Studies in the Miliuseae. V. Review of the taxonomic history of a polyphyletic 'tribe'

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Abstract

J.B. Mols & P.J.A. Keβler (Nationaal Herbarium Nederland, Universiteit Leiden Branch, P.O. Box 9514, 2300 RA Leiden, The Netherlands. Email: Mols@NHN.LeidenUniv.NL) 2003. Studies in the Miliuseae. Review of the taxonomic history of a polyphyletic 'tribe'. Telopea *10(1): 113–124*. This article addresses the problem of the classification of the genera in the Annonaceae. The former classifications within the family based on subsets of morphological data are not congruent with each other and cannot be used to make a clear and uniform classification of the family or subsets of genera. This problem is illustrated here for the tribe Miliuseae Hook.f. & Thomson, which has been defined by a special stamen type (miliusoid) uncommon in the Annonaceae. It is argued that this tribe is not monophyletic and is not "recognised" as such in the classifications using different morphological data sets. The polyphyly of the tribe is also indicated by preliminary molecular studies. Future studies, combining molecular and morphological data, are outlined.

Introduction

The Annonaceae Juss. are a pantropical family consisting of about 130 genera and 2300 species. Most of the species occur primarily in tropical lowland rainforest. The family is often represented by high numbers of individuals and species. In Asia alone about 60 genera and about 1000 species can be found.

The Annonaceae are clearly delimited (e.g. Fries 1959; Keβler 1993) and are regarded monophyletic in all studies including those based on molecular data (Qiu et al. 2000). In contrast to distinct family boundaries, the classification on subfamilial level is unresolved. The genera in the Annonaceae are notoriously difficult to define and to classify into "natural groups". Over the years different authors have subdivided the family into formal and informal groups based on several character sets, such as pollen, flowers, fruits, etc. (e.g. Hutchinson 1923, 1964; Sinclair 1955; Fries 1959; Walker 1971; Setten & Koek-Noorman 1992; Heusden 1992; Keβler 1993; Koek-Noorman et al. 1997). A comparison of these classifications reveals a diversity of conflicting conclusions.

The problem of the classification within the Annonaceae is here illustrated by discussing the position of the genera included in the tribe Miliuseae Hook.f. & Thomson (sub Saccopetaleae) according to Keβler (1993), namely *Alphonsea* Hook.f. & Thomson, *Mezzettia* Becc., *Mezzettiopsis* Ridl., *Miliusa* A.DC., *Orophea* Blume, *Phoenicanthus* Alston and *Platymitra* Boerl. Various alternative character sets that might delimit the tribal boundaries are also discussed. This article is one of a series of studies on this tribe. In previous publications in this series the tribal name Saccopetaleae (articles I–IV) has been used, but a study of the nomenclature has revealed that the correct name should be Miliuseae. This tribe has been chosen as it is the object of the Ph.D. study of the first author.

Miliuseae

The first time the Annonaceae were subdivided was by Dunal (1817). At the time only eight genera (excluding *Kadsura* Juss., now placed in the Schisandraceae) were recognised and, based on fruit characters only, were classified into three groups.

The tribe Miliuseae was first described by Hooker and Thomson in 1855 in their *Flora Indica* and included the genera *Miliusa*, *Saccopetalum* Benn. (= *Miliusa*), and *Alphonsea*. The circumscription of the tribe was based on the following characters: carpels free, petals valvate, stamens loosely imbricate and anthers visible in flower (not concealed). The authors stressed that the tribe differs from the other tribes in the structure of the stamens. In present day taxonomy of the family, this type of stamen is referred to as the miliusoid stamen. Generally the stamens in the Annonaceae are characterized by being narrowly oblong to oblanceoloid with a short filament, two thecae and a shield-like apical prolongation of the connective (**Fig. 1**) (Heusden, 1992). In the miliusoid stamen this apical prolongation is not shield-like and is either small-discoid and not extending over the anthers or absent (**Fig. 2**). The same characters used by Hooker and Thomson are still used to define this tribe. Keβler (1993) recognises a *Miliusa*-group based on sepals and petals being valvate, stamens and carpels few, and connective without a prolonged specialized apex.

