

Systematics and Leaf Architecture of the Gunneraceae

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I. Abstract

Cladistic and phenetic analyses of leaf and other morphological characters of *Gunnera* strongly support monophyly of the genus, with the Saxifragaceae s.str. as the closest sister group. This morphologically based phylogeny provides a more coherent understanding of the evolutionary history of *Gunnera* than do recent phylogenetic hypotheses based on genetic data sets with Myrothamnaceae as the sister group. Simple, crenate, palinactinodromously veined leaves lacking freely ending veinlets and tricolpate, tectate-perforate pollen with a reticulate exine indicate a shared ancestry. Within the genus *Gunnera* all six traditionally recognized subgenera are monophyletic, as supported by leaf architectural apomorphies. The monotypic subgenus *Ostenigunnera* is the sister group to the other five subgenera, which can be divided into two principal lineages. One lineage includes the subgenera *Milligania* and *Misandra*, characterized by a prostrate stoloniferous habit with small, low-rank leaves and exclusively unisexual flowers, whereas the other lineage includes the subgenera *Perpensum*, *Pseudo-Gunnera*, and *Panke*, all of which possess at least some hermaphroditic flowers and larger, high-rank leaves. When the phylogeny of the subgenera is considered in light of biogeography and the fossil record, a number of cladogenetic events can be explained by continental vicariance in the Late Cretaceous. The African *Perpensum* became distinct from the other large-leaved lineage with the separation of the African continent ca. 90 Ma. The two small-leaved lineages, the subgenera *Milligania* and *Misandra*, split with the separation of New Zealand from Western Gondwana, about 80 Ma. *Pseudo-Gunnera* became isolated from *Panke* prior to this time, when *Panke* fossils occur in North America. *Gunnera* probably arose out of an early herbaceous radiation of tricolpate eudicots having close affinity to the basal Saxifragaceae, especially the genus *Chrysosplenium*.

II. Introduction

A. OVERVIEW OF *GUNNERA* TAXONOMY

Gunnera is a dicotyledonous genus of 40–60 species, ranging in habit from small, stoloniferous herbs to fleshy-stemmed, rhizomatous herbs with enormous leaves (Schindler, 1905; Mora-Osejo, 1984; Bergman et al., 1992) (Fig. 1). Species of *Gunnera* are successful colonizers of disturbed sites and poor soils in subtropical or wet temperate regions in the Southern Hemisphere. This is due in part to a unique intracellular mutualism with nitrogen-fixing cyanobacteria (Silvester & Smith, 1969; Osborn et al., 1991). Research in recent years has elucidated details of the incorporation, metabolic transport, specificity, and interdependence of *Gunnera* and its symbionts (Towata, 1985; Zimmerman & Bergman, 1990; Bergman et al., 1992; Johansson & Bergman, 1992, 1994; Osborn et al., 1992; Stock & Silvester, 1994).

Gunnera is one of the oldest living angiosperm genera, with a fossil record of characteristic palynomorphs that appear as early as the Turonian (Late Cretaceous), ca. 93 Ma (Brenner, 1968; Jarzen & Dettmann, 1989; ages follow Geological Society of America, 1996) and a current distribution that can be related to the breakup of Gondwanaland (Mora-Osejo, 1984; Fuller, 1995a; Wanntorp & Wanntorp, 2003). The macrosystematics of this genus have been problematic (Doyle & Scogin, 1988a, 1988b; Bergman et al., 1992; Baum, 1994), although recent work has suggested that *Gunnera* is part of an early radiation of tricolpate (eudicot) angiosperms (Fuller 1995a, 1995b; Wilkinson, 1998; Angiosperm Phylogeny Group, 2003; Hilu et al., 2003; Soltis et al., 2003). Genetic sequence data of several *Gunnera* species have been subjected to cladistic analyses and have provided the first systematic assessment of phylogenetic relationships of subgenera within *Gunnera* (Wanntorp et al., 2001, 2002). This

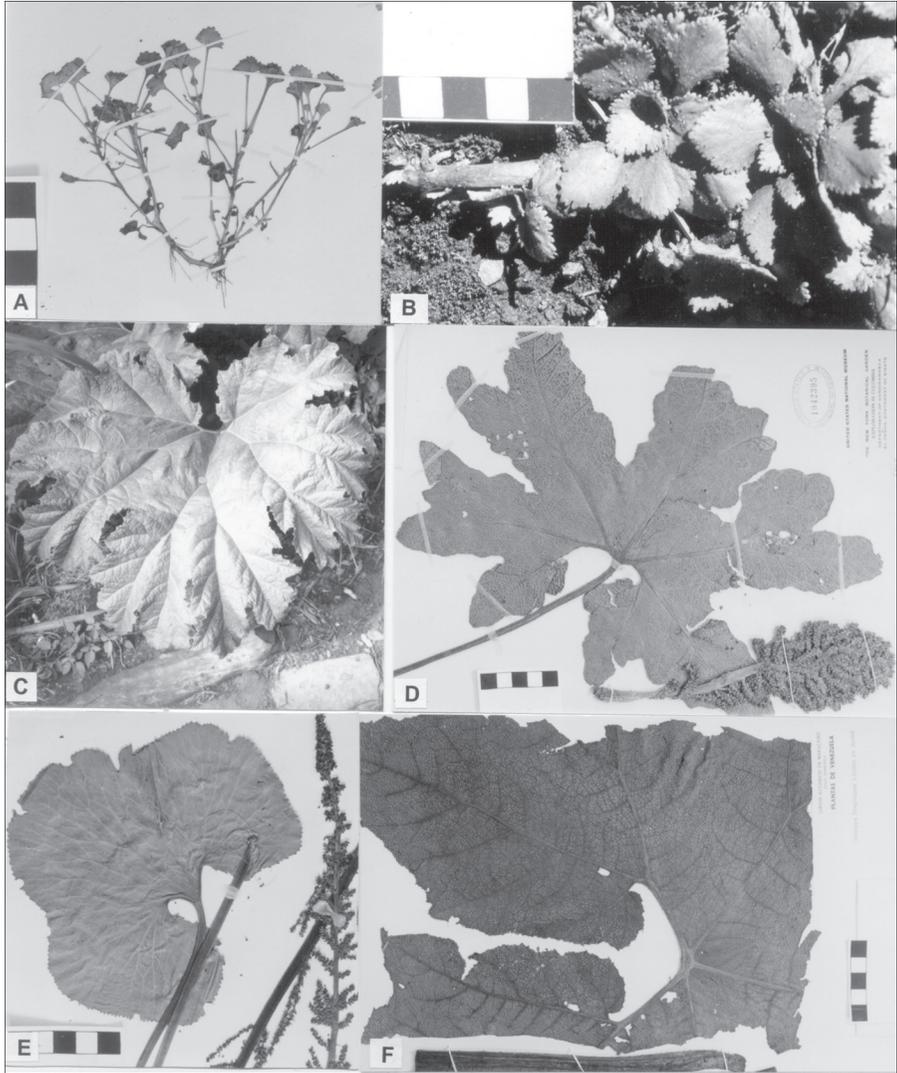


Fig. 1. Growth habit and leaf form in *Gunnera*. For this and succeeding figures, collection data can be found in Table III, unless otherwise specified. **A.** *Gunnera herteri*. Note the bifurcating axes and the production of axes in axils (Herter 22697b [US]). **B.** *Gunnera hamiltonii*. Note the dense rosettes of leaves formed by compression of decussately opposite pairs, and the thick, fleshy stolon. Living specimen in Strybing Botanical Garden, San Francisco. **C.** *Gunnera chilensis*. Note the twice-bifurcating midvein and multiple orders of lobes (akroteria). The great size of this leaf is apparent from the tiny appearance of the U.S. quarter (approximately 2.5 cm diameter) placed on its midvein. Living specimen in Strybing Botanical Garden, San Francisco. **D.** *Gunnera pilosa*. Note the alveolar leaf texture (Pennell 2660 [US]). **E.** *Gunnera perpensa*. Note three incipient lobes (akroterion cohorts) (Bourrell 2731 [CAS]). **F.** *Gunnera brephoega*, showing the reiterative, excurrent branching that forms the lobate base.

Table I
Affinities of *Gunnera* proposed or implied by various authors

| Proposed affinity | Source(s) |
|------------------------------|---|
| Haloragaceae/ Haloragales | Bentham & Hooker, 1865; de Candolle, 1868; Engler & Prantl (Peterson, 1893); Schindler, 1905; Hutchinson, 1973; Cronquist, 1981; Heywood, 1993 |
| Urticales | Jussieu, 1789; Bartling, 1830; Endlicher, 1837 |
| Araliaceae | Lindley, 1846 |
| Umbellales | Gibbs, 1974 |
| Onagraceae | Gray, 1854; Gibbs, 1974; Doyle & Scogin, 1988a, 1988b |
| Vitaceae | Behnke, 1981; Thorne, 1992 |
| Cornaceae | Thorne, 1992 |
| Connaraceae | Behnke, 1986 |
| Eucryphiaceae | Behnke, 1986 |
| Balanophoraceae | Hooker, 1856; Hansen, 1980; Mabberley, 1993 |
| Saxifragaceae | Huber, 1963; Takhtajan, 1980, 1983; Dahlgren, 1983; Doyle & Scogin, 1988a, 1988b |
| Hamamelidaceae | Chase et al., 1993 |
| Cercidiphyllaceae | Chase et al., 1993; Sytsma & Baum, 1996 |
| Platanaceae | Chase et al., 1993 |
| Trochodendrales | Chase et al., 1993 |
| Myrothamnaceae | Hoot et al., 1999; Soltis et al., 2000, 2003; Wanntorp et al., 2001, 2002 |

provides a basis for discussing trends in morphological evolution within *Gunnera* (Wanntorp et al., 2003, 2004) and its historical biogeography (Wanntorp & Wanntorp, 2003). The present article contributes additional evidence for phylogenetic relationships on the basis of detailed leaf morphological studies but differs in some of its conclusions from recent genetic studies.

Numerous affinities have been suggested for *Gunnera* (Table I). Linnaeus defined *Gunnera* on the basis of the African species *G. perpensa* and associated it with genera of the Orchidaceae and with *Forstera* (Stylidiaceae) (Linné 1767, 1787). Other early workers placed it in the Araliaceae (Lindley, 1846) or Urticales (Jussieu, 1789; Bartling, 1830; Endlicher, 1837), an affinity suggested by Airy Shaw in Willis (1966). Meissner (1836–1843) and Endlicher (1837) placed *Gunnera* in its own family, a circumscription followed by several modern taxonomists (Takhtajan, 1980, 1983; Cronquist, 1981, 1988; Thorne, 1992) and this article. However, Bentham and Hooker (1865), de Candolle (1868), Engler and Prantl (Peterson, 1893), and Schindler (1905) supported assignment within the family Haloragaceae, an assignment that remains the consensus in recent taxonomic and floristic sources (e.g., Macbride, 1959; Allan, 1961; Standley & Williams, 1963; Davis, 1966; Meijden & Caspers, 1971; Hutchinson, 1973; Moore, 1983; Mora-Osejo, 1984; Osborn et al., 1991; Heywood, 1993). An important character in allying them is the distinctive pseudo-polystelic vascular system of *Gunnera*, which has been claimed to indicate a monostelic, aquatic ancestry, such as from the Haloragaceae (Scott, 1891; Arber, 1920; Batham, 1943; Osborn et al., 1991).

However, striking differences between *Gunnera* and haloragaceous taxa have been noted by numerous researchers (e.g., Praglowski, 1970; Orchard, 1975; Behnke, 1986; Wilkinson, 1998). Orchard (1975: 27), based on the floral anatomy of Haloragaceae, considered the floral vasculature of *Gunnera* “so reduced as to be useless for comparison with Haloragaceae.” Nevertheless, Gunneraceae has remained associated with Haloragaceae in much of the taxonomic literature by placement in the same order (Takhtajan, 1969; Hutchinson, 1973; Cronquist, 1981, 1988), despite a long list of character differences between the two families. The highly autapomorphic nature of *Gunnera* was highlighted by its “wild-card” status in genetic studies

(cf. Chase et al., 1993; Baum, 1994; Hoot et al., 1999; Soltis et al., 2000). *Gunnera* was elevated to its own order by some (Dahlgren, 1983; Takhtajan, 1997; Soltis et al., 2003), but, although this emphasizes its unique nature, such a placement does little to reveal its evolutionary affinities.

Several nonhaloragaceous assignments also have been proposed. Assignment to the Saxifragales (Huber, 1963; Takhtajan, 1980, 1983; Dahlgren, 1983) received support from analyses of leaf phenolics and other phytochemicals (Doyle & Scogin, 1988a, 1988b; Doyle, 1990) and is accepted in this article. As part of Haloragaceae, *Gunnera* has often been placed in the Myrtales, alongside Onagraceae (Emberger, 1960; Melchior, 1964; Soó, 1975; Moore, 1993). Although there are phytochemical similarities between *Gunnera* and Onagraceae (Gibbs, 1974; Doyle & Scogin, 1988b; Doyle, 1990), Haloragaceae and *Gunnera* differ from Myrtales in numerous morphological and anatomical characters (Corner, 1976; Doyle & Scogin, 1988b; Conti et al., 1996). Cladistic analyses of *rbcL*, 18S, and *atpB* sequence data also argue against this placement (Chase et al., 1993; Conti et al., 1996; Hoot et al., 1999; Soltis et al., 2000). On the basis of chemical similarities, Gibbs (1974) suggested placement in the Umbellales, although additional data countered this (Doyle & Scogin, 1988b). Thorne (1989, 1992) put Gunneraceae near Vitaceae, Haloragaceae, and Cornaceae within the Cornales. Orchard (1975) supported the affinity of Haloragaceae and Cornaceae on the basis of floral and wood anatomy but excludes *Gunnera*. On the basis of sieve-tube plastids, Behnke (1981) associated *Gunnera* with Vitaceae and Leeceae, and later suggested a close affinity to Connaraceae and Eucryphiaceae (Behnke, 1986). A relationship has also been suggested between Balanophoraceae, *Gunnera* and Haloragaceae on the basis of an epigynous, valvate perianth with opposite stamens; a single, pendulous ovule; and seeds with a strongly adhering, thin testa, and cellular endosperm (Hooker, 1856; cf. Hansen, 1980; Mabberly, 1993). Most older classifications agree in placing *Gunnera* in the subclass Rosidae (or Thorne's Rosanae), an idea supported by the presence of mutualistic Mycorrhizae in *Gunnera petaloidea* (Koske et al., 1992). However, the rosid status of *Gunnera* now seems unlikely in light of recent genetic studies that fail to support the placement of *Gunnera* near *Myriophyllum* (Haloragaceae), nor within clearly rosid groups (Chase et al., 1993; Morgan & Soltis, 1993; Qiu et al., 1993; Soltis et al., 1993, 2000, 2003; Sytsma & Baum, 1996; Soltis & Soltis, 1997; Hoot et al., 1999; Angiosperm Phylogeny Group, 2003; Hilu et al., 2003). The other taxa suggested above were also distant.

As molecular-based phylogenies have multiplied, *Gunnera* has found a prominent place in the phylogeny of the lower angiosperms with tricolpate pollen (i.e., the eudicots). Early *rbcL* analyses put *Gunnera* as a sister group to a caryophyllid-rosid-asterid clade derived from a paraphyletic basal hamamelid grade. Among the closest taxa to *Gunnera*, according to the *rbcL* study, are *Trochodendron*, *Tetracentron*, *Nelumbo*, *Platanus*, and *Hamamelis* (Chase et al., 1993). On this basis our leaf architectural studies included basal hamamelid groups for detailed morphological studies (Fuller 1995a, 1995b; this article). Combining *rbcL* and ITS sequences, Soltis and Soltis (1997) placed *Gunnera* with some magnoliid taxa, on a branch outside a broadly saxifragalean-hamamelidalean group.

Recent analyses, including additional molecular data sets, have strengthened the likelihood of evolution early within the tricolpate angiosperms and have proposed the hypothesis of a sister-group relationship with Myrothamnaceae (Hoot et al., 1999; Soltis et al., 2000, 2003; Hilu et al., 2003). A placement near "Berberidopsidales" or Buxaceae is also suggested by recent genetic analyses. Caution is warranted here because long terminal branch length often makes cladistic placement unstable (Baum, 1994) and leads to a more basal placement of such terminal taxa (Sytsma & Baum, 1996). Because the fossil record suggests that *Gunnera* has been a distinct lineage for at least 93 million years, considerable anagenesis and apomorphy acquisition is likely.

The implications of tricolpate pollen in *Gunnera* have never been seriously considered. Recent phylogenetic studies suggest that the tricolpate and tricolpate-derived pollen of higher dicotyledons are monophyletic, forming a "eudicot clade" (Doyle & Hotton, 1991; Chase et al., 1993; Albert et al., 1994; Doyle et al., 1994; Crane et al., 1995; Sytsma & Baum, 1996; Hoot et al., 1999; Soltis et al., 2000, 2003; Hilu et al., 2003), if the tricolpate pollen of Illiciales, which shows radial arrangement of colpi within the pollen tetrad, is excluded as being an independent, convergent origin (Huynh, 1976; Donoghue & Doyle, 1989; Doyle & Hotton, 1991). Tricolpate pollen is largely absent from the traditional Rosidae, with the tricolporate state considered primitive in the subclass (Dickison, 1989). Notable exceptions are some Saxifragaceae, namely some *Saxifraga* s.l., especially section *Micranthes*, and *Chrysosplenium* (Ferguson & Webb, 1970; Heusser, 1971; Gupta & Sharma, 1986), some Podostemaceae, and *Gunnera* (Erdtman, 1966).

The fossil record indicates that tricolpate pollen originated after, and evolved from, monosulcate forms, and preceded polycolpates and all porate forms (Doyle, 1969; Doyle & Hickey, 1976; Hickey & Doyle, 1977; Traverse, 1988; Doyle & Hotton, 1991). Doyle and Hickey (1976) suggested that tricolpate pollen represents an advance, allowing more efficient release of recognition proteins and pollen tubes. The multiple origins of tricolpate-derived lineages suggest that there are adaptive reasons for advancing beyond the tricolpate toward the porate condition. This directional evolution is strongly supported by the fossil record in which, after the early Aptian, tricolpates quickly spread from the equatorial zone to all latitudes (Brenner, 1976; Hickey & Doyle, 1977; Lidgaard & Crane, 1988), and stratigraphically higher, tricolpate-derived forms increase in relative proportion to tricolpate types (Doyle, 1969; Hickey & Doyle, 1977; Traverse, 1988). There are no documented reversals of this trend, so on this basis alone it seems highly unlikely that *Gunnera* belongs with the Haloragaceae (s.str.) with its polycolpate and porate pollen forms resembling the fossil form-genus *Normapolles* (Praglowski, 1970).

Pollen of this level of advancement originates in the late Cenomanian, considerably after tricolpate pollen (Doyle, 1969; Hickey & Doyle, 1977; Traverse, 1988; Kedves, 1989). Thus the derivation of *Gunnera* from monostelic Haloragaceous ancestors (Scott, 1891; Arber, 1920) would imply major reversals in pollen morphological evolution, since nonmonostelic, terrestrial species like *Haloragodendron* or *Glischrocaryon* (compared with *Gunnera* by Meijden & Caspers, 1971) with hexacolpate, pectectate pollen and secondary growth (Orchard, 1975), would have to be considered ancestral in such a scheme. In fact, putatively primitive species of *Gunnera* do have a limited vascular cambium in their rhizome but none in their stolons or petioles (Batham, 1943), making a fully monostelic ancestry difficult to accept. Similarly, the spherical, triporate pollen of *Myrothamnus* suggests a later evolutionary development than do the pollen characters of *Gunnera*.

Recent genetic studies linking *Gunnera* and *Myrothamnus* as sister groups (Soltis et al., 2000, 2003; Hilu et al., 2003) raise interesting evolutionary questions but fail to offer insight into the evolutionary origins of the Gunneraceae and its phylogenetic relationship to large-scale trends in dicot evolution. Although this pairing has arisen in recent studies, caution is warranted due to concerns over long branch length attraction as well as anatomical and morphological characters. As noted by Wilkinson (2000), there is little anatomical or morphological evidence for a close relationship, which is confirmed by leaf architectural, pollen, and other characters discussed below. Were the hypothesis of a *Gunnera-Myrothamnus* clade to be accepted, their highly divergent morphologies and the specialization of *Myrothamnus* would mean that the ancestral morphological character states and the evolutionary origins of either group were not clear.

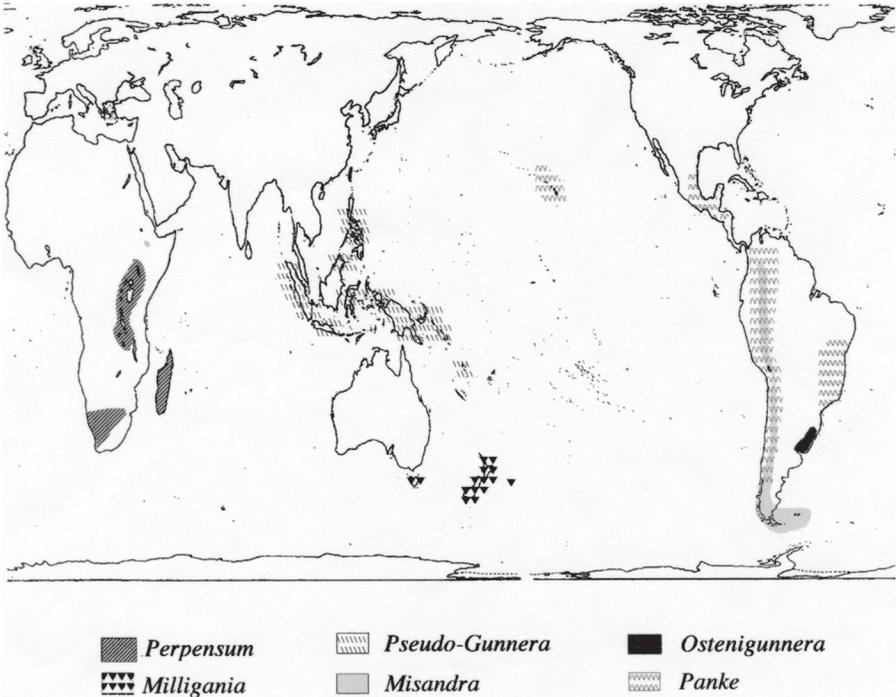


Fig. 2. General distribution of *Gunnera* subgenera. Data from Meijden (1975) and Mora-Osejo (1984).

B. GENERIC CIRCUMSCRIPTION AND SUBGENERA

Schindler (1905) defined *Gunnera* largely on the basis of fruit and floral morphology. *Gunnera* has panicles of "reduced" flowers, sometimes unisexual, consisting of two bithecal anthers with subtending sepals and/or a single unilocular pistil with two long, feathery stigmas. Its fruits are single-seeded drupes with small, cordate embryos. When a new species, *G. herteri*, which lacked long stigmas, was added to the genus (Osten, 1932; Mattfeld, 1933), it became clear that the presence of *Nostoc* in specialized glands is also a defining character of the whole family, although this was not shown to be a symbiotic adaptation until later (Silvester & Smith, 1969).

