



Systematic implications of seed coat diversity in Gaultherieae (Ericaceae)

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The seed morphology of 90 samples from 83 species of tribe Gaultherieae (*Chamaedaphne*, *Diplycosia*, *Eubotryoides*, *Eubotrys*, *Gaultheria* and *Leucothoe*) and relatives in tribes Andromedeae (*Andromeda* and *Zenobia*) and Vaccinieae (*Satyria*) was investigated with stereoscopic and scanning electron microscopy. Seeds exhibit variation in shape, colour, size, wing, hilum region, primary ornamentation and epidermal cells. Non-metric multidimensional scaling (NMDS) analysis based on selected seed characters supports the affinities of some groups within Gaultherieae at various taxonomic levels. Seed characters corroborate the delimitation of *Andromeda*, *Chamaedaphne*, *Leucothoe*, *Satyria* and *Zenobia* and *Gaultheria* series *Trichophyllae*, series *Hispidulae*, section *Amblyandra* and section *Brossaeopsis*. Parsimony optimization of seed characters onto a previously published phylogenetic estimate of Gaultherieae reveals that small seeds have evolved from larger seeds and an areolate seed coat has evolved from a reticulate seed coat. Optimization also suggests that several seed character states are synapomorphies or potential synapomorphies for some major clades of Gaultherieae. Seeds of Gaultherieae from East Asia, temperate North America and the Pacific are more diverse than those from tropical America. Samples from the eastern Himalaya possess the highest variation in seed morphology. The wing and bulging edge cells observed in seeds of *Leucothoe* suggest dispersal by wind. © 2010 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2010, 162, 477–495.

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INTRODUCTION

The amphi-Pacific tribe Gaultherieae (Ericaceae), consisting of *Chamaedaphne* Moench (one species), *Eubotryoides* (Nakai) Hara (one species), *Eubotrys* Nutt. (two species), *Leucothoe* D. Don (five species) and the three genera of the wintergreen group, i.e. *Diplycosia* Blume (c. 90 species), *Gaultheria* L. (c. 130 species) and *Tepuia* Camp (seven species), form a monophyletic

group within subfamily Vaccinioideae (Ericaceae; Powell & Kron, 2001; Kron *et al.*, 2002; Waselkov & Judd, 2008; Bush *et al.*, 2009). The distribution of *Gaultheria* (including *Pernettya* Gaud.) is as wide as that of the entire tribe. *Chamaedaphne* is widely distributed throughout the cool temperate and subarctic regions of the Northern Hemisphere. *Leucothoe* is distributed in eastern Asia and North America. *Eubotryoides* is native to Japan, whereas *Eubotrys* is found in eastern North America. *Diplycosia* and *Tepuia* are endemic to the Indo-Malaya region and the Guayana Highlands of South America, respectively.

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Studies based on DNA sequences (Powell & Kron, 2001; Kron *et al.*, 2002; Bush *et al.*, 2009) have provided a phylogenetic estimate for Gaultherieae. The most recent and comprehensive study included all currently recognized genera and 51 samples (Bush *et al.*, 2009). This study has confirmed both the monophyly of Gaultherieae and its sister-group relationship to Andromedeae (*Andromeda* L. and *Zenobia* D. Don). All genera except *Gaultheria* are monophyletic. *Eubotryoides* and *Leucothoe* form the first-diverging clade and a clade comprising *Chamaedaphne* and *Eubotrys* is sister to the wintergreen clade (*Diplycosia*, *Gaultheria* and *Tepuia*). *Diplycosia* and *Tepuia* are both nested within *Gaultheria*.

Although progress has been made elucidating the phylogeny of Gaultherieae, the morphological basis for the phylogenetic structure inferred from molecular data is still unclear. The most recent classification of *Gaultheria* emphasized characters of the calyx, fruit and inflorescence (Middleton, 1991a), but this classification is based on overall similarity. Bush *et al.* (2009) found that all these characters are homoplasious to various degrees and that a reassessment of their utility is needed. Lu *et al.* (2009) studied pollen morphology in the context of the phylogenetic estimate of Kron *et al.* (2002). They found that pollen data support the sister relationships of both *Satyria* Klotzsch and some species of *Vaccinium* L. (e.g. *V. macrocarpon* Aiton and *V. meridionale* Sw.) and *Chamaedaphne* and *Eubotrys* in Kron *et al.* (2002). Reclassification of Gaultherieae must await a comprehensive assessment of likely morphological synapomorphies for its major clades.

Although the potential value of seed characters in systematics has been emphasized (Elisens & Spencer, 1983; Barthlott, 1984), only general observations have thus far been made on the systematic utility of the seeds of Gaultherieae. Stevens (1970, 1971) found seed characters to be useful in his subfamilial and tribal treatment of Ericaceae. Judd (1979) observed that the cells of the seed coat in Gaultherieae and other genera ('*Gaultheria* group' *sensu* Stevens, 1970) are variable in shape and thickness. Middleton (1991b) discussed the significance of seed size, seed wing presence and cell shape of the seed coat for the generic relationships of Andromedeae *sensu* Stevens (1970), which included Gaultherieae. A few scanning electron microscopy (SEM) photomicrographs of seeds of *Tepuia* and Neotropical members of *Gaultheria* were presented by Luteyn (1995). None of these studies was, however, conducted in a molecular phylogenetic context.

Here, we survey seed morphology in Gaultherieae with SEM and stereoscopic microscopy (SM) to assess the potential of seeds for providing characters of systematic value in the classification of the tribe. A

non-metric multidimensional scaling (NMDS) analysis, widely used in testing the similarity of categorical phenotypes or traits (e.g. Meyer *et al.*, 1992; Kent, 2001) is applied. We further map selected seed characters observed from the survey onto a DNA sequence-based phylogenetic estimate of Gaultherieae to infer likely synapomorphies for major clades. We also hypothesize modes of seed dispersal based on various morphological features.

MATERIAL AND METHODS

Mature seeds of 90 samples representing 83 species (including 14 varieties) of six out of the seven genera of Gaultherieae, the two genera of Andromedeae and one representative genus (*Satyria*) of Vaccinieae were taken from fresh material in the field or dried specimens from the herbaria of the Kunming Institute of Botany (KUN) and the California Academy of Sciences (CAS; Appendix). All genera of Gaultherieae were sampled except *Tepuia*, seeds of which were unavailable to us. Because of the large size of the study group, among-taxon variation was emphasized over within-taxon variation. Within-species variation was assessed to some extent by sampling the seeds of the varieties within some species. Moreover, a random sample of multiple accessions for seven other species (data not shown) suggested a general lack of infraspecific variation. Classification follows Middleton (1991a) for *Gaultheria* and Waselkov & Judd (2008) for *Eubotryoides*, *Eubotrys* and *Leucothoe*.

The method of Martínez-Ortega & Rico (2001) was applied to remove alien or wax deposits adhering to the seed surface. Seed samples were immersed in purified water for 2 days so that any artifactual ruga on the seed surface caused by desiccation could be minimized, after which they were placed in a 1 : 1 solution of chloroform and methanol for 48 h, dehydrated through an ethanol series (70, 90 and 100%) and finally treated with xylene for 3 days. For SEM, seeds were mounted on double-sided sticky conducting tape on the stub and sputter-coated with gold palladium. Most samples were imaged with a KYKY-10000B scanning electron microscope (Science Instrument Company, Beijing, China). Several samples were imaged with an LEO 1450VP (Leo Corporation) scanning electron microscope. Adobe Illustrator CS2 and Adobe Photoshop CS2 were used to edit figures.

Seed size, shape and colour were observed under SM. Twenty seeds of each sample were measured in transverse and longitudinal directions (length and width at the longest and widest axes; Fig. 1A). Seed epidermal cells were also measured in this manner (Fig. 1B), with minimal and maximal cell measure-

ments taken from each seed to indicate the general range of epidermal cell size of each species.

Sixteen seed characters were scored for Gaultherieae (Tables 1 and 2). Of these, 14 (characters 1–14 in Table 1) were employed in an NMDS analysis. The characters ‘Suture between epidermal cells’ and ‘Threads attached to muri’ were excluded because of their instability. The NMDS plot was constructed with the PAST 1.81 software package (Hammer, Harper & Ryan, 2007). Gower’s similarity index was used for this analysis because it resulted in a lower stress index than any other index available in the program.

Seed character evolution was inferred by mapping each character onto the most recent molecular phylogenetic tree of Gaultherieae (Bush *et al.*, 2009) with parsimony optimization via the computer program MacClade 4.08 (Maddison & Maddison, 1992). Thirty-eight taxa that we sampled for seed characters were also sampled in the study of Bush *et al.* (2009). Thus, the phylogenetic tree of Bush *et al.* (2009) was modified accordingly by excluding taxa not available in our study. The data for *Tepuia* were extracted from Luteyn (1995). We highlighted the characters with the least homoplasy, i.e. seed shape (overall), lateral shape, size, presence or absence of a wing, shape of the hilum region and primary ornamentation. Two character states from the NMDS analysis are treated as polymorphic in the optimization analysis (i.e. ‘reticulate laterally + areolate dorsiventrally’ is changed to the two character states ‘reticulate’ and ‘areolate’ and ‘obliquely pyramidal and trapezoidal in the same fruit’ is changed to ‘obliquely pyramidal’ and ‘trapezoidal’). Seed character terminology generally follows that of Corner (1976), Barthlott (1981, 1984), Takahashi (1993), Arias & Terrazas (2004), Liu, Lin & He (2004) and Oh *et al.* (2008).

RESULTS

DESCRIPTION OF SEED MORPHOLOGY

Seeds of the 90 samples in our study exhibit wide variation in shape, colour, size, wing, shape of the hilum region and, particularly, primary ornamentation and epidermal cell characters [including epidermal cell shape, murus thickness, secondary ornamentation (the outer paracidal wall and anticline wall) and suture between epidermal cells] and specialized structure in some species.

Seed shape

Seed shape in Gaultherieae can be categorized as follows: globose or near so, subglobose or nearly ellipsoid, obliquely pyramidal, trapezoidal in outline, ellipsoid–mucronate or hippocrepiform–angular.

Seeds of *Diplycosia viridiflora* DC (Fig. 1C) and *G. nummularioides* D. Don are globose or nearly so. Subglobose or nearly ellipsoid seeds occur mainly in *Chamaedaphne* (Fig. 1D), most species of *Eubotrys* (Fig. 1E), most species of *Gaultheria* section *Pernettya* (Gaud.) Middleton (Fig. 1F), *Satyria* (Fig. 1G) and *Andromeda* (Fig. 1H), whereas obliquely pyramidal seeds occur in most species of *Gaultheria* (Fig. 1I), some species of *G.* section *Pernettya*, *Zenobia* and *Diplycosia memecyloides* Stapf (Fig. 1J). Some species of *Gaultheria* commonly have both obliquely pyramidal and trapezoidal seeds in the same fruit, such as *G. erecta* Vent. (Fig. 1K) and *G. straminea* R.C.Fang (Fig. 1L). Ellipsoid–mucronate seeds are only found in *Eubotryoides*, *Eubotrys* and *Leucothoe* (Fig. 1M) and hippocrepiform–angular seeds are unique to *Diplycosia sphenophylla* Sleumer (Fig. 1N).

