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Exploring the evolution of humus collecting leaves in drynarioid ferns (Polypodiaceae, Polypodiidae) based on phylogenetic evidence

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Abstract. Most species of the paleotropic fern genera *Aglaomorpha* and *Drynaria*, together constituting a monophyletic clade (drynarioid ferns), possess humus-collecting structures as an adaptation to their epiphytic life form. Humus-collectors are either present as a specialized foliar structure (external leaf dimorphism) or as a specialized leaf part (internal dimorphism). Apart from these basic patterns there are several forms of reduction and an internal fertile – sterile dimorphism in *Aglaomorpha*. We present a phylogeny of drynarioid ferns based on morphological and molecular (cpDNA) markers. The genus *Aglaomorpha* was found to be monophyletic, whereas *Drynaria* is likely to be a paraphyletic assemblage including a grade of Himalayan to Southern Chinese taxa basal to *Aglaomorpha*. The evolution of humus-collectors is reconstructed by plotting their character state changes onto the obtained phylogeny. Despite the complex morphological pattern across species, evolution of drynarioid humus-collecting structures can be reconstructed postulating a simple sequence of character state changes based on only a few elementary processes.

Keywords: Dimorphism, drynarioid ferns, epiphytism, nest leaves, phylogeny, Polypodiaceae, *Aglaomorpha*, *Drynaria*.

Introduction

Access to mineral nutrients limits the vigor of epiphytic plants, and several adaptive strategies to improve nutrient availability have been described (Benzing 1990, Zotz and Hietz 2001, Laube and Zotz 2003). One of these strategies is the housing of ants occurring in some flowering plants such as *Myrmecodia* Jack and some ferns such as *Lecanopteris* Reinw. (Benzing 1990, Gay 1993). Collecting humus is another successful strategy found in angiosperms and ferns. These plants impound litter in basket-like structures (nests) created through particular leaf arrangements. Nest-gardens harboring a diverse microfauna are known from Bromeliaceae and some ferns such as *Asplenium nidus* L. (Benzing 1990). In some ferns, the nest is formed by one or a few leaves that are arranged on a long creeping rhizome. The petiole of these leaves is short to absent and their lamina has a broad base that is pressed to the stem of the host-tree. This form of nests is found in *Microsorum linguiforme* (Mett.) Copel. and *M. musifolium* Blume. A further variation of the strategy is found in some ferns by differentiating some

leaves into humus-collectors, whereas the other leaves carry out the usual functions of photosynthesis and spore production (trophosporophylls). This kind of external leaf dimorphism (holodimorphism) is found in two genera of polygrammoid ferns, *Drynaria* (Bory) J.Sm. (Fig. 1A) and *Platycerium* Desv. Recent phylogenetic studies have shown that these two genera are not closely related (Schneider et al. 2002, 2004b, unpubl. data). Whereas the sister clade of *Platycerium*, the genus *Pyrrosia* Mirbel, does not show any humus-collecting structures, *Drynaria,* together with the genus *Aglaomorpha* Schott, forms a clade that displays different kinds of humus collecting structures. In *Drynaria*, these are always specialized leaves distinct from other leaves by the short to absent petiole, the lack of sporangia, and a short period of photosynthetic activity (Fig. 1A).

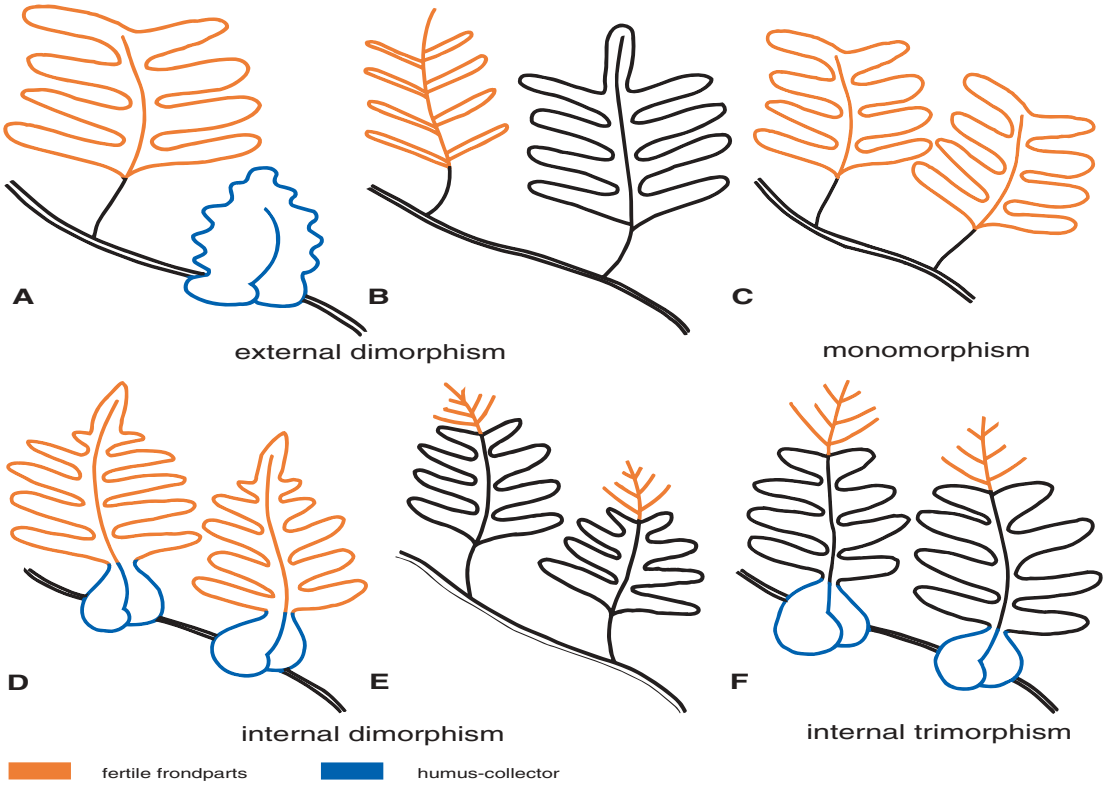

Fig. 1. Morphological types in drynarioid ferns. These schematical line drawings summarize the morphological types that may be found in drynarioids. All pictures show two fronds connected by a rhizome fragment. Except for the rhizome, black lines correspond to sterile, assimilating structures. Fertile structures (orange) are generally assimilating, although often of little importance in this function due to surface reduction. Humus collectors (blue) are more or less rapidly desiccating and assimilate only in the initial stages of their development. External humus collectors dry out more rapidly than internal collectors. Humus collectors are generally sterile. **A** External dimorphism with humus collecting nest leaves is found in most species of *Drynaria*. **B** External dimorphism with fertile and sterile fronds both green and absence of humus collectors is found in *Aglaomorpha parkinsonii*. **C** Strictly internally and externally monomorphic leaves occur in *Drynaria parishii* and facultatively (rarely) in *D. mollis*, *D. rigidula*, and *D. sinica*. **D** Externally monomorphic, but internally dimorphic leaves with basal humus collectors are the condition found in *Aglaomorpha coronans* and *A. heraclea*. **E** Externally monomorphic, but internally dimorphic leaves without humus collectors are found some species of *Aglaomorpha*, e.g. *A. hieronymi*. **F** Externally monomorphic, but internally trimorphic leaves occur in *Aglaomorpha*, e.g. *A. meyeniana*

The cells of humus collectors die after a few months but the dead leaves remain on the plant showing a very slow decay. With the exception of *D. parishii* (Bedd.) Bedd., humus collectors are found in all species of *Drynaria*, but their development is facultative in some. In *Aglaomorpha*, the humus collecting structures are not formed by specialized leaves, instead they are part of the trophosporophyll (Fig. 1D, 1F). This corresponds to an internal leaf dimorphism (hemidimorphism) with a broadened and sessile basal part serving as humus collector. Many species of *Aglaomorpha* display an internal trimorphism, because the upper part of the lamina bears sporangia in its strongly modified apical part only (Fig. 1F). The dimorphism between fertile and sterile lamina parts, with the exception of two species, is always present. The variability of humus collecting structures within the drynarioid ferns, *Aglaomorpha* and *Drynaria*, is intriguing and provokes questions about the evolution of humus collectors in these ferns. Roos (1985) established a phylogeny of drynarioids, based on morphological data but without taking into account any putative relatives as an outgroup. In the present study, we address the evolution of these ferns considering morphological data and chloroplast DNA sequence data, but also rooting the phylogeny with the selligueoid ferns, that have been shown to be the closest relatives of drynarioids (Schneider et al. 2002, 2004a, 2004b). This allows us to explore the origin of humus collectors in their various modifications in drynarioid ferns.

Materials and methods

Morphological data set (MORPH). We sampled a morphological data set for all described species of the genera *Aglaomorpha* and *Drynaria* with the exception of *A. nectarifera* (Baker in Becc.) M.C. Roos. No material was seen from this species that has been collected only once. The morphological data set is based on Roos (1985). The original binary coding was translated into a multi-state coding scheme when applicable. Polymorphic state assignments were allowed. Several characters were excluded for reasons of non-independence, redundancy, high intraspecific variability, or ambiguous definition of states for quantitative characters. We retained 110 unordered equally weighted characters (Appendix 1). Six species of selligueoid ferns, one species of *Arthromeris* J.Sm. and five species of *Selliguea* Bory, were scored to create a multitaxon-outgroup using our own observations and data from Hovenkamp (1998). Characters pertaining to taxa with particular structures such as humus collectors were scored as missing for taxa that lack the particular structure (Maddison 1993). Specimens for morphological studies were obtained from three herbaria (P, L, GOET) and from living material cultivated in the Botanical Garden of the University of Göttingen.