Gunnera is traditionally divided into six subgenera that are largely geographically distinct (Fig. 2). Schindler (1905) identified five subgenera on the basis of size, biogeography, and general floral habit, to which Mattfeld (1933) added the monotypic subgenus *Ostenigunnera*. *Milligania* incorporates 6 (to 11) species of creeping, stoloniferous herbs from New Zealand and one species from Tasmania, which are often dioecious, although the species *G. cordifolia*, *G. strigosa*, and *G. monoica* are monoecious (Schindler, 1905; Cheeseman, 1925; Allan, 1961; Webb et al., 1988; Table II). *Misandra* includes three species of prostrate, stoloniferous herbs, all dioecious, found in South America. *Pseudo-Gunnera* is erect and stoloniferous, producing panicles of unisexual female flowers basally and perfect, proterandrous flowers apically. The sole species of this subgenus is *G. macrophylla*, found in New Guinea, the Philippines and scattered on some volcanic islands of Melanesia (Meijden, 1975). *Perpensum* contains a single

Table IIThe division of *Gunnera* species into subgenera following different authors

| Schindler, 1905 | Maclaughy, 1917 | Mattfeld, 1933 | Meijden and Caspers, 1971 |
|-------------------------------|---|--|--|
| <i>Milligania</i> (9 spp.) | <i>Milligania</i> | <i>Milligania</i> | <i>Gunnera</i> |
| <i>Misandra</i> (3 spp.) | <i>Misandra</i> | <i>Misandra</i> | section: <i>Misandra</i> |
| <i>Pseudo-Gunnera</i> (1 sp.) | <i>Pseudo-Gunnera</i> (including <i>Perpensum</i>) | <i>Pseudo-Gunnera</i> | section: <i>Gunnera</i> (including <i>Milligania</i> , <i>Pseudo-Gunnera</i> , <i>Perpensum</i>) |
| <i>Perpensum</i> (1 sp.) | | <i>Perpensum</i> | |
| <i>Panke</i> (19 spp.) | <i>Panke</i> | <i>Panke</i> | section: <i>Panke</i> |
| | | <i>Ostenigunnera</i> (1 sp., discovered 1929) | <i>Ostenigunnera</i> |

nonstoloniferous, erect species, *Gunnera perpensa*, found in South Africa, Madagascar, and East Africa (Lowry & Robinson, 1988). Inflorescences in *Perpensum* are like those in *Pseudo-Gunnera*. *Panke* consists of more than 19 species in South America and the Hawaiian and Juan Fernández Islands (Mora-Osejo, 1984; Doyle, 1990). These species produce laminae up to 2 meters across, supported by erect petioles that arise near the apex of a pachycaul. Inflorescences are large (up to 2 meters long) panicles of hermaphroditic, but occasionally, unisexual, flowers. The subgenus *Panke* lacks stolons. The number of species in *Panke* and *Milligania* is problematic because of a high degree of gross morphological variability within and between populations, as well as interspecific hybridization (Palkovic, 1974, 1978; Webb et al., 1988; Doyle, 1990; Pacheco et al., 1991).

Several additional subgeneric classifications have been suggested (Table II). To Schindler's treatment, given above, Mattfeld (1933) added the monotypic subgenus *Ostenigunnera*, incorporating the newly discovered species *Gunnera herteri* (Osten 1932), distinctive for its minuscule size, small (but otherwise stenopalynous) pollen (Pragłowski, 1970; Jarzen, 1980), cauline growth without stolons, and axillary spikes with proterandrous male flowers apically and clusters of female (occasionally bisexual flowers) basally. Maclaughy (1917) published a slightly different classification of *Gunnera*, subsuming *G. perpensa* into the subgenus *Pseudo-Gunnera*. More recently, it has been suggested that only two subgenera should be recognized, the monospecific *Ostenigunnera* and *Gunnera* comprising all other species within three sections, *Panke*, *Misandra*, and *Gunnera* (i.e., *Perpensum*, *Pseudo-Gunnera*, and *Milligania*) (Meijden & Caspers, 1971; Meijden, 1975). However, this article will use the subgeneric divisions of Schindler (1905) as emended by Mattfeld (1933).

None of these earlier taxonomic works was explicit in proposing a phylogeny within the genus *Gunnera*. Most authors have pointed to *Milligania* as the most basal subgenus (Schindler, 1905; Batham, 1943; Mora-Osejo, 1984; Bergman et al., 1992), whereas the possible basal status of *Ostenigunnera* is implied in the work of others (Bader, 1961; Meijden & Caspers 1971). More recent morphological evidence (Fuller, 1995a, 1995b; Wilkinson, 1998) and genetic data (Wanntorp et al., 2001, 2002) strongly indicate the basal status of *G. herteri*. The cladistic analysis of ITS, *rbcL*, and *rps16* data sequences of 22 species of *Gunnera* by Wanntorp et al. (2002) led to their proposed phylogeny, in which the large-leafed *G. macrophylla* is sister group to small-leafed *Milligania* and *Panke* is descended from small-leafed *Misandra*. These analyses were based on the assumption of *Myrothamnus* as the sole outgroup. Given the importance of outgroup selection for polarizing character states within *Gunnera*, the present study considered a wide range of potential outgroups in order to find the best morphological sister group for analyzing phylogenetics within *Gunnera*.

III. Materials and Methods

A. CLEARED LEAF PREPARATION

In order to study foliar morphology, leaf clearings were made following the procedure of Foster (1952) with modifications from Hickey (1973). Most specimens were obtained from herbarium sheets, although *Gunnera insignis* came from material preserved in FAA solution and fresh leaves of *G. hamiltonii* were collected from a botanical garden (Strybing Botanical Garden, San Francisco). Clearing was done with 5% NaOH solution followed by acetolization and further clearing with 5% sodium hypochlorite. Chloral hydrate 250% solution was used as mordant, and staining was done with an ethanol solution of 1% safranin O, produced by EM Scientific, Cherry Hill, NJ. Specimens were mounted in a 50–60% concentration of Canada balsam in xylene, from Anchemia Scientific, Montreal. Specimens were flattened with a photographic roller and mounted with an identification label between slides of optical glass. All specimens were added to the National Cleared Leaf Collection (NCLC), currently housed at Yale University (Table III).

The process differed to some degree among specimens. Some specimens (*Gunnera killipiana*, *G. herteri*) were clear enough after NaOH treatment that the acetic acid and sodium hypochlorite steps were skipped. Bleaching was then followed by an additional rinse. Specimens of the subgenus *Milligania* species consistently retained opaque blotches that were removed by soaking in 5% chromium trioxide solution for a period of 10 minutes to an hour.

B. LEAF ARCHITECTURAL ANALYSIS

The systematic use of leaf morphology has been demonstrated in paleobotanical and recent systematic studies (e.g., Dickson, 1973; Hickey & Wolfe, 1975; Wolfe, 1989; Hickey & Taylor, 1991; Taylor & Hickey, 1992; Gornall et al., 1998). Leaf architecture seemed a particularly useful approach to *Gunnera* because this genus encompasses a wide range of leaf size, form, and venation pattern. Meanwhile, variation in fertile structures is rather limited, as the minute, single-seeded drupes and paniculate inflorescences are conservative throughout the genus. We examined cleared leaf material from 18 *Gunnera* species for leaf architectural characters. In addition to the cleared leaves, herbarium specimens were consulted for confirmation of many characters. For the larger-leaved species only small portions could be cleared, and these had to be supplemented by observation of herbarium specimens. Owing to the large size of leaves in subgenus *Panke* species, herbarium specimens are often only partial leaves or have been folded repeatedly. That made it difficult to assess characters of overall leaf form and the course of its primary venation. We therefore consulted as many specimens, published drawings, and photographs as possible (e.g., St. John, 1946, 1957; Mora-Osejo, 1984). In these largest leaves, the highest order of venation (sixth or seventh) proved difficult to observe because they are poorly lignified and do not stain well.

The basic system used to describe venation character states derives from Hickey (Hickey, 1973, 1977, 1979; Hickey & Wolfe, 1975) and conforms to the codification of the Leaf Architecture Working Group (Ash et al., 1999). Terms and concepts that were not in the original system (Hickey, 1979) are discussed here. In addition, a few concepts developed during the course of this study are discussed in the description of *Gunnera* leaf architecture (see “Results” and “Discussion”; also Fig. 3). Basal lateral primary veins and basal secondary veins form a spectrum and can all be considered *agrophic veins* (approximately equivalent to pectinal veins sensu Spicer, 1986), which are characterized by giving rise to a succession of higher-order, excurrent, adaxial veins. (These are equivalent in their course and behavior to secondary veins.)

Table III
Specimens examined, including leaf clearings and herbarium sheets

| Family or subgenus | Species | Figure No. | OTU | Herbarium material ^{a, b} |
|--|--|----------------|-------|---|
| Gunneraceae | | | | |
| <i>Milligania</i> | <i>Gunnera cordifolia</i> (Hook. f.) Hook f. | | | R. C. Gunn s.n. (YU) |
| | <i>G. dentata</i> Kirk | 4E, 5A | G DEN | ex Herb Kirk 365 (US), NCLC 6923 |
| | <i>G. monoica</i> Raoul | 3D, 9C | G MON | Fosberg 30750 (US), Fosberg 30777 (US), Fuller and Jones 310 (YU), Fuller and Jones 311 (YU), L. Cockayne 4537, NCLC 6900 |
| <i>Misandra</i> | <i>G. prorprens</i> Hook. f. | 4C | G PRO | Fosberg 30731 (US); Walker 4869 (US), NCLC 6917 |
| | <i>G. strigosa</i> Sol. (GSTR) | 4D, 7C | | Walker 4420 (US), NCLC 6918 |
| | <i>G. hamiltonii</i> Kirk ex W. Ham. | 1B, 4F | G HAM | Fuller 95-1 (YU), NCLC 6927 |
| | <i>G. lobata</i> Hook. f. | 4A, 9D | G LOB | Goodall 2303 (US), NCLC 69157; Moore 2062 (US) |
| | <i>G. magellanica</i> Lam | 4B, 4G | G MAG | Banks and Solander (US): US 1232964, NCLC 6915 Sleumer 1048 (US), NCLC 6916 |
| <i>Ostenigunnera</i> <i>Panke</i> | <i>G. herteri</i> Osten | 1A, 5B, 7A | G HER | Herter 22697b (US), NCLC 6914; anon. (US): US 2104785 |
| | <i>G. brephogea</i> Linden et André | 1F | G BRE | B. T. 542 (NY), NCLC 6890; Bunting 11.674 (NY), Wiggins 10993-A (NY) |
| <i>G. chilensis</i> Lam. <i>G. insignis</i> Oerst. | | 1C, 6C, 6D | G CHI | Betreund 12556 (UC) |
| | | 5C, 6B, 8C, 9F | G INS | Crosby 11436 (NY), Lent 277 (NY), Hill 17747 (NY), Gomez 19719 (NY), Skog 1301 (US), Hickey 5031 (YU): Standley 37583 (?) |
| | | | | |
| <i>G. killipiana</i> Lundell <i>G. manicata</i> Linden <i>G. mexicana</i> Brandegees <i>G. petaloidea</i> Gaudich | | | G KIL | Steyermark 49835 (US), NCLC 6919; Breedlove 9000 (US) |
| | | | G MAN | Pabst 77112 (NY), Krapovickas 23066 (NY), Reitz 2.669 (US), NCLC 1870 |
| | | | G MEX | Martinez 3230 (CAS); Reischfeld 1481 (NY), NCLC 6899; Neal and Harrr s.n. (YU) |

| | | | |
|---|----------------|--------|--|
| <i>G. pilosa</i> Kunth | ID, 8B, 8D | (GPIL) | Ewan 16628 (US), St. John 20525 (NY), NYBG 6911; Pennell 2660 (US), Penninton 5508 (US), Barriga 13232 (NY) |
| <i>G. talamancana</i> H. Weber & L. E. Mora | | GTAL | Lent 118 (NY), NCLC 6912; NYBG 650206-04 (NY), NCLC 6920; Rodriguez 437 (UC) |
| <i>G. perperum</i> | 1E, 6A, 9B | GPER | Ash 2843 (US), NCLC 6922; Strey 7960 (US), Bourell 2731 (CAS) |
| <i>Pseudo-Gunnera</i> | 3C, 8A, 9A, 9E | GMAC | Hoogland & Pullen 5591 (US), NCLC 6921; Barker 67544 (US), Bartlett 8532 (US), Fuller and Doyle 216 (YU), Fuller and Doyle 217 (YU), Fuller and Doyle 219 (YU) |
| Other families | | | |
| Aextoxicaceae | | | Unknown (US): US 2343533, NCLC 503 |
| Berberidopsidaceae | | | Philippi s.n. (US): US 1391551, NCLC 967 |
| Buxaceae | 10H, 11A | PACH | Kearney 108 (US), NCLC 4655 |
| Cercidiphyllaceae | 10G | CERC | Nikko s.n.: US 1314386, NCLC 4115 |
| Connaraceae | | CNES | Rock 769 (US), NCLC 263 |
| Cornaceae | | AUCU | Wilson 6440 (US), NCLC 2744 |
| | | | Eyerdan 10530 (US), NCLC 3795 |
| Euchryphiaceae | | GRIS | Philippi 1084 (US), NCLC 3793 |
| Haloragaceae | | EUER | Arthur 5111 (US), NCLC 3193 |
| | | | McDonald s.n. (US): US 2634123, NCLC 1863 |
| | | | Pritzel 836 (US), NCLC 1861 |
| Hamamelidaceae | 10A | PROS | Chandler s.n. (US): US 983503, NCLC 1869 |
| | | DISA | Wilson 7743 (US), NCLC 805 |
| | 10D | HAMA | Palmer 4441 (US), NCLC 6343 |
| | | LIQU | Shimada and Tanaka 3298 (US), NCLC 816 |
| Myrothamnaceae | | | Decary 10246 (US), NCLC 826 |
| Onagraceae | 10B | LOPE | Rose and Painter 6692 (US), NCLC 1908 |

Table III, continued

| Family or subgenus | Species | Figure No. | OTU | Herbarium material ^{a, b} |
|---------------------------|---|-------------|------|--|
| Other families, continued | | | | |
| Penthoraceae | <i>Penthorum sedoides</i> L. | | | Burton s.n. (YU), NCLC 6717 |
| Platanaceae | <i>Platanus occidentalis</i> L. | 10E | PLAT | Hickey s.n. (YU), NCLC 6344A |
| Saxifragaceae | <i>Astilbe japonica</i> A. Gray | | | Nanokawa s.n. (US): US 206082, NCLC 3488 |
| | <i>Chrysoplenium alternifolium</i> L. | 7F | CHRY | Hall 3068 (US), NCLC 3530 |
| | <i>C. grayanum</i> Maxim. | | CHRY | Togasi 702 (US), NCLC 3538 |
| | <i>C. griffithii</i> Hook. f. & Thomas | | CHRY | Rock 4979 (US), NCLC 3539 |
| | <i>Jepsonia parryi</i> (Torrey) Small | | | C. C. Parry s.n. (YU) |
| | <i>Leptarrhena pyrifolia</i> (D. Don) Ser. | | | M. McCluskey 117 (YU) |
| | <i>Lithophragma affinis</i> A. Gray | | | Brewer 1146 (YU) |
| | <i>Mitella breweri</i> A. Gray | | | Brewer 1679 (YU) |
| | <i>Peltiphylum peltatum</i> (Torrey) Eng. | | | Bolander 4986 (YU) |
| | <i>Saxifraga mertensiana</i> Bong. | | | Cusick 3321 (US), NCLC 3468 |
| Tetracentraceae | <i>S. (Micranthes) eriophora</i> S. Watson | 7B, 7D, 11C | MICR | Moran 15406 (US), NCLC 3458 |
| | <i>S. (Micranthes) nivalis</i> L. | | MICR | Coville and Kearney 1108 (US), NCLC 3466 |
| | <i>S. sarmentosa</i> L. | | SAXI | Tsu 1679 (US), NCLC 3508 |
| | <i>Sullivantia oregana</i> S. Watson | | | T. J. Howell s.n. (YU) |
| | <i>Tolmiea menziesii</i> (Pursh) Torrey & A. Gray | | | Bolander 4792 (YU) |
| | <i>Tetradentron sinense</i> Oliv. | 10f, 11B | TETR | Oliver s.n. (US): US 596944, NCLC 184 |
| | <i>Trochodendron ratioides</i> Siebold & Zucc. | | TROC | Unknown (US): US 1271620, NCLC 174 |
| | <i>Ampelopsis heterophylla</i> Blume | | AMPE | Barnes 20191 (US), NCLC 172 |
| | <i>Vitis inconstans</i> Miq. | 10C | VITI | Li Hao-min 13169 (YU), NCLC 6452 |

^a For specimens without collector numbers, herbarium serial numbers are given after the colon, if possible.

^b Key: (CAS) = California Academy of Science; (US) = U. S. National Herbarium; (YU) = Yale Herbarium; (NY) = New York Botanical Garden; (UC) = University of California at Berkeley.

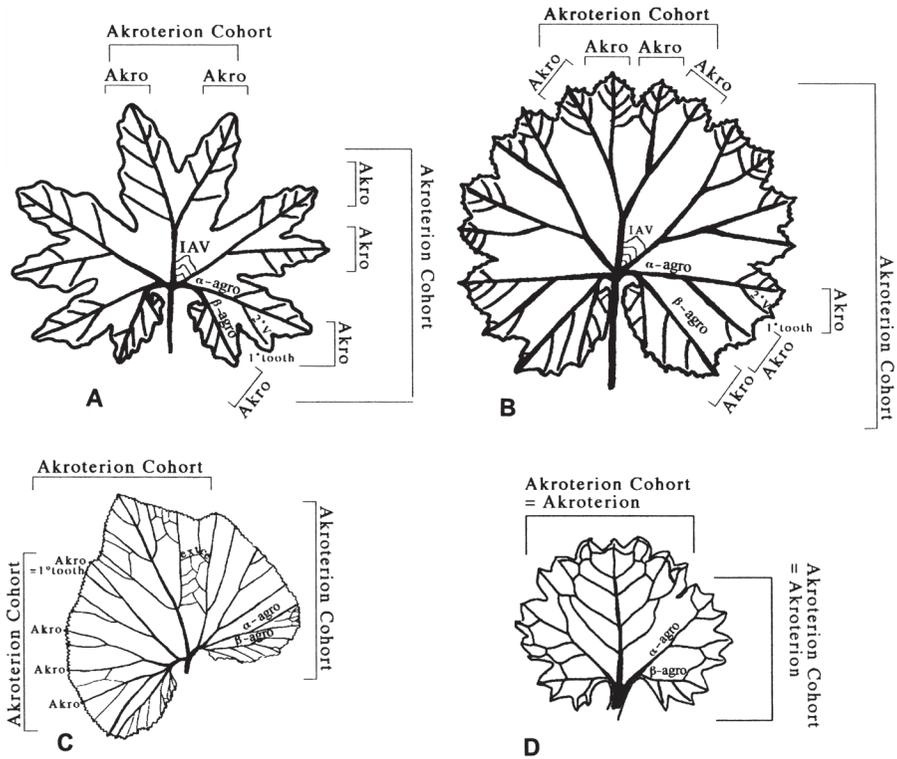


Fig. 3. Examples of akroteria (akro), akroterion-pairs, akroterion-cohorts. Two orders of agrophic (agro) veins are labeled as α -agro and β -agro. The other labels are: IAV = interangular veins; 2° V = secondary vein; 1° tooth = primary tooth; ext = external direction (in C); co = counterexternal direction (in C). **A.** *Panke* leaf of the pedate type, with a repand margin. **B.** *Panke* leaf of the orbicular type, with a fully developed, nested hierarchy of akroteria. **C.** *Gunnera macrophylla*, with incipient akroterion cohorts. **D.** *Gunnera monoica*, showing equivalent units.

Often the basal-most excurrent branch of an agrophic vein behaves in a similar fashion, giving rise to a series of lateral veins. In this case, different orders of agrophics can be distinguished by Greek-letter prefixes, as alpha (the first), beta (the next), and so forth (Fig. 3). Tertiary veins can be divided on the basis of the position of their insertion and the orientation of their course (following Pole, 1991; see Fig. 3C). One set of tertiary veins runs to the leaf margin. Among these tertiary veins, the externals originate excurrently on the abaxial (basal) side of secondary veins, and the *counterexternals* originate on the adaxial (apical) side of the secondaries. These are distinguished from reticulate or ramifying tertiaries, and joining tertiaries (cf. Pole, 1991), which include percurrent veins and interangular veins (sensu Pole, 1991). These terms can also be usefully applied to some of the second- and third-order venation in *Gunnera*.

Venation within marginal teeth was described with terms adopted from Hickey and Taylor (1991), distinguishing the *principal vein* from the *conjunctals*, which converge with the principal vein toward the tooth apex. The *admedial* is a vein that originates just beneath the tooth and runs beneath or toward the sinus of the tooth but does not become the principal vein of another tooth. *Accessories* are higher-order veins within a tooth framed by the principal, admedial, and

conjunctal veins. In addition to describing venation, leaf rank was useful for determining the level of leaf organization and evolutionary sequences (Hickey, 1977; cf. Doyle & Hickey, 1976; Hickey & Doyle, 1977; Hickey & Taylor, 1991). Trichomes were also examined following the guidelines and terminology of Theobald et al. (1979). Crystalline inclusions visible in cleared leaf tissue were classified on the basis of the terminology of Radford et al. (1974). Epidermal structure was described following the terminology of Dilcher (1974). However, epidermal characters could not be assessed for several specimens in which the epidermis appeared to have been damaged by the clearing process.

C. PHYLOGENETIC ANALYSIS

The characters obtained from study of cleared leaves formed the core for a cladistic analysis of the phylogeny within *Gunnera*. In addition to 39 foliar characters derived from this study, a literature survey produced 11 pollen characters as well as 15 other characters relating to growth habit, inflorescence morphology, reproductive anatomy, and sieve tube anatomy (see Appendix). Although most of the second two groups of characters are considered common to the entire genus (cf. Cronquist, 1981; Wilkinson, 1998, 2000), most have only been studied in a limited number of species, especially in subgenera *Panke*, *Pseudo-Gunnera*, and *Milligania*. Even though we have coded these on the basis of the available data, there remains the possibility that more than one state of the character exists within the genus.

Certain difficulties were encountered in coding the characters. Some characters intergrade along a spectrum that made dividing them into distinct character states somewhat arbitrary. For example, the common leaf margin in *Gunnera* consists of convex-convex teeth, forming a single order of crenations. Some species have a fully developed second order of teeth, but many others have only an occasional secondary tooth. Coding the latter as either singly or doubly crenate unnecessarily biases cladistic analyses toward one group or another. Another solution would be to establish a separate state for intermediate conditions. This worked for some characters, but in other cases, as with marginal configuration, this would have unnecessarily divided species with essentially the same character state. In such cases arbitrary criteria had to be established. For example, leaves were only considered to have two orders of dentition if the majority of teeth had secondary teeth associated with them.

Several of the characters considered were dependent on the presence of another character (e.g., the characters of primary venation that were applicable only to palmately veined leaves, or characters describing venation within marginal teeth were necessarily absent from entire-margined species). Following the reasoning of Hickey and Taylor (1991), such characters were assigned separate, not-applicable (N/A) states for each taxon. This makes each character a unique autapomorphy for the terminal taxa and thus will not link taxa on the basis of absences, treated as shared-derived characters by the cladistic algorithm.