The Miliuseae *sensu* Keβler includes six (seven in the original publication) genera which can be determined using the following key and diagnostic descriptions.

Key to the genera in the Miliuseae

1a. Outer and inner petals of (almost) equal length **2**

b. Outer petals much smaller than inner petals **5**

2a. Sepals connate at the base, inner petals mitreform **3**

b. Sepals free, inner petals not mitreform **4**

3a. Stamens 6 or 9, ovule(s) 1–2, monocarps up to 1 cm in diameter .. **Phoenicanthus**

b. Stamens 20–35, ovules c. 10, monocarps 1 to 5 cm in diameter .... **Platymitra**

4a. Inner petals saccate at the base, stamens 15–60, carpel(s) 1–15, monocarps stipitate .... **Alphonsea**

b. Inner petals not saccate at the base, stamens 9–21, carpel 1, monocarps sessile .... **Mezzettia**

5a. Sepals and outer petals similar in size and shape **6**

b. Sepals and outer petals similar in shape but outer petals much larger, inner petals clawed at base .... **Orophea (incl. Mezzettiopsis)**

6a. Sepals free, inner petals saccate, glandular tissue along middle vein .. **Miliusa s.s.**

b. Sepals connate at the base, inner petals not saccate, glandular ring at base .... **Miliusa p.p. (thought to be a new genus)**

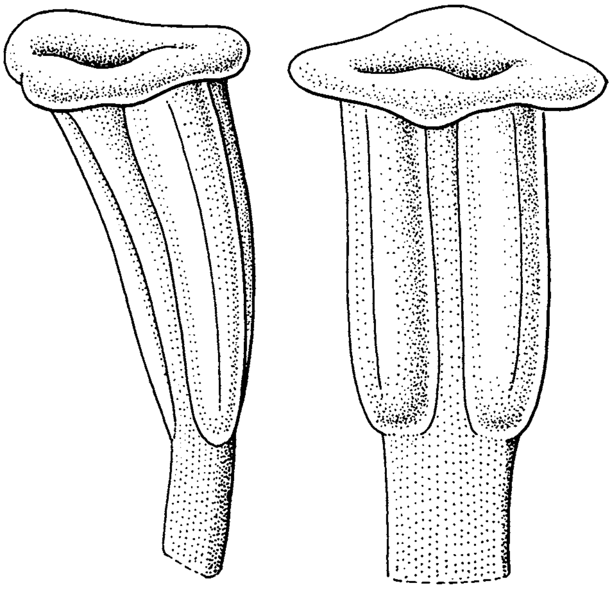

Fig. 1. Stamens of *Phaeanthus nutans* Hook.f. & Thomson (*KEP/FRI 12307 (Whitmore)*). Drawing from Mols & Keßler (2000) by J. van Os.

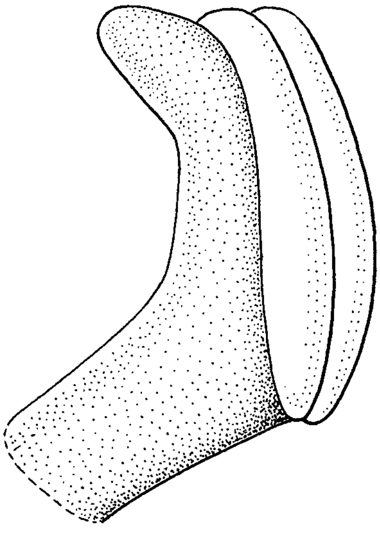

Fig. 2. Stamen of *Miliusa velutina* (Dunal) Hook.f. & Thomson (*Kerr 1078*). Drawing from and by Heusden (1992) (sub *Miliusa villosa*) redrawn and adapted by J. van Os.

Alphonsea Hook.f. & Thomson, Fl. Ind. 1: 152 (1855).

Trees or shrubs; flowers bisexual; sepals 3; petals 6, subequal, inner petals slightly saccate at the base; stamens 15–60, miliusoid; carpel(s) 1 to 15; ovules few, biseriate; monocarps globose to cylindrical, stipitate. Twenty-five species found from mainland Asia (India, Thailand) to Indonesia and the Philippines. Recently revised by Keβler (1996).

Mezzettia Becc., Nuovo. Giorn. Bot. Ital. 3: 187 (1871).

