

Phylogenetic relationships of Cyrillaceae and Clethraceae (Ericales) with special emphasis on the genus *Purdiaea* Planch.

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Abstract

The phylogenetic relationships and systematic position of the three genera of Cyrillaceae (Ericales), *Cyrilla*, *Cliftonia*, and *Purdiaea*, were investigated by jackknife analysis of a combination of DNA sequences from the plastid genes *atpB*, *ndhF*, and *rbcl*. The results show that *Cyrilla* and *Cliftonia* together are the sister group of Ericaceae, whereas *Purdiaea* groups with *Clethra* of Clethraceae. Together, *Clethra* and *Purdiaea* form the sister group of (*Cyrilla*+*Cliftonia*)+Ericaceae. It is concluded that *Purdiaea* should be moved to Clethraceae. A cladistic analysis based on morphological data was performed to investigate relationships among the species of *Purdiaea*. The results indicate that *Purdiaea belizensis* from Central America is sister to all other species of the genus, and that *Purdiaea nutans* from northern South America is sister to the remaining group of species which are all Cuban endemics, among which *P. cubensis* from Pinar del Rio in western Cuba is sister to the eight species occurring in the Oriente province in eastern Cuba.

Key words: Clethraceae, Cyrillaceae, DNA, Ericales, morphology, phylogeny

Introduction

In a recent paper on phylogenetic relationships among families of the Ericales (Anderberg et al. 2002), a monophyletic group formed by the six ericoid families Actinidiaceae, Roridulaceae, Sarraceniaceae, Clethraceae, Cyrillaceae, and Ericaceae was identified. The first three of these families constituted the sister group of the remaining three among which Clethraceae was found to be sister to Cyrillaceae+Ericaceae. Cyrillaceae, in its traditional sense, has three genera. *Cyrilla* L. is found from southern North America to northern South America. In this genus a number of species has been described, but Thomas (1960) recognized only one variable species. *Cliftonia* Gaertn. f. has a single species in southeastern North America, and *Purdiaea* Planch. has 11 species in South and Central America, most of which grow in eastern Cuba. The study of Anderberg et al. (2002) included only *Cyrilla* as representative of Cyrillaceae. Since this genus is different in some respects from the two other genera in the family, e.g. in having haplostemonous flow-

ers, more detailed sampling was needed for a better understanding of phylogenetic relationships within Cyrillaceae and with other families in this clade. Many authors (e.g. Cronquist 1981, Dahlgren 1989, Takhtajan 1997) have pointed to the similarity between Clethraceae and Cyrillaceae, and particularly the genera *Clethra* L. and *Purdiaea* share a number of features, which Anderberg (1993) discussed as possible symplesiomorphic similarities. Such character states include the presence of inverted, versatile, poricidal anthers and sympetalous corollas (Fig. 1 A–C), that stand in contrast to the non-inverted anthers opening with slits, and to the choripetalous flowers of *Cyrilla* and *Cliftonia* (Fig. 1 D–E), although most *Clethra* also have choripetalous flowers. Mabblerley (1997) listed Clethraceae as a synonym of Cyrillaceae, including *Clethra* as a fourth genus of Cyrillaceae, and it is noteworthy that one species of *Purdiaea*, viz. *P. belizensis* (A. C. Smith & Standley) J. L. Thomas was originally described as a second genus of Clethraceae, albeit with some hesitation (*Schizocardia* Smith & Standley 1932, Standley & Record 1936, Thomas 1961).

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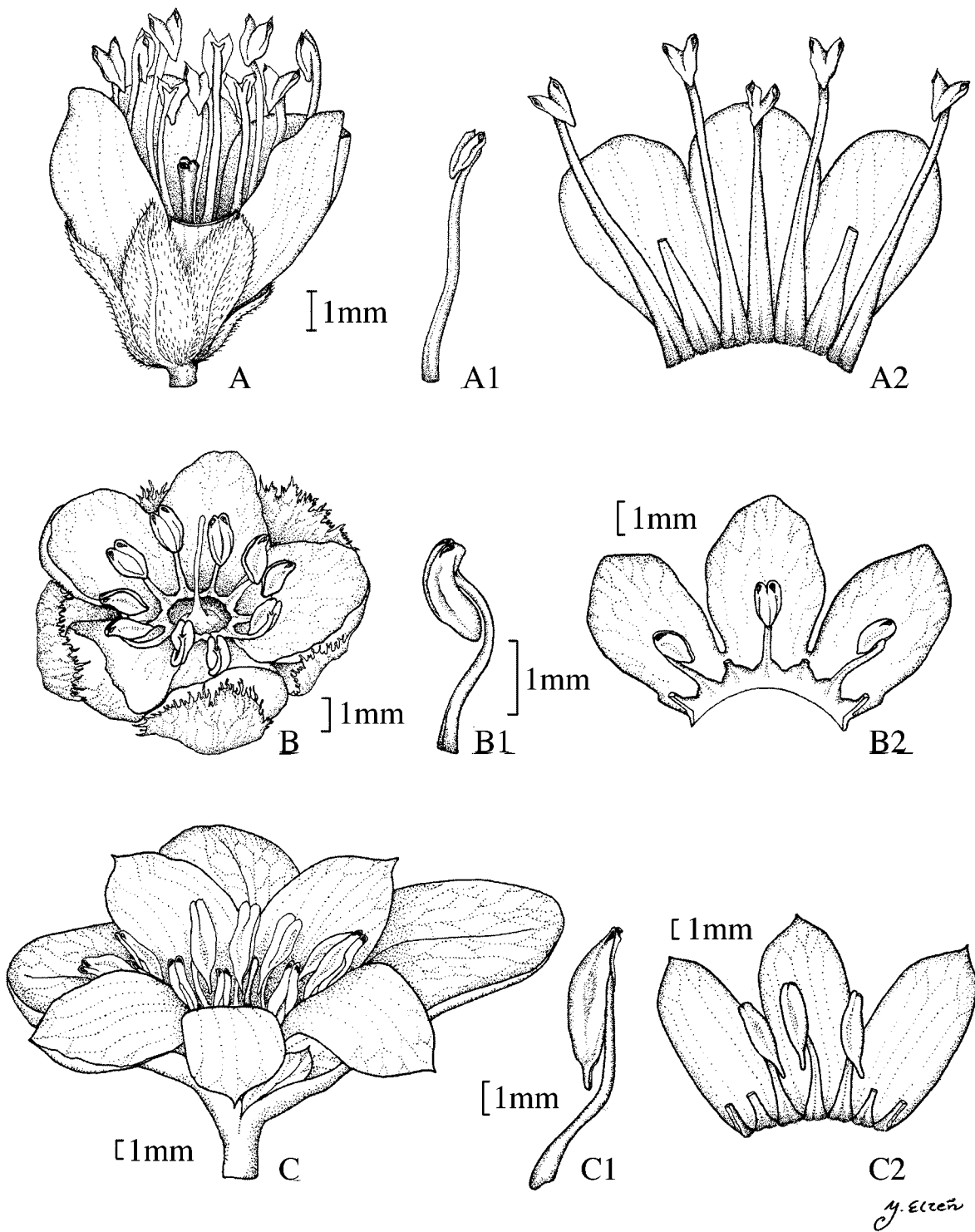


