as the *D. filix-mas–D. affinis* group, remains unresolved. Much of the complexity has been attributed to hybridization and polypoidization (Hoshizaki & Wilson, 1999).

In the present study we focus on the origins of the European *Dryopteris* polyploid taxa, and on resolving their relationships to diploid species. We use the single-copy nuclear region *pgiC* to test previously proposed hypotheses based on cytological and chemical studies (Fig. 1; Table 1). The *pgiC gene* has recently been used in several phylogenetic analyses

Table 1. List of European *Dryopteris* species (Blockeel, 2006) and hypothesized evolutionary origins of the non-diploid species.

Species	Ploidy level	Mode of origin	Parental species	Reference
Dryopteris aemula (Aiton) Kuntze	2*x*	diploid		Manton (1950)
Dryopteris affinis (Lowe) Fraser-Jenk.	2*x*, 3*x*	apomictic di-/triploid		Fraser-Jenkins (1980)
Dryopteris aitoniana Pic.Serm.	2*x*	diploid		Goldblatt & Johnson (1991)
Dryopteris carthusiana (Vill.) H.P. Fuchs[a]	4*x*	tetraploid		Manton (1950)
		allotetraploid	?	Walker (1955)
		allotetraploid	*intermedia* × “*semicristata*”	Wagner (1971)
		allotetraploid	*intermedia* × *tokyensis*	Widén & Britton (1985)
		allotetraploid	*intermedia* × *ludoviciana*	Hickok & Klekowski (1975)
		allotetraploid	*intermedia*[b] ×? (or *ludoviciana*)	Gibby & al. (1978)
		allotetraploid	*azorica* or *maderensis* × ?	Gibby (1983)
Dryopteris caucasica (A. Braun) Fraser-Jenk. & Corley	2*x*	diploid		Jalas & Suominen (1972)
Dryopteris corleyi Fraser-Jenk.	4*x*	allotetraploid	*aemula* × *oreades*	Jiménez & al. (2009)
Dryopteris crispifolia Rasbach, Reichst. & G. Vida.	4*x*	allotetraploid	*aemula* × *azorica*	Widén & al. (1975)
		allotetraploid	*aemula* × *azorica*	Gibby & al. (1977)
		allotetraploid	*aemula* × *azorica*	Gibby (1985)
Dryopteris cristata (L.) A. Gray	4*x*	tetraploid		Manton (1950)
		allotetraploid	?	Walker (1955)
		allotetraploid	*ludoviciana* × ?	Walker (1969)
		allotetraploid	*ludoviciana* × “*semicristata*”	Wagner (1971)
		allotetraploid	*ludoviciana* × *goldiana*	Hickok & Klekowski (1975)
		allotetraploid	*ludoviciana* × ?	Gibby & Walker (1977)
		allotetraploid	*ludoviciana* × ?	Gibby & al. (1978)
Dryopteris dilatata A. Gray[c]	4*x*	allotetraploid	?	Manton & Walker (1954)
		allotetraploid	?	Walker (1955)
		allotetraploid	*expansa* × *azorica*	Widén & al. (1970)
		allotetraploid	*expansa* × *azorica*	Gibby & Walker (1977)
		allotetraploid	?	Fraser-Jenkins (1982)
		allotetraploid	*expansa* × *azorica*	Gibby (1983)
Dryopteris expansa (C. Presl) Fraser-Jenk. & Jermy	2*x*	diploid		Jalas & Suominen (1972)
Dryopteris filix-mas (L.) Schott	4*x*	allotetraploid	*oreades*[d] × ?	Manton (1950)
		allotetraploid	*oreades*[d] × *villarii*/*pallida* complex	Widén & al. (1971)
		autotetraploid	*marginalis*	Wagner (1971)
		allotetraploid	*oreades*[d] × *caucasica*	Fraser-Jenkins & Corley (1972)

Table 1. Continued.

Species	Ploidy level	Mode of origin	Parental species	Reference
		allotetraploid	*oreades*[d] × *caucasica*	Fraser-Jenkins (1976)
Dryopteris fragrans (L.) Schott	2*x*	diploid		Jalas & Suominen (1972)
Dryopteris gorgonea J.P. Roux	?	polyploid	?	Roux (2004)
Dryopteris guanchica Gibby & Jermy	4*x*	tetraploid		Gibby & al. (1977)
		allotetraploid	*aemula* × *maderensis*	Gibby & al. (1978)
		allotetraploid	*aemula* × *maderensis*	Gibby (1983)
Dryopteris intermedia subsp. *azorica* (Christ) Jermy	2*x*	diploid		Gibby & Walker (1977)
Dryopteris intermedia subsp. *maderensis* (Alston) Fraser-Jenk.	2*x*	diploid		Gibby & Walker (1977)
Dryopteris liliana Golitsin	2*x*	diploid		Davis (1988)
Dryopteris oligodonta (Desv.) Pic.Serm.	2*x*	diploid		Gibby & al. (1977)
Dryopteris oreades Fomin	2*x*	diploid		Jalas & Suominen (1972)
Dryopteris pallida (Bory) C. Chr. ex Maire & Petitm.	2*x*	diploid		Jalas & Suominen (1972)
Dryopteris remota (A. Braun ex Döll) Druce	3*x*	apomictic triploid		Manton (1950)
		apomictic triploid	*affinis* sbusp. *affinis* × *expansa*	Gibby & Walker (1977)
		apomictic triploid		Schneller & al. (1998)
Dryopteris submontana (Fraser-Jenk. & Jermy) Fraser-Jenk.	4*x*	tetraploid	?	Jalas & Suominen (1972)
Dryopteris tyrrhena Fraser-Jenk. & Reichst.	4*x*	allotetraploid	*oreades*[d] × *pallida*	Fraser-Jenkins & al. (1975)
Dryopteris villarii (Bellardi) Woyn. ex Schinz & Thell.	2*x*	diploid		Jalas & Suominen (1972)

[a] Synonymous to *D. spinulosa* (O.F. Müll.) Watt.
[b] Including *D. azorica* (Christ) Alston and *D. maderensis* Alston.
[c] Synonymous to *D. austriaca* auct.
[d] Synonymous to *D. abbreviata* (DC.) Newman.

and studies of hybridization of flowering plants (Ford & al., 2006; Choong & al., 2008; Russel & al., 2010; Kamiya & al., 2011). However, Schuettpelz & al. (2008) reported unsuccessful amplifications for cheilanthoid ferns. We present the first case study using *pgiC* sequence data for addressing phylogenetic questions in ferns (Ishikawa & al., 2002). The present study also provides first insight into the evolutionary relationships and genetic diversity among populations of the arctic-alpine diploid *D. fragrans* (L.) Schott.

■ MATERIALS AND METHODS

Taxon sampling. — Taxon sampling for the *pgiC* analyses was targeted towards species hypothesized to be involved in the evolutionary origins of European polyploids (Table 1). A total of 56 accessions representing 24 mainly European and North American species of *Dryopteris* were sampled (Appendix). The sampling included 15 of the 22 European species of the genus (Blockeel, 2006). In addition to fresh material collected in the Azores, Madeira, the Canary Islands, Finland, and Sweden, tissue from well-preserved herbarium specimens located in H were used. For the *trnL-F* analyses corresponding species were sampled, although for fewer accessions, and sequences were complemented with previously published sequences from GenBank (Wang & al., 2003; Geiger & Ranker, 2005; Lu & al., 2005; Chang & al., 2009). Voucher information for all included accessions is given in the Appendix.

DNA extraction, amplification, cloning, and sequencing procedures. — Total genomic DNA was extracted from silica-dried material and/or herbarium specimens following the DNeasy Plant Protocol for Isolation of DNA from

Fresh Plant Leaves Using the Mixer Mill MM 300 (Quiagen, Hilden, Germany). Initial sequences for the *pgiC* nuclear region were obtained via PCR amplification using the primers 14F and 16R from Ishikawa & al. (2002). More specific primers 14F2 (GAGTGTTTGGAATGTTTCATTCCT) and 16R2 (CAAAGWCAATTTCACCAGCTTCA) were subsequently designed based on sequences from *Dryopteris intermedia* subsp. *azorica* (FR728950) and an unpublished sequence of *Dryopteris carthusiana* (Vill.) H.P. Fuchs. The amplified region of *pgiC* corresponds to part of exon 14, intron 14, exon 15, intron 15, and part of exon 16 of the *Arabidopsis thaliana* X69195 sequence (see Ishikawa & al., 2002 for a comparative alignment). Primers for the PCR amplification of the *trnL-F* plastid region were obtained from Taberlet & al. (1991).

