

## *Gomphocalyx* and *Phylohydrax* (Rubiaceae): sister taxa excluded from Spermaceace s.s., featuring a remarkable case of convergent evolution\*

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The genera *Gomphocalyx* and *Phylohydrax* (Rubiaceae) have been invariably placed in the tribe Spermaceace s.s. based on the uni-ovulate ovary locules and pluri-aperturate pollen grains. Sequence data from the *rps16* intron and the *rbcL* gene here presented exclude *Gomphocalyx* and *Phylohydrax* from Spermaceace s.s. The two genera are closely related and their nearest relatives are found among members of the former tribe Hedyotideae. This position may be surprising at first, because the growth form of *Phylohydrax* is similar to some observed in Spermaceace s.s. A detailed survey of morphological and anatomical characters shows, however, that the character states of the two genera are largely consistent with the here-proposed position in Hedyotideae. The creeping growth form, the uni-ovulate ovaries and the pluri-colporate pollen grains of *Gomphocalyx* and *Phylohydrax* are the result of convergence between this pair of genera and Spermaceace s.s. The taxonomic position of *Lathraeocarpa*, sometimes considered closely related to *Gomphocalyx*, is also discussed.

**KEYWORDS:** convergent evolution, *Gomphocalyx*, *Hydrophylax*, *Phylohydrax*, Rubiaceae, Spermaceace.

### INTRODUCTION

The present paper deals mainly with two genera of the family Rubiaceae, *Gomphocalyx* Baker and *Phylohydrax* Puff. These taxa have been placed in Spermaceace as traditionally delimited (e.g., Schumann, 1891; Robbrecht, 1988). Although Bremer (1996) and Bremer & Manen (2000) argue that the tribe Spermaceace should be emended to include most of the tribes Hedyotideae, Triainolepideae, Knoxieae and Manettieae, the Spermaceace as traditionally conceived are still monophyletic (Andersson & Rova, 1999; Terrell & Wunderlin, 2002). In what follows, we will refer to it as Spermaceace s.s. Morphologically, this clade is mainly characterized by uni-ovulate ovary locules and pluri-aperturate pollen grains.

*Gomphocalyx herniarioides* Baker is a procumbent to decumbent herb (Figs. 1b, 3) endemic to Madagascar. In the protologue of the genus, Baker (1887) considered it close to *Hydrophylax* L. f. and *Ernodea* Sw., two members of Spermaceace s.s. This position seems supported by the pluri-zonocolporate pollen grains and uni-ovulate locules of *Gomphocalyx*. Capuron (1973), however, supposed a relationship with the monogeneric tribe

Lathraeocarpeae, and Piesschaert (2001) even proposed merging *Lathraeocarpa* Bremek. with *Gomphocalyx*. Indeed, the two taxa are somewhat similar by having one ascending ovule per locule, rather undifferentiated pluri-zonocolporate pollen, and indehiscent fruits. Furthermore, *Gomphocalyx* and *Lathraeocarpa* share the peculiar character of having a calyx with eight lobes, while the corolla is 4-lobed.

*Phylohydrax* was established by Puff (1986) to accommodate one African and one Madagascan species of the genus *Hydrophylax* (placed in Spermaceace s.s. by Robbrecht, 1988). The two species of this genus are pioneers of maritime dune vegetation, and show a differentiation into long creeping shoots and short flowering shoots (Figs. 1c, 2). *Phylohydrax madagascariensis* (Roem. & Schult.) Puff is endemic to the eastern coast of Madagascar (but was recently also introduced in Tanzania), and *P. carnososa* (Hochst.) Puff is restricted to the east coast of continental Africa. The position of the genus in Spermaceace s.s. has never been questioned (Puff, 1986). This is not surprising, since the species have uni-ovulate ovary locules and pluri-zonocolporate pollen grains. Moreover, a number of species from Spermaceace s.s. have growth forms similar to the ones

\*This article is dedicated to our good friend, colleague, and co-author, Lennart Andersson, who died unexpectedly while this paper was in press.

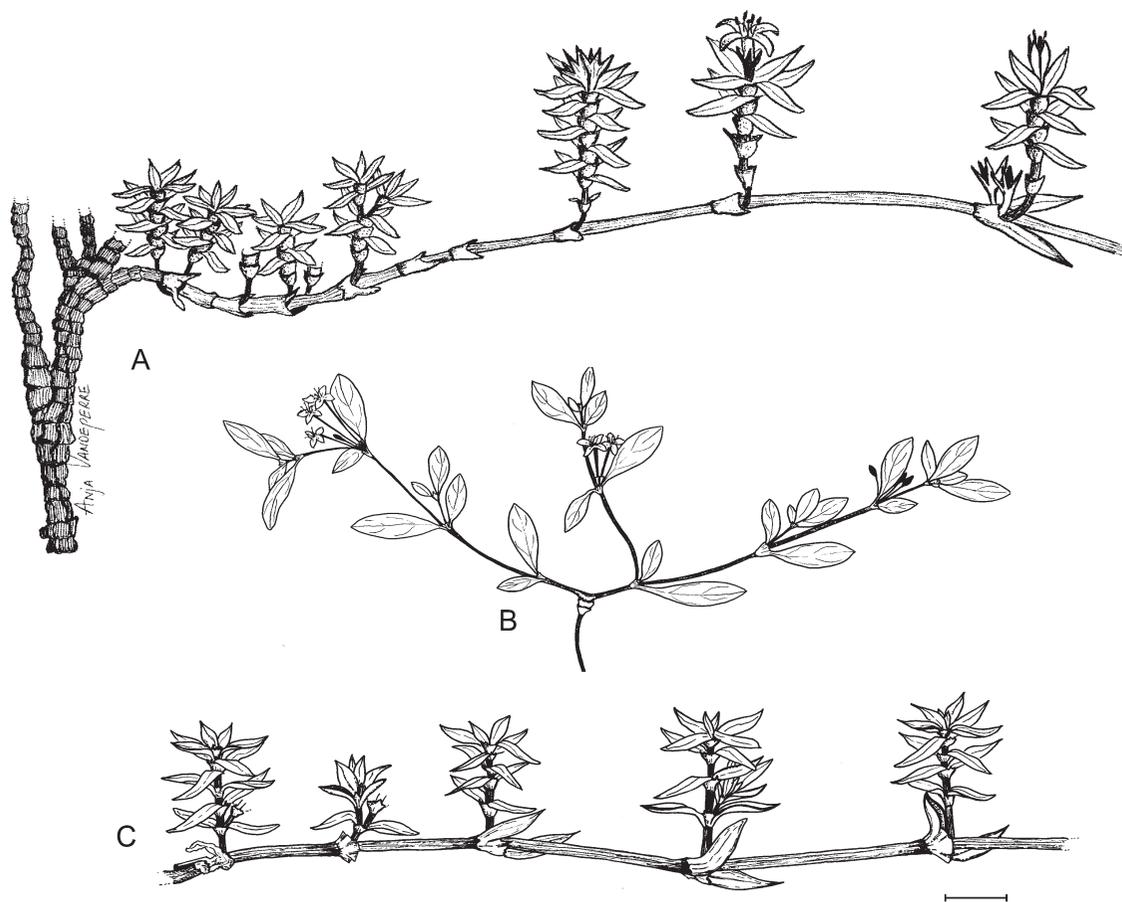


Fig. 1. Growth form. A, *Hydrophyllax maritima* (Cooray 69011707R, Sri Lanka, BR); B, *Gomphocalyx herniarioides* (De Block & al. 569, Madagascar, BR); C, *Phylohydrax madagascariensis* (De Block & al. 640, Madagascar, BR). Scale bar = 1 cm.



Figs. 2–3. Photographs of *Phylohydrax madagascariensis* (2) and *Gomphocalyx herniarioides* (3). Photos by Steven Dessein and Petra De Block, respectively.

observed in *Phylohydrax*. In particular, the growth form of *Hydrophyllax maritima* L. f. is very similar (Fig. 1a), being the main reason why the species of *Phylohydrax* were once placed in that genus.

We included *Gomphocalyx* and *Phylohydrax* in a phylogenetic study of Spermacoceae based on DNA sequence variation. Rather to our surprise, it turned out that these two are sister taxa, and that they do not belong to Spermacoceae s.s. but instead fall among taxa from the former tribe Hedyotideae. The present study presents this molecular evidence and identifies morphological and anatomical features consistent with (1) their exclusion from Spermacoceae s.s. and (2) their new position among members of the former tribe Hedyotideae. Therefore, a comparative morphological and anatomical study of the poorly known *Gomphocalyx herniarioides* is presented. The taxonomic position of *Lathraeocarpa*, a Madagascan genus sometimes linked with *Gomphocalyx*, is also discussed.

## MATERIALS AND METHODS

**Morphological investigations.** — The morphological study is based on herbarium material from BR, K, MO, and P. *Phylohydrax madagascariensis* and *Gomphocalyx herniarioides* were also studied in the field, and pickled material collected by Petra De Block was available to investigate leaf, ovary, and seed anatomy.

Methods follow normal practice of herbarium taxonomy (De Vogel, 1987). The distribution map was produced using iMap (Schols & al., 2001); the specimens used are listed in Appendix 1. Pollen data are based on observations of acetolysed pollen grains, which were obtained following Reitsma's "wetting agent" method (1969) as outlined by Dessein & al. (2002).

Anatomical sections were made from tissues embedded in 2-hydroxyethyl methacrylate (GMA). The material was first dehydrated by putting the samples in increasing ethanol concentration (2 hours 70%, 2 hours 96%, 2 hours 100%). They were then transferred to the infiltration medium for 36 hours (100 ml Technovit 7100 resin and 1 gr of Hardener, i.e., dibenzoyl peroxide). Finally, the tissues were placed in the molds and covered with the embedding solution (15 parts of infiltrating solution and 1 part of Hardener II, dimethyl sulfoxide).

**Molecular methods.** — Some *rps16* intron sequences were obtained following the protocols described by Andersson & Rova (1999). Others, produced at the Laboratory of Plant Systematics (K. U. Leuven), were obtained by the methods described below.

Total genomic DNA was extracted from leaf tissues of fresh, silica-dried or herbarium material. The isolation method followed the procedure as described by Geuten & al. (2004). The buffer consisted of 2% CTAB, 2% PVP-40, 2% 2-mercaptoethanol (pH 9.5) and 4 mM DIECA and 5 mM ascorbic acid (Chase & Hills, 1991). The extract was washed two times with ethanol 76% - NH<sub>4</sub>Ac. The *rps16* intron was amplified on a DNA Thermal cycler 480 or a GeneAmp 9700 (Applied Biosystems) thermal cycler using the *rpsF* and *rpsR2* primers described by Oxelman & al. (1997). Taq polymerase used was supplied by Amersham Pharmacia Biotech or Fermentas. Invariably, 25 µl PCR reactions were run. Denaturation (95°C, 2 min) was followed by 25–35 cycles of denaturation at 95°C (30 sec), primer annealing at 55–57°C (1 min), and extension at 72°C (2 min), ending with a final extension for 7 min at 72°C. The amplification products were purified with Qiaquick (Qiagen) spin columns according to the manufacturer's instructions.

