

# A decade of uncertainty: Resolving the phylogenetic position of *Diclinanona* (Annonaceae), including taxonomic notes and a key to the species

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**Abstract** The molecular phylogenetic placement of *Diclinanona* (Annonaceae) has been debated in the literature for a decade. On the basis of morphological studies the genus was thought to be related to genera now all placed in subfam. Annonoideae. This early hypothesis was supported by the first phylogenetic analyses of Annonaceae. However, more recently a placement in subfam. Malmeoideae was hypothesised based on an analysis of more plastid data, thus contradicting older but also new morphological findings and previous phylogenetic work. The current study uses newly sequenced plastid data for two species of *Diclinanona* to show that the earlier hypothesised placement was correct and discusses the (little) anatomical and morphological data on *Diclinanona* that is available in a phylogenetic framework. Furthermore, an online revision of the three species of *Diclinanona* is presented in order to update the taxonomic knowledge of this genus.

**Keywords** Annonaceae; *Diclinanona*; morphology; phylogenetic placement; South America; taxonomic revision

**Supplementary Material** The Electronic Supplement (Taxonomic treatment) is available in the Supplementary Data section of the online version of this article at <http://www.ingentaconnect.com/content/iapt/tax>

## ■ INTRODUCTION

Some morphologically easily distinguishable taxa are notoriously difficult to pin down phylogenetically. This can be the result of insufficient data, analytical artefacts such as long-branch attraction (Graybeal, 1998) or the result of evolutionary phenomena, such as rapid radiations (Givnish & Sytsma, 1997: 89). Phylogenetic trees provide insight into the speciation events that have generated present-day species diversity (Hennig, 1966). When this information is combined with ecological and geographical characteristics of species, hypotheses on the causes of speciation in a particular taxon can be generated (Barracough & al., 1998). Finally, with DNA sequence data the relative timing of speciation events can be deduced (Hey, 1992) and hence also rates of speciation (Barracough & Nee, 2001). Difficult-to-place taxa form a problem because their omission from a phylogenetic tree might drastically change the results of such analyses.

Phylogenetic trees can be used to reconstruct the history of the tropical rainforest biome, such as within the Amazonian region in South America (Rull, 2008; Hoorn & al., 2010). In order to understand the history of this biome, the history of

its constituent taxa also needs to be understood. Important tropical rainforest families such as Annonaceae Juss. (Richardson & al., 2004; Couvreur & al., 2011b; Erkens & al., 2012; Pirie & Doyle, 2012) or Arecaceae Bercht. & J.Presl (Couvreur & al., 2011a; Baker & Couvreur, 2013a, b; Couvreur & Baker, 2013) have therefore been focal points of phylogenetic work. An almost fully resolved plastid phylogeny of Annonaceae has been published, representing all but 15 genera (Chatrou & al., 2012b). The few omissions are primarily due to lack of good quality material for DNA extraction for some of the smaller genera. However, one genus was excluded from several recent studies (Couvreur & al., 2011b; Chatrou & al., 2012b; Erkens & al., 2012; Pirie & Doyle, 2012) because of an on-going debate on its placement: the small genus *Diclinanona* with only three species (Maas & al., 2011).

The molecular phylogenetic position of *Diclinanona* Diels has been debated in literature over the past decade but the mystery of its placement is much older. Diels in 1927 described *D. tessmannii* Diels as belonging to the new genus but in the same publication described *D. calycina* (Diels) R.E.Fr. as *Xylopia calycina* Diels. In 1959 Fries placed *Diclinanona* in his “*Xylopia* Gruppe” (a group now known to be polyphyletic).

Hutchinson (1964) had similar problems placing the genus. Walker (1971) placed *Diclinanona* in the “*Hexalobus* tribe” of the “*Annona* subfamily” together with genera such as *Monodora* Dunal, *Uvariastrium* Engl. and *Hexalobus* A.DC. Although he did place *Diclinanona* in the appropriate subfamily, the exact relationship to the other genera remained unclear. One of the first major molecular phylogenetic analyses of Annonaceae (Richardson & al., 2004) positioned *Diclinanona* in the clade that since 2012 is known as subfam. Annonoideae Raf. A few years later, however, in a more detailed analysis of Annonoideae, Erkens & al. (2009) claimed that *Diclinanona* should be placed in subfam. Malmeoideae Chatrou & al. based on newly generated sequence data. A year after that Huysmans & al. (2010) challenged Erkens & al.’s claim with a study on orbicules. The absence of orbicules was thought to predict an affiliation of *Diclinanona* with Annonoideae, and thus supporting the placement hypothesised by Richardson & al. (2004). Lastly, Chatrou & al. (2012b) positioned *Diclinanona* in the Annonoideae based on unpublished sequence data.

This study provides the best estimate of the molecular phylogenetic placement of *Diclinanona* available today. As an online electronic supplement a revision of its three species (including a list of exsiccatae) is presented in order to update the taxonomic knowledge of this genus.

## ■ MATERIALS AND METHODS

**Taxon and character sampling.** — Analyses were based on the dataset of Chatrou & al. (2012b), which is a supermatrix containing 193 species, representing 95 of 109 genera of Annonaceae, and seven additional species as outgroups. The plastid markers *rbcL*, *trnL* intron, and *trnL-F* spacer were sampled for all 200 species. Six plastid markers, *matK*, *ndhF*, *trnT-L*, *trnSG*, *psbA-trnH*, and *atpB-rbcL*, were sampled for 56 selected taxa that are placeholders for clades within Annonaceae. As a rule the placeholder taxa were chosen to represent early-diverging lineages of clades within the family, and crown nodes of these clades as far as known. For further details on taxon sampling we refer to Chatrou & al. (2012b).