Trees; flowers bisexual; sepals 3; petals 6, inner petals shorter than outer petals; stamens 9–21, miliusoid with truncate dilated apex; carpel 1; ovules 2, biseriate; monocarps globose, sessile. Four species found from Peninsular Malaysia to the Moluccas. Recently revised by Heijden and Keβler (1990). Doubtfully included in the Miliuseae *sensu* Keβler.

Miliusa A.DC., Mem. Anon. (preprint): 37 (1832).

Shrubs or trees; flowers bisexual or unisexual; sepals 3; petals 6, outer petals similar to sepals, inner petals much longer and saccate at the base; stamens few to numerous, miliusoid; carpels few to numerous; ovule(s) 1–10, biseriate; monocarps globose to oblong, stipitate. About 25 to 30 species from mainland Asia (India, China) to New Guinea and Australia. Several species previously included in *Miliusa* differ from the general perception of the genus. The main differences are that the inner petals bases are not saccate in these species but are thickened forming a glandular ring, the sepals are slightly connate at the base and the leaf bases are amplexicaule. These species are thought to form a new genus (to be described) and are referred to in the key as *Miliusa* p.p.. The species of the Flora Malesiana area and Australia have recently been revised (Mols and Keβler, in prep.).

Orophea Blume, Bijdr. Fl. Ned. Ind.: 18 (1825).

Small trees; flowers bisexual; sepals 3; petals 6, inner petals longer than outer ones, inner petals clawed or spoon-shaped; stamens 3–12, miliusoid; carpels 3–18; ovule(s) 1– 6, uni- or biseriate; monocarps globose or cylindrical, stipitate. Fifty species subdivided into two subgenera *Orophea* and *Sphaerocarpon* (latter includes former genus *Mezzettiopsis* Ridl., Kew Bull.: 389 (1912)). Found from mainland Asia (India, China) to the Sunda Islands and the Philippines. The genus has been revised by Keβler (1988a, 1990) and Leonardia and Keβler (2001).

Phoenicanthus Alston in Trimen, Handb. Fl. Ceylon 6: 6 (1931).

Trees; flowers bisexual; sepals 3, slightly connate; petals 6, subequal, inner petals mitreform and concave at the base; stamens 6 or 9, miliusoid with obtuse apex; carpel(s) 1–3; ovule(s) 1–2; monocarps globose, sessile. Two species endemic to Ceylon (Huber, 1985).

Platymitra Boerl., Cat. Pl. Phan. 1: 33 (1899).

Trees; flowers bisexual, ramiflorous; sepals 3, connate at base; petals 6, subequal, inner petals mitreform; stamens 20–35, miliusoid; carpel(s) 1–3; ovules 10, biseriate; monocarps globose to ovoid, sessile. Two species found from Thailand to Java and the Philippines. The genus has been revised by Keβler (1988b).

Classifying the Miliuseae

In the next paragraphs and accompanying Table 1 the position of each genus in the tribe in twelve classifications is shown. These twelve classifications are not all that are known for the family, but are the most widely used or of particular interest in the study of the Miliuseae. The classifications are not always easy to compare because in some cases a genus is missing from a classification simply because it had not been described at the time, did not occur in the area the treatment was dealing with, or because no sufficient data were available. If a genus has been placed in synonymy the most recent circumscription of the accepted genus is recognised.

Reichenbach 1837: After the work by Dunal (1817), the number of genera in the family rose sharply to twenty-four (including some non-Annonaceous genera). The Annonaceae (sub Annoneae) were treated as a tribe of the Ranunculaceae, and based on flower characters primarily three groups were recognised: the Cardiopetaleae, Guatterieae and Annonariae, with the latter group subdivided into three smaller groups, Uvarieae, Bocageae, Annoneae genuinae. The only members of the Miliuseae *sensu* Keβler known up to this point were *Miliusa* and *Orophea*, placed in the Cardiopetaleae because the petals were united at the base in *Miliusa* (in the type specimen, discovered to be genetically atypical, the inner petals were united in some flowers).