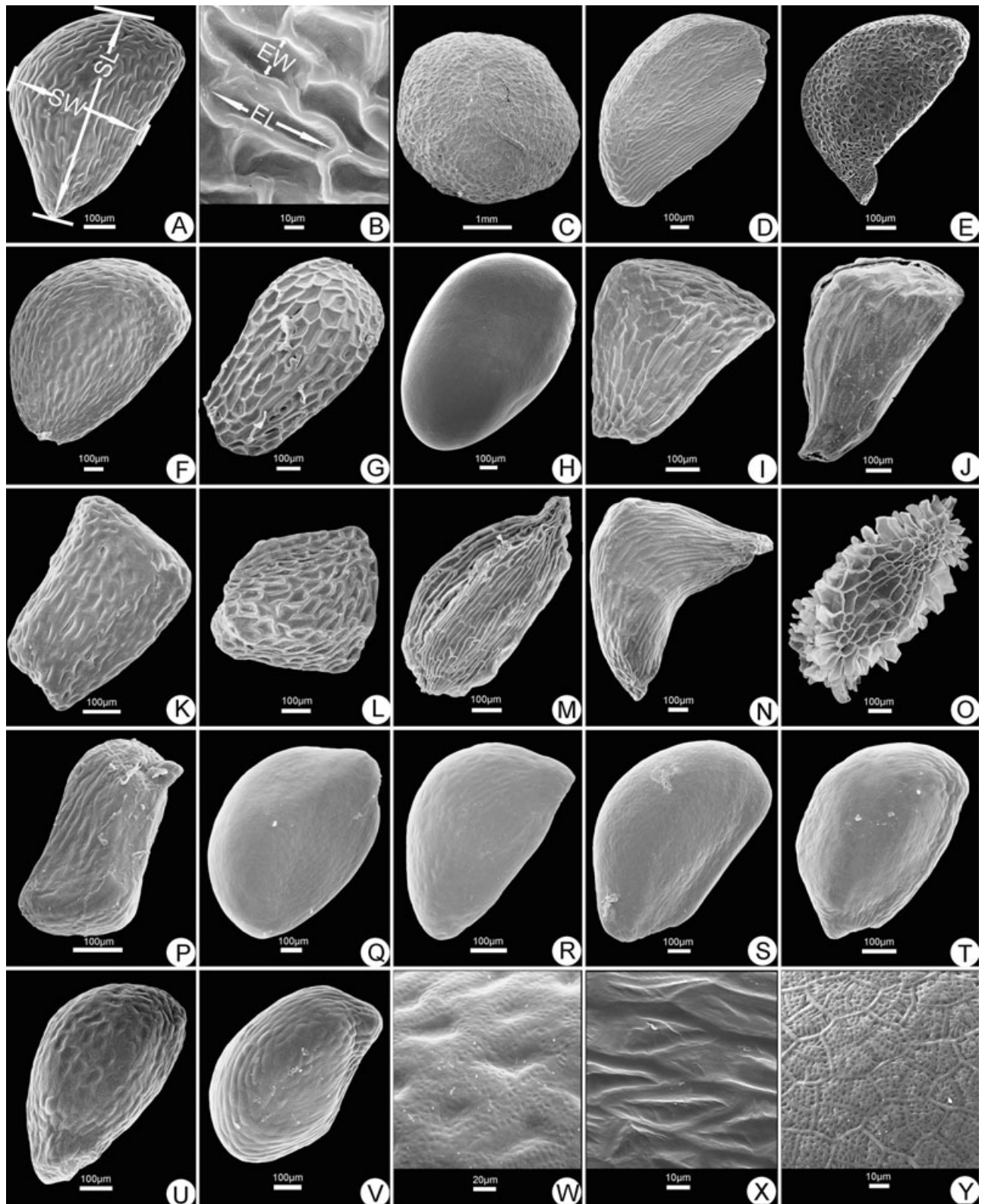
Seeds with two concave regions laterally can result in a thick dorsal region and a thin ventral region (Fig. 1A). Seeds of this type occur in almost all samples studied, except *Eubotryoides grayana* Maxim., *Eubotrys recurva* (Buckl.) Britt. and species of *Leucothoe*. These taxa possess distinctively flat seeds (Fig. 1M, O).

Seed colour

Most seeds are glossy and are either yellow, light brown, mid-brown or dark brown. *Chamaedaphne*, *Diplycosia*, most species of *G.* section *Pernettya* and *Zenobia* possess mid-brown or dark brown seeds, whereas most other species of *Gaultheria* and those of *Andromeda* and *Satyria* have yellow or light brown (rarely mid-brown) seeds. All *Leucothoe* seeds are yellow. Seeds of *Eubotrys* are yellow except those of *Eubotrys racemosa* Nutt. var. *elongata* (Small) Small, which are dark brown.

Seed size

All examined seeds range from 0.37×0.26 to 1.98×0.84 mm, except *D. viridiflora*, which has seeds $4.42\text{--}5.74 \times 2.55\text{--}3.55$ mm (Fig. 1C). *Gaultheria wardii* Marq. & Airy Shaw var. *elongata* R.C.Fang has the smallest seeds, $0.30\text{--}0.44 \times 0.20\text{--}0.32$ mm (Fig. 1P). It is difficult to calculate seed volume because of the irregularity of seed shape. Here, we employ seed length to represent seed size. For testing the general evolutionary trend of seed size, we divided seed length into four categories, as mean values in mm: (1) small (≤ 0.6); (2) medium–small (> 0.6 and < 1.0); (3) medium–large (> 1.0 and < 5.0); (4) large (≥ 5.0). Small to medium–small seeds occur in most species of *Eubotrys* (those of *E. recurva* are medium–large) and most species of *Gaultheria*, whereas medium–large to large seeds occur in *Chamaedaphne*, *Diplycosia*, some species of *Gaulth-*



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Figure 1. Seeds of Gaultherieae and related genera. A, *Gaultheria itoana*; SL, measure of seed length, SW, measure of seed width. B, *G. discolor*; EL, measure of epidermal cell length, EW, measure of epidermal cell width. C, *Diplycosia viridiflora*, showing globose shape. D, *Chamaedaphne calyculata*, showing subglobose shape and regulate–reticulate ornamentation. E, *Eubotrys racemosa*, showing distinct protuberant hilum region and reticulate ornamentation. F, *G. mucronata*, showing subglobose, laterally concave shape. G, *Satyria panurensis*, showing nearly ellipsoid shape and reticulate ornamentation. H, *Andromeda polifolia*, showing nearly ellipsoid shape. I, *G. foliolosa*, showing obliquely pyramidal shape. J, *D. memecyloides*, showing obliquely pyramidal shape. K, *G. erecta*, showing trapezoidal shape. L, *G. straminea*, showing trapezoidal shape. M, *Leucothoe keiskei*, showing ellipsoid–mucronate shape. N, *D. sphenophylla*, showing hippocrepiform–angular shape. O, *L. davisiae*, showing laterally flattened shape. P, *G. wardii* var. *elongata*, showing smallest size, a distinctly protuberant hilum region and projections on seed surface. Q, *G. hispidula*. R, *G. humifusa*. S, *G. suborbicularis*, showing faintly reticulate ornamentation. T, *G. hypochlora*, showing ornamentation that is faintly reticulate laterally + areolate dorsiventrally. U, *G. griffithiana*, showing ornamentation that is reticulate laterally + areolate dorsiventrally. V, *G. trichophylla*, showing areolate ornamentation. W, *G. pumila*. X, *Zenobia pulverulenta*, showing narrowly and shortly elongate epidermal cells. Y, *A. polifolia*, showing suborbicular, square or nearly so, or polygonal epidermal cells.

Table 1. Seed characters and character states of the Gaultherieae

Seed characters	Character states
1 Shape	0 globose or nearly so, 1 subglobose or nearly ellipsoid, 2 obliquely pyramidal, 3 trapezoidal, 4 obliquely pyramidal and trapezoidal in the same fruit, 5 ellipsoid–mucronate, 6 hippocrepiform–angular
2 Shape laterally	0 concave, 1 flattened
3 Colour	0 yellow, 1 light brown, 2 mid-brown, 3 dark brown
4 Size (represented by seed length, mean value, mm)	0 small (≤ 0.6), 1 medium-small (> 0.6 and < 1.0), 2 medium-large (> 1.0 and < 5.0), 3 large (≥ 5.0)
5 Wing	0 unwinged; 1 dorsiventrally winged; 2 laterally winged; 3 laterally winged with bulging cells
6 Hilum region	0 not protuberant, 1 indistinctly protuberant, 2 distinctly protuberant
7 Seed primary ornamentation	0 rugulate–reticulate, 1 reticulate, 2 faintly reticulate, 3 faintly reticulate laterally + areolate dorsiventrally, 4 reticulate laterally + areolate dorsiventrally, 5 areolate
8 Projections on surface	0 absent, 1 present
9 Epidermal cell shape	0 narrowly and shortly elongate, 1 suborbicular, square or nearly so, or polygonal, 2 elongate, 3 sublinear, 4 nubiform
10 Murus thickness	0 thin-walled, 1 moderately thick-walled, 2 thick-walled
11 Periclinal wall	0 convex, 1 concave, 2 mixed convex and concave
12 Periclinal wall ornamentation	0 faintly granulate, 1 granulate, 2 verrucate, 3 foveolate, 4 reti-rugulate, 5 reticulate
13 Anticlinal wall	0 convex, 1 concave, 2 mixed convex and concave
14 Anticlinal wall ornamentation	0 psilate to faintly granulate, 1 raised-cross-banded (part of the anticlinal wall raised discontinuous, and crossing over the suture sporadically in various places along the length of the wall), 2 verrucate, 3 scalariform, 4 foveolate, 5 reticulate
15 Suture between epidermal cells	0 absent, 1 present
16 Threads attached to muri	0 absent, 1 present

eria, *Satyria* and *Zenobia*. Large seeds characterize *Leucothoe* and *Andromeda*.

Seed primary ornamentation

Seed primary ornamentation in Gaultherieae can be categorized as follows: regulate–reticulate, reticulate, faintly reticulate, faintly reticulate laterally +

areolate dorsiventrally, reticulate laterally + areolate dorsiventrally and areolate. Only *Chamaedaphne calyculata* (L.) Moench possesses regulate–reticulate ornamentation (Fig. 1D). Reticulate ornamentation occurs in all species of *Andromeda* (Fig. 1H), *Eubotrys* (Fig. 1E), *Satyria* (Fig. 1G) and *Zenobia*, and in most samples of *Diplycosia*, *Gaultheria* and *Leucothoe*. The

