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Flower morphology and biology of Myrsine laetevirens, structural and evolutionary implications of anemophily in Myrsinaceae

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This study deals with the phenology, pollination biology and floral morphology of *Myrsine laetevirens*, a neotropical dioecious tree. In Punta Lara (Argentina), its flowering period occurs during January-February. Both pistillate and staminate flowers are small, with a yellowish-green perianth and produce neither nectar nor odour. Staminate flowers have five stamens and a vestigial gynoecium while pistillate flowers, have non-functional anthers and a conspicuous stigma. The floral characteristics of staminate and pistillate plants are related to the syndrome of anemophily. Fruit set in inflorescences covered with mesh bags and observations prove that animals are not involved in the pollination process. A provisional cladistic analysis of Myrsinaceae shows that dioecy evolved as one of the most recent apomorphies of *Myrsine* and is part of the anemophilous syndrome.

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Introduction

Almost nothing is known about the floral ecology of the mainly tropical Myrsinaceae. It has been traditionally assumed (Mez 1902) that this family containing about 1250 species and 39 genera (Mabberley 1987) is entomophilous throughout. However, by focusing on one species in the remnant river margin rain forest near La Plata (Argentina) it turned out that *Myrsine laetevirens* (Mez) Arechav. was wind pollinated. This finding posed interesting evolutionary and ecological questions.

On one side, anemophily is a very unusual pollination syndrome among the relatives to the Myrsinaceae. Among the Subclass Dillenidae anemophily is of isolated occurrence, for example in the Salicaceae (*Populus*) and Ericaceae (*Philippia*). This case may thus provide insight into how and why this pollination system could have arisen in a mainly zoophilous plant group. The existence of anemophily in tropical rain forest trees is also a much debated question born in recent research in tropical ecosystems trying to find a link between dioecious trees bearing small inconspicuous flowers either with pollination by generalist insects or with anemophily (Bawa & Opler 1975; Bawa 1980; Beach 1981).

Intending to shed light on both questions an account on flower structure of a species of the little known genus *Myrsine* is given here together with an experimental demonstration of anemophily. In order to demonstrate how and where the anemophilous floral traits of *Myrsine* could have arisen within the family, data were mapped on a provisional phylogeny of the Myrsinaceae.

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Material and methods

Field observations were carried out mainly from September 1992 to October 1994 in the marginal rain forest of Punta Lara, Argentina (32°47' S; 58°1' W). Additional observations were performed in 1996.

The reproductive cycle of *Myrsine laetevirens* was studied during these two years. The pollination biology was studied in 10 plants (8 pistillate and 2 staminate). To demonstrate wind pollination fruit set was determined in 248 pistillate inflorescences: 30 unbagged in a open area of the forest, 30 unbagged in areas overgrown by vegetation, 36 bagged with coarse mesh (1.42 × 1.42 mm) bags, 30 bagged with fine mesh (0.2 × 0.2 mm) bags, 60 bagged with paper bags, 30 bagged with coarse mesh bags and hand pollinated, 32 bagged with paper bags and hand pollinated. All inflorescences were bagged before anthesis and fruits were counted at the end of the season.

To detect osmophores, fresh intact flowers were immersed in a tap water solution of neutral red (1:10 000) for about 2 hours (Vogel 1990). The presence of nectar was tested with the glucose reactive stripes (Diastix®, Bayer). Stigmatic receptivity was determined with a 3% hydrogen peroxide solution by considering the bubbling on the stigma as indicator of receptivity (Osborn et al. 1988).

Samples for optical microscopy were fixed in 3:1 (absolute ethyl alcohol: acetic acid) and embedded in Paraplast. Polysaccharides were located using the PAS method (Pearse 1985).

Morphology and dimensions of pollen grains were studied using acetolysed material (Erdtman 1952). Morphological terminology follows Faegri & Iversen (1964) and Blackmore et al. (1992). Some pollen grains were directly removed from the anthers, coated with gold and observed by scanning electron microscope (Jeol JSM-T 100).

To reveal pollen histochemical features, the following stainability tests were performed: iodine - potassium iodide for detecting starch grains, Coomassie-brilliant blue for proteins (Gahan 1984) and the Nile Blue-fluorescence method for lipids (Pearse 1985).

Pollen grain viability was determined with fluorescein diacetate/ propidium iodine vital staining (Greissl 1989) and with acid fuchsin stain on fixed material.

The number of pollen grains per anther was counted using a hemocytometer (Kearns & Inouye 1993).

The cladistic analysis of Myrsinaceae was based on personal observations and published accounts by Agostini (1980), Allan (1961), Anderberg & Ståhl (1995), Chandler (1911), D'Arcy (1973), de Witt (1957), Gentry (1993), Hosaka (1940), Janzen (1970), Larsen & Hu (1991, 1992, 1995), Lundell (1964, 1966a, b, 1971, 1976, 1981a, b, 1982), Mennechet (1902), Mez (1902, 1903), Pipoly (1983, 1987, 1992a, b, 1993), Rao (1971), Sleumer (1986, 1987a, b, 1988a, b, c), Smith (1973), Ståhl (1987, 1989, 1990, 1991, 1992, 1993, 1995), Stone (1988a, b, 1989, 1990, 1991a, b, 1992a, b, c, 1993a, b), Taton (1980) and Urban (1913).

Characters used for the cladistic analysis are shown in Table 1.

The data matrix (Table 2) was runned with the program Hennig86 (Farris 1988) using commands mhennig*, bb* and a successive weighting procedure. Character distributions were analysed with the program Clados (Nixon 1992).

Outgroup: Based on the study of Anderberg & Ståhl (1995) about Primulales phylogeny, Theophrastaceae was chosen as the sister group of Myrsinaceae.

Ingroup: In this study, 32 genera of Myrsinaceae were considered the terminal taxa. *Cybianthus* Mart. was treated in the sense of Agostini (1980) and Pipoly (1987). *Ardisia* Sw. was considered in its broader sense, *i.e.*, including *Parardisia* Nayar & Giri (Stone 1993a), *Tetrardisia* Mez (Larsen & Hu 1995) and the genera segregated by Lundell (1964, 1966a, 1971, 1981a, b, 1982).