Fig. 1. Flower morphology. A: *Clethra alnifolia*. A1: Stamen. A2: Part of opened flower with two stamens removed. B: *Purdiaea belizensis*. B1: Stamen. B2: Part of opened flower with four stamens removed. C: *Purdiaea nutans*. C1: Stamen. C2: Part of opened flower with four stamens removed. - A: Brumbach 8605 (S). B: Monro & Cafferty 2615 (MO). C: Harling & Andersson 22028 (S).

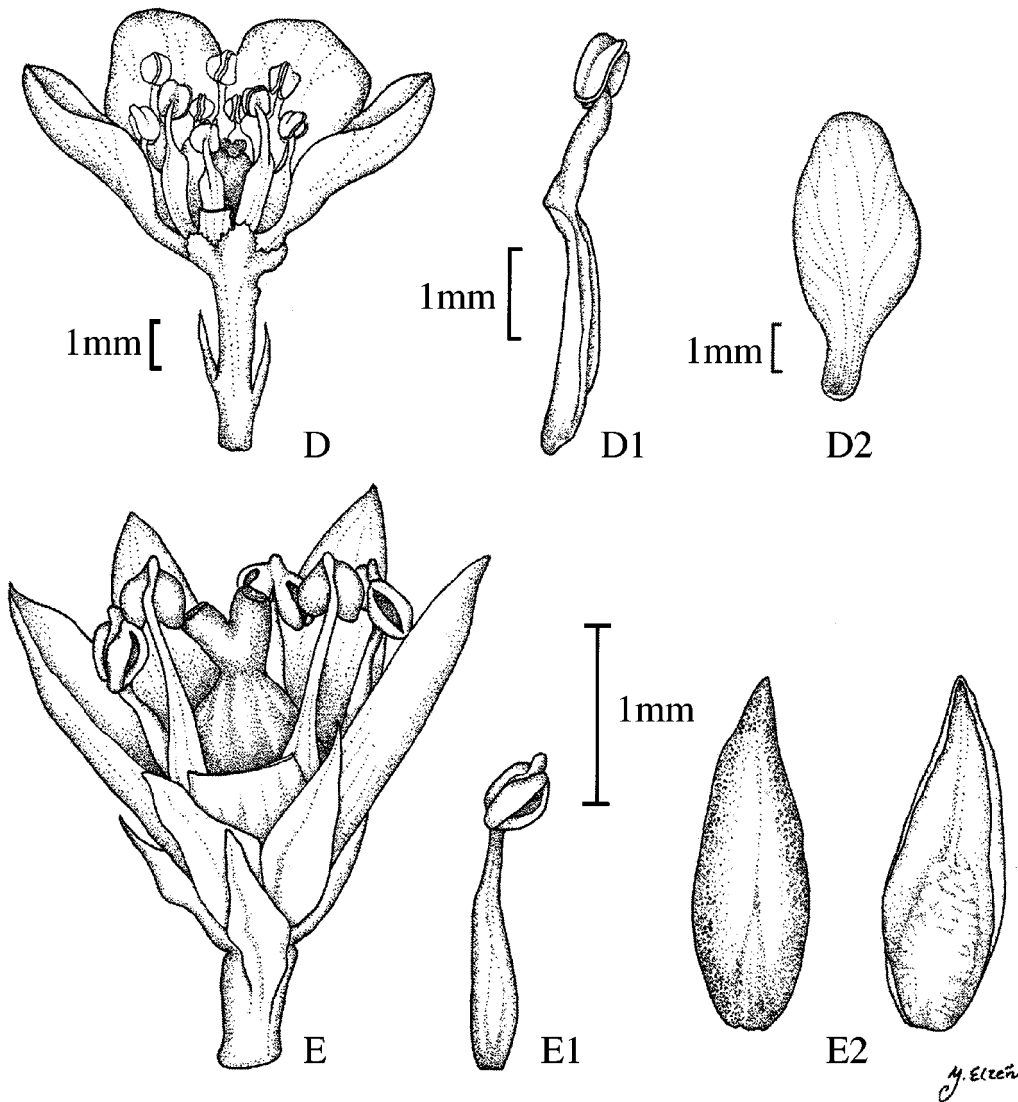


Fig. 1 (continued). Flower morphology. D: *Cliftonia monophylla*. D1: Stamen. D2: Petal. E: *Cyrilla racemiflora*. E1: Stamen. E2: Petals. - D: Biltmore herbarium 5979b (S). E: Segel & Thompson 193 (S).