Table 2. Seed characters of Gaultherieae and related genera

No.	Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Seed size (length × width, mm)	Epidermal cell size (length × width, μm)
1	<i>Chamaedaphne calyculata</i>	1	0	3	2	0	0	0	0	3	2	1	2	0	3	1	0	1.23 ± 0.09 × 0.67 ± 0.06	29.4–29.4 × 8.9–58.8
2	<i>Diphycosia memecyloides</i>	2	0	2	1	0	1	1	0	3	0	1	4	0	0	1	0	0.96 ± 0.11 × 0.49 ± 0.09	100.0–260.0 × 15.0–60.0
3	<i>D. sphenophylla</i>	6	0	2	2	1	1	1	0	3	0	1	4	0	1	1	0	1.16 ± 0.09 × 0.41 ± 0.06	15.8–52.6 × 36.8–40.0
4	<i>D. viridiflora</i>	0	1	3	3	0	0	5	0	5	0	0	2	1	0	1	0	5.08 ± 0.66 × 3.05 ± 0.50	40.0–380.0 × 10–180.0
5	<i>Eubotryodes grayana</i>	5	1	0	1	3	2	1	0	3	0	1	3	0	4	0	0	0.89 ± 0.07 × 0.46 ± 0.10	61.9–357.1 × 23.8–57.1
6	<i>Eubotrys racemosa</i>	1	0	0	1	0	2	1	0	1	1	1	5	0	5	1	1	0.71 ± 0.12 × 0.45 ± 0.11	13.3–50.0 × 5.0–33.3
7	<i>E. racemosa</i> var. <i>elongata</i>	1	0	3	0	0	2	1	0	1	0	1	5	0	5	0	0	0.53 ± 0.06 × 0.34 ± 0.09	12.0–60.0 × 10.0–40.0
8	<i>E. recurva</i>	5	1	0	2	3	2	1	0	1	0	1	3	0	4	1	0	1.98 ± 0.14 × 0.79 ± 0.11	25.0–108.3 × 12.5–70.8
9	<i>Gaultheria acuminata</i>	4	0	0	0	0	0	1	0	0	2	1	0	0	3	0	1	0.53 ± 0.09 × 0.37 ± 0.07	6.7–83.3 × 6.5–33.3
10	<i>G. alnifolia</i>	3	0	1	1	0	0	1	0	2	1	1	0	0	2	0	0	0.71 ± 0.12 × 0.49 ± 0.06	26.9–126.9 × 7.7–50.0
11	<i>G. arnoea</i>	1	0	0	1	0	1	4	0	2	1	2	0	2	0	0	0	0.64 ± 0.05 × 0.44 ± 0.04	*
12	<i>G. anastomasans</i>	4	0	3	1	0	0	1	0	1	1	1	0	0	3	0	0	0.74 ± 0.08 × 0.51 ± 0.10	24.0–200.0 × 12.0–36.0
13	<i>G. antipoda</i>	1	0	1	0	0	0	1	0	2	1	1	0	0	3	0	1	0.56 ± 0.08 × 0.36 ± 0.04	21.4–142.9 × 10.7–50.0
14	<i>G. borneensis</i>	1	0	2	0	0	1	1	0	2	1	1	1	0	3	1	0	0.60 ± 0.05 × 0.32 ± 0.04	20.9–144.2 × 6.9–23.3
15	<i>G. bracteata</i>	4	0	1	0	0	0	1	0	2	2	1	1	0	0	0	0	0.54 ± 0.06 × 0.36 ± 0.07	14.7–132.4 × 7.4–35.3
16	<i>G. brevistipes</i>	4	0	2	0	0	0	4	0	2	0	2	2	2	0	0	0	0.55 ± 0.07 × 0.39 ± 0.07	25.0–132.5 × 6.3–37.5
17	<i>G. buxifolia</i> var. <i>buxifolia</i>	4	0	1	0	0	0	1	0	0	2	1	0	0	0	0	0	0.60 ± 0.07 × 0.47 ± 0.06	17.2–137.9 × 5.17–24.1
18	<i>G. cardiosepala</i>	1	0	1	1	1	1	3	0	2	0	2	2	2	2	0	0	0.71 ± 0.15 × 0.42 ± 0.06	12.5–140.6 × 4.7–34.4
19	<i>G. codonantha</i>	2	0	1	0	0	0	1	0	4	0	1	0	0	0	0	1	0.55 ± 0.06 × 0.33 ± 0.04	22.5–137.5 × 7.5–37.5
20	<i>G. cuneata</i>	1	0	0	0	0	0	1	0	0	2	1	1	0	3	1	0	0.59 ± 0.06 × 0.36 ± 0.05	11.6–93.0 × 4.7–23.3
21	<i>G. discolor</i>	3	0	1	0	0	0	1	0	2	1	0	2	2	4	0	0	0.46 ± 0.03 × 0.33 ± 0.07	36.6–189.8 × 9.9–53.3
22	<i>G. doctropoda</i>	1	0	0	1	1	1	3	0	4	0	2	2	2	4	0	0	0.66 ± 0.03 × 0.46 ± 0.02	48.1–166.7 × 18.5–44.4
23	<i>G. domingensis</i>	1	0	2	0	0	0	1	0	0	2	1	0	0	3	0	1	0.57 ± 0.04 × 0.39 ± 0.06	15.5–96.9 × 6.1–27.3
24	<i>G. dumicola</i> var. <i>aspera</i>	1	0	0	2	0	0	1	0	4	2	1	0	0	3	0	0	1.03 ± 0.07 × 0.34 ± 0.08	*
25	<i>G. dumicola</i> var. <i>dumicola</i>	4	0	1	0	0	0	1	0	4	0	1	0	0	3	0	1	0.54 ± 0.09 × 0.34 ± 0.04	17.6–147.5 × 11.7–41.2
26	<i>G. dumicola</i> var. <i>petanoneuron</i>	2	0	0	0	0	0	1	0	4	1	1	0	0	0	1	0	0.56 ± 0.07 × 0.36 ± 0.04	29.9–116.7 × 9.9–59.9
27	<i>G. dumicola</i> var. <i>pubipes</i>	4	0	1	2	0	0	1	0	4	2	1	0	0	0	1	1	1.05 ± 0.06 × 0.65 ± 0.02	10.0–133.3 × 10.0–40.0
28	<i>G. erecta</i>	4	0	2	0	0	0	1	0	0	2	1	0	0	0	0	1	0.49 ± 0.08 × 0.40 ± 0.10	11.8–101.1 × 2.9–35.3
29	<i>G. eriophylla</i> var. <i>mucronata</i>	4	0	1	0	0	1	1	0	0	2	1	0	0	3	0	0	0.57 ± 0.08 × 0.43 ± 0.05	8.3–110.0 × 3.3–33.3
30	<i>G. foliolosa</i>	4	0	2	0	0	0	1	0	2	0	1	0	0	3	0	0	0.55 ± 0.05 × 0.42 ± 0.05	26.7–150.0 × 6.7–36.7
31	<i>G. fragrantissima</i>	4	0	0	0	0	0	1	0	2	1	1	2	0	2	1	0	0.51 ± 0.07 × 0.32 ± 0.05	19.5–97.6 × 4.9–31.7
32	<i>G. glomerata</i>	4	0	2	0	0	0	1	0	2	2	1	3	0	0	0	1	0.51 ± 0.03 × 0.33 ± 0.06	13.9–94.4 × 2.8–30.6
33	<i>G. gracilis</i>	4	0	1	1	0	0	1	0	1	1	1	4	0	0	0	1	0.71 ± 0.08 × 0.60 ± 0.04	27.3–45.5 × 7.5–36.4
34	<i>G. griffithiana</i>	1	0	1	1	0	2	4	0	1	1	2	2	2	2	0	0	0.68 ± 0.12 × 0.39 ± 0.08	14.7–114.7 × 8.8–52.9
35	<i>G. griffithiana</i> var. <i>insignis</i>	4	0	2	0	0	0	1	0	1	0	1	0	0	0	0	1	0.57 ± 0.04 × 0.45 ± 0.05	23.5–64.7 × 2.9–26.5
36	<i>G. hispida</i>	2	0	0	0	0	0	1	0	2	1	1	1	0	3	0	0	0.60 ± 0.04 × 0.38 ± 0.03	17.4–126.5 × 2.9–26.5
37	<i>G. hispida</i>	1	0	0	1	1	1	2	0	4	1	1	2	0	2	0	0	0.97 ± 0.05 × 0.69 ± 0.03	13.5–183.8 × 5.4–51.4
38	<i>G. hookeri</i>	4	0	2	0	0	0	1	0	1	1	1	0	0	0	1	1	0.58 ± 0.13 × 0.34 ± 0.05	7.1–107.1 × 4.8–30.9
39	<i>G. howellii</i>	4	0	3	0	0	0	4	0	4	0	2	2	0	0	0	0	0.54 ± 0.09 × 0.41 ± 0.08	12.2–73.2 × 4.9–24.4
40	<i>G. humifusa</i>	1	0	1	0	0	1	2	0	0	2	0	1	0	0	1	0	0.57 ± 0.05 × 0.37 ± 0.06	39.5–118.4 × 7.9–50.0
41	<i>G. hypochlora</i>	1	0	1	0	1	1	3	0	2	0	2	2	2	3	0	1	0.59 ± 0.04 × 0.41 ± 0.02	60.3–310.3 × 12.1–65.5
42	<i>G. insipida</i>	4	0	1	0	0	0	1	0	2	0	1	3	0	0	0	0	0.46 ± 0.03 × 0.36 ± 0.04	23.5–142.1 × 5.9–47.1
43	<i>G. itatiaiae</i>	4	0	1	1	0	0	1	0	2	1	1	2	0	2	1	0	0.82 ± 0.13 × 0.59 ± 0.10	20.8–166.7 × 6.3–45.8
44	<i>G. itoana</i>	2	0	1	1	0	1	1	0	0	2	1	0	0	3	1	0	0.66 ± 0.05 × 0.42 ± 0.02	11.4–128.6 × 2.9–37.1