Multistate characters 16, 18 and 30 were treated as non- additive. The presence of hydroxybenzoquinone derivatives is considered here as a synapomorphy that supports the monophyly of Myrsinaceae (Ogawa & Natori 1968; Hegnauer 1969).

Results

Phenology

All observed plants are dioecious and their flowering and fruiting phenology is shown in Fig. 1. Both staminate and pistillate plants have their flowering periods in summer (5/1-5/3) with a flowering maximum between January and early February. The flowering of the pistillate plants extends for 2-3 weeks after the flowering of staminate plants has finished. The fruiting period extends throughout late summer, autumn and winter (10/3-30/8). There was no evidence of vegetative reproduction during the entire period of observation.

Inflorescence structure

Both staminate and pistillate flowers are borne in short axillary racemes of 3-15 flowers. Each pedicel bears a pilose bract. Anthesis in a given inflorescence is mostly simultaneous.

Table 1. Characters used for the cladistic analysis of the Myrsinaceae and their codification (0 = plesiomorphic; 1, 2 = apomorphic states).

1-Habit	0-trees or shrubs
	1-suffrutices or herbs
2-Scandent stems	0-absent
	1-present
3-Leaves	0-lacking subepidermal sclereids
	1-with subepidermal sclereids
4-Inflorescences	0-lateral
	1-terminal
5-Inflorescences	0-simple (racemes, corymbs, umbels)
	1-compound (panicles)
6-Basal rachis (brachyblast-like) with sterile bracts	0-absent
	1-present
7-Bracteoles in floral pedicel	0-absent
	1-present
8-Sepals	0-almost free at base
	1-united
9-Corolla	0-sympetalous
	1-petals free or almost free at base
10-Corolla aestivation	0-imbricate
	1-valvate
11-Adaxial petal surface	0-without capitate trichomes
	1-with capitate trichomes
12-Petals	0-entire
	1-emarginate
13-Petals	0-longer than sepals or subequal
	1-smaller than sepals
14-Stamen/ perianth ratio	0-1/1 or less
	1-greater than 1/1
15-Stamen	0-with filaments
	1-without filaments
16-Filament attachment	0-at base of corolla tube
	1-at about the middle of corolla tube
	2-filament fused to and decurrent on lower part of the corolla
17-Anthers	0-not versatile
	1-versatile
18-Anthers	0-initially forming a cone, but then free
	1-free
	2-fused in a cone
19-Anther dehiscence	0-longitudinal
	1-poricidal
20-Anther locule	0-not septate
	1-septate
21-Apical anther appendages	0-absent
	1-present
22-Ovary position	0-superior
	1-inferior to intermediate
23-Ovules	0-in three or more series
	1-in one or two series
24-Style	0-shorter to or as long as ovary
	1-longer than ovary
25-Stigma	0-capitate, conical or lobed
	1-punctate or truncate
26-Endocarp	0-not sclerified
	1-sclerified
27-Fruit	0-many seeded
	1-one seeded
28-Seed	0-with endosperm
	1-without endosperm
29-Cotyledons	0-free
	1-connate
30-Breeding system	0-hermaphrodite
	1-monoecious
	2-dioecious

Table 2. Data matrix for the cladistic analysis of Myrsinaceae

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Theophrastaceae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aegiceras	0	0	1	1	?	0	0	0	0	0	0	1	0	0	0	0	1	1	0	1	0	0	0	1	1	0	1	1	1	0
Amblyanthus	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	1	0	0	1	1	0	0	0
Amblyanthopsis	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0
Antistrophe	0	0	0	0	0	0	0	0	0	?	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0
Ardisia	?	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	1	?	0	0	?	1	1	1	1	0	0	0
Badula	0	0	0	0	?	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0
Conandrium	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	0	1	1	1	0	0	0
Cybianthus	0	0	?	1	?	0	0	?	0	?	1	0	0	0	?	2	0	1	?	0	0	0	1	0	1	1	1	0	0	?
Discocalyx	0	0	0	0	?	0	0	1	0	0	0	1	0	0	1	?	0	1	0	0	0	0	1	0	0	1	1	0	0	2
Elingamita	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	?	1	1	1	0	0	0
Embelia	0	1	0	?	?	0	0	0	1	0	1	0	0	?	?	2	0	1	0	0	0	0	1	0	0	1	1	0	0	2
Emblemantha	1	0	0	0	0	0	0	?	0	?	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1	1	0	0	0
Fittingia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	2
Geissanthus	0	0	0	1	1	0	0	?	0	?	0	0	0	?	0	2	1	1	0	0	0	0	1	?	1	1	1	0	0	2
Grenacheria	0	1	0	?	?	0	0	0	0	0	1	0	0	0	0	2	0	1	0	0	0	0	1	0	0	1	1	0	0	2
Heberdenia	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	2	0	1	0	0	0	0	0	1	1	1	1	0	0	0
Hymenandra	0	0	0	0	?	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	1	1	1	1	1	0	0	0
Labisia	1	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	1	0	1	1	1	1	1	0	0	0
Loheria	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0	0	2
Maesa	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	0	1	1	1	0	0	2
Myrsine	0	0	0	0	0	1	0	0	?	0	0	0	0	0	?	0	0	1	0	0	0	0	1	0	0	1	1	0	0	2
Oncostemon	0	0	0	0	0	0	0	0	0	0	0	?	0	0	1	0	0	2	0	0	0	0	1	0	0	1	1	0	0	0
Parathesis	0	0	0	?	1	0	0	0	0	1	1	1	0	0	0	0	?	1	?	0	0	0	1	1	1	1	1	0	0	0
Pleiomeris	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	1	1	1	1	0	0	0
Sadiria	0	0	0	0	?	0	0	0	0	?	0	0	0	0	1	0	0	1	0	0	1	0	1	1	1	1	1	0	0	0
Solonia	0	0	0	?	?	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1	0	0	0
Stylogyne	0	0	0	?	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	?
Systellantha	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	?	0	0	0	1	1	1	1	1	0	0	1
Tapeinosperma	0	0	0	?	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0
Vegaea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	0	0
Wallenia	0	0	0	?	?	0	0	1	0	0	0	?	0	1	0	2	1	1	0	0	0	0	1	0	0	1	1	0	0	2