Molecular data set (MOLEC). Sequences of four cpDNA markers – two coding regions (*rbc*L, *rps*4) and two non-coding regions (*trn*L-F IGS, *rps*4-*trn*S IGS) – were generated for 11 of the described 16 species of *Drynaria*, and for 13 of the 15 described species of *Aglaomorpha*. In addition, we sampled the same outgroup as in the morphological data set. Material for DNA extraction was provided from field collections by colleagues, or obtained from cultivated material of various botanical gardens, and from herbarium specimens. A list of vouchers and Genbank accession numbers is given in Appendix 2. Total DNA was extracted from silica dried or fresh leaf material using the Invisorb® Spin Plant Mini Kit (Invitek) and following the manufacturer's protocol with lysis time extended to 60 min. The extracts were used directly for PCR. PCR primers have been described in previous publications: *rbc*L (Haufler and Ranker 1995, Haufler et al. 2003), *rps*4 + *rps*4-*trn*S IGS (Nadot et al. 1995, Smith and Cranfill 2002), and *trn*L-F IGS (Taberlet et al. 1991). Reactions were carried out in a volume of 25 µl, 10 nM dNTP, 20 nM Mg, 0.01 fM primer, 4% (v/v) DMSO, 0.25 u Taq (SilverStar, Eurogentec), and 0.5–2 µl DNA. Following initial denaturation at 94 °C, 30 cycles of 94 °C 1 min, 49 °C 1 min, 72 °C 2 min were run for *rbc*L and of 94 °C 15 s, 52 °C 30 s, 72 °C 1 min for *trn*L-F and *rps*4 + *rps*4-*trn*S. Fragments were purified using the GFX™ DNA and Gel Band Purification Kit (Amersham Biosciences) following the manufacturer's instructions. Purified DNA was directly used for sequencing with the BigDye®

Terminator Cycle Sequencing Kit v3.1 (ABI Prism) and analyzed on an ABI 3100 Genetic Analyzer (Applied Biosystems). Sequences were edited with Chromas 2.23 [http://www.technelysium.com.au], assembled with SeqAssem [http://www.user.gwdg.de/~dhepper/seqassem/index.htm], and aligned manually in Bioedit [http://www.mbio.ncsu.edu/BioEdit/bioedit.html] and MacClade 4.0 (Maddison and Maddison 2000). Ambiguously aligning zones in the spacer regions were excluded prior to phylogenetic analyses. Four non-overlapping indels within the two spacer regions were scored as binary characters and added to the morphological data set in combined analyses with the indel regions excluded from the molecular data set. cpDNA markers were checked for biases in their base-pair frequency. The data matrix is available from the corresponding author upon request.

Total evidence data set (TOEVI). A combined data set was compiled based on the morphological and the molecular data sets. No material for molecular analysis was available for six species. The morphological data for these species were included in this data set. Analyses were performed with and without these six species.

Phylogenetic analyses. Maximum parsimony analyses (MP) were performed for the three data sets (MORPH, MOLEC, TOEVI) with PAUP* 4.0b10 (Swofford 2000) using the heuristic search mode with TBR branch swapping, 1000 random replicates, and MULPARS on. Results of these analyses were summarized as strict consensus trees if more than one most parsimonious tree was found. All characters were treated as equally weighted and unordered. Non-parametric bootstrap trees (Felsenstein 1985) under maximum parsimony (BS-MP) were calculated with 10000 replicates performing heuristic searches, TBR, and ten random additions for each replicate. BS-MP analyses were performed for each of the four partitions of the molecular data set to search for heterogeneity between the partitions (Johnson and Soltis 1998). TreeRot 2.0 (Sorenson 1999) was employed to calculate decay values.

Maximum likelihood analyses (ML) were carried out with PAUP* 4.0b10 for the molecular data set (MOLEC). The implemented model (GTR + I + Γ) was selected applying the Akaike-information-criterion (AIC) using Modeltest (Posada and Crandall 1998). We carried out analyses with alternative models to explore the influence of model parameters on the recovered topology. A non-parametric bootstrap under maximum likelihood (BS-ML) was carried out with 1000 bootstrap replicates performing a heuristic search, TBR, and ten random additions for each replicate.

Bayesian inference of phylogeny (BY) was carried out with MrBayes 3.0 (Huelsenbeck and Ronquist 2001). Four chains were run for ten million generations under the GTR + I + Γ model, sampling every 1000th generation and excluding the burn-in period.

Testing for the monophyly of *Drynaria*. Several tests were employed to infer the compatibility of the two alternative phylogenetic hypotheses found with the morphological and molecular data in the three data sets. The two hypotheses were compared for all data sets (MORPH, MOLEC, TOEVI) including morphological data via a Templeton test (TT) and a Kishino-Hasegawa (KH-MP) test under MP as implemented in PAUP*. Additionally, the Kishino-Hasegawa test (KH-ML) and Shimodaira-Hasegawa test (SH) were carried out under ML for the molecular data (Goldman et al. 2000). Taxa with incomplete data (two or less cpDNA markers sequenced) were excluded from these tests. We also performed a test for monophyly (MON-TE) as described in O'Donnell et al. (2001). The length of the tree was calculated with the monophyly of *Drynaria* constrained and compared to the tree lengths of 1000 unconstrained maximum parsimony bootstrap replicates. The test is interpreted as not significant if the tree length of the constrained tree is equal to or less than that of the shortest tree from the longest 1% of the trees derived from 1000 unconstrained MP bootstrap replicates.

Reconstruction of character evolution. Character state changes were reconstructed using MacClade 4.0 (Maddison and Maddison 2000) for each of the three phylogenetic hypotheses recovered with the three data sets (MORPH, MOLEC, and TOEVI). ACCTRAN and DELTRAN optimizations were compared with and without ordering character states. In addition, a weighting scheme was applied that reflects the evolutionary steps proposed in the model of humus-collector evolution as outlined in the discussion. The weighting scheme reflects a cost-matrix assigning to each character state change an individual cost value. The fit of the model of humus-collector

evolution was explored by comparison of the reconstruction obtained with and without implementing the weighting scheme.

Results

Phylogenetic hypothesis based on morphology. 108 of the 110 morphological characters proved to be parsimony informative for drynarioids. The MP analyses of the MORPH data set (Fig. 2) resulted in 114 most parsimonious trees with the following parameters: tree length 487 steps, CI = 0.3018, HI = 0.6982, RI = 0.6149, RC = 0.1856. Both *Aglaomorpha* and *Drynaria* are found to be monophyletic. The *Drynaria* clade is poorly resolved. *D. fortunei* (Kunze ex Mett.) J.Sm. is indicated as sister to the remainder of the genus, but BS support is lacking for this position. Three clades with some BS support are found within *Drynaria*: (1) *D. bonii* to *D. quercifolia*, (2) *D. delavayi* to *D. sinica*, and (3) *D. parishii* & *D. propinqua*. The *Aglaomorpha* clade is completely resolved but most clades lack BS support. *A. coronans* (Wall. ex Mett.) Copel. is the basal taxon followed by *A. heraclea* (Kunze) Copel. Four clades are found: (1) *A. brooksii* & *A. splendens*, (2) *A. drynarioides* to *A. meyeniana*, (3) *A. cornucopia* & *A. novoguineensis*, and (4) *A. acuminata* to *A. latipinna*.

Phylogenetic hypothesis based on molecular data (maximum parsimony analyses). Of 2498 included sequence positions 192 are parsimony informative for drynarioid ferns. No discordance was detected among the four partitions of the molecular data. MP analyses of the MOLEC data set resulted in 396 most parsimonious trees (Fig. 3) with the parameters: tree length 552 steps, CI = 0.6881, HI = 0.3111, RI = 0.8752, RC = 0.7198. *Aglaomorpha* is found to be monophyletic, whereas *Drynaria* is paraphyletic. *Drynaria* includes two clades with bootstrap support: (1) *D. laurentii* to *D. volkensii*, and (2) *D. bonii* to *D. sparsisora* to which *D. rigidula* (Sw.) Bedd. is sister. *Drynaria fortunei*, *D. sinica* Diels, and *D. mollis* Bedd. form a grade at the base of *Aglaomorpha*. Within *Aglaomorpha*, *A. meyeniana* Schott is found in a basal position followed by *A. coronans*. The remaining lineages are born in a large polytomy, but three clades receive support: (1) *A. heraclea* & *A.* x *leporella*, (2) *A. cornucopia* to *A. novoguineensis*, and (3) *A. hieronymi* to *A. pilosa*. Excluding taxa from the analysis for which only one or two out of four cpDNA markers were sequenced did not influence tree topology and supported clades.

Phylogenetic hypothesis based on total evidence data set. The total-evidence approach was applied despite the MP analyses indicated inconsistencies (see above) between the MOLEC and MORPH data set. However, the two conflicting phylogenetic hypotheses lack bootstrap support > 75%. MP analyses of the TOEVI data set resulted in two most parsimonious trees (Fig. 4) with the parameters: 1068 steps, CI = 0.4427, HI = 0.5573, RI = 0.7309, RC = 0.4147. *Aglaomorpha* is found to be monophyletic, whereas *Drynaria* is paraphyletic. *D. fortunei* and the *D. delavayi* – *D. sinica* clade form a grade at the base of *Aglaomorpha*, but the branches receive no BS support. The remaining species of *Drynaria* form a monophylum with a limited BS support (75%) that includes three supported clades: (1) *D. bonii* to *D. sparsisora*, (2) *D. parishii* & *D. propinqua*, and (3) *D. laurentii* to *D. willdenowii*. *A. coronans* is basal in the *Aglaomorpha* clade followed by *A. meyeniana*. *Aglaomorpha heraclea* and *A.* x *leporella* form a strongly supported clade. Three other supported clades are found: (1) *A. brooksii* & *A. splendens*, (2) *A. cornucopia* & *A. novoguineensis*, and (3) *A. acuminata* to *A. latipinna*. Excluding taxa from the analysis for which no molecular data were available did not influence tree topology and supported clades.