To this published supermatrix we added sequences of *rbcL*, *trnL* intron and *trnL-F* spacer, and *psbA-trnH* for two species of *Diclinanona*: *D. calycina* (Diels) R.E.Fr. (voucher specimen: *M.D. Pirie & al. 116*, Peru (U)) and *D. tessmannii* Diels (voucher specimen: *P.J.M. Maas & al. 8198*, Peru (U)). For *D. calycina* we also sequenced *matK*. GenBank numbers for the sequences used in this study are for *D. calycina*: *rbcL*: KC196270\*, *matK*: KC196271\*, *trnL-F*: KC196272\*, and *psbA-trnH*: KC196269\*; for *D. tessmannii*: *rbcL*: AY841611 and *trnL-F*: AY841689 (an asterisk indicates a sequence newly generated for this study; the *rbcL* and *trnL-F* sequences for *D. tessmannii* we re-sequenced for confirmation). For the third species, *D. matogrossensis* Maas, only five herbarium sheets are available (collected between 1963 and 1985). We assume that the placement of the genus can be determined on the basis of the two other species; *D. matogrossensis* was not included in this part of the study.

**DNA extraction, amplification and sequencing.** — Protocols for DNA extraction, PCR and sequencing are identical to those used before in Annonaceae (Erkens & al., 2008; Chaowasku & al., 2012). Individual sequences were aligned with the supermatrix of Chatrou & al. (2012b) using Mesquite v.2.75 (Maddison & Maddison, 2011). Indel characters for the *Diclinanona* sequences were coded in agreement with the indel coding of the entire dataset, which followed the “simple indel coding” method of Simmons & Ochoterena (2000).

**Phylogenetic analyses.** — We adopted a two-stage strategy for our phylogenetic analysis. During a first round of maximum likelihood and maximum parsimony bootstrap analyses, we analysed the entire dataset of Chatrou & al. (2012b), supplemented with the two species of *Diclinanona*. These analyses were designed to optimize efficiency (rather than depth of the analyses) with the aim to (1) examine possible incongruences among the data partitions and (2) determine the subfamily of Annonaceae to which the *Diclinanona* species belong. Maximum parsimony bootstrap analyses were performed using PAUP\* v.4.0a136 (Swofford, 2002), and consisted of a large number of bootstrap replicates (50,000), while minimizing the thoroughness of searches and computing time per bootstrap replicate by limiting the number of random addition sequence replicates to one, saving a single tree (following Müller, 2005). Maximum likelihood bootstrap analyses implemented the efficient and rapid heuristic bootstrap described by Stamatakis & al. (2008), included in the programme RAxML v.7.0.4 (Stamatakis, 2006). Both the maximum parsimony and maximum likelihood analyses placed *Diclinanona* in subfam. Annonoideae tribe Annoneae Endl. (results not shown). Subsequently, taxon sampling focused on Annonoideae by excluding most of the Malmeoideae (except *Annickia pilosa* (Exell) Setten & Maas, *Klarobelia inundata* Chatrou, *Piptostigma mortehani* De Wild., and *Maasia discolor* (Diels) Mols & al.). All species of the two basal subfamilies Anaxagoreoideae and Ambavioideae were retained in the second stage of analyses. This reduced the number of included Annonaceae species and outgroups to 95, permitting in-depth tree inference while avoiding computationally over-demanding analyses. The dataset of 95 species was analysed using maximum parsimony, maximum likelihood and Bayesian inference methods.

Maximum parsimony analyses were performed using PAUP\* v.4.0a136 (Swofford, 2002), and character states were specified as unordered and equally weighted (Fitch parsimony; Fitch, 1971). The search strategy consisted of 25,000 replicates of random addition sequence, saving a single tree per replicate, using the heuristic search option, tree bisection–reconnection (TBR) branch swapping, and the accelerated transformation (ACCTRAN) criterion. The resulting trees were subsequently used as starting trees and swapped to completion.

Maximum parsimony bootstrap analyses consisted of 5000 bootstrap replicates. As the depth of the tree search and the number of trees saved per resampling replicate are the two primary factors governing the accuracy of bootstrapping procedures (Freudenstein & Davis, 2010), we increased the number of random addition sequence (RAS) replicates as well as the number of trees saved per RAS replicate while minimizing the

thoroughness of searches and computing time per bootstrap replicate by limiting the number of random addition sequence replicates to one, saving a single tree (Müller, 2005).

MrBayes v.3.2 (Ronquist & al., 2012) was used for Bayesian inference of phylogenetic trees. Following Chatrou & al. (2012b) the data were partitioned into three subsets: the combined protein coding regions, the combined intron and spacer regions, and the combined binary coded indel characters. The substitution models for the first two data partitions were estimated during the MCMC runs (so-called “model jumping”) by sampling across model space and integrating over all possible models. A gamma distribution for among-site rate variation, and a proportion of invariant sites were assumed, separately for the coding and non-coding partitions. The binary model (Iset coding = variable) was applied to the coded (presence/absence) indel characters. Analyses consisted of three independent runs of 10 million generations, with four chains per run, sampling every 1000th generation. The resulting output was used to examine convergence of runs. In particular, we examined chain swap information, as reported by MrBayes v.3.2, ESS values (using Tracer v.1.5; Rambaut & Drummond, 2009), the cumulative clade posterior probabilities and the comparison of split probabilities for paired MCMC runs (both using AWTY; Nylander & al., 2008).