45	<i>G. lanigera</i>	4	0	0	0	1	1	0	0	2	1	1	2	0	2	0	1	0.58 ± 0.06 × 0.44 ± 0.07	14.3–74.3 × 4.3–20.0
46	<i>G. leucocarpa</i> var. <i>pingbienensis</i>	2	0	2	1	0	0	1	0	1	1	1	1	0	0	1	0	0.72 ± 0.12 × 0.44 ± 0.09	15.4–96.2 × 7.7–46.2
47	<i>G. leucocarpa</i> var. <i>yunnanensis</i>	2	0	2	1	0	0	1	0	1	1	1	1	0	0	1	0	0.73 ± 0.15 × 0.43 ± 0.07	15.4–105.1 × 7.7–51.3
48	<i>G. longibracteolata</i>	4	0	1	0	0	0	4	0	4	0	2	1	2	0	1	0	0.50 ± 0.07 × 0.34 ± 0.06	17.5–127.5 × 15.0–50.0
49	<i>G. macrostigma</i>	4	0	1	1	1	0	5	0	4	0	0	3	1	0	0	0	0.70 ± 0.09 × 0.52 ± 0.10	22.7–159.1 × 9.1–59.1
50	<i>G. megalodonata</i>	1	0	2	1	0	1	1	0	2	0	2	1	2	0	0	0	0.76 ± 0.08 × 0.42 ± 0.04	20.0–160.0 × 8.0–40.0
51	<i>G. mucronata</i>	1	0	2	2	1	0	1	0	0	2	1	1	0	3	1	0	1.03 ± 0.07 × 0.80 ± 0.10	27.3–136.4 × 4.5–61.4
52	<i>G. myrsinoides</i>	1	0	2	0	0	1	0	0	2	1	1	0	0	0	0	0	0.60 ± 0.09 × 0.41 ± 0.07	42.9–134.3 × 2.9–40.0
53	<i>G. myrtilloides</i>	1	0	3	1	0	1	1	0	2	1	1	0	0	3	0	0	0.88 ± 0.12 × 0.58 ± 0.08	26.1–117.4 × 6.5–43.5
54	<i>G. nummularioides</i>	0	0	3	0	0	0	5	0	4	0	0	0	1	2	0	0	0.47 ± 0.05 × 0.35 ± 0.04	18.4–123.7 × 6.6–26.3
55	<i>G. ovatifolia</i>	0	0	1	1	0	1	1	0	4	0	1	2	0	2	0	0	0.67 ± 0.05 × 0.37 ± 0.07	24.1–151.7 × 8.6–48.3
56	<i>G. phillyraeefolia</i>	4	0	1	1	0	0	1	0	1	0	1	1	0	2	1	0	0.79 ± 0.14 × 0.52 ± 0.07	31.8–140.9 × 4.5–45.5
57	<i>G. praticola</i>	4	0	0	1	1	0	1	0	0	1	1	0	0	0	0	1	0.61 ± 0.03 × 0.46 ± 0.04	13.8–113.8 × 10.3–44.8
58	<i>G. procumbens</i>	4	0	1	2	0	0	5	0	4	0	0	1	1	0	0	0	1.16 ± 0.11 × 0.84 ± 0.09	22.3–105.3 × 13.2–46.1
59	<i>G. pseudonotabilis</i>	4	0	1	0	0	1	1	0	4	1	1	2	0	0	0	0	0.43 ± 0.08 × 0.32 ± 0.06	19.2–66.7 × 2.2–26.7
60	<i>G. pumila</i>	1	0	1	1	1	0	1	0	1	2	1	3	0	4	0	0	0.87 ± 0.14 × 0.46 ± 0.08	7.1–80.9 × 2.4–28.6
61	<i>G. purpurea</i>	4	0	1	0	0	0	4	0	0	0	2	2	0	0	1	0	0.58 ± 0.09 × 0.42 ± 0.05	43.2–117.9 × 12.6–42.1
62	<i>G. pyrrolaeifolia</i>	4	0	1	0	0	0	1	0	1	1	1	0	0	0	0	1	0.59 ± 0.05 × 0.44 ± 0.04	21.9–125.0 × 7.8–59.4
63	<i>G. reticulata</i>	2	0	1	1	0	0	1	0	2	1	1	0	1	0	0	1	0.71 ± 0.08 × 0.43 ± 0.06	19.2–123.1 × 3.8–42.3
64	<i>G. rigida</i>	4	0	1	0	0	0	5	0	1	0	0	1	1	0	0	0	0.51 ± 0.04 × 0.35 ± 0.04	6.2–69.2 × 2.3–21.5
65	<i>G. schultesii</i>	4	0	1	1	0	0	1	0	2	1	0	0	0	0	1	0	0.62 ± 0.04 × 0.43 ± 0.09	13.8–96.6 × 3.4–41.4
66	<i>G. sclerophylla</i> var. <i>sclerophylla</i>	2	0	1	1	0	0	1	0	1	1	1	0	0	0	1	0	0.77 ± 0.06 × 0.49 ± 0.07	24.0–104.0 × 4.0–60.0
67	<i>G. semi-infera</i>	3	0	0	0	0	0	1	0	0	2	1	0	0	0	0	1	0.52 ± 0.10 × 0.41 ± 0.07	10.8–81.1 × 4.1–29.7
68	<i>G. shallon</i>	4	0	2	2	0	1	1	5	0	2	1	2	1	0	0	0	1.02 ± 0.12 × 0.62 ± 0.08	22.2–166.7 × 5.6–27.8
69	<i>G. sinensis</i>	1	0	1	0	1	1	1	0	0	2	1	0	2	1	0	0	0.55 ± 0.06 × 0.34 ± 0.08	33.3–116.7 × 13.3–40.0
70	<i>G. straminea</i>	4	0	1	0	0	0	1	0	2	1	1	1	0	0	1	0	0.52 ± 0.07 × 0.37 ± 0.04	20.6–102.9 × 7.4–52.9
71	<i>G. strigosa</i> var. <i>strigosa</i>	2	0	2	0	0	0	1	0	1	1	1	0	0	0	0	0	0.47 ± 0.03 × 0.27 ± 0.06	*
72	<i>G. suborbicularis</i>	1	0	0	2	1	1	2	0	4	1	1	3	0	2	0	0	1.03 ± 0.05 × 0.68 ± 0.06	30.3–74.5 × 4.2–18.4
73	<i>G. tomentosa</i>	4	0	1	0	0	0	1	0	2	1	0	0	0	0	0	0	0.58 ± 0.09 × 0.44 ± 0.04	11.1–108.3 × 4.2–33.3
74	<i>G. trichophylla</i>	1	0	1	1	1	2	5	0	1	0	0	2	1	0	0	0	0.69 ± 0.06 × 0.49 ± 0.04	25.8–225.8 × 16.1–45.2
75	<i>G. trigonoelada</i>	4	0	0	0	0	0	1	0	1	1	1	1	0	0	0	0	0.48 ± 0.07 × 0.37 ± 0.05	12.5–100.0 × 30.0–50.0
76	<i>G. vaccinioides</i>	1	0	0	1	0	0	1	0	0	2	1	1	0	0	1	1	0.75 ± 0.06 × 0.50 ± 0.06	23.3–150.0 × 3.3–20.0
77	<i>G. wardii</i>	1	0	1	0	1	2	5	1	4	0	0	2	1	2	0	0	0.45 ± 0.07 × 0.33 ± 0.05	*
78	<i>G. wardii</i> var. <i>elongata</i>	1	0	2	0	0	2	5	1	3	0	0	2	1	2	0	1	0.37 ± 0.07 × 0.26 ± 0.06	*
79	<i>Leucothoe axillaris</i>	5	1	0	2	2	2	5	0	1	0	0	0	1	0	0	0	1.33 ± 0.13 × 0.63 ± 0.14	35.1–122.8 × 23.4–93.6
80	<i>L. davisiac</i>	5	1	0	2	3	2	1	0	2	0	1	5	0	2	0	0	1.12 ± 0.14 × 0.53 ± 0.06	21.4–139.3 × 11.5–71.4
81	<i>L. fontanesiana</i>	5	1	0	2	2	2	5	0	1	0	0	3	1	4	0	0	1.31 ± 0.19 × 0.64 ± 0.08	55.0–185.0 × 25.0–100.0
82	<i>L. griffithiana</i>	5	1	0	2	3	2	1	0	2	1	0	1	5	0	3	0	1.23 ± 0.11 × 0.81 ± 0.12	17.6–147.1 × 5.9–29.4
83	<i>L. keiskei</i>	5	1	0	2	2	2	1	0	3	1	0	5	0	5	1	0	1.22 ± 0.13 × 0.33 ± 0.07	16.7–290 × 8.3–33.3
84	<i>Andromeda polifolia</i>	1	0	1	2	0	0	1	0	1	0	1	2	0	0	0	0	1.03 ± 0.07 × 0.68 ± 0.04	17.0–25.0 × 10.0–25.0
85	<i>A. polifolia</i> var. <i>glaucoaphylla</i>	1	0	1	2	0	0	1	0	1	0	1	5	0	0	0	0	1.12 ± 0.06 × 0.82 ± 0.11	18.0–27.0 × 11.0–23.0
86	<i>Zenobia pulverulenta</i>	3	0	2	1	0	0	1	0	0	2	1	1	0	0	1	0	0.89 ± 0.15 × 0.54 ± 0.10	23.8–142.9 × 11.9–57.1
87	<i>Satyria meiantha</i>	1	0	1	2	0	0	1	0	1	0	1	3	0	4	0	0	1.15 ± 0.21 × 0.52 ± 0.04	52.0–104.0 × 20.0–80.0
88	<i>S. panamensis</i>	1	0	1	2	0	0	1	0	1	0	1	3	0	4	0	0	1.27 ± 0.12 × 0.52 ± 0.03	5.7–50.8 × 5.6–36.7
89	<i>S. panurensis</i>	1	0	0	1	0	0	1	0	2	0	1	3	0	4	0	0	0.69 ± 0.10 × 0.43 ± 0.07	30.8–164.1 × 25.6–71.8
90	<i>S. warszewiczii</i>	1	0	0	1	0	0	1	0	2	0	1	3	0	4	0	0	0.77 ± 0.14 × 0.46 ± 0.09	31.8–136.4 × 11.4–59.1

*Boundary between epidermal cells too indistinct to measure.

Figure 2. Seeds of Gaultherieae and related genera A, *Leucothoe axillaris*, showing areolate ornamentation and suborbicular, square or nearly so, or polygonal cells. B, *Gaultheria insipida*, C, *Satyria warszewiczii*, showing elongate epidermal cells and foveolate periclinal wall ornamentation. D, *Eubotryoides grayana*, showing sublinear epidermal cells. E, *Leucothoe keiskei*, showing sublinear epidermal cells and reticulate periclinal wall ornamentation. F, *G. codonantha*, showing nubiform epidermal cells. G, *G. dumicola* var. *dumicola*, showing nubiform epidermal cells and faintly granulate periclinal wall ornamentation. H, *G. cuneata*, showing granulate periclinal wall ornamentation and scalariform anticlinal wall ornamentation. I, *G. mucronata*, showing a distinct suture between epidermal cells. J, *G. ovatifolia*, showing verrucate periclinal wall ornamentation. K, *Diplycosia memecyloides*, showing reti-rugulate periclinal wall ornamentation. L, *D. sphenophylla*, showing raised-cross-banded anticlinal wall ornamentation. M, *G. nummularioides*, showing verrucate anticlinal wall ornamentation. N, *L. griffithiana*, showing scalariform anticlinal wall ornamentation. O, *Eubotrys racemosa* var. *elongata*, showing reticulate anticlinal wall ornamentation. P, *Eubotryoides grayana*. Q, *L. fontanesiana*. R, *L. griffithiana*, showing a distinctly protuberant hilum region. S, *G. wardii*, showing projections on seed surface (black arrow). T, *G. pyrolifolia*, showing thin interweaving surface threads. U, *L. axillaris*, showing seed outline. V, *G. amoena*, showing epidermal cells. W, *Eubotrys recurva*. X, *G. praticola*. Y, *G. macrostigma*, showing the outline of a seed.

seed coats of *G. hispidula* Muhl. (Fig. 1Q), *G. humifusa* Rydb. (Fig. 1R) and *G. suborbicularis* W.W.Sm. (Fig. 1S) are faintly reticulate. Faintly reticulate laterally + areolate dorsiventrally ornamentation occurs in three species of *G.* series *Trichophyllae* Airy Shaw (*sensu* Middleton, 1991a): *G. cardiosepala* Hand.-Mazz., *G. dolichopoda* Airy Shaw and *G. hypochlora* Airy Shaw (Fig. 1T). Samples of some Asian species of *Gaultheria* (e.g. *G. brevistipes* (C.Y.Wu & T.Z.Hsu) R.C.Fang, *G. griffithiana* Wight (Fig. 1U), *G. longibracteolata* R.C.Fang, *G. purpurea* R.C.Fang and *G. howellii* (Sleumer) D.J.Middleton) have reticulate laterally + areolate dorsiventrally ornamentation. Areolate ornamentation occurs in some species of *Diplycosia*, *Gaultheria* (particularly in the Asian species, e.g. *G. trichophylla* Royle in Figure 1V; also in *G. macrostigma* (Colenso) D.J.Middleton from New Zealand) and *Leucothoe*.