Phylogenetic hypothesis based on molecular data (maximum likelihood analyses). ML analysis resulted in a tree with a maximum likelihood of log = −7074.95 (Fig. 5). The topology is identical to that found in the MP analyses of the same data set (Fig. 3). BS-ML and BY supported the same clades with two exceptions in which BY supports clades with a

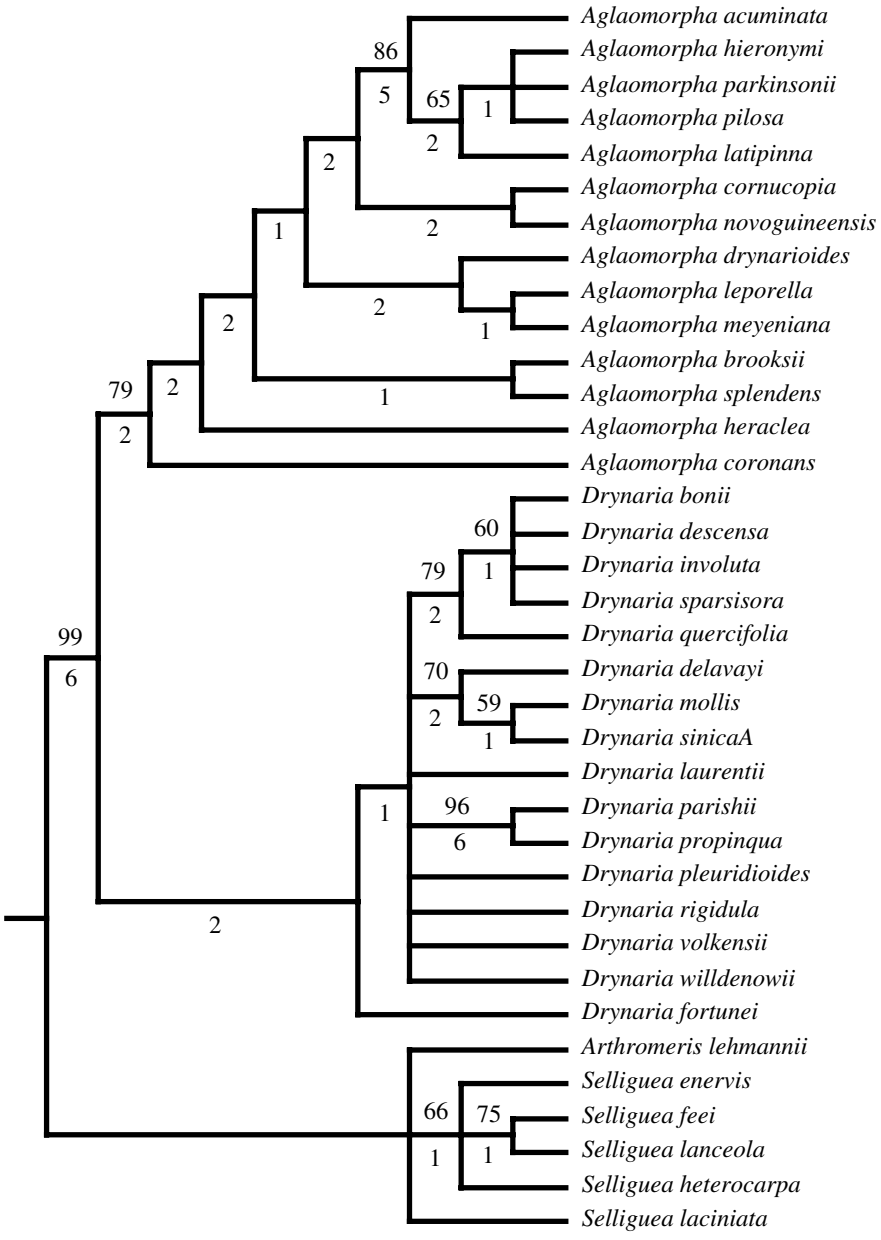

Fig. 2. Phylogenetic hypothesis (MP) based on the morphological data set (MORPH). Strict consensus tree of 36 most parsimonious trees. Bootstrap values $\geq$ 75% are given above and decay indices below branches

BS support below 75%. The *A. hieronymi* to *A. pilosa* clade shows a remarkable long branch separating it from its sister taxon *A. acuminata* (Willd.) P.H.Hovenkamp.

Comparison of results obtained with the different data sets. Besides tests that are implemented in PAUP* such as TT, KH, and SH, we performed a test for monophyly of groups described by O'Donnell et al. (2001). All employed tests showed that the morphological (MORPH) and combined (TOEVI) data sets do not reject the hypothesis of a paraphyletic *Drynaria* (Table 1). Several tests found significant differences in the cpDNA data set (MOLEC) for the monophyly versus the paraphyly hypothesis rejecting the monophyly hypothesis.

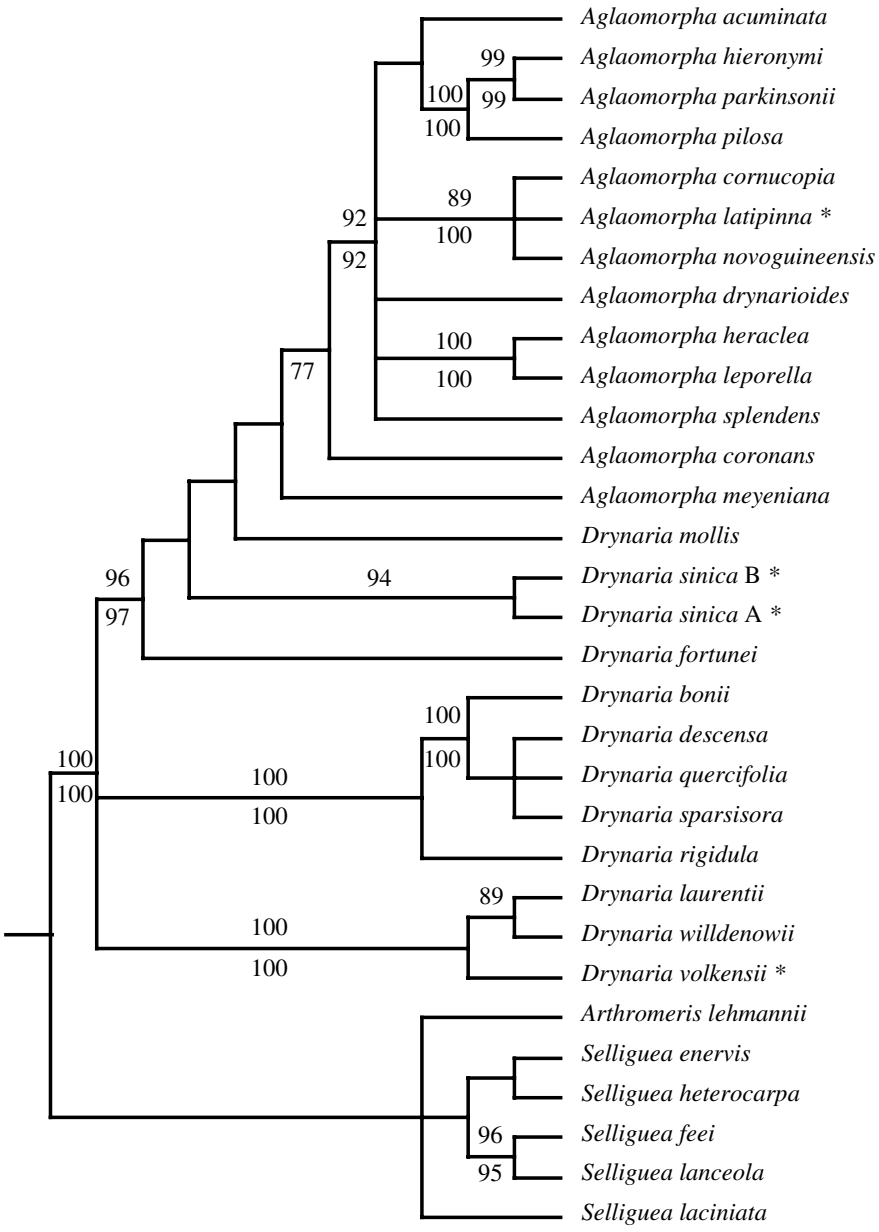

Fig. 3. Phylogenetic hypothesis (MP) based on the cpDNA sequence data set (MOLEC). Strict consensus tree of 396 most parsimonious trees. Bootstrap values $\geq$75% given above branches were calculated for all taxa, whereas bootstrap values below branches were calculated excluding taxa for which sequences of at least one cpDNA marker are missing (*)

However, the monophyly test of O'Donnell et al. (2001) failed to reject the monophyly hypothesis with the molecular data set.

Reconstruction of the evolution of humus collecting leaves based on morphology, cpDNA markers, and combined data. In all three data sets we consistently found evidence for a single origin of the external dimorphism of humus-collectors versus trophosporophylls (Fig. 1A) that is present in all except one species of *Drynaria* (Fig. 6). cpDNA data (MOLEC) indicate this character state as a synapomorphy of the drynarioids and at the same time as the plesiomorphic state within the drynarioid ferns.