Maximum likelihood analyses were run using RAxML (Stamatakis, 2006) on the CIPRES portal (<http://www.phylo.org/portal2>; Miller & al., 2010). The data were analysed using the same partitions as for the Bayesian inference, with exclusion of the indel characters that cannot be analysed by RAxML. The search for the tree with the highest likelihood was conducted by running 200 alternative runs on different starting trees. Confidence values were obtained using the standard non-parametric bootstrapping approach (the “-b” option), with 1000 bootstrap replicates, consequently ruling out the option to include invariant sites into the model.

**Revisionary work.** — Herbarium material was investigated from the following herbaria: F, G, GH, K, L, MO, NY, P, U, US, WAG, and WIS. Measurements, colour indications and descriptions of surface structures are based on dried material, unless stated otherwise. We have indicated the density of hair cover according to the following gradations: densely, rather densely and sparsely.

## ■ RESULTS

**Phylogenetic analyses.** — The dataset consisted of 7965 characters, including 130 indel characters. The first stage of maximum likelihood and maximum parsimony analyses to examine possible conflict between data partitions all produced consistent results, showing *Diclinanona* to belong to (clades within) subfam. Annonoideae. For that reason, the data of the individual partitions were concatenated for further analyses, and taxon sampling was limited to focus on phylogenetic relationships within Annonoideae.

The three different methods of phylogenetic inference all agreed on phylogenetic relationships, and on the position of

*Diclinanona*. The maximum parsimony analyses produced 288 shortest trees (tree length 8630; consistency index, CI = 0.608; retention index, RI = 0.718).

The three MrBayes runs converged on similar results. For each run, the proportion of successful state exchanges indicated good mixing among chains. After discarding 2.5 million generations (25%) as burn-in, ESS values were all well above 200. The “compare” command in AWTY showed that split frequencies of the three pairwise comparisons were strongly correlated. The cumulative split probabilities displayed straight lines for the post burn-in generations, indicating that the analyses had reached a likelihood-plateau. Estimates of the model probabilities indicated substantial uncertainty with regard to the preferred substitution model. In each run, the posterior probability of five different models exceeded the reporting threshold of 0.05. The model gtrsubmodel [123421] received the highest probability (run 2,  $P = 0.548$ ).

Clade support values were similar for all inference methods. The two accessions of *Diclinanona* were retrieved as a maximally supported clade within tribe Annoneae, sister to a clade consisting of *Annona* R.E.Fr., *Disepalum* Hook.f., and *Asimina* Adans. (Fig. 1). The latter node received the lowest support values, which nevertheless was well supported (bootstrap values of 90% and 95% in maximum parsimony and maximum likelihood analyses, respectively, and a posterior probability of 1.0 from Bayesian inference).

**Revisionary work.** — The revisionary work maintained the three recognized species of *Diclinanona* as distinct entities. Their circumscriptions have been updated and a list of exsiccatae has been created. There exist a few aberrant specimens of *D. calycina* which may be extreme forms of this species or possibly represent an undescribed species. The complete revision and list of exsiccatae are presented in the Electronic Supplement. For convenience a generic circumscription and key to the species are included after the discussion of the phylogenetic results.

## ■ DISCUSSION

**Phylogenetic placement.** — The ongoing discussion on the molecular phylogenetic placement of *Diclinanona* within Annonaceae has lasted almost a decade. Erkens & al. (2009) attributed the placement by Richardson & al. (2004) in Annonoideae to dubious *trnL-F* and *rbcl* sequences, convinced that their own newly generated sequences were correct in placing it in Malmeoideae. However, re-sequencing of *D. calycina* in this study (in two independent labs) yielded sequences that matched the earlier sequences of Richardson & al. (2004) and not those of Erkens & al. (2009). This means that not Richardson & al. but Erkens & al. were in error. The published sequences by Erkens & al. (*psbA-trnH*: EF179314; *ndhF*: EF179291; *trnS-G*: EF179333; *atpB*: EF179256) were therefore removed from GenBank.

The current analysis showed *Diclinanona* as a maximally supported clade within tribe Annoneae, sister to a clade consisting of *Annona*, *Disepalum* and *Asimina* (Fig. 1). All authors feel confident that this result is solid.

**Some morphological interpretations.** — The current placement of *Diclinanona* fits with published morphological data. The Annonaceae are taxonomically well known (for a summary see Chatrou & al., 2012a, b) but the (little) anatomical and morphological data on *Diclinanona* always lacked a phylogenetic interpretation.