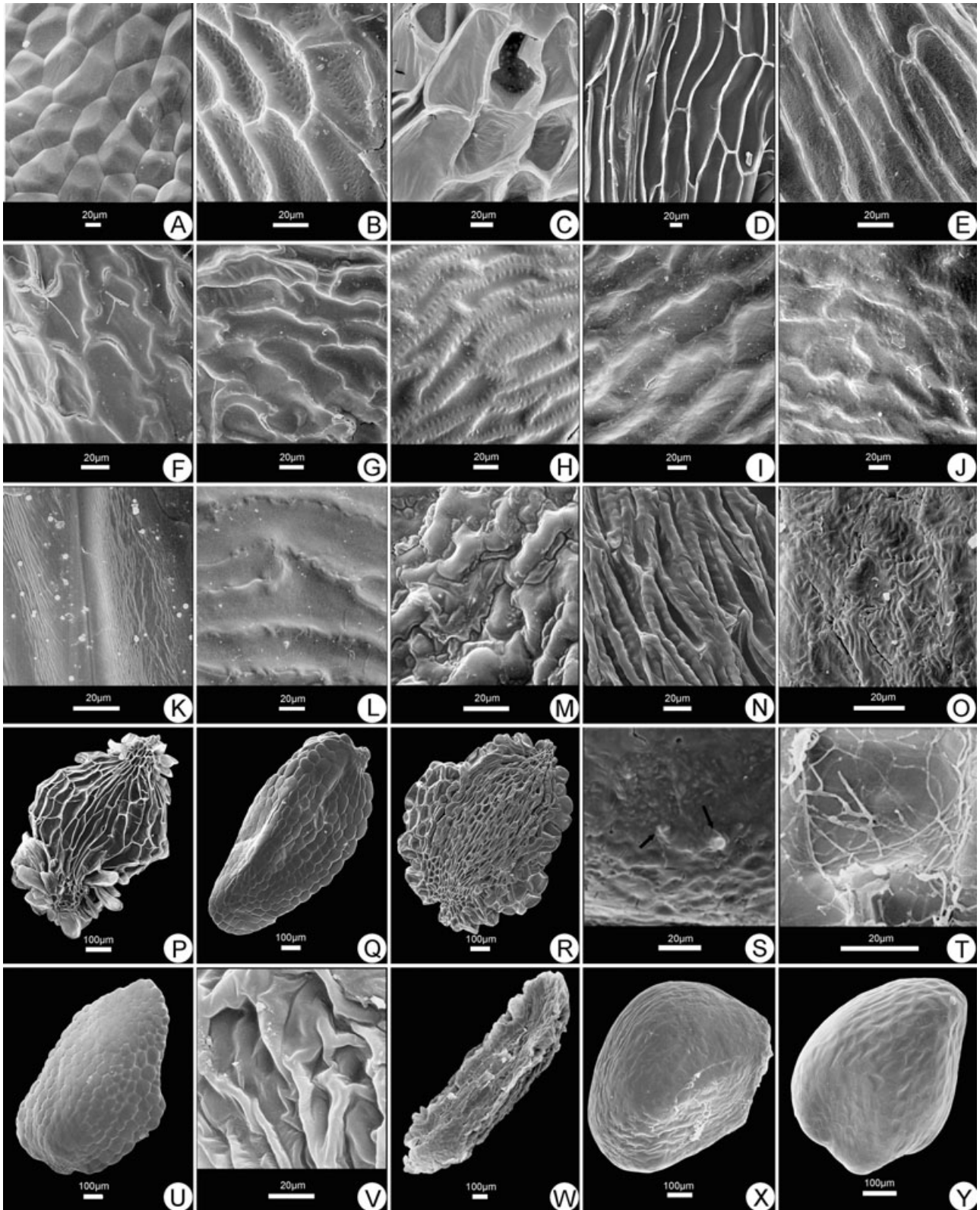
Epidermal cell characters

The epidermal cells of the seed coats in Gaultherieae exhibit variation in cell shape, cell size, murus wall thickness, the presence or absence of a suture between cells and in the pattern (convex or concave) and ornamentation of the cell walls. The shape of epidermal cells can be categorized as follows: narrowly and shortly elongate; suborbicular, square or nearly so, or polygonal; elongate; sublinear; and nubiform. Narrowly and shortly elongate cells occur in some species of *Gaultheria* [e.g. *G. pumila* (L.f.) D.J.Middleton (Fig. 1W)] and *Zenobia* (Fig. 1X). *Andromeda* (Fig. 1Y), *Eubotrys*, *Zenobia*, many species of *Gaultheria* and some species of *Diplycosia*, *Leucothoe* (Fig. 2A) and *Satyria* have suborbicular, square or nearly so, or polygonal cells. Elongate cells occur in some species of *Gaultheria* (Fig. 2B), *Satyria* (Fig. 2C) and some species of *Leucothoe*, whereas sublinear cells occur in *Chamaedaphne* (Fig. 1D), most species of *Diplycosia* (Fig. 1N), *Eubotryoides* and *Leucothoe* (Fig. 2D, E) and *G. wardii* var. *elongata*

(Fig. 1P). Nubiform cells only occur in some species of *Gaultheria* (Fig. 2F, G).

Epidermal cell size is not correlated with seed length (Table 2). In general, all epidermal cells sampled are (long axis) 5.7–80.0 × (short axis) 2.2–180.0 μm. Murus wall thickness can be categorized as follows: thin-walled (lumen width >> murus width, e.g. Fig. 2D, G), medium-walled [lumen width ≈ 2(–5) × murus width, e.g. Fig. 1B] and thick-walled (lumen width < 2 × murus width, e.g. Fig. 1W, X). Wall thickness is usually correlated with epidermal cell shape and seed colour. Sublinear and nubiform shapes are often thin-walled. Cells with suborbicular, square or nearly so, polygonal or elongate shapes are often thin- or medium-walled, whereas narrowly and shortly elongate cells are thick-walled. Thin-walled cells tend to be lighter coloured (e.g. *Leucothoe*, with yellow seeds, and some Asian species, with light brown seeds), whereas thick-walled cells tend to be darker coloured (e.g. *G.* section *Pernettya*, with light brown to dark brown seeds). Most samples possess a distinct suture between epidermal cells, as in *Chamaedaphne*, *Diplycosia*, *Leucothoe*, *Zenobia* and some species of *Gaultheria* (Fig. 2H, I). Seeds with reticulate ornamentation have concave periclinal walls and convex anticlinal walls, whereas those with areolate ornamentation have convex periclinal walls and concave anticlinal walls.

Periclinal wall ornamentation can be categorized as faintly granulate (*G. dumicola* W.W.Sm. var. *dumicola*, Fig. 2G), granulate (*G. cuneata* Bean, Fig. 2H), verrucate (*G. ovatifolia* A.Gray, Fig. 2J), foveolate (*G. insipida* Benth. and *Satyria warszewiczii* Klotzsch, Fig. 2B, C), reti-rugulate (*D. memecyloides*, Fig. 2K) and reticulate (*Leucothoe keiskei* Miq., Fig. 2E). Anticlinal wall ornamentation can be categorized as psilate to faintly granulate (*G. insipida* and *G. codonantha* Airy Shaw, Fig. 2B, F), raised-cross-banded (*D. sphenophylla*, Fig. 2L), verrucate (*G. nummularioides*, Fig. 2M), scalariform (*G. cuneata* and *Leucothoe griffithiana* C.B.Clarke, Fig. 2H, N), foveo-



late (*G. pumila*, Fig. 1W) and reticulate (*L. keiskei* and *E. racemosa* var. *elongata*, Fig. 2E, O).

Specialized structures

The hilum region of most samples is flat or concave (Fig. 1G, H, I). It is distinctly protuberant in *Eubotrys* (Fig. 1E), *Eubotryoides* (Fig. 2P), all species of *Leucothoe* (e.g. Fig. 2Q, R) and a few species of *Gaultheria* [*G. griffithiana* (Fig. 1U), *G. trichophylla* (Fig. 1V) and *G. wardii* Marq. & Airy Shaw (Figs 1P, 2S)] and indistinctly protuberant in, for example, *Gaultheria* series *Trichophyllae*. Seeds with a dorsiventral wing occur in *D. sphenophylla* (Fig. 1N), the Asian species of *Gaultheria* [especially in series *Trichophyllae* (Fig. 1V)] and some species of section *Pernettya*, whereas *Eubotryoides grayana* (Fig. 2P) and all species of *Leucothoe* [e.g. *L. fontanesiana* (Steud.) Sleumer, Fig. 2Q and *L. griffithiana*, Fig. 2R] possess a lateral wing. The lateral wing often contains bulging cells (Figs 1O, 2P, R). *Eubotryoides grayana* (Fig. 2P) has distinctly larger bulging cells in the hilum and chalaza region. Unique projections on the seed surface are restricted to *G. wardii* (Figs 1P, 2S). The seeds of some samples (e.g. *G. hookeri* C.B. Clarke; *G. pyrolifolia* Hook. f. ex C.B. Clarke; Fig. 2T) have many thin interweaving threads above the lumen, which appear to emanate from the seed surface.

NMDS ANALYSIS

Stress index NMDS values between 0.05 and 0.2 suggest that the distribution of points on the NMDS plot accurately estimates the actual distances between the studied samples (Clarke & Warwick, 1994). The stress index of our analysis was 0.163, suggesting that the NMDS plot is reliable. On the coordinate 1/2 plot, almost half of the species sampled (mainly those in *Gaultheria* and *Zenobia*) cluster near the Coordinate Origin (CO) in quadrant III and form a domain (Fig. 3). The species of *Andromeda*, *Chamaedaphne* and *Satyria* are relatively close but located around the fringe of this domain. Samples of *Eubotrys* in our study (except *E. recurva*) are located in a distinct region of the plot. Species of *Diplycosia*, *Gaultheria* and *Leucothoe* are more variable than those of other genera. The three samples of *Diplycosia* are scattered among quadrants I, II and IV. The species of *Leucothoe* occur in quadrants I and IV, but in a distinct region of the plot. Samples of some sections of *Gaultheria* group near each other on the NMDS plot. Among the species of *G.* section *Brossaea* (L.) Middleton, the largest section of *Gaultheria*, most species from subsection *Dasyphyta* Middleton overlap with those from subsection *Botryphoros* Middleton. They mostly occur in quadrant III. The species of *G.* section *Monoanthea* Middleton mostly occur in

quadrant III, but two species, *G. amoena* A.C.Sm. and *G. nummularioides*, are distantly located in quadrants I and II. The species of *G.* section *Amblyandra* Airy Shaw occur near the CO in quadrant I. Compared with other species of *Gaultheria*, those of *G.* section *Chiogenopsis* Middleton are more distantly located in quadrants I and IV. The species of *G.* section *Pernettya* mostly occur in quadrant III; two species occur in quadrants I and II.

CHARACTER EVOLUTION

Evolutionary patterns of six seed characters with the least homoplasious changes have been studied. For the seed shape character, there are 16 equally parsimonious reconstructions. Subglobose or nearly ellipsoid seed shape could be the ancestral condition of the Gaultherieae + Andromedeae clade. The ellipsoid–mucronate shape is gained along the branches to *Leucothoe* + *Eubotryoides* in clade VI and *Eubotrys recurva* in clade V. The evolution of seed shape is equivocal throughout much of the wintergreen clade (I + II + III + IV) as inferred from parsimony optimization. In eight reconstructions, obliquely pyramidal shape is a synapomorphy for the wintergreen clade. In all reconstructions it is a synapomorphy for clade III, clade IV and a subclade (some of the South American taxa) of clade I, respectively (Fig. 4A).

Flattened seeds are a synapomorphy for clade VI (*Eubotryoides* + *Leucothoe*) and an autapomorphy for *Eubotrys recurva*. Flattened seeds evolved prior to the origin of the wintergreen group and were later lost. A concave pattern is a synapomorphy for the wintergreen clade (Fig. 4B).

Seeds of the most recent common ancestor of Gaultherieae are inferred to have generally larger size (at least seed length > 0.6 mm). Small seeds (seed length < 0.6 mm) are gained along the branches to clades I, II and III, with reversals to medium–small seeds (> 0.6 mm, < 1.0 mm) along the branches to some species in these three clades and to medium–large seeds (> 1.0 mm) along the branch to *G. mucronata* Hook. & Arn. Small seed size is a synapomorphy for clade I + II in six equally-parsimonious reconstructions and a synapomorphy for clade I + II + III in two reconstructions (Fig. 4C).