Endlicher 1839: Endlicher dealt with a similar number of genera, but the tribal division was somewhat different. Based also on stamen and carpel characters three tribes were recognised: Bocageae, Xylopieae and Annoneae with several genera not allocated to any of these three tribes. The main difference with Reichenbach's system was that the two Miliuseae genera were now placed in the Bocageae, based on having a finite number of stamens. A first indication of the problems concerning the classification was already shown here. The genus *Hyalostemma,* placed in *Miliusa* in modern classifications, could not be accommodated under Endlicher's system.

Hooker and Thomson 1855: As mentioned before, the tribe Miliuseae was first established in the Flora Indica. Based on flower characters alone, Hooker and Thomson recognised six tribes: Annoneae, Uvarieae, Miliuseae (sub Saccopetaleae), Mitrephoreae, Xylopieae and Guatterieae. This classification was based on the plants occurring in India and predominantly dealt with the Asian Annonaceae. But many of the tribal names and characters adopted have been used in later stages as a precursor for the general classification of the Annonaceae. The Miliuseae, defined as having free carpels, valvate petals, and imbricate, miliusoid stamens consisted of *Miliusa, Saccopetalum* (= *Miliusa)*, and *Alphonsea*. The only other genera of Miliuseae *sensu* Keβler classified by Hooker and Thomson were *Orophea* and *Phoenicanthus* (sub *Orophea obliqua*). These genera were placed in the Mitrephoreae, which was defined by having free carpels, valvate petals, inner petals clawed at the base, stamens densely packed together and anthers concealed. This last character state did not apply to *Orophea* and *Phoenicanthus*. The clawed base of the inner petals of *Orophea* (and *Phoenicanthus*) was the reason this genus was placed in the Mitrephoreae, but the stamen characters suggest it should have been placed in the Miliuseae.

Hooker and Thomson 1872: Only slight variations were made from the previous treatment. Five tribes were recognised with the Annoneae included in the Xylopieae. The genus *Guatteria* Ruiz & Pav. was no longer recognised as Asiatic, and the tribe Guatterieae had been renamed Unoneae. The description of the Miliuseae was modified slightly to allow for the fact that the petals could be valvate or imbricate. The stamen characters were again thought to be of more importance and so *Orophea* and *Phoenicanthus* (as *Bocagea obliqua* and *B. coriacea*) were included in the tribe. Additionally, the genus *Lonchomera* Hook.f. & Thomson was first described and placed in the *Miliuseae*, this genus is now considered a synonym for *Mezzettia*. This meant that

Table 1. Overview of the position of the genera included in the Miliuseae sensu Keβler in different classifications. For additional explanation see text (– = not described at time of treatment, N.I. = genus not included in the treatment, N.A. = genus could not be accommodated in the treatment).

	Alphonsea Hook. f. & Thomson	***Mezzettia*** Becc.	***Mezzettiopsis*** Ridl.	***Miliusa*** A.DC.	***Orophea*** Blume	***Phoenicanthus*** Alston	***Platymitra*** Boerl.
Keβler (1993)	Miliuseae	Miliuseae	Miliuseae	Miliuseae	Miliuseae	Miliuseae	Miliuseae
Reichenbach (1837)	–	–	–	Cardiopetaleae	Cardiopetaleae	–	–
Endlicher (1839)	–	–	–	Bocageae	Bocageae	–	–
Hooker & Thomson (1855)	Miliuseae	–	–	Miliuseae	Mitrephoreae	Mitrephoreae	–
Hooker & Thomson (1872)	Miliuseae	Miliuseae	–	Miliuseae	Miliuseae	Miliuseae	–
Ridley (1922)	Miliuseae	Miliuseae	N.I.	Miliuseae	Mitrephoreae	N.I.	–
Sinclair (1955)	Miliuseae	Unoneae	Miliuseae	Miliuseae	Miliuseae	N.I.	Miliuseae
Hutchinson (1923/1964)	Unoneae Xylopineae Hexapetaleae	Unoneae Xylopineae Hexapetaleae	Miliuseae	Miliuseae	Miliuseae & Unoneae Xylopineae Hexapetaleae	(Miliuseae)	Unoneae Xylopineae Hexapetaleae
Fries (1959)	Unoneae Desmos-group	Unoneae Polyalthia-group	Unoneae Orophea-group	Unoneae Polyalthia-group	Unoneae Orophea-group	N.I.	Unoneae Orophea-group
Walker (1971)	Malmea-subfamily Uvaria-tribe	Malmea-subfamily Uvaria-tribe	Malmea-subfamily Uvaria-tribe	Malmea-subfamily Uvaria-tribe	Malmea-subfamily Uvaria-tribe	N.I.	Malmea-subfamily Uvaria-tribe
Setten & Koek-Noorman (1992)	Group 12	Group 5	Group 8	N.A.	Group 8 or 11	N.A.	Group 9
Heusden (1992)	Sageraea-group	N.A.	Mitrephora-group	Miliusa-group	Mitrephora-group	Sageraea-group	Mitrephora-group
Koek-Noorman et al. (1997)	Cluster A	Cluster D	Cluster D	Cluster C	Cluster D	Cluster D	Cluster A