Flower morphology

The flowers are mostly complete, heterochlamydeous, pentamerous and greenish-yellow (5% of the flowers are 4- or 6-merous) (Figs 13-17). The pedicels are 2-3 mm in length. Petals and sepals contain secretory cavities and bear marginal trichomes with a 2-celled head (Fig. 2). The petals have a smooth epidermis (Fig. 3) and are fused at base. Each petal lobe bears one sessile and longitudinally dehiscent anther (Figs 5, 6, 15, 15', 17,17'). In pistillate flowers anthers are vestigial and do not produce pollen (Fig. 6). The ovary is superior, one-locular, and contains 3-4 ovules sunken into a central placenta. The style is very short and the stigma is relatively large, 5-lobed and papillose (Fig. 4). Staminate flowers bear a vestigial gynoecium (Fig. 17'). Neither nectar, nectaries nor osmophores could be detected.

Pollen morphology

The monadic pollen has a dusty appearance when released. The pollen grains are isopolar, sphaeroidal and 4-(3-5) colporate (Fig. 7), circular in polar view and circular to elliptic in equatorial view. The equatorial diameter is 18 μm (± 1.5 μm, N=35), the polar diameter is 17 μm (± 1.5 μm, N=41), the colpus length is 11.2 μm (±

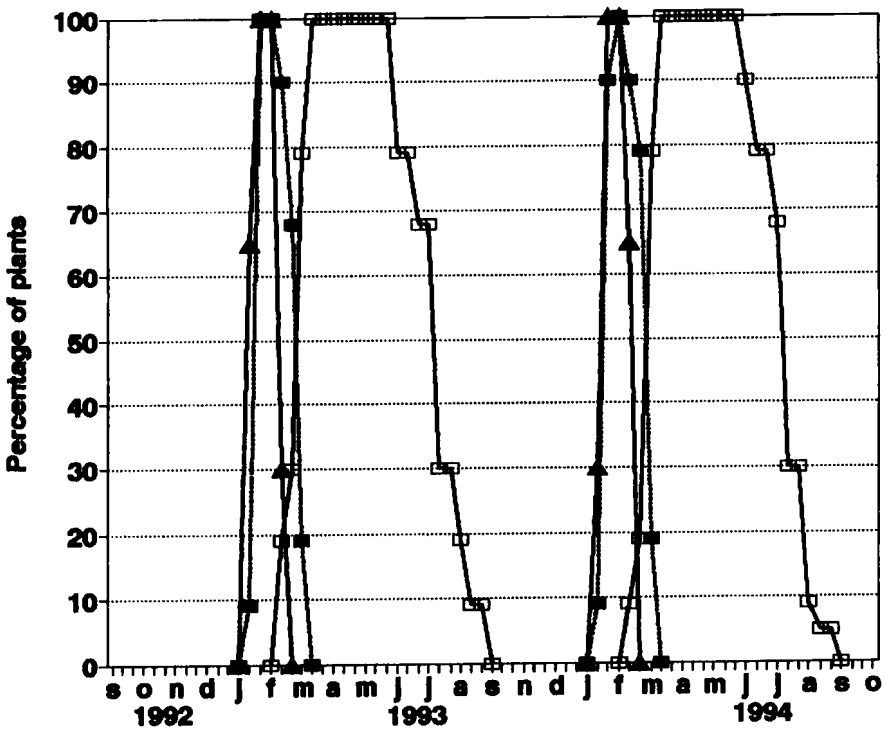


Fig. 1. Percentage of *Myrsine laetevirens* plants bearing pistillate flowers (dotted line, black square), staminate flowers (unbroken line, black triangle) and fruits (unbroken line, open square) at Punta Lara during 1993-1994.

0.5 μm, N=40). The exine is tectate and ca 1 μm thick. The tectum is scabrate with small perforations (Fig. 8). The aperture membrane is scabrate, except at the os, where it is psilate (Fig. 9).

Histochemical features of pollen

The pollen grains contain starch (Fig. 10) and pollenkitt can be observed as scanty, small lipidic drops on the pollen walls (Fig. 12).

Pollen viability

From the examination of 1 000 pollen grains, 79.5% were vital with the fluorescent dye (Fig. 11) and 90.8% stained with acid fuchsin.

P/O ratio

Each anther contained 46 798 (± 4 368, N=102) pollen grains. Therefore a pentamerous staminate flower produced a mean of 233 990 pollen grains. The number of ovules per pistillate flower varied between 3 and 4, with a mean of 3.35. Thus the P/O ratio is ca. 69 847.

Flower stages

Anthesis of pistillate flowers lasts 10 days and consists of 4 different stages:

Stage 1, the perianth is partially open and the empty vestigial anthers are dehiscent (Fig. 13 and 13'). The whitish green stigma has a weak reaction to hydrogen peroxide.

Stage 2, the open perianth extends horizontally (Fig. 14 and 14') and the bubbling reaction on the stigma is stronger.

Stage 3, the perianth is folded downwards so that the stigma is completely exposed (Fig. 15 and 15'), and the strongest bubbling reaction can be observed.

Stage 4, the perianth is partially withered, the stigma turns black and its reaction to hydrogen peroxide is negative.

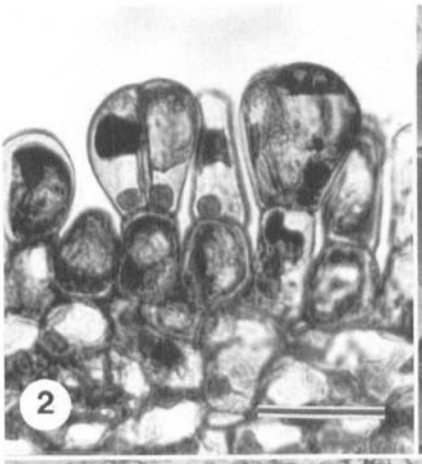
The anthesis of the staminate flowers lasts 5 days, and 3 different stages can be discerned:

Stage 1, the perianth is partially open but the anthers are still closed (Fig. 16 and 16').