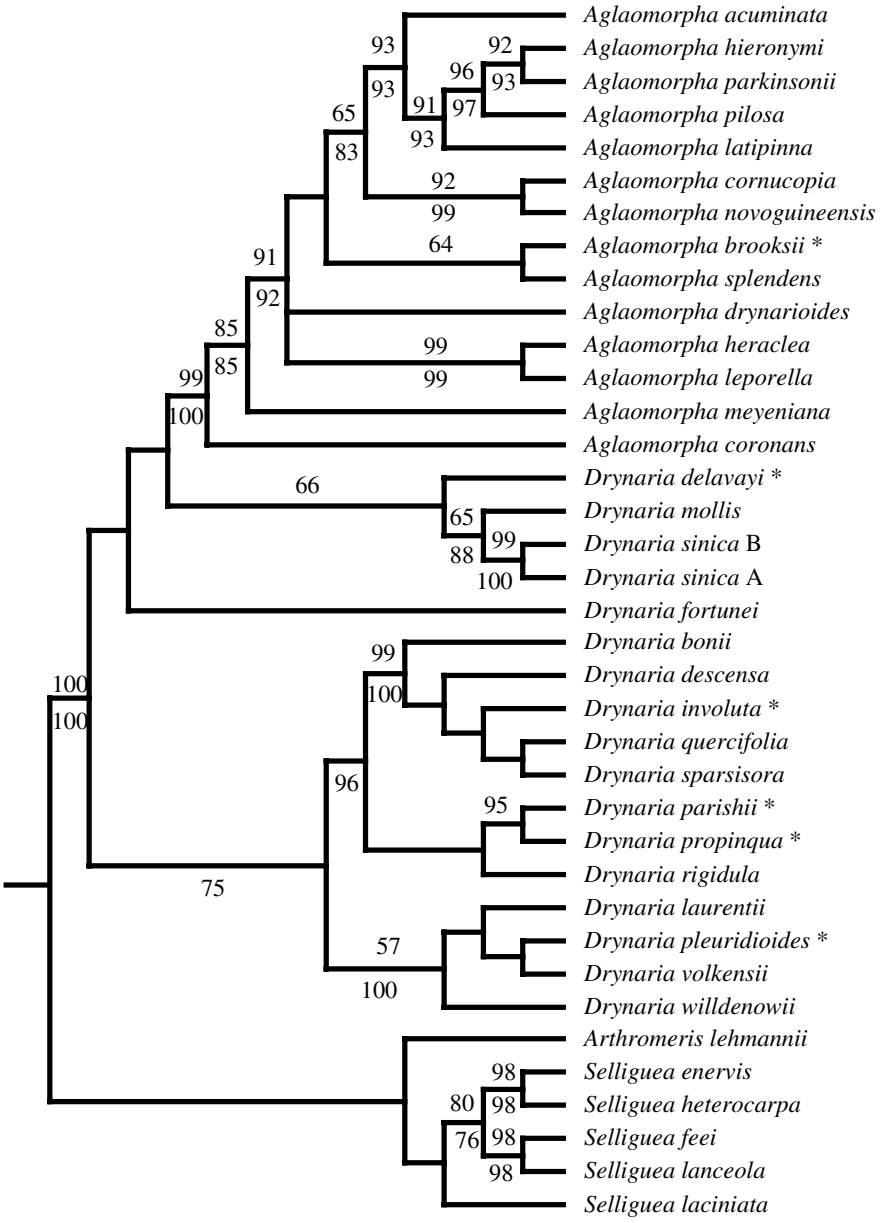

Fig. 4. Phylogenetic hypothesis (MP) based on the combined data set (TOEVI). Strict consensus tree of two most parsimonious trees. Bootstrap values $\geq$75% given above branches were calculated including all taxa, whereas bootstrap values below branches were calculated excluding taxa for which only morphological data were available (*)

In contrast, morphological data (MORPH) alone give ambiguous results because they cannot reject the alternative hypothesis of this character state as a synapomorphy of the genus *Drynaria*. The data set combining morphological and cpDNA evidence (TOEVI) does not support the latter hypothesis. All three data sets suggest that the internal dimorphism of humus-collectors versus trophosporophylls is a synapomorphy of *Aglaomorpha*. A humus collecting part is found in all lineages of *Aglaomorpha* with the exception of the terminal clade including *A. acuminata* to *A. parkinsonii*. Here, internal dimorphism is restricted to a basal trophophyll

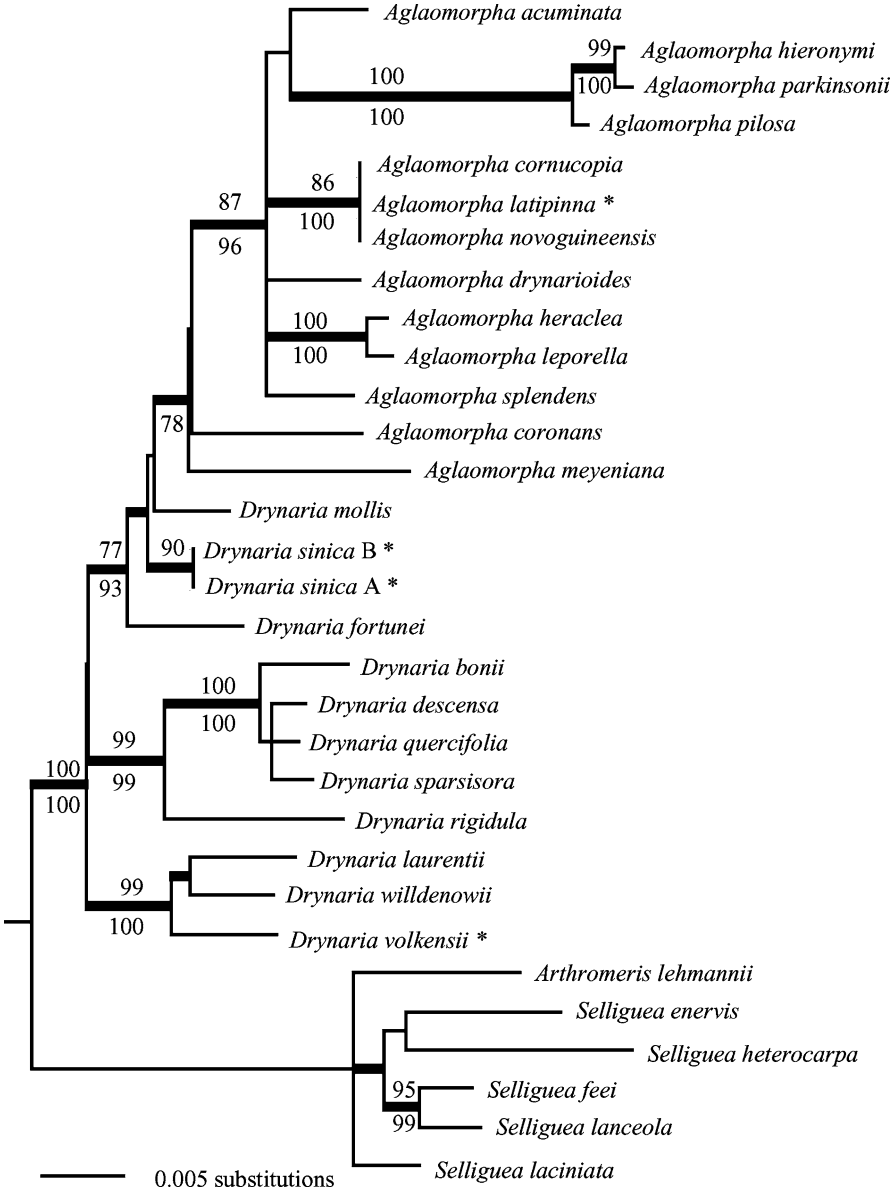

Fig. 5. Phylogenetic hypothesis (ML, BY) based on the cpDNA sequence data set (MOLEC). Phylogram recovered in a maximum likelihood analysis with the GTR + I + Γ model applied. Branch lengths correspond to the estimated amount of substitutions. Thick branches indicate posterior values of $p \geq 0.95$ in Bayesian analyses with all taxa included. Values of ML bootstrapping (≥75%) are given above and below branches. Values above branches were found in BS-ML analyses including all taxa, values below branches were found in analyses excluding taxa with incomplete cpDNA data (*)

and an apical sporophyll. The ancestors of these taxa possessed a humus-collecting part at the base of the leaves that has been lost in the evolutionary sequence. A single species of this terminal clade, *A. parkinsonii*, evolved independently an external leaf dimorphism with a trophophyll and a sporophyll. Both of these hypotheses were recovered with all three data sets. A certain ambiguity persists concerning the transition between the *Drynaria* type of external dimorphism (humus collectors as independent leaves) and the *Aglaomorpha* type of internal

Table 1. Results of statistical tests for *Drynaria* monophyly versus paraphyly. The employed data sets are MORPH = morphological data, MOLEC = cpDNA sequence data, and TOEVI = cpDNA & morphological data. The following tests were conducted: TT = Templeton test, KH-MP = Kishino-Hasegawa test under maximum parsimony, KH-ML = Kishino-Hasegawa test under maximum likelihood, SH = Shimodaira-Hasegawa test, MON-TE = monophyly test according to O'Donnell et al. (2001). Hypotheses are abbreviated as follows: mono = *Drynaria* is monophyletic, para = *Drynaria* is paraphyletic. With the according data set the alternative hypothesis is significantly different from the preferred hypothesis at $p \leq 0.01$ (*)

Data set	Test	Preferred hypothesis	p-Value
MORPH	TT	Mono	0.344
MOLEC	TT	Para	0.004*
TOEVI	TT	Para	0.359
MORPH	KH-MP	Mono	0.207
MOLEC	KH-MP	Para	0.003*
TOEVI	KH-MP	Para	0.251
MOLEC	KH-ML	Para	0.011*
MOLEC	SH-ML	Para	0.011*
MOLEC	MON-TE	Para	>0.01
TOEVI	MON-TE	Para	>0.01

dimorphism or trimorphism (humus collectors at the base of other leaves). This ambiguity is caused by the notable variation of leaf structures in *Aglaomorpha*: An internal dimorphism with a humus collector at the base of a trophosporophyll is found in *A. coronans* only, whereas most species with the exception of the terminal *A. acuminata* to *A. parkinsonii* clade display a trimorphism with a leaf having a humus collector at its base, a trophophyll in the middle, and a sporophyll at the apex. [The terms sporophyll and trophophyll will be retained for internally dimorphic or trimorphic leaves, even though they then refer only to parts of the leaf and not to independent foliar structures as in taxa with externally dimorphic leaves.] Reconstructions using unordered and equally weighted characters resulted in a fully resolved scenario using DELTRAN character optimization, but the character state at the base of *Aglaomorpha* was equivocal if ACCTRAN character optimization was applied. Fully resolved character reconstructions were obtained by using either ordered characters or a weighting scheme reflecting a model of character evolution for ACCTRAN and DELTRAN optimization. These reconstructions indicate that *A. coronans* displays an intermediate state between the external dimorphism of *Drynaria* and the internal trimorphism found in the closely related species of *Aglaomorpha*. The internal dimorphism is based on differentiation of the lamina in a basal humus-collector and an upper trophosporophyll. The application of DELTRAN and ACCTRAN does not alter any other reconstruction of character evolution and the same scenario was obtained for phylogenetic hypotheses that include only taxa with existing cpDNA data.