Dideoxy sequencing was conducted using the BigDye dye terminator cycle sequencing protocol (from Applied Biosystems) with addition of 5% DMSO. The

sequencing products were purified by an isopropanol precipitation (modified from protocol supplied by Applied Biosystems). The sequencing reactions were analyzed with an ABI PRISM™ 310 Genetic Analyzer. Sequences were verified and edited with the Staden Package.

GenBank accession numbers with voucher specimen and country of origin are given in Appendix 2. Sequences were aligned manually. Some regions of the *rps16* intron could not be aligned with confidence and were deleted from the final matrices. Indel coding for the *rps16*-intron followed the criteria as described by Andersson & Rova (1999).

## RESULTS

**Molecular evidence.** — While analyzing ITS sequences of members of Spermaceae s.s., we noticed that *Gomphocalyx* and *Phylohydrax* were nested among the outgroup taxa. Since only a few ITS sequences are available for the subfamily Rubioideae, it was impossible to find the closest relatives of the two genera based on these data alone. For this reason, *rps16* intron sequences were determined as well. These sequences were then combined with sequences available in GenBank. A few preliminary analyses with representatives of the major groups of Rubiaceae included showed that the closest relatives of the two genera were amongst former Hedyotideae. The final matrix then also contains numerous taxa from Spermaceae alliance with a few taxa from the *Psychotria* L. alliance as outgroup (see Appendix 2). The aligned *rps16* intron matrix had 897 characters, 470 of which were constant, 178 variable but uninformative, and 249 informative. The cladistic analysis was done with PAUP\* 4.0b10 (Swofford, 2002). A jackknife analysis of 1000 replicates was performed with the following parameters: 37% of characters deleted in each replicate; each replicate with 20 random addition sequence replicates; 5 trees saved in each replicate, and TBR branch swapping. A combined *rbcL* and *rps16* intron matrix was also analyzed. Only those taxa for which the two genes were available were retained in this second matrix, except for *Gomphocalyx* for which only the *rps16* intron sequence is available. The same search procedure as described for the *rps16* intron matrix was performed. Of the 2315 characters, 1615 were constant, 329 were variable but uninformative, and 371 were informative.

The tree (Fig. 4) derived from the *rps16* intron sequences is largely in agreement with the cladogram published by Andersson & Rova (1999). The emended tribe Knoxieae sensu Andersson & Rova (including the Hedyotideae-*Pentas* group, Triainolepideae, and the gen-

era *Carphalea* and *Otiophora*) forms the sister group of a clade that comprises Anthospermeae, Danaideae, the Hedyotideae-*Hedyotis* group, Manettieae, Paederieae, Rubieae, and Spermaceae s.s.

The tree based on *rps16* intron and *rbcL* sequences (Fig. 5) is similar to the tree published by Bremer and Manen (2000), which was based on *rps16*, *rbcL* and *atpB-rbcL* spacer data. In contrast to the latter study, however, Paederieae, Theligoneae, Anthospermeae, Rubieae, and Argostemmateae do not form a monophyletic group but appear unresolved as four groups that form a polytomy together with Spermaceae sensu Bremer & Manen. Apparently, the support for this group in the study of Bremer & Manen (2000) is mainly based on the *atpB-rbcL* spacer data.

The main difference between the two trees here presented lies in the position of emended Knoxieae. In the combined analysis, this group no longer takes a basal position, but is the sister group of a clade comprising most genera of the former tribe Hedyotideae and Spermaceae s.s. However, the basal position in the *rps16* analysis had only ambiguous support.

In the two analyses, most genera traditionally included in Spermaceae form a monophyletic group within a clade that comprises members of the former tribe Hedyotideae. This is not the case for *Phylohydrax* and *Gomphocalyx*. They come out as sister taxa in a strongly supported clade containing inter alia *Conostomium* (Stapf) Cufod. and *Amphiasma* Bremek., two genera

endemic to mainland Africa, formerly placed in Hedyotideae and considered closely related to *Oldenlandia* L. (Bremekamp, 1952; Verdcourt, 1976).

#### Morphology and anatomy of *Gomphocalyx*.

— Whereas *Phylohydrax* was rather well documented by Puff (1986), not a single study has been devoted to the morphology and anatomy of *Gomphocalyx*. Therefore, detailed morphological and anatomical data are here provided for *Gomphocalyx* with reference to character states observed in the Spermaceae s.s. and Hedyotideae. These data will then be confronted with the molecular evidence. Morphological data of the main taxa under discussion are summarized in Table 1.

**Growth form.** — *Gomphocalyx herniarioides* is a prostrate or decumbent herb, sometimes forming dense mats (Figs. 1b, 3). The green or reddish-brown stems radiate from the somewhat woody base and are up to 50 cm long (usually shorter). They are much branched, round in cross-section, and finely ribbed. Stems are usually glabrous, although just below the stipule sheath the ribs may be scabrid. The primary root is well-developed and often somewhat woody. The species has always been described as annual, but as many specimens studied have a somewhat woody base and taproot, it is probably perennial.

This type of growth form is frequently observed among members of Hedyotideae and is not uncommon in Spermaceae s.s. either.

**Leaves and stipules.** — Leaves are decussate,

**Table 1. Character states as observed in *Gomphocalyx*, *Lathraeocarpa*, *Phylohydrax*, *Hydrophylax*, and Spermaceae s.s. for some selected characters.**

Characters	<i>Gomphocalyx</i>	<i>Lathraeocarpa</i>	<i>Phylohydrax</i>	<i>Hydrophylax</i>	Spermaceae s.s.
<b>Growth form</b>	procumbent or decumbent (?) perennial herbs	subshrubs	perennial herbs with differentiation into creeping long shoots and short erect shoots	perennial herbs with differentiation into creeping long shoots and short erect shoots	procumbent, decumbent or erect; mostly annual or perennial herbs, but also shrubs and lianas
<b>Leaf</b>	amphistomatic	unknown	amphistomatic	hypostomatic	hypostomatic and amphistomatic
<b>Leaf mesophyll</b>	differentiated	unknown	palisades only	differentiated	differentiated
<b>Heterostyly</b>	present	present	present	absent	absent in most taxa, sometimes present
<b>Calyx tube</b>	reduced	reduced	well-developed	reduced	reduced
<b>Number of calyx lobes</b>	8	8	4, reduced to colletar-tipped outgrowths	4	0, 2, (3–)4, 8
<b>Corolla tube</b>	narrowly cylindrical	± cylindrical	funnel-shaped	funnel-shaped	(narrowly) cylindrical, funnel-shaped
<b>Ovule position</b>	attached near base of septum	attached near base of septum	attached near base of septum	attached just below middle of septum	attached near or below middle of septum, rarely near base
<b>Nectary disk</b>	2-lobed	cylindrical	cylindrical	2-lobed	2-lobed or cylindrical
<b>Stigma</b>	2-armed	4-armed	2-armed	subcapitate	often subcapitate, rarely 2-armed
<b>Fruit type</b>	dry and indehiscent	drupes	dry and indehiscent	dry and indehiscent	often capsules or schizocarps, sometimes berries or drupes, rarely dry and indehiscent
<b>Seed</b>	obovoid, ellipsoid or pyriform without ventral groove	unknown	ellipsoid without ventral groove	ellipsoid with ventral groove	variably shaped, ventral groove present, though sometimes wide and obscure, absence of groove uncertain in <i>Schwendenera</i>
<b>Pollen type</b>	pluri-zonocolporate	pluri-zonocolporate	pluri-zonocolporate	pluri-zonocolporate	pluri-zonocolporate, pluri-zonopo(ro)-rate, pluri-pantopo(ro)rate, rarely 3-colporate
<b>Pollen sexine</b>	(micro-)reticulate with granules	(micro-)reticulate with granules	(micro-)reticulate with granules	perforate with microspines	mostly perforate, (micro-)reticulate or psilate with granules or microspines

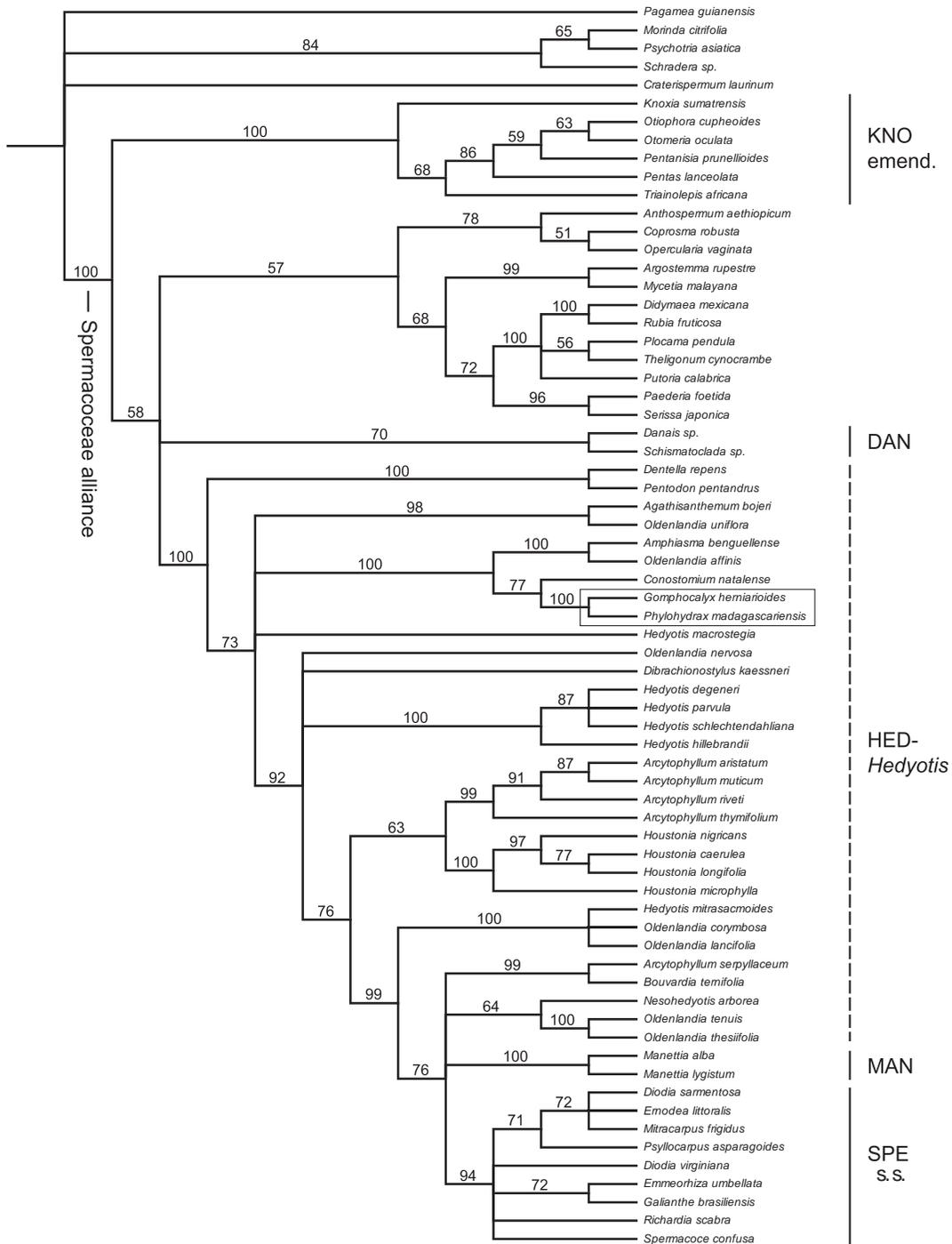


Fig. 4. Phylogeny of Spermacoceae alliance estimated on basis of *rps16* intron sequences. Numbers above branches are jackknife support values. DAN = Danaideae; HED-Hedyotis = part of former Hedyotideae including *Hedyotis*; MAN = Manettieae; SPE = Spermacoceae; KNO = emended *Knoxieae* sensu Andersson & Rova.

sessile or rarely short-petiolate, and connate with the stipule base forming a sheath around the stem as observed within most members of the Spermacoceae sensu Bremer & Manen. Leaf-blades are elliptic to narrowly elliptic, sometimes slightly ovate or obovate,

rarely almost linear. They are mostly dark-green, turning yellowish-green when dried. They are 0.4–2.7 cm long and 0.1–0.8 cm wide, glabrous above and below or sparsely beset with scabrid hairs above. In most specimens, the leaves are distinctly anisophyllous (Fig. 1b).