If the observed similarity between *Purdiaea* and *Clethra* is symplesiomorphic, the former would probably be the sister group of the two other genera in the Cyrillaceae. In the present investigation, our aims were to test the position of the family in relation to the surrounding families identified by Anderberg et al. (2002), the monophyly of Cyrillaceae, and the relationships within genus *Purdiaea*.

Materials and methods

Taxa

We added eight new sequences from three species (*Purdiaea nutans* Planch., *P. stenophylla* Grieseb., and *Cliftonia mono-*

phylla (Lam.) Britt.) to the plastid gene data set (*atpB*, *ndhF*, and *rbcL*) of a previous study (Anderberg et al. 2002), comprising a larger sample from all families recognized in the order Ericales by APG (1998) plus Sladeniaceae and Pentaphragmaceae. Two mitochondrial genes (*atp1* and *matR*) that were also included in the study by Anderberg et al. (2002) did not give PCR products with *Purdiaea*, and could therefore not be used in the present study. The morphological investigations were based on herbarium material from the following herbaria, abbreviated as in Holmgren et al. (1990): BM, BP, GH, MO, NY, S. Requests for loans of herbarium material were also sent to three Cuban herbaria, but no replies were obtained.

Molecular methods

DNA was extracted from leaves taken from herbarium specimens [*Purdiaea nutans*, Harling and Andersson 22028 (S)], ma-

terial collected in silica gel [*Purdiaea stenopetala* Grieseb., Cuba, Gutiérrez & Nilsson 9 (S)], or fresh leaves [*Cliftonia monophylla*, Anderson 19705 (S)]. Leaves were ground with a Mini-Bead Beater (BioSpec Products, Bartlesville, Oklahoma, U.S.A.) and subsequently treated with the DNEasy plant DNA extraction kit from Qiagen (Qiagen Inc., Valencia, California, U.S.A.), following the manufacturer's protocol. PCR was performed with 10 μ mol/l primers in 25- μ l reactions using "Ready-to-go" PCR beads from Pharmacia Biotech (Amersham Pharmacia Biotech, Uppsala, Sweden) following the manufacturer's standard protocol and suggested thermal cycling profile, generally 95 °C for 5 min, followed by 35 cycles of 95 °C 30 s, 50 °C 30 s, 72 °C 1 min 30 s, and finally 72 °C for 8 min.

For sequencing reactions the "Big Dye Terminator Sequencing" kit (Applied Biosystems, Warrington, Cheshire, UK) was used, and fragments were separated on an ABI377 from Applied Biosystems. Primers used for PCR and for sequencing were the same as those described by Källersjö et al. (2000). Sequence fragments were assembled with the Staden software (Staden et al. 1998), and aligned manually with the AssemblyLign software (Oxford Molecular Group Inc., Campbell, California, U.S.A.). Alignment was unproblematic with only a few small gaps in *ndhF*. The new sequences have been submitted to GenBank (Accession numbers AY082692-AY082699).

Phylogenetic analyses

Two different data sets were analyzed. First an analysis of the molecular data was analyzed to find the systematic positions of *Purdiaea* and *Cliftonia* in relation to *Cyrilla*, *Clethra* and other groups of the Ericales. Based on the results of this initial analysis, a second analysis based on morphological data was performed to investigate species relationships in *Purdiaea*.

The molecular data from a combination of *atpB*, *ndhF*, and *rbcL* sequences was analyzed with parsimony jackknifing (Farris et al. 1996) using the computer software "Xac" (Farris 1997, Källersjö et al. 1998) with the following settings: 1000 replications, each with branch-swapping and ten random addition sequences. For this analysis, *Cornus* L. was used as outgroup, because it belongs to one of the major clades of the Asteridae that is a potential sister group of Ericales (Anderberg et al. 2002). Gaps

found in the *ndhF* sequences were treated as missing information. Analyses were performed with all codon positions included, as this has been demonstrated to give the best resolution and a higher number of supported groups than analyses of first and second codon positions, or of transversions only (Källersjö et al. 2000).

The morphological data set (Table 1) was also analysed with the "Xac" software, using the same settings as described above. Species delimitation in Cuban *Purdiaea* followed Berazain & Rodrigues (1992). The morphological analysis used *Cyrilla* and *Cliftonia* as outgroup, based on the results of the analysis of molecular data. One species of *Clethra* (*C. alnifolia* L.) was also included. A parsimony analysis was also performed on the morphological data set, using Hennig86 (Farris 1988) and the following options: mh* (constructing several initial cladograms by adding taxa in several different sequences, and retaining the shortest cladogram of each) with a subsequent bb* (generating multiple equally parsimonious cladograms). All characters were coded as non-additive (cc-).

Results

The jackknife analysis of the molecular data, with the three genes *atpB*, *ndhF*, and *rbcL* in combination, resulted in a tree (Fig. 2) with the same basic tree topology as the one obtained for the three plastid genes by Anderberg et al. (2002), and congruent with their more resolved five-gene tree. The close relationship between the families Actinidiaceae, Sarraceniaceae, Roridulaceae as sister to a clade with Clethraceae, Cyrillaceae, and Ericaceae received 67% jackknife support, and the clade with Clethraceae, Cyrillaceae, and Ericaceae got 89% support. In the present analysis, the two species of *Purdiaea* and the two *Clethra* species formed a monophyletic group with 88% support, and on the next higher node *Cyrilla* and *Cliftonia* grouped together (100% support) forming the sister group of Ericaceae (84% support).