The resulting data matrix (Table IV) was the basis for several analyses using PAUP 3.1 (Swofford, 1993). Trees were further explored with MacClade (Maddison & Maddison, 1992). In light of more recent phylogenetic work on *Gunnera* and the proposed sister-group relationship with *Myrothamnus*, leaf architectural characters were examined on *Myrothamnus*, and this taxon was added to the matrix. Identical analyses were performed (now with PAUP 4.0) with the addition of *Myrothamnus* to test its effects on topology. Although the matrix includes 36 terminal taxa, a cladistic analysis of the entire data set was problematic. Several of the outgroups considered are distant, often derived groups (cf. Cronquist, 1981; Takhtajan, 1983; Thorne, 1992; Heywood, 1993). Necessarily, many intermediate taxa were excluded, as well as significant apomorphies of many outgroup taxa. Because the analysis concentrated on characters of

Table IV
Data matrix for phylogenetic analysis

| OTUs | Character states |
|-------|---|
| | 111111111122222222233333333344444444455555555566666666 |
| | 12345678901234567890123456789012345678901234567890123456789012345 |
| ----- | |
| GHER | 12000021101203130001300201030041000600010111000020001112121010200 |
| GMON | 10012012320200030001301201022011301200010011000020411112121010222 |
| GSTR | 10002012320200030001301201022011301200010011000020411112121010222 |
| GDEN | 10002002310310010001301101022011301400010011000020411112121020222 |
| GPRO | 10002002310300010001301001022011301200010011000020411112121020222 |
| GHAM | 10002001311310010001301001022001311400010011000020411112121020222 |
| GMAG | 32100022220212030010311011032011401100010011000020011112121020203 |
| GLOB | 12112012020202010010301011032011401100010011000020011112121020203 |
| GPER | 32100023331201001001402200032111121400010011000020001112121010200 |
| GMAC | 32400023330201002000422000032111121301?10011000020011112121010204 |
| GKIL | 42433523540422002010023000012211621311010011000020301112121000225 |
| GNLS | 42433423540422002010423000012111621311010011000020301112121000225 |
| GPIL | 3442402343040300200042302001211292131110011000020301112121010225 |
| GBRE | 44433223440401002000423000002111621301010011000020301112121010225 |
| GTAL | 34424023430401002000023020012112A2131110011000020301112121010225 |
| GMEX | 42433223540422002010023000012211221311010011000020301112121000225 |
| GMAN | 4442342354042100200042300000221152131110011000020301112121000225 |
| GCHI | 4442342354022200201042300001221122131110011000020301112121010225 |
| MICR | 12000021101203110001300200030441102000010111000011400012010100221 |
| CHRY | 12000021101203130001300000030041000E0001011100001?000?1?000103200 |
| SAXI | 32202022100203130001300000020040114400010012106111410011000103221 |
| PROS | 00001202056210110001000204021301114500026122002021000001000011010 |
| LOPE | 0030130200702211001100230003130100110005122321323?200001210024000 |
| TROC | 0330300200201000210110300302211021070001011100012020001100110110 |
| TETR | 10201032001001001121102003032101210800010111000110200001?0?101110 |
| PLAT | 110113140104022010012234000323111134000101110000?2?2000001?1?010010 |
| CERC | 1020313202100100112120200213540071090001000001120200001011122200 |
| HAMA | 01301002000411202121203402030011700D000101110?1?0200001000101010 |
| DTSA | 10206612015020201122202227256823800B00010011000110200001000121010 |
| CNES | 003067060590210021112030363477349?140005001200?01?200111100020?10 |
| AMPE | 1320180202300020210120301200261041220003100100?2?2?200101?00105010 |
| VITI | 13221412020001202101223002002611612G0003100100?2?2?200101?00100010 |
| AUCU | 00301306004101021122102200034111610A000512143041?200112001023000 |
| GRIS | 1020130400B100002121202235005411710F0003005110501?0?0?12?01113000 |
| PACH | 100000140112001111011020020320101113000502?2107200000?11?01011010 |
| MYRO | 01001120000401140101001102030310010H00040125208220200001300120100 |

^a For taxa abbreviations, see Table III; for character state definitions, see Appendix.

Gunnera and then looked for comparable characters in the outgroups, the data are not necessarily appropriate for resolving higher-level relationships between outgroups.

In order to mitigate these problems the analysis was broken down into several steps. First, the least likely outgroups were removed on the basis of absolute and mean patristic distances from representative *Gunnera* species (Table V). The more similar outgroup taxa, including a basal Hamamelid outgroup and a rosid outgroup, were run using two outgroup rooting strategies. The data matrix was further reduced by using six *Gunnera* species to represent the ingroup. Both of these analyses were rerun with the addition of *Myrothamnus*. The resulting data sets could be searched using the branch-and-bound algorithm, which finds the absolutely most parsimonious trees. On the basis of the above analyses the most likely sister groups of

Table VPairwise patristic distances between outgroup taxa and representative *Gunnera* species^a

| | GHER | GPRO | GLOB | GPBR | GMAC | GCHIL | Mean |
|------|-------|-------|-------|-------|-------|-------|-------|
| CHRY | 0.177 | 0.516 | 0.516 | 0.452 | 0.541 | 0.694 | 0.482 |
| MICR | 0.277 | 0.492 | 0.554 | 0.462 | 0.562 | 0.677 | 0.504 |
| PLAT | 0.557 | 0.574 | 0.574 | 0.492 | 0.567 | 0.607 | 0.561 |
| PACH | 0.534 | 0.534 | 0.586 | 0.5 | 0.579 | 0.759 | 0.582 |
| TROC | 0.6 | 0.554 | 0.646 | 0.569 | 0.625 | 0.646 | 0.606 |
| SAXI | 0.508 | 0.538 | 0.615 | 0.6 | 0.625 | 0.754 | 0.606 |
| TETR | 0.571 | 0.603 | 0.635 | 0.524 | 0.629 | 0.73 | 0.615 |
| GRIS | 0.607 | 0.623 | 0.656 | 0.574 | 0.65 | 0.754 | 0.644 |
| CNES | 0.705 | 0.623 | 0.656 | 0.607 | 0.683 | 0.672 | 0.657 |
| CERC | 0.646 | 0.646 | 0.646 | 0.585 | 0.688 | 0.769 | 0.663 |
| AUCU | 0.641 | 0.672 | 0.688 | 0.562 | 0.667 | 0.781 | 0.668 |
| HAMA | 0.597 | 0.645 | 0.677 | 0.645 | 0.705 | 0.758 | 0.671 |
| VITI | 0.721 | 0.656 | 0.656 | 0.656 | 0.667 | 0.689 | 0.674 |
| PROS | 0.585 | 0.631 | 0.723 | 0.646 | 0.75 | 0.815 | 0.691 |
| AMPE | 0.738 | 0.639 | 0.672 | 0.689 | 0.75 | 0.787 | 0.712 |
| DISA | 0.708 | 0.677 | 0.708 | 0.692 | 0.797 | 0.815 | 0.732 |
| LOPE | 0.688 | 0.703 | 0.688 | 0.719 | 0.81 | 0.828 | 0.739 |
| MYRO | 0.569 | 0.677 | 0.708 | 0.661 | 0.734 | 0.831 | 0.697 |

^a Abbreviations as in Table III.

Saxifragaceae and Platanaceae were used as outgroups to root an analysis of all *Gunnera* species.

IV. Results

A. SYSTEMATIC LEAF ARCHITECTURE OF GUNNERACEAE

Gunnera (Linné, 1767) is a genus of herbaceous plants producing simple leaves ranging in size from less than 1 cm across in *G. herteri* (Fig. 1A) to more than 2 meters across in *G. magnifica* (St. John, 1957). The wide range in size is accompanied by a range in texture from membranaceous in *G. herteri* to coriaceous in the subgenera *Pseudo-Gunnera* and *Panke*, as well as in the species *G. hamiltonii* (Fig. 1B–1D), with all other species being basically chartaceous. There is also a correlated increase in leaf rank (sensu Hickey, 1977; Hickey & Doyle, 1977); i.e., the regularity in vein organization through the hierarchy of vein orders. The veins in *G. herteri* are highly irregular; i.e., low first rank, where the course of any given secondary vein is not predictable from that of another (Fig. 5B). Other small-leaved species, such as in the subgenera *Milligania* and *Misandra*, are low second rank, where the course of secondary veins is similar from one vein to the next, but higher-order veins are highly irregular (Figs. 3D, 4G). The subgenera *Perpensum* (Fig. 6A) and *Pseudo-Gunnera* (Figs. 3C, 8A) are high second rank, having tertiary veins that are fairly consistent in angle of origin and course. The subgenus *Panke* (Figs. 6B, 8B, 8C) has third-rank leaves, in which regularity in arrangement can be found up to fourth- or fifth-order veins. The fossil record of early angiosperms indicates a general evolutionary trend toward increasing leaf rank (Hickey & Doyle, 1977). The spectrum of leaf rank in *Gunnera* therefore suggests a polarity for the venation characters of the genus, with the smaller-leaved, low-rank subgenera being more basal.

The thick, coriaceous leaves of the large-leaved subgenera (*Pseudo-Gunnera* and *Panke*) also have an alveolar texture, in which veins on the lower (abaxial) surface are highly prominent,

forming alveolae between them (Fig. 1D). These same veins are highly impressed on the upper (adaxial) surface, forming a colliculate (“hilly”) texture (Fig. 1E, 1F). In these alveolar species four or more vein orders show high relief. In *Perpensum* only the first and second orders, and sometimes the third, have such relief and can be considered “subalveolar.” Finally, in *Misandra* lower surface primary veins are highly prominent, whereas secondary veins are sometimes slightly so.

Most *Gunnera* species are heterophyllous, producing petiolate leaves as well as bracteose, cauline leaves that are sessile on the rhizome, although these cauline leaves have sometimes been confused with stipules (cf. Cronquist, 1988). They have usually been considered discrete leaves, which are often improperly called “ligules” or “squamae rhizomatis” (Schindler, 1905; Palkovic, 1974). An ontogenetic relationship of these structures with leaves is argued by Mora-Osejo (1984) and is clearly supported by the descriptive study of Wanntorp et al. (2004). In the subgenus *Panke* the venation in these “ligules” appears to be a highly reduced form of that in the normal, petiolate leaves. The species *G. herteri* and *G. perpensa* are isophyllous and lack these cauline leaves (cf. Osten, 1932; Humbert, 1950; Wanntorp et al., 2004). The leaf architecture described below is that of petiolate leaves.

Lamina are basically ovate (Fig. 1A–1E), to reniform-ovate and in many species more properly termed “reniform” (Figs. 1D, 1E, 3C), to essentially orbicular in many *Panke* (Figs. 6, 9). The leaf apex is rounded or acute (subgenus *Milligania*, and sometimes *Pseudo-Gunnera*), with the basically rounded apex in *Panke* becoming emarginate or deeply embayed (Figs. 1D, 1E, 3A, 3B). The leaf base is decurrent or lobate (Fig. 1F).

Although there is a wide range in marginal form within *Gunnera*, it is possible to suggest an underlying order that unites the variations in a hypothetical developmental spectrum (Fig. 3). In *Panke* species with orbicular leaves, there are marginal indentations between primary veins. Although the projections thus formed might be termed “lobes,” we will refer to one of these projections as an *akroterion* (from the Greek, meaning a small peninsula). This new term is necessary to allow a comparison between lobes and teeth. Lobes have been defined on the basis of depth of indentation toward the midvein (Hickey, 1979; Ash et al., 1999), whereas teeth refer to much smaller and strictly marginal portions of the leaf. An akroterion allows both kinds of structures to be considered in relation to each other and to venation.

An akroterion is the smallest lobe or marginal projection in which a primary vein or agrophic secondary vein terminates and which is set apart laterally from other primary veins by embayments of the margin. An akroterion cohort consists of all the akroteria that are produced by marginal bifurcation of one of the basal primary veins of the leaf. A primary vein may end in a tooth, but an akroterion is the full lobe with all of the secondary ramifications of a single primary branch. An akroterion cohort may consist of only a single akroterion (Fig. 3D), or a pair of them if the primary bifurcates on its way to the margin (Fig. 3A). Additional primary branches produce cohorts with more akroteria (Fig. 3B). For example, in the subgenus *Panke* multiple akroteria can be grouped into cohorts. In *Panke* species with a pedate margin (*Gunnera pilosa*, *G. talamancana*), cohorts may include only pairs of akroteria (Fig. 3A). In orbicular-leaved *Panke* (Fig. 3B) akroterion cohorts usually consist of numerous akroteria. The basic number of akroterion cohorts appears to be three. In nonlobed, but large-leaved, species—i.e., *G. macrophylla* and *G. perpensa*—akroterion cohorts are still present (Fig. 3C). In such species the leaf can often be divided into three zones, each of which is served by a cohort of primary veins sharing a common basal origin. The boundaries of these akroterion cohorts are marked by only the slightest indentation or notch in the margin. In this example akroteria are merely the marginal teeth in which the primary veins terminate. Thus, although these leaves are not lobed, they may still be compared with those of *Panke*, and it appears that the akroteria of *Panke* are homologous to primary marginal teeth of *Perpensum* and *Pseudo-Gunnera*.

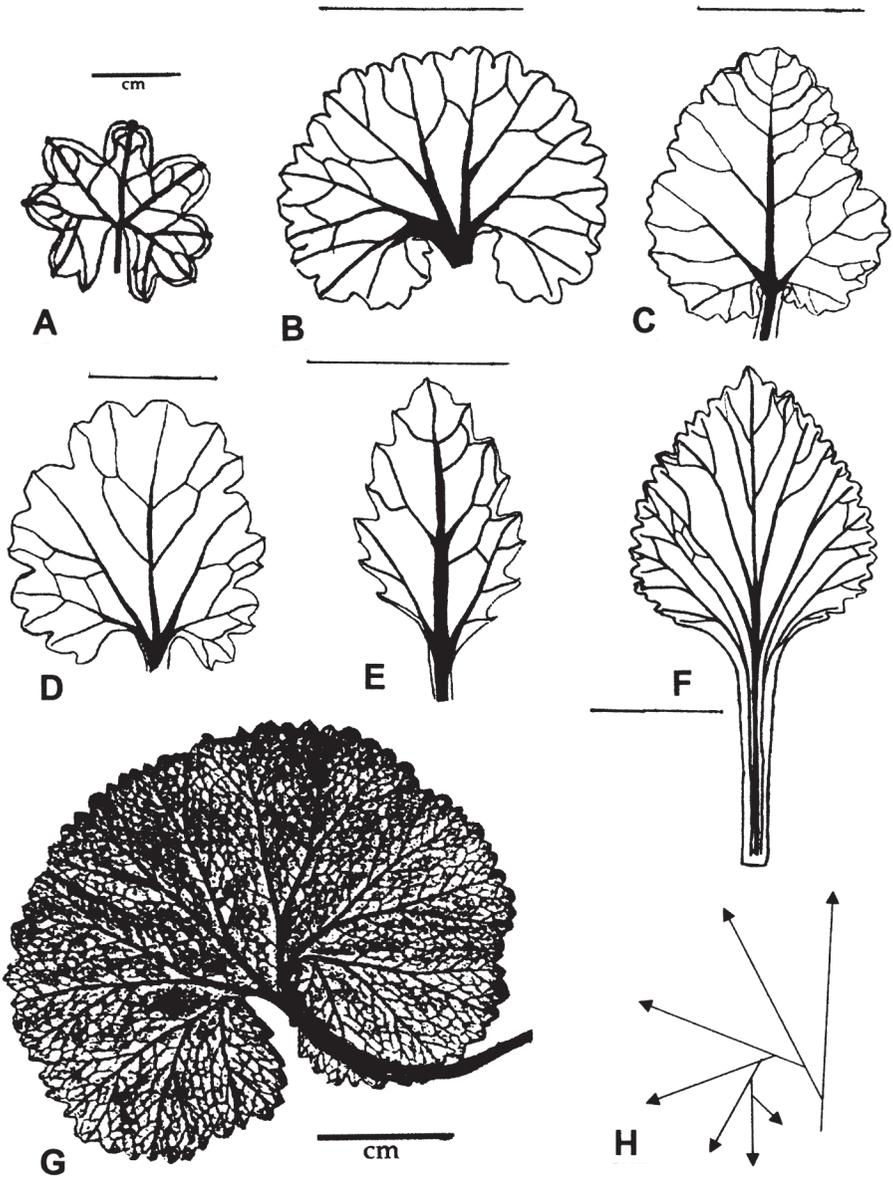


Fig. 4. Primary venation of *Gunnera*, mainly small-leaved taxa. All scale bars = 1 cm. **A.** *Gunnera (Misandra) lobata* (Goodall 2303 [US]). **B.** *Gunnera (Misandra) magellanica*. Note the bifurcation of the midvein from the base (Sleumer 1048 [US]). **C.** *Gunnera (Milligania) prorepens* (Walker 4869 [US]). **D.** *Gunnera (Milligania) strigosa*. **E.** *Gunnera (Milligania) dentata*. **F.** *Gunnera (Milligania) hamiltonii*. **G.** *Gunnera (Misandra) magellanica*, complete foliar venation from a flatbed scan (Meia 7969 [US]). **H.** Schematic representation of basal lateral venation in *G. perpensa* and larger species that produces a lobate base through reiterated, excurrent branching.

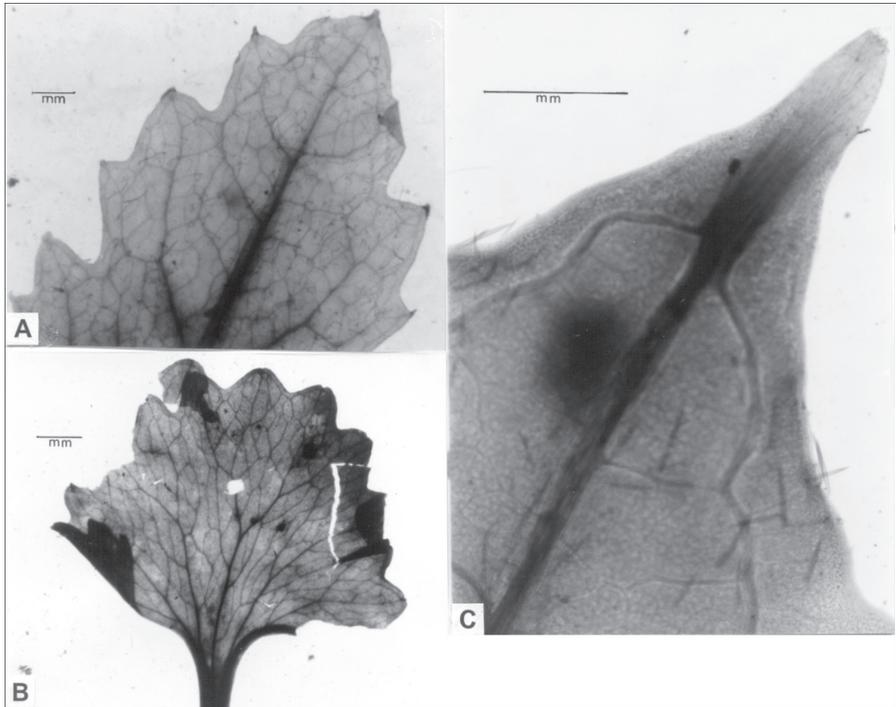


Fig. 5. Marginal tooth venation in *Gunnera*. **A.** *Gunnera dentata*, leaf clearing, showing low rank, reticulate venation, and chloranthoid teeth with dark-staining apical processes. **B.** *Gunnera herteri*, leaf clearing showing low rank, reticulate venation with a twice-bifurcating midvein, and chloranthoid teeth with dark-staining apical processes. **C.** *Gunnera insignis*, an elongate marginal tooth, with vascular strands splaying into the tooth apex in chloranthoid fashion (Hickey 5081 [YU]).

The small leaves of the subgenera *Milligania* and *Misandra* can also be described using this concept. Most of these species lack lobes, although akroteria can be distinguished. In *Gunnera monoica* each primary vein is contained within a shallow lobe, which is therefore an akroterion (Fig. 3D). In other species of *Milligania* the akroteria are less clearly distinguishable although the teeth terminating agrophic veins tend to be larger than those supplied by lower-order teeth. These akroteria can still be grouped into akroterion cohorts, of which there are three in the unlobed leaves of *G. strigosa*, *G. dentata*, and *G. prorepens* (Figs. 3D, 4D, 4E). Since these akroteria are formed around single primary veins (or strong basal secondary veins), they are also equivalent to the akroterion cohorts of larger species. The marginal “lobes” in *G. lobata* are enlarged crenations. Sometimes these are akroteria in their own right (Fig. 4A, apical tooth), whereas on other specimens there are additional secondary teeth (as in the basal akroteria of Fig. 4A). Although *G. lobata* has five akroteria, these can be grouped into three akroterion cohorts because there are three distinct veins at the leaf base. In *G. magellanica* (Fig. 4B, 4G), *G. herteri* (Fig. 5B), and *G. cordifolia* akroteria are not distinct from teeth; primary veins are terminated by marginal crenations, but these are not set apart from other portions of the margin.

Thus by relating margin patterns with the constituent venation it is possible to identify related patterns of venation development and leaf form that may be elaborated or simplified in the course of phylogeny. The akroterion cohort present in the subgenus *Milligania* species is

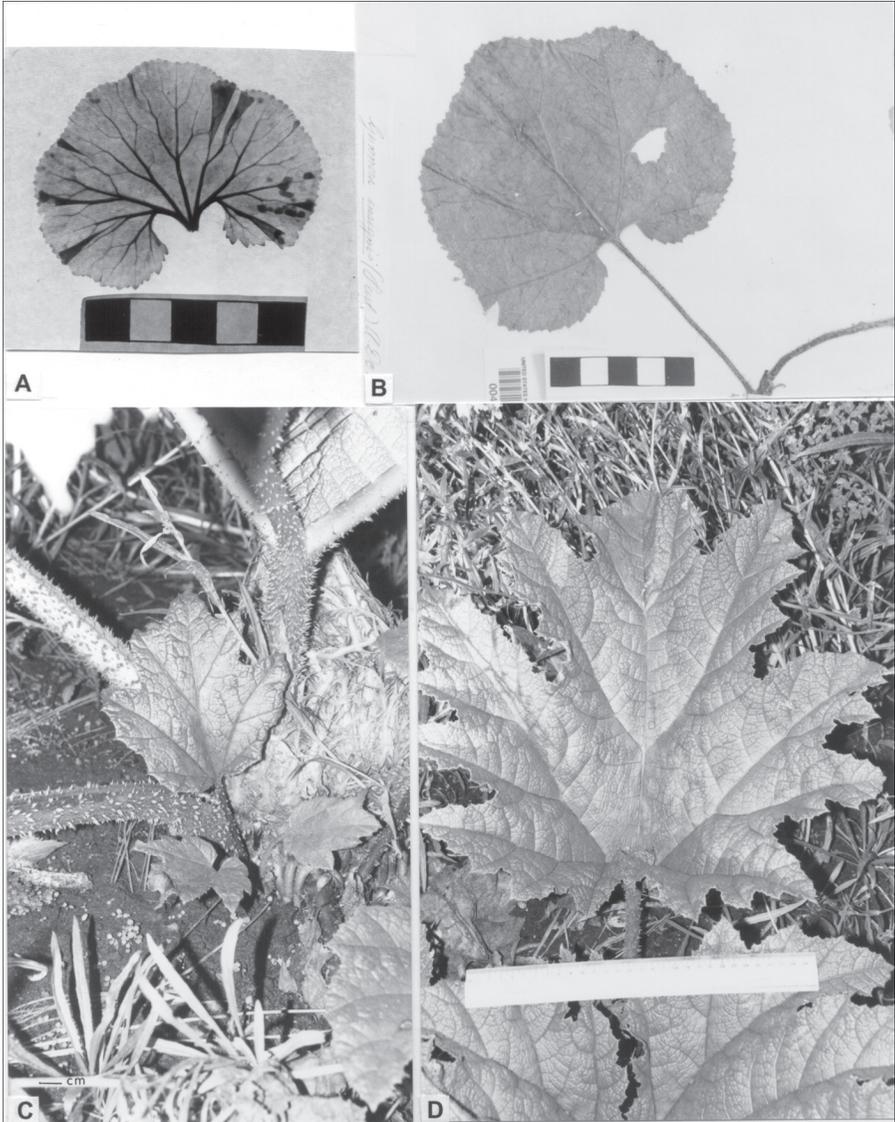


Fig. 6. Primary venation of large *Gunnera*. Scale bars = 1 cm. **A.** *Gunnera perpensa*, a cleared leaf showing a trichotomous midvein (Ash 2843 [US]). **B.** *Gunnera insignis*, juvenile leaf, resembling in form and venation those of species in subgenera *Perpensum* and *Pseudo-gunnera* (Standley 37583 [US]). **C.** *Gunnera chilensis*, leaf of a young plant, showing actinodromous-pinnate venation. **C** and **D** were taken in the Strybing Botanical Garden, San Francisco. **D.** *Gunnera chilensis*, new leaf on mature plant, showing a trichotomous midvein pattern with the right-hand (and more basal) branch beginning to out-grow the left-hand branch, thus suggesting that the bifurcating midvein syndrome develops gradually during later organogenesis. The ruler is 30 cm long.