PCR amplifications of the *pgiC* and *trnL-F* were performed using Phusion enzyme (Finnzymes, Vantaa, Finland)

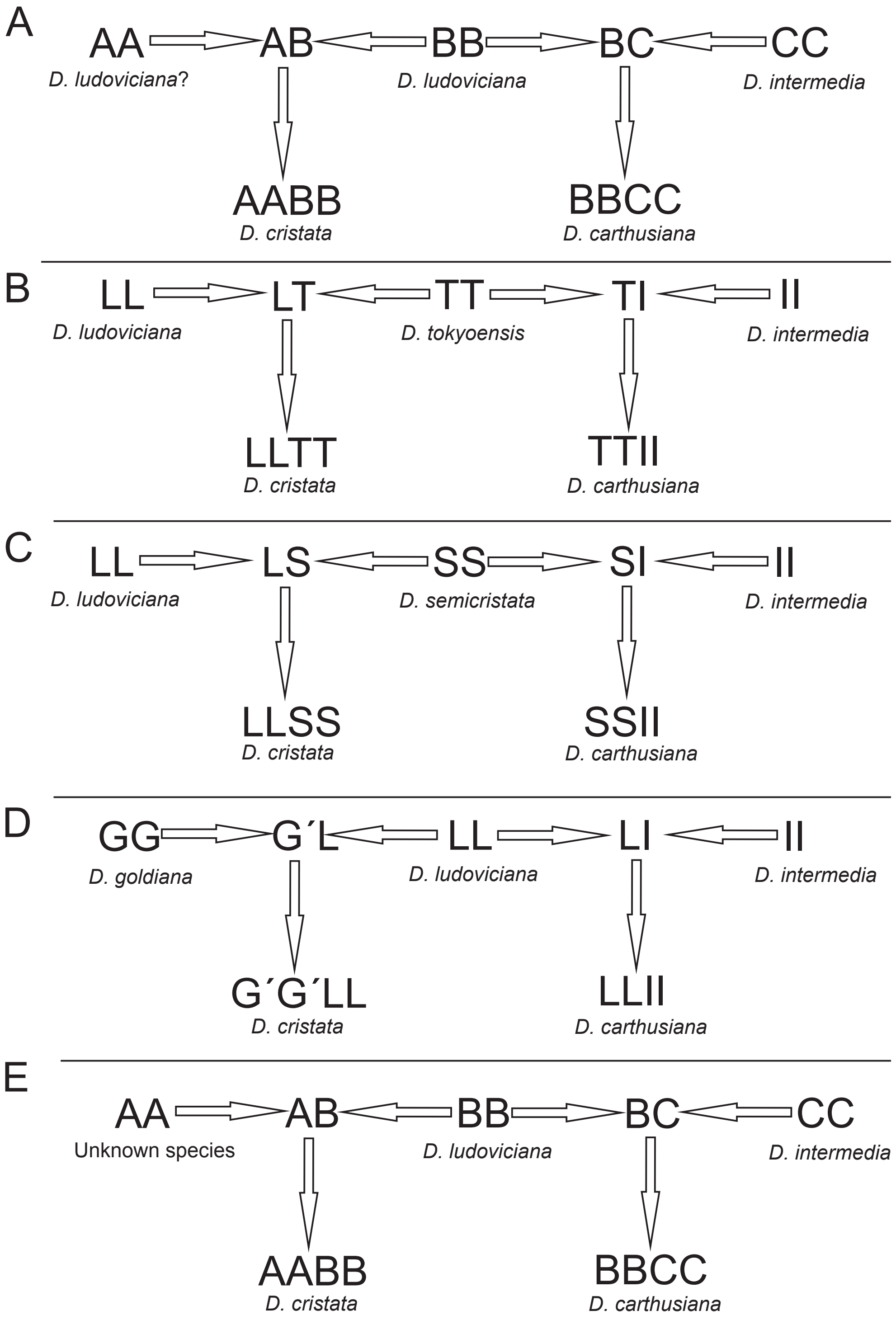

Fig. 1. Parentage of the allotetraploids *Dryopteris cristata* and *D. carthusiana* as hypothesized by different workers. **A,** Gibby & Walker (1977); **B,** Hickok & Klekowski (1975); **C,** Widén & Britton (1985); **D,** Wagner, (1977) and Stein & al. (2010); **E,** present analysis. See text for details. Notation as used by the original authors.

in 20 µl reactions with the following reagents: template DNA 1 µl, 5× Phusion HF Buffer 4 µl, 10 mM dNTP 0.5 µl each, 10 µM forward and reverse primers 0.5 µl each, Phusion enzyme 0.2 µl. The thermal cycling profile was set to 98°C for 2 min, (98°C for 30 s, 60°C for 30 s, 72°C for 1 min) × 40 cycles, 72°C for 7 min. PCR products were cleaned using Montage PCR Plates (Millipore Corporation, Billerica, Massachusetts, U.S.A.) and submitted to direct sequencing using the amplification primers. All sequencing was performed at Macrogen (Seoul, Republic of Korea).

When direct sequencing of *pgiC* resulted in chromatograms showing overlapping peaks and/or length variation, cloning was done using the Topo TA Cloning Kit for Sequencing (Invitrogen Corporation, Carlsbad, California, U.S.A.). As a rule, between 5 and 10 colonies were sequenced and used in combination with the direct sequence to identify paralogs and/or alleles. Amplifications of clone colonies were performed using Taq DNA polymerase (ABgene, Epson, U.K.) in 20 µl reactions using the supplied standard protocol and primers M13F and M13R. PCR products were cleaned using Montage PCR Plates (Millipore Corporation) and submitted for sequencing.

Sequence assembly, editing, and phylogenetic analyses. — Sequences were edited and assembled using the Staden Package (Staden & al., 2000), and aligned manually in Seaview v.4.0 (Gouy & al., 2010). Aligned datasets comprised 663 (*pgiC*) and 903 characters (*trnL-F*). Inferred gaps were coded separately as binary characters (Simmons & Ochoterena, 2000) and treated as morphological partitions in the phylogenetic analyses. A total of 24 unambiguous gaps were inferred in the *pgiC* alignment and 11 in the *trnL-F* alignment. Tree topology and support were assessed for each dataset separately using a Bayesian Markov Chain Monte Carlo (MCMC) approach (Larget & Simon, 1999) implemented in MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). Each dataset comprised a nucleotide partition and a morphological (inferred gaps) partition. Each partition was allowed partition-specific parameters (Ronquist & Huelsenbeck, 2003; Nylander & al., 2004). The nucleotide substitution model for each dataset was selected based on a corrected Akaike information criterion (AIC) as calculated using MrAIC v. 1.4.4 and PHYML v.2.4 (Guindon & Gascuel, 2003; Nylander, 2004). The HKY+gamma model was selected for the *pgiC* data, and the GTR+gamma was selected for the *trnL-F* data. The Mk model (Lewis, 2001) was used for the morphological (inferred gaps) partition in each analysis. Rate heterogeneity across characters was accounted for by assuming a discrete gamma distribution.

Bayesian analyses were run for 10 million generations with four MCMC chains in two independent and parallel runs. Following a "burn-in" phase of 5 million generations, we sampled trees and parameters every 1000th generation resulting in a posterior distribution of 5000 trees and parameter estimates for each run. Convergence was assessed by comparing the standard deviation of split frequencies between the different runs. After confirming convergence, the posterior trees were pooled and used to calculate majority-rule consensus trees and Bayesian posterior probabilities. For the purpose of comparison with non-parametric bootstrapping (Alfaro & al., 2003), we consider nodes with posterior probabilities equal to or greater than 0.95 to be well supported.

Resulting trees from the *trnL-F* analyses were rooted using outgroup taxa from the genus *Arachnioides* Blume (Geiger & Ranker, 2005). Trees from the *pgiC* analyses were rooted following the results of the *trnL-F* analyses.

■ RESULTS

The phylogeny based on plastid *trnL-F* was poorly resolved (Fig. 2) and the *trnL-F* data was analyzed primarily to infer the maternal copies of *pgiC* of the allopolyploid species (Fig. 2).