The jackknife analysis of the morphological data resulted in a tree with well resolved basal nodes but with more or

Table 1. Data matrix used for cladistic analysis of morphological data. *Cyrilla* and *Cliftonia* were used as outgroup following the results of the analysis of molecular data. The characters are discussed in Appendix 1. The resulting jackknife tree is shown in Fig. 3.

<i>Cyrilla racemiflora</i> L.	1?000	?01?0	10010	01011	11100	00111	1
<i>Cliftonia monophylla</i> (Lam.) Britt.	1?110	?1??0	10010	01011	11100	10131	1
<i>Clethra alnifolia</i> L.	00000	?00?0	00000	01000	00?00	00000	0
<i>Purdiaea belizensis</i> (Smith & Standl.) Thomas	1??00	?0100	01100	10100	00001	11120	1
<i>Purdiaea cubensis</i> Urb.	1??11	00000	01102	10100	00011	1112?	1
<i>Purdiaea ekmanii</i> Marie-Vict.	01?11	11010	01101	?0100	00011	1112?	1
<i>Purdiaea microphylla</i> Britt. & Wils.	02?11	11011	01101	00100	00011	11120	1
<i>Purdiaea moaensis</i> Marie-Vict.	02?11	01010	01101	00100	00011	11120	1
<i>Purdiaea nipensis</i> Marie-Vict.	02?11	01010	01102	10100	00011	1112?	1
<i>Purdiaea nutans</i> Planch.	1??10	?0000	01102	10100	00011	1112?	1
<i>Purdiaea ophiticola</i> Vict.	1??11	11010	01102	?0100	00011	1112?	1
<i>Purdiaea shaferi</i> Britt.	1??11	01010	01101	10100	00011	11120	1
<i>Purdiaea stenopetala</i> Grieseb.	1??11	11010	01101	00100	00011	1112?	1
<i>Purdiaea velutina</i> Britt. & Wils.	01?11	11010	01101	00100	00011	11120	1