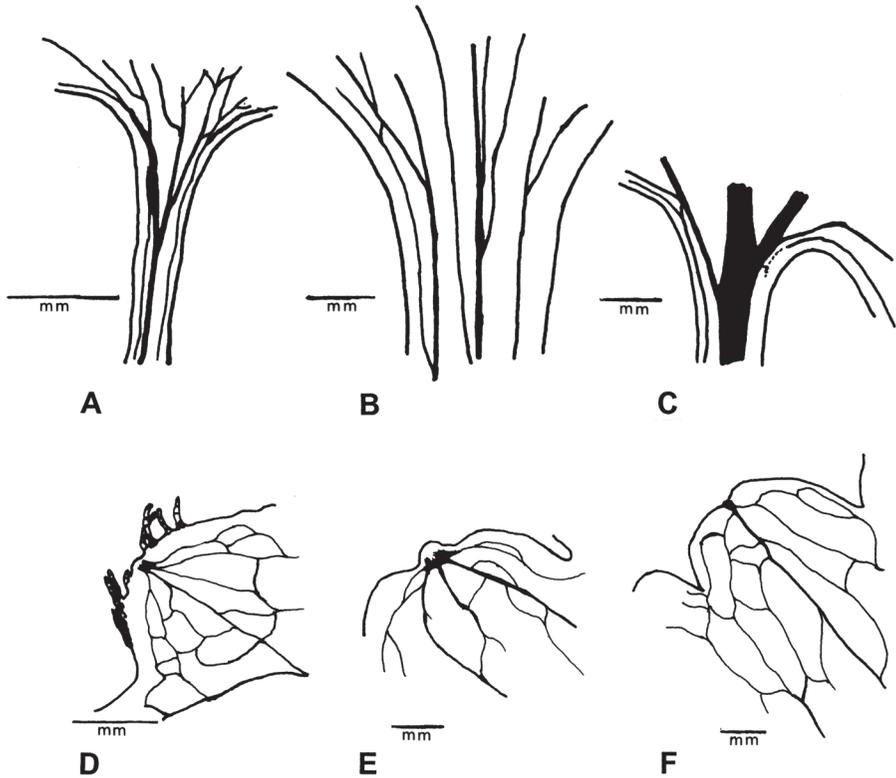


Fig. 7. A–C. Petiolar venation, showing the probable homology between *Gunnera herteri* and *Saxifraga* (*Micranthes*). **A.** *Gunnera herteri*, petiolar and basal laminar venation (Herter 22697b [US]). **B.** *Saxifraga* (*Micranthes*) *eriophora*. **C.** *Gunnera strigosa*, showing vestiges of the same morphology. **D–F.** Marginal tooth venation in Saxifragaceae, comparable to that in Gunneraceae. **D.** *Saxifraga eriophora*, a marginal tooth, with connivent conjunctals. Note the uniseriate trichomes. Primary venation of this species is represented in Fig. 11C. **E.** *Chrysosplenium griffithii*, a marginal tooth with joining conjunctals, tending toward connivent due to pronounced splaying. **F.** *Chrysosplenium alternifolium*, a marginal tooth showing the oppositely joining, chloranthoid syndrome.

equivalent to an akroterion cohort in the subgenus *Perpensum*, *Pseudo-Gunnera*, or *Panke*, except that in the latter taxa this cohort has been expanded through the reiteration of akroteria and filled out with more teeth (Fig. 3). The akroteria in *Panke*, which in some cases are clearly demarcated lobes, are the elaborated equivalents of the primary marginal teeth in *Perpensum*, *Pseudo-Gunnera*, or small-leaved species. This suggests a developmental spectrum in which higher-order veins ending in small marginal teeth are promoted to lower-order (primary) veins forming large lobes, resulting in a form of peramorphosis (cf. Alberch et al., 1979; Niklas, 1994). These relationships of leaf form and venation could be the result of reduction if the polarity is assumed to be from larger-leaved species toward the small; recapitulation (peramorphosis) is congruent with the directionality of evolution implied by leaf ranking and outgroup comparison (see “Phylogenetic Analysis within the Gunneraceae” below).

Primary venation in *Gunnera* is basically palinactinodromous, except in some species of the subgenus *Milligania*. A number of pinnately veined species occur in *Milligania* (Fig. 4C, 4E),

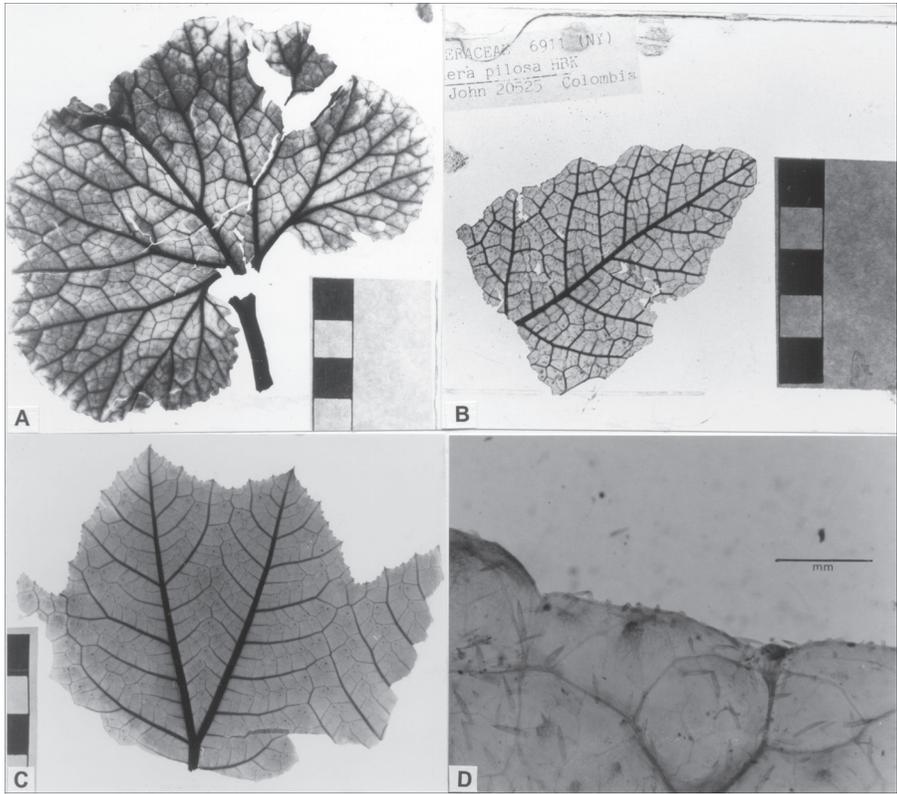


Fig. 8. Venation of large *Gunnera*. Scale bars = 1 cm in A–C. **A.** *Gunnera macrophylla*, a cleared leaf, showing a trichotomous midvein and a reticulate network of external and counterexternal veins (Hoogland and Pullen 5591 [US]). **B.** *Gunnera pilosa*, a cleared leaf, apex of akroterion, with a repand margin. Note the tertiary vein network, including some external-counterexternal craspedodromous pairs as well as some percurrent veins (St. John 20525 [NY]). **C.** *Gunnera insignis*, leaf clearing, akroterion pair. Note the series of interangular veins between split primaries, succeeded apically by connivent secondary veins that are joined by a reticulum of tertiary veins. **D.** *Gunnera pilosa*, a marginal gland showing derivation from a tooth by its oppositely joining, chloranthoid tooth-venation syndrome. Same specimen as in **B**; scale bar = 1 mm.

although their basal, agrophic secondaries are considerably thicker than the more apical secondaries. These agrophic secondaries are clearly homologous with the lateral primaries of actinodromous species, such as *G. monoica* and *G. strigosa* (Figs. 3D, 4D). *Gunnera hamiltonii* is intermediate between the palinactinodromous and the pinnate condition, because it has strong basal lateral veins that arise from distinct lateral petiole veins, although these are somewhat weaker than the midvein; nonbasal venation is like that in pinnate species (Fig. 4F). In addition, *Milligania* includes *G. cordifolia*, which is palinactinodromous. In the monotypic subgenera *Perpensum* and *Pseudo-Gunnera*, the primary veins fork numerous times to form a reticulum of primaries (Figs. 3C, 6A).

The course of the primary veins shows characteristic trends that distinguish *Gunnera* from most of the other taxa examined in this study. The basic primary venation appears to consist of three primary veins at the leaf base. In *G. herteri* these arise sub-basally by two rapid bifurcations (Figs. 5B, 7A), whereas in *G. hamiltonii* they derive from separate, lateral petiolar veins

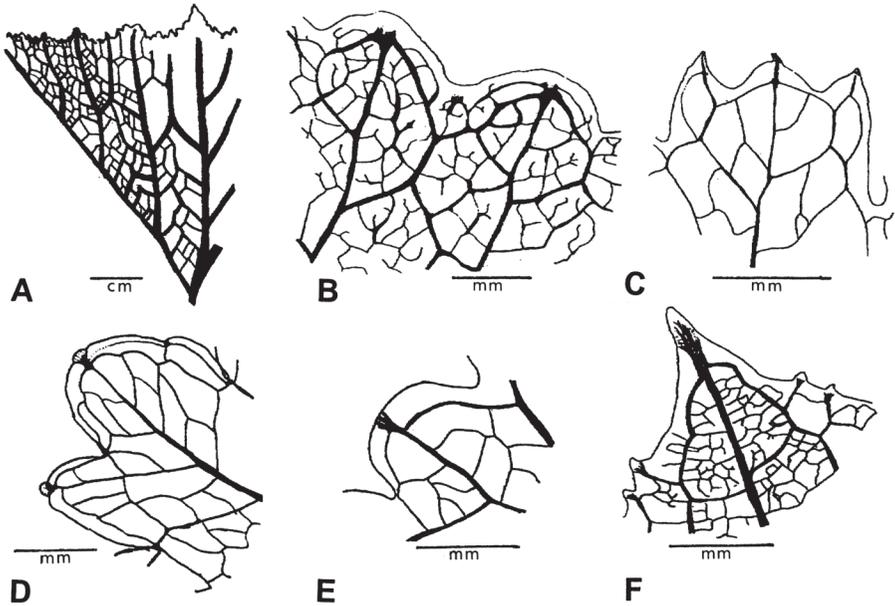


Fig. 9. Marginal venation in *Gunnera*. **A.** Fine venation of *G. macrophylla* (Hoogland and Pullen 5591 [NY]). **B.** *Gunnera perpensa*, marginal venation. Note the freely ending marginal veinlets, including an incipient sinus gland (Ash 2843 [US]). **C.** *Gunnera monoica*, a marginal tooth, with secondary teeth. **D.** *Gunnera lobata*, primary and secondary marginal teeth. Note the glandular sinus characteristic of *Misandra* (L. Cockayne 4537 [NY]). **E.** *Gunnera macrophylla*, a marginal tooth with alternately joining conjunctals (Hoogland and Pullen 5591 [NY]). **F.** *Gunnera insignis*, a marginal primary tooth with higher-order teeth, tending toward an acuminate shape (Hickey 5081 [YU]).

(Fig. 4F). In *Panke* and some species of *Milligania*, the two bifurcations have moved nearly opposite one another, giving the appearance of a basal trichotomy, although the sympodial origin of this trichotomy is often still detectable (e.g., Fig. 4C, 4D). The lateral primaries bifurcate again above the base, giving rise either to the five basal primary veins of palinactinodromous species or to strong secondaries (in *Milligania*, with the exception of *G. dentata*, and *G. hamiltonii*, this bifurcation has been greatly reduced or lost). These basal, lateral primaries (or basal secondaries in pinnate species) are agrophic veins; i.e., they produce a comblike series of lower-order veins toward the leaf margin (Fig. 3). Thus the α -agrophic sometimes bifurcates to produce an abmedial β -agrophic. This bifurcation process can be reiterative for as many as five orders of agrophics (*Perpensum*), as successive orders of external veins (sensu Pole, 1991) develop to form a basally directed spiral of veins (Fig. 4H). It is this reiterative venation that forms the earlike flap of laminar tissue at the lobate leaf base (Figs. 1D, 1F, 3A, 3B), which is most pronounced in the larger-leaved species. Some species of *Panke* have peltate leaves or include peltate populations (e.g., *G. peltata*, *G. insignis*, *G. kuaiensis* Rock (see Palkovic, 1974; Doyle, 1990). This study did not include any peltate leaves.

The midvein is often dichotomous in some sections of the genus. In dichotomous species (in subgenus *Panke* and *Gunnera herteri*) the midvein bifurcates between one-quarter and one-half of the distance from the lamina base to the apex. Often each of the resulting primaries bifurcates again (Figs. 1C, 3B). The midvein bifurcates once in the deeply lobed, pedate species (e.g., *G. pilosa*, *G. talamancana*, *G. brephogea*, Figs. 1D, 3A), and twice in species with

generally larger, orbicular leaves (e.g., *G. insignis*, *G. killipiana*, *G. manicata*). Bifurcation of the midvein reaches an extreme in *G. magellanica*, in which there is no midrib but rather two medial primaries that bifurcate at the leaf base (Fig. 4B, 4G). Other species (e.g., *G. perpensa*, *G. macrophylla*) show a simple midvein with two pronounced subopposite lateral veins (sensu Pole, 1991) of the same, or nearly the same, thickness that arise from the midvein and then curve toward the leaf apex (Fig. 6A). This suggests that the bifurcating midvein of *Panke* is derived from a simple midvein by splitting the midvein and strengthening the lateral veins. This surmise is supported by venation in leaves of young plants of *Panke* that have actinodromous-pinnate venation with similarities in the pattern of their midveins to *G. macrophylla*, *G. perpensa*, and subgenus *Milligania* (Fig. 6B–6D).

Secondary veins are reticulate or craspedodromous and often curved, although some are slightly recurved (*Gunnera dentata*, *G. hamiltonii*, Fig. 4E, 4F). Secondaries usually originate at angles ranging from 35° to 55°, but more obtuse angles occur in some species of the subgenus *Panke*. Members of *Panke* have a succession of interangular veins between primary veins (four in *G. pilosa*; six or seven in *G. insignis* (Fig. 6B) and *G. killipiana*), whereas other subgenera have significantly fewer. Intersecondaries are composite (*Panke* and *G. macrophylla*) or lacking. Tertiary and higher-order veins are reticulate and usually orthogonal. In *G. perpensa* and *G. macrophylla* (Fig. 8A) secondaries from adjacent primaries merge into a single vein running to the leaf margin that equals the strength of its two source veins (Figs. 6A, 8A). Third- (and sometimes fourth-) order veins follow a similar pattern. Due to repeated reticulations in *G. perpensa*, veins near the margin are extremely attenuated, and different vein orders are almost indistinguishable on the basis of size (Fig. 9B). In the subgenus *Pseudo-Gunnera* the veins are reticulate and converge in the same pattern as do those in *G. perpensa* (Figs. 8A, 9A). However, primary veins are stouter toward the margin. These thicker primaries may be necessary to support the larger leaves of *G. macrophylla*. In *Panke* some opposite tertiary veins join, but secondaries do not (Fig. 8C). Instead, adjacent secondaries run toward each other and are joined by a series of third-order veins that ramify between them (Fig. 8B).

Areoles range from imperfect to well developed and are predominately quadrangular. In smaller-leaved subgenera (*Ostenigunnera*, *Milligania*, *Misandra*) areoles tend to be elongated radially, as though radiating away from the leaf base (Fig. 5B, 5G). Areoles generally lack freely ending veinlets. However, some veinlets occur in a minority of the areoles of large leaves (subgenus *Panke*, also in *Gunnera perpensa* and *G. macrophylla*); these are simple and usually curved. Marginal venation is generally looped, although some freely ending, hooked veins occur in *G. perpensa* (Fig. 9B).

The leaf margin is always toothed, with generally crenate or dentate projections. The basic tooth shape is convex-convex (Type A1, Hickey, 1979) although some acuminate teeth are found. In *Milligania* the margin is dentate in two orders, with *Gunnera dentata* (Figs. 4E, 5A) and *G. hamiltonii* (Fig. 4F) tending toward serrate, and the second-order teeth of these species are generally convex-concave. Each tooth has a glandular, tylate apex. *Ostenigunnera*, *Milligania*, and *Misandra* have dark-staining apical processes (Fig. 5A, 5B) that are distinct from the deciduous papillae on many rosoid teeth (e.g., Haloragidaceae). Teeth of *Milligania* also have a small apical foramen.

The teeth of *Gunnera* are basically of the chloranthoid type, with conjunctal veins merging with the principal vein of the tooth oppositely or nearly so—i.e., in the manner of *Chloranthus* (Figs. 5A, 5B, 9B–9D)—but in some cases alternately—i.e., like those of *Ascarina* (Figs. 5C, 9E, 9F). In the putatively advanced subgenus *Panke*, tooth venation sometimes parallels the rosoid configuration, as the conjunctal veins remain distinct (i.e., connivent) and splay before joining, although some vascular strands always join the principal (e.g., *G. mexicana*, *G. insignis*, Figs. 5C, 9F).

The principal vein of the teeth has a direct, central course that terminates abruptly (truncate) or splays. Associated with the principal vein is an admedial vein, as well as conjunctals and sometimes reticulate accessories. The admedial vein is generally of a lower vein order than the principal, although in *Gunnera herteri*, *G. strigosa*, and *G. prorepens* the admedial and principal veins are of the same order. In some teeth *G. perpensa* has two successive pairs of conjunctals (Fig. 9B). The epidermal tissue on the teeth often has suberized, open stomata, which appear black in cleared specimens. These are rarely found elsewhere on the leaf. The sinuses of *Gunnera* are round and unbraced, except in the subgenus *Misandra*, which has glandular sinuses into which admedial and accessory veins converge and give rise to conjunctals (Fig. 9D). Otherwise, venation of the sinus consists of the conjunctals, admedials, and sometimes branches from them. In *G. perpensa* the sinus is subtended by a branch from a conjunctal vein. Sometimes this vein produces freely ending veinlets in the direction of the margin, as though to an incipient gland in the sinus (Fig. 9B).

In species of the subgenus *Panke* the margin usually has four or five orders of teeth. In some species "teeth" do not protrude far enough from the margin to have sinuses (sensu Hickey & Taylor, 1991). The resultant uneven margin is neither truly dentate nor entire but can be termed "repand" (Figs. 1D, 8C, 8D). These processes are clearly teeth, on the basis of their glandularity, venation, and homology with teeth on orbicular-leafed *Panke* (Fig. 8B). In some species the teeth become acuminate-acuminate (e.g., *Gunnera killipiana*, *G. insignis*, *G. manicata*, and *G. mexicana*).

Except for *Gunnera herteri*, *Gunnera* has unicellular, glandular hairs. These trichomes are ensiform with acuminate tips, but a few notable exceptions include the long, slender hairs of *G. dentata* and *G. lobata*. An occasional long, slender trichome is interspersed among the wider ones on *G. manicata* leaves. Of special note are the trichomes of *G. lobata*, which appear to have long, thin, black inclusions within the hair. The placement of trichomes is variable, but they are always found along the leaf margin and on veins of the abaxial surface. Often trichomes also occur along veins of the adaxial surface and in adaxial areoles, but less densely than on the lower surface. Only in *G. dentata* and *G. perpensa* were trichomes found in the areoles of the lower surface. The base of trichomes is socketed in a ring of epidermal cells that otherwise appear unspecialized. Some species of subgenus *Panke* also possess multiseriate and capitate trichomes, although it is unclear how widespread these are because they have only been reported for *G. kauaiensis* Rock (Wilkinson, 1998, 2000).

In addition to trichomes, *Gunnera macrophylla* and *Panke* species possess multicellular laminar processes often called "colleters" (Soloreder, 1908; Palkovic, 1974; Mora-Osejo, 1984). These have not been noted on any of the other subgenera or outgroups examined. These glandular processes are multicellular, round growths of tissue at the junction of high-order veins (fourth or fifth order). Soloreder (1908: 337) describes them as "more or less distinctly hemispherical warts, which consist of a considerable number of epidermal cells, arranged in longitudinal section, in the form of a fan, and adjoined internally by a few isodiametric parenchymatous cells." Colleters stain more darkly than does the surrounding leaf tissue. A second kind of process, which is nonglandular, is also found on the upper surface of *Gunnera* leaves. These "pseudo-colleters" are conical growths that arise in areoles and are subtended by sixth- or seventh-order veins. Although these two kinds of processes have both been considered colleters (Mora-Osejo, 1984), they are not functionally or evolutionarily homologous because no transitional states were observed. Wilkinson (1998, 2000) outlines a wide range of variation in epidermal processes, but the clearest division is between glandular "colleters" and nonglandular "pseudo-colleters."

Epidermal characters could not be observed in cleared specimens of all species. Nevertheless, the epidermis usually consists of five- or six-sided cells, which are elongate with undulate

walls. In larger species (subgenera *Pseudo-Gunnera*, *Panke*) these cells are interspersed with polygonal cells that are prevalent along major veins. In a number of species stomata occur on either surface, often in clusters, especially on marginal teeth. Stomates are anomocytic, possibly the plesiomorphic state for angiosperms (Doyle et al., 1994) and therefore not likely to be phylogenetically informative for the placement of *Gunnera*. Some leaves have crystalline inclusions. Druses were noted in the subgenera *Perpensum* and *Pseudo-Gunnera* and in *G. monoica*. *Gunnera dentata* has sandy inclusions. Druses were reported for *G. herteri* by Mattfeld (1933).