all in all five of seven genera included in the Miliuseae *sensu* Keβler were placed in this tribe, with the other two not yet recognised or described. The genus *Dendrokingstonia* (Hook.f. & Thomson) Rauschert (sub *Kingstonia* Hook.f. & Thomson), with imbricate sepals and petals was included in the tribe. This genus is now usually regarded as related to the genus *Uvaria* L. and allies.

Ridley 1922: In his treatment of Malayan Annonaceae Ridley recognised the same tribes as Hooker and Thomson based on mostly the same floral characters. The main difference was that Ridley again put the emphasis on petal shape instead of the stamens when dealing with *Orophea* and placed it back in the Mitrephoreae. The miliusoid stamen was in fact not mentioned as a defining character for the Miliuseae that included *Miliusa, Alphonsea, Mezzettia* and *Dendrokingstonia* (sub *Kingstonia*). Ridley included *Phaeanthus* Hook.f. & Thomson in the Miliuseae because it also had small sepals and outer petals and large inner petals, this difference between the two whorls of petals becoming the main character to place all genera together. In *Mezzettia* this difference is only slight, the inner petals often being smaller than the outer.

Sinclair 1955: This treatment dealt also only with the Annonaceae of the Flora of Peninsular Malaysia, but is regarded as one of the main references dealing with Asian Annonaceae as it is the most complete overview of the family in the region. The tribes used were the same as Ridley's with a slight difference in the composition of the tribes. *Platymitra* had been described in the meantime and was added to the tribe. Furthermore Sinclair stated (p. 175) that the stamen characters are probably better suited to be used than the corolla characters to base the relationships between the genera in the Miliuseae, this in contrast with the other tribes. He thought that sexual organs are more likely to be static and less liable to variation over time. For this reason *Orophea* was transferred (back) to the Miliuseae, and Sinclair noted that, based on petal structure, *Orophea* and *Platymitra* were considered to be closely linked to the Mitrephoreae. The stamens of *Mezzettia* on the other hand were not regarded to be miliusoid and the genus was transferred to the Unoneae. The stamens of *Mezzettia* are slightly different as the apex is truncately dilated, but presently are regarded by us to be more related to the miliusoid type than any other.

Hutchinson 1923 & 1964: Hutchinson (1923) used the ideas of Hooker and Thomson for the entire family (nearly 100 genera and more than 1000 species). But using the same characters for all Annonaceae genera resulted in an artificial classification. Hutchinson recognised two subfamilies Annonoideae and Monodoroideae. Within the Annonoideae three tribes Uvarieae, Miliuseae and Unoneae were recognised. The latter was divided into two subtribes Annonineae and Xylopineae, the latter of which was divided into three series Hexapetalae, Tetrapetalae and Tripetalae (group A, B, and C in Hutchinson, 1964). *Miliusa, Orophea* and *Mezzettiopsis* were in the Miliuseae based on the valvate petals and a large differentiation between the inner and outer petals together with the Asian genera *Phaeanthus*, *Trivalvaria* Miq., *Marsypopetalum* Scheff., the American genera *Heteropetalum* Benth. and *Cymbopetalum* Benth. as well as the African genera *Piptostigma* Oliv. and *Brieya* De Wild. (now included in *Piptostigma*). The genera *Mezzettia, Platymitra* and *Alphonsea* were placed in the Unoneae, subtribe Xylopineae, series Hexapetalae, based on having 6 petals, the outer petals similar or larger than the inner petals and free carpels. The miliusoid stamen character was only used to define the genera *Mezzettiopsis*, *Miliusa*, *Orophea* and *Saccopetalum*. This classification is artificial because *Orophea* was placed in two tribes, the Miliuseae and the Unoneae, subtribe Xylopineae, series Hexapetalae. The 1964 treatment only differed by the addition of some (not all!) newly described genera. To the Miliuseae *Fenerivia* Diels (Africa) and *Anomianthus* Zoll. (Asia) were added, with the latter placed also in the tribe Uvarieae.