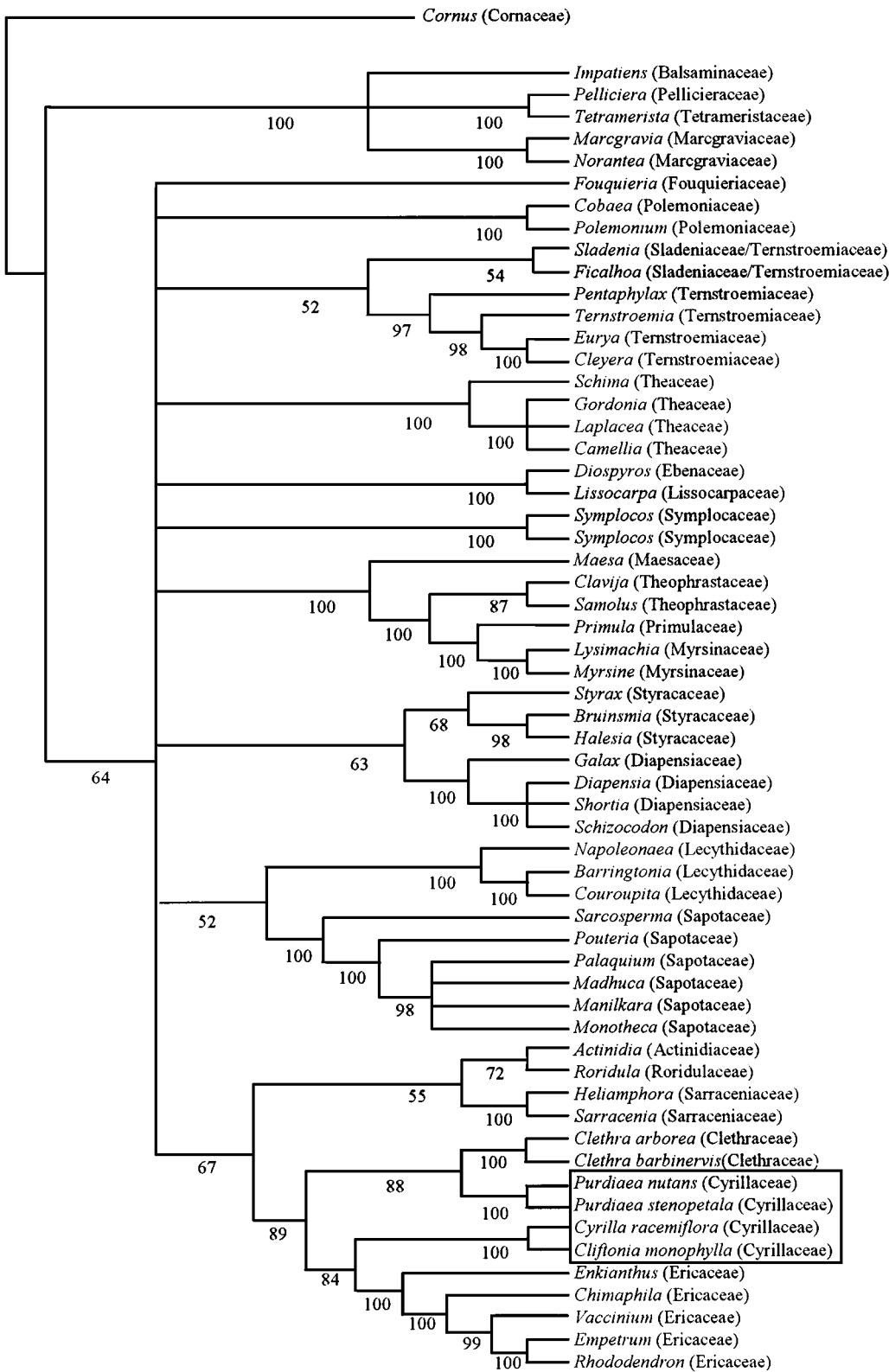


Fig. 2. Tree based on jackknife analysis of a combination of DNA sequences from the chloroplast genes *atpB*, *ndhF*, and *rbcl*. Only groups with jackknife support >50% are shown. *Purdiaea* is the sister group of *Clethra*, whereas *Cyrilla* and *Cliftonia* together is the sister group of Ericaceae. The boxed area shows the traditional circumscription of Cyrillaceae (e.g. Thomas 1960, Cronquist 1981, Takhtajan 1997).

less unresolved relationships between the Cuban endemic species (Fig. 3). In the jackknife analysis of morphological data, *Purdiaea* is monophyletic (99% support) with *P. belizensis* as sister to all other species (92% support). On the next higher node, *P. nutans* is sister to the rest of the species (64% support), and *P. cubensis* Urb. is sister to the remaining Cuban endemic species (85% support) which form an unresolved polychotomy. The Hennig86 analysis of the morphological data resulted in 36 equally parsimonious cladograms (44 steps, consistency index 0.79, retention index 0.83). The strict consensus tree (not shown) has exactly the same topology as the jackknife tree in Fig. 3.

Discussion

Our results show that the Cyrillaceae is not a monophyletic group as presently circumscribed (e.g. Thomas 1960, Cronquist 1981, Takhtajan 1997), since *Cyrilla* and *Cliftonia* together form the sister group of Ericaceae (Fig. 2), whereas the third genus, *Purdiaea*, is sister to the hitherto monogeneric Clethraceae. Therefore, *Purdiaea* is moved from Cyrillaceae to Clethraceae, a family now comprising two genera instead of one. The sample of species from *Clethra* included in the molecular analysis is small in relation to the number of recognized species in that genus, but the two included taxa (*Clethra arborea* Ait. and *C. barbinervis* Sieb & Zucc.) represent both subgenera (Sleumer 1967), and since they form a clade, it is reasonable to believe that *Clethra* is monophyletic. The genus *Clethra* is morphologically rather homogeneous, and its morphological homogeneity strengthens the conclusion from the

molecular data that it is monophyletic. One potential synapomorphy diagnosing *Clethra* could be a three-carpellate gynoecium, but the number of carpels forming the ovary varies in surrounding groups; five to three in Actinidiaceae, five in Sarraceniaceae, three in Roridulaceae, and primitively five in Ericaceae, e.g. in *Enkianthus* Lour. and the pyroloid taxa (Anderberg 1993, Kron & Chase 1993). *Enkianthus* is the sister group of all other Ericaceae, including the pyroloids, epacrids and empetrids (i.e. former families Pyrolaceae, Epacridaceae, and Empetraceae; Anderberg 1993, Kron & Chase 1993), and has retained several character states that are found in the surrounding families but not in other Ericaceae.

With the present new results, it is interesting to try and understand why earlier workers did not group *Purdiaea* with *Clethra*, but with *Cyrilla* and *Cliftonia*. One reason may be that the Cyrillaceae has been taken to comprise taxa with indehiscent fruits, seeds without seed coat, and with an ovary wall acting as the functional seed coat (Thomas 1960, Johri et al. 1992). Most taxa of the Clethraceae-Cyrillaceae-Ericaceae clade (Fig. 2) are actually characterized by having seeds with more or less reduced seed coat. In *Clethra* the seed coat is one-layered and much reduced, and in many other genera the seed coat is highly reduced, e.g. in the pyroloid and the strongly mycotrophic monotropoid taxa of Ericaceae (Johri et al. 1992). The loss of seed coat in *Purdiaea* and *Cyrilla-Cliftonia* may be a result of fruits becoming indehiscent, and is probably a parallelism in the two lineages.

Morphological features that diagnose a clade with *Clethra* and *Purdiaea* are difficult to pinpoint without

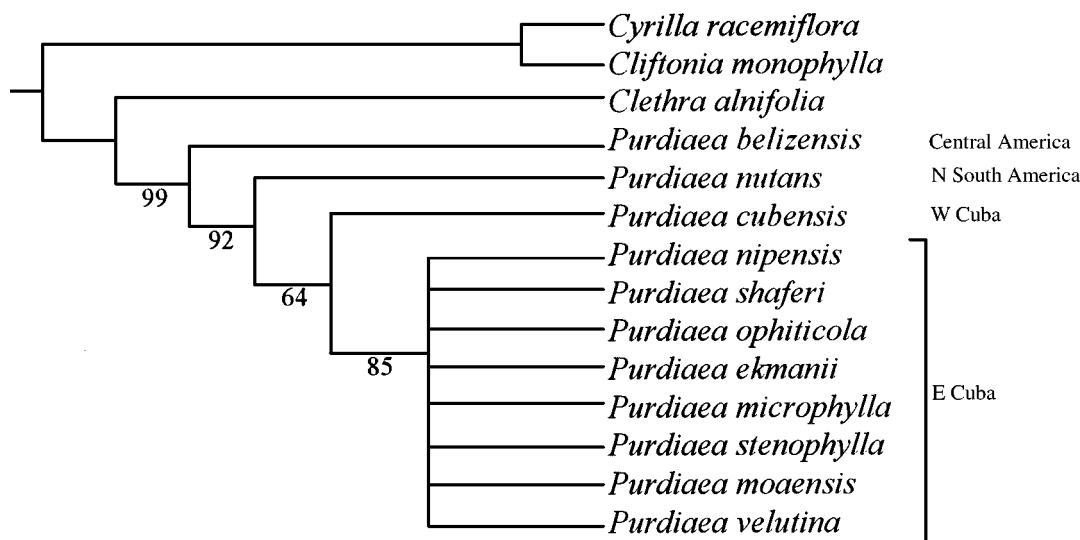


Fig. 3. Tree based on jackknife analysis of morphological data in Table 1. Only groups with jackknife support >50% are shown. The monophyly of *Purdiaea* is supported, with *P. belizensis* as sister to the other species in the genus, and with *P. nutans* as sister to the Cuban endemic species. The tree topology is identical to that of the strict consensus tree from the parsimony analysis.