Ostenigunnera is the most distinct subgenus of *Gunnera*. *Gunnera herteri* is a cauline, rhizomatous perennial with isophyllous, opposite leaves. Vegetative axes and inflorescences are produced in the leaf axils. In addition, axes sometimes bifurcate (Fig. 1A). This species also has very low rank, membranous, widely ovate leaves with a decurrent bases, rounded apex, and marginal crenations (Fig. 5B). Although loosely palinactinodromous, the weak and reticulating primaries approach a flabellate condition. The midvein usually bifurcates twice before reaching the leaf apices. Secondary and tertiary veins of *G. herteri* branch at random angles to form a reticulum of irregular, imperfect areoles. Higher-order veins are lacking, as are trichomes. In the petiole, two thin but distinct veins run alongside the stout medial vein. These enter the base of the lamina and curve outward between the basal-most primary and the basal leaf margin (Fig. 7A). Here each soon bifurcates, with one branch joining the basal primary and the other tapering out into the laminar tissue. The course, branching, and position of this vein resemble the lateral primaries in herbaceous Saxifragaceae (see below), which are persistent in the petiole as distinct veins (Fig. 7B), as well as in the fossil taxon from the Aptian of Koonwarra, Australia described by Taylor and Hickey (1990). This vein provides negligible vasculature to *G. herteri* and probably represents a vestigial or incipient form of the three-veined petiole of an ancestral taxon. The homologous status of this character is further supported by its occurrence in *G. hamiltonii* (Fig. 4F), as well as by vestiges in *G. strigosa* (Fig. 7C).

B. THE SEARCH FOR SISTER GROUPS: COMPARATIVE LEAF ARCHITECTURE

Despite older taxonomic opinion (see Table I), *Gunnera* shows little affinity with Haloragaceae in its leaf architecture. In *Proserpinaca* (Haloragaceae), leaves are narrowly ovate with an acute apex, a decurrent base, and a serrate margin (Fig. 10A). The basic venation of this genus is pinnate with a massive primary vein. This suggests that leaf width might have been reduced in response to an aquatic habit while the midrib remained stout. This inference would seem to argue against a relationship to small-leaved *Gunnera* in which the midvein is more moderately proportioned. Secondaries in *Proserpinaca* are craspedodromous, sinuous, and marginal, arising at narrowly acute angles and becoming more obtuse apically. The tertiaries and quaternaries form a reticulum of irregular areoles lacking freely ending veinlets. Marginal venation is looped. Although *Proserpinaca* can be compared to *G. dentata* and aspects of the subgenus *Milligania* or *G. herteri* generally, the pinnate species in *Gunnera* suggest an actinodromous ancestry, whereas *Proserpinaca* leaves are strongly pinnate and suggest reduction from a true pinnate ancestry. In terms of the angles of origination and curvature of secondaries and intersecondaries, *Proserpinaca* shows similarities with the venation of Penthoraceae, which has been allied with Haloragaceae based on genetic data (Morgan & Soltis, 1993; Soltis & Soltis, 1997). *Proserpinaca* trichomes are multiserial, whereas those in *Haloragis* are uniserial and multicellular, three cells long and with a socket ring of particularly small cells. Teeth of *Proserpinaca* are strikingly different from those of *Gunnera* in their straight-straight margins, lack of accessory veins, deciduous papillate apices, and clearly rosoid venation syndrome of nonjoining, connivent conjunctals (Fig. 10A).

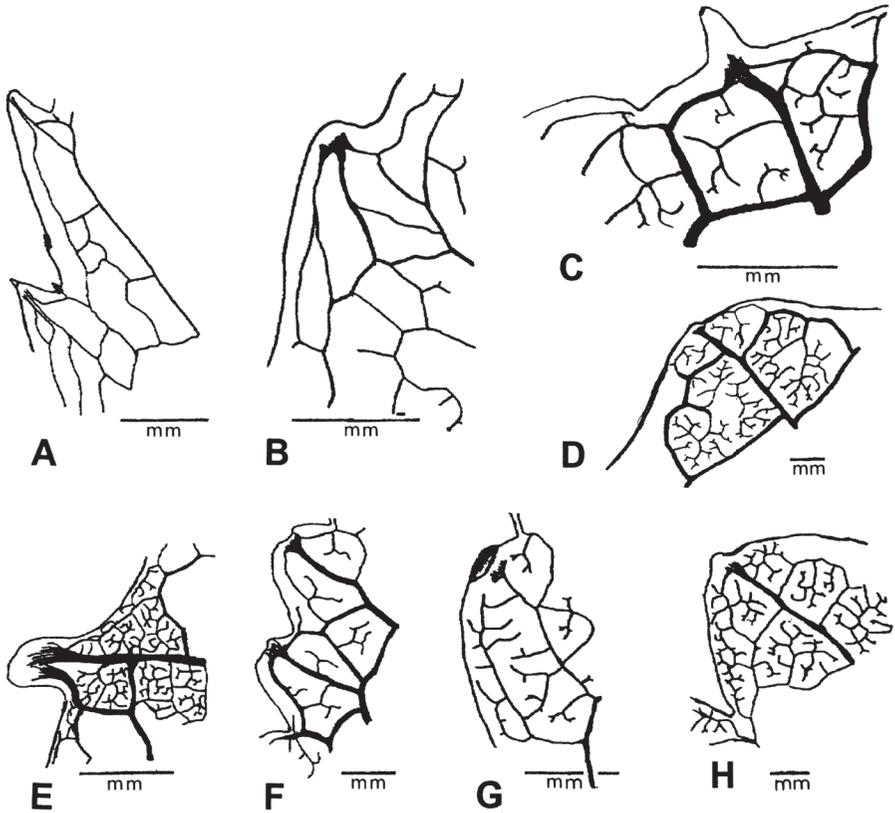


Fig. 10. Venation in selected putative outgroups. **A.** *Proserpinaca palustris* (Haloragaceae), marginal venation, showing connivent conjunctals and a papillate apex characteristic of the rosoid syndrome. **B.** *Lopezia trichota* (Onagraceae), a marginal tooth with connivent conjunctals and a deflected principal vein. **C.** *Vitis inconstans* (Vitaceae), showing a “vitioid” tooth, in which the principal vein is deflected and the conjunctals are two of different orders. The conjunctal veins are basically conniving, although one or a few vascular strands join the principal vein. **D.** *Hamamelis virginiana* (Hamamelidaceae), a marginal tooth showing low-order accessory venation with one joining conjunctal. **E.** *Platanus occidentalis* (Platanaceae), a marginal tooth, with connivent, rosoid venation. **F.** *Tetracentron sinense*, a marginal tooth with the chloranthoid syndrome. The teeth in *Trochodendron* are similar. **G.** *Cercidiphyllum japonicum* (Cercidiphyllaceae), a marginal tooth, with a large foramen. The principal vein splays; true conjunctal venation is lacking. Compare to *Hamamelis* (Fig. 10D). **H.** *Pachysandra procumbens*, a marginal tooth and the chloranthoid vein syndrome, with high-order accessories. Compare to *Hamamelis* (Fig. 10D).

Onagraceae show some possible affinities with Haloragaceae but none with *Gunnera*. The basic venation is pinnate and craspedodromous in narrowly ovate leaves. *Lopezia* has simple, linear, freely ending veinlets within well-developed, quadrangular areoles. Marginal teeth are of a modified rosoid type, characterized by convergent, splaying veins but in which the course of the principal vein is deflected by a thickened apical conjunctal (Fig. 10B). However, *Lopezia* does have epidermal cells with undulate walls and unicellular trichomes like those in *Gunnera*.

Vitaceae was examined through both *Vitis* (Fig. 10C) and *Ampelopsis*. The two genera share ovate leaves with acute apices, cordate bases, and basally originating agrophic veins (primaries

in *Vitis*) that give rise to a second pair of agrophics (for *Ampelopsis* see Hickey, 1977, Pls. 39.4, 40.2). Areoles are imperfect and irregular and contain simple, freely ending veinlets. Leaves are often alveolar. *Ampelopsis* has angular, glandular sinuses. Secondaries are craspedodromous; tertiaries, percurrent. Teeth have a characteristic venation that may be termed "vitoid" and appears to be chloranthoid derived (Fig. 10C). Alternate or subopposite conjunctals bifurcate just before joining the principal vein, which splays out into an epithemous glandular tooth apex. Admedial veins are of a higher order than is the principal vein of the tooth. There are two orders of accessory veins. Epidermal cells are hexagonal, straight walled, and subsodiametric. Leaves of the Vitaceae are most similar to the large-leaved *Gunnera*, a polarity that, if accepted, would imply the evolution of small *Gunnera* by reduction.

Pachysandra of the Buxaceae shows similarities to *G. herteri* and some species of the subgenus *Milligania*, with its ovate, crenate, decurrent-based leaf, its unicellular trichomes, and its teeth with alternately joining conjunctals (Fig. 11A). In addition, epidermal cells are isodiametric and straight walled. However, its principal veins originate suprabasally. In addition, incomplete areoles with numerous freely ending veinlets and spiked marginal venation clearly set the leaves of *Pachysandra* apart. However, marginal and tooth venation is similar to that of some Hamamelidaceae (Fig. 10D).

Aucuba and *Griselinia* were chosen as cornalean representatives because of their marginal teeth, although the cornaceous affinity of *Aucuba* is problematic (Eyde, 1988; Chase et al., 1993; Morgan & Soltis, 1993; Angiosperm Phylogeny Group, 2003). Its teeth are clearly rosoid with connivent, splaying conjunctals. The teeth in *Griselinia*, when present (most species in the genus are entire), are reduced to a simple, spinose principal vein with nonbracing, unplayed conjunctal veins. Such teeth have been suggested to be a derived rosoid type (Hickey & Wolfe, 1975). Leaves of both genera are pinnate, semicraspedodromous with incomplete areolation, and lack agrophic veins. Epidermal cells are isodiametric and straight walled.

The leaf architecture of the "basal Hamamelids" has been more extensively studied and illustrated than have many groups due to their paleobotanical interest (e.g., Hickey, 1977; Crane, 1989; Wolfe, 1989). In general, families in this "grade taxon" share some features with Gunneraceae, but most of these features are widespread and seem more likely to be symplesiomorphies. Leaves are often ovate, with round or truncate bases and sharply acute leaf apices (acuminate in *Trochodendron*).

The pinnate-brochidodromous venation of *Trochodendron* shows affinities with acrodromous venation in that its secondaries are concentrated near the leaf base. The acrodromous venation of *Tetracentron* consists of five primaries that originate from the petiole (Fig. 11B). This is notably different from *Gunnera*, in which the petiole gives rise to three basic primary veins, of which the lateral two provide the fourth and fifth primaries at the lamina base. The marginal teeth have chloranthoid venation (Fig. 10F), with oppositely joining conjunctals, but lack a gland (cf. Hickey & Wolfe, 1975). Unlike *Gunnera*, the sinus venation consists of the conjunctal and its branch. Areolation is imperfect or incomplete (*Tetracentron*), irregular in shape, and encloses once-branched, freely ending veinlets. *Trochodendron* has stellate hairs, whereas *Tetracentron* lacks hairs. The leaves of *Trochodendron* and *Tetracentron* contain stellate idioblasts. Epidermal cells are hexagonal and isodiametric with straight cell walls. Stomata, confined to the underside of the leaves, are laterocytic (Endress, 1993c), thus of a more derived form than in Gunneraceae.

Cercidiphyllum shows few affinities with *Gunnera*. Its ovate leaf, with a truncate-cordate base, acute apex, and crenate margin, has acrodromous venation. Secondaries are brochidodromous. Areolation is incomplete, with multiply branched veinlets. In modern leaves the common condition is for the tooth to have a large apical foramen beneath which "conjunctal" veins

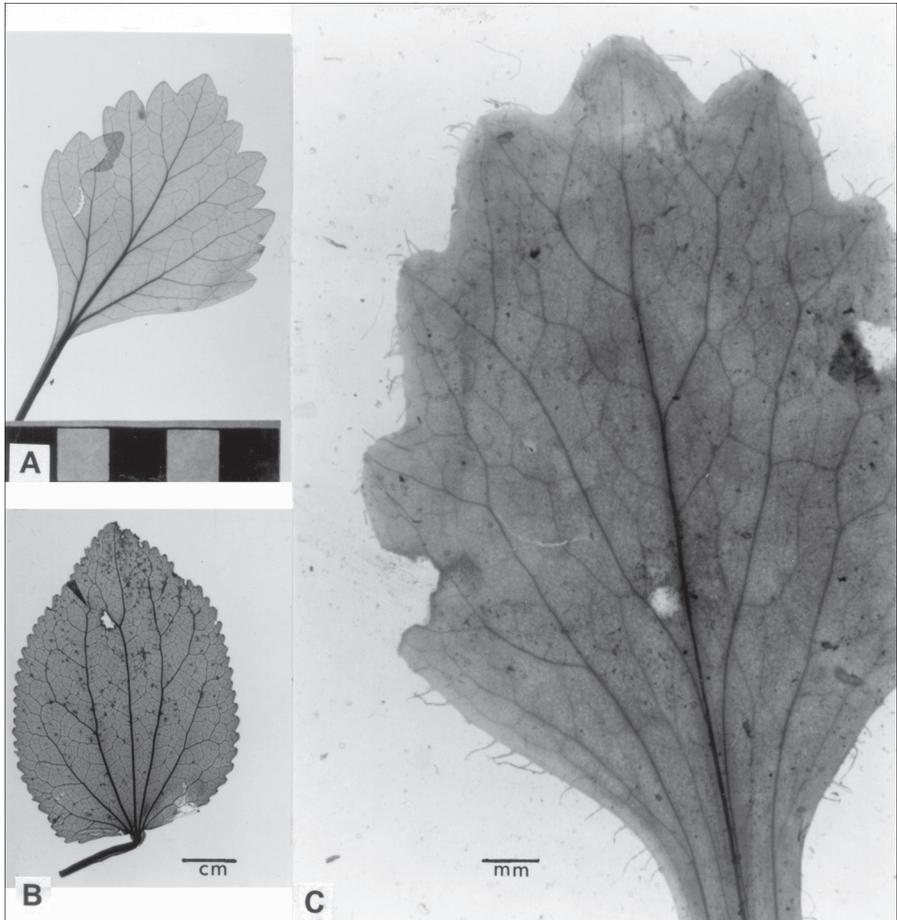


Fig. 11. Cleared leaves of putative outgroups. **A.** *Pachysandra procumbens* (Buxaceae), a cleared leaf, showing actinodromous-pinnate venation. **B.** *Tetracentron sinense* (Tetracentraceae), a cleared leaf, with basic acrodromous-brochidodromous venation. **C.** *Saxifraga* (*Micranthes*) *eriophora*, a cleared leaf. Other species in this subgenus and many *Chrysplenium* show the same basic venation pattern. Compare to *Gunnera herteri* and *G. hamiltonii* (Figs. 4F, 5B).

converge but do not connive with the principal vein (Fig. 10G). However, in long-shoot and sucker-shoot leaves of living *Cercidiphyllum*, as well as in many fossil leaves, oppositely joining conjunctals are present. Fossil evidence indicates that well-developed opposite conjunctals fusing with the principal are common in leaves that appear to be related to *Cercidiphyllum* (cf. Chandrasekharam, 1974) and that the modern condition of short-shoot leaves evolved after the Paleocene. Trichomes are lacking. Anomocytic stomata are found on the underside of leaves (Endress, 1993b), whereas the remaining epidermal cells are like those in *Tetracentron* and *Trochodendron*.

To encompass the full range of leaf variation in Hamamelidaceae, *Liquidambar*, *Disanthus*, and *Hamamelis* were examined, either of the latter two suggested to be at the primitive end of the

family (Cronquist, 1968; Schwarzwalder & Dilcher, 1991; Endress, 1993a). The venation of *Disanthus* consists of acrodromous primaries with brochidodromous secondaries, suggesting affinities with *Cercidiphyllum* and *Tetracentron*. Unfortunately, *Disanthus* lacks marginal teeth for comparison. *Hamamelis* has teeth with only a single, high-order conjunctal vein joining the splaying principal vein on its basal side (Fig. 10D), not unlike those in *Pachysandra*. *Hamamelis* has pinnate venation with strong basal secondaries (agrophic veins), which suggests an actinodromous ancestry (cf. Wolfe, 1989). Stomata are paracytic (Endress, 1993a), whereas epidermal cells are undulate-elongate. Areoles are incomplete, with twice-branched, freely ending veinlets.

Platanus shows general similarities with *Gunnera*, especially with subgenus *Panke*, in having actinodromous primaries and lobes that are homologous with teeth (akroteria). However, the primaries in *Platanus* originate suprabasally. Like some species of *Panke*, *Platanus* has craspedodromous secondaries, percurrent tertiaries, and an orthogonal reticulum of higher orders including imperfect, quadrangular areoles, although *Platanus* is of higher rank than even the most regular *Gunnera*. *Platanus* is also somewhat alveolar with three orders of adaxially impressed, abaxially prominent veins. However, it differs in the four- to six-vein orders of alveolarity in *Gunnera*. *Platanus* also differs in that its freely ending veinlets are twice branched. Epidermal cells are isodiametric and straight walled with five or six sides. The marginal teeth show affinities with the rosoid tooth because the conjunctal veins connive with the principal vein and splay out at the tooth apex without merging with the principal vein (Fig. 10E; cf. Wolfe, 1989). Some authors have indeed suggested a relationship between Platanaceae and the ancestors of the rosoid subclass (Hickey & Wolfe, 1975; Crane, 1989; Endress, 1993a); others derive *Platanus* within the Hamamelidaceae (Schwarzwalder & Dilcher, 1991) or near basal Hamamelids such as the Trochodendrales (Thorne, 1992; Chase et al., 1993; Endress, 1993a; Kubitzki, 1993a; Loconte, 1996). A hypothesis of a close relationship with *Gunnera* would imply that small, low-rank *Gunnera* groups were derived through reduction from larger, more regularly organized leaves.

A number of other suggested taxa can easily be removed from consideration. The long, ovate, entire, pinnately veined leaflets of Eucryphiaceae, with straight, freely ending veinlets and no marginal teeth, offer few features that compare to those of *Gunnera*. A similar situation holds for Connaraceae (cf. Dickison, 1973). The Urticales were discounted because of their third-rank, pinnate venation with percurrent tertiaries and their derived pollen.

The Berberidopsidales of recent genetic phylogenies (e.g., Soltis et al., 2000; Angiosperm Phylogeny Group, 2003) were considered on the basis of *Berberidopsis* and *Aextoxicon*, which are highly divergent from one another. *Berberidopsis* has ovate leaves, with pinnate, semicraspedodromous venation. The basal two pairs of secondaries represent agrophic veins that originate at narrowly acute angles, whereas secondaries farther along the midrib originate at a wide-acute angle. The single, multistranded petiole vein is flanked by two separately sheathed strands, indicating derivation from a three-veined petiole. *Berberidopsis* leaves are of high second rank, with reticulate-random tertiaries, imperfect areolation, and multiply branched, freely ending veinlets. Marginal crenations are nonglandular with tapering principal veins and alternately joining conjunctals. These features are compatible with a basal eudicot derivation and with Takhtajan's (1997) placement of them at the base of the Violales, or near the Dilleniales.

Aextoxicon, by contrast, has toothless, elliptical leaves with slightly emarginate (retuse) apices and strongly decurrent bases. Venation is pinnate and brochidodromous. Its leaves are highly ordered and of low fourth rank, with regularly orthogonal quaternary veins, well-developed areolation, and occasional freely ending veinlets. These characters are more in keeping with placement in Celastrales (Cronquist, 1981) and show no affinities with *Berberidopsis*, *Gunnera*, or other "basal Hamamelids" examined.

C. MYROTHAMNACEAE

Genetic analyses have recently suggested a relationship between *Gunnera* and Myrothamnaceae (Hoot et al., 1999; Soltis et al., 2000, 2003; Angiosperm Phylogeny Group, 2003; Hilu et al., 2003). This hypothesis was accepted without further consideration in the analyses of Wanntorp et al. (2001, 2002), and *Myrothamnus* genetic sequences only were used to polarize the character states in their *Gunnera* cladograms. Cleared leaves of *Myrothamnus moschatus* Baill., as well as published morphological and anatomical studies (Zavada & Dilcher, 1986; Endress, 1989; Carlquist, 1990; Kubitzki, 1993b), were examined to code equivalent characters for cladistic analysis and identify possible homologies.

Few characters show similarities between *Myrothamnus* and *Gunnera*. Although *Myrothamnus* has three petiole veins, in common with *G. herteri* and some species of the subgenus *Milligania* (Fig. 7A), this trait is likely to be a plesiomorphy of many early eudicots and early angiosperms, as indicated by its occurrence in the fossil record since the Barremian, 135 Ma (Taylor & Hickey, 1990; also Takhtajan, 1969: 51). The venation of *Myrothamnus* is of higher rank than that of *Gunnera* (low- to mid-second rank). Its areoles have simple, freely ending veinlets. The marginal teeth are rosoid with conniving, but not joining, conjunctals. The leaf tissue contains very fine, sandy, crystalline inclusions. As discussed above, the pollen of the Myrothamnaceae is triaperturate, has a clavate exine deriving from reduced columellae and a foot layer, and has no thickening near the apertures (Zavada & Dilcher, 1986).

D. SAXIFRAGACEAE

Saxifragaceae s.str., delimited as an essentially herbaceous family (Hideux & Ferguson 1976; Takhtajan, 1983; Thorne, 1992; Morgan & Soltis, 1993; Soltis et al., 1993; Soltis & Soltis, 1997), provides the most likely candidates for comparison to *Gunnera*. We thus made an attempt to examine some of the more basal taxa in this family for leaf architecture. A wide range of additional genera was examined as herbarium material to establish widespread leaf architectural characters, including probable symplesiomorphies. Initially, taxa were selected on the basis of pollen, assuming that the tricolpate condition was more basal, for reasons argued in the introduction, as well as for their low leaf rank, for which the fossil record also suggests directionality (Doyle & Hickey 1976; Hickey & Doyle, 1977). Tricolpate pollen with reticulate exines occurs in *Saxifraga*, section *Micranthes*, and in the genus *Chrysosplenium* (Ferguson & Webb, 1970; Heusser, 1971; Hideux & Ferguson, 1976; Gupta & Sharma, 1986). These taxa also have lower-rank leaves than have most of the family.

Astilbe, often considered to be basal within the family (Savile, 1975; Soltis et al., 1993; but not in the more recent analyses of Soltis et al., 2001a), has tricolporate, reticulate pollen (cf. Hideux & Ferguson, 1976) and higher-rank leaves (high second rank) than does *Gunnera*. *Astilbe* teeth differ from those of *Gunnera* in having alternately conniving conjunctals that splay at the tooth apex alongside the principal vein to produce a typical rosoid tooth. In some teeth a few vascular strands in the conjunctals join the principal vein, thus suggesting an evolutionary relationship (derivation) with chloranthoid teeth. In addition, *Astilbe* has a compound leaf with pinnately veined leaflets. Several recent genetic studies, which have elucidated well-supported clades within the Saxifragaceae, have failed to offer consistent resolution of basal relationships within the family (Soltis et al., 1993, 1996; Johnson & Soltis, 1994, 1995; Soltis et al., 2001a).

In some analyses *Chrysosplenium* is an early branch within the family or within the "heuchroid" clade of the family; *Saxifraga*, section *Micranthes*, also sometimes falls low within this group. The position of *Chrysosplenium* and a proposed sister relationship with *Peltoboykinia*

is more weakly supported in a comparative analysis of multiple gene data than are most of the clades in this family (Soltis et al., 2001a). Genetic data strongly indicate that *Micranthes* is distinct from the rest of *Saxifraga*, which implies that shared morphological features of the two groups are symplesiomorphies, perhaps of the entire family (Soltis et al., 1996). Within *Saxifraga* s.str., the section *Irregulares* and *S. mertensiana* are primitive (Soltis et al., 1993: fig. 1; 1996, 2001a; Johnson & Soltis 1995: fig. 3) and were thus chosen to represent basal leaf architecture for *Saxifraga* s.str.. Thus, cleared leaves of *Micranthes*, *Irregulares*, *S. mertensiana*, and *Chrysosplenium* were examined in detail. The large number of similar venation characters suggests that these taxa retain numerous leaf architectural plesiomorphies, many of which are also shared with Gunneraceae.