Stage 2, the perianth opens and the dehisced anthers are exposed, thus releasing the dusty pollen (Fig. 17 and 17').

Stage 3 the perianth withers weakly and the anthers have been emptied.

The hydrogen peroxide test on the vestigial stigma is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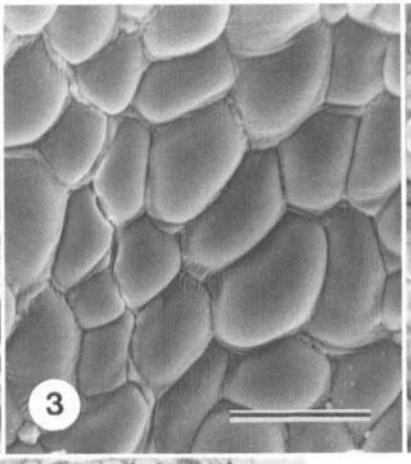
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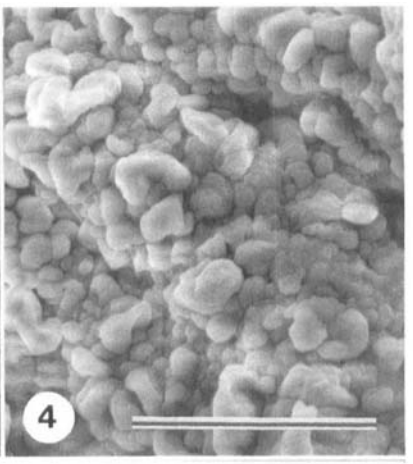
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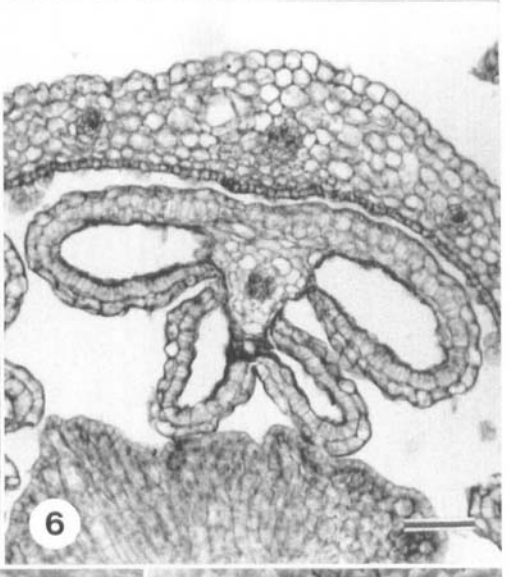
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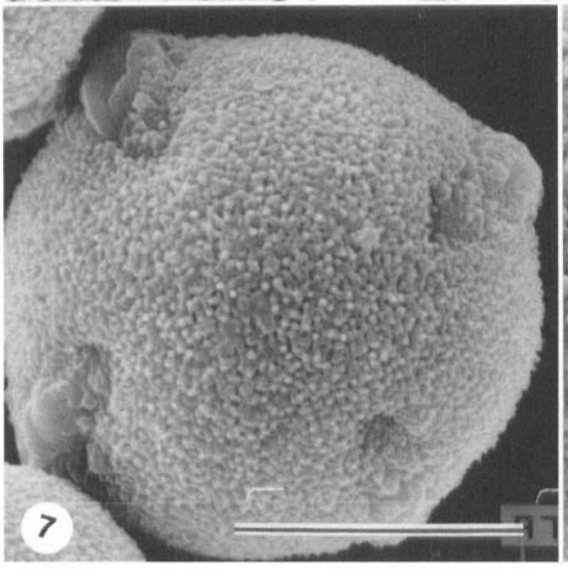
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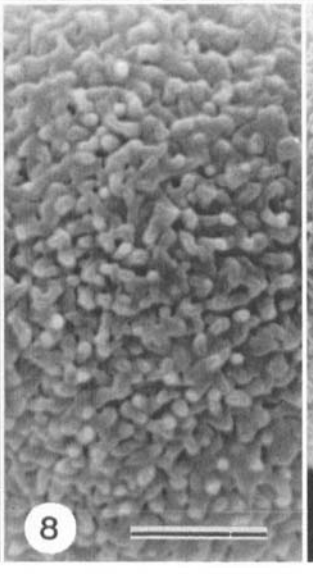
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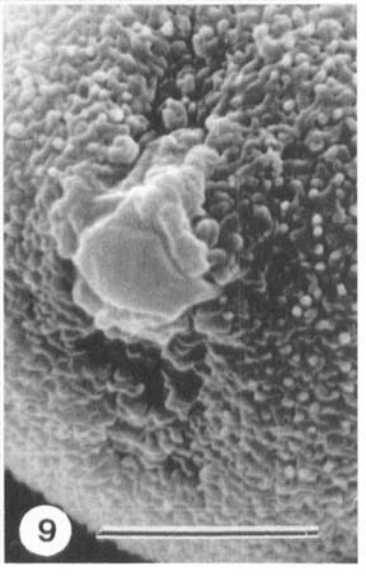
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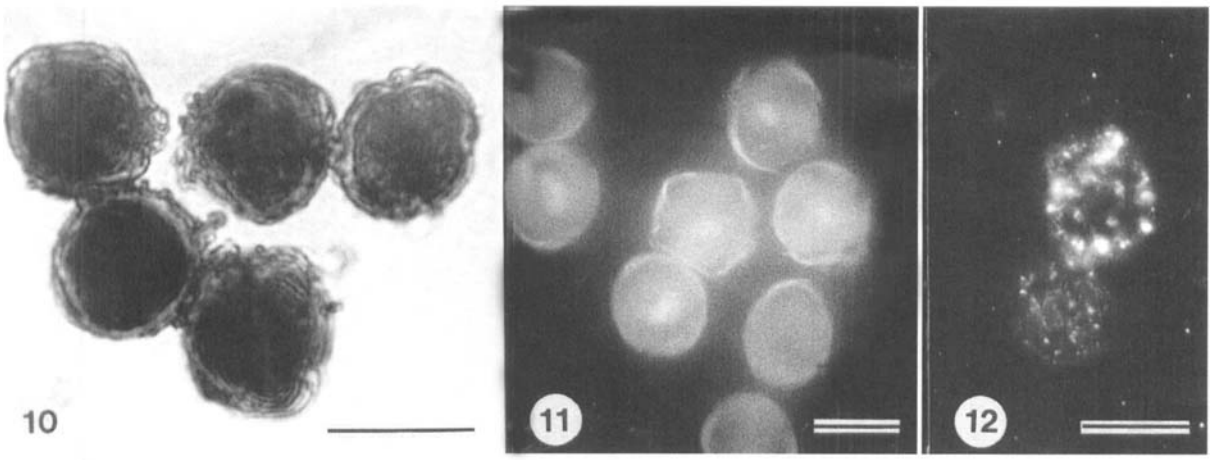