Phylogenetic analyses of the *pgiC* dataset resulted in a relatively well-supported tree (Fig. 3). Of the polyploids sampled, *Dryopteris carthusiana*, *D. cristata* (L.) A. Gray, and *D. guanchica* Gibby & Jermy clearly showed maternal and paternal copies within different clades indicating that they have allopolyploid origins. Tetraploid *D. dilatata* A. Gray was grouped with diploid *D. expansa* (C. Presl) Fraser-Jenk. & Jermy and *D. campyloptera* (Kunze) Clarkson, a putatively allotetraploid species from North America (Gibby, 1977). However, none of the three included accessions had multiple sequence copies that would indicate an allopolyploid origin. *Dryopteris affinis* (Lowe) Fraser-Jenk. and *D. filix-mas* (L.) Schott were grouped together without clear species boundaries in the *pgiC* phylogeny; like in *D. dilatata* there were no signs of multiple sequence copies in the tetraploid *D. filix-mas*. In the present phylogeny both accessions of *D. intermedia* subsp. *maderensis* were united in a well-supported clade and clearly different from other specimens of *D. intermedia*. The positions of *D. intermedia* and *D. intermedia* subsp. *azorica* were less clear. One *D. intermedia* accession was grouped with *D. carthusiana* and the other in an unresolved clade with *D. crispifolia* Rasbach & al. *Dryopteris intermedia* subsp. *azorica* joined with two *D. crispifolia* samples with low support. The polyploid origin of *D. crispifolia* remained unclear. One accession, *Väre 16351*, showed no sign of multiple copies, indicating an autopolyploid origin. The second one, *Väre 16320*, had two different copies but the possibility that these represent different alleles cannot be ruled out. *Dryopteris remota* (A. Braun ex Döll) Druce was successfully amplified only for *trnL-F* (Fig. 2) and needs further study with a nuclear marker for progenitor searching.

■ DISCUSSION

***Dryopteris carthusiana* and *D. cristata*. —** Based on cytogenetic evidence Walker (1955, 1961, 1969) and Gibby & Walker (1977) put forth the hypothesis that *Dryopteris ludoviciana* (Kunze) Small represents a common diploid progenitor of both *D. cristata* and *D. carthusiana* (Fig. 1). Gibby & Walker (1977) hypothesized that *D. intermedia* is one diploid progenitor of *D. carthusiana* and that *D. ludoviciana* or another unknown diploid species is the second progenitor. The unknown species was called "*semicristata*" by Wagner (1971). Hickok & Klekowski (1975) presented a reinterpretation of relationships

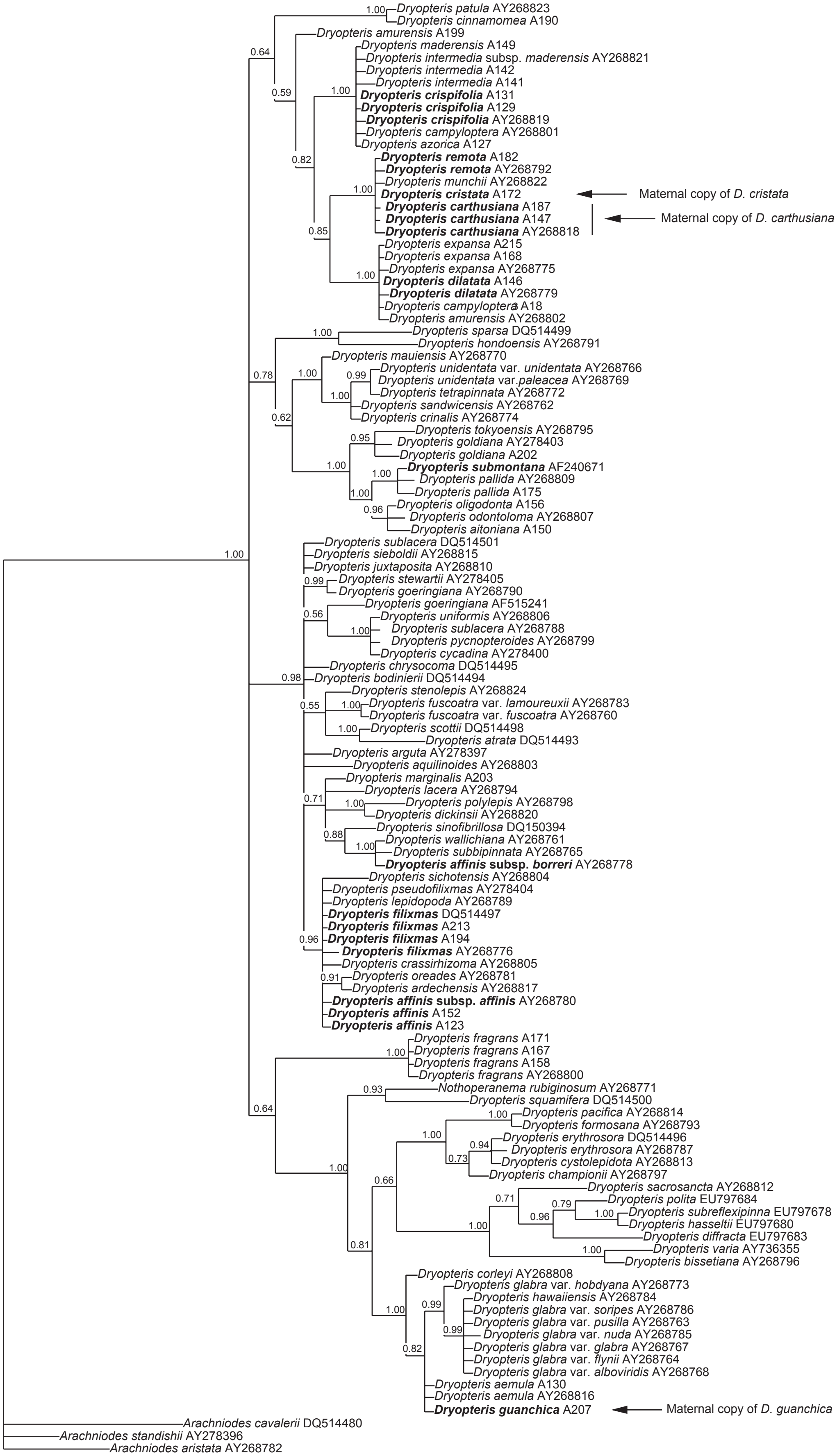

Fig. 2. Majority-rule consensus tree of the sampled *Dryopteris* species inferred from Bayesian analysis of the plastid *trnL-F* region.