Members of the Saxifragaceae examined have ovate (to reniform) leaves that are palinactinodromous with three distinct veins entering the lamina base from the petiole (Fig. 11C). The primaries form a loose reticulum with the secondaries and tertiaries. The lateral primaries dichotomize as they near the margin, not unlike those of *Gunnera herteri*. Areoles are imperfect and irregular in shape, but usually radially elongated. They lack freely ending veinlets. Teeth are convex-convex, with an outline intermediate between crenate and serrate.

The venation of saxifragaceous teeth includes conjunctals, admedials, and a single order of reticulating accessories. The tooth apices have round tylate processes and principal veins with truncate to slightly bulbous terminations, similar to those in the subgenera *Ostenigunnera* and *Milligania*; marginal veins are looped. An unusual feature of these teeth is that the conjunctals can be formed from extensions or branches of subadjacent secondary veins. *Micranthes* has multicellular, uniseriate hairs along its margin, as do many *Saxifraga* spp. (Gornall, 1986). *Irregulares* and *S. mertensiana* both have multiseriate trichomes; *Chrysosplenium* lacks trichomes. *Chrysosplenium* and *Saxifraga* have chloranthoid teeth with oppositely converging and joining conjunctal veins (Fig. 7D–7F). Some *Chrysosplenium* species show a tendency toward conjunctals that splay before joining (Fig. 7F), which is typically the case in section *Micranthes* (Fig. 11C) and *Gunnera*. In the saxifragaceous taxa surveyed, the principal vein of some teeth is joined by two pairs of conjunctal veins. Epidermal cells in *Chrysosplenium* and *Micranthes* are elongate with undulate cell walls, like those found in *Gunnera*. Stomata are anomocytic (Moreau, 1984). This survey is sufficient only to indicate a strong general affinity of Saxifragaceae with Gunneraceae. A broader study of the leaf architecture of the Saxifragaceae is necessary in order to fully elucidate its affinities with *Gunnera*.

A conspicuous feature across several genera of Saxifragaceae is the tendency to develop three lobes or three leaf areas that can be understood as akroteria, as described in the previous section on the leaf architecture of *Gunnera*. These can be observed widely across representatives of many saxifragaceous genera, including *Saxifraga* s.str., *Sullivantia*, *Jepsonia*, *Heuchera*, *Peltiphyllum*, *Leptarrhena*, *Mitella*, and *Tolmiea*. This suggests that the basic ontogenetic pathway of these leaf features is shared across Saxifragaceae and Gunneraceae and that lobes have evolved multiple times by this pathway.

Two relictual species in southern South America are putatively basal within *Chrysosplenium* (Hara, 1957). Both have opposite leaves with a bifurcating axis, producing additional axes and inflorescences in the axils of leaves. In this habit they (and other *Chrysosplenium*) resemble *Gunnera herteri* (Fig. 1A). They are isophyllous; i.e., lacking sessile cauline leaves, which are common among most Saxifragaceae and Gunneraceae but are also lacking in *G. herteri* and *G. perpersa*. Another possible plesiomorphy of *Chrysosplenium* and *Gunnera* is the growth of axillary inflorescences that do not overtop the foliage. Hara (1957) considered this feature as peculiar to *C. micrantha*, but the same habit is found in *G. herteri* and *G. monoica*. Although recent genetic work has placed the South American *C. valdivicum* Hook as a derivative of a

terminal East Asian clade (Soltis et al., 2001b), morphological features point to a very ancient, relictual status for the other South American species, *C. micrantha*.

E. CLADISTIC ANALYSES

In order to streamline the cladistic analysis presented here, some of the 15 outgroup taxa were eliminated on the basis of their mean patristic distances from *Gunnera* (Table V). The nearest sister group of *Gunnera* was determined by searching among outgroup taxa with averaged patristic distances from *Gunnera* of under 0.6 when rooted by more distant taxa. These analyses included a single representative of each of the *Gunnera* subgenera. This approach allowed *Gunnera* to be rooted in any of its subgenera and also adduced cladistic support for the closest sister group. Because the polarization of characters is crucial, analyses were performed using both basal hamamelid and rosoid outgroups. The most parsimonious trees were then explored by including other taxa and minimizing the additional tree length. In response to the recent hypothesis of a sister relationship with *Myrothamnus*, these analyses were run again with the addition of *Myrothamnus*.

Using a basal Hamamelid outgroup, represented by *Hamamelis*, *Tetracentron*, and *Cercidiphyllum*, eight equally parsimonious trees were found in a branch-and-bound search. Their consistency indices ranged from 0.593 to 0.62. The tree with the highest consistency index is reproduced as Figure 12, which also shows the frequency of occurrence of each branch among all eight trees. In these, *Gunnera* forms a strong clade with the Saxifragaceae. When two trees in which *Gunnera* is not monophyletic and which have the lowest consistency indices are disregarded, all other trees support this Gunneraceae-Saxifragaceae clade. *Gunnera herteri* is the basal taxon in the *Gunnera* clade, with the rest of *Gunnera* (corresponding to the subgenus *Gunnera* sensu Meijden & Caspers 1971) forming a well-supported clade.

Among the outgroups, *Pachysandra* is the next-closest clade, followed by *Platanus*, although the placement of *Pachysandra* is the most variable aspect of these trees. With the addition of *Myrothamnus*, the Saxifragaceae-Gunneraceae clade remains strongly supported in the most parsimonious tree, and *Myrothamnus* groups with Hamamelidaceae. However, the varied polarization of characters in this set of trees made the ingroup topology of Gunneraceae differ among them, and Saxifragaceae became paraphyletic (Fig. 13).

The Saxifragaceae-Gunneraceae clade was also supported in analyses using rosoid outgroups. This search included several traditional sister groups, as well as *Pachysandra*. *Platanus* was defined as the outgroup, because it was the closest taxon to the Saxifragaceae-Gunneraceae clade in the first analysis. In the initial branch-and-bound search, a monophyletic saxifragaceous clade was the sister group to *Gunnera* supported by 16 apomorphies. *Pachysandra* formed the next branch. When *Griselinia* and *Aucuba* were added, the shortest tree separated the *Pachysandra*-Saxifragaceae-Gunneraceae clade from a rosoid clade including well-supported Cornaceae and Vitaceae clades (Fig. 14). With the addition of *Myrothamnus*, all of four equally parsimonious trees in branch-and-bound search grouped Saxifragaceae and *Gunnera* with *Pachysandra* as the next branch, whereas *Myrothamnus* was distant. All of the above analyses strongly support Saxifragaceae as the nearest sister group to a monophyletic Gunneraceae, whereas the Cornaceae, Haloragaceae, and Vitaceae are all very distant and form a separate rosoid group.

The cladistic relationship between Saxifragaceae and Gunneraceae is supported by a consistent set of apomorphies from the above analyses as well as by other shared traits between the two groups. The most striking difference between Gunneraceae and Saxifragaceae is in floral morphology. Whereas the latter usually has perfect, pentamerous flowers, *Gunnera* flowers have two or four parts and are all highly simplified. However, Saxifragaceae share with *Gunnera* the presence of two styles. In *Chrysosplenium* these are extremely short and arise from connate

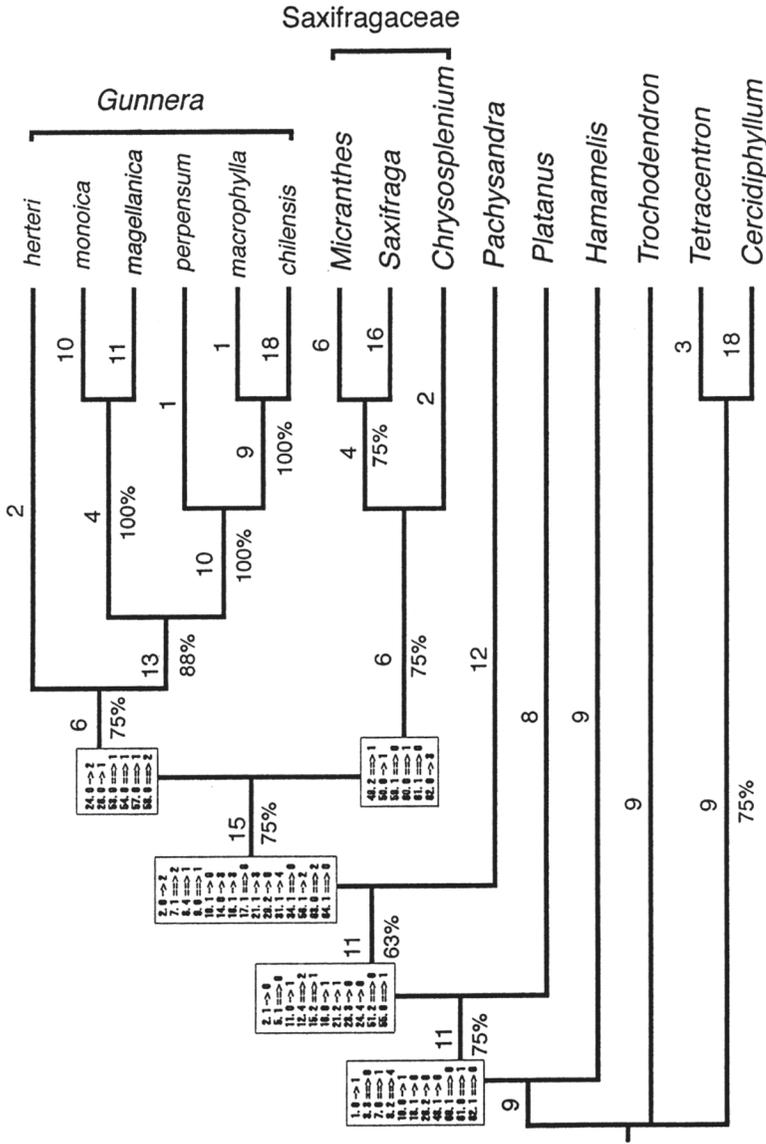


Fig. 12. Cladogram showing *Gunnera* as the sister group of Saxifragaceae, rooted by basal hamamelids. One of eight equally parsimonious trees having the highest consistency index (0.62). Branch lengths are shown above branches. Numbers below branches represent frequency occurrence of the branch out of all eight trees. Character-state changes for major branches are given in boxes. Unambiguous changes/apomorphies are indicated by \Rightarrow ; \Rightarrow indicates that changes may have undergone reversal within the crown group or may have arisen in parallel within the crown group.

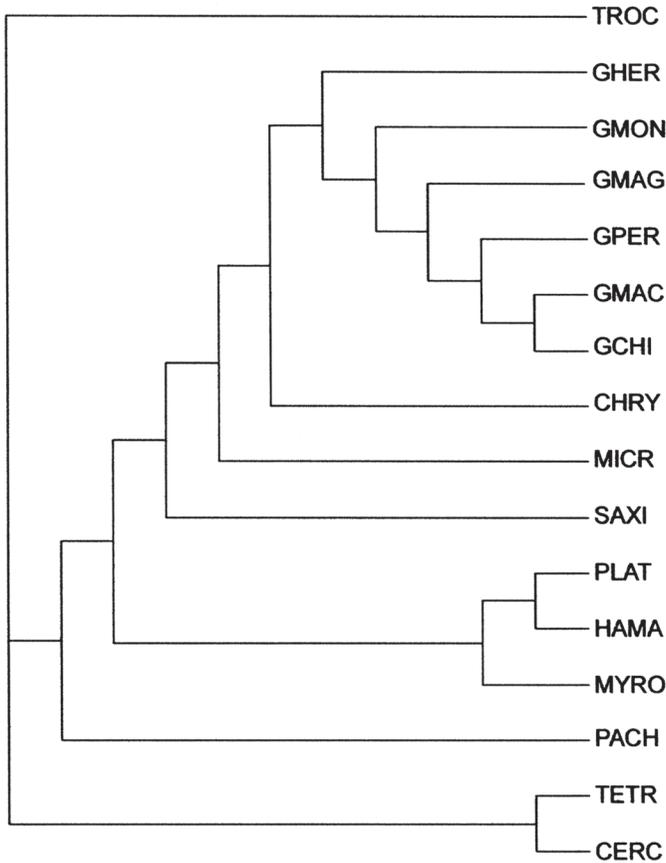


Fig. 13. The most parsimonious tree produced by adding *Myrothamnus* (MYRO) to a cladistic analysis of Gunneraceae, Saxifragaceae, and basal hamamelids. Note that in this analysis Saxifragaceae becomes paraphyletic.

locules. In *G. herteri* the styles are also extremely small. However, in *Gunnera* they arise from a single, uniovulate locule. The two styles suggest the evolution of the *Gunnera* flower partially through reduction, as does the unitegmic ovule. As with about half the genera of Saxifragaceae, including *Chrysosplenium* (Savile 1975; Soltis et al., 1993), *Gunnera* has parietal placentation. Savile (1975) has pointed out that flowers within the Saxifragaceae are often morphologically specialized for particular dispersal mechanisms.

Gunnera inflorescences would seem to be specialized for wind pollination and the dispersal of visibly exposed, red drupes. *Gunnera* flowers are reduced to essentially two parts, although they sometimes have four. The tetramerous, apetalous flowers of *Chrysosplenium* are likely to be plesiomorphic, as opposed to the perfect pentamerous flowers of the rest of the family. This is in line with recent recognition that tetramerous or dimerous flowers are basic to the basal eudicots (Soltis et al., 2003; Zanis et al., 2003). As suggested for Proteaceae, Buxaceae, and Papaveraceae (Soltis et al., 2003: 467), two decussately opposite dimerous whorls form a superficially tetramerous flower. This process of doubling merosity through the merging of

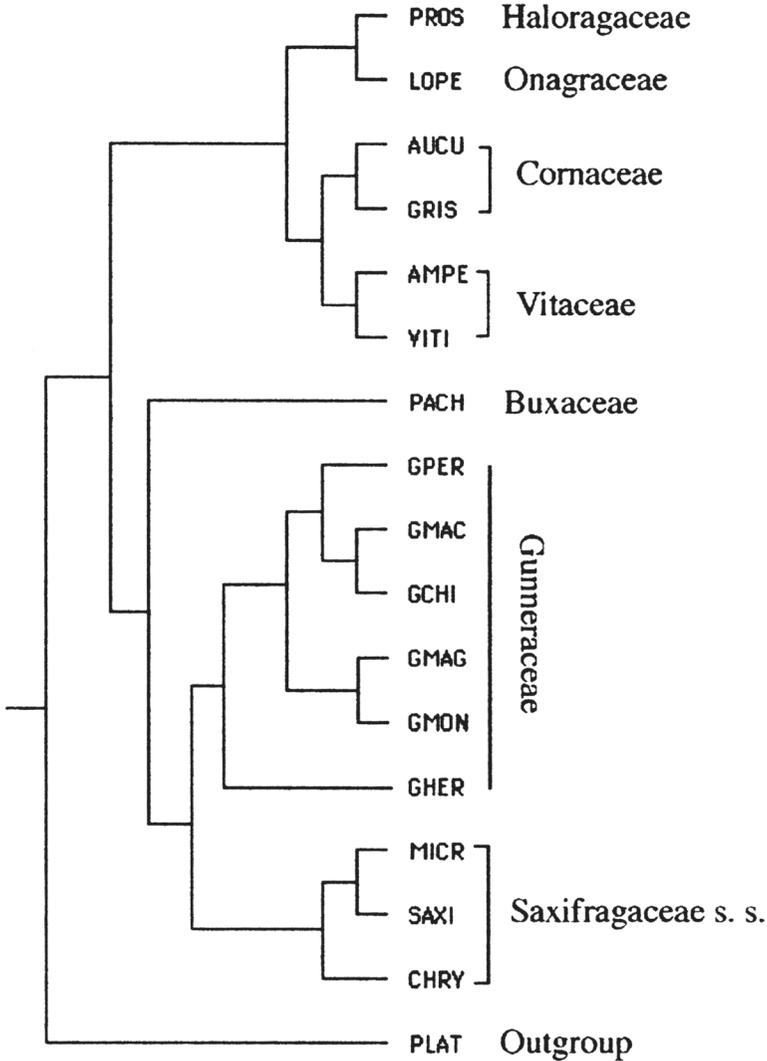


Fig. 14. Representative parsimonious tree using rosid outgroups. A branch-and-bound search, conducted using Vitaceae, Haloragaceae, and Onagraceae, yielded a single most parsimonious tree. Using MacClade, this tree was explored by selectively adding rosid taxa and minimizing tree length increase. In all cases, the Gunneraceae-Saxifragaceae clade remains strongly supported. This tree is one of two equally parsimonious trees of length 293 with a consistency index of 0.60 and a retention index of 0.58. In the other tree the Haloragaceae-Onagraceae clade emerges below *Pachysandra*.

sequential whorls can be understood through the anthion concept developed to describe the homologies of early angiosperm inflorescence evolution (Hickey & Taylor, 1996); indeed, the earliest fossil flowers are essentially dimerous, consisting of spikes of decussately opposite, single ovules subtended by bracts (Taylor & Hickey, 1990, 1992).

F. PHYLOGENETIC ANALYSIS WITHIN THE GUNNERACEAE

In order to examine systematic relationships below the genus level, 18 *Gunnera* species were analyzed, with Saxifragaceae as a monophyletic outgroup, through a branch-and-bound search (Fig. 15). Four equally parsimonious trees resulted, with CI = 0.694, RI = 0.834, and a length of 164. The only differences between the trees were in the topology within Saxifragaceae and the placement of *G. brephogea* within the *Panke* lineage, either at the base or in a position between a pedate-repand lineage (*G. pilosa* and *G. talamancana*) and larger, orbicular-leafed lineage. Rerunning our data matrix with *Myrothamnus* as outgroup to all *Gunnera* species yielded 12 equally parsimonious trees, including four with higher-consistency indices that are identical to our analysis in subgenus placement. The remainder of this discussion refers to our analysis that places Saxifragaceae as the outgroup, because this provides a more coherent polarization of characters for considering morphological evolution.

Although traditional taxonomy places the subgenus *Milligania* at the base of *Gunnera* (cf. Schindler 1905; Meijden & Caspers, 1971), all of the above analyses support *G. herteri* as the most basal extant species, as suggested also by genetic research (Wanntorp et al., 2001). All other species share a monophyletic ancestry, characterized by 13 apomorphies, such as unicellular trichomes, straight “thichotomous” midveins, the presence of agrophic veins, orthogonal tertiaries, pollen more than 25 μm , and the presence of ligules (lost in subgenus *Perpensum*).

Above the node of *G. herteri*, the genus can be divided into two lineages, one with unisexual flowers (the “*Prorepens*” clade in Fig. 16), the other often hermaphroditic with enlarged leaves (the “megaphyll” clade in Fig. 16). The grouping of the subgenera *Misandra* and *Milligania* has also been suggested by Wilkinson (2000) on the basis of an anatomical review. Nevertheless, *Milligania* shows some distinct apomorphies, such as glandular sinuses, in leaf-venation patterns. *Milligania* appears to have additional phytochemical apomorphies, as suggested by the fact that only leaves from this subgenus of *Gunnera* proved difficult to clear (see “Materials and Methods” above). By contrast, the phylogeny proposed by Wanntorp et al. (2001, 2002) raises problems. When we forced the cladogram into the subgeneric relationships of *Milligania-Pseudo-Gunnera* and *Misandra-Panke*, tree length was 180, 16 steps less parsimonious than the scheme proposed here. In addition to numerous homoplasies in leaf architecture, this topology requires two convergent origins of unisexual/dioecious flowers (Fig. 17).

Milligania is further divisible into a pinnately veined lineage (*G. dentata*, *G. prorepens*, *G. hamiltonii*) and an actinodromously veined lineage (*G. monoica*, *G. strigosa*, and probably *G. cordifolia*). The two groups within *Milligania* were also recognized by Schindler (1905) in his diagnosis on the basis of fruit shape; clavate fruits are restricted to the actinodromous species.

Gunnera macrophylla (subgenus *Pseudo-Gunnera*) is the sister group of the subgenus *Panke*, sharing with it alveolarity, colleters, orthogonal fifth-order veins, and quadrangular areoles but differing in the course of its primary venation, marginal akroteria, and apparently independent development of the stoloniferous habit. Stolons in the subgenera *Milligania* and *Misandra* are axillary and presumably derived from axes and have adventitious root primordia below their apices (Wanntorp et al., 2004). The lack of root primordia (Wanntorp et al., 2004) in *G. macrophylla* may represent a distinctive evolutionary character. Whereas the stolons in

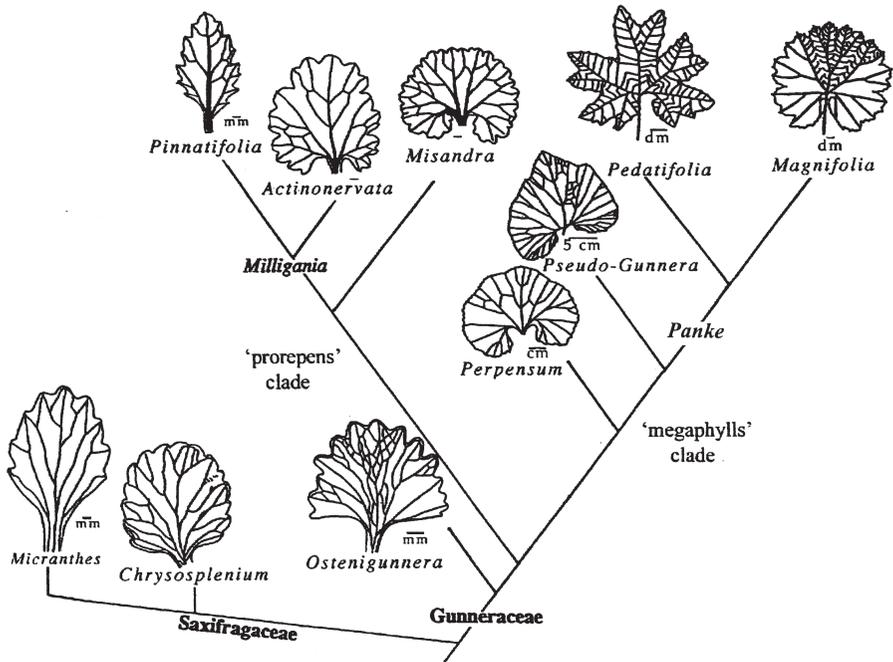


Fig. 16. Phylogram representing the evolution of leaf architecture in Gunneraceae.

Milligania and *Misandra* are subtended by foliose leaves, those in *Pseudo-Gunnera* are not and appear to substitute for leaves (authors' observation). In this regard, Meijden and Caspers (1971) made the intriguing observation that adventitious roots are produced on the undersides of *G. macrophylla* leaves. Thus the stolons in *Pseudo-Gunnera* may be derived from leaves!