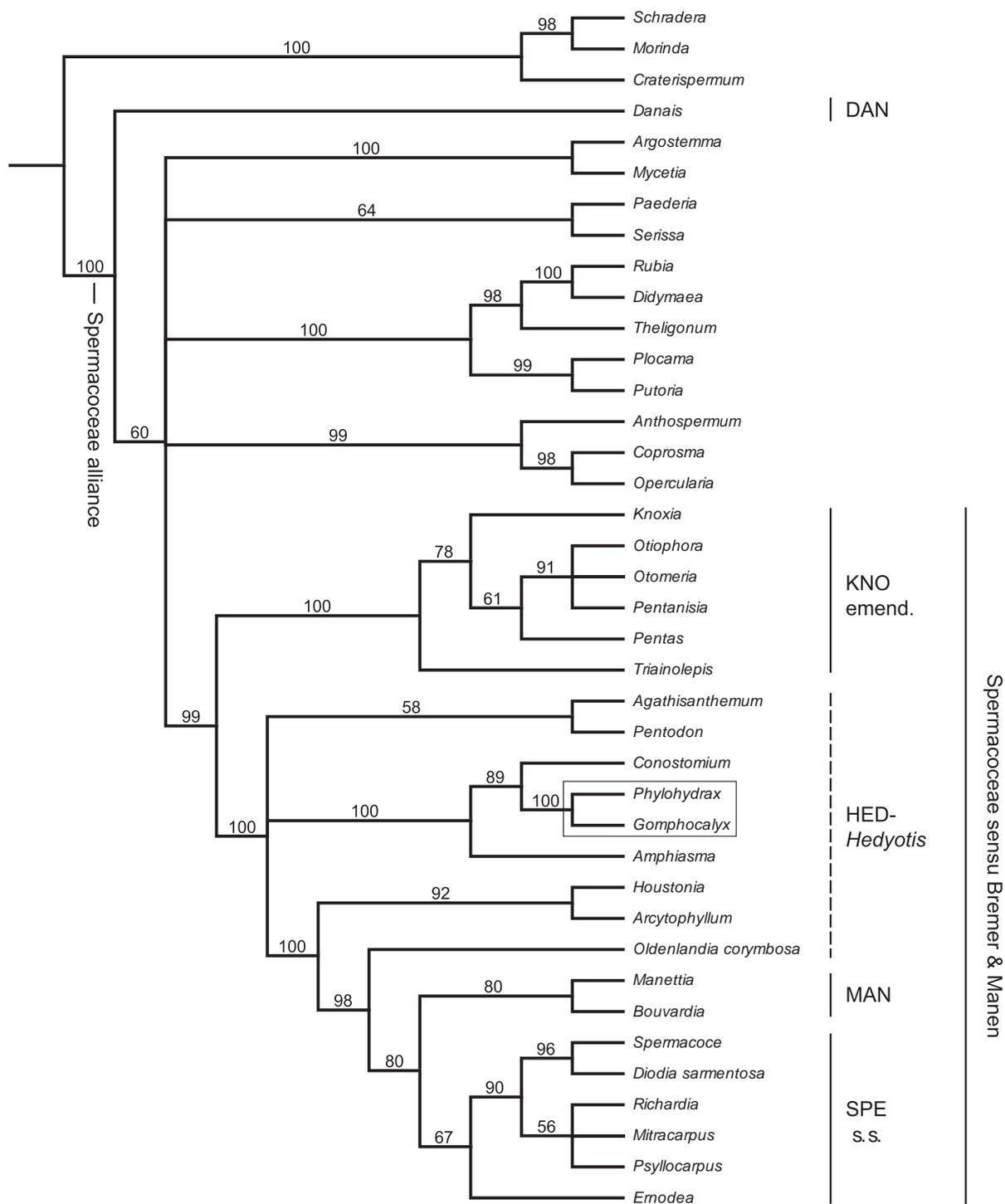
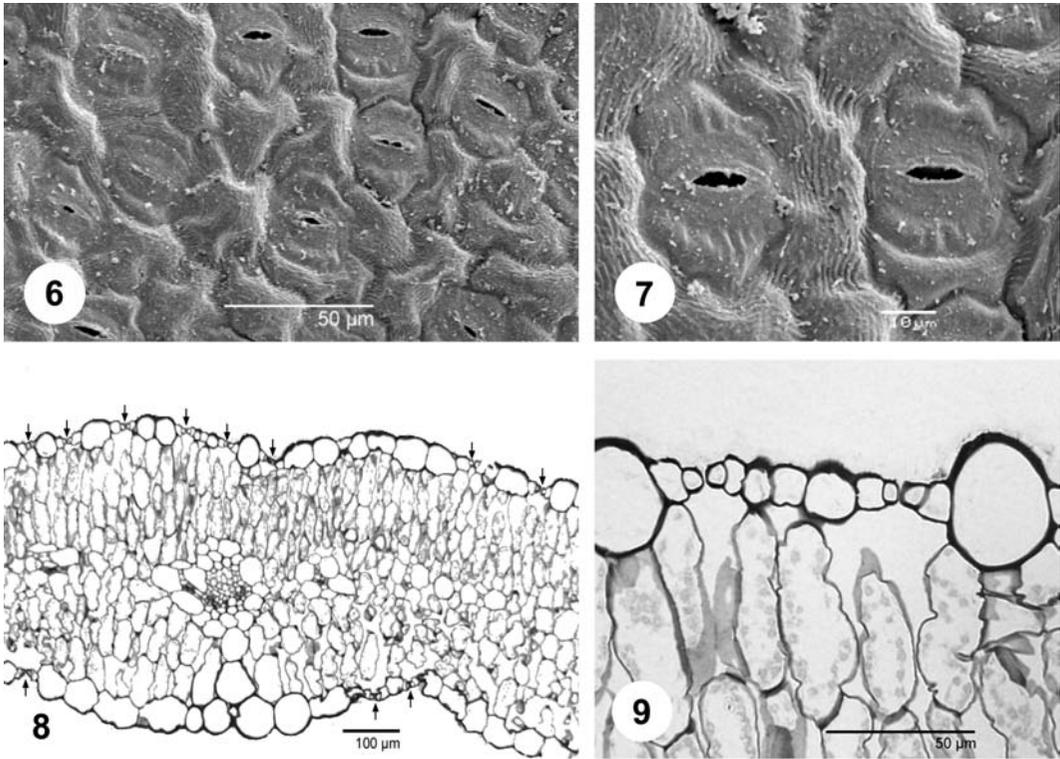


Fig. 5. Phylogeny of Spermaceace alliance estimated on basis of *rps16* intron and *rbcL* data. Numbers above branches are jackknife support values. Abbreviations as in Fig. 4.

The margins are flat and minutely scabrate (often hardly visible on older leaves). The leaf apex is (broadly) acute to almost obtuse or slightly cuspidate; the base is variable, often gradually narrowed, sometimes attenuate, rounded or acute. The main vein is not very prominent

and has up to 5 glabrous secondary veins on each side. The stipules are almost truncate. The stipule sheath is cup-shaped, 0.5–2 mm long, almost glabrous, and papery when dried. The stipule margin is irregular, sometimes crowned with a few short fimbriae, 0.2–1 mm long and



Figs. 6–9. Leaf morphology and anatomy of *Gomphocalyx herniarioides*. 6, detail of upper surface of leaf blade showing stomata; 7, detail of two stomata; 8, LM photo of cross-section through leaf with stomata (arrows); 9, LM photo of cross-section through leaf showing two neighboring stomata. All from *De Block & al.* 569, Madagascar (BR).

colleter-tipped.

**Leaf anatomy.** — The somewhat fleshy leaves have a rather large-celled epidermis and a mesophyll made of 2–3 layers of palisade parenchyma and several layers of loosely arranged spongy cells (Fig. 8). Raphide-containing idioblasts are present, preferentially in between the spongy and palisade layer. Tanniferous idioblasts are absent. The vascular bundles lack obvious bundle sheaths and are inconspicuous, with the thicker median bundle comprising a few xylem and phloem cells only (Fig. 8).

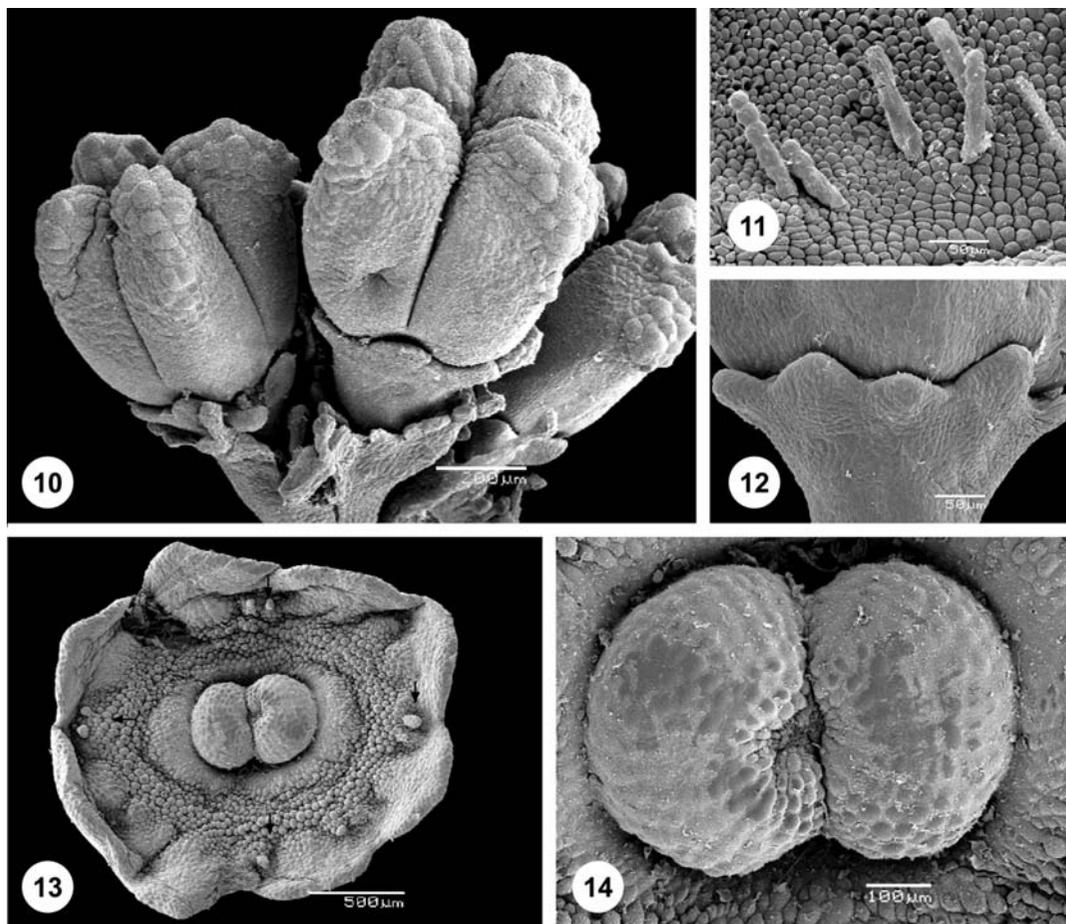
Stomata are paracytic (Figs. 6, 7, 9), as often observed in Rubiaceae (see also Dessein & al., 2001). The leaves are clearly amphistomatic, with a higher density of stomata on the upper surface (Fig. 8). Amphistomatic leaves are considered uncommon in Rubiaceae. It has been observed, however, in several *Spermacoce* species (Holm, 1907; Dessein, 2003), in *Houstonia caerulea* L., and in *Phylohydrax*.