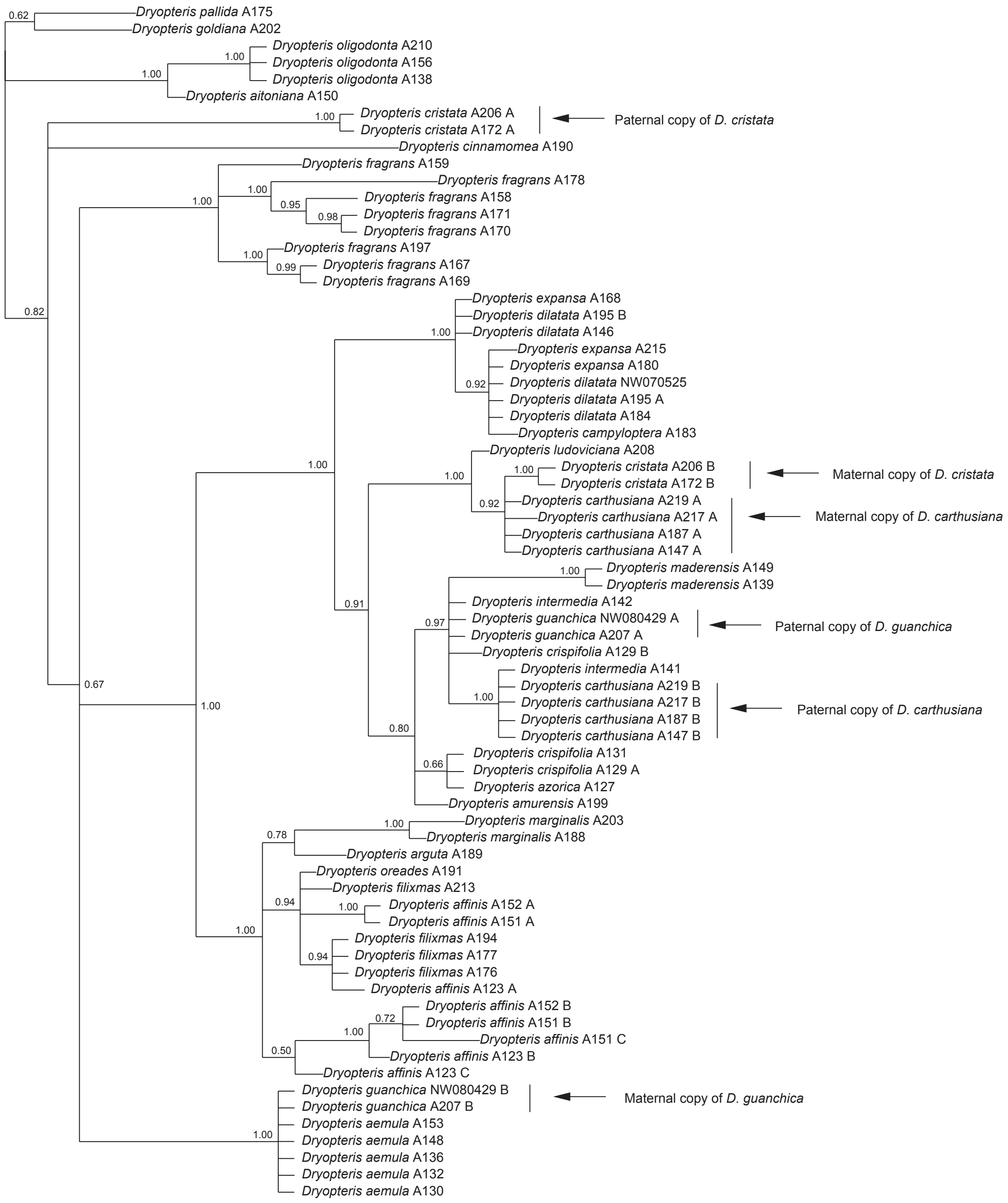

Fig. 3. Majority-rule consensus tree of sampled *Dryopteris* species inferred from Bayesian analysis of the nuclear *pgiC* gene.

of *Dryopteris* species occurring in the Appalachian Mountains in North America. They contested that the existence of "*D. semicristata*" is not necessary or plausible to explain the origin of polyploid species. They hypothesized that the parents of *D. carthusiana* are *D. intermedia* and *D. ludoviciana*, and that the parents of *D. cristata* are *D. ludoviciana* and *D. goldiana* (Hook. ex Goldie) A. Gray (Hickok & Klekowski, 1975). Based on morphological similarities and distributions, Carlson & Wagner (1982) suggested that *D. tokyoensis* (Matsum. ex Makino) C. Chr., an Asian diploid, could be the missing "*D. semicristata*". This hypothesis was supported by studies of phloroglucinol derivatives (Widén & Britton, 1985). However, morphological character state prediction (Werth, 1989) and isozyme studies by Werth (1991) rejected this theory. Werth (1991) was in favor of the "*D. semicristata*" hypothesis, which was supported also by a leaf indumenta study by Viane (1986). Werth (1990) proposed *D. semicristata* to be typified by an illustration based on extrapolation of its morphological features and photographic documentation of its cytological and biochemical attributes. Petersen & Fairbrothers (1983) reviewed the morphological, cytological, and chemical studies handling the relationships of North American *Dryopteris* species. Based on flavonol concentrations they suggested that the missing diploid genome of "*semicristata*" is more closely related to *D. intermedia* than it is to *D. marginalis* (L.) A. Gray, another North American diploid species. A recent study based on isozymes and chloroplast sequences support the "*semicristata*" hypothesis (Stein & al., 2010). The parentage of the allotetraploids *D. cristata* and *D. carthusiana* as hypothesized by different workers is presented in Fig. 1.

Our *pgiC* analysis provides support for an allopolyploid origin of both *Dryopteris carthusiana* and *D. cristata* (Fig. 3). The results are congruent with the hypothesis suggesting that *D. intermedia* and *D. ludoviciana* are the diploid progenitors of *D. carthusiana* (Fig. 1). Our results also indicate that *D. ludoviciana* represents the shared genome and is the ancestor of both *D. cristata* and *D. carthusiana*. These results are in disagreement with the "*D. semicristata*" hypothesis.

The second diploid progenitor of *Dryopteris cristata* remains unclear. The paternal copy of *D. cristata* is in an unresolved position (Fig. 3). *Dryopteris goldiana* (in the hypothesis by Hickok & Klekowski, 1975), *D. tokyoensis* (Widén & Britton, 1985), or unknown species (Wagner, 1971; Gibby & Walker, 1977) cannot be ruled out as paternal species of *D. cristata*.

***Dryopteris guanchica* and *D. crispifolia*. —** Chemotaxonomic and morphological studies support the hypothesis that *Dryopteris guanchica* is an allotetraploid species derived from hybridization between *D. aemula* Kunze and *D. intermedia* subsp. *maderensis* (Widén & al., 1975; Gibby & al., 1978; Gibby, 1979; Fraser-Jenkins, 1982). The present study generally supports this hypothesis although the results do not unequivocally point to *D. intermedia* subsp. *maderensis* as one of the diploid progenitors. The distribution of *D. guanchica* is restricted to Spain, Portugal, and the Canary Islands (Fraser-Jenkins, 1982). The origin of this species remains unclear as one of the putative ancestors, *D. intermedia* subsp. *maderensis*, is absent in the current range of *D. guanchica*. On the other hand, in Madeira where both assumed ancestors are found, *D. guanchica* does not occur nowadays (Fraser-Jenkins, 1982).

The progenitors of *Dryopteris crispifolia* are hypothesized as *D. intermedia* subsp. *azorica* and *D. aemula* based on cytological, morphological, and chemical data (Widén & al., 1975; Gibby & al., 1977, 1978; Gibby, 1985). If this hypothesis is valid, *D. crispifolia* is genomically similar to *D. guanchica* (Fraser-Jenkins, 1982). Gibby (1985) stated that both *D. guanchica* and *D. crispifolia* resemble *D. aemula* morphologically, and that the differences between *D. guanchica* and *D. crispifolia* can be attributed to the differences between *D. intermedia* subsp. *azorica* and *D. intermedia* subsp. *maderensis*. *Dryopteris crispifolia* occurs sympatrically with *D. aemula* and *D. intermedia* subsp. *azorica* in the Azores (Fraser-Jenkins, 1981).

Our *pgiC* phylogeny does not support *Dryopteris aemula* as a diploid progenitor of *D. crispifolia* (Fig. 3). *Dryopteris crispifolia* samples are united in a weakly supported clade with both *D. intermedia* and *D. intermedia* subsp. *azorica*, but there is no support for a *D. aemula* relationship. Based on the *pgiC* results, the allotetraploid status of *D. crispifolia* remains uncertain. One of the accessions shows no sign of having multiple *pgiC* copies, as would be expected from an allotetraploid, and although two copies are identified in the second accession, they are highly similar and may well represent different alleles rather than copies from homeologous loci. Additional data is required to resolve the polyploid origin of *D. crispifolia*.

***Dryopteris dilatata* and *D. filix-mas*. —** *Dryopteris dilatata* is hypothesized to be an allotetraploid species based on evidence from chromatography and cytological studies (Manton & Walker, 1954; Widén & al., 1970; Gibby, 1983). The parents of *D. dilatata* are hypothesized to be *D. expansa* (Walker, 1955; Gibby & al., 1977) and *D. intermedia* subsp. *azorica* (Widén & al., 1970; Gibby, 1983). Surprisingly, results of the present molecular study show no signs of homeologous loci of *pgiC* in *D. dilatata*. The sequences of *D. dilatata* unequivocally group together with *D. expansa* (Fig. 3) and *D. campyloptera*, a putatively allotetraploid species from North America (Gibby, 1977). Sigel (2008), in her study based on *trnL-F*, *rps4-trnS* cpDNA sequences, *gapCp* nuclear DNA sequences, and amplified fragment length polymorphisms, was unable to identify the other progenitor of *D. dilatata*, in addition to *D. expansa.* She discussed the possibility that a European maternal progenitor could be an extinct species genetically similar to *D. intermedia*. Our results, using direct sequencing of *pgiC* in *D. dilatata* do not support this hypothesis, and indicate an autopolyploid origin of *D. dilatata*. Different accessions of *D. dilatata* are not even grouped as monophyletic, but show alternate relationships to different accessions of *D. expansa*, and this indicates that *D. dilatata* may result from multiple autopolyploid origins. This possibility should be investigated further.