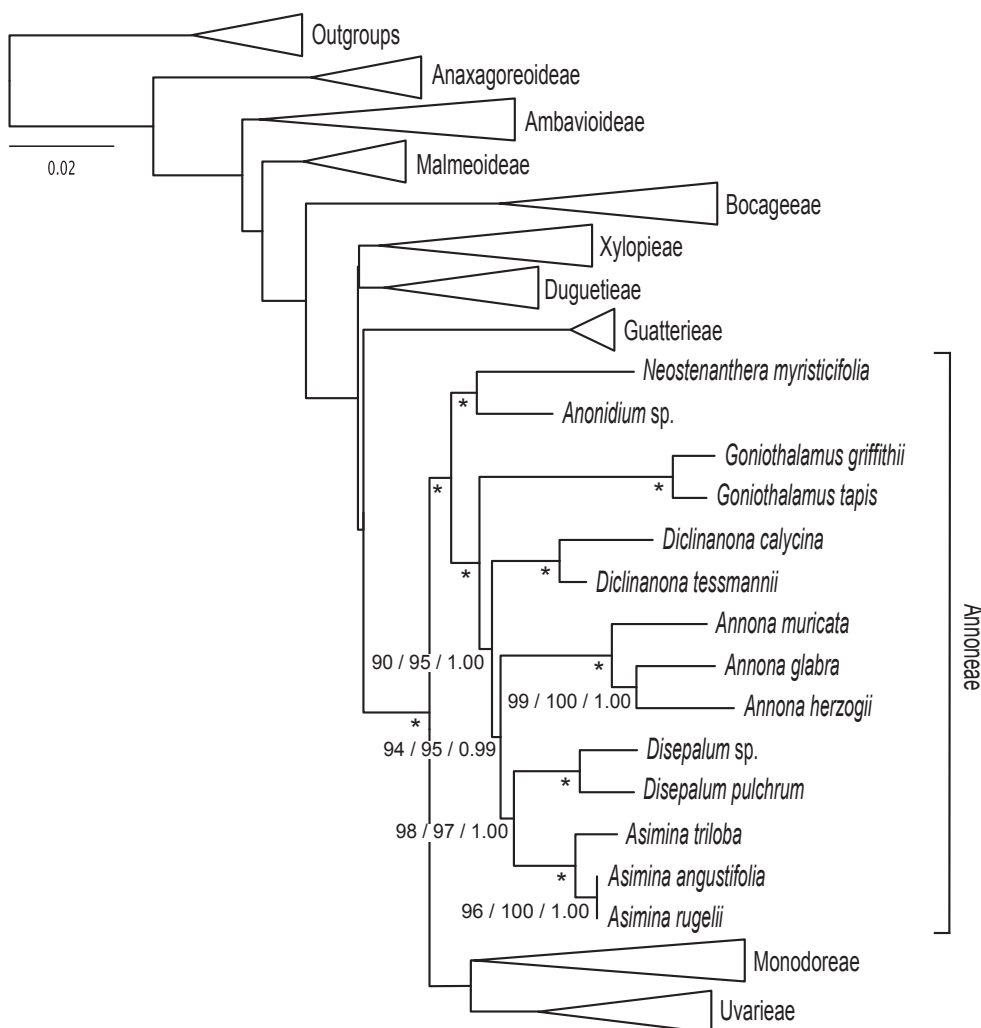
Huysmans & al. (2010) demonstrated a strong phylogenetic signal in the presence/absence of orbicules. The phylogenetic placement of *Diclinanona* in tribe Annoneae is consistent with the absence of orbicules and confirms the predictive value of this character for classification in Annonaceae (Huysmans & al., 2010).

Annonaceae are among the families with wood that is easy to recognize even to the naked eye because of the typical concentric parenchymatic bands (Koek-Noorman & Westra, 2012). Within the family, however, it is very difficult to distinguish groups or genera because of the homogeneous wood structure (e.g., Ter Welle & Du, 2003). A notable feature of *Diclinanona* wood is the presence of oil cells in the rays (Koek-Noorman & Westra, 2012: figs. 19E–F, 20A). However, the genus is not unique in this characteristic, since it has been found in many, only distantly related, genera such as *Cyathocalyx* Champ. ex

Hook.f. & Thomson, *Cymbopetalum* Benth., *Guatteria* Ruiz. & Pav., *Monodora*, or *Xylopia* L.

Inflorescences of *Diclinanona* resemble those in several other, not necessarily related genera, e.g., *Unonopsis* R.E.Fr. and *Xylopia*, found in the Malmeoideae and Annonoideae clades, respectively (Chatrou & al., 2012b). Morphological studies of inflorescences were made by R.E. Fries (1911, 1919, 1959), and later by Weberling & Hoppe (1996); those of *Unonopsis* and the related small genera *Bocageopsis* R.E.Fr. and *Onychopetalum* were recently discussed by Maas & al. (2007). However, inflorescence structure in relation to phylogeny in Annonaceae is largely unexplored terrain.

Inflorescences of Annonaceae are determinate, i.e., the axis is always terminated by a flower. The other possibility, found in numerous other angiosperm families, is that the axis does not develop a terminal flower and thus retains its potential to grow on: the indeterminate or racemose inflorescence. An inflorescence form often found in Annonaceae is the rhipidium (“fan”), a monochasial cyme where a second flower develops from the axil of the lower bract of a bibracteate pedicel (very common in Annonaceae), also with a bibracteate pedicel. This second flower may, in turn, develop a third-order flower in the same way



**Fig. 1.** Best-scoring maximum likelihood tree resulting from the RAxML analysis. Clades other than the clade containing *Diclinanona* (tribe Annoneae) have been collapsed. Numbers at nodes indicate parsimony bootstrap percentages, maximum likelihood bootstrap percentages, and Bayesian clade credibility values, respectively. Stars indicate maximum clade support in all three methods.