**Inflorescence.** — The inflorescences of *Gomphocalyx* are few- to many-flowered with all the axes short, resulting in a dense cluster of flowers (Fig. 10). The cluster is ca. 0.3–0.5 cm wide in fruiting stage. The bracts are stipuliform, membranous and bear colleter-tipped setae, which rarely exceed 1 mm. The inflorescences are initially terminal, but already during anthesis they are

pushed aside by a developing shoot in one of the axils, which lengthens the stem. Consequently, the inflorescences appear axillary. These so-called pseudo-axillary inflorescences are frequently observed in Spermaceae s.s. but are also found in Hedyotideae.

**Flowers.** — *Gomphocalyx* has heterostylous, 4-merous flowers (not 5-merous as stated in the protologue of the genus). The calyx tube is reduced to a rim, or cup-shaped and then up to ca. 0.4 mm high. It is glabrous or ciliate, with 1 or 2 colleters on the inside between the calyx lobes (Fig. 13). The number of lobes is basically 8 (fruits with 7 lobes are sometimes observed), and the lobes are (broadly) triangular and 0.2–0.5(–1.5) mm long (Figs. 12–13). They are usually glabrous or ciliate, rarely densely hairy. The 8-partite condition of the calyx is probably derived from an originally 4-merous calyx, since larger and smaller lobes alternate with each other. Four interstitial lobes, becoming as large as the four true lobes, seem to have developed in *Gomphocalyx*.

The corolla tube is narrowly cylindrical and very variable in length, ranging from 4 up to 16 mm. Most corolla tubes are 8–12 mm long, and 0.3–1 mm wide, the narrowest point being at or just above the base and the broadest point at the mouth. The corolla is glabrous throughout, or scabrate on the nerves outside and sparsely or more densely pubescent with short hairs inside (Fig.



Figs. 10–14. Flower morphology of *Gomphocalyx herniarioides*. 10, flower buds: note the thickening of the distal part of corolla lobes; 11, inner surface of upper part of corolla; 12, detail of calyx morphology showing five of the eight minute calyx lobes: note the alternation of larger lobes with smaller 'interstitial' ones; 13, top view of ovary showing minute calyx lobes, colleters between the lobes, and nectary disc; 14, detail of bilobed nectary disc. All from *De Block & al.* 569, Madagascar (BR).

11). The corolla lobes are elliptic to narrowly triangular, 2–5 mm long, and glabrous except for broad thick hairs at the tips outside (sometimes obscure) and a few hairs at the base inside. As the corolla tube, they are white or pale pink with a red streak at the base of each lobe.

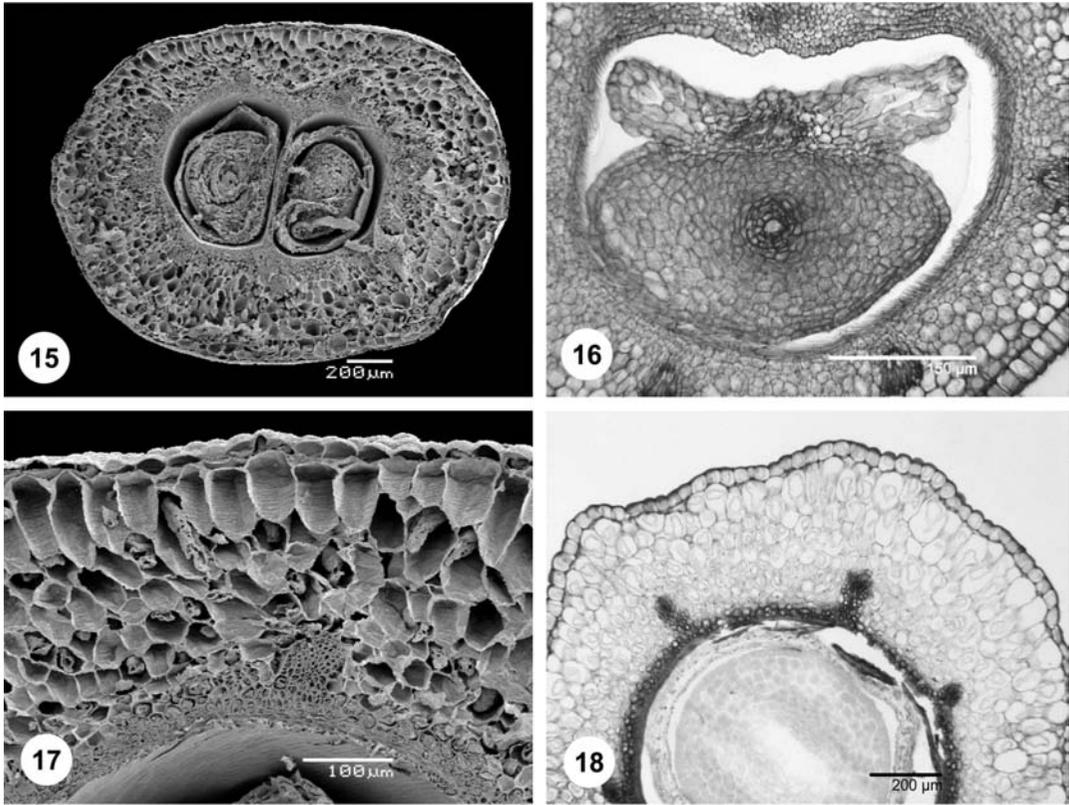
The anthers are dorsifixed, with the filament attached near the base. They are elliptic to narrowly elliptic in outline, 0.6–1.2 mm long and open with longitudinal slits. In the longistylous form only the upper parts of the anthers are exserted. In brevistylous flowers the whole anthers are exserted for 0.5–1.5 mm. The anthers appear white, yellow-green or brown (colour presumably correlated with the maturity of the pollen grains). The filaments are glabrous and white or pale purple.

The ovary is 2-locular (Fig. 15; sometimes one of the locules is aborted in fruit), obovoid to obconic, usually glabrous and (yellow) green. In young ovaries, the vascular bundles can be observed through the exocarp; on dried material they sometimes appear as ribs. Each ovary

locule has one ovule (Figs. 15–16). The placenta is inserted at the base of the septum. Neither the ovule, nor the placenta show any kind of specialized outgrowth. The white style is included in the brevistylous form and exserted for ca. 1.5–2(–3) mm in the longistylous form. In the longistylous morph, the stigma has two somewhat spreading papillose to shortly hairy lobes up to 1 mm long. In the brevistylous morph, the stigma lobes are connivent and sometimes somewhat longer. The nectary disc is found on top of the ovary, surrounding the base of the style. It is clearly bilobed, sunken into the ovary tissue at the early stages, but the central part is distinctly raised at later ones (Figs. 13, 14).

All of the flower characters observed in *Gomphocalyx*, except the uni-ovulate locules, are also observed among members of Spermacoeceae s.s. and Hedyotideae. Uni-ovulate locules have never been reported from the latter group.

**Pollen.** — The pollen grains are 8–10-zono-



Figs. 15–18. Ovary and fruit wall of *Gomphocalyx herniarioides*. 15, transverse section through ovary/young fruit showing relatively thick wall; 16, LM photo of young locule in cross-section: notice the placenta and the developing ovule; 17, detail of ovary wall; 18, LM photo of cross-section through young fruit. All from De Block & al. 569, Madagascar (BR).

colporate, suboblate with an equatorial diameter of 45–55  $\mu\text{m}$ . The ectocolpi are short; the endoaperture is an endocingulum. The tectum is (micro-)reticulate, evenly covered with granules (Figs. 19–22). The inner nexine surface is granular. This pollen type is clearly more common in Spermaceae s.s. than in Hedyotideae, in which 3-colporate pollen grains predominate (cf. Dessein & al., 2000; Huysmans & al. 2003).

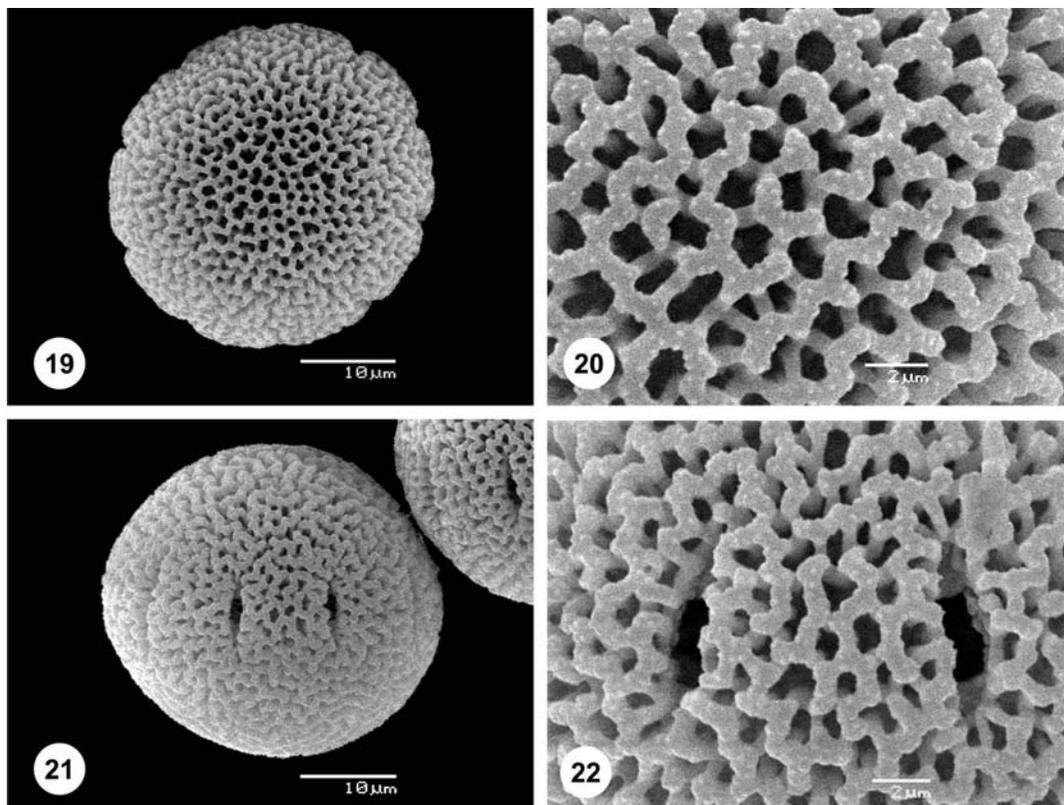
**Fruits.** — The fruits are obconical (Fig. 23), often abruptly narrowed towards the base, 1.75–2.25  $\times$  1.25–2 mm (including the beak), indehiscent, and crowned by the minute calyx lobes. Towards the lower part of the fruit, ribs are often distinctly visible. The fruit is comprised of three distinct layers: a one-layered exocarp, a thick and rather undifferentiated mesocarp and a thin but sclerenchymatous endocarp (Figs. 17, 18). The vascular bundles are initiated in the mesocarp (Fig. 16), but after outgrowth of the ovary into a fruit, they are positioned tight to the endocarp (Fig. 18).