a detailed analysis of the morphological character states of the entire ericoid clade. Some features that make *Clethra* different from *Purdiaea* are clearly symplesiomorphies, and some of the character states in *Purdiaea* are clearly autapomorphies. It seems reasonable to assume that at least some of the similarities that Anderberg (1993) considered to be symplesiomorphic similarities between *Clethra* and *Purdiaea* – e.g. presence of inverted, versatile, and poricidal anthers – could prove to be synapomorphies. The small sized pollen grains with an exine bridge at the colpus (Zhang & Anderberg in press) could also be a synapomorphy that diagnoses the *Clethra-Purdiaea* clade. Some other characters may also be worthy of discussion in this context.

Flower pedicels in *Clethra* and *Purdiaea* lack bracteoles, but bracteoles are present in both *Cyrilla* and *Cliftonia*. It is possible that absence of bracteoles also diagnoses the *Clethra-Purdiaea* clade, but it is difficult to ascertain which of the two conditions constitutes the derived state at this particular level. We note that Actiniaceae have bracteoles but that the morphologically specialized Sarraceniaceae and Roridulaceae do not. Ericaceae is variable, and both in *Enkianthus* and the py-

roid taxa bracteoles are wanting, indicating that Ericaceae are primitively without bracteoles.

In *Clethra*, *Purdiaea*, *Cliftonia*, and most Ericaceae, the flowers are diplostemonous, whereas *Cyrilla* and some derived Ericaceae have haplostemonous flowers. *Cyrilla* and *Cliftonia* both have choripetalous flowers, and this is also the case in most *Clethra*, although at least some species are distinctly sympetalous like *Purdiaea* and the genera of the surrounding families. The anthers are inverted in *Purdiaea* and *Clethra* and open with pores in the proximal part of the anther. In *Cliftonia* and *Cyrilla* the anthers are not inverted, and they open with longitudinal slits, like in *Enkianthus* of Ericaceae.

Relationships within *Purdiaea*

The jackknife analysis of the morphological data gives robust support for the monophyly of the lower nodes within a monophyletic *Purdiaea*. *P. belizensis* is sister to all other *Purdiaea*, and *P. nutans* is sister to the rest on the next higher level (Fig. 3). *P. belizensis* differs in several respects from other *Purdiaea*, and was originally described as the only member of the genus *Schizocardia* that was considered to be a second genus of Clethraceae (Smith & Standley 1932). This is particularly interesting in the light of the present results where *Purdiaea* is found to be sister to *Clethra*. When writing his monograph, Thomas (1960) did not know of *Schizocardia belizensis*, and considered *P. nutans* from northern South America to be the most primitive species. In our analysis *P. nutans* is sister to the remaining species on the next higher node above *P. belizensis*, thus corroborating Thomas' view. The year after his monograph on Cyrillaceae was published, Thomas transferred *Schizocardia belizensis* to *Purdiaea* because of the specialized features it shared with that genus (Thomas 1961). Record (1932) had earlier found that the wood anatomy of *Schizocardia belizensis* differed in several respects from that of *Clethra*, and the shared presence of character states such as sepals of unequal size, an ovary with five carpels and five locules each containing a single seed, a single style with unbranched stigma, and dry indehiscent fruits were strong indications to suggest a close relationship between *Schizocardia belizensis* and *Purdiaea*.

The leaves in *Clethra* are pinnately veined, and the same is true for the *Purdiaea* species on the lowermost nodes, *P. belizensis* and *P. nutans*. Both of these have secondary veins arising from the mid-vein and sometimes running some distance parallel to the primary vein, whereas all other species of *Purdiaea* have secondary veins initiating from the leaf base, which according to our analysis constitutes an apomorphic condition in this genus. Like *Clethra*, *Purdiaea belizensis*, *P. nutans*, and *P. cubensis* also have a fine reticulate anastomosing venation pattern between the main veins, but this is not the case in the other species.

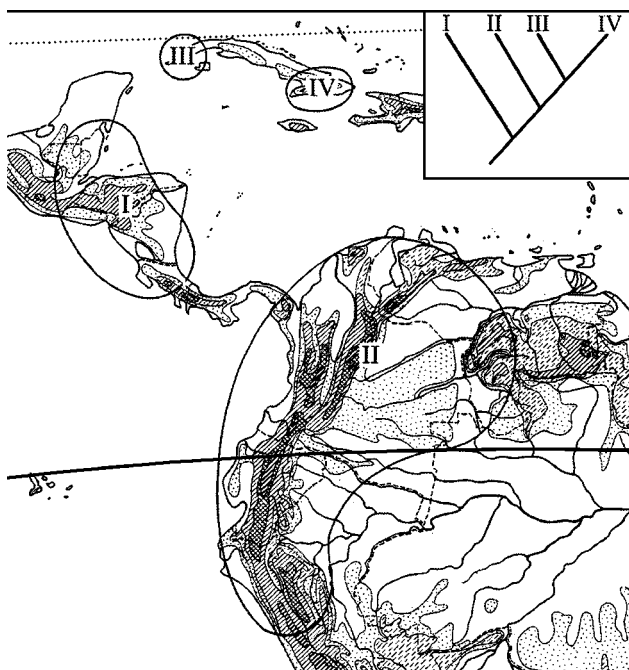


Fig. 4. Vicariance patterns in the genus *Purdiaea* based on the tree of Fig. 3. A Central American area (Area I with *P. belizensis*) is sister to an eastern area (Areas II, III, IV) in which a continental South American area (Area II with *P. nutans*) is sister to an area comprising Cuba (Area III with *P. cubensis*, and area IV with all the other Cuban endemic species).