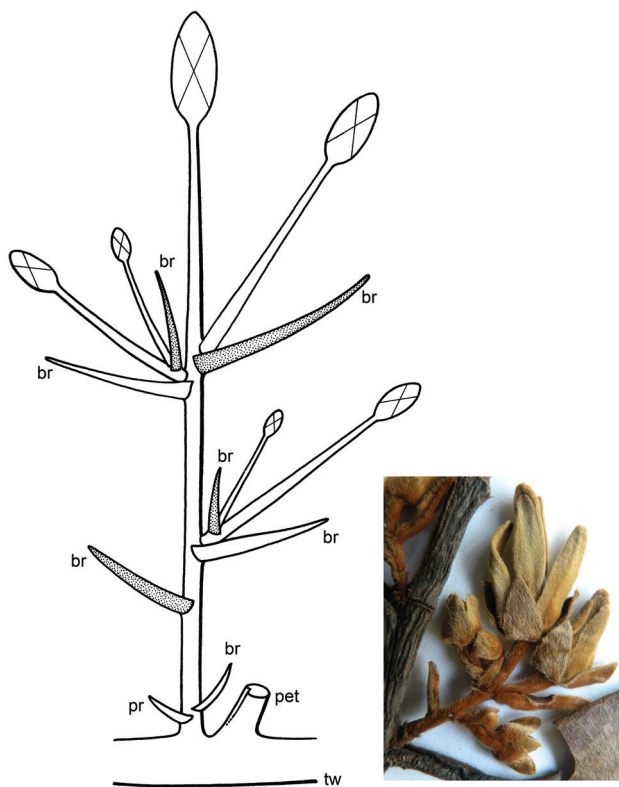
(and this process can be reiterated a number of times in various genera). Characteristically, in a rhipidium all flowers develop in one plane (Fries, 1911, 1919). In *Diclinanona*, as in, among others, *Unonopsis*, well-developed inflorescences combine both cymose and racemose aspects and can be called mixed inflorescences. Here the axis initially is an indeterminate shoot (rachis) which after a varying number of internodes is terminated by a rhipidium. At the same time, or with a slight delay due to suppression by the apical flower, rhipidia (sometimes reduced to only the first flower) develop from the axils of bracts on the rachis. Figure 2 gives a schematic representation of an inflorescence of this kind, termed a thyrsoid (Weberling & Hoppe, 1996). Note that in *Diclinanona* the pedicel has only one bract, not two as in many Annonaceae (see also Fries, 1959: 13ff.).

The vast majority of species of Annonaceae have bisexual flowers from which unisexual flowers have evolved, e.g., twice in tribe Miliuseae, in *Pseuduvaria* Miq. and in a clade consisting of *Sageraea* Dalzell, *Winitia* Chaowasku, and *Stelechocarpus* Hook.f. & Thomson (Su & Saunders, 2006; Chaowasku & al., 2014). A few species have separate male and female flowers, and are usually dioecious, even though monoecy does occur. More common is the occurrence of androdioecy: bisexual and male flowers on different individuals of the same species. This phenomenon has evolved a number of times on all continents

(Couvreur & al., 2009; Saunders, 2010). In the Neotropics, it mainly occurs in tribe Malmeeae Chatrou & R.M.K.Saunders (Chatrou & al., 2012b). Genera such as *Klarobelia* Chatrou and *Pseudomalmea* Chatrou consist exclusively of androdioecious species (Chatrou, 1998). The distribution of flower types in herbarium specimens of well-collected species and scattered field observations suggest that in these species, male individuals are more frequent than bisexual individuals. It is not always known whether rarely collected species are unisexual and dioecious, or androdioecious. Chatrou & Pirie (2003) published a new species of *Ephedranthus* S.Moore, while having observed male individuals only and tentatively assumed the species to be androdioecious, like congeneric species that have been observed more frequently and for which androdioecy has been established (e.g., Lopes & al., 2014).

This has a bearing on the flowers of *Diclinanona*. Flowering material of the three species is limited, and we mostly avoided destructive sampling of scarce flowers on herbarium sheets. We dissected a number of flowers as far as the available material permitted. Our dissections revealed only male flowers, and not a single bisexual or female flower was encountered. In one collection of *D. calycina* three flowers were analysed and all appeared to be male (D.M. Johnson, pers. comm.). Given the more frequent occurrence of androdioecy vs. unisexual and dioecious species in Annonaceae, it would be tempting to hypothesise that species of *Diclinanona* are androdioecious. It should be added immediately here that Spichiger & al. (1989) investigated a number of individuals of *D. tessmannii* all occurring in the Arboretum of Jenaro Herrera (Loreto, Peru), and saw either only male flowers or female flowers, with individuals with male flowers more common than such with female flowers. It is evident that dioecism also occurs in *Diclinanona*. Unlike the Neotropical tribe Malmeeae, *Diclinanona* does not belong to a more species-rich clade that mainly consists of androdioecious species, which would strengthen this hypothesis. Within Annonaceae other androdioecious species are five species of *Annona* L., formerly placed in *Raimondia* Saff. (Westra, 1995). Also *Anonidium* Engl. & Diels is androdioecious with bisexual flowers with a reduced number of stamens (Le Thomas, 1969). Given the phylogenetic relationships between *Annona*, *Anonidium* and *Diclinanona* as found here, it can be ruled out that all androdioecious species make up a clade. It underscores the need for more collections containing flowers, and for observations on the frequency of different flower types in populations of species of *Diclinanona*.