Already Manton (1950) hypothesized that *Dryopteris filix-mas* was an allotetraploid species and she identified *D. oreades* Fomin as one of the parental species. Later workers have followed Manton (1950) in recognizing *D. filix-mas* as an allotetraploid, and *D. caucasica* (A. Braun) Fraser-Jenk. & Corley (Frazer-Jenkins & Corley, 1972) or some species from the *D. villarii*/*D. pallida* complex (Widén & al., 1970) have been suggested to be the second parental species.

There is no support in our analyses for an allotetraploid origin of *D. filix-mas*, and there are no signs of homeologous *pgiC* loci in the four accessions for which *pgiC* was sequenced. Wagner (1971) investigated the cytology of naturally occurring hybrids between *D. filix-mas* and *D. marginalis* from Canada and Eastern U.S.A. and considered *D. filix-mas* to be an autopolyploid, with *D. marginalis* as the single parental species. Fraser-Jenkins (1976) argued against this interpretation. He suspected *D. filix-mas* from Eastern U.S.A. and from Europe to be quite different species, and found support for this view in chemical analyses carried out by Widén & Britton (1971). Our analyses only include accessions of *D. filix-mas* from Finland, Sweden, and Russia and cannot address this possibility but there is no support in our analyses for *D. marginalis* as the single parental species of an autotetraploid *D. filix-mas* (Fig. 3).

***Dryopteris fragrans*. —** *Dryopteris fragrans* is a diploid species (Jalas & Suominen, 1972). The *pgiC* phylogeny reveals large genetic variance within species throughout the distribution area. The genetic diversity indicated from *pgiC* within *D. fragrans* is much larger than that of other included species of *Dryopteris*. Interestingly, the Finnish specimens collected in four different locations in Kevo (see Appendix) are divided into two well-supported clades. In the first clade, two Finnish specimens group with a Mongolian sample. The two other Finnish specimens group together with specimens from eastern Russia and Chukotka. This suggests that either (1) Finnish populations of *D. fragrans* represent a refugial population (Kalliola, 1937), (2) *D. fragrans* arrived at least twice to Finland, or (3) occurrence of remarkable genetic differentiation due to sexual reproduction in the Finnish population.

The next closest occurrences of *Dryopteris fragrans* are in the Ural Mountains and eastern Greenland. According to Kalliola (1937) *D. fragrans* is a continental species, occurring in areas where annual changes in temperature are at least 25°C or more (Kalliola, 1937). Kalliola presumed that the localities of *D. fragrans* in Kevo are relicts of a larger distributional range of the species during the more severe continental climate following the last glaciation. A larger sampling of *D. fragrans* across its geographical range may provide greater resolution of its evolutionary history.

■ ACKNOWLEDGEMENTS

We thank the three anonymous referees for their insightful comments and linguistic corrections, and many colleagues for sending material. The Georg and Ella Ehrnroot Foundation and the Nordenskiöld Foundation are thanked for funding the project.

■ LITERATURE CITED

Alfaro, M.E., Zoller, S. & Lutzoni, F. 2003. Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. *Molec. Biol. Evol.* 20: 255–266.

Blockeel, T.L. 2006. *The liverworts, mosses and ferns of Europe*. Colchester: Harley Books.

Carlson, T.M. & Wagner, W.H., Jr. 1982. The North American distribution of the genus *Dryopteris*. *Contr. Univ. Michigan Herb.* 15: 141–162.

Chang, H.M., Chiou, W.L. & Wang, J.C. 2009. Molecular evidence for genetic heterogeneity and the hybrid origin of *Acrorumohra subreflexipinna* from Taiwan. *Amer. Fern J.* 99: 61–77.

Choong, C.Y., Wickneswari, R., Norwati, M. & Abbott, R.J. 2008. Phylogeny of *Hopea* (Dipterocarpaceae) inferred from chloroplast DNA and nuclear *pgiC* sequences. *Molec. Phylog. Evol.* 48: 1238–1243.

Davis, P.H. (ed.). 1988. *Flora of Turkey and the East Aegean Islands*, vol 10. Oxford: The Alden Press.

Ford, V.S., Lee, J., Baldwin, B.G. & Gottlieb, L.D. 2006. Species divergence and relationships in *Stephanomeria* (Compositae): *pgiC* phylogeny compared to prior biosystematic studies. *Amer. J. Bot.* 93: 480–490.

Fraser-Jenkins, C.R. 1976. *Dryopteris caucasica* and the cytology of its hybrids. *Fern Gaz.* 11: 263–267.

Fraser-Jenkins, C.R. 1980. *Dryopteris affinis*: A new treatment for a complex species in the European pteridophyte flora. *Willdenowia* 10: 107–115.

Fraser-Jenkins, C.R. 1981. *Dryopteris* (Pteridophyta: Dryopteridaceae) on Pico Island in the Azores. *Arquipélago, Sér. Ci. Nat.* 2: 83–101.

Fraser-Jenkins, C.R. 1982. *Dryopteris* in Spain, Portugal and Macaronesia. *Bol. Soc. Brot.* 55: 175–336.

Fraser-Jenkins, C.R. 1986. A classification of the genus *Dryopteris* (Pteridophyta: Dryopteridaceae). *Bull. Brit. Mus. (Nat. Hist.), Bot.* 14: 183–218.

Fraser-Jenkins, C.R. & Corley, H.V. 1972. *Dryopteris caucasica*—An ancestral diploid in the male fern aggregate. *Brit. Fern Gaz.* 10: 221–231.

Fraser-Jenkins, C.R., Reichstein, T. & Vida, G. 1975. *Dryopteris tyrrhena* nom. nov.—A misunderstood western Mediterranean species. *Fern Gaz.* 11: 177–198.

Geiger, J.M.O. & Ranker, T.A. 2005. Molecular phylogenetics and historical biogeography of Hawaiian *Dryopteris* (Dryopteridaceae). *Molec. Phylog. Evol.* 34: 392–407.

Gibby, M. 1977. The origin of *Dryopteris campyloptera*. *Canad. J. Bot.* 55: 1419–1428.

Gibby, M. 1979. Palaeoendemism and evolution in Macaronesian *Dryopteris*. Pp. 347–358 in: Bramwell, D. (ed.), *Plants and islands*. London: Academic Press.

Gibby, M. 1983. The *Dryopteris dilatata* complex in Macaronesia and the Iberian peninsula. *Acta Bot. Malac.* 8: 59–72.

Gibby, M. 1985. Hybridization and speciation in the genus *Dryopteris* (Pteridophyta: Dryopteridaceae) on Pico Island in the Azores. *Pl. Syst. Evol.* 149: 241–252.

Gibby, M. & Walker, S. 1977. Further cytogenetic studies and a reappraisal of the diploid ancestry in the *Dryopteris carthusiana* complex. *Brit. Fern Gaz.* 11: 313–324.

Gibby, M., Jermy, A.C., Rasbach, H., Rasbach, K, Reichstein, T. & Vida, G. 1977. The genus *Dryopteris* in the Canary Islands and Azores and the description of two new tetraploid species. *Bot. J. Linn. Soc.* 74: 251–277.

Gibby, M., Widén, C.-J. & Widén, H.K. 1978. Cytogenetic and phytochemical investigations in hybrids of Macaronesian *Dryopteris* (Pteridophyta: Aspidiaceae). *Pl. Syst. Evol.* 130: 235–252.

Goldblatt, P. & Johnson, D.E. 1991. Index to plant chromosome numbers 1988–1989. *Monogr. Syst. Bot. Missouri Bot. Gard.* 40: 1–238.

Gouy, M., Guindon, S. & Gascuel, O. 2010. SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molec. Biol. Evol.* 27: 221–224.

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

Haufler, C.H.1987. Electrophoresis is modifying our concepts of evolution in homosporous pteridophytes. *Amer. J. Bot.* 74: 953–966.

Haufler, C.H. 2002. Homospory 2002: An Odyssey of progress in pteridophyte genetics and evolutionary biology: Ferns and other homosporous vascular plants have highly polyploid chromosome numbers, but they express traits following diploid models and, although capable of extreme inbreeding, are predominantly outcrossing. *BioScience* 52: 1081–1093.

Hickok, L.G. & Klekowski, E.J. 1975. Chromosome behavior in hybrid ferns: A reinterpretation of Appalachian *Dryopteris*. *Amer. J. Bot.* 62: 560–569.

Hoshizaki, B.J. & Wilson, K.A. 1999. The cultivated species of the fern genus *Dryopteris* in the United States. *Amer. Fern J.* 89: 1–100.