Figs 10-12. *Myrsine laetevirens*. –Fig. 10. Pollen grain stained with IKI, bar: 20 mm. –Fig. 11. Fluorescence of pollen grain by fluorescein-diacetate/propidium iodide method, bar: 20 mm. –Fig. 12. Fluorescent lipid drops (pollenkitt) using a Nile Blue staining method, bar: 20 mm.

negative during all stages of anthesis and the perianth does not fold back in any stage.

Flower visitors

No visits were observed either on pistillate nor on staminate flowers during the period of observations. (Every day, 3 hs in rotative periods of observation between 6 AM and 12 PM during the flowering period).

Experimental demonstration of anemophily

The fruit is a fleshy one-seeded drupe. Fruit set with different treatments is given in Table 3. The decrease in fruit set in wind-protected inflorescences is very marked and proportional to the mesh diameter. On the other hand pollination is a condition for fruit development since none of the flowers enclosed in paper bags produced fruits.

Phylogeny

The cladistic analysis of Table 2 gave one most parsimonious cladogram (Fig. 18) with 240 steps, a consistency index of 0.75 and a retention index of 0.84.

One seeded-fruits, which is a character often associated with anemophily, is a basal condition in Myrsinaceae (excluding *Maesa*) (Fig. 21). Other character states associated with anemophily (dioecy, free anthers and conspicuous stigmas), according to the present phylogeny, evolved as synapomorphies of increasingly restricted groups (Figs 19, 20 and 22).

Discussion

Our lack of knowledge about the floral biology of *Myrsine* was already pointed out by Stone (1992b). Until now, there were no direct observations on pollination in Myrsinaceae, so conclusions about its pollination biology have been drawn from indirect evidences. The structural resemblance between the flowers of *Ardisia* and *Solanum* lead Mez (1902) and Knuth (1905) to presume the same kind of pollination, i.e. by pollen collecting bees. Mez (1902) further pointed out the resemblance between the flowers of *Cybanthus* and *Ribes* suggesting pollination by flies, and that the showy and fragrant flowers of *Aegiceras* would also be pollinated by insects. Finally Mez (1902) concludes that "doubtlessly all species in the family are entomophilous on account of flower structure" (translation).

Regarding *Myrsine*, Knuth (1905) cites evidence indicating that *M. divaricata* A. Cunn. and *M. urvillei* A.

Figs 2-9. Flowers and pollen of *Myrsine laetevirens*. – Fig. 2. Trichomes in petal margin, bar: 50 mm. – Figs 3 - 4. SEM micrographs of the inner petal surface and the papillose stigma, resp., bars: 50 mm. – Figs. 5 - 6. Transversal sections of a functional anther (from staminate flower) and a vestigial anther (from pistillate flower), bars: 100 mm. – Figs 7-9. SEM micrographs of pollen grain, bars: 7 and 9: 10 mm; 8: 2.5 mm.

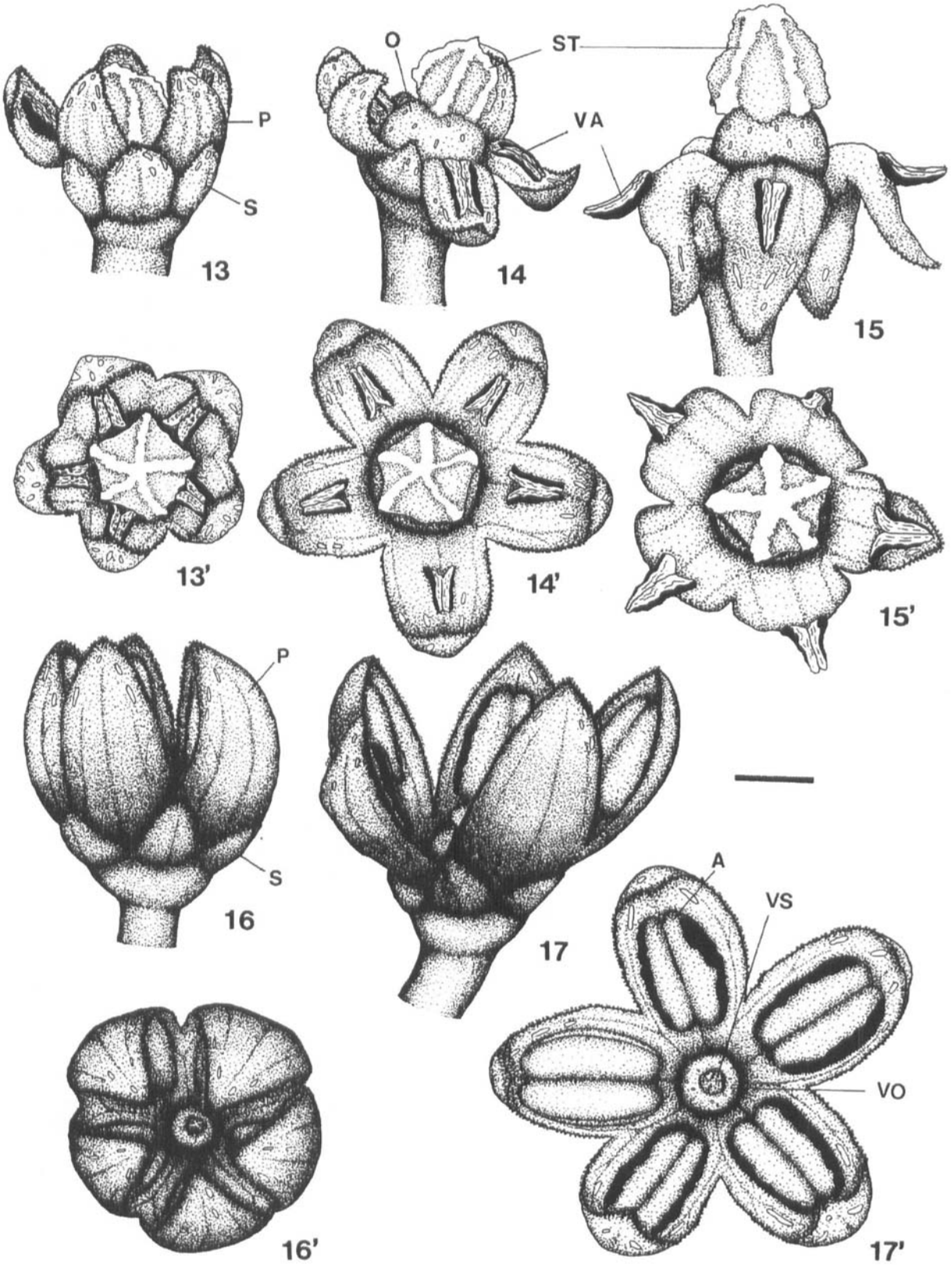
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Table 3. Fruit set in *Myrsine laetevirens*.