Discussion

Monophyly versus paraphyly of *Drynaria*. The genus *Drynaria* is found to be monophyletic with the morphological data set only, whereas the molecular and total evidence data set suggest paraphyly of this genus. The bootstrap values for the grade of *Drynaria* basal to *Aglaomorpha* are below 50% with the combined data set indicating a strong conflict between cpDNA data and morphological data. The fit of the two hypotheses – (1) *Drynaria* is monophyletic, and (2) *Drynaria* is paraphyletic – to the molecular and morphological data set has been tested. Several tests were employed to explore the support of the two hypotheses by

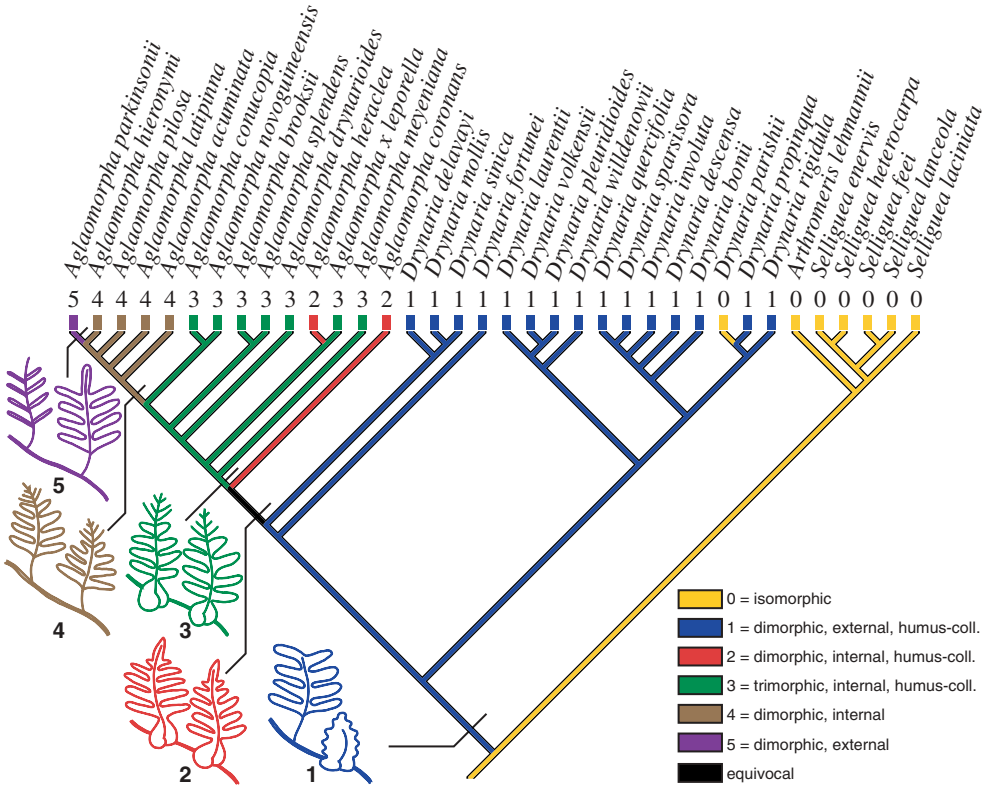

Fig. 6. Reconstruction of the evolution of humus-collectors in drynarioid ferns. Leaf characters are plotted onto the strict consensus tree of the two most parsimonious trees found with the combined data set (TOEVI). Characters were treated as unordered. Both, ACCTRAN and DELTRAN optimizations have been used indicating branches for which the reconstruction was equivocal. Schematic line drawings illustrate the morphotypes found in drynarioid ferns

the given data. Taking into account that tests such as KH tend to be conservative (Goldman et al. 2000), all employed tests indicate that the currently available data fit best with the hypothesis of a non-monophyletic *Drynaria*, but they are insufficient to reject the alternative hypothesis of a monophyletic *Drynaria*. Additional data have to be generated to solve this issue and it will be particularly interesting to employ nucleotide sequence data of nuclear genes. These data may also improve the resolution and support of all clades within the drynarioids.

Relationships of *A. latipinna* and *A.* x *leporella*. Morphological and molecular data provide inconsistent estimates for the phylogenetic position of *Aglaomorpha latipinna* (C.Chr.) M.C.Roos and *A.* x *leporella* (Goebel) C.Chr. The latter taxon is found to be sister of *A. heraclea* with the molecular data set, but morphological evidence indicates *A. meyeniana* being the closest relative of *A.* x *leporella*. A conflict between morphological and cpDNA data is to be expected for *A.* x *leporella*, because this taxon is known to be of hybrid origin between two diploid species of *Aglaomorpha* (Goebel 1928, Roos 1985). Our data indicate *A. heraclea* as one and probably *A. meyeniana* as the other parent. Chloroplasts are usually inherited uniparentally, and thus their origin is not obscured by recombination (Gastony and Yatskievych 1992, Vogel et al. 1998). In most derived ferns the chloroplasts are inherited from the taxon contributing the egg cell. Hence, under the assumption that this also applies to *Aglaomorpha*, *A. heraclea* is

likely to be the maternal taxon. Morphological similarities of the sporophyte support *A. meyeniana* as the other parent, perhaps the taxon contributing the sperm cell. If this is true, the information inherited from the sperm cell has considerable impact on the development of sporophyte characters in these ferns. Our findings are not concordant with Roos (1985) who suggested *A. coronans* x *A. splendens* as hybrid formula, however, not without ambiguity and based on morphological data only. When reconstructing the evolution of humus collectors, the position of *A. heraclea* in molecular and combined topologies requires one additional state change compared to the morphological tree, which is unavailable for plotting character evolution to avoid circular reasoning. This inconsistency is without consequence for the proposed evolutionary scenario.

Only a few hybrids are known for the drynarioids and nearly all are only known from cultivation. Hybridization and perhaps introgression are well documented for temperate ferns, but existing data indicate a lower frequency of these processes in epiphytic tropical ferns (Haufler et al. 2000, Haufler 2002). The hypothesis of rare hybridization of epiphytes in their native environment is supported by the scarcity of hybrids reported in extensive taxonomic studies of various groups of polygrammoid ferns (e.g. Roos 1985; Hovenkamp 1986, 1998). The inclusion of *A.* x *leporella*, only known from cultivation, has demonstrated that the applied approach would likely detect hybridization events by observing conflicts between cpDNA and morphological reconstructions. As discussed in other studies, phylogenetic reconstruction based on cpDNA is not directly influenced by introgression because chloroplast are inherited uniparentally – likely maternal – in derived ferns (Gastony and Yatskievych 1992, Vogel et al. 1998).

Aglaomorpha latipinna is another species with conflicting positions in phylogenetic hypotheses based on cpDNA data and morphological data, respectively. Sporophyte morphology is very similar to species of the *A. hieronymi* – *A. pilosa* clade, but cpDNA indicates close relationships to *A. cornucopia* (Copel.) M.C.Roos and *A. novoguineensis* (Brause) C.Chr. Unfortunately, we were only able to generate a *trn*L-F IGS sequence from a herbarium specimen of *A. latipinna* and further data are needed to verify the signal from this single cpDNA marker. No indication exists that this species is of hybrid origin. The spores are regularly developed which is often not the case in hybrids and their offspring. However, it is known that some hybrids can generate diploid spores by modifying the pathway of spore development (Manton 1950, Lovis 1977). Thus, intact spores can still be found in ferns that originated from a hybridization event many generations ago. Further DNA data are needed to confirm the position of this species.

The evolution of humus collectors. Plotting the observed character states of leaf-morphology onto the phylogenetic hypothesis obtained by combined analyses of the data sets (TOEVI), we generated a hypothesis for the evolution of leaves in drynarioid ferns (Fig. 6). The same sequence of character evolution was recovered using the combined morphology and cpDNA data set (total evidence) and the cpDNA data set alone. The only difference is the interpretation of the status of the internal dimorphism found in *A. coronans*. The total evidence data set suggests this morphological type as a putative intermediate state, whereas it is likely to represent a further modification in the reconstructions based on cpDNA alone. The MOLEC data set does not contain characters derived from morphology and the cpDNA tree can hence be regarded as a hypothesis of phylogeny independent from the characters plotted on this tree. This approach avoids putative problems of circular interpretation. However, the combined data set has the advantage that all available information is integrated (de Queiroz 2000). Only two out of 108 parsimony informative characters in the morphological matrix are based on the presence and structure of humus collectors. It gives us some confidence in our reconstruction, that

both data sets support the same sequence of character evolution (de Queiroz 2000). The advantage of using the combined topology lies in the possibility of including all drynarioid taxa in the scenario, notably those for which no molecular data are available at present. Using morphological data alone, we were unable to reject the suggested model of character evolution, but other models would need to be considered if the morphological topology reflects the true phylogeny.

The scenario (Fig. 7) is based on six steps including only one that is not observed in the reconstruction (Fig. 6). The basal taxa of the selligueoid outgroup have isomorphic leaves lacking any internal dimorphism, but a few species of selligueoids show an external leaf dimorphism, which is common in polygrammoid ferns and may have evolved independently several times. In most cases of dimorphism the functions of photosynthesis and spore production are performed by different leaves (external dimorphism) or by different parts of the leaf (internal dimorphism). The photosynthetic active leaf is called trophophyll, whereas the spore producing leaf is called sporophyll. In polygrammoid ferns, the sporophyll often retains some photosynthetic activity.

Character state 1 (external dimorphism, nest leaves and trophosporophylls) appears to be plesiomorphic for the extant taxa of drynarioid ferns. This condition may have evolved from the hypothetical state (external dimorphism with differentiation into sporophyll and trophophyll) by transformation of the trophophyll into a humus-collector. The sporophyll has to overtake photosynthetic functions. We then call this structure a trophosporophyll. As mentioned above, sporophylls rarely lose photosynthetic activity completely in polygrammoid ferns. The humus-collector differs from regular trophophylls by exhibiting early cell death, and persistence of the dead leaf. In most species of *Drynaria*, humus-collectors are always present, whereas a few species develop humus-collectors only facultatively (observed in *D. mollis*, *D. sinica*, and *D. rigidula*), and one species (*D. parishii*) completely lacks humus collectors. Expression of humus collectors in *Drynaria* might be dependent on ecological conditions and cultivation. For example, the frequently cultivated species *D. rigidula*, regularly develops humus collectors, but some cultivated individuals never form them.

Aglaomorpha is characterized by variable leaf morphology. All phylogenetic reconstructions advocate an internal leaf dimorphism with a basal humus-collector and an apical trophosporophyll as a synapomorphy of the lineage. Total evidence and morphology alone suggest that *A. coronans* displays a transitional character state by showing an internal dimorphism. The other species show a trimorphism by differentiation of three components in each leaf: basal humus-collector, central trophophyll, and apical sporophyll. These leaves have a very short to nearly absent petiole. The humus-collector is deleted in the *A. acuminata* clade and the leaves are composed of only two components, a basal trophophyll and an apical sporophyll. The humus-collector is replaced by a long petiole that resembles those found in *Drynaria* trophosporophylls and other polygrammoid ferns. This appears to be a true reversal. In addition, the resulting internal dimorphism is similar to leaf dimorphisms found in other polygrammoid ferns. *Aglaomorpha parkinsonii*, displaying an external dimorphism with a sporophyll and a trophophyll, is remarkable because this represents the condition hypothesized to be ancestral for extant drynarioid ferns. Here is a remarkable example of evolutionary reversal and a nearly circular evolution of the leaf-morphology of these ferns. Both hypotheses, a monophyletic *Drynaria* or a paraphyletic *Drynaria*, are congruent with the described scenario.