Seeds of *Diclinanona calycina* and *D. tessmannii* were studied by Van Setten & Koek-Noorman 1992: 44, fig. 13C–E). The seeds of the two species appear to be rather different (Fig. 3). The seeds of *D. calycina* are characterized by a raised raphe, a small hilum surrounded by a rib, and a transversely grooved coat. Those of *D. tessmannii* have a large hilum without a rib and a smooth seed coat. Here, the raphe is not raised. The seeds of *D. matogrossensis* were not included in the study by Van Setten and Koek-Noorman as they were not known to them. As can be seen in Fig. 3, the seeds of *D. matogrossensis* are quite similar to those of *D. tessmannii* albeit somewhat smaller in size. In both *D. tessmannii* and *D. matogrossensis*



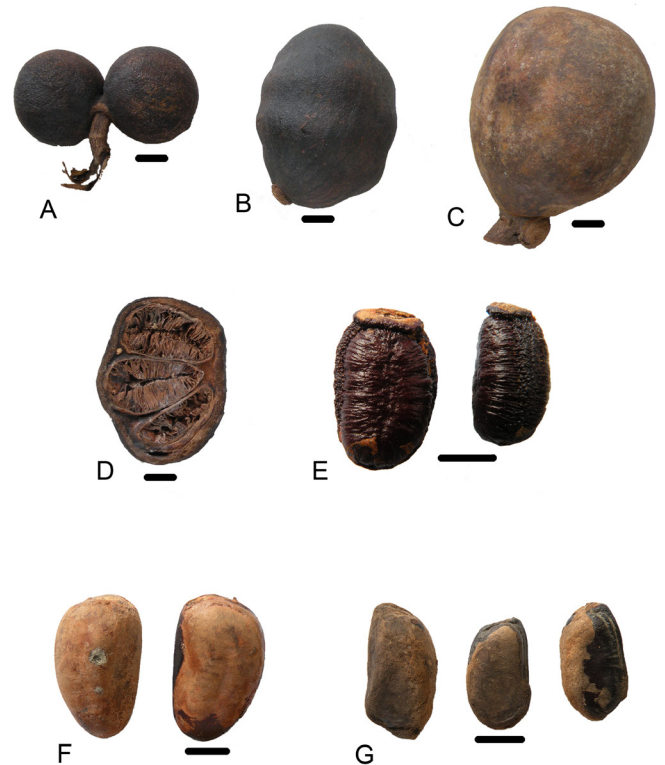
**Fig. 2.** Schematic representation of a thyrsoid inflorescence of *Diclinanona calycina* (drawn after *M. Rimachi* Y. 473, U, as shown in inset); br, bract (bracts blank: fallen off, bracts stippled: present); pet, petiole of supporting leaf; pr, prophyll or lowermost bract; tw, twig. Further explanation given in text.

the seeds are covered with a powdery, brownish, scurfy layer which is absent in *D. calycina*.

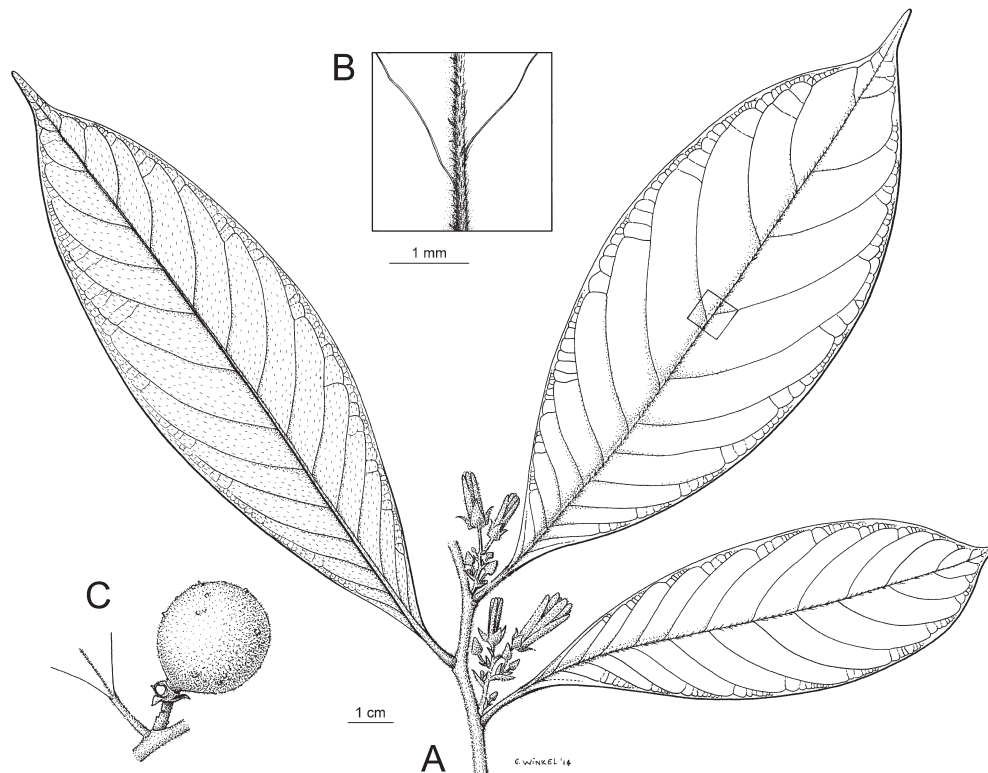
**Key to the species of *Diclinanona*.** — As part of this phylogenetic study also the taxonomic circumscription of the genus and its species has been studied. The last revision of *Diclinanona* dates back to Fries in 1934 although Maas & al. provided a small update on *D. calycina* (Fig. 4) and *D. tessmannii* (Fig. 5) in 1993 together with the description of *D. matogrossensis*. Since then this is the first revision of the genus. In the electronic supplement a revision of all three species (including a list of exsiccatae) is presented in order to update the taxonomic knowledge of this genus. Here we only present a generic circumscription and a key to the three species.