Treatment	% fruit set	SD	n
Open pollination			
open area	69.3	11.4	30
overgrown area	32.2	7.6	30
Bagged			
coarse mesh	42.4	20	36
fine mesh	1.9	5.2	30
paper	0	0	60
Bagged (paper bags) and hand pollinated			
	92.0	7	32

DC. of New Zealand are probably wind pollinated based on the presence of dusty pollen, small unisexual flowers and the lack of nectar and odour in both species. However, no experimental evidence was presented.

General observations on the flower morphology of *Myrsine* were made by Mez (1902), who demonstrated dioecy for *M. laetevirens*. More recent works repeatedly report the presence of perfect flowers in *M. laetevirens* (Digilio & Legname 1966; Cabrera & Zardini 1978; Burkart 1979). However, all plants analysed in the present work are dioecious as previously indicated by Mez (1902).

Reproductive features of *M. laetevirens*, such as dioecy, small flowers with small perianths, absence of nectaries or osmophores, reduced number of ovules, high production of small, smooth, and dry pollen grains, and a large stigmatic surface are well known as characteristics of anemophily (Faegri & van der Pijl 1979; Niklas 1985). Also according to Baker & Baker (1979) starch storage in pollen grains is associated with wind pollination. However, the heterochlamydeous perianth and the vestigial anthers in the pistillate flowers are probably remnants of zoophilous ancestors.

Fruit set in inflorescences covered with coarse and fine mesh bags and the flower observations in the field proved that animals are not involved in the pollination process. The relative decrease in fruit set in these inflorescences may be due to the bag meshes which possibly disrupted air currents as pointed out by Dafni (1992).

Since there is great uniformity in the flower and pollen morphologies in all species of *Myrsine* (Mez 1902; Vasanthy & Pocock 1981; Fonnegra & Melhem 1986), anemophily is perhaps the dominant pollination syndrome in this pantropical genus containing about 200 species.

The present evidence further supports the idea that anemophily is not necessarily rare in tropical forests (Bawa & Crisp 1980; Bullock 1994; Lewis 1986). Though anemophily is assumed to be rare or absent in tropical rain forest species (Whitehead 1969; Regal 1982) there are several reports indicating the presence of wind pollination in such ecosystems (Bawa & Crips 1980, Bullock 1994). No fewer than 21 neotropical woody families are assumed wind pollinated (Lewis 1986).

The "Bawa Hypothesis" (Bawa & Opler 1975; Bawa 1980; Beach 1981) predicts that there is a high correlation between dioecious tropical species with small, white or pale yellow-green flowers and pollination by small generalist insects. The present data, though restricted to one species, may be extrapolated to other species of the same genus and do not support this hypothesis. In any case, statistical data at community level are needed to draw any conclusion. Recently, the existence of this correlation has been extensively debated (Renner & Feil 1993; Bawa 1994).

Fruit features and previous observations (Montaldo, pers. comm.; Roldán et al. 1993), indicate that the fruits of *M. laetevirens* are dispersed by birds. This is in accordance with the observation of Givnish (1980) and Fox (1985) who have suggested that dioecy should be predominant in those anemophilous plants that produce fleshy fruits dispersed by animals. Dioecy has also been reported to be associated with fleshy fruits in general (Bawa 1980; Givnish 1980; Fox 1985).

According to published accounts (Mez 1902; Lundell 1966a, 1971, 1976; Sleumer 1988c; Stone 1989, 1990, 1991b) members of the Myrsinaceae that are more basal in the present provisional phylogeny, such as *Ardisia*, *Antistrophe* and *Hymenandra*, show the same type of flower, i.e. relatively large and conspicuous flowers, bearing connivent, large and almost sessile anthers. Such features agree with the well known *Solanum*-type flowers, which are pollinated by specialized females of wild bees that buzz the anthers to extract pollen (Buchmann 1983). The similarity between *Ardisia* and *Solanum* was already pointed out and a studied species of *Ardisia* (Mainz Bot. Garden) could effectively have its anthers emptied with the vibration of a tuning fork (440 Hz) (Cocucci unpubl.).