Ishikawa, H., Watano, Y., Kano, K., Ito, M. & Kurita, S. 2002. Development of primer sets for PCR amplification of the *pgiC* gene in ferns. *J. Pl. Res.* 115: 65–70.

Jalas, J. & Suominen, J. (eds.). 1972. *Atlas Florae Europaeae. Distribution of vascular plants in Europe*, vol. 1, *Pteridophyta (Psilotaceae to Azollaceae).* Helsinki: The Committee for Mapping the Flora of Europe & Societas Biologica Fennica Vanamo.

Jiménez, A., Quintanilla, L.G., Pajarón, S. & Pangua, E. 2009. Genetic variation in allotetraploid *Dryopteris corleyi*

(Dryopteridaceae) and its diploid parental species in the Iberian Peninsula. *Amer. J. Bot.* 96: 1880–1886.

Kalliola, R. 1937. *Dryopteris fragrans* (L.) Schott, ein für Europa neuer Farn. *Ann. Bot. Soc. Zool.-Bot. Fenn. "Vanamo"* 9: 1–56.

Kamiya, K., Gan. Y.Y., Lum, S.K.Y., Khoo, M.S., Chua, S.C. & Faizu, N. 2011. Morphological and molecular evidence of natural hybridization in *Shorea* (Dipterocarpaceae). *Tree Genet. Genomes* 7: 297–306.

Larget, B. & Simon, D.L. 1999. Markov Chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molec. Biol. Evol.* 16: 750–759.

Lewis, P.O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Syst. Biol.* 50: 913–925.

Linder, C.R. & Rieseberg, L.H. 2004. Reconstructing patterns of reticulate evolution in plants. *Amer. J. Bot.* 91: 1700–1708.

Lu, J.M., Li, D.Z., Gao, L.M., Cheng, X. & Wu, D. 2005. Paraphyly of *Cyrtomium* (Dryopteridaceae): Evidence from *rbcL* and *trnL-F* sequence data. *J. Pl. Res.* 118: 129–135.

Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.

Manton, I. & Walker, S. 1954. Induced apogamy in *Dryopteris dilatata* (Hoffm.) A. Gray and *D. filix-mas* (L.) Schott emend. and its significance for the interpretation of the two species. *Ann. Bot. (Oxford)* 18: 377–383.

Montgomery, J.D. & Paulton, E.M. 1981. *Dryopteris* in North America. *Fiddlehead Forum* 8: 25–31.

Moran, R.C. 2004. *A natural history of ferns.* Portland: Timber Press.

Nylander, J.A.A. 2004. MrAIC.pl: Program distributed by the author. Uppsala University: Evolutionary Biology Centre.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves-Aldrey, J.L. 2004. Bayesian phylogenetic analyses of combined data. *Syst. Biol.* 53: 47–67.

Petersen, R.L. & Fairbrothers, D.E. 1983. Flavonols of the fern genus *Dryopteris*: Systematic and morphological implications. *Bot. Gaz.* 144: 104–109.

Ramsey, J. & Schemske, D.W. 2002. Neopolyploidy in flowering plants. *Annual Rev. Ecol. Syst.* 33: 589–639.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Roux, J.P. 2004. *Dryopteris gorgonea* (Pteropsida: Dryopteridaceae), a new species from the Cape Verde Islands. *Bothalia* 34: 32–35.

Russell, A., Samuel, R., Klejna, V., Barfuss, M.H., Rupp, B. & Chase, M.W. 2010. Reticulate evolution in diploid and tetraploid species of *Polystachya* (Orchidaceae) as shown by plastid DNA sequences and low-copy nuclear genes. *Ann. Bot.(Oxford)* 106: 37–56.

Schneller, J., Holderegger, R., Gugerli, F., Eichenberger, K. & Lutz E. 1998. Patterns of genetic variation detected by RAPDs suggest a single origin with subsequent mutations and long-distance dispersal in the apomictic fern *Dryopteris remota* (Dryopteridaceae). *Amer. J. Bot.* 85: 1038–1042.

Schuettpelz, E., Grusz, A.L., Windham, M.D. & Pryer, K.M. 2008. The utility of nuclear *gapCp* in resolving polyploid fern origins. *Syst. Bot.* 33: 621–629.

Sigel, E. 2008. Polyphyly of a polyploid species: An inquiry into the origin of *Dryopteris campyloptera* and *Dryopteris dilatata* (Dryopteridaceae). M.Sc. thesis, University of Vermont.

Simmons, M.P. & Ochoterena, H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Syst. Biol.* 49: 369–381.

Soltis, D.E. & Soltis, P.S. 1993. Molecular data and the dynamic nature of polyploidy. *Crit. Rev. Pl. Sci.* 12: 243–273.

Soltis, D.E. & Soltis, P.S. 1999. Polyploidy: Recurrent formation and genome evolution. *Trends Ecol. Evol.* 14: 348–352.

Staden, R., Beal, K.F. & Bonfield, J.K. 2000. The Staden Package 1998. *Meth. Molec. Biol.* 132: 115–130.

Stein, D.B., Hutton, C., Conant, D.S., Haufler, C.H. & Werth, C.R. 2010. Recontructing *Dryopteris* "*semicristata*" (Dryopteridaceae): Molecular profiles of tetraploids verify their undiscovered diploid ancestor. *Amer. J. Bot.* 97: 998–1004.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.

Thompson, J.D. & Lumaret, R. 1992. The evolutionary dynamics of polyploid plants: Origins, establishment and persistence. *Trends Ecol. Evol.* 7: 302–307.

Viane, R.L.L. 1986. Taxonomical significance of the leaf indument in *Dryopteris* (Pteridophyta): I: Some North American, Macaronesian, and European taxa. *Pl. Syst. Evol.* 153: 77–105.

Wagner, W.H., Jr. 1971. Evolution of *Dryopteris* in relation to the Appalachians. Pp. 147–192 in: Holt, P.C. (ed.), *The distributional history of the biota of the Appalachians*, part 2, *Flora*. Blacksburg: Virginia Polytechnical Instiute and State University.

Walker, S. 1955. Cytogenetic studies in the *Dryopteris spinulosa* complex—I. *Watsonia* 3: 193–209.

Walker, S. 1961. Cytogenetic studies in the *Dryopteris spinulosa* complex II. *Amer. J. Bot.* 48: 607–614.

Walker, S. 1969. Identification of a diploid ancestral genome in the *Dryopteris spinulosa* complex. *Brit. Fern Gaz.* 10: 97–99.

Walker, T.G. 1966. A cytotaxonomic survey of the pteridophytes of Jamaica. *Trans. Roy. Soc. Edinburgh* 66: 169–237.

Walker, T.G. 1984. Chromosomes and evolution in pteridophytes. Pp. 101–141 in: Sharma, A.K. & Sharma, A. (eds.), *Chromosomes in evolution of eukaryotic groups*, vol. 2. Boca Raton: CRC Press.

Wang, M.L., Chen, Z.D., Zhang, X.C., Lu, S.G. & Zhao, G.F. 2003. Phylogeny of the Athyriaceae: Evidence from chloroplast *trnL-F* region sequences. *Acta Phytotax. Sin.* 41: 416–426.

Werth, C.R. 1989. A study of the morphological attributes of the hypothetical diploid fern *Dryopteris* "*semicristata*". *Amer. J. Bot.* 76: 208.

Werth, C.R. 1990. Typification of an extinct species without fossil evidence: The case of *Dryopteris* "*semicristata*". *Amer. J. Bot.* 77: 164.

Werth, C.R. 1991. Isozyme studies on the *Dryopteris* "*spinulosa*" complex, I: The origin of the log fern *Dryopteris celsa*. *Syst. Bot.* 16: 446–461.

Widén, C.-J. & Britton, D.M. 1971. Chemotaxonomic investigations on the *Dryopteris cristata* complex in North America. *Canad. J. Bot.* 49: 1141–1154.

Widén, C.J. & Britton, D.M. 1985. Phloroglucinol derivative of *Dryopteris tokyoensis* and the missing genome in *Dryopteris cristata* and *Dryopteris carthusiana* (Dryopteridaceae). *Ann. Bot. Fenn.* 22: 213–218.

Widén, C.-J., Lounasmaa, M., Vida, G. & Reichstein, T. 1975. Die Phloroglucide von drei *Dryopteris*-Arten von den Azoren sowie zwei Arten von Madeira und den Kanarischen Inseln zum Vergleich. *Helv. Chim. Acta* 58: 880–904.