Indehiscent fruits are rarely observed in both Spermaceae s.s. and Hedyotideae, but are present in *Phylohydrax* (Fig. 26). A well-developed beak (outgrowth of the endocarp) is characteristic for many genera

of Hedyotideae.

**Seeds.** — Seeds are obovoid to ellipsoid or somewhat pyriform, 1–1.5  $\times$  0.5–0.8 mm, white. A ventral groove is absent. The seed-coat is parenchymatous without thickenings and somewhat reticulate in surface view (Figs. 24, 25). This seed type is not found in Spermaceae s.s. nor in Hedyotideae, but is present in *Phylohydrax* (Figs. 27, 28). Seeds of Spermaceae s.s. invariably have a more or less distinct ventral groove, while in Hedyotideae the seeds are much smaller and angular.

**Distribution and habitat.** — *Gomphocalyx* is mainly collected from sandy soils (one collection mentions limestone) in thorn forests, dunes and beaches up to 850 m high. Most collections come from two areas in the Western and Central part of Madagascar (Fig. 29): Majunga (Northwest Madagascar) and the area between Tulear (Southwest Madagascar) and Isalo (Central Madagascar). Only one collection comes from Ambovombe on the southern tip of Madagascar. The occurrence of the species in this part of the island needs confirmation through additional collections. As the species is widespread, it is not endangered.



Figs. 19–22. Pollen morphology of *Gomphocalyx herniarioides*. 19, polar view of 10-zonocolporate pollen grain; 20, detail of apocolpium showing (micro-)reticulate tectum covered with small granules; 21, equatorial view of pollen grain; 22, detail of mesocolpium and colpi. All from De Block & al. 569, Madagascar (BR).

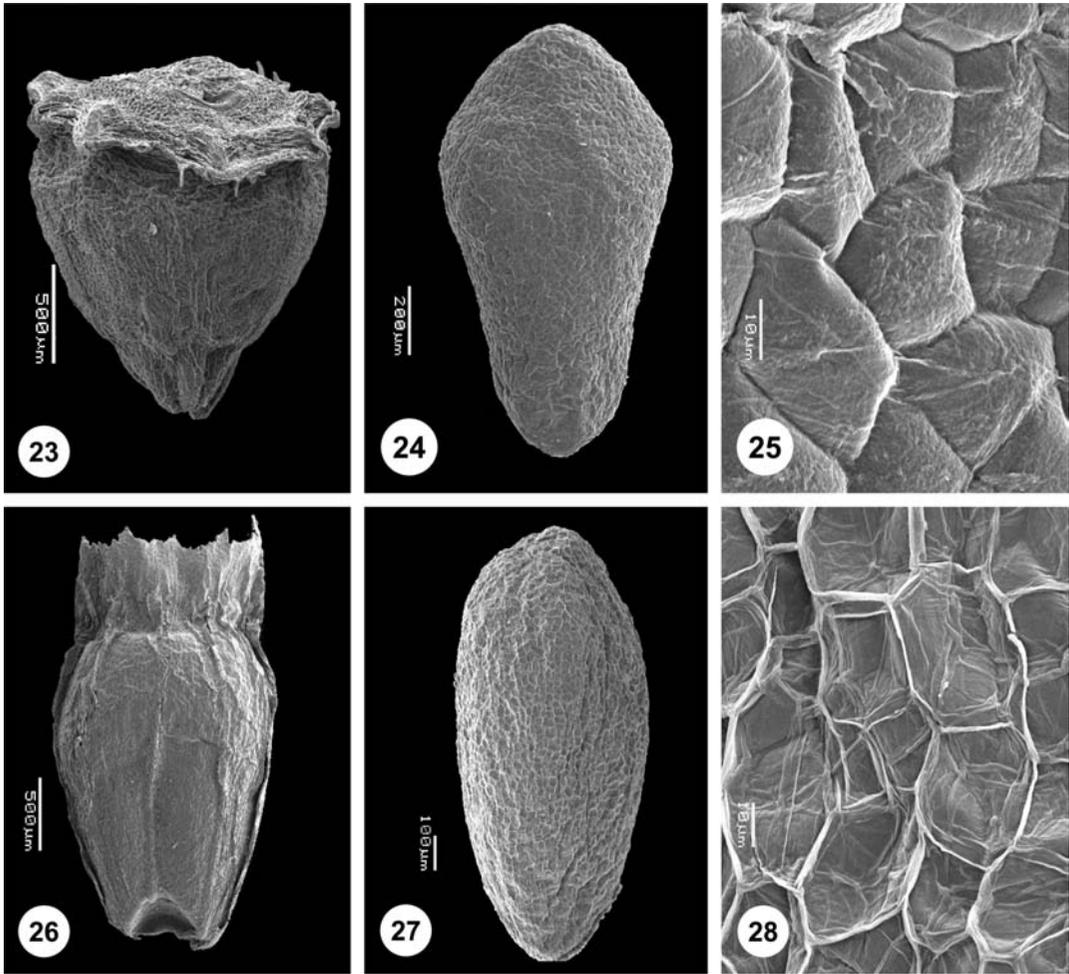
## DISCUSSION

The molecular results are surprising in two ways. First, a close relationship between *Gomphocalyx* and *Phylohydrax* has rarely been proposed in the literature. Second, their position in the vicinity of *Amphasma* and *Conostomium* away from Spermacoceae s.s., seems at first sight in conflict with morphological and anatomical data. In the following paragraphs, we identify characters that do in fact indicate (1) a close relationship between *Phylohydrax* and *Gomphocalyx* and (2) their new position amidst former Hedyotideae genera. We also discuss the synonymy between *Lathraeocarpa* and *Gomphocalyx* as proposed by Piesschaert (2001).

**Close relationship between *Phylohydrax* and *Gomphocalyx*.** — Several morphological characters support a close relationship between the two genera, consistent with molecular data. In our opinion, the most conspicuous and essential similarities lie in flower, seed, and pollen characters. In both genera the ovary has two carpels with a single ovule per locule, with the micropyle pointing downwards. The placenta is attached near the base of the septum and there are no outgrowths associated with the placenta or ovule. Flower morphology is

rather different in the two species: funnel-shaped in *Phylohydrax*, narrowly cylindrical in *Gomphocalyx*. In both the calyx lobes are small: eight minute calyx lobes in *Gomphocalyx* and a lacerate calyx vaguely showing two longer and two shorter lobes in *Phylohydrax*. Complete heterostyly is present in both genera, and the style ends in two papillose or shortly hairy stigmatic lobes. In both genera seeds are round in cross-section (there is no kind of ventral groove); the seed-coat is only weakly coloured, and the exotesta is parenchymatous and obscurely reticulate. Pollen grains are basically identical in the two genera, ca. 45–60 µm in diameter [Puff (1986) gives  $E = 30\text{--}40$  µm for *Phylohydrax* pollen grains, but this is contradicted by our observations], with 8–12 short colpi situated at the equator [8–10 colpi in *Gomphocalyx* and 10–12 colpi in *Phylohydrax*], and a (micro-)reticulate tectum evenly covered with granules. Other similarities are the amphistomatic leaves, the papery stipular sheath with very short appendages only, and the dry indehiscent fruits.

Certainly, none of the enumerated characters represent synapomorphies for the two genera and only a few of them are useful at a rank higher than the species level. We will feed these doubts by listing the differences



Figs. 23–28. Fruit and seed morphology of *Gomphocalyx herniarioides* (23–25) and *Phylohydrax madagascariensis* (26–28). 23, lateral view of an almost mature fruit; 24, dorsal view of seed; 25, detail of seed coat surface; 26, lateral view of mature fruit; 27, dorsal view of seed; 28, detail of seed coat surface. Figs. 23–25 from De Block & al. 790, Madagascar (BR); Figs. 26–28 from Cours 2885, Madagascar (P).

between the two genera. The most obvious difference between *Gomphocalyx* and *Phylohydrax* lies in the growth form. Although the two species are essentially creeping herbs forming dense mats, *Phylohydrax* has a distinct differentiation into vegetative long creeping stems and short erect ones that bear the flowers. *Gomphocalyx* has no such shoot differentiation. Since growth forms are most likely to be influenced by parallel evolution, not too much attention should be paid to the dissimilarity, however. More important is the fact that fruit wall anatomy is different. In *Phylohydrax* a relatively thick sclerenchymatous endocarp is present, the mesocarp is relatively thin, the exocarp comprises only one cell layer with the vascular traces situated just underneath the exocarp. In *Gomphocalyx*, the fruit wall is superficially similar, but the mesocarp is more extensive, and the vascular traces lie close to the endocarp. Other differences are found in the nectary disc morphology

(bilobed vs. entire), leaf anatomy (differentiated into spongy and palisade parenchyma vs. palisade parenchyma only), and calyx tube (reduced vs. collar-like calyx).

In conclusion, it is clear that morphology does not provide unambiguous evidence to call a close relationship between *Gomphocalyx* and *Phylohydrax*. On the other hand there are no characters that contradict such a relation. Indeed, for very specialized genera such as *Gomphocalyx* and *Phylohydrax* it is often difficult to determine close relatives based on morphology alone.

