

Seed coat micromorphology of *Gordonia* sensu lato (including *Polyspora* and *Laplacea*; Theaceae)

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Abstract. Species of *Gordonia* s.l. are characterized by having seeds with prominent flattened apical wings. However, recent molecular phylogenetic studies show that this concept of *Gordonia* is not monophyletic, with species occurring in two tribes of Theaceae. We examine seed coat micromorphology of 14 species of *Gordonia* s.l., including representatives from all proposed lineages, and ten species from six genera from all three tribes of Theaceae. We observed that seeds from Gordonieae, including two species of *Gordonia* s.l., have irregularly protruding groups of cells on the seed coat that appear to be unique in the family. Seeds of Theeae, including all remaining species of *Gordonia* s.l., lack protruding cells and include testa cells that are isodiametric to elongate. Seeds of Stewartieae lack protrusions and elongate testa cells, and often have sculpting visible below the seed coat. Seeds of *Gordonia* s.l. from Gordonieae appear significantly smaller than species placed in Theeae. These results may help to infer relationships of fossilized seeds previously identified as *Gordonia*.

Key Words: *Gordonia*, *Laplacea*, *Polyspora*, Theaceae, seed morphology, SEM.

Gordonia J. Ellis is a genus with 20–65 species distributed in the Americas and Asia, with the type species *G. lasianthus* (L.) J. Ellis as the sole representative in the continental U.S.A. (Stevens et al., 2004; Mabberley, 2008). This concept of the genus dates to Keng (1980) who combined species from several other taxa, most notably *Polyspora* Sweet and *Laplacea* Kunth, based largely on their shared fruit and seed morphology. Fruit of all species assigned to *Gordonia* s.l. are ovoid to subglobose capsules with a columella present. The basal part of the seed containing the embryo is ovoid and asymmetric, with a single prominent, flattened apical wing that is usually at least as long as or longer than the embryo. Seeds of other genera of Theaceae vary, but all lack the prominent characteristic wing seen in *Gordonia* s.l. (Keng, 1962; Wang et al., 2006).

Recent molecular phylogenetic studies have shown that *Gordonia* s.l. is not monophyletic (Prince & Parks, 2001; Yang et al., 2004), with species formerly assigned to it occurring either in Gordonieae or Theeae, two of three monophyletic tribes of the family (Prince & Parks, 2001; Yang et al., 2004). The type, *G. lasianthus*, and another species from the Americas, *G. brandegeei* H. Keng, are included in

Gordonieae. The remaining taxa sampled are in Theeae and include species from Asia and tropical America. Although relationships within Theeae are not well resolved, there appear to be two lineages of species formerly placed in *Gordonia* s.l. within this tribe. Prince and Parks (2001) recommend resurrection of two older names for these taxa, *Polyspora* and *Laplacea*, and some authors have already formally transferred some species to *Polyspora* (Yang et al., 2004; Orel et al., 2012). More densely sampled phylogenetic analyses will be necessary to clarify relationships and place as yet unsampled species. Here, we follow Prince and Parks (2001) and assume that *G. brandegeei* and *G. lasianthus* are the only species of *Gordonia* s.l. in Gordonieae, and that all remaining species are in Theeae, closely related to the clades provisionally identified as *Polyspora* or *Laplacea*.

The polyphyly of *Gordonia* s.l. suggests that the prominently winged seeds of these species may be an example of convergent evolution, and raises the possibility that subtle differences may exist that distinguish seeds of unrelated species. Here, we examine the micromorphology of seeds from 48 specimens representing at least 24 species of Theaceae, with representatives from all

three tribes, using Scanning Electron Microscopy (SEM) in a low vacuum mode with backscatter detector. This mode of SEM has not been used extensively in botanical studies; however, previous studies of seed coat micromorphology have revealed detail not observed under the more commonly used high vacuum mode of SEM (Whitlock et al., 2010; Zona et al., 2012). We test the following predictions: (1) seeds of *Gordonia* s.l. in Gordonieae differ in micromorphology from seeds of *Gordonia* s.l. (i.e., *Polyspora* and *Laplacea*) in Theeae; and (2) members of each of the three tribes of Theaceae are characterized by similar seed coat micromorphology.

Materials and methods

Seeds were obtained from field collections by the first author or from herbarium specimens from the Arnold Arboretum (A), the Fairchild Tropical Botanic Garden (FTG) and The New York Botanic Garden (NY). We examined seeds from 48 specimens representing 24 species of Theaceae (Table 1), with representatives of all three tribes and six of the nine genera recognized by Prince and Parks (2001). Seeds from 14 species of *Gordonia* s.l. were examined, including both species hypothesized to be in Gordonieae. All other specimens of *Gordonia* s.l. examined were assumed to be in Theeae following Prince and Parks (2001) and Prince (2007), including species previously referred to *Polyspora* or *Laplacea*. Seeds of three specimens were