Widén, C.-J., Sorsa, V. & Sarvela, J. 1970. *Dryopteris dilatata* s.lat. in Europe and the island of Madeira, a chromatographic and cytological study. *Acta Bot. Fenn.* 91: 1–30.

Wíden, C.-J., Vida, G., Euw, J. von & Reichstein, T. 1971. Die Phloroglucide von *Dryopteris villarii* (Bell.) Woynar und anderer Farne der Gattung *Dryopteris* sowie die mögliche Abstammung von *D. filix-mas* (L.) Schott. *Helv. Chim. Acta* 54: 2824–2850.

Wood, T.E., Takebayashi, N., Barker, M.S., Mayrose, I., Greenspoon, P.B. & Riesberg, L.H. 2009. The frequency of polyploid speciation in vascular plants. *Proc. Natl. Acad. Sci. U.S.A.* 106: 13875–13879.

Appendix. Species, extraction numbers and voucher information for newly reported sequences, and GenBank accession numbers of the DNA sequences used in this paper (*pgiC*; *trnL-F*, respectively).

Arachniodes aristata (G. Forst.) Tind.; –; AY268782[a]. ***Arachniodes standishii*** (T. Moore) Ohwi; –; AY278396. ***Arachniodes cavalerii*** (Christ) Ohwi; –; DQ514480. ***Dryopteris aemula*** (Aiton) Kuntze; A130; The Azores, São Miguel; *Väre 16353* (H 1733681); FR728934; FR731965. A132; The Azores, São Miguel; *Väre 16353* (H 1733681); FR728935; –. A136; Spain, Liendo; *Alejandre 1791-89* (H 1655113); FR728936; –. A148; Madeira, Ribeiro Frio; *Väre 16544* (H 1733370); FR728937; –. A153; Madeira, Eucumenia; *Väre 16582* (H 1733410); FR728938; –. –; AY268816[a]. ***Dryopteris affinis*** (Lowe) Fraser-Jenk.; A123; The Azores, São Miguel; *Väre 16196* (H 1733526); FR728939; FR731966. A123; The Azores, São Miguel; *Väre 16196* (H 1733526); FR728940; –. A123; The Azores, São Miguel; *Väre 16196* (H 1733526); FR728941; –. A151; Madeira, Ribeiro Frio; *Väre 16539* (H 1733365); FR728942; –. A151; Madeira, Ribeiro Frio; *Väre 16539* (H 1733365); FR728943; –. A151; Madeira, Ribeiro Frio; *Väre 16539* (H 1733365); FR728944; –. A152; Madeira, Pico de Areiro; *Väre 16577* (H 1733381); FR728945; FR731967. A152; Madeira, Pico de Areiro; *Väre 16577* (H 1733381); FR728946; –. –; AY268780[a]. –; AY268778[a]. ***Dryopteris aitoniana*** Pic. Ser.; A150; Madeira, Ribeiro Frio; *Väre 16527* (H 1733706); FR728947; FR731968. *Dryopteris amurensis* Christ; A199; Japan, Hokkaido; *Alanko 70810* (H 1659792); FR728948; FR731990. –; AY268802[a]. ***Dryopteris aquilinoides*** (Desv.) C. Chr.; –; AY268803[a]. ***Dryopteris ardechensis*** Fraser-Jenk.; –; AY268817[a]. ***Dryopteris arguta*** (Kaulf.) Maxon; A189; USA, California; *Fraser-Jenkins 13546* (H 1667357); FR728949; –. –; AY278397. ***Dryopteris atrata*** (Wall) Ching; –; DQ514493. ***Dryopteris azorica*** (Christ) Alston; A127; The Azores, São Miguel; *Väre 16360* (H 1733689); FR728950; FR731969. ***Dryopteris bissetiana*** (Baker) C. Chr.; –; AY268796[a]. *Dryopteris bodinieri* (Christ) C. Chr.; –; DQ514494. ***Dryopteris campyloptera*** (Kunze) Clarkson; A183; Canada, Quebec, Evain; *Martineau* (H 1686002); FR728951; FR731970. –; AY268801[a]. ***Dryopteris carthusiana*** (Vill.) H.P. Fuchs; A147; Finland, Dragsfjärd; *Väre 16439* (H 807588); FR728952; FR731970. A147; Finland, Dragsfjärd; *Väre 16439* (H 807588); FR728953; –. A187; Finland, Tammisaari; *Väre 17577* (H 812819); FR728954; FR731972. A187; Finland, Tammisaari; *Väre 17577* (H 812819); FR728955; –. A217; Canada, Quebec, Saulaie; *Deshaye 91-528* (H 1700973); FR728956; –. A217; Canada, Quebec, Saulaie; *Deshaye 91-528* (H 1700973); FR728957; –. A219; Russia, Hogland; *Uotila 38970* (H 1662195); FR728958; –. A219; Russia, Hogland; *Uotila 38970* (H 1662195); FR728959; –. –; AY268818[a]. ***Dryopteris championii*** (Benth.) C. Chr.; –; AY268797. ***Dryopteris chrysocoma*** (Christ) C. Chr.; –; DQ514495[a]. ***Dryopteris cinnamonea*** (Cav.) C. Christens.; A190; Mexico, Chihuahua; *Fraser-Jenkins 13499* (H 1667361); FR728960; FR731991. ***Dryopteris corleyi*** Fraser-Jenk.; –; AY268808[a]. ***Dryopteris crassirhizoma*** Nakai; –; AY268805[a]. ***Dryopteris crinalis*** (Hook. & Arn.) C. Chr.; –; AY268774[a]. ***Dryopteris crispifolia*** Rasbach, Reichst. & G.Vida; A129; The Azores, São Miguel; *Väre 16320* (H 1733648); FR728961; FR731973. A129; The Azores, São Miguel; *Väre 16320* (H 1733648); FR728962; –. A131; The Azores, São Miguel; *Väre 16351* (H 1733679); FR728963; FR731974. –; AY268819[a]. ***Dryopteris cristata*** (L) A. Gray; A172; Russia, Jaakkima; *Uotila 42353* (H 720395); FR728964; FR731975. A172; Russia, Jaakkima; *Uotila 42353* (H 720395); FR72865; –. A206; *Martineau* (H 1685981); Canada, Quebec, Arntfield; FR728966; –. A206; *Martineau* (H 1685981); Canada, Quebec, Arntfield; FR728967, –. ***Dryopteris cycadina*** (Franch. & Sav.) C. Chr.; –; AY278400. ***Dryopteris cystolepidota*** (Miq.) C. Chr.; –; AY268813[a]. ***Dryopteris dickinsii*** (Franch. & Sav.) C. Chr.; –; AY268820[a]. ***Dryopteris diffracta*** (Baker) C. Chr.; –; EU797683[b]. ***Dryopteris dilatata*** A. Gray; A146; Italy, Torino; *Camoletto* (H 1589411); FR728968; FR731976. A184; Finland, Sipoo; *Kytövuori 96-212* (H 746589); FR728969; –. A195; Estonia, Tartu; *Alanko 80312a* (H 1677094); FR728970; –. A195; Estonia, Tartu; *Alanko 80312a* (H 1677094); FR728971; –. NW070525; Sweden, Uppland; *N. Wikström 070525* (S); FR728972; –. –; AY268779[a]. ***Dryopteris erythrosora*** (Eat.) Kuntze; –; AY268787[a]. –; DQ514496. ***Dryopteris expansa*** (C.Presl) Fraser-Jenk. & Jermy; A168; Finland, Kevo; *Väre & Juslén Ke11* (live collection); FR728973; FR731977. A180; Germany, Westerwald; *Kalheber* (H 1690229); FR728974; –. A215; Finland, Enontekiö; *Väre 14923* (H 745989); FR728975; FR731978. –; AY268775[a]. ***Dryopteris filix-mas*** (L.) Schott; A176; Sweden, Uppland; *Uotila 40320* (H 1684191); FR728976; –. A177; Finland, Tammisaari; *Väre 17578* (H 812820); FR728977; –. A194; Russia, Karelia, Lake Onega; *Uotila 45197* (H 811003); FR728978; FR731979. A213; Finland, Enontekiö; *Väre 14935* (H 746001); FR728979; FR731980. –; AY268776[a]. –; DQ514497. ***Dryopteris formosana*** (Christ) C. Chr.; –; AY268793[a]. ***Dryopteris