Fries 1959: This treatment is generally seen as the standard work of the Annonaceae on a global scale. It is based primarily on floral characters, but more characters were used, probably producing a better classification, especially for the South-American genera, as Fries revised almost all the Neotropical species known at that time. Fries also recognised two subfamilies: the Annonideae and the Monodoroideae. The Annonideae were subdivided into three tribes and 15 groups, the Uvarieae (with 5 groups), the Unoneae (with 9 groups), and the Tetramerantheae. All genera now in the tribe Miliuseae *sensu* Keβler, based on having valvate petals, were placed in the Unoneae but subdivided into three groups. *Miliusa* and *Mezzettia* were placed in the Polyalthia-group with eight other genera. This group was recognised based on having petals in 2 whorls, flowers having bracts, apocarpous fruits, inner petals not enclosing the sexual organs and touching each other at the base, and axillary inflorescences. *Orophea (*including *Mezzettiopsis*) and *Platymitra* were placed in the Orophea-group, which, although similar to the Polyalthia-group, differed in having inner petals that enclose the sexual organs being fused at the tip to form a dome. In this group twelve additional genera were included. The genus *Alphonsea* was placed in the Desmos-group together with four other genera. This group differs from the Polyalthia-group in having leaf-opposed inflorescences. As can be concluded from this short summary the interpretation of the characters is different and some are used at another level as in previous treatments.

Walker 1971: Walker introduced a major change in the general classification trend within the family. He used pollen morphology to classify the Annonaceae into three subfamilies, the Malmea, Fusaea and Annona subfamilies. The first and last of these subfamilies were subdivided further; the Malmea subfamily into three tribes, the Malmea tribe, Uvaria tribe and Guatteria tribe, and the Annona subfamily into four tribes, Hexalobous tribe, Asimina tribe, Annona tribe and Cymbopetalum tribe. The Miliuseae were placed in the Uvaria-tribe which was defined by having solitary globose pollen grains which are apolar, radiosymmetric and inaperturate of medium-size to large with well-developed to reduced columellae and often verrucate exine. Based on these characters the tribe included c. 60 genera, including all genera in the Miliuseae *sensu* Keβler (except *Phoenicanthus* which was not included in the treatment).

Setten and Koek-Noorman 1992: These authors studied fruit and seed characters, and recovered 16 groups from the observed variation patterns. This suggested a different set of relationships among the genera. Unfortunately only four of seven genera in the Miliuseae *sensu* Keβler were accommodated in these groups. *Mezzettia* was placed in group 5 because it has large monocarps with two lateral seeds with thick walls and stout lamellate ruminations in often more than 4 parts. *Mezzettiopsis* (= *Orophea*) was placed in group eight based on a low number of pitted lateral seeds with spiniform ruminations. This group was said to be closely related to the genus *Polyalthia* and the members of group 9. This latter group included *Platymitra* and was different from 8 in having its seeds in 2 rows. *Alphonsea* was accommodated in group 12 which was defined by having lamellate rumination in four parts with a smooth seed wall and having no oil cells in the endosperm. This group was also said to be closely related to *Polyalthia*. *Phoenicanthus* was not accommodated, as data available was not sufficient to suggest any placement. Fruit and seed characters are very diverse in *Miliusa* and *Orophea*. *Orophea* was found to consist of two sections one of which could be placed in group 8 (together with *Mezzettiopsis*) and the other in group 11. *Miliusa* was so diverse that the authors did not accommodate the genus, but proposed it should be placed in the centre of the scheme connecting the groups, together with *Polyalthia,* with which it also shares some features.

Heusden 1992: In this study the flower characters were partly reevaluated, new characters were used and the significance of more traditional characters to the classification of the genera was reinterpreted. Nineteen groups were recognised and the members of the Miliuseae *sensu* Keβler were distributed across three groups. *Mezzettia* could not be accommodated in any of the nineteen groups. *Miliusa* was included in the Miliusa-group along with *Marsypopetalum* and *Phaeanthus*. The sepals and outer petals being of similar size and shape characterized this group. The author indicated that this group was closely related to the Mitrephora-group, which included the genera *Orophea*, *Mezzettiopsis*, and *Platymitra*, and was defined by valvate sepals, two whorls of petals that are of slightly different size and shape and mostly several lateral ovules. The genera *Alphonsea* and *Phoenicanthus* were placed in the Sageraea-group based on a large number of broadly defined characters. Heusden also stated that this group was ill-defined but the genera could not be placed better in any group other than together. Some connection was shown with the *Miliusa*-group as *Alphonsea* has similar stamens and carpels to the genera in this group. The author concluded that the groups could be recognised only through a combination of several character states but in many instances the genera did not fall neatly into any particular group.

Koek-Noorman et al. 1997: This study combined the two previous datasets and performed a cluster analysis on the data thus making it the first treatment to use both flower and fruit/seed characters to group the genera within the family. The phenetic analysis clustered *Alphonsea* and *Platymitra* together in group A. *Miliusa* was placed in group C forming in this group a cluster with *Mitrephora* (Blume) Hook.f. & Thomson and *Desmopsis* Saff. All other genera in the Miliuseae *sensu* Keβler were placed in group D, along with *Phoenicanthus* (based on flower characters only) and *Mezzettia,* and *Orophea* and *Mezzettiopsis* closely also clustering together.

This comparison of earlier classifications clearly shows that when switching the emphasis from one character (state) or data set to another the composition of resulting groups or tribes changes considerably. Our conclusions are therefore similar to those of Koek-Noorman et al. (1990). No single character set gives a clear picture of the relationships of the genera within the family. One needs to combine the different datasets (e.g. fruit, pollen, flower, etc.) into a complete data set, which should be used in a phylogenetic analysis. Doyle and Le Thomas (1994, 1996) performed such an analysis of 79 morphological characters. This data set produced 180 most parsimonious trees belonging to two islands, which showed a high level of homoplasy, and therefore the relationships between the groups obtained remained unclear. However in the rooting of these cladograms and in the formation of the major clades the pollen characters were phylogenetically most informative. This idea was strengthened in their later studies (Doyle and Le Thomas, 1997) because of the phylogenetic differences found between the cladograms before and after removal of the pollen data from the overall morphological data set in the analysis. It must be stated that their studies only included c. 36 out of 130 genera of the Annonaceae worldwide, which means that many possibly morphologically intermediate genera were omitted. Because only one genus (*Miliusa*) of the *Miliuseae sensu* Keβler was included this study has not been used in the previous comparison of classifications.

Preliminary ideas based on molecular data

In recent years research has been more directed towards the use of molecular data to determine relationships within the family. Zuilen (1996) was the first to use molecular data but she only dealt with one genus in the family. Bygrave (2000) and Chatrou et al. (in prep.) have made phylogenetic analyses based on respectively *rbc*L and *rbc*L & *trn*L-F DNA sequence data. Although many of the groupings found are not well supported and the overall resolution of parts of the cladograms obtained is rather poor, the cladograms have shown some interesting aspects. When dealing with such a "primitive" family within the angiosperms, the closest relative of a certain genus might not be found on the same continent. For instance, a close relative of the Asiatic genus *Miliusa* appears to be the Central American genus *Sapranthus* Seeman. Classifications on a regional scale will not identify these possible relationships. The importance of this has already been referred to by other authors (e.g. Setten & Koek-Noorman 1992; Heusden 1992; Koek-Noorman et al. 1997; Keβler 1993).

Furthermore even though the resolution in the end clades is poor, these early molecular studies provide a basis for future research. They indicate larger subsets of genera that can be the subjects of further research into the relationships between these genera. A preliminary phylogenetic analysis based on *rbc*L and *trn*L-F DNA sequence data (Chatrou et al., in prep.) suggests that the Miliuseae *sensu* Keβler are not monophyletic and that the closest relatives of *Miliusa* might be any of the following genera: *Fitzalania* F.Muell., *Ancana* F.Muell., *Sapranthus*, *Neo-uvaria* Airy Shaw, *Sageraea* Dalzell, *Enicosanthum* Becc., *Alphonsea*, *Platymitra*, *Phaeanthus*, *Popowia* Endl. and *Polyalthia* Blume p.p. Of these genera only *Alphonsea* and *Platymitra* are included in the Miliuseae *sensu* Keβler and all genera except *Sapranthus* have an Asiatic distribution. Only some *Polyalthia* species ended up in this clade. The few species of *Polyalthia* included (c. 10 of 150) in the analyses (Bygrave, 2000; Chatrou et al., in prep.) were divided over several clades. Not surprisingly the genus *Polyalthia* is regarded as polyphyletic, because based on morphology alone it was already considered to be an ill-defined genus.

In none of the classifications examined and surveyed above, is the molecular grouping of these twelve genera closest to *Miliusa* suggested or found. For instance different stamen types can be found (miliusoid, various stamens with a shield-like prolongation of the connective, etc.), some genera have two whorls with similar petals, while others have similar sepals and outer petals, and also the fruits come in many different shapes and sizes.

To solve this question a combined analysis of molecular and morphological data needs to be performed. Doyle et al. (2000) performed such analysis by combining Bygrave's *rbc*L data and Doyle and Le Thomas' morphological data. In their article the morphological characters were plotted on the cladogram based on the *rbc*L data and the datasets were combined and phylogenetically analyzed. Only the taxa included in the studies by Doyle and Le Thomas were used, all other genera studied by Bygrave were omitted. Of the possible relatives of *Miliusa* based on the work by Chatrou et al. (in prep.) mentioned previously, *Ancana*, *Sapranthus*, several *Polyalthia* species and *Miliusa* itself were included. The combined analysis showed a better resolution than the cladogram based solely on molecular data and *Miliusa*, *Sapranthus, Ancana* and several *Polyalthia* species ended up in one clade (the miliusoids) in the consensus cladogram of each of the three islands. This corroborates the preliminary ideas based on the work by Chatrou et al. (in prep.). When looking at the morphological characters it seems that the pollen characters again are phylogenetically most informative. It would be interesting to see whether the other genera thought to be closely related to *Miliusa* would also end up in this miliusoid clade based on morphology and molecular data.

Conclusions

From the evidence presented here, it is clear that classifications of the Annonaceae, based on subsets of morphological data are not satisfactory as they do not recognise monophyletic groups unambiguously. In debating the status of the tribe Miliuseae it seems evident that the Miliuseae as variously recognised by different authors is not a monophyletic group, which also seems to be supported by the molecular data. It is envisaged that the tribe in the present circumscription of Keβler does not comprise a natural group.

In order to find out which morphological characters are phylogenetically informative one must not look at the subsets of morphological data independently but rather combine them to a complete data set, preferably supplemented with molecular data. Within the Annonaceae several such studies are now being carried out.

In our further studies we will keep a focus on the genera included in the tribe Miliuseae *sensu* Keβler. By using molecular data we want to establish whether this tribe is really polyphyletic. For this purpose a number of genera will be included (up to 30, from Asia, Central-America and Africa) apart from the genera included in the Miliuseae. Based on the work by Bygrave (2000) we will select which genera are the most relevant in elucidating the relationships. If the tribe is really polyphyletic we will then further study the clade containing the genus *Miliusa* s.s., because this genus has recently been studied morphologically by the authors (Mols & Keβler, in prep.). In this further study on the *Miliusa*-clade more taxa will be added, from *Miliusa* as well as related genera, and when needed, additional genes will be sequenced. At the same time a morphological data set will be constructed for the genera included in the *Miliusa*-clade by combining the available morphological subsets supplemented with additional data scored from literature and by a survey of herbarium collections. By combining the molecular data set and the complete morphological data set (when congruent), and performing a combined phylogenetic analysis we hope to find which morphological characters (states) are phylogenetically most informative. This information might then be used in further revealing the relationships between the Annonaceae as a whole.

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