No functional nectaries are present in the *Solanum*-

Figs 13-17. Different flower stages of *Myrsine laetevirens*. – Figs 13-15. Pistillate flower – Figs 16-17. Staminate flower. – (Numbers with apostrophe indicate the same flower shown from a frontal view). A = anther, O = ovary, P = petal, S = sepal, ST = stigma, VA = vestigial anther, VO = vestigial ovary, VS = vestigial stigma. Bar: 1 mm. M.S. Otegui del.

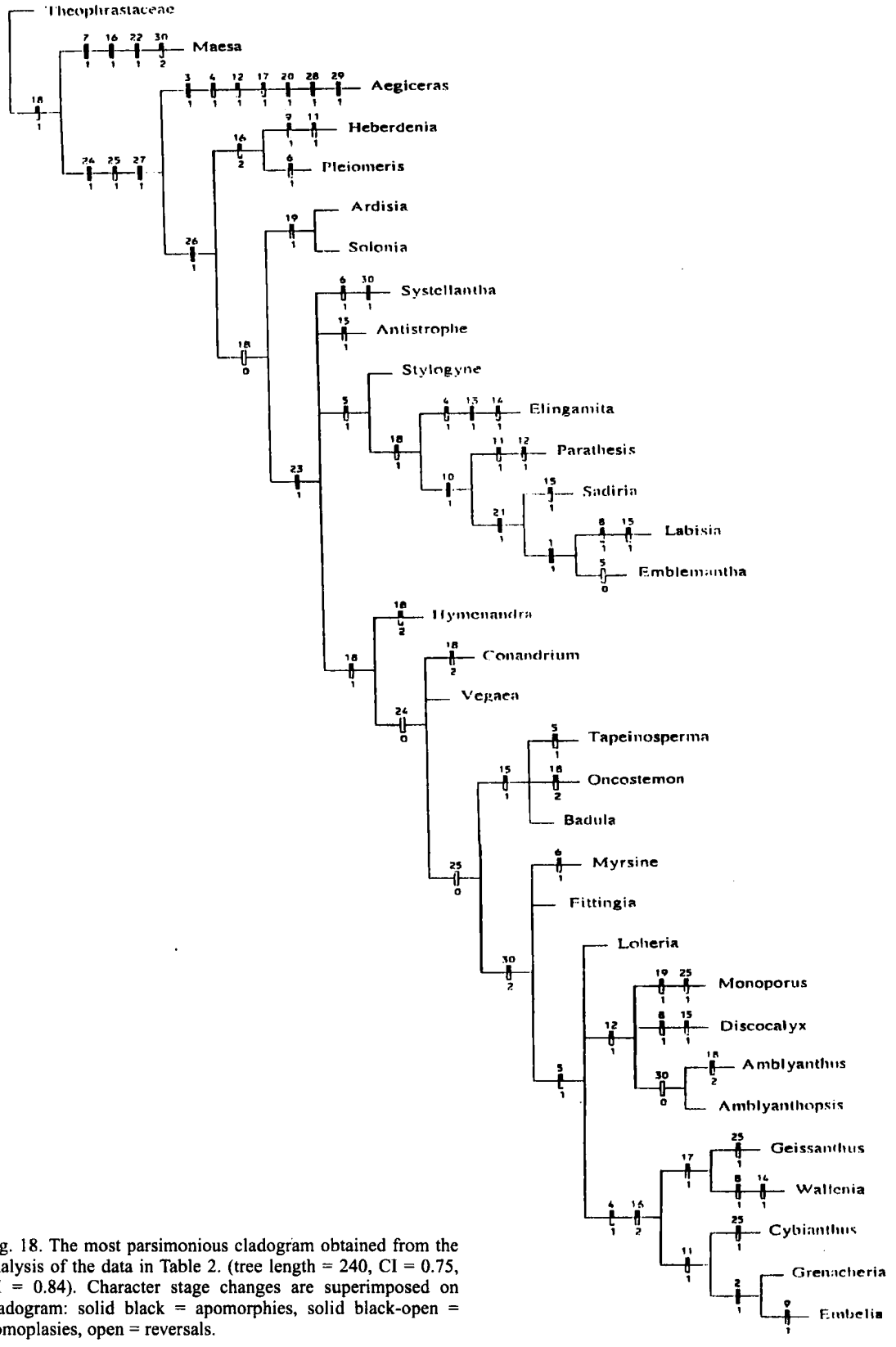
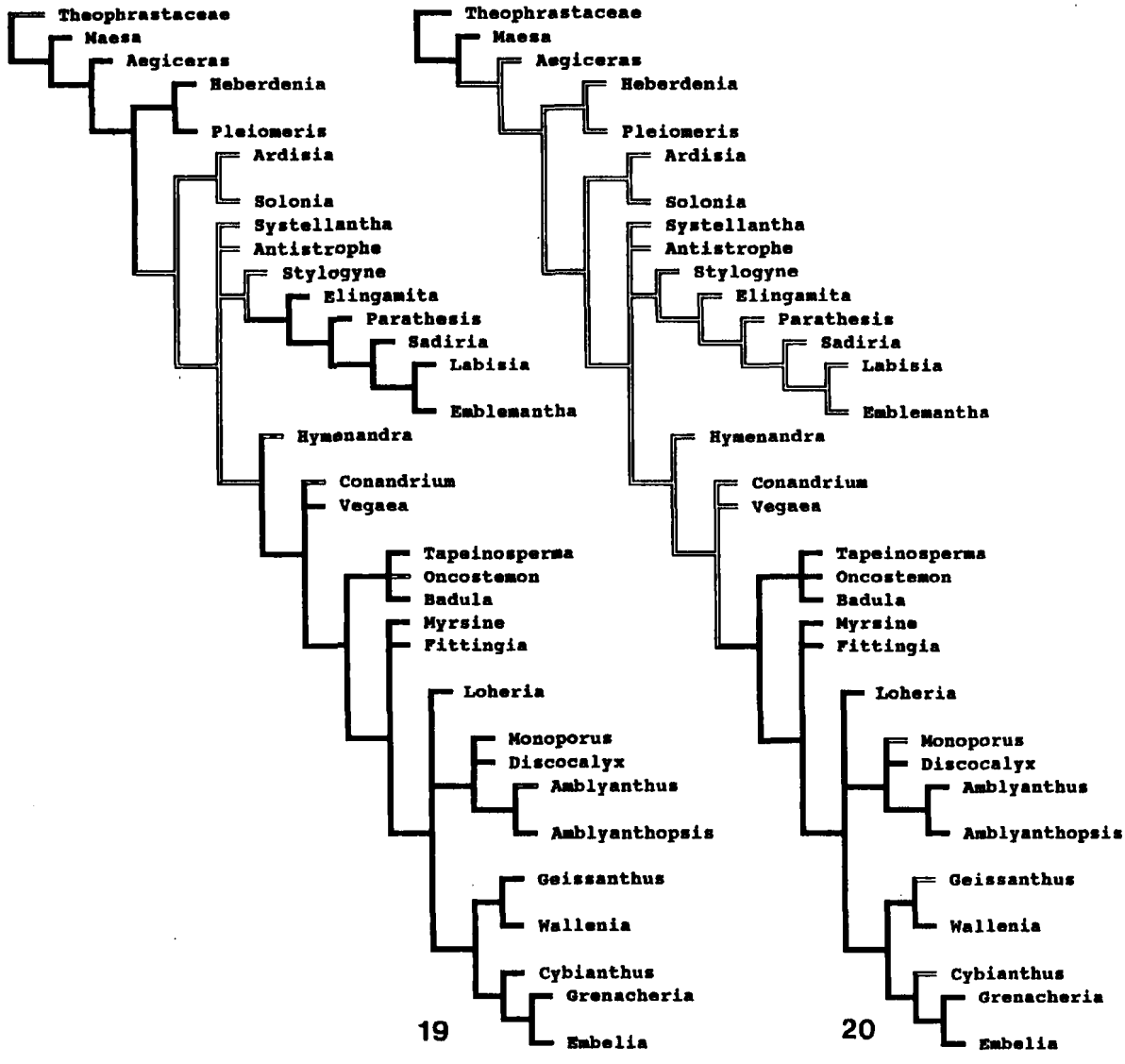