Unwinged seeds are inferred as the ancestral state in the Gaultherieae + Andromedeae clade, whereas seed wings with lateral bulging cells are gained along the branches to clade VI and the branch leading to *E. recurva*. Seed wings with lateral bulging cells are subsequently lost in *Leucothoe fontanesiana*. A dorsiventral wing is derived in some species of the wintergreen group (*G. pumila* and *G. sinensis* Anth.). Only two equally parsimonious reconstructions exist in seed wing evolution. A dorsiventral wing has

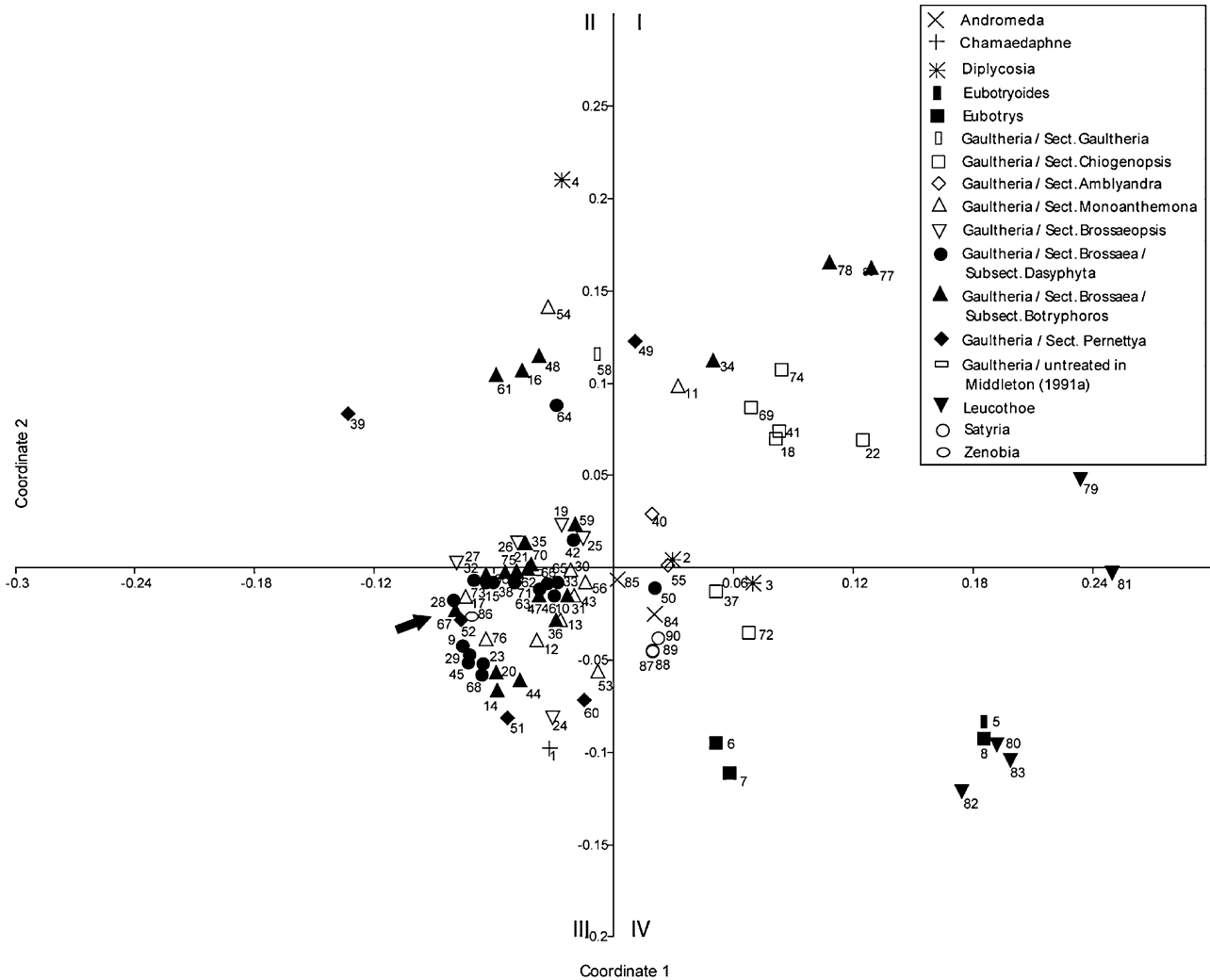


Figure 3. Non-metric multidimensional scaling (NMDS) plot of 14 seed characters sampled for 83 species of Gaultherieae and related genera. The number of each sample is the same as that in Table 1. The black arrow shows the location of most samples of Gaultherieae, in quadrant III.

evolved four or five times from the unwinged state. The presence of a dorsiventral wing is a synapomorphy for the clade comprising three *Gaultheria* species from section *Pernettya* in clade I (Fig. 4D).

A planar (not protuberant) hilum region is inferred to be the ancestral condition in the Gaultherieae + Andromedeae clade and a distinctly protuberant hilum is gained along the branches leading to clade VI (*Leucothoe*), *Eubotrys*, *G. griffithiana* and *G. wardii*. An indistinctly protuberant hilum region has evolved several times within the wintergreen group (Fig. 4E).

There are 12 equally parsimonious reconstructions of seed primary ornamentation evolution. The most recent common ancestor of Gaultherieae is inferred to have had reticulate primary ornamentation. The

areolate ornamentation is gained independently along the branches leading to clades II and IV and to *L. fontanesiana* (in clade VI), with reversals to reticulate ornamentation in some members of clade II (*G. dumicola*, *G. fragrantissima* Wall. and *G. hookeri*) and clade IV (*G. leucocarpa* Bl.). Areolate ornamentation has evolved three to six times from a reticulate ornamentation. A unique change to the faintly reticulate ornamentation from an equivocal state occurs in *G. hispidula*, whereas another unique change to the regulate–reticulate ornamentation from reticulate ornamentation occurs in *C. calyculata*. In four reconstructions, areolate ornamentation is a synapomorphy for clade II. In nine reconstructions, areolate ornamentation is a synapomorphy for clade IV (Fig. 4F).

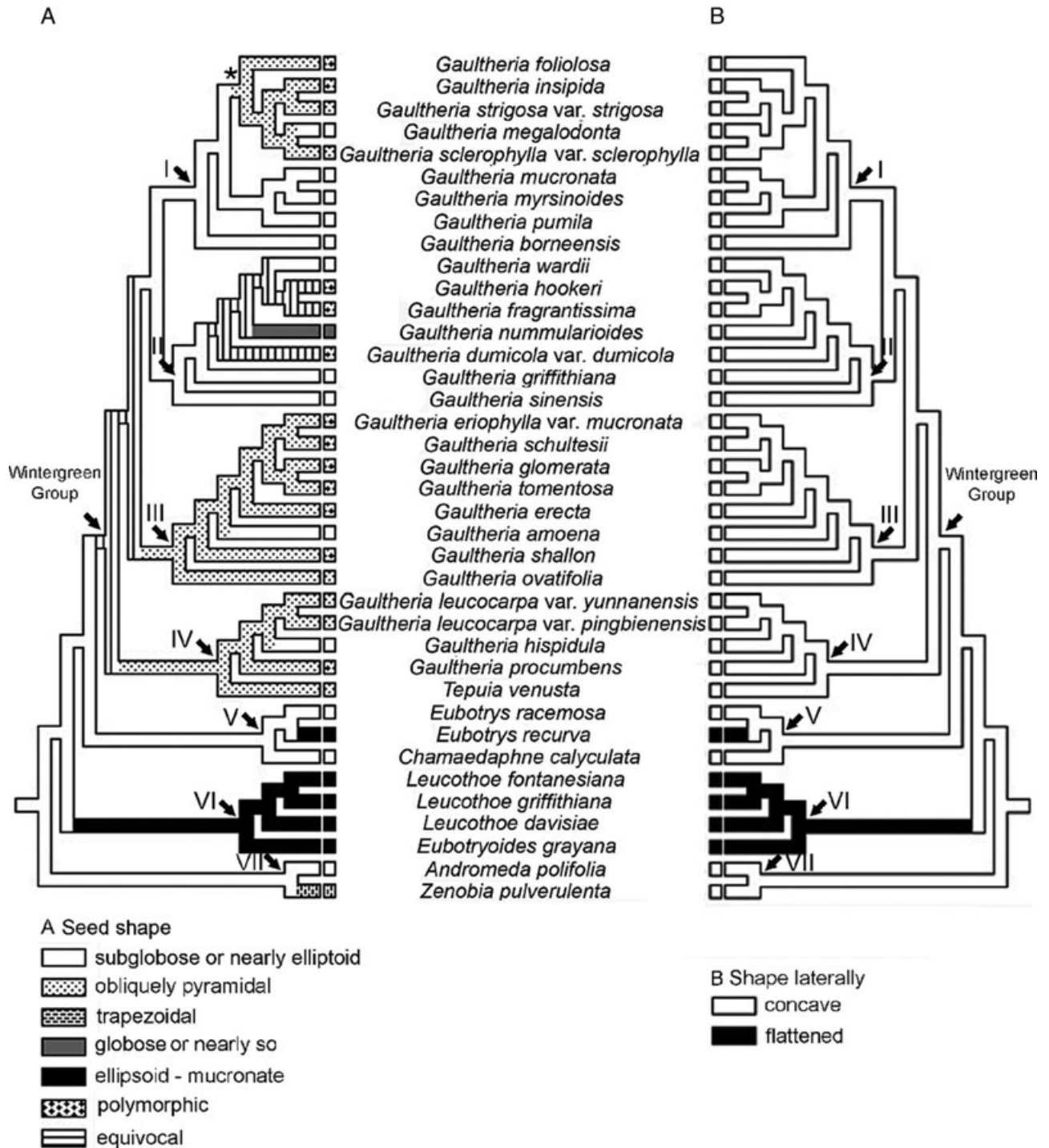


Figure 4. Seed characters of 38 samples of Gaultherieae and related genera plotted on the revised molecular phylogenetic tree of Bush *et al.* (2009). A, seed shape [character 1 in Table 1; some of South American taxa in clade I are denoted by * (see text)]. B, shape laterally (character 2 in Table 1). D, seed wing (character 5 in Table 1, section *Pernettya* in clade I denoted by *). E, hilum region (character 6 in Table 1). F, seed primary ornamentation (character 7 in Table 1); data for *Tepuia* from Luteyn (1995).

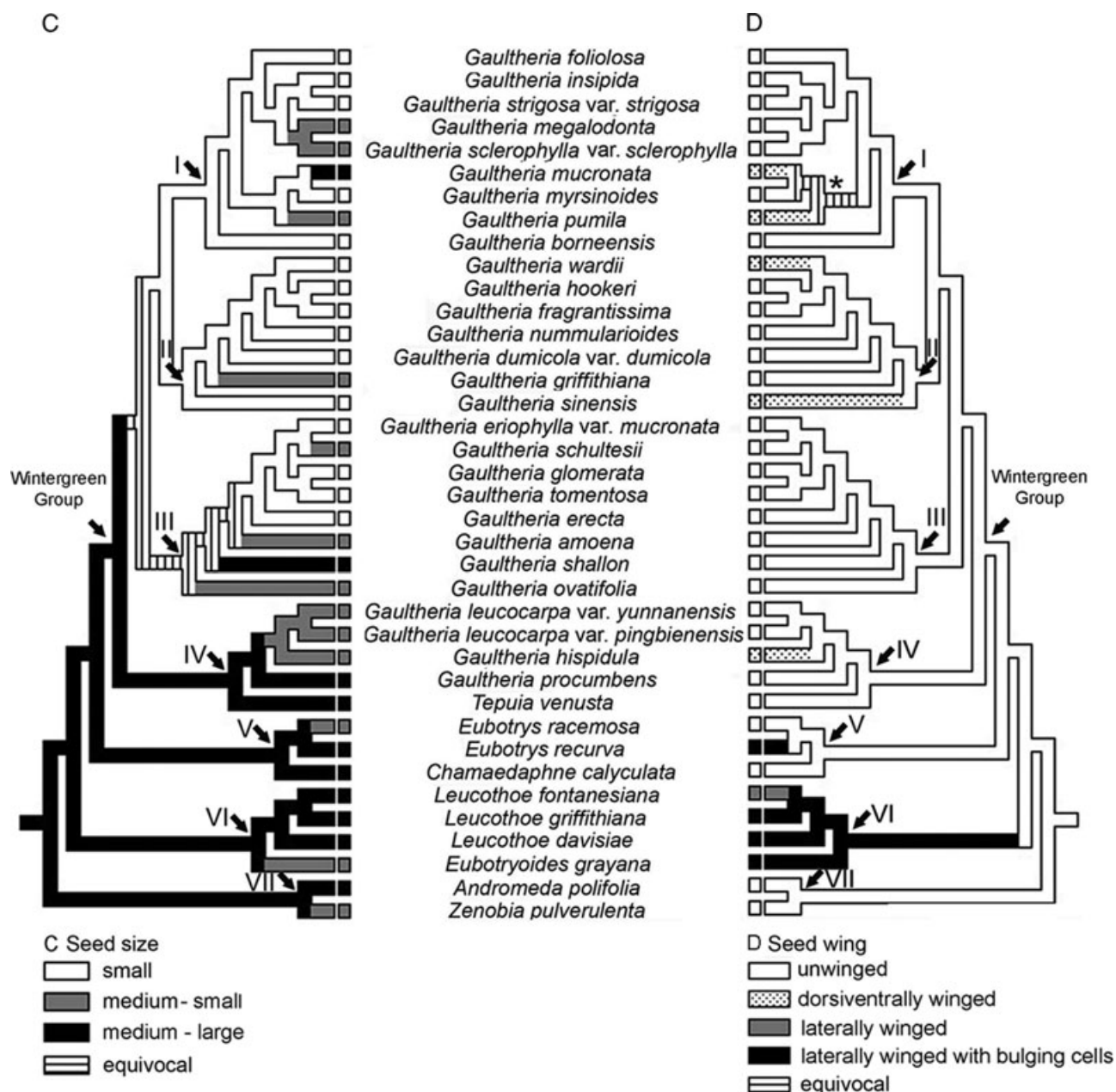


Figure 4. Continued

DISCUSSION

DELIMITATION OF THE GENERA OF GAULTHERIEAE AND RELATED GENERA

Andromeda, *Chamaedaphne*, *Satyria* and *Zenobia* each have unique or consistent seed characters of significance for their generic delimitation. Seeds of *Andromeda* are characterized by the combination of nearly ellipsoid shape, light brown colour, medium-large size and cells of the seed coat that are regularly polygonal and uniquely subdivided into four or

five cells, each with delicate muri (Fig. 1H, Y). *Chamaedaphne* is unique in its regulate-reticulate primary ornamentation with distinct sublinear epidermal cells (Fig. 1D). Species of *Satyria* are characterized by the combination of an ellipsoid seed shape and foveolate ornamentation of periclinal and anticlinal walls and a unique thin membrane covering the concave lumen of each epidermal cell (Fig. 2C). Seeds of *Zenobia* are characterized by the combination of a broad ventral region and thick-walled, narrowly and shortly elongate epidermal cells (Fig. 1X).