In short, the complex pattern of humus-collecting structures in drynarioid ferns can be described with a relatively simple evolutionary scheme based on a sequential change of character states. Only one hypothetical step is needed and evolution can be described as follows: (1) segregation of developmental pro-

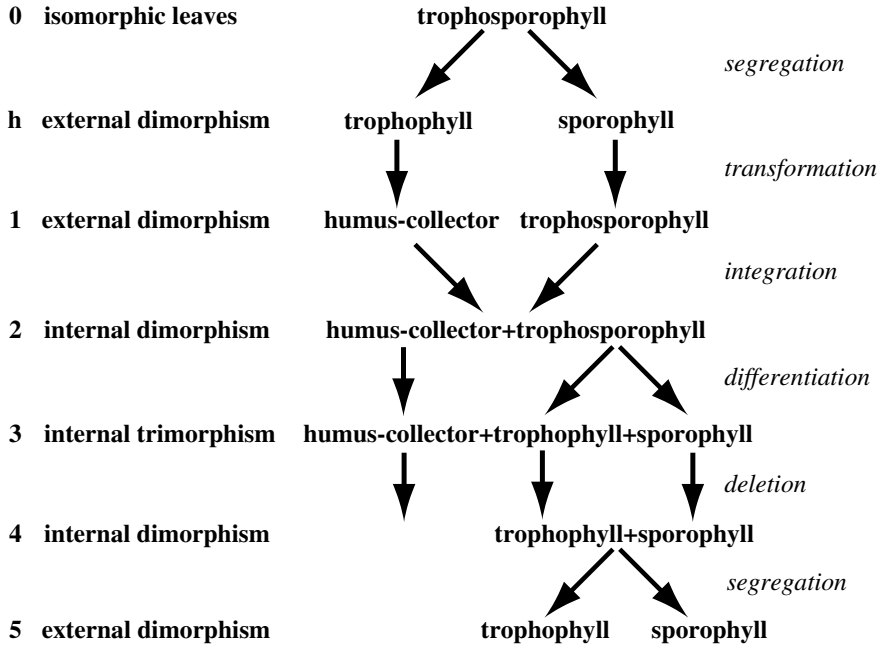

Fig. 7. Scheme of the evolution of humus-collectors in drynarioid ferns. The scheme takes into account the sequence of character state changes reconstructed using maximum parsimony (see Fig. 6). Italics indicate evolutionary processes. The numbers **0** to **5** correspond to character states in Fig. 6; **h** indicates a hypothetical state that is not observed in the phylogeny

grams to be expressed in different leaves, (2) transformation of an existing leaf by altering the developmental program, (3) integration of developmental programs of different leaves in a single leaf, (4) differentiation of components within a structure, for example the trophophyll versus sporophyll parts of the lamina, and (5) deletion of entire structures (Fig. 7).

Humus collecting is a putative key innovation because the improved access to mineral nutrients may provide an adaptive advantage in epiphytic habitats. Hence, its appearance may have been a crucial event inciting the original radiation of drynarioid ferns, but this interpretation does not fit with the observed loss of these structures in the diverse *A. acuminata* clade. However, the diversification of this clade might have been triggered by the colonization of new habitats or new geographical areas (Janssen and Schneider, unpubl. res.). Furthermore, the selligueoid ferns – the sister clade of drynarioids – display a larger species diversity than the drynarioids despite the absence of humus collectors. These ferns show a broad ecological range including epiphytic, epipetric, and terrestrial growth, which may account for their species diversity.

Taxonomic implications. cpDNA sequence data suggest that the genus *Drynaria* is paraphyletic. As this result is still unconfirmed and further studies are needed to generate a sufficiently robust phylogeny, we prefer not to propose a new classification based on the current results. Future studies may provide additional evidence that may lead to the merging of *Aglaomorpha* and *Drynaria* into a unigeneric classification of drynarioid ferns. This would be consistent with our findings that no morphological characters allow to distinguish unambiguously both currently accepted genera. *Aglaomorpha* is found to be monophyletic in our study, which strongly supports Roos' (1985) interpretation of the genus. We do not find any evidence for the resurrection of several small genera Roos included in

Aglaomorpha. Our sampling is insufficient to consider any problems of species delimitation.

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Appendix 1. Morphological characters and data matrix (MORPH). The descriptive terminology of character states follows Roos (1985)

1. General morphology

1. Dimorphism of fertile and sterile fronds or frondparts: 0. absent, 1. holodimorphism, 2. internal dimorphism
2. Nest leafs: 0. absent, 1. present

2. Foliage fronds

– General morphology

3. Lobation: 0. entire, 1. pinnatifid, 2. once pinnate, 3. bipinnate or more complex
4. Frond base or stalk: 0. frond base dilated, unstalked, 1. more or less conspicuously winged stalk, 2. unwinged stalk
5. Pinna shape: 0. equally wide throughout, 1. at least some pinnae with clear basal constriction
6. Relative size of pinnae: 0. clearly decreasing in size and width towards apex. 1. more or less equally sized all over
7. Apical pinna: 0. aborted, 1. present and straight
8. Contraction of fertile pinnae: 0. absent, 1. present
9. Shape of contracted fertile pinnae: 0. string of beads, 1. linear, margin more or less sinusoid
10. Margin of pinnae: 0. serrate, 1. entire
11. Cross-section of petiole/costa: 0. round, 1. flattened to invaginated

– Venation

12. Hydathodes: 0. all free veins terminated by a hydathode, 1. free veins partly terminated by a hydathode, 2. free veins never terminated by a hydathode
13. Lime scales: 0. absent, 1. present
14. Nectaries: 0. absent, 1. present
15. Position of nectaries: 0. acroscopic, 1. basiscopic, 2. acroscopic and basiscopic
16. Secondary veins: 0. straight, 1. more or less zigzagging, 2. not prominent
17. Orientation of free veinlets: 0. excurrent and recurrent, 1. predominantly excurrent, 2. running diffusely
18. Shape of costal areoles: 0. all regular, 1. irregular
19. Presence of costal areoles: 0. present in all frondparts, 1. at least partly absent
20. Branching points of costular tertiary vein and secondary vein: 0. both branching points always clearly distant from primary vein, 1. bordering part of basal secondary veins always shortened, 2. irregular
21. Included venation of primary areoles: 0. containing 0–1 veins, 1. containing always 2 or more veins, 2. variable
22. Branching of included veins in costal areoles: 0. unbranched, 1. unbranched to once dichotomous, 2. more than once dichotomous present
23. Anastomosing included veins: 0. absent, 1. present
24. Subdivision of primary areoles: 0. undivided to once divided, 1. 2–4 secondary areoles, 2. some primary areoles divided into more than 4 secondary areoles
25. Number of primary areoles in areole layering: 0. always 3 or more primary areoles between adjacent secondary veins, 1. occasionally less than 3 primary areoles between adjacent secondary veins
26. Bordering venation of primary areoles: 0. always distinct, 1. (sometimes) indistinct
27. Shape of primary areoles: 0. always regularly shaped, 1. irregularly shaped present
28. Included venation of secondary areoles: 0. largely empty (or with simple veins only), 1. once or more dichotomous veins present
29. Diplodesmic venation: 0. absent, 1. present