The subgenus *Panke* can tentatively be divided into two main groupings, based on whether their leaves are orbicular or pedate. However, resolution of evolutionary relationships within *Panke* is likely to be complicated by reticulate evolution because hybridization between these leaf groups appears to be frequent (Palkovic, 1978; Doyle, 1990; Pacheco et al., 1991). In the pedate species, the margin is sinuous (repand) and has incipient or poorly developed teeth, whereas the orbicular group has leaves with four or five orders of teeth. The groups may also be distinguished by the presence of deciduous sepals in the orbicular assemblage, as opposed to persistent sepals in the pedate species (cf. Schindler, 1905). *Gunnera mexicana*, *G. killipania*, and *G. insignis* have round, glandular colleters only and lack pseudo-colleters, which supports the suggested closeness of these species (Palkovic, 1974). *Gunnera manicata* and *G. chilensis*, a close paraphyletic pair in this analysis, both have thick, succulent infructescence axes (Schindler, 1905) and striate cuticles (Wilkinson, 1998, 2000). More refined systematic studies of the largest-leaved, orbicular species of *Panke* are surely required, but it is likely that hybridization and reticulate evolution will make conventional parsimony analysis ineffective.

Our cladistic analysis supports aspects of previous taxonomic treatments of *Gunnera* while providing a firm basis for an improved infrageneric taxonomy (Table II). All five subgenera of Schindler (1905) and the sixth proposed by Mattfeld (1933) are monophyletic. The division of Meijden and Caspers (Meijden & Caspers, 1971; Meijden, 1975), in which *G. herteri* is distinguished from the rest of the genus as its own subgenus, is clearly supported, because *G. herteri*

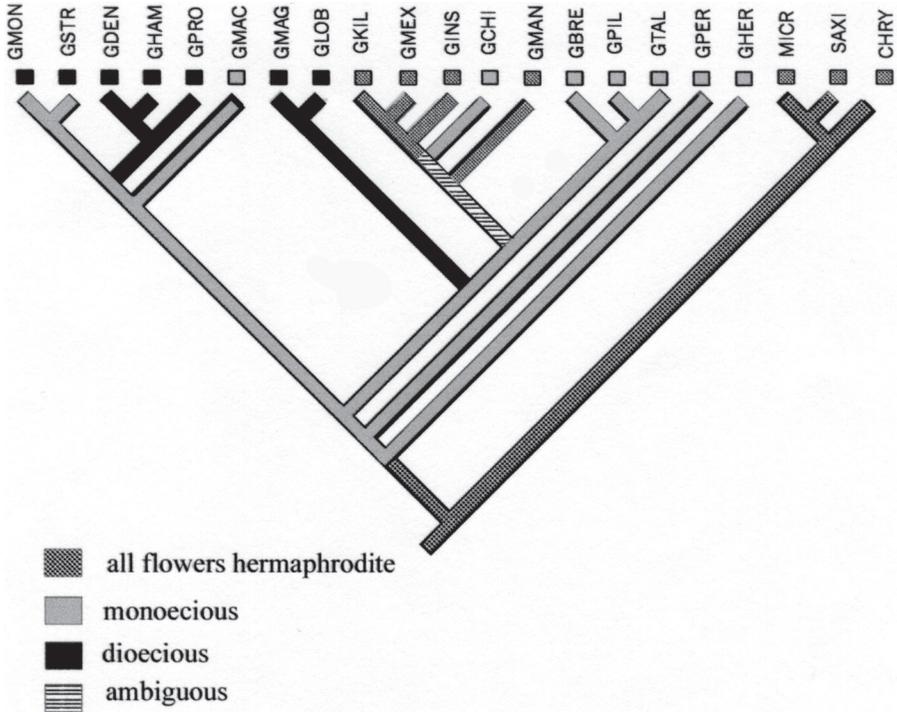


Fig. 17. Ingroup topology of Wanntorp et al., which is 16 steps less parsimonious than the topology supported by the present study. Superimposed on this cladogram are the states for character 61, flowering habit. This is one of several characters that are forced into homoplasy when this topology is used.

represents a distinct lineage sharing an ancestry with a lineage containing all other extant species (also supported by the genetic studies of Wanntorp et al., 2001, 2002). However, the sectional divisions within the subgenus *Gunnera* of Meijden and Caspers are problematic, because their section *Gunnera* is clearly polyphyletic. The combination of *G. perpensia* and *G. macrophylla* by Maclaughy (1917), though a paraphyletic grouping, was perceptive of their close affinity and shared ancestry, particularly evident from leaf architecture. As regarded by previous taxonomists, the subgenus *Panke* is a monophyletic clade with many apomorphies in leaf architecture, growth habit, and inflorescences.

V. Discussion

A. EVOLUTIONARY TRENDS

Gunnera appears to have undergone a large amount of morphological evolution in its leaves and growth habit, while its fruit and flower morphology have remained largely conservative. The basal position of *G. herteri* is supported by its incipient polystely, with three steles in the stems reported by Mattfeld (1933) and four steles in young stems becoming a complete ring in mature specimens examined by Wilkinson (2000). Other small-leaved species are mildly polystelic, as are those of the subgenera *Milligania* and *Misandra* (Batham, 1943; Wilkinson, 1998, 2000). Larger species have many steles; e.g., 59 in *G. perpensia* and hundreds in species

of the subgenus *Panke* (Batham, 1943; Wilkinson 1998, 2000). Our analyses suggest that ancestral *Gunnera* was a small, sympodially branching, cauline herb, producing axes in the axils of its leaves, lacking stolons, and with isophyllous, opposite leaves. Leaves were low rank, were ovate with a rounded and crenate apex, had a decurrent base with three distinct veins in the petiole, and had reticulate, palinactinodromous primary venation. They had radially elongated, irregular, shared areoles lacking freely ending veinlets. Leaf tissue probably contained druses. Many of these characters may be plesiomorphies shared with the ancestor of herbaceous Saxifragaceae (Figs. 15, 16, 18).

Gunnera evolved by the acquisition of a number of important apomorphies, such as the development of a physiologically complex intracellular symbiosis with *Nostoc* cyanobacteria (cf. Bergman et al., 1992) and the production of paniculate, anemophilous inflorescences with unisexual male flowers apically, and female or hermaphroditic flowers basally. The primitively microreticulate, tricolpate pollen of basal Saxifragaceae developed the bulging mesocolpia distinctive of *Gunnera* (cf. Praglowski, 1970; Jarzen, 1980).

From this stock the subgenus *Gunnera* evolved unicellular trichomes, reniform leaves with bases becoming lobate, a "trichotomous" midvein, moderate-sized primaries, reticulate orthogonal tertiaries, teeth with admedial veins of a lower order than the principal vein, and secondaries originating at increasingly obtuse angles apically. The descendent lineage is also united in having larger pollen. This clade diverged into two lineages: the *Prorepens* clade, which remained of small stature and tended to become dioecious, and the "Megaphyll" clade, which tended to increase its leaf size and rank (Figs. 16, 18), as well as the frequency of hermaphroditic flowers and the number of steles within its stem. This clade shows a trend toward increasing leaf rank, from low second rank in the *Prorepens* clade, toward higher second rank in the members of the basal megaphyll clade, and finally to third-rank leaves in the subgenus *Panke* (Figs. 16, 18). This coherent increase in leaf rank differs from that implied in the phylogenies of Wanntorp et al. (2002). Within the small-leaved *Prorepens* clade, the subgenus *Misandra* developed glandular sinuses and well-developed areolation. The subgenus *Milligania* evolved narrower, ovate, actinodromously veined leaves, with the conjunctal veins of the teeth often joining the principal vein alternately. The split between *Milligania* and *Misandra* is also supported by aspects of the *Nostoc* symbiosis. In *Gunnera monoica* of subgenus *Milligania*, mature *Nostoc* colonies appear to be highly productive (Stock & Silvester, 1994). This is not the case in *G. magellanica* of subgenus *Misandra*, in which the more basal and mature colonies of cyanobionts show less nitrogenase activity (Söderbäck et al., 1990). This is mirrored by evidence that *Milligania* seems to control its symbiont to such a degree that the cyanobionts lose the ability to synthesize the full range of photosynthetic proteins (Silvester, 1976), whereas in *G. magellanica* there has been no such loss (Söderbäck & Bergman, 1992).

The Megaphyll clade evolved toward larger, thicker leaves that culminated in the Latin American radiation of *Panke* (Fig. 15, 16). This clade is characterized by the modification of the lobate leaf base, probably as an expansion of the leaf as basal lateral primaries grew from the petiole at angles increasingly obtuse to the midvein. This seems to have been accomplished by a more basal branching from the petiole vein and an expansion of the foliar part of the leaf, in which the primary veins that form the margin (without laminar tissue) are derived evolutionarily from the petiole. Marginal teeth lost the dark-staining apical process, although hints of a clear glandular, tylate process remain in *G. perpersa*. Tooth venation developed a second order of reticulate accessories. From this inferred ancestor there evolved an even larger-leaved clade, in which alveolarity and colleters, as well as quadrangular areoles and an orthogonal reticulum of quinary veins, developed. The alveolarity is caused by the thickening of successive vein orders, probably for structural reasons related to larger leaves. Druses were again lost. This trend toward leaf expansion produced the massive leaves, as well as the fleshy stems (pachycauls), of *Panke*.

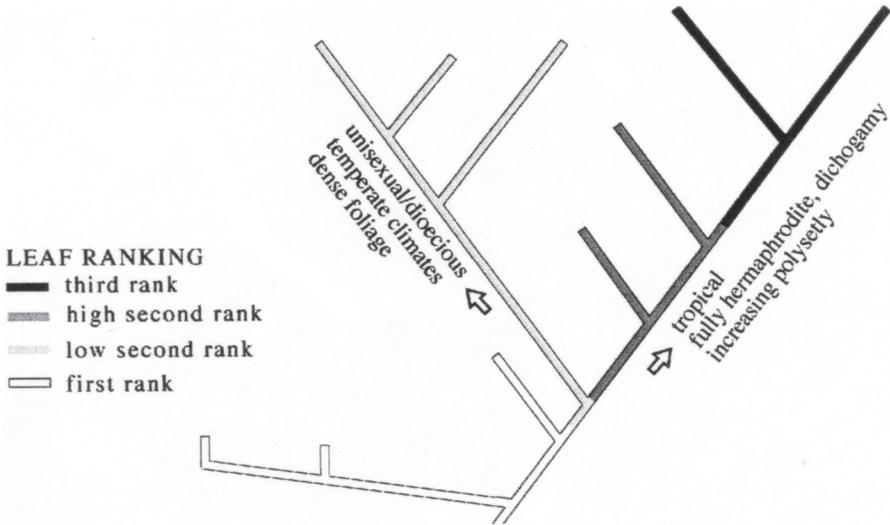


Fig. 18. Same phylogram as Fig. 16, with indication of directions of evolution in leaf ranking, flowering habit, and climatic zone. Leaf rank can be seen to stratify in this phylogeny, as it does generally in the fossil record for angiosperms.

Of particular interest is the evolution of the primary venation pattern and the akroteria in *Gunnera*. The midvein patterns of more-derived lineages probably evolved through midvein bifurcation and the promotion of secondary veins to the status of primaries. Trichotomies in the subgenus *Milligania* and in *G. lobata* are actually a pair of opposite secondaries arising from the midvein at moderately acute angles. In larger leaves (e.g., *G. perpensa*, *G. macrophylla*), these secondary veins were promoted to primary thickness, perhaps for structural reasons. These trichotomous midribs actually represent two quick bifurcations in immediate proximity. A similar near-trichotomy is found in young *Panke* leaves, which suggests evolutionary recapitulation (Fig. 6A–6C). The lowermost of these branches then gets promoted to the primary order and subsequently outgrows the more apical secondary branch, producing the bifurcation seen in mature *Panke* leaves.

The large pedate lobes of some species of the subgenus *Panke* may have allowed for great expansion in their size, providing greater photosynthetic area and the ability to overshadow smaller, weedy competitors. Size increase was accomplished by expanding the akroteria—i.e., marginal teeth at the ends of primary veins—followed by the formation of secondary, and then higher-order, teeth. The orbicular-leafed clade of *Panke* may be the result of the secondary venation system expanding between primaries through intercalary growth to fill out the increased space between marginal primaries as the leaf expanded. This is suggested by the increased number of interangular veins (i.e., secondary veins that joined and fused) in these species. This mode of marginal growth produced relatively shallow lobes and more distinct, higher-order teeth.

B. IMPLICATIONS FOR HISTORICAL BIOGEOGRAPHY

Our phylogenetic analysis suggests possible stages in the biogeographical history of *Gunnera* (Fig. 19). Mora-Osejo (1984) accounted for the Gondwana distribution of *Gunnera* by

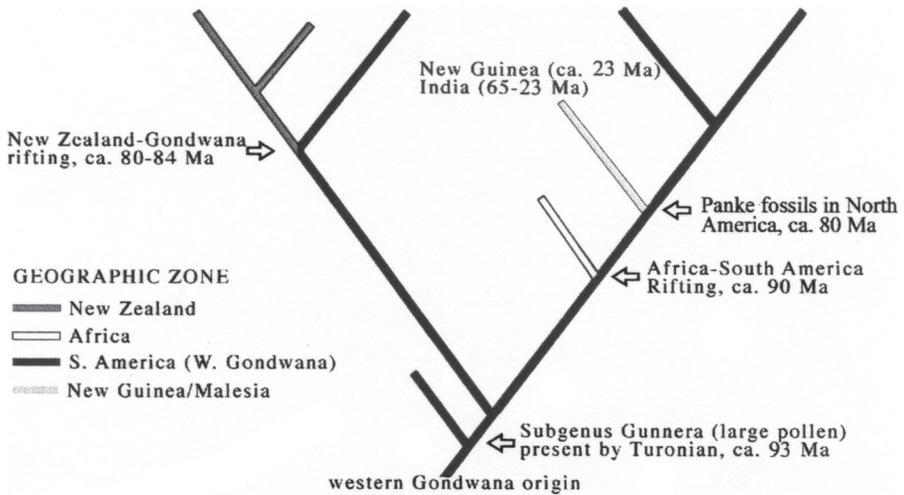


Fig. 19. *Gunnera* phylogeny indicating probable minimum ages for branches on the basis of inferences from vicariance biogeography. Typology is the same as for the Gunneraceae branch of Fig. 16.

suggesting a radiation out of Antarctica. Our phylogeny harmonizes with biogeographical patterns and the sequence of continental drift separation in both Australasia and South America. On the other hand, Wanntorp and Wanntorp (2003) produce a less parsimonious vicariance pattern by proposing a mixture of vicariance and long-distance dispersal. Two major nodes in their scheme are not accounted for by commonly accepted scenarios for continental vicariance. In the case of *G. macrophylla*, they propose long-distance dispersal from New Zealand to New Guinea and Malaya and a parallel dispersal of small-leaved *G. cordifolia* from New Zealand to Australia (Tasmania). In the case of South American clades, they propose dispersal from South America to North America and then a return dispersal of *Panke*.

The earliest confirmed fossil record of *Gunnera* comes from pollen found in rocks of Turonian Age (Late Cretaceous) in Peru, ca, 93 Ma (ages from Geological Society of America, 1996). This supports an origin for the genus in West Gondwanaland (Brenner, 1968; Jarzen, 1980; Jarzen & Dettmann, 1989). The Peruvian pollen falls into the size range of all *Gunnera* species except those of the subgenus *Ostenigunnera* (Belsky et al., 1965). This suggests that it derives from the core *Gunnera* clade above the node of *Ostenigunnera*, placing a minimum age on that node.

By the Campanian Age of the Late Cretaceous, post-*Ostenigunnera*-grade pollen occurs in Antarctica, New Zealand, Australia, and Africa. The node between the subgenus *Milligania* (in New Zealand and Tasmania) and the subgenus *Misandra* (centered in Tierra del Fuego) is datable by the separation of New Zealand from western Gondwanaland in the early Campanian, 80–84 Ma (Tulloch & Kimbrough, 1989; Laird, 1993). Australia also began to move away from Antarctica at about this time, but probably after New Zealand (Smith et al., 1994; Swenson et al., 2001). Campanian *Gunnera* pollen from Australia shows exine sculpturing similar to that of *Milligania* (Jarzen & Dettmann, 1989; Wanntorp et al., 2004), and it may be that the lineage of this group was by this time distinct from its Tierra del Fuego counterpart, which differs in exine sculpturing. This hypothesis would place *G. cordifolia* near the base of *Milligania* just above the *Prorepens* node. As noted above, several general features of leaf morphology

shared with *Ostenigunnera* suggest that this species is quite primitive. Indeed, Schindler (1905) separated *G. cordifolia* from other species of *Milligania* in his diagnosis. However, its clavate fruit form and sometimes monoecious flowering habit suggest its placement near *G. monoica* and *G. strigosa*. *Gunnera* pollen is absent from Australia after the Paleocene and does not reoccur there until the Pliocene (Jarzen & Dettmann, 1989). Thus it is possible that *G. cordifolia* represents a Neogene redispersal event from New Zealand.

The subgenus *Perpensum* became distinct in the Campanian at more or less the same time as *Milligania*. South America and Africa were clearly separated by 90 Ma (Turonian), when Tethyan marine taxa reached the South Atlantic (Raven & Axelrod, 1974; Pitman et al., 1993; but for an estimate of 105 Ma see McLoughlin, 2001). This is when the ancestors of *Gunnera perpensa* would have been isolated. The post-*G. perpensa* Megaphyll clade would then have evolved in South America–Antarctica. Fossil pollen from the Late Cretaceous of Antarctica shows exine sculpturing similar to that of *G. macrophylla* (Jarzen & Dettmann, 1989; Wanntorp et al., 2004). This pollen disappears from the Antarctic record in the earliest Tertiary, probably because of climatic cooling, which allowed only temperate species to spread between South America and Australia during the early Tertiary (Raven & Axelrod, 1975). This places the latest possible period for cladogenesis between the subgenera *Pseudo-Gunnera* and *Panke* at the end of the Cretaceous, although additional evidence suggests an earlier divergence.

Recently discovered megafossils of highly alveolar, palinactinodromous leaves from the Campanian–Maastrichtian of Wyoming and Montana show affinities with *Panke* (Fuller & Hickey, unpubl., cited in Wilkinson, 1998). Its earliest occurrence is in the Judith River Formation, dating to 80 Ma (Hicks, 1993; Hicks et al., 1995). This is consistent with evidence from the pollen record (Jarzen, 1980; Jarzen & Dettmann, 1989) and implies that the separation of *Panke* and *Pseudo-Gunnera* had already occurred on the South America–Antarctica landmass.

Although North and South America were separate, a selective dispersal corridor via islands was provided by the Caribbean plate (Raven & Axelrod, 1974, 1975; Pitman et al., 1993; Smith et al., 1994). The modern occurrence of *Panke* in Hawaii and the Juan Fernández Islands indicates that this subgenus is prone to bird dispersal. *Gunnera macrophylla* is similarly found on oceanic islands today, including the young, volcanic Vanuatu archipelago. It probably reached its current Malaysian–New Guinean range by long-distance dispersal across the Indian Ocean via islands. *Gunnera macrophylla*-like pollen did not reach New Guinea until the Neogene, ca. 23 Ma (Jarzen & Dettmann, 1989). This was a time of global cooling that drove many austral tropical species northward (Raven & Axelrod 1972, 1974, 1975; Mercer, 1983). This pollen type was present, however, on the Indian subcontinent as it rafted northward during the early Tertiary (Jarzen & Dettmann, 1989).

C. ECOLOGICAL TRENDS

Given the great age of *Gunnera*, its apparent ecological conservatism is significant. Before the evolution of mutualism with *Nostoc*, the ancestors of *Gunnera* probably grew in a habitat much like that of its modern sister groups. Webb and Gornall (1989) suggested that the primitive *Saxifraga* inhabited damp areas on the margin of temperate forests. *Chrysosplenium* can be found in such habitats today and is particularly associated with damp, shady areas and stream banks within forests, especially gymnosperm-dominated forests (Hara, 1957; Ohwi, 1965; Yuzepchuk, 1971; Savile, 1975; Hickman, 1993). From the stream- or pond-edge of mid–Late Cretaceous gymnosperm-dominated forests, the ancestors of *Gunnera* may have spread to more open areas while still requiring damp conditions.

With the establishment of the *Nostoc* symbiosis, *Gunnera* was able to invade poor, sandy soils, such as the waterlogged, sandy-paludal habitat where *G. herteri* grows today (cf. Osten, 1932).

This ability to thrive in poor soils may have been aided by an additional symbiosis with mycorrhizae, although this has been reported only from the highly derived *G. petaloidea* (Koske et al., 1992). Some species radiated onto permanently damp sand dunes (e.g., as in *Misandra*, *Ostenigunnera*, and some species of *Milligania*), while others remained on stream banks (*Perpensum* and some species of *Milligania* and *Misandra*). Once the constraint of soil nutrients was overcome through mutualism, the clade appears to have evolved toward larger size in plants and leaves, which could have been advantageous in overtopping competition. The availability of sunlight in open, disturbed areas, as along waterways, may have aided this directionality.

Movement toward tropical zones would have made available more areas with moisture levels conducive to the radiation of subgenus *Gunnera*, especially the *Pseudo-Gunnera-Panke* clade. Although increasing leaf size would have been selected for in warmer climes, smaller, more densely foliate forms were probably favored in the colder, austral zones. High transpiration rates may have restricted near-equatorial populations to higher altitudes (Bader, 1961; Jarzen, 1980) where areas of landslides as well as cliffs provided islands of open habitat for colonization. Increasingly sturdy and erect stems that produced leaves only near the apex (i.e., the pachycauls of *Panke*), as well as thick petioles, would have been necessary to support massive leaves that provided yet more photosynthetic area. At the same time, such stems were useful in resisting burial due to slumping and alluviation. The subgenus *Pseudo-Gunnera* developed an alternative strategy for coping with frequent burial in tropical mountains (where it is found today in New Guinea) through the production of numerous stolons and the ability to produce adventitious roots from leaves. The increasingly thick and succulent petioles in the line leading to *G. manicata* acquired thorns or hardened processes, perhaps in response to large herbivores.

D. IMPLICATIONS FOR MACROSYSTEMATICS: A HERBACEOUS RADIATION OF EUDICOTS?

No evidence was noted in this study of a compound-leafed ancestry for *Saxifraga*, *Chrysosplenium*, or *Gunnera*. The taxa with which these genera grouped in cladograms were consistently groups with simple leaves, such as *Pachysandra*. This raises the possibility that this group represents a distinct "rosid" radiation separate from much of the traditional subclass Rosidae with its possible sapindopoid or Cunoniaceae origins (Hickey & Wolfe, 1975; Takhtajan 1980, 1983; Cronquist, 1981; Dickison, 1989). This conclusion is similar to that implied by recent genetic analyses (Soltis et al., 2000, 2003; Angiosperm Phylogeny Group, 2003; Hilu et al., 2003). A number of the apomorphies linked *Gunnera* and Saxifragaceae in the cladistic analyses. These include low rank, palinactinodromous leaves that lacked fourth and higher order veins or freely ending veinlets and possessed chloranthoid teeth. Such leaves are reminiscent of those of early angiosperm fossils and represent character states that may precede the evolution of the trochodendroid hamamelids (cf. Hickey & Doyle, 1977; Taylor & Hickey, 1992). Further research may identify a more basal outgroup for the Gunneraceae-Saxifragaceae clade.