Biogeography

Purdiaea belizensis grows in Central America (Belize to Panama), *P. nutans* in north-western South America, in Venezuela, Colombia, Ecuador, and Peru. All the remaining species are Cuban endemics, and all but *P. cubensis* grow in the eastern part of the island. The serpentine areas in the Oriente have a high biodiversity with many local endemic species (Borhidi 1996), and this is also the case in *Purdiaea*, but the phylogenetic relationships between the eastern species are yet unclear. Except for the species occurring in eastern Cuba all the other species are allopatric (Fig. 4).

The tectonic processes in the Caribbean have been much debated (see Borhidi 1996 for references). Although the presence of a Caribbean plate has been recognized to have existed from the middle Jurassic to the Upper Oligocene, the composition of the flora of the Greater Antilles is usually explained in terms of migration via land bridges from Honduras and Yucatan during the Oligocene, and via later connections to the Lesser Antilles. Only by the end of the Miocene, South American elements are said to have immigrated into Cuba via the Lesser Antilles (Borhidi 1996). The evergreen shrubland, pine-oak woodland, and coniferous-laurel forests in Cuba have also been interpreted as relict areas from the Madrean-Tethyan vegetation that developed in the Miocene and Eocene, when the Greater Antilles were part of a larger continuous landmass. If the accepted opinion on the origin of the flora is correct, then the biogeographical patterns in *Purdiaea* are difficult to explain only in terms of vicariance, without involving some dispersal event. If the ancestor of the Cuban species came from South America via the Lesser Antilles, all representatives must have become extinct in that area. On the other hand, if the ancestor of the Cuban endemics came by migration from Central America during or after the Oligocene, it would be more likely that the Cuban clade was phylogenetically more closely related to *P. belizensis* than to *P. nutans*. If, however, the origin of *Purdiaea* is older than the Tertiary, then its diversification could be a result of vicariance. Our analysis shows a separation of a Central American area, in which *P. belizensis* later evolved, from an eastern area including Cuba and a north-western continental South American area covering Colombia, Venezuela, Peru and Ecuador. The eastern area was subsequently broken up, with *P. nutans* evolving in the continental part of the area and the ancestor of the Cuban endemics evolving in the Antilles. The Cuban population was later divided into a western and an eastern population, the former in Pinar del Rio subsequently evolving into *P. cubensis*, the latter eventually giving rise to all the species that today inhabit the Oriente province.

Today, *Clethra* is partly sympatric with *Purdiaea*, but no cladistic analysis of *Clethra* has been performed, thus

there is no data on phylogenetic relationships. Within the genus, *Clethra* sect. *Cuellaria* as defined by Sleumer (1967) has a wide distribution in Central America and north-western South America, and two species of the genus reach eastern Cuba and Jamaica. Another species grows in Macaronesia and could be a relict from the Madrean-Tethyan vegetation.