Fig. 18. The most parsimonious cladogram obtained from the analysis of the data in Table 2. (tree length = 240, CI = 0.75, RI = 0.84). Character stage changes are superimposed on cladogram: solid black = apomorphies, solid black-open = homoplasies, open = reversals.



Figs 19-20. Characters mapped on the cladogram. The character states associated with anemophily are shown as solid black lines. – Fig. 19. Character 18: 0-anthers initially forming a cone, but then free (outline); 1-anthers free (solid black line); 2-anthers fused in a cone (striped line). – Fig. 20. Character 25: 0-stigma capitate, conical or lobed (solid black line); 1-stigma punctate or truncate (outline).

type flowers, since pollen is the only reward offered (Buchmann 1983; Vogel 1978). Among Primulales, a nectar disc seems to be primarily absent and being secondarily present only in some Primulaceae (Vogel 1986). Instead, glandular trichomes that secrete nectar or oils may be present in many Primulaceae and basal members of Primulales, such as *Jaquinia* of the Theophrastaceae (Vogel 1986). Trichome nectaries are also present in *Aegiceras* of the Myrsinaceae but are allegedly absent in the rest of the Myrsinaceae, i.e. in Myrsinaceae s. str. (Vogel 1986). On sepals and petals of *Myrsine*, trichomes similar to those producing nectar in other genera of Primulales can be found, but in the present study no secretory activity could be revealed during anthesis.

The more advanced clades in our provisional phylogeny of the Myrsinaceae are characterized by small flowers and include several genera that are not anemophilous, such as *Conandrium*, with a staminal tube within which the anthers are hidden, *Embelia*,

Figs 21-22. Characters mapped on the cladogram. The character states associated to anemophily are shown as solid black lines. – Fig. 21. Character 28: 0-fruit many seeded (outline); 1- fruit one-seeded (solid black line). – Fig. 22. Character 30: 0-hermaphrodite (outline); 1-monoecious (striped line); 2-dioecious (black line).

Fittinga and *Discocalyx* with conspicuous and fragrant flowers (Sleumer 1987b, 1988a). These plants could be pollinated by small insects and would reward them with pollen when being hermaphrodite or otherwise be pollinated by deceit. In tropical forests, about one third of the dioecious zoophilous angiosperm species are pollinated by deceit (Renner & Feil 1993). Other genera of the Myrsinaceae sharing the anemophilous features of *Myrsine* are *Loheria* and *Wallenia*. However, more data are needed to ascertain that these genera are also wind pollinated.

In the present phylogeny of the Myrsinaceae, dioecy evolved as one of the most recent apomorphies of *Myrsine* that is part of the anemophilous syndrome. However, in several examples of wind pollinated plants, dioecy is believed to antedate the evolution of anemophily (Bawa 1980). In any case, the evolution of dioecy in the Myrsinaceae may have opened new evolutionary possibilities both for anemophilous and entomophilous clades.

The presence of a large stigma in all the dioecious taxa of Myrsinaceae (except *Cybanthus*) suggests that

dioecy is related to a inefficient mode of pollen transfer in which pollen is unprecisely positioned on the vectors. Thus entailing considerable pollen loss. Interestingly, flowers of the *Solanum*-type, such as those present in the possible ancestors of *Myrsine*, have to divert a considerable part of their pollen resources to the rewarding of the pollinators. The production of surplus pollen in such flowers is a possible precondition favouring the evolution of pollination systems with considerable pollen loss. Such conditions are present in wind pollinated flowers or allotropic flowers (i.e. flowers available for pollination by many generally unspecialized vectors) and would mean similar selective pressures. The socalled intrasexual competition postulated by Bawa (1980) may be operating in such systems.

However, to draw any further conclusions studies are needed especially on woody tropical species with inconspicuous flowers. The currently much debated correlation between dioecy and pollination mode is partly fuelled by this lack of data.

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