fragrans*** (L.) Schott; A158; Russia, Chukotka; *Solstad & Elven 05/0090* (H 1733476); FR728986; FR731981. A159; USA, Alaska, Anchorage; *Solstad 05/1317* (H 1733477); FR728985; –. A167; Finland, Kevo, Linkkapahta; *Väre & Juslén Ke2* (live collection); FR728987; FR731982. A169; Finland, Kevo, Kenesjärvi; *Väre & Juslén Ke12* (live collection); FR728980; –. A170; Finland, Kevo, Jesnalvarri*; Väre & Juslén Ke15* (live collection); FR728981; –. A171; Finland, Kevo, Kotkapahta; *Väre & Juslén Ke18* (live collection); FR728982; FR731983. A178; Russia, Habarovsk; *Alanko 31010* (H 1137070); FR728983; –. A197; Mongolia, Ulan Bator; *Pinter* (H 1556772); FR728984; –. –; AY268800[a]. ***Dryopteris fusco-atra*** var. ***fusco-atra*** (Hillebr.) W.J. Rob.; –; AY268760[a]. ***Dryopteris fusco-atra*** var. ***lamoureuxii*** Fraser-Jenk.; –; AY268783[a]. ***Dryopteris glabra*** (Brack.) Kuntze var. *alboviridis* (W.H. Wagner) D.D. Palmer; –; AY268768[a]. ***Dryopteris glabra*** var. ***flynnii*** D.D. Palmer; –; AY268764[a]. ***Dryopteris glabra*** var. ***glabra****;* –; AY268767[a]. ***Dryopteris glabra*** var. ***hobdyana*** (W.H. Wagner) D.D. Palmer; –; AY268773[a]. ***Dryopteris glabra*** var. ***nuda*** (Underw.) Fraser-Jenk.; –; AY268785[a]. ***Dryopteris glabra*** var. ***pusilla*** (Hillebr.) Fraser-Jenk.; –; AY268763[a]. ***Dryopteris glabra*** var. ***soripes*** (Hillebr.) Herat ex Fraser-Jenk.; –; AY268786[a]. ***Dryopteris goeringiana*** (Kunze) Koidz.; –; AY268790[a]. –; AF515241[c]. ***Dryopteris goldiana*** (Hook.) A. Gray; A202; USA, Vermont; Zika 1984 (H 1469873); FR728988; FR731984. –; AY278403. ***Dryopteris guanchica*** Gibby & Jermy; A207; Portugal, Euskadi, Coiros; *Zúñiga & Alejandre 161. 4-89* (H 1555132); FR728989; FR731992. A207; Portugal, Euskadi, Coiros; *Zúñiga & Alejandre 1614-89* (H 1555132); FR728990; –. NW080429; Canary Islands, Teneriffe; *N. Wikström 080429* (S); FR728991; –. NW080429; Canary Islands, Teneriffe; *N. Wikström 080429* (S); FR728992; –. ***Dryopteris hasseltii*** (Blume) C. Chr.; –; EU797680[b]. ***Dryopteris hawaiiensis*** (Hillebr.) W.J. Rob.; –; AY268784[a]. ***Dryopteris hondoensis*** Koidz.; –; AY268791[a]. ***Dryopteris intermedia*** (Muhl. ex Willd.) Gray; A141; Canada, Quebec, Evain; *Martineau* (H 1685994); FR728993; FR731993. A142; USA, New Hampshire; *Alanko 96215* (H 1695737); FR728994; FR731994. ***Dryopteris intermedia*** (Muhl. ex Willd.) Gray subsp. ***maderensis*** Fraser-Jenk.; –; AY268821[a]. ***Dryopteris juxtaposita*** Christ; –; AY268810[a]. ***Dryopteris lacera*** (Thunb.) Kuntze; –; AY268794[a]. ***Dryopteris lepidopoda*** Hayata; –; AY268789[a]. ***Dryopteris ludoviciana*** (Kunze) Small; A208; USA, South Carolina; *Leonard 2144* (H 1125133); FR728995; –. ***Dryopteris maderensis*** Alston; A139; Madeira, Santana; *Alanko 109381* (H 1722763); FR728996; –. A149; Madeira, Ribeiro Frio; *Väre 16531* (H 1733354); FR728997; FR731985. ***Dryopteris marginalis*** (L.) A. Gray; A188; Canada, Quebec, Mont Orford; *Brisson 80358* (H 1467485); FR728998; –. A203; Canada, Quebec, Mont Bellevue; *Brisson 78625* (H 1250429); FR728999; FR731986. ***Dryopteris mauiensis*** C. Chr.; –; AY268770[a]. ***Dryopteris munchii*** A. Reid Smith; –; AY268822[a]. ***Dryopteris odontoloma*** (Beddome) C. Chr.; –; AY268807[a]. ***Dryopteris oligodonta*** (Desv.) Pic. Ser.; A138; Canary Islands, Teneriffe; *Alanko 74198* (H 1676846); FR729000; –. A156; Canary Islands, Teneriffe; *Kurtto & Helynranta* (H 1733475); FR729001; FR731987. A210; Canary Islands, Teneriffe; *Alanko 74281* (H 1676852); FR729002*;* –. ***Dryopteris oreades*** Fomin; A191; France, Auvergne, Puy de Dôme; *Kukkonen 13904* (H 166776); FR729003; –. –; AY268781[a]. ***Dryopteris pacifica*** (Nakai) Tag.; –; AY268814[a]. ***Dryopteris pallida*** (Bory) C. Chr. ex Maire & Petitm.; A175; Greece, Epirus; *Räsänen* (H 1691248); FR729004; FR731988. –; AY268809[a]. ***Dryopteris patula*** (Sw.) L. Underw.; –; AY268823[a]. ***Dryopteris polita*** Rosenst.; –; EU797684[b]. ***Dryopteris polylepis*** (Franch. & Sav.) C. Chr.; –; AY268798. ***Dryopteris pseudofilix-mas*** (Fée) Rothm.; –; AY278404[a]. ***Dryopteris pycnopteroides*** (Christ) C. Chr; –; AY268799[a]. ***Dryopteris remota*** (A. Braun ex Doell) Druce; –; AY268792[a]. A182; France, Auvergne, Cantal; *Kukkonen 13917* (H1667753); FR731989; –. ***Dryopteris sacrosancta*** Koidz.; –; AY268812[a]. ***Dryopteris sandwicensis*** (Hook. & Arn.) C. Chr.; –; AY268762[a]. ***Dryopteris scottii*** (Bedd.) Ching; –; DQ514498. ***Dryopteris sichotensis*** V. Komarov; –; AY268804[a]. ***Dryopteris sieboldii*** (Van Houtte ex Mett.) Kuntze; –; AY268815[a]. ***Dryopteris sino-fibrillosa*** Ching; –; DQ150394. ***Dryopteris sparsa*** (D. Don) Kuntze; –; DQ514499. ***Dryopteris squamifera*** Ching & S.K. Wu; –; DQ514500. ***Dryopteris stenolepis*** (Baker) C. Chr.; –; AY268824[a]. ***Dryopteris stewartii*** Fraser-Jenk.; –; AY278405. ***Dryopteris subbipinnata*** W.H. Wagner & Hobdy; –; AY268765[a]. *Dryopteris sublacera* Christ; –; AY268788[a]. –; DQ514501. ***Dryopteris submontana*** (Fraser-Jenk. & Jermy) Fraser-Jenk.; –; AF240671. *Dryopteris subreflexipinna* M. Ogata; –; EU797678[b]. *Dryopteris tetrapinnata* W.H. Wagner & Hobdy; –; AY268772[a]. ***Dryopteris tokyoensis*** (Matsum. & Makino) C. Chr.; –; AY268795[a]. ***Dryopteris unidentata*** var. ***paleacea*** (Hillebr.) Herat ex Fraser-Jenk.; –; AY268769[a]. ***Dryopteris unidentata*** var. ***paleacea*** (Hillebr.) Herat ex Fraser-Jenk.; –; AY268769[a]. ***Dryopteris unidentata*** var. ***unidentata*** (Hook. & Arn.) C. Chr.; –; AY268766[a]. ***Dryopteris uniformis*** (Makino) Makino; –; AY268806[a]. ***Dryopteris varia*** Kuntze; –; AY736355[d]. ***Dryopteris wallichiana*** (Spreng.) Hyl.; –; AY268761[a]. ***Nothoperanema rubiginosum*** Smith & Palmer; –; AY268771[a].

---

[a] Geiger & Ranker (2005); [b] Chang & al. (2009); [c] Wang & al. (2003); [d] Lu & al. (2005).