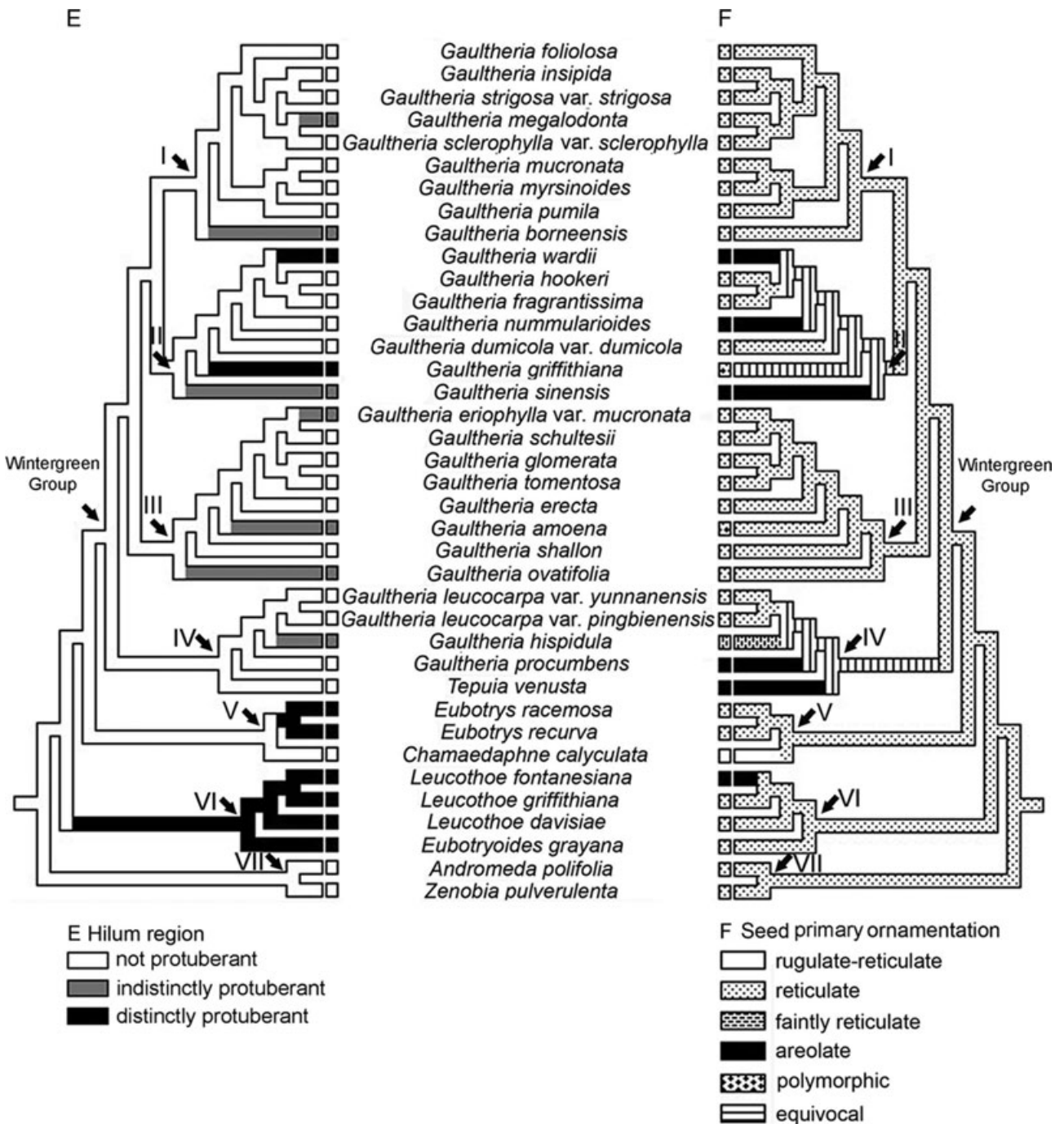


Figure 4. Continued

Diplycosia, *Gaultheria* and *Leucothoe* each exhibit wide variation in seed characters. Locations of *Diplycosia* on the NMDS plot are relatively far from one another. Section *Pernettya* is located mainly near quadrant III. Members of *Leucothoe* are the earliest-diverging taxa within Gaultherieae (Bush *et al.*, 2009). Seed morphology in this genus exhibits the highest diversity among all the seeds studied, even

although it is a small genus with only five species. It still can be distinguished by a set of seed characters: yellow seed colour, medium–large seed size, distinctly protuberant hilum and thin-walled sublinear cells. The NMDS locations of the species of *Leucothoe* are relatively isolated from other genera, especially *L. axillaris* D. Don (no. 79, Fig. 2U) and *L. fontanesiana* (no. 81, Fig. 2Q).

INFRAGENERIC AFFINITIES IN *GAULTHERIA*
AND *LEUCOTHOE*

Gaultheria seed morphology provides evidence for the affinities of some sections of *Gaultheria sensu Middleton (1991a)*

Series *Trichophyllae* in section *Chiogenopsis*, a small-leaved group with solitary flowers occurring in alpine areas of East Asia, is characterized by the following combination of seed characters: subglobose shape, yellow to light brown colour, presence of a dorsiventral wing, a more or less protuberant hilum region, laterally reticulate and dorsiventrally areolate or completely areolate primary ornamentation, verrucate periclinal wall, and the lack of a suture between epidermal cells. The close affinities among the species of this series are supported by their close locations in quadrant I of the NMDS plot (Fig. 3, nos 18, 22, 41, 69 and 74).

Gaultheria hispidula (Fig. 1Q) and *G. suborbicularis* (Fig. 1S) are both from series *Hispidulae* Airy Shaw of section *Chiogenopsis*. They have a disjunct distribution (Eastern Asia and North America) and are distinct in fruit colour (the former is white and the latter red). Their seed similarity is demonstrated by their close proximity in the NMDS plot (nos 37 and 72). These two species have seeds that are similar to those of series *Trichophyllae*, such as subglobose seed shape, a dorsiventral wing, a protuberant hilum region and verrucate periclinal cell walls, but the most recent molecular study by Bush *et al.* (2009) shows that the two series have distant phylogenetic positions. The main difference between the seeds of these two groups is that those of series *Hispidulae* have a reticulate primary ornamentation and a distinctly nubiform cell shape, whereas those of series *Trichophyllae* usually have an areolate primary ornamentation and polygonal cell shape.

Gaultheria ovatifolia (Fig. 2J) and *G. humifusa* (Fig. 1R), both of section *Amblyandra* and native to the western USA, cluster near each other on the NMDS plot (in quadrant I, near the CO, nos 40 and 55). The seeds of both species have a light brown colour, an indistinctly protuberant hilum region, a nubiform epidermal cell shape and granulate periclinal wall ornamentation.

Half of the species of *Gaultheria* are included in section *Brossaea* (Middleton, 1991a). This section consists of two subsections, *Dasyphyta* and *Botryphoros*. Subsection *Dasyphyta* is distributed nearly throughout the Americas, whereas subsection *Botryphoros* ranges from Asia to Australia and temperate South America. The species of subsection *Dasyphyta* are located closer to each other on the NMDS plot than those of subsection *Botryphoros*. High seed diversity occurs in the Himalayan species of *Botryphoros*; these species are mainly characterized by an areolate

primary ornamentation and are scattered across quadrant I.

The species of section *Monoanthemona* are in close proximity in the NMDS plot, except for *G. amoena* (no. 11, Fig. 2V) and *G. nummularioides* (no. 54, Fig. 2M). Most samples have a reticulate primary ornamentation and faintly granulate periclinal wall.

All samples of *G.* section *Brossaeopsis* Airy Shaw are consistent in their reticulate primary ornamentation and nubiform epidermal cell shape and are restricted to quadrant III in the NMDS plot (nos 19, 24, 25, 26 and 27).

Chamaedaphne, Eubotryoides, Eubotrys and Leucothoe

Molecular evidence (*matK*, *ndhF* and internal transcribed spacer) supports a clade comprising *Leucothoe davisiae* Torr., *L. fontanesiana*, *L. griffithiana* and *Eubotryoides grayana* as the first-diverging lineage within Gaultherieae, and a second-diverging clade of *Eubotrys racemosa* Nutt. + *E. recurva* and their sister lineage *Chamaedaphne* (Bush *et al.*, 2009). Seed of these genera exhibit wide diversity in seed characters. However, consistency in all observed seed characters except periclinal and anticlinal wall ornamentation [faintly granulate in *L. axillaris* (Fig. 2U) vs. foveolate in *L. fontanesiana* (Fig. 2Q)] exists in this first-diverging lineage. This similarity is reflected by their close proximity to each other in the NMDS plot.

The clade comprising *E. racemosa* (Fig. 1E) and *E. recurva* (Fig. 2W) has a distinct seed shape, seed size and epidermal cell characters. *Eubotrys racemosa* possesses a subglobose or nearly ellipsoid and concave shape, medium–small size and reticulate periclinal/anticlinal wall ornamentation and lacks wings, whereas *E. recurva* has an ellipsoid–mucronate and flattened shape, medium–large size, foveolate periclinal/anticlinal wall ornamentation and wings. *Eubotryoides grayana* (no. 5, Fig. 2P) and *L. keiskei* (no. 83, Fig. 1M), both of which are endemic to Japan, are close to *E. recurva* (no. 8, Fig. 2W), *L. davisiae* (no. 80, Fig. 1O) and *L. griffithiana* (no. 82, Fig. 2R) in the NMDS plot. They all have an ellipsoid–mucronate shape, yellow colour, medium to large size, flattened shape, a distinctly protuberant hilum region and thin-walled cells. As indicated by mapping characters onto the molecular phylogenetic tree, the seed similarities between *E. recurva* and the taxa of the *Eubotryoides* + *Leucothoe* clade appear to result from either convergent evolution (e.g. Fig. 4A, B, D, E) or symplesiomorphies (e.g. Fig. 4C, F).

POTENTIAL SYNAPOMORPHIES FOR THE MAIN CLADES
OF THE GAULTHERIEAE

Some seed character states were found to be likely synapomorphies for the main clades of Gaultherieae.