– Anatomy

30. Hypodermis of sterile foliage fronds: 0. absent, 1. adaxially, 2. adaxially and abaxially

			10			20	30				40				50		
Aglaomorpha acuminata	202210	1	11100	1111	0	10222111101	121	2	1001	11	1112	1	??	1?	???	?????	??00
Aglaomorpha brooksii	201010	1	11110	0110	0	10221121101	12(01)	2	1102	13	0112	1	??	1?	???	?????	??00
Aglaomorpha coronans	001000	1	0?100	0110	2	1012111110(01)	021	2	000(12)	0?	?102	0	10	1?	???	?????	??01
Aglaomorpha cornucopia	20100?	1	10100	0110	2	10221121101	12(01)	2	100(01)	10	0112	1	??	1?	???	?????	??11
Aglaomorpha drynarioides	201010	1	11100	0110	(02)	11211121101	121	2	100(12)	11	1112	1	??	1?	???	?????	??00
Aglaomorpha heraclea	001000	1	0?110	0110	2	11221121101	011	2	1102	0?	?002	0	31	1?	???	?????	??00
Aglaomorpha hieronymi	201100	1	11110	1111	0	10222111101	111	(12)	0001	11	1112	1	??	1?	???	?????	??00
Aglaomorpha latipinna	201100	1	11100	1111	0	10122111101	121	2	1001	10	0112	1	??	1?	???	?????	??00
Aglaomorpha leporella	201010	1	11100	0110	(02)	11221111100	121	2	1002	13	0112	1	??	1?	???	?????	??00
Aglaomorpha meyeniana	201000	1	10100	011(01)	(02)	10112121101	121	2	100(12)	10	0112	1	??	1?	???	?????	??1?
Aglaomorpha novoguineensis	201000	1	11110	0110	0	10122111101	121	2	1002	10	0112	1	??	1?	???	?????	??00
Aglaomorpha parkinsonii	101110	1	11100	1111	0	10111111101	111	2	1011	0?	?102	1	10	1?	???	?????	??00
Aglaomorpha pilosa	201100	1	11110	1111	0	10222111101	121	2	1011	12	0112	1	??	1?	???	?????	??00
Aglaomorpha splendens	201000	1	11110	0110	(02)	11221121101	121	2	1012	12	0112	1	??	1?	???	?????	??00
Drynaria bonii	111111	0	0?112	0100	2	10221111000	001	2	1000	0?	?000	0	21	12	012	1?00(01)	1011
Drynaria delavayi	111100	0	0?012	0111	2	0012101010(01)	000	0	1000	0?	?10(01)	1	00	00	101	11001	1101
Drynaria descensa	111111	0	0?102	0101	2	1022111111(01)	001	1	1000	0?	?00(02)	0	21	12	012	1200(01)	1111
Drynaria fortunei	111100	1	0?00(01)	0111	2	00122111101	000	1	0000	0?	?101	(01)	10	0(12)	012	11200	1010
Drynaria involuta	111111	0	0?102	0100	2	10220121001	001	2	1000	0?	?10(02)	0	21	12	012	1?21(012)	1111
Drynaria laurentii	111200	0	0?002	0120	2	10221121000	000	1	1002	0?	?10(012)	1	00	11	001	12112	1110
Drynaria mollis	111100	1	0?101	011(12)	1	00200001100	000	0	0000	0?	?10(01)	0	00	00	101	0100(01)	0011
Drynaria sinica	111100	(01)	0?012	011(01)	2	00201011100	000	0	1000	0?	?102	0	00	00	101	0100(12)	0101
Drynaria parishii	001111	0	0?001	0110	2	0000?011100	00(01)	0	1000	0?	?101	0	00	1?	???	?????	??11
Drynaria pleuridioides	111100	0	0?110	0101	2	10112111001	000	1	1002	0?	?102	1	00	11	111	00001	0100
Drynari propinqua	11111(01)	0	0?001	0110	2	00200011100	000	0	1010	0?	?101	(01)	00	10	111	11012	1111
Drynaria quercifolia	111011	0	0?112	0100	2	11211121000	001	2	1002	0?	?10(02)	(01)	21	12	01(12)	12112	1111
Drynaria rigidula	11221(01)	0	0?012	0110	2	00021011100	00(01)	1	100(12)	0?	?10(01)	(01)	00	1(12)	102	12001	1111
Drynaria sparsisora	111111	0	0?112	0100	2	10211121010	001	2	100(01)	0?	?00(02)	(01)	21	1(12)	01(12)	1221(12)	1111
Drynaria volkensii	111101	0	0?011	0111	2	10112121000	000	1	1001	0?	?10(01)	1	00	10	101	01011	0100
Drynaria willdenowii	111100	0	0?002	0110	2	10221121110	000	1	100(12)	0?	?102	1	00	11	102	12001	1111
Arthromeris lehmannii	002210	1	0?110	00?0	0	00011100001	0??	?	????	0?	?01?	0	2(01)	0?	???	?????	??11
Selliguea enervis	0002??	?	??110	00?2	2	?1??????11?	01?	?	????	0?	??0?	0	10	0?	???	?????	??01
Selliguea feei	0002??	?	??110	00?2	2	?1??????11?	02?	?	????	11	1?0?	?	0?	0?	???	?????	??01
Selliguea heterocarpa	0002??	?	??110	00?2	2	?1??????11?	00?	?	????	11	1?0?	?	0?	1?	???	?????	??01
Selliguea laciniata	001201	1	0?010	00?0	2	00210000110	0??	?	????	0?	?00?	0	10	1?	???	?????	??01
Selliguea lanceola	0002??	?	??112	00?2	2	?1??????11?	01?	?	????	1(01)	1?0?	?	0?	0?	???	?????	??00

	60	70	80					90			100			110
	.	.	.					.			.			.
Aglaomorpha acuminata	01111?01(01)	10000?010000010		1	3	1	0	110(01)	00(01)	011	111	00(12)	100?	001
Aglaomorpha brooksii	010111011	00000?010011?10		1	3	1	0	111(01)	011	102	??0	004	1111	000
Aglaomorpha coronans	00110100?	?0000?000100000		1	3	1	0	1111	000	?12	?20	004	1011	000
Aglaomorpha cornucopia	011110011	00000?0101010??		1	1	1	?	111?	011	121	111	01(234)	0?10	000
Aglaomorpha drynarioides	010121111	11100?010001?00		1	3	1	0	1111	000	?02	?20	004	1011	000
Aglaomorpha heraclea	010121111	10000?000000010		1	(23)	1	0	1111	011	102	?20	004	1111	000
Aglaomorpha hieronymi	011111010	101010010100000		1	0	0	1	100(01)	001	011	011	00(01)	0?01	001
Aglaomorpha latipinna	011111010	11000?010000010		1	0	0	1	110(01)	001	011	?11	001	1001	001
Aglaomorpha leporella	010100011	01000?000000000		1	0	1	0	1111	000	?02	??0	003	0?10	000
Aglaomorpha meyeniana	100100011	01000?000000000		1	3	1	0	1111	000	?02	?20	004	1011	000
Aglaomorpha novoguineensis	010121011	00100?010011010		1	3	0	0	1111	001	111	111	00(234)	0?00	00?
Aglaomorpha parkinsonii	01111101(01)	110010011011000		2	0	0	1	100(01)	001	011	111	00(01)	1001	001
Aglaomorpha pilosa	010111010	100011011000000		1	0	0	1	110(01)	001	011	?11	001	1001	001
Aglaomorpha splendens	010112011	11100?010010011		1	3	1	0	111(01)	011	102	120	004	1111	000
Drynaria bonii	00010000?	?0000?020011011		2	2	2	1	1111	001	011	010	00(12)	0?00	000
Drynaira delavayi	001121011	10010?000101100		1	3	1	0	0?11	000	?10	010	101	0?01	010
Drynaria descensa	00010000?	?0000?020001011		2	2	2	0	111(01)	001	121	010	002	0?00	000
Drynaria fortunei	001111011	10000?000110011		2	3	1	0	1111	001	011	010	001	0?00	001
Drynaira involuta	00010000?	?0000?000101011		2	2	2	0	1111	001	120	010	001	0?01	001
Drynaria laurentii	10110100?	?1000?12110001(01)		2	3	1	1	1110	101	001	?10	103	0?00	000
Drynaria mollis	100111011	11000?010100100		1	3	1	0	0?11	000	?10	?10	102	0?00	00?
Drynaria sinica	001121011	11000?010101100		1	0	1	0	1111	000	?10	110	101	0?00	00?
Drynaria parishii	101122111	00000?011101010		2	0	0	1	1110	101	011	?11	001	0?01	001
Drynaria leuridioides	00111100?	?0000?100001?00		2	3	1	1	1111	101	011	0?0	102	0?01	111
Drynaria propinqua	101102011	10000?111101010		2	0	0	1	1110	101	011	?10	00(01)	0?0?	000
Drynaria quercifolia	00010000?	?0000?020101011		(12)	3	1	0	1011	001	012	?10	10(234)	1000	000
Drynaria rigidula	10011200?	?0000?121101001		2	3	1	1	1110	001	011	000	10(12)	0?01	101
Drynaria sparsisora	00010000?	?0000?020111011		2	2	2	1	1111	011	12(12)	110	00(234)	1000	000
Drynaria volkensii	01111100?	?0000?120111011		0	2	1	0	1110	001	101	?10	102	0?00	000
Drynaria willdenowii	0010?100?	?0000?120000000		2	3	1	0	1111	001	001	000	102	0?0?	011
Arthromeris lehmannii	0??0???11	?0000?0??????01		2	0	1	0	???0	0?1	000	110	01?	??0?	???
Selliguea enervis	0?00???11	?0000?0??????11		2	0	1	0	???0	0?1	010	110	010	??01	1??
Selliguea feei	0?00???11	?0000?0?????01		2	2	(01)	2	????	??0	?20	110	010	??01	0??
Selliguea heterocarpa	1?00?0?11	?0000?0?????11		2	3	1	(02)	????	??1	110	110	010	??01	1??
Selliguea laciniata	0?00???11	?0000?0??????11		(12)	0	1	0	???0	0?1	010	110	010	??0(01)	1??
Selliguea lanceola	1?00???11	?0000?0??????01		2	2	1	2	????	??1	020	110	010	??01	1??

31. Colour of epidermis cells after astrablue – safranine staining: 0. blue, 1. red
32. Marginal sclerenchymatous strand: 0. ill-developed (< 10 cells), 1. moderately developed (10–20 cells), 2. well-developed (> 25 cells)
33. Development of vein sheath: 0. conspicuous, clearly sclerenchymatous, 1. inconspicuous, slightly sclerenchymatous
34. Vascular bundles in primary veins: 0. one, 1. two or three
35. Sclerenchymatous strands in vascular bundle: 0. absent, 1. present
36. Number of vascular bundles in petiole/costa: 0. 3–6, 1. 7–10, 2. > 11

– Sori

37. Form of sori: 0. round to elliptical sori, 1. coenosori
38. Shape of coenosori: 0. round to elliptic, 1. linear, 2. quadrangular, 3. irregular quadrangular
39. Coenosori crossing secondary veins: 0. absent, 1. present
40. Soriferous costal areoles: 0. absent, 1. present
41. Distribution of sori relative to frond surface: 0. entire abaxial surface, 1. upper 2/3 or less of abaxial surface
42. Differentiation of sori distribution from basal to apical pinnae: 0. indifferent or from the middle to both ends, 1. from costa towards apex, 2. from apex towards costa
43. Distribution of sori relative to veins: 0. on branching points of 1–4 veins, 1. on branching points of more than 4 veins
44. Main distribution pattern of sori: 0. one row parallel to primary veins, 1. one row parallel to secondary veins, 2. two rows parallel to secondary veins, 3. two rows parallel to costal tertiary veins
45. Number of sori in primary areoles: 0. one, 1. two or more
46. Position relative to surface: 0. sori superficial, 1. sori (slightly) pustulate

3. Base fronds (nest leafs)

– General morphology

47. Lobation: 0. up to 2/3 or more of width, 1. up to 1/2 of width, 2. entire to sinusoid
48. Margin: 0. entire (to somewhat crenulate), 1. more or less regularly denticulate
49. Index: 0. > 1,5, 1. ≤ 1,5

– Venation

50. Secondary veins: 0. straight, 1. more or less zigzagging, 2. not prominent
51. Shape of costal areoles: 0. all regular, 1. irregular
52. Branching points of costular tertiary vein and secondary vein: 0. both branching points always clearly distant from primary vein, 1. bordering part of basal secondary veins always shortened, 2. irregular
53. Included venation of primary areoles: 0. containing 0–1 veins, 1. containing always 2 or more veins, 2. variable
54. Anastomosing included veins: 0. absent, 1. present
55. Subdivision of primary areoles: 0. undivided, 1. two secondary areoles, 2. 3–4 secondary areoles
56. Shape of primary areoles: 0. always regularly shaped, 1. irregularly shaped present
57. Included venation of secondary areoles: 0. always empty, 1. containing (mostly simple) included veins