*Diclinanona* Diels in Notizbl. Bot. Gart. Berlin-Dahlem 10: 174. 1927 – Type: *Diclinanona tessmannii* Diels

Trees; young twigs covered with appressed, simple hairs, becoming glabrous with age. *Leaves* distichous, simple, entire, long-petiolate, exstipulate; lamina medium-sized, narrowly elliptic to narrowly obovate, or elliptic to obovate, leaf index varying from 2.5–3.8, chartaceous, not verruculose, base acute, sometimes attenuate, apex acuminate, upper side sparsely covered with simple, appressed or erect hairs to glabrous, lower side sparsely covered with appressed hairs, or densely covered



**Fig. 3.** Fruits and seeds of *Diclinanona*. **A**, fruits/monocarps of *D. calycina* (C.A. Cid Ferreira & al. 7925, U); **B**, fruit/monocarp of *D. tessmannii* (D.C. Daly & al. 5083, U); **C**, fruit/monocarp of *D. matogrossensis* (B. Maguire & al. 56530, U); **D**, monocarp of *D. tessmannii* in longitudinal section (J. Aronson & P.E. Berry 674, U); **E**, seeds of *D. calycina* (P.E. Berry 2194, U); **F**, seeds of *D. tessmannii* (J. Aronson 684, U); **G**, seeds of *D. matogrossensis* (B. Maguire & al. 56530, U). — Scale bars = 10 mm.



**Fig. 4.** *Diclinanona calycina*. **A**, part of flowering twig; **B**, detail of primary vein showing indument on lower side (A and B after L.H.P. Martins & al. 28, U); **C**, monocarp (after P.A.C.L. Assunção 82, U).