The clade comprising *Eubotryoides* + *Leucothoe* (clade VI) is strongly supported by the following synapomorphies: ellipsoid–mucronate shape, flattened structure, presence of a lateral wing and a distinctly protuberant hilum region. The latter is also a synapomorphy for the genus *Eubotrys* in clade V. An ellipsoid–mucronate shape, laterally flattened shape and presence of a lateral wing with bulging cells are autapomorphies for *E. recurva*. An obliquely pyramidal seed shape was found to be a potential synapomorphy for the winter-green group (clade I + II + III + IV). Small seed size is a potential synapomorphy for the clade comprising clades I + II or the clade comprising clades I + II + III, which are composed of East Asian (clade II) and most American (clades I, III) species of *Gaultheria*. Areolate primary ornamentation is a potential synapomorphy for clade II (East Asian taxa) and clade IV (some *Gaultheria* + *Tepuia*), respectively.

The shape of seed epidermal cells was suspected to be an important taxonomic character in Gaultherieae by Stevens (1970), Judd (1979) and Middleton (1991b). Our study confirms the taxonomic importance of this character at both the interspecific (e.g. species within *Leucothoe*) and infraspecific levels (e.g. *G. dumicola* and its varieties); see Table 2. However, it shows little phylogenetic significance at the deeper nodes in the phylogeny of Gaultherieae because of high levels of ambiguity in character state reconstruction.

COMMENTS ON SEED MORPHOLOGY AND ITS ECOLOGICAL SIGNIFICANCE

Besides the systematic significance of seed morphology, we found that various aspects of seed structure are correlated with the geographic distribution of species in Gaultherieae. Species from eastern Asia, North America and the Pacific are more diverse than those from Central and South America in seed shape, presence or absence of a wing, primary ornamentation and epidermal cell ornamentation. Species in the eastern Himalaya, with approximately 15% of the species in the tribe, are particularly diverse.

Middleton (1991b) postulated that effective and long-range dispersal in *Gaultheria* is particularly important for these pioneer plants. Seeds of most species of Gaultherieae are minute (only 0.37×0.26 to 1.98×0.84 mm in size), dry and numerous in each fruit, and thus have an 'r'-selected strategy of reproduction (Pianka, 1970). For instance, two Asian pioneer plants, *G. fragrantissima* and *G. sinensis* could have high seed agility.

Variation in the shape of seeds is most often related to differing modes of their dispersal (Harper, Lovell & Moore, 1970) and thus the high seed variation observed here suggests that there are different adaptations for seed dispersal among the members of

Gaultherieae. Species of *Leucothoe* have winged seeds and a dry fruit, a probable adaptation for wind dispersal (Middleton, 1991b). The laterally positioned bulging cells in some seeds of *Leucothoe* documented here may enhance this adaptation. Species of Gaultherieae with fleshy fruit are generally thought to be dispersed by birds, insects or mammals (Airy Shaw, 1941; Sleumer, 1967; French, 1992; Duthie, Gibbs & Burns, 2006). Nonetheless, Middleton (1991c) had a contrary opinion that animals might not disperse *Gaultheria* because many fruits were observed to be old and shrivelled on the plants. The fruit of most of these species is dehiscent (a capsule), the fleshiness being confined to the surrounding persistent calyx. Some of the species from windswept alpine areas, such as those of *G. series Trichophyllae*, *G. praticola* C.Y.Wu (Fig. 2X) and *G. macrostigma* (Fig. 2Y), were found to possess a dorsiventral wing, suggesting that these species may be dispersed at least in part by wind.

One species with a thin fruit wall, *G. wardii* C. Marquand & Airy Shaw, has projections on its seeds. These projections, like those observed in other plant groups (e.g. Harper *et al.*, 1970) may enhance dispersal through attachment to the body of passing animals. Alternatively, the projection could serve as a method to avoid predation.

We found that several species, such as *G. hookeri* and *G. pyrolifolia*, have interweaving threads that appear to emanate from the seed surface, even although these seeds were treated chemically to remove surface impurities prior to examination under microscopy. The function of these threads is unknown, but could be related to seed dispersal.

Satyria spp. possess a membranous layer covering the lumen of each epidermal cell. The function of this membrane is unknown, but could be an adaptation for water dispersal or the regulation of environmental conditions, such as humidity.

CONCLUSION

Our study demonstrates that the seeds of Gaultherieae display high diversity in shape, colour, size, wing, hilum region, seed primary ornamentation and epidermal cell characters, and some species have specialized structures. Based on the NMDS analysis, seed characters provide insight into intergeneric and infrageneric delimitation within the Gaultherieae. Seeds of *Andromeda*, *Chamaedaphne*, *Satyria* and *Zenobia* are all unique or consistent at the genus level, whereas those of *Diplycosia*, *Gaultheria* and *Leucothoe* exhibit wide variation. *Leucothoe* displays the highest diversity in seed coat among all genera studied even although it is a small genus. Seed morphology also provides evidence for some infrageneric affinities of *G. sensu* Middleton (1991a). Seed similarities occur

among each of the samples of series *Trichophyllae*, series *Hispidulae*, *G. ovatifolia* and *G. humifusa* in section *Amblyandra*, subsection *Dasyphyta* (section *Brossaea*), section *Monoanthea* (except for *G. amoena* and *G. nummularioides*) and section *Brossaeopsis*, whereas high seed diversity occurs in the Himalayan species of subsection *Botryphoros* (section *Brossaea*). A monophyletic group comprising *L. davisiae*, *L. fontanesiana*, *L. griffithiana* and *Eubotryoides grayana* is also consistent in all observed seed characters except periclinal and anticlinal wall ornamentation. The two species of *Eubotrys* have divergent seed types and *E. recurva* converges on *Leucothoe* in most seed characters. Seed similarities between *E. recurva* and the taxa of *Eubotryoides* + *Leucothoe* appear to result from convergent evolution. Some character states were detected to be likely synapomorphies for some clades of Gaultherieae, such as ellipsoid-mucronate shape, flattened structure, presence of a lateral wing and a distinctly protuberant hilum region for *Eubotryoides* + *Leucothoe*, the last of these for the monophyletic genus *Eubotrys* and an obliquely pyramidal seed shape for the wintergreen group. In our study, epidermal cell shape shows little phylogenetic significance at the deeper nodes in the phylogenetic tree for Gaultherieae. Eastern Asian, North American and the Pacific species are more diverse than those from Central and South America in seed shape, wing presence vs. absence and coat ornamentation. Seed variation in our study implies that there are different adaptations for seed dispersal. For instance, a dorsoventral wing suggests that these species may at least in part be dispersed by wind. The membrane covering the lumen of each epidermal cell in *Satyria* spp. could be an adaptation for water dispersal or the regulation of humidity. Projections on seed surface of *G. wardii* may enhance dispersal or deter predation. Because of their reproductive and dispersal functions, seeds characters may provide insights into biogeographical studies of Gaultherieae.

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 3. *D. sphenophylla* Sleumer, *J. H. Beaman* 9842 (CAS);
 4. *D. viridiflora* DC., *S. G. Tang* 6946 (CAS);
 5. *Eubotryoides grayana* Maxim., *H. EHOK* 92 (CAS);
 6. *Eubotrys racemosa* Nutt., *W. R. Dudley* s.n. (CAS);
 7. *E. racemosa* Nutt. *elongata* (Small) Small, *29 April 1941, POS.* (CAS);
 8. *E. recurva* (Buckl.) Britt., *J. A. Churchill* 93905 (CAS);
 9. *Gaultheria acuminata* Cham. & Schldtl., *M. Sousa S. et al.* 9367 (CAS);
 10. *G. alnifolia* (Dunal) A.C.Sm., *L.J.Dorr et al.* 5122 (CAS);
 11. *G. amoena* A.C.Sm., *J. L. Luteyn & H. Mogollon* 15333 (CAS);
 12. *G. anastomosans* (L.) H.B.K., *J. L. Luteyn et al.* 13014 (CAS);
 13. *G. antipoda* G Forst., *May 1959, J. D. Reid* s.n. (CAS);
 14. *G. borneensis* Stapf, *Y. Ando et al.* 497 (KUN);
 15. *G. bracteata* (Cav.) G.Don, *J. L. Luteyn & E. Cotton* 11316 (CAS);
 16. *G. brevistipes* (C.Y.Wu & T.Z.Hsu) R. C. Fang, *L. Lu et al.* LL-07300;
 17. *G. buxifolia* Willd. var. *buxifolia*, *L. J. Dorr & L. C. Barnett* 5613 (CAS);
 18. *G. cardiosepala* Hand.-Mazz., *L. Lu* 05-16;
 19. *G. codonantha* Airy Shaw, *Qinghai-Xizang Expedition Team* 73-831 (KUN);
 20. *G. cuneata* Bean, *S. D. Zhang & L. Lu* 1543;
 21. *G. discolor* Nutt. ex Hook., *K. M. Feng* 7376 (KUN);
 22. *G. dolichopoda* Airy Shaw, *H. Li et al.* 22005 (KUN);
 23. *G. domingensis* Urb., *M. Colella et al.* 1365 (CAS);
 24. *G. dumicola* W.W.Sm. var. *aspera* Airy Shaw, *Qinghai-Xizang Expedition Team* 9751 (KUN);
 25. *G. dumicola* W.W.Sm. var. *dumicola*, *G. D. Tao* 012813 (KUN);
 26. *G. dumicola* W.W.Sm. var. *petanoneuron* Airy Shaw, *Gao Expedition Team* 8215 (KUN);
 27. *G. dumicola* W.W.Sm. var. *pubipes* Airy Shaw, *Bijiang Expedition Team* 0086 (KUN);
 28. *G. erecta* Vent., *J. L. Luteyn et al.* 10596 (CAS);
 29. *G. eriophylla* (Pers.) Sleumer ex Burt var. *mucronata* (J.Rémy) Luteyn, *J. L. Luteyn & L. J. Dorr* 13645 (CAS);
 30. *G. foliolosa* Benth., *L. J. Dorr & L. C. Barnett* 5807 (CAS);
 31. *G. fragrantissima* Wall., *L. Lu* 05-11;
 32. *G. glomerata* (Cav.) Sleumer, *J. L. Luteyn et al.* 8006 (CAS);

APPENDIX

VOUCHER INFORMATION OF SAMPLES USED
IN THIS STUDY

Gaultherieae

1. *Chamaedaphne calyculata* (L.) Moench, *R. McVaugh* 8747 (CAS);

33. *G. gracilis* Small, R. L. Wilbur & D. E. Stone 10498 (CAS);
34. *G. griffithiana* Wight, L. Lu 05-12;
35. *G. griffithiana* Wight var. *insignis* R.C.Fang, *Qingzang Expedition* 73-781 (KUN);
36. *G. hispida* R.Br., C. T. White 8289 (CAS);
37. *G. hispidula* Muhl., *Frere Rolland-Germain* 6256 (CAS);
38. *G. hookeri* C.B.Clarke, L. Lu et al. LL-07091;
39. *G. howellii* (Sleumer) D.J.Middleton, T. W. J. Taylor G. 22 (CAS);
40. *G. humifusa* Rydb., 9 December 1942, M. Kerr s.n. (CAS);
41. *G. hypochlora* Airy Shaw, H. Li et al. 16876 (KUN);
42. *G. insipida* Benth., J. L. Luteyn et al. 7379 (CAS);
43. *G. itatiaiae* Wawra, B. Rambo 35227 (CAS);
44. *G. itoana* Hayata, C790A (CAS);
45. *G. lanigera* Hook., J. L. Luteyn 13419 (CAS);
46. *G. leucocarpa* Bl. var. *pingbienensis* C.Y.Wu & T. Z.Hsu, Y.M.Shui 43522;
47. *G. leucocarpa* Bl. var. *yunnanensis* (Franch.) T.Z.Hsu & R.C.Fang, L. Lu Anning-01;
48. *G. longibracteolata* R.C.Fang, L. Lu et al. 06-01;
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56. *G. phillyreaefolia* (Pers.) Sleumer, R. De Barba 1606 (CAS);
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60. *G. pumila* (L.f.) D.J.Middleton, A. Donat 295 (CAS);
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