4. Sporangia and spores

58. Perispore: 0. psilate, 1. verrucate
59. Spines: 0. absent, 1. present
60. Baculae: 0. absent, 1. present
61. Spherical bodies (globules): 0. absent, 1. present
62. Exospore: 0. smooth, 1. verrucate

5. Indument

63. Laminar glandular trichomes: 0. absent, 1. present
64. Number of cells per laminar glandular trichomes: 0. > 90% 2-celled, 1. > 10% 3-celled, 2. more celled present (and then always > 10% 3-celled)
65. Number of cells per receptacular hairy paraphyses: 0. > 75% 3- or less-celled, 1. > 25% 4- or more-celled, 2. > 25% 5- celled (and then always > 25% 4- or more celled)
66. More than two glandular cells per receptacular hairy paraphysis: 0. absent, 1. present
67. Laminar acicular trichomes: 0. absent, 1. present
68. Density of laminar acicular trichomes: 0. tomentose, 1. inconspicuously set
69. Position of laminar acicular trichomes: 0. apical tufts on abscission vein, 1. scattered throughout
70. Thick glandular trichomes with hyaline end cell: 0. absent, 1. present

71. Deviating sporangia: 0. absent, 1. present
72. Glandular sporangial paraphyses: 0. absent, 1. present
73. Acicular sporangial paraphyses: 0. absent, 1. present
74. Position of acicular sporangial paraphyses on capsule: 0. one-sided, 1. two-sided
75. Receptacular scaly paraphyses: 0. absent, 1. present

6. Scales

– Frond scales

76. Attachment: 0. basifix only, 1. pseudopeltate present, 2. peltate present
77. Marginal protuberances: 0. absent, 1. present
78. Marginal glandular trichomes: 0. absent, 1. present
79. Lignified cell walls in frond scales: 0. always absent, 1. (sometimes) present
80. Apex of frond scales: 0. (long) filiform, 1. acuminate to obtuse

– Rhizome scales

81. Insertion: 0. sunken in invaginations, 1. on pustulate protrusions
82. Colour: 0. light brown, 1. dark to blackish
83. Surface: 0. dull, 1. shiny
84. Attachment: 0. basifixed only, 1. basifixed to (at least some) pseudopeltate, 2. peltate
85. Shape: 0. triangular/ovate only, 1. additionally rounded cone-shaped, 2. additionally rounded and spathulate, 3. additionally filiform
86. Exposition: 0. adpressed, 1. spreading, 2. upper part perpendicular
87. Margin: 0. teeth, 1. short to long protuberances
88. Glandular indument: 0. absent, 1. present
89. Position of glandular indument: 0. near the base, 1. overall
90. Distinct apical glandular trichome: 0. absent, 1. present
91. Position of marginal protrusions: 0. uniseriate, 1. biseriate
92. Marginal protrusions composed of more than one cell: 0. absent, 1. present
93. Insertion of marginal indument: 0. marginally only, 1. marginally and abaxially
94. Lignified cell walls: 0. inconspicuous, 1. conspicuous
95. Distribution of lignified cells: 0. around point of attachment, 1. forming a midrib
96. Apex: 0. long (to short) filiform, 1. acute to acuminate (to short filiform), 2. rounded to acute

7. Rhizome

– General morphology

97. Diameter: 0. thin (<1 to 2 cm), 1. intermediate (1 to 3 cm), 2. thick (2 to >3 cm)
98. Wax layer: 0. absent, 1. present
99. Growth habit: 0. encircling bole many times, crusts when terrestrial, 1. spirally climbing (or linearly creeping), 2. encircling bole once (ring shaped basket)
100. Insertion of fronds: 0. less than 10 cm apart, 1. more than 20 cm apart
101. Persistent naked rhachises: 0. absent or rare, 1. present and many
102. Phyllopodia: 0. absent, 1. present

– Anatomy

103. Number of vascular bundles: 0. 10–15, 1. 15–20, 2. 20–30, 3. 30–40, 4. $>$ 40
104. Auxiliary vascular bundles: 0. absent, 1. present
105. Postition of auxiliary vascular bundles: 0. throughout, 1. ventrally
106. Stele type: 0. perforated dictyostele (to polycyclic dictyostele p.p.), 1. polycyclic dictyostele (drynariopsis-type)
107. Bundle sheath: 0. absent, 1. present
108. Sclerenchyma strands in cortex: 0. absent, 1. present
109. Idioblastic strands in roots: 0. absent, 1. present
110. Relative size of vascular bundles: 0. all more or less equally sized, 1. four larger sized bundles dorsally

Appendix 2. Source and voucher information for the taxa included in the molecular data set. Accession numbers for sequences created during this study are in bold face. Voucher information does apply to these sequences only. Most material was taken from cultivated plants. Where available, the garden accession number is indicated. The *rps*4 sequences include the *rps*4-*trn*S IGS region. Two *rbc*L sequences are partial only (*)

Species	Source	Genbank Accession Number			Voucher
		*rbc*L	rps4	trnL-*trn*F	
Aglaomorpha acuminata (Willd.) Hovenkamp	cult. Heidelberg	**AY529147**	**AY529172**	**AY459176**	–
Aglaomorpha coronans (Wall. ex Mett.) Copel.	cult. Heidelberg (105656)	AF470349	**AY459184**	**AY529463**	–
Aglaomorpha cf. *cornucopia* (Copel.) M.C.Roos	cult. München	**AY529148**	**AY529173**	**AY529464**	Janssen 2255 (GOET)
Aglaomorpha drynarioides (Hook.) M.C.Roos	cult. Berlin (239-27-90-33)	**AY529149**	**AY529174**	**AY529465**	Janssen 2256 (GOET)
Aglaomorpha heraclea (Kunze) Copel.	cult. Göttingen	**AY529150**	**AY529175**	**AY529466**	Janssen 2249 (GOET)
Aglaomorpha hieronymi (Brause) Copel.	cult. Heidelberg (100798)	**AY529151***	**AY529176**	**AY529467**	Hagemann 2601 (HEID)
Aglaomorpha latipinna (C.Chr.) M.C.Roos	Indonesia, Irian Jaya	–	–	**AY529468**	J.-M. Mangen 2230 (L)
Aglaomorpha x leporella (K.I.Goebel) C.Chr.	cult. Heidelberg (106062)	**AY529152**	**AY529177**	**AY529469**	Janssen 2253 (GOET)
Aglaomorpha meyeniana Schott	cult. Göttingen	**AY529153**	**AY459185**	**AY529470**	Janssen 2260 (GOET)
Aglaomorpha novoguineensis (Brause) C.Chr.	cult. Berlin (178-10-86-33)	**AY529154**	**AY529178**	**AY529471**	Janssen 2254 (GOET)
Aglaomorpha parkinsonii (Baker) Parris et M.C.Roos	cult. Göttingen	**AY529155***	**AY529179**	**AY529472**	Janssen 2259 (GOET)
Aglaomorpha pilosa (J.Sm. ex Hook. et Bauer) Copel.	cult. Berlin (239-09-90-33)	**AY529156**	**AY529180**	**AY529473**	Janssen 2258 (GOET)
Aglaomorpha splendens (J.Sm. ex Hook. et Bauer) Copel.	private cult. (C. Alford, Florida)	**AY529157**	**AY529181**	**AY529474**	A. R. Smith s.n. (UC)
Drynaria bonii Christ	cult. Berlin (234-28-97-83)	**AY529158**	**AY529182**	**AY529475**	Janssen 2248 (GOET)
Drynaria descensa Copel.	cult. Heidelberg (106187)	**AY529159**	**AY529183**	**AY529476**	Schneider s.n. (GOET)
Drynaria fortunei (Kunze ex Mett.) J.Sm.	cult. Göttingen	**AY529160**	**AY529184**	**AY529477**	Janssen 2252 (GOET)

Appendix 2 (Continued)

Species	Source	Genbank Accession Number			Voucher
		*rbc*L	rps4	trnL-*trn*F	
Drynaria laurentii (Christ ex de Wild. & T.Durand) Hieron.	cult. Selby (97-0378)	**AY529161**	**AY529185**	**AY529478**	A. R. Smith s.n. (UC)
Drynaria mollis Bedd.	cult. Göttingen	**AY529162**	**AY529186**	**AY529479**	Janssen 2257 (GOET)
Drynaria quercifolia (L.) J.Sm.	cult. Göttingen	**AY529165**	**AY529187**	**AY529480**	Janssen 2247 (GOET)
Drynaria rigidula (Sw.) Bedd.	cult. Berlin (082-04-97-50)	**AY529166**	**AY529188**	**AY529481**	Janssen 2251 (GOET)
Drynaria sinica Diels (A)	China, Xizang	**AY529163**	–	–	G. Miehe & U. Wuendisch 94-155-28
Drynaria sinica Diels (B)	China, Xizang	**AY529164**	–	–	G. & S. Miehe 98-13402 (GOET)
Drynaria sparsisora (Desv.) T.Moore	cult. Tübingen	**AY529167**	**AY529189**	**AY529482**	Janssen 2246 (GOET)
Drynaria volkensii Hieron.	Tanzania, Kilimandjaro	**AY529168**	**AY529190**	–	Hemp A 3635 (UBT)
Drynaria willdenowii (Bory) T.Moore	cult. Berlin (239-15-90-33)	**AY529169**	**AY529191**	**AY529483**	Janssen 2250 (GOET)
Arthromeris lehmannii (Mett.) Ching	Taiwan	AY096198	AY096216	**AY459177**	Cranfill TW-77 (UC)
Selliguea enervis (Cav.) Ching	Java	AY096200	AY096218	**AY459178**	Wilson 2893 (UC)
Selliguea feei Bory	Java	**AY529170**	**AY529192**	**AY459179**	Wilson 2862 (UC)
Selliguea heterocarpa (Blume) Blume	Malaysia	**AY459172**	AY362619	**AY459180**	Jaman 5897 (UC)
Selliguea laciniata (Bedd.) Hovenkamp	Malaysia	**AY529171**	**AY529193**	**AY529484**	Jaman 5894 (UC)
Selliguea lanceola (Mett.) E.Fourn.	New Caledonia	**AY459173**	**AY459186**	**AY459181**	Munzinger et al. 1253 (P)

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