***Gomphocalyx* and *Phylohydrax* ruled out from Spermaceae.** — Molecular data are conclusive to exclude *Gomphocalyx* and *Phylohydrax* from Spermaceae s.s., but is there also morphological evidence for this? To answer this question we will first evaluate the characters that were used to classify the two genera in the tribe Spermaceae s.s., i.e., (1) the presence of pluri-zonocolporate pollen grains, and (2) an ovary with

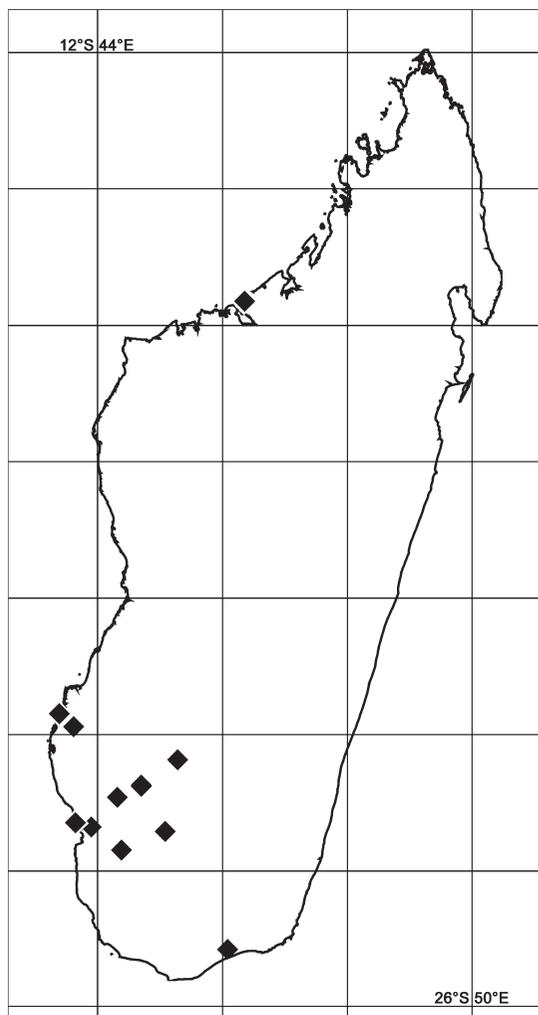


Fig. 29. Distribution of *Gomphocalyx herniarioides*.

only a single ovule per locule. A closer look at these characters will prove that the pollen argument is rather weak because of the presence of convergent evolution, and that the ovary features do not hold when more detailed gynoeceal characters are taken into account.

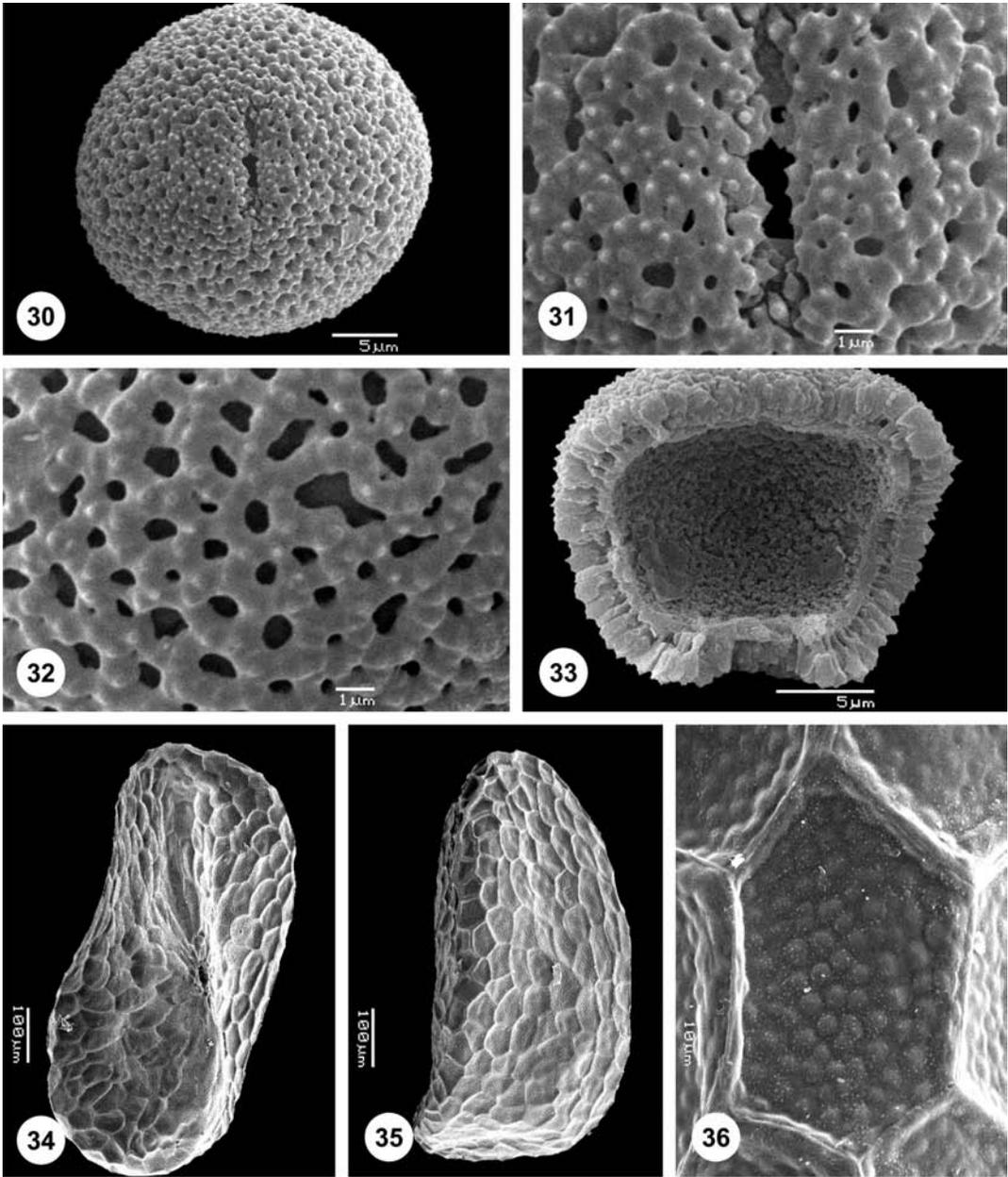
One of the main characteristics of Spermacoceae s.s. is the presence of pluri-aperturate pollen. Indeed, almost all members of the clade have pollen grains with more than three colpi or pores, and those with three colpi have a very peculiar morphology with a margo of granules around the ectoapertures only observed in this clade (Dessein & al., 2002). Pluri-aperturate pollen grains are not restricted to Spermacoceae s.s., however. The tribe Rubieae, for example, is characterized by 5–13-zonocolpate pollen and Theligoneae by 4–8-zonoporate pollen (Huysmans & al., 2003). In other tribes, the pluri-aperturate pollen condition is not a tribal characteristic, but is observed in a few species or genera only. In Psychotrieae, for example, the number of apertures is

variable but rarely more than 5. In *Geophila repens* (L.) I. M. Johnst., however, a genus belonging to the same tribe, up to 150 pores were counted (Piesschaert & al., 1999; Vanthournout, 2002). In the Hedyotideae-*Hedyotis* group, several genera show a tendency to develop more than three apertures. In *Neanotis* W. H. Lewis, for example, pollen grains are very similar to what is observed in *Gomphocalyx* and *Phylohydrax*: pollen is (5–)6–12-zonocolporate, the colpi are short, and the tectum is (micro-)reticulate (Lewis, 1966). Pluri-zonocolporate pollen is also found in *Lathraeocarpa* (Figs. 37–40), which is further discussed below. In short, pluri-aperturate pollen grains are not uncommon outside Spermacoceae s.s.

Uni-ovulate locules are found in numerous genera and tribes and originated several times in the evolution of Rubiaceae. In most members of the subfamily Rubioideae, the ovules are erect with an inferior micropyle and attached to the base or middle of the septum. In some tribes, formerly placed in the subfamily Antirheoideae (Robbrecht, 1988), the micropyle is superior and the ovules are pendulous. The former condition characterizes Spermacoceae s.s. The ovules are attached to the septum typically just below its middle (*Richardia* L. being the only known exception with the ovules attached near the base). This is not the case for *Phylohydrax* and *Gomphocalyx*, where the ovules are attached near the base of the septum, which is the most common condition in other uni-ovulate taxa (e.g., Paederieae, Psychotrieae). Hence, the attachment of the ovule near the base of the septum corroborates the exclusion of the two genera from Spermacoceae s.s.

Additional morphological support to reject a close relationship between *Gomphocalyx-Phylohydrax* and Spermacoceae s.s. is found in the absence of a ventral groove in the seeds of the former two genera. In Spermacoceae s.s., such a groove (often very deep and narrow, more rarely wide and shallow) is almost omnipresent (see also Terrell & Wunderlin, 2002). Note that the seeds of *Hydrophylax* have a distinct ventral groove, and that they are attached near the middle of the septum (Puff, 1986). Any doubt about the position of *Hydrophylax* within Spermacoceae s.s. on the basis of these grounds is therefore not justified.

A position outside Spermacoceae s.s. is thus fairly well supported morphologically. But is there also support for the new position of the genera amidst taxa of Hedyotideae known as closely related to *Oldenlandia*? Before attempting to answer this question, it should be noted that the current molecular sampling of Hedyotideae is insufficient. In our opinion, a close relationship between the African genus *Conostomium* and *Phylohydrax-Gomphocalyx*, as suggested by our molecular data, is most unlikely and is the result of this poor

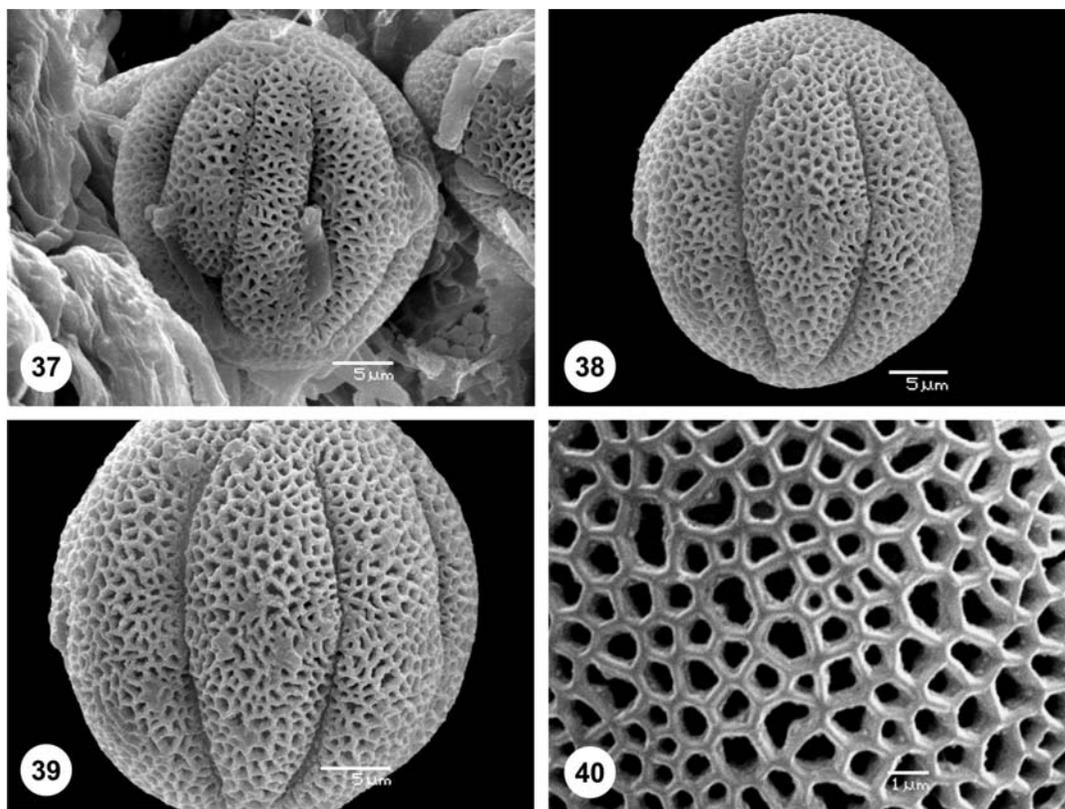


Figs. 30–36. Pollen and seed morphology of *Conostomium*. 30, equatorial view of 3-zonocolporate pollen grain; 31, detail of colpus; 32, detail of apocolpium showing microreticulate tectum with granules; 33, view of inside of pollen grain showing granular nexine and layers of the pollen wall; 34, ventral view of seed: note position of hilum; 35, dorsal view of seed; 36, detail of seed coat surface. Figs. 30–33 from *Chase 6039*, Zimbabwe (BR). Figs. 34–36 from *Brenan & al. 1844*, Kenya (BR).

sampling. Indeed, *Conostomium* is a small genus of herbaceous plants that is endemic to tropical Africa (see Bremekamp, 1952; Dessein, 1998; Scheltens, 1998). It is characterized by pluri-ovulate placentas, capsular fruits with a distinct beak, and 3-zonocolporate pollen grains with a perforate to microreticulate tectum (Figs. 30–33). The seeds are very small and the outer tangential wall of the seed coat cells is punctate (Figs. 34–36). There is not a single morphological character known by us that would

support a close relationship between *Conostomium* and *Phylohydrax-Gomphocalyx*.