The tricolpate pollen of some Saxifragaceae, as well as that of *Gunnera*, suggests that they occupy a basal position among eudicots in light of evidence for the monophyletic origin of tricolpate pollen (Doyle & Hotton, 1991; Chase et al., 1993; Crane et al., 1995; Sytsma & Baum 1996; Hoot et al., 1999; Soltis et al., 2000, 2003). Dickison (1989) suggested that among the plesiomorphies shared by rosids and basal hamamelids are trilacunar, three-trace nodes that may be related to the three-veined petiole of the basal Saxifragaceae-Gunneraceae. Although Dickison saw the rosid-hamelid ancestor as having several ovules per locule, recent phylogenetic and anatomical studies suggest that primitive carpels may have been unilocular (Taylor & Hickey, 1992; Crane et al., 1995; Hickey & Taylor, 1996; Taylor & Kirchner, 1996). If so, then unilocular taxa like the tricolpate

Gunnera, *Chrysosplenium*, or the tricolporate *Astilbe*, may fall near the base of the rosid-hamamelid clade, or the “core eudicots” (sensu Soltis et al., 2003). There is increasing evidence for an herbaceous ancestry for the eudicots (Donoghue & Doyle, 1989; Doyle & Hotton, 1991; Taylor & Hickey, 1992; Hickey & Taylor, 1996), which may therefore warrant the placement of herbaceous groups near the base of the eudicots. Paleobotanical considerations of eudicot origins need to incorporate a search image of small, low-rank herbaceous leaves like those of the subgenus *Ostenigunnera* and the genera *Chrysosplenium* and *Saxifraga*.

VI. Conclusions

Gunnera has long proved difficult to place systematically. The same consistent suite of fertile characters that so well designates the monogeneric Gunneraceae has offered little evidence for direct comparison with other taxa. The very simple flowers have led to superficial comparisons with groups such as Haloragaceae and Balanophoraceae, which may represent convergence through reduction (cf. Hooker, 1856). On the other hand, leaves vary greatly within *Gunnera*, and the study of leaf architecture provides a robust data set both for examining the phylogenetics within *Gunnera* and for determining its higher-order affinities. Ontogenetic evidence within *Gunnera* is congruent with a phylogenetic trend that sees species with large, elaborately veined leaves develop from ancestors having small, poorly organized leaves. The resultant hypothesis of *Gunnera* phylogeny is congruent with the biogeographical distribution of its subgenera and with the separation times of the Gondwana continents (Figs. 16, 19). It seems that the ecological range of *Gunnera* and its two major clades, the *Prorepens* clade and the Megaphyll clade, was fully established during its early radiation by the Turonian and has remained remarkably consistent. Thus *Gunnera* palynofossils should prove to be useful environmental indicators even in the distant past.

Leaf architecture and its ontogeny in *Gunnera* strongly suggest a relationship with the lower, herbaceous Saxifragaceae (Figs. 12, 13). For this reason we support placement of Gunneraceae in the order Saxifragales (as recognized by Takhtajan, 1983, 1997). The sister relationship with Saxifragaceae has implications for the evolutionary polarization of traits within that family. This implies a much greater antiquity for Saxifragaceae than has previously been proposed (Savile, 1975; Benton, 1993; Soltis et al., 2001a, 2001b). Both the pollen record and paleobiogeography indicate that the Gunneraceae was well established by the Turonian (93 Ma), so the ancestors of modern Saxifragaceae should be sought in some prior age. *Gunnera* represents one of yet another of the evolutionary directions taken by a basal herbaceous angiosperm radiation that included the eudicots beginning in the early-mid Cretaceous.

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IX. Appendix: Characters and Character States

Characters and character states for the data matrix (Table IV) used to produce the cladograms are set forth below. Discussion is provided for those taxa showing more than one state, or when additional explanation is warranted. Leaf architectural characters and their states are mostly derived from Hickey (1973, 1979). Additional character states are defined and, if derived from another source, the citation is given after the character state description. For those characters for which data are not based on the authors' own study, citations follow the character-state descriptions.

A. FOLIAR MORPHOLOGY: FORM

1. General laminar form: (0) elliptical; (1) ovate; (2) obovate; (3) reniform, distinguished from ovate in that the width of the leaf exceeds its length; (4) orbicular, distinguished from elliptical by having width and length approximately equal.

2. Apex: (0) acute; (1) obtuse; (2) rounded; (3) acuminate; (4) embayed, with indentation at the apex equal to, or greater than, one-third of the leaf length.

3. Base: (0) decurrent to sublobate; (1) lobate; (2) truncate to cordate; (3) rounded; (4) lobate-auriculate, having ear-shaped lobes of the lamina developing toward the petiole and partially enclosing the lobate base.

4. Lobes or akroteria: (0) lobes absent, akroteria only present as marginal teeth; (1) lobed, akroteria indented one-quarter of the distance to the midvein; (2) pedate, having two orders of lobes or akroterion pairs; (3) having primary akroteria as large, convex-convex teeth, with secondary akroteria as marginal teeth: akroteria are indented generally less than one-quarter of the distance to the midrib and therefore do not qualify as lobes.

For discussion of the terms in these character states, see the section "Systematic Leaf Architecture of Gunneraceae" above, and Fig. 3. *Gunnera brephogea* is borderline between states 2 and 3, with some specimens falling into either character state. This demonstrates the arbitrariness of the division into distinct categories of what is in fact an evolutionary, and probably ontogenetic, spectrum. *Gunnera brephogea* has been coded as state 2, because this strengthens its position as intermediate between the "pedate" and "orbicular" lineages of the subgenus *Panke*.

5. Margin: (0) serrate; (1) crenate, or dentate, with one order of teeth; (2) dentate in two orders; (3) dentate in three or more orders; (4) dentate with secondary akroteria as broad, first-order teeth, the margins of which bear higher-order akroteria that resemble teeth in venation but lack sinuses; thus the margin of the broad, first-order teeth is essentially repand; (5) entire.

States 1 and 2 are merely points along a spectrum of marginal form in *Gunnera*. In order to assign this state, leaves were assumed to have a single order of teeth unless a substantial majority of teeth were associated with second-order teeth. Thus the existence of small numbers of secondary teeth does not warrant assignment to state 2.

In *Gunnera pilosa* and *G. talamancana*, high-order teeth are incipient (state 4). They are apparent by their glandularity and venation pattern but project barely, if at all, beyond the margin. These repand margins have a tendency to be revolute.

6. Tooth form, first-order teeth: (0) acuminate-convex (type D1); (1) convex-convex (type A1); (2) straight-straight (type B2); (3) convex-concave (type C1); (4) acuminate-acuminate (type A4); (5) concave-concave (type C3); (6) *Disanthus* N/A; (7) *Cnestis* N/A; (8) acuminate-straight (type D2).

In determining tooth form, only that portion of the leaf margin separated from other teeth by sinuses was considered.

B. VENATION CHARACTERS

7. Primary venation type: (0) pinnate; (1) actinodromous; (2) palinactinodromous, but with basal origination of three primaries; (3) acrodromous.

Palmate and pinnate venation types can intergrade and thus represent points along a morphocline. This is particularly evident in the subgenus *Milligania*. For this reason, character 8, state 2 (below) includes pinnately veined leaves.

8. Mode of origination of lateral primaries or basal secondaries: (0) three distinct veins in the petiole; (1) basal with lateral primaries forming a margin along a lobate base; (2) basal, with only one vascular bundle visible in the petiole, including pinnately veined leaves with a pair of strong, basally originating secondaries; (3) suprabasal; (4) *Griselinia* N/A; (5) *Aucuba* N/A; (6) *Cnestis* N/A.

9. Course of midrib: (0) straight, unbranched; (1) straight, reticulating, becoming indistinct from higher-order veins; (2) midrib lacking (i.e., dichotomizing from the base); (3) straight, trichotomous approximately halfway toward the apex, or with oppositely arising secondaries forming a virtual trichotomy; (4) straight, dichotomizing once below the apex; (5) straight, dichotomizing twice below the apex.

10. Number of agrophic veins present on each side of lamina: (0) absent; (1) 1; (2) 2; (3) 4; (4) 5 or 6; (5) N/A, true pinnate venation.

11. Primary vein development (including agrophic veins in craspedodromous leaves): (0) marginal; (1) reticulate; (2) *Trochodendron* N/A (A); (3) *Ampelopsis* N/A; (4) *Griselinia* N/A; (5) *Disanthus* N/A; (6) *Proserpinaca* N/A; (7) *Lopezia* N/A; (8) *Aucuba* N/A; (9) *Cnestis* N/A.

12. Secondary vein, type and course: (0) brochidodromous; (1) semicraspedodromous; (2) craspedodromous, branched or sinuous, includes reticulate; (3) craspedodromous or marginal, recurved; (4) craspedodromous or marginal, uniformly curved.

13. Secondary vein, angle of origination: (0) moderately acute, 45°–65°; (1) narrowly acute, <45°; (2) widely acute, >65°.

14. Variation in angle of secondary origination: (0) upper veins more obtuse; (1) uniform; (2) upper veins more acute; (3) angle irregular.

15. Tertiary pattern: (0) reticulate-orthogonal; (1) reticulate-random; (2) percurrent.

16. Quaternary course: (0) orthogonal; (1) random reticulate; (2) ramified admedial; (3) fourth order lacking.

17. Quintenary course: (0) fifth-order veins absent; (1) random; (2) orthogonal.

18. Marginal veins: (0) looped; (1) incomplete.

19. Areolation: (0) imperfect; (1) well developed; (2) incomplete.

20. Areole shape: (0) quadrangular; (1) irregular.

21. Freely ending veinlets: (0) simple, linear or curved; (1) branched once; (2) branched more than once; (3) veinlets lacking; (4) veinlets occasional.

The absence of freely ending veinlets in *Gunnera* was noted by Palkovic (1974). In leaves of the larger species, particularly in subgenus *Panke*, veinlets are found in a small minority of areoles.

22. Alveolarity of laminar tissue: (0) none or slightly prominent major veins; (1) subalveolar, with prominent veins on abaxial surface; (2) alveolar.

This character refers to the texture of the leaf formed by prominent veins and surrounding impressed areas of laminar tissue on the abaxial surface. On the adaxial surface, the veins are highly impressed and around small hills of laminar tissue, which thus appears colliculate. Alveolarity within Gunneraceae is distinctive in this character because prominent or impressed veins include the penultimate vein order present (e.g., the fifth or sixth order), whereas in other taxa that sometimes show alveolarity, accentuation of the veins is usually restricted to the

third (or fourth) order; e.g., Vitaceae, Platanaceae. The presence of pseudo-colleters (character 39 below) in many species of *Gunnera* greatly exaggerates their alveolarity.

23. Leaf rank (Hickey, 1977: appendix; Hickey & Taylor, 1991): (0) first rank; (1) low second rank ($2r^0-2r^1$); (2) high second rank ($2r^2-2r^3$); (3) third rank.

This character represents the general organizational regularity of the leaf. It is significant because it makes less likely the derivation of very irregularly veined leaves from highly ordered leaves under normal mesic conditions. Because Gunneraceae show a broad spectrum of rank, from the low first-order leaves of *Gunnera herteri* to the highly reiterative venation of subgenus *Panke*, this character should elucidate trends toward greater or lesser rigidity in venation pattern. In general, leaf rank tends to reflect the general size of the leaf within *Gunnera*.

24. Crystalline inclusions in leaf tissue: (0) absent; (1) sandy; (2) druses; (3) raphides, as well as other forms; (4) framboids.

For a discussion of oxalate crystals in *Saxifraga*, see Gornall (1986). Druses are reported for *Gunnera herteri* by Mattfeld (1933).

C. MARGINAL TEETH, GLANDS, AND SINUSES

25. Glands: (0) on teeth; (1) on teeth and in sinuses; (2) marginal; (3) absent.

26. Apical termination of marginal teeth: (0) tylate; (1) tylate with dark-staining apical process; (2) foramenate; (3) simple; (4) papillose; (5) spinose; (6) *Cnestis* N/A; (7) *Disanthus* N/A.

27. Principal vein: (0) central and direct; (1) eccentric, running to one side of the axis of symmetry of the tooth; (2) *Disanthus* N/A; (3) *Cnestis* N/A.

28. Termination of principal vein: (0) tapered; (1) bulbous; (2) truncate; (3) splayed; (4) *Cnestis* N/A; (5) *Disanthus* N/A.

29. Suite of veins associated with the principal vein of the tooth: (0) no admedial, conjunctals and accessories only; (1) admedial and conjunctals only; (2) admedial, conjunctals, and one order of reticulate accessories; (3) admedial, conjunctals, and reticulate accessories in more than one order; (4) admedial, conjunctals, and freely ending accessories; (5) admedial and freely ending accessories, true conjunctals lacking; (6) *Disanthus* N/A; (7) *Cnestis* N/A.

30. Conjunctal veins: (0) joining and fusing with the principal vein, opposite; (1) joining and fusing with the principal vein, alternate; (2) incipiently connivent, with only a few strands merging; (3) connivent with the principal (i.e., running alongside it and splaying or ending concurrently in tooth epithem); (4) convergent but remaining separate; (5) only one conjunctal, joining; (6) "vitioid" tooth venation, in which alternate conjunctals split just before meeting the principal vein, with the admedial portion of the conjunctal joining the principal while the exmedial portion connives; (7) *Cnestis* N/A; (8) *Disanthus* N/A; (9) *Griselinia* N/A.

Although character state 0 occurs in a minority of modern *Cercidiphyllum* leaves, it is indicated as the ancestral state in the fossil record (see the discussion in the text). However, recoding our cladistic matrix for this character would not affect the placement of *Cercidiphyllum*.

31. Strength of the admedial: (0) same order as the principal vein; (1) lower order than the principal vein; (2) N/A *Disanthus*; (3) N/A *Cnestis*; (4) absent.

32. Sinus shape: (0) angular; (1) rounded; (2) rounded sinuses between first-order teeth, sinuses between the higher order lacking; (3) N/A *Disanthus*; (4) N/A *Cnestis*.

33. Source of sinus venation: (0) conjunctal; (1) branch from conjunctal; (2) combinations of conjunctal and its branch; (3) combination of conjunctal and admedial; (4) convergent, thickened, merging admedials; (5) combination of admedial and branch from admedial; (6) combination of branches from admedial and conjunctal; (7) branch from admedial; (8) *Disanthus* N/A; (9) *Gunnera pilosa* N/A; (A) *Gunnera talamancana* N/A.

D. EPIDERMAL CHARACTERS

34. Epidermal cell morphology (Dilcher, 1974): (0) elongate cells and deeply undulate outline; (1) isodiametric, pentagonal-hexagonal, straight to round walls; (2) hexagonal with straight walls, interspersed with irregularly shaped, sinuously walled cells.

35. Trichomes (Theobald et al., 1979): (0) absent; (1) unicellular; (2) uniseriate, multicellular; (3) multicellular-stellate; (4) multiseriate.

36. Trichome placement: (0) on the margin only; (1) on the lower surface veins and the margin; (2) on veins of both surfaces and margin; (3) on veins of both surfaces, margin, upper surface, and areoles; (4) on the veins and areoles of both surfaces, plus the margin; (5) on the veins, areoles, and sinuses; (6) *Gunnera herteri* N/A; (7) *Trochodendron* N/A; (8) *Tetracentron* N/A; (9) *Cercidiphyllum* N/A; (A) *Aucuba* N/A; (B) *Disanthus* N/A; (C) *Ascarina* N/A; (D) *Hamamelis* N/A; (E) *Chrysosplenium* N/A; (F) *Griselinia* N/A; (G) on the petiole only; (H) *Myrothamnus* N/A.

37. Petiolar processes: (0) thorns or processes lacking; (1) thorns or processes present.

This character does not include trichomes. Thorns on the petioles of *Panke* species often extend onto the primary veins.

38. Colleters, circular glandular processes emerging from laminar tissue of the leaf's upper surface, at the intersection of fourth- and fifth-order veins, secretory: (0) absent; (1) present.

Colleters are unique to the *Gunnera* subgenera *Panke* and *Pseudo-Gunnera* and have been discussed by numerous authors (Solereeder, 1908; Palkovic, 1974; Mora-Osejo, 1984; Wilkinson, 1998).

39. Pseudo-colleters, conical, nonglandular processes emergent from the upper surface of leaf tissue in the midst of areoles or subtended by seventh- or higher-order venation: (0) absent; (1) present.

These have often been noted as a variant form of colleter (Solereeder, 1908; Palkovic, 1974; Mora-Osejo, 1984), but because these are not glandular they are not true colletes (cf. Wilkinson, 2000).

E. POLLEN

The sources for characters 38 through 47 are as follows: *Gunnera* (Praglowksi, 1970; Jarzen, 1980; Jarzen & Dettmann, 1989), *Proserpinaca* (Praglowksi, 1970); *Ascarina* (Walker & Walker, 1984), *Aucuba* (Chao, 1954), *Griselinia* (Heusser, 1971) *Cercidiphyllum*, Trochodendrales, Hamamelidaceae (Zavada & Dilcher, 1986; Hufford & Crane, 1989; Endress, 1993a, 1993b, 1993c); *Platanus* (Kubitzki, 1993a); *Lopezia* (Patel et al., 1984); *Saxifraga* (Erdtman, 1966; Ferguson & Webb, 1970); *Chrysosplenium* (Heusser, 1971; Gupta & Sharma, 1986); *Cnestis* (Dickison, 1979), Vitaceae (Erdtman, 1966); *Myrothamnus* (Zavada & Dilcher, 1986; Kubitzki, 1993b; Wanntorp et al., 2004). In some cases character states were based on photographs in the above publications.

40. Form: (0) monosulcate; (1) tricolpate; (2) bicolpate; (3) tricolporate; (4) triporate; (5) periporate.

41. Protruding apertures: (0) absent; (1) present.

42. Pollen size: (0) medium (25–40 μm); (1) small (10–24 μm); (2) large (>40 μm).

43. Aperture shape: (0) elliptical furrow (i.e., aperture termini round); (1) lenticular (i.e., aperture termini pointed); (2) circular.

The potential systematic utility of this character is noted by Hufford and Crane (1989).

44. Nonapertural exine sculpturing (Walker & Walker, 1984): (0) reticulate, beaded, spinulose, or verrucate; (1) reticulate, smooth, without tectal spinules; (2) tectum with insular protrusions; (3) rugulate, imperforate, with viscin threads; (4) psilate; (5) clavate.

45. Nonapertural exine structure (Walker & Walker, 1984): (0) tectate perforate to semitectate; (1) tectate imperforate; (2) intectate.

46. Columellae and foot layer: (0) both present; (1) both absent.

47. Nonapertural exine lumina: (0) polygonal; (1) round; (2) *Proserpinaca* N/A; (3) *Lopezia* N/A; (4) *Aucuba* N/A; (5) *Griselinia* N/A; (6) *Saxifraga* N/A; (7) *Pachysandra* N/A; (8) *Myrothamnus* N/A.

48. Apertural sculpturing: (0) smooth to finely verrucate; (1) coarsely granulate; (2) pore, no sculpturing.

49. Endexine: (0) thick, under apertures only; (1) endexine throughout, thickened under apertures; (2) endexine throughout, not thickened under apertures; (3) endexine absent under apertures.

50. Pollen maturity at shedding: (0) 2-celled state; (1) 3-celled state.

F. MISCELLANEOUS, REPRODUCTIVE, AND ANATOMICAL CHARACTERS

51. Growth habit: (0) cauline herb, with bifurcating axis, opposite, axillary leaves without stolons; (2) tree or shrub; (3) pachycaul; (4) rhizomatous herb, with leaves basal; (5) woody vine (Schindler, 1905; Ohwi, 1965; Spongberg, 1972; Cronquist, 1981; Webb & Gornall, 1989; Endress, 1993a, 1993b, 1993c; Heywood, 1993; Kubitzki, 1993a, 1993b). (Note the absence of state 1 for this character, both here and in the matrix in Table IV.)

52. Stolons: (0) absent; (1) present.

We have coded all stolons the same, despite indications that stolons in *Misandra* and *Milligania* differ from those of *Pseudo-Gunnera*, because the latter are not subtended by foliose leaves and lack adventitious root primordia (see the discussion in the text).

53. Symbiotic *Nostoc* cyanobacteria: (0) absent; (1) present.

The *Nostoc* symbiosis is unique to *Gunnera* among angiosperms (Bergman et al., 1992).

54. Sieve-element plastid types: (0) plastids containing starch crystals only (S-type); (1) plastids containing proteins (P-type) (Behnke, 1981, 1986, 1991).

55. Stipules: (0) present; (1) absent.

Gunnera possesses axillary scales, called "ligules" or "rhizome scales" (Schindler, 1905), which are sometimes considered to be stipulate (cf. Cronquist, 1981, 1988). However, Mora-Osejo (1984) argued for their derivation from reduced leaves and did not consider them to be stipules. Examination of the ligules in *G. chilensis* suggests that they may represent developmentally reduced leaves. Study of the minute "ligules" in the smaller and presumably more primitive species indicates that they are indeed cataphylls (Wanntorp et al., 2003).

56. Ovule: (0) orthotropous, bitegmic; (1) anatropous or hemianatropous, bitegmic; (2) anatropous, unitegmic (Davis, 1966; Palkovic, 1974; Orchard, 1975; Corner, 1976; Cronquist, 1981; Dahlgren & Thorne, 1984; Webb & Gornall, 1989; Kubitzki, 1993b).

57. Embryo-sac development: (0) monosporic, 8-nucleate (*Polygonum* type); (1) tetrasporic, 16-nucleate (*Peperomia* type); (2) *Oenothera* type; (3) bisporic (*Allium* type). Same sources as for character 56.

58. Nucellus: (0) crassinucellar; (1) tenuinucellar. Same sources as for character 56.

59. Endosperm development: (0) nuclear; (1) cellular. Same sources as for character 56.

60. Number of seeds per fruit: (0) 1; (1) many. Same sources as for character 56.

61. Flowering habit: (0) all flowers hermaphroditic; (1) monoecious (mixture of unisexual and some hermaphroditic flowers); (2) dioecious (Schindler, 1905; Ohwi, 1965; Meijden & Caspers, 1971; Spongberg, 1972; Palkovic, 1978; Cronquist, 1981; Lowry & Robinson, 1988; Webb & Gornall, 1989; Endress, 1993a, 1993b, 1993c; Heywood, 1993; Kubitzki, 1993a, 1993b; Takhtajan, 1997).

62. Inflorescence type: (0) panicle or compound spike; (1) spike or raceme; (2) dense racemose head; (3) corymb; (4) solitary flower; (5) cyme. Same sources as for character 61.

Platanus has been coded as a panicle on the basis of the likely derivation of its inflorescence (Kubitzki, 1993a). *Myrothamnus* is also considered a reduced panicle (Kubitzki, 1993b). *Chrysosplenium* often has a single flower, but, because some species have compound inflorescences of the corymb type (Ohwi, 1965), we have coded this as basic to the genus, assuming that the solitary flowers are a reduced derivative.

63. Embryo shape: (0) straight, cylindrical; (1) undifferentiated or with slight cotyledons; (2) with two lobes, obcordate. Same sources as for character 61.

64. Phyllotaxis: (0) opposite; (1) alternate; (2) whorled.

65. Sessile, cauline leaves (sometimes improperly called "ligules" on *Gunnera*): (0) absent; (1) like other leaves, but smaller and sessile; (2) small, straplike; (3) small, shieldlike; (4) budlike; (5) pinnatifid to lacinate.

Study indicates these to be cataphylls (Mora-Osejo, 1984; Wanntorp et al., 2003); cf. character 55 above.