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Appendix 1

Characters used for the morphological analysis of relationships in *Purdiaea*

1. Young stems hairy (0); Young stems glabrous (1). Some species of *Purdiaea* have persistently hairy young stems, whereas others have glabrous or glabrescent stems, being puberulent when young. The hairs may be of different kinds (see character 2).
2. Young stems with stellate trichomes (0); Young stems with long, silky, appressed trichomes (1); Young stems with short, rigid, erect trichomes (2). The young stems of *P. moaensis* Marie-Vict., *P. nipensis* Marie-Vict., and *P. microphylla* Britton & P. Wilson are finely pubescent or velutinous but become glabrous with age. The trichomes are clearly different from the long, silky, appressed trichomes found in *P. velutina* Britton & P. Wilson and *P. ekmanii* Marie-Vict.
3. Wood parenchyma abundant and diffuse (0); Wood parenchyma less abundant and more often collectively found adjacent to vessel elements (1). The difference in xylem parenchyma in *Cyrilla* and *Cliftonia* was noted by Thomas (1960), who observed that the xylem parenchyma is relatively abundant and diffuse in *Cyrilla*, usually occurring as apotracheal strands, whereas in *Cliftonia* it is less abundant but more often found adjacent to vessel elements than in *Cyrilla*. The condition in *Purdiaea* is unclear, but at least *P. belizensis* has been investigated in detail (Record 1932) and is described as having both abundant and diffuse wood parenchyma, as well as tangential strands touching the vessels. Solitary vessels occur both in *Clethra* and *Cyrilla*, whereas *Cliftonia* and *Purdiaea* have pore multiples in the xylem (Metcalfe & Chalk 1950, 1989; Thomas 1960).
4. Leaves petiolate (0); Leaves sessile or almost sessile (1).
5. Leaves pinnately veined (0); Leaves with two or more lateral veins running parallel to the leaf margin (1). The leaf venation is pinnate in *Cyrilla* and *Cliftonia*, and similar to that of *Clethra*. Leaf venation is often diagnostic at the species level (Thomas 1960; Rodrigues & Berazain 1991, 1992; Berazain & Rodrigues 1992; Valentin et al. 1992), especially in the genus *Purdiaea*. Most species of *Purdiaea* have one to five pairs of strongly arcuate lateral veins which run longitudinally more or less parallel to the margin of the leaf. In *P. belizensis* and *P. nutans* the leaves are basically pinnate without or with very indistinct lateral veins from the base.
6. Two or more of the longitudinal lateral leaf veins consistently arising near the middle of the leaf (0); All the longitudinal lateral veins initiating from the leaf base (1).
7. Leaves with fine, reticulate venations between the main veins (0); Leaves without fine, reticulate venations between the main veins (1).
8. Racemes terminal (0); Racemes axillary (1).
9. Racemes with an apparent transition zone between vegetative and reproduction shoots (0); Racemes without a transition zone (1). Thomas (1960) noted that the racemes of *Purdiaea nutans* and *P. cubensis* show a zone of transition from a vegetative to a reproductive shoot, i.e. a change in the morphology of the shoot axis, and often a transition from leaves to large bracts to smaller bracts. This is also the case in *P. belizensis*, but in all other species of *Purdiaea* this transition is usually quite abrupt.
10. Racemes longer than 2.5 cm (0); Racemes shorter than 2.5 cm (1).
11. Bracteoles absent on the pedicel (0); A pair of bracteoles present on the pedicel (1).
12. Sepals not conspicuously increasing in size (0); Sepals increasing in size during the maturation of the fruit (1).
13. Sepals equal in size (0); Sepals unequal in size (1). All *Purdiaea* have sepals that are distinctly unequal in size.
14. Sepals quincuncial (0); Sepals imbricate (1). In *Purdiaea* the sepals are different in size and quite different from those found in *Cyrilla*, *Cliftonia*, and *Clethra*. The two exterior sepals completely enclose two interior sepals, and all but one edge of the middle sepal. Therefore the lowermost of the two exterior sepals is called, according to its position in the spiral, the first sepal, and the other exterior sepal is referred to as the second sepal. Thomas (1960) considered the form and arrangement of the sepals in *Purdiaea* to be a modified quincuncial type of sepal insertion that is found also in *Clethra*.
15. First sepal (see character 14) almost equal in size to the second sepal (0); First sepal distinctly different in size from the second sepal (1); First sepal nearly twice as long as the second sepal (2).
16. Sepals foliaceous at maturity (0); Sepals scarious at maturity (1).
17. Petals basally connate (0); Petals free (1).
18. Petals with only one main vein and two very small lateral veins (0); Petals with numerous small, longitudinal, repeatedly branched veins (1).
19. Anthers inverted at anthesis. Filaments attached to the ventral side of the anther, with anther lobes free above the point of attachment (0); Anthers not inverted. Filaments attached to the dorsal side of the anther, with the lobes of the anthers free below the point of attachment (1).
20. Anthers oblong with a caudate base, opening with pores (0); Anthers ovoid without a caudate base, opening with slits (1).
21. Pollen grains usually smaller than 20 μm in equatorial diameter (0); Pollen grains usually larger than 25 μm in equatorial diameter (1).
22. Pollen grains oblate with a rhombic outline in equatorial view (0); Pollen grains spherical with a slightly oblate-spherical outline in equatorial view (1). The pollen grains of *Purdiaea* are flattened, and only the upper half of the pollen can be seen if viewed in equatorial view. Additionally, there is always a strongly protruding marginal bridge at the ora, giving the pollen a flattened rhomboid outline in equatorial view. A similar type of pollen is found in *Clethra* although these are less flattened. In *Cyrilla* and *Cliftonia* the pollen grains are spherical with a slightly oblate-spherical outline in equatorial view.
23. Pollen surface sculpture variously rugulate (0); Pollen surface almost smooth (1). The pollen surface in *Cyrilla* and

Cliftonia is generally perfectly smooth without any appreciable variations of the sculpturing. Also in *Clethra* the pollen surface is almost smooth (e.g. *C. alnifolia*) or slightly verrucose (*C. bodinieri*). In *Purdiaea*, the pollen surface sculpturing is variously rugulate, giving rise to an entirely different appearance compared to the other genera. The surface sculpturing seems to be diagnostic for different species, but it is difficult to find discrete character states. Only *P. belizensis* differs significantly by having sharp ridges.

- 24. Pollen surface lacking very small whitish granules (0); Pollen surface with minute white granules (1).
- 25. Stigma lobed (0); Stigma entire (1).
- 26. Ovary locules with three or many ovules (0); Each ovary locule with a single ovule (1).
- 27. Ovules anatropous (0); Ovules pendulous-orthotropous (1). The ovules are anatropous in *Cyrilla* and *Cliftonia*,

but in *Purdiaea* they are attached to the funiculus in an orthotropous manner, and were described as pendulous-orthotropous by Thomas (1960).

- 28. Fruit a loculicidal capsule (0); Fruit indehiscent (1). The fruits of *Clethra* are loculicidal capsules (Sleumer 1967), whereas those of *Cyrilla*, *Cliftonia* and *Purdiaea* are indehiscent, and the fruit type was referred to as a caryopsis by Thomas (1960). The fruit of *P. belizensis* was described as being a five-locular capsule by Smith & Standley (1932), but Thomas (1961) referred to it as indehiscent. In the material we have seen, the fruit has the aspect of a capsule but does not seem to open.
- 29. Fruit globose, smooth (0); Fruit bisulcate or trisulcate (1); Fruit 3- to 5-ribbed (2). Fruit 3- to 5-winged (3).
- 30. Fruit variously hairy (0); Fruit glabrous (1).
- 31. Seed coat present (0); Seed coat absent (1).