Whatever the relatives of *Phylohydrax* and *Gomphocalyx* are within Hedyotideae, two discontinuities in the series of morphological character states will have to be explained. The first one concerns the uni-ovulate condition of both *Gomphocalyx* and *Phylohydrax*, the second one the pluri-aperturate pollen grains. Indeed, almost all taxa of Hedyotideae have pluri-ovulate ovaries



Figs. 37–40. Pollen morphology of *Lathraeocarpa decaryi*. 37, equatorial view of unacetolysed pollen grain; 38, equatorial view of pollen grain; 39, detail of equatorial zone; 40, detail of mesocolpium showing (micro-)reticulate tectum. All from *Decary 9508*, Madagascar (P).

and the number of apertures of the pollen grains rarely exceeds five. One exceptional taxon should be mentioned, however, namely the Asian genus *Neanotis*. As already mentioned above, *Neanotis* has pluri-zonocolporate pollen grains that are very similar to those of *Gomphocalyx* and *Phylohydrax*. What is more interesting, however, is that this genus also shows a clear reduction trend in the number of seeds per locule. In mature fruits, only one or two seeds are present and the fruits often remain indehiscent. In the absence of sequence data, it would be premature to hypothesize a close relationship between *Phylohydrax*-*Gomphocalyx* and *Neanotis*, but *Neanotis* shows at least that the evolutionary tendency to develop pluri-aperturate pollen grains and uni-ovulate ovaries is present in Hedyotideae. This makes more plausible the hypothesis that *Phylohydrax* and *Gomphocalyx* developed directly from a Hedyotideae ancestor and that character states similar to representatives of Spermaceae s.s. were obtained via convergent evolution.

**Remarkable convergent evolution.** — The above observations bring up new questions about the ecological and functional significance of morphological changes. The similar growth form observed in *Phylo-*

*hydrax* and *Hydrophylax* is easily explained by similar growth conditions. In fact, several other pioneers of maritime dune vegetation show parallel adaptations. Within Rubiaceae, “*Diodia*” *vaginalis* Benth. (Spermaceae s.s.; the position of this species in *Diodia* is doubtful; Dessein, 2003) from West-Africa also has a similar habit, while fruit and pollen morphology indicate that it is not closely related to *Hydrophylax* or *Gomphocalyx*-*Phylohydrax*.

More intriguing is the observation that in several evolutionary lineages of Rubiaceae a reduction in the number of seeds and an augmentation of the number of apertures in the pollen grains is present, and that those character states are often correlated. More fine-tuned phylogenies and detailed ecological studies that estimate the fitness of species in their environment may help reveal the triggers of such apparently related morphological tendencies.

**Another strange Madagascan genus: *Lathraeocarpa*.** — As mentioned in the introduction, a few authors have proposed a close relationship between *Lathraeocarpa* and *Gomphocalyx*, and Piesschaert (2001) even proposed merging the two genera. *Lathraeocarpa* comprises two subshrubby species, which are

endemic to the southernmost part of Madagascar. It is another deviant genus, mainly characterized by ternate leaves, truncate stipules (with one or a few teeth) fused with the petioles, calyx lobes in number twice the number of corolla lobes, heterostyly, tetralocular gynoecea with one basal ovule per locule, drupaceous fruits, and pluri-zonocolporate pollen grains (Figs. 37–40). In the protologue of *Lathraeocarpa*, Bremekamp (1957) dismissed any affinity between the novelty and Spermaceae s.s., because (1) the ovules are attached around the middle of the septum in Spermaceae (and not at the base), (2) the fruits are capsules or dicocous fruits (and not drupes), and (3) the pollen grains of Spermaceae lack pores. The first statement is here confirmed. Now that *Gomphocalyx* and *Phylohydrax* are excluded from Spermaceae s.s., all genera left in the clade, except for *Richardia*, have their ovules attached around the middle of the septum. Bremekamp's other two criteria are more problematic. In fact, several genera of Spermaceae s.s. do have indehiscent fruits that tend to be fleshy (e.g., the neotropical genera *Ernodea* and *Tortuella* Urb.), and the combination of colpi and pores is, e.g., found in *Scandentia* E. L. Cabral & Bacigalupo, another neotropical genus. There is, however, another character that makes a real affinity between *Lathraeocarpa* and Spermaceae s.s. doubtful, viz., the absence of a ventral groove in the developing *Lathraeocarpa* seeds.

A position near *Gomphocalyx* seems at first sight more convincing. The two genera have a similar stipule, calyx, and pollen morphology (pollen of *Lathraeocarpa* is 7–8-zonocolporate with E 41–50 µm). Some differences, however, obscure the picture and some characters point to an affinity between *Lathraeocarpa* and *Triainolepis* Hook. f., an Afro-Madagascan member of emended Knoxieae. The main reason for questioning a relationship between *Gomphocalyx* and *Lathraeocarpa* is that the ovary of the latter genus is tetralocular. Furthermore, the fruits of *Lathraeocarpa* are fleshy and not dry as in *Gomphocalyx*, and in *Lathraeocarpa decaryi* Bremek., the pyrene is surrounded by eight strands of thin-walled cells, very similar to the fruit wall anatomy observed in some *Triainolepis* species (see Bremekamp, 1957; Piesschaert, 2001). The subshrubby habit of *Lathraeocarpa* is also much closer to the shrubby habit of *Triainolepis* than to the herbaceous habit of *Gomphocalyx*. The fusion of *Lathraeocarpa* and *Gomphocalyx* as proposed by Piesschaert (2001) is not corroborated by our current knowledge. Once more, we will have to wait until molecular data become available to determine the position of *Lathraeocarpa* more confidently.

## ACKNOWLEDGEMENTS

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#### Appendix 1. Specimens studied of *Gomphocalyx herniarioides*.

**Madagascar.** **Fian:** Route Nat. 7, Isalo-Sakaraha, forêt de Zombitse, *L. Allorge* 2097 (P); Plateaux et vallées de l’Isalo à l’ouest de Ranohira, *H. Humbert* 28740 (P), 28703 (P), 19581 (P). **Maha:** Majunga, 3 km N of the city, on the beach, *P. De Block & al.* 790 (BR); Belamonty forest, Fok. d’Antsanitia, 2 km from coast, *P. De Block & F. Rakotonasolo* 812 (BR); Majunga (Pointe du Caïman); *H. Humbert* 7171 (P); Environs de Majunga, *H. Humbert* 4076 (P); Sables des dunes de Majunga au soleil, *H. Perrier de la Bâthie* 3503 (P); Majunga, *H. Poisson* 46 (P). **Toli:** Anakao vers Beheloka, *L. Allorge* 2276 (P); environs de Tuléar, *M. J. Bosser* 10409 (P); Sakaraha, *M. J. Bosser* 9135 (P); on RN 7 from Toliara towards “La Table”, ca. 10 km from Toliara, *P. De Block & al.* 569A & B (BR); Morombé, *R. Decary* 18743 (P); Ambovombe, *R. Decary* 3489 (P); Ferme de Mahabo, *J. Dequaire* 27096 (P); Bas Mangoky, *Descoings* 784 (P); Côte ouest de Madagascar, De Manombo à Morondava, *Grandidier s.n.* (P); Forêt de Zombitsy (Sakaraha); aux confins des bassins du Fiherenana et de l’Onil, *H. Humbert & al.* 29667 (P); environs de Tuléar: delta du Fiherenana, *H. Humbert & H. Perrier de la Bâthie* 2453 (P); environs de Sakaraha, forêt du Zombitsy, *M.-R. Keraudren* 492 (P); N of Tuléar, Lac Ihotry, *P.B. Phillipson* 3049 (MO, P); Bassin de Fiherenana, *H. Perrier de la Bâthie* 3893 (P); Vallée du Fiherenana, *H. Perrier de la Bâthie* 16673 (P); Tuléar, *H. Perrier de la Bâthie* 12831 (P); Benenitra, *H. Perrier de la Bâthie* 12741 (P); “La Table”, Tuléar, *M. Petit* 23 (P); Route de Betsioky à Sakamena, *H. Poisson* 427, 436 (P); Beza Mahafaly special reserve, NE of Betsioky, parcell 2, “pink trail”, *T. Willing* 61 (P). **Without locality or locality not traced:** *R. Baron* 4585 (P00267333), 4782 (P00267332); *M. R. Decary* 18741 (P); *M. Grévé* 63, 304, 331 (P); *A.-M. Homolle* 1567 (P); *C. Rutenberg s.n.* (P00267331).