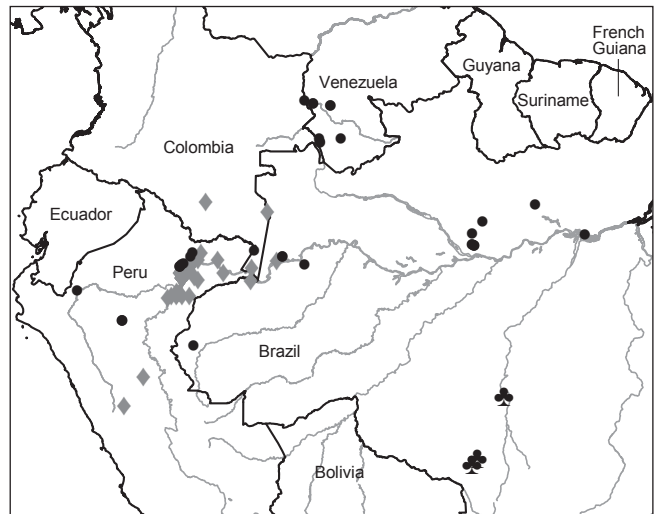
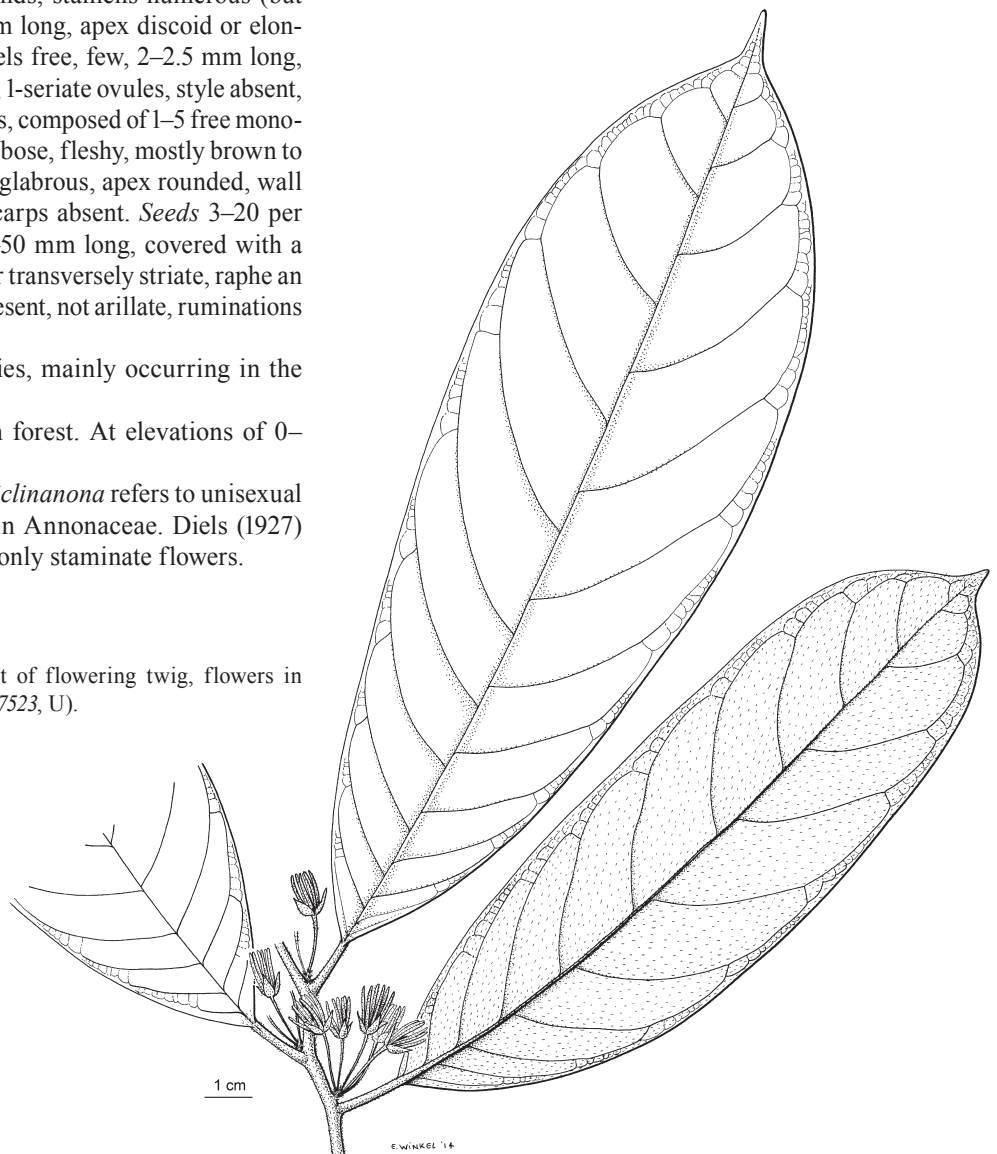
with erect hairs, venation brochidodromous, primary vein impressed above, secondary veins distinct, 9–18 on either side of primary vein, impressed above, tertiary venation reticulate to percurrent, impressed above. *Inflorescences* in axils of leaves or on leafless branchlets, 1–10-flowered, thyrsoidal or dithyrsoidal (Weberling & Hoppe, 1996), i.e., raceme- or panicle-like with the axis ending in a terminal flower, comparatively lax in *D. calycina*, (often) more or less umbellately clustered in *D. tessmannii*, and reduced to 1–3 flowers in *D. matogrossensis*; pedicels 3–40 mm long; articulated at base, bract 1 below articulation, small, <10 mm long, present at flowering time or soon falling off, or (possibly) lacking, rarely foliaceous. Indument of flower parts composed of simple hairs. *Flower buds*: ovoid to rhombic or narrowly rhombic. Flowers actinomorphic, unisexual or bisexual, perianth consisting of one whorl of 3 sepals and two whorls of 3 petals; sepals valvate, free or basally connate, much smaller than the petals; petals green, cream to white, valvate, free, linear to narrowly ovate, 8–30 mm long, equal or distinctly unequal, inner base of inner petals hollow and with 2 marginal glands; stamens numerous (but in bisexual flowers few), 1–2 mm long, apex discoid or elongate, glabrous or papillate; carpels free, few, 2–2.5 mm long, ovary 1-locular with 3–20, lateral, 1-seriate ovules, style absent, stigma spheroid. *Fruit* apocarpous, composed of 1–5 free monocarps; monocarps ellipsoid to globose, fleshy, mostly brown to black, 20–90 mm diam., mostly glabrous, apex rounded, wall 3–10 mm thick; stipes of monocarps absent. *Seeds* 3–20 per monocarp, lateral, ellipsoid, 15–50 mm long, covered with a brownish, scurfy layer, smooth or transversely striate, raphe an indistinct to distinct rib, hilum present, not arillate, ruminations in 2–4 equal parts.

*Distribution.* – Three species, mainly occurring in the Amazon region (Fig. 6).

*Habit and Ecology.* – Rain forest. At elevations of 0–1600 m.

*Note.* – The generic name *Diclinanona* refers to unisexual flowers, a very rare condition in Annonaceae. Diels (1927) when describing this genus saw only staminate flowers.

**Fig. 5.** *Diclinanona tessmannii*. Part of flowering twig, flowers in young stage (after E. Lleras & al. P17523, U).



**Fig. 6.** Geographical distribution of *Diclinanona calycina* (●), *D. tessmannii* (◆), and *D. matogrossensis* (▲).

1. Lower side of leaves densely to rather densely covered with erect hairs; monocarps ca. 20-seeded ..... *D. matogrossensis*
1. Lower side of leaves rather densely to sparsely covered with appressed hairs; monocarps 3–10-seeded ..... 2
2. Monocarps 20–40 mm long; upper side of leaves shiny with densely hairy primary vein and flat to slightly impressed secondary veins; inner petals slightly longer than outer petals, 2–5 mm wide; seeds 15–20 mm long ..... *D. calycina* – Fig. 4
2. Monocarps 60–90 mm long; upper side of leaves dull with glabrous primary vein and strongly impressed secondary veins; petals of inner and outer whorls equal, 1–2 mm wide; seeds 20–50 mm long ..... *D. tessmannii* – Fig. 5

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