## Appendix 2. Vouchers for sequences used in the cladistic analyses with GenBank accessions.

Taxon, Voucher data, *rbcl*, *rps16* intron

**Agathisanthemum Klotzsch:** *A. bojeri* Klotzsch, South Africa, Transvaal, *Dahlstrand 1625* (GB), AF002752; Tanzania: *Bremer 3060* (UPS), Z68787.  
**Amphiasma Bremek.:** *A. benguelense* (Hiern) Bremek., Angola, Moçamedes district: *Kers 3350* (S), AF002753; *A. luzuloides* (K. Schum.) Bremek., Tanzania: *Iversen & al. 87694* (UPS), AJ288594. **Anthospermum L.:** *A. aethiopicum* L., South Africa: *Dahlstrand 416* (GB), AF257897; *A. herbaceum* L. f., Tanzania: *Bremer 3093* (UPS), X83623. **Arctophyllum Willd. ex Schult. & Schult. f.:** *A. aristatum* Standl., Ecuador: *Hekker & Hekking 10335* (GB), AF333348; Ecuador: *Bremer & al. 3371* (UPS), AJ288595; *A. muticum* (Wedd.) Standl., Colombia, Cundinamarca: *Andersson & al. 2195* (GB), AF002754; *A. riveti* Danguy & Cherm., Ecuador: *Harling & Andersson 22232* (GB), AF333362; *A. serpyllaceum* (Schltdl.) Terrell, Mexico: *Stafford & al. 203* (MO), AF333364; *A. thymifolium* (Ruiz & Pav.) Standl., Ecuador: *Ståhl 4481* (GB), AF333366. **Argostemma Wall.:** *A. hookeri* King, Malaysia: *Wanntorp s.n.* (S), Z68788; *A. rupestre* Ridl., Sarawak: *Bremer & Bremer 1675* (S), AF002756. **Bouvardia Salisb.:** *B. glaberrima* Engelm., Cultivated: *Forbes s.n.* (S), X83626; *B. ternifolia* (Cav.) Schltld., Cultivated: *S2928* (BR), AF002758. **Conostomium (Stapf) Cufod.:** *C. natalense* (Hochst.) Bremek., South Africa, Transvaal: *Dahlstrand 1346* (GB), AF002760; *C. quadrangulare* (Rendle) Cufod., Ethiopia: *Puff & Kelbessa 821222* (UPS), Z68792. **Coprosma J. R. Forst. & G. Forst.:** *C. pumila* Hook. f., Cultivated: *Forbes s.n.* (S), X87146; *C. robusta* Raoul, Cultivated: *Andersson 2206* (GB), AF002735. **Craterispermum Benth.:** *C. laurinum* (Poir.) Benth., R.D.Congo: *Lejoly 86/207* (BR), AF331646, AF331645. **Danais Comm. ex Vent.:** *D. xanthorrhoea* (K. Schum.) Bremek., Tanzania: *Bremer 3079* (UPS), Z68794; *D. sp.*, Madagascar: *Davis 1003* (K), AF331648. **Dentella J. R. Forst. & G. Forst.:** *D. repens* (L.) J. R. Forst. & G. Forst., Australia: *Andersson 2262* (GB), AF333370. **Dibrachionostylus Bremek.:** *D. kaessneri* (S. Moore) Bremek., Kenya, Nairobi: *Strid 2598* (GB), AF002761. **Didymaea Hook. f.:** *D. alsinoides* (Cham. & Schltdl.) Standl., Cultivated: *Keller 1901* (CAS), Z68795. *D. mexicana* Hook. f., Mexico, México: *Hultén 1259* (S), AF004047. **Diodia L.:** *D. sarmentosa* Sw., French Guiana: *Andersson & al. 2071* (GB), AF002762; Puerto Rico: *Taylor 11749* (MO), AJ288600; *D. virginiana* L., USA: *Vincent 4296* (GB), AY764288. **Emmeorhiza Pohl ex Endl.:** *E. umbellata* (Spreng.) K. Schum., Trinidad: *Hummel s.n.* (GB), AY764289. **Ernoidea Sw.:** *E. littoralis* Sw., Cuba, National Botanical Garden: *Rova & al. 2286* (GB), AF002763; Cuba: *Kiehn s.n.* (WU), AJ288601. **Galianthe Griseb.:** *G. brasiliensis* (Spreng.) E. L. Cabral & Bacigalupo, Argentina: *Vanni & Radovancick 996* (GB), AY764290. **Gomphocalyx Baker:** *G. herniarioides* Baker, Madagascar: *De Block & al. 569* (BR), AY764291. **Hedyotis L.:** *H. degeneri* Fosberg, Cultivated: *Wood 5062* (PTBG), AF333371; *H. hillebrandii* (Fosberg) W. L. Wagner & D. R. Herbst, Hawaii: *Harrison-Gagne s.n.* (GB), AF002765; *H. macrostegia* Stapf, Sabah: *Wallander 6* (GB), AF002767; *H. mitrasacmoides* F. Muell., Australia: *Andersson 2260* (GB), AF333372; *H. parvula* (A. Gray) Fosberg, Cultivated: *Perlman 12783* (GB), AF333375; *H. schlechendahliana* Steud., Cultivated: *Lorence 8021* (PTBG), AF333376. **Houstonia L.:** *H. caerulea* L., USA: *Vincent & Lammers s.n.* (GB), AF333379; cultivated, AJ288604; *H. longifolia* Gaertn., USA: *Yatskievych 96-49* (MO), AF002766; *H. microphylla* Willd., Ecuador: *Molau & al. 2665* (GB), AF333377; *H. nigricans* (Lam.) Fernald, USA: *Yatskievych 96-92* (MO), AF333373. **Knoxia L.:** *K. platycarpa* Arn., Sri Lanka: *Lundqvist 11302* (UPS), AF288631; *K. sumatrensis* (Retz.) DC., India, Bihar: *Dahlstrand s.n.* (GB), AF002738. **Manettia Mutis ex L.:** *M. alba* (Aubl.) Wernh., French Guiana: *Andersson & al. 1917* (GB), AF002768; *M. luteorubra* (Vell.) Benth., Cultivated: *Bremer 2716* (UPS), Z68803; *M. lygistum* (L.) Sw., Colombia, Cauca: *Andersson & al. 2128* (GB), AF002769. **Mitracarpus Zucc. ex Schult. & Schult. f.:** *M. frigidus* (Willd. ex Roem. & Schult.) K. Schum., French Guiana: *Andersson & al. 1995* (GB), AF002770; *M. villosus* (Sw.) Cham. & Schltdl., Sri Lanka: *Fagerlind 810* (S), AJ288632. **Morinda L.:** *M. citrifolia* L., Cultivated: *Bremer 3106* (UPS), X83651; Cultivated at Dept. of Botany, Stockholm University, acc. n° SU-C-88.38: no voucher, AF001442. **Mycetia Reinw.:** *M. malayana* (G. Don) Craib, Cultivated: *Andersson 2219* (GB), AF002771; Cultivated: *Larsen & al. 42486* (AAU, UPS), Z68806. **Nesohedyotis (Hook. f.) Bremek.:** *N. arborea* (Roxb.) Bremek., Cultivated: *Chase 2915* (K), AF003607. **Oldenlandia L.:** *O. affinis* (Roem. & Schult.) DC., South Africa, Transvaal: *Dahlstrand 1661* (GB), AF003608; *O. corymbosa* L., Gabon: *Andersson & Nilsson 2263* (GB), AF333381; Tanzania: *Bremer 3075* (UPS), X83655; *O. lancifolia* (Schumach.) DC., French Guiana: *Andersson & al. 1996* (GB), AF003609; *O. nervosa* Hiern, Gabon: *Andersson & Nilsson 2326* (GB), AF333382; *O. tenuis* K. Schum., Guyana: *Jansen-Jacobs & al. 41* (U), AY764293; *O. thesiifolia* (A. St.-Hil.) K. Schum., Brazil: *Harley 15514* (U), AY764294; *O. uniflora* L., USA: *Godfrey 57268* (GB), AY764295. **Opecularia Gaertn.:** *O. vaginata* Juss., Australia: *Nordenstam & Anderberg 627* (S), AF257936; Australia: *Bremer & Gustafsson 25* (UPS), Z68809. **Otiophora Zucc.:** *O. cupheoides* N. E. Br., South Africa, Transvaal: *Hilliard & Burt 14307* (S), AF002742; South Africa: *Bremer 3805* (UPS), AJ288613. **Otomeria Benth.:** *O. oculata* S. Moore, Kenya, Samburu district: *Newbould 29015* (S), AF002743; Ethiopia: *Puff 821222-2/1* (K), AJ288614. **Paederia L.:** *P. foetida* L., Cultivated: Without voucher (acc. No. 19763869), AF004065; Japan: *Ehrendorfer 930830-0801*, AF332373. **Pagamea Aubl.:** *P. guianensis* Aubl., French Guiana: *Andersson & al. 2015* (GB), AF002744. **Pentanisia Harv.:** *P. longituba* (Franch.) Oliv., Ethiopia: *Puff & Kelbessa 821220* (UPS), Z68812; *P. prunelloides* (Eckl. & Zeyh.), South Africa, Eastern Cape: *Dahlstrand 1972* (GB), AF002745. **Pentas Benth.:** *P. lanceolata* (Forssk.) Defl., Cultivated: *Andersson 2248* (GB), AF002750; Cultivated: *Bremer 2702* (S), X83659. **Pentodon Hochst.:** *P. pentandrus* (K. Schum. & Thonn.) Vatke, Zanzibar: *Sundström 2* (GB), AF003612; Tanzania: *Bremer 3082* (UPS), X83660. **Phylohydrax Puff:** *P. carnosa* (Hochst.) Puff, South Africa: *Bremer 3783* (UPS), AJ288615; *P. madagascariensis* (Willd. ex Roem. & Schult.) Puff, Madagascar: *De Block & al. 640* (BR), AY764292. **Plocama Aiton:** *P. pendula* Aiton, Cultivated: *Bremer 3321* (UPS), AF004071; Canary Islands: *Andreasen 1* (UPS), Z68816. **Psychotria L.:** *P. asiatica* L., Cultivated at Royal Botanic Garden, Edinburgh: without voucher (accession number 19780778A), AF369854. **Psyllocarpha Mart. & Zucc.:** *P. asparagoides* Mart. & Zucc., Brazil, Bahia: *Harley & al. 20077* (AAU), AF003611; *P. laricoides* Mart. & Zucc., Brazil: *Andersson & al. 355750* (UPS), AJ288619. **Putoria Pers.:** *P. calabrica* (L. f.) DC., Cultivated at Royal Botanic Gardens, Kew: without voucher, AF004072; Unknown: *Jonsell 4216* (UPS), AJ288620. **Richardia L.:** *R. scabra* L., Colombia: *Andersson & al. 2073* (GB), AF003614; Cultivated: *Bremer 2744* (UPS), Z68820. **Rubia L.:** *R. fruticosa* Aiton, Cultivated: *Andersson 2224* (GB), AF004078; *R. tinctorum* L., Cultivated: *Bremer 3300* (UPS), X83666. **Schismatoclada Baker:** *S. sp.*, Madagascar: Antananarivo province: *Philipspon1608* (S), AF003618. **Schradera Vahl:** *S. sp.*, Colombia: Cauca: *Andersson & al. 2107*, AF003617; *S. subandina* K. Krause, Ecuador: *Clark & Watt 783* (UPS), Y11859. **Serissa Comm. ex A. Juss.:** *S. japonica* (Thunb.) Thunb., Cultivated: *Andersson 2202* (GB), AF004081; Cultivated: *Bremer 2717* (UPS), Z68822. **Spermacoce L.:** *S. confusa* Rendle ex Gillis, Colombia: *Andersson & al. 2074* (GB), AF003619; *S. hispida* L., Sri Lanka: *Wanntorp & al. 2667* (S), AJ288623. **Theligonum L.:** *T. cynocrambe* L., Spain, Málaga: *Dept. of Systematic Botany 1298* (GB), AF004087; Cultivated: *Struwe 1004* (UPS), X83668. **Triainolepis Hook. f.:** *T. africana* Hook. f., Kenya: *Lorence 5553* (PTBG), AF129276; *T. hildebrandtii* Vatke, Kenya: *Bally 13258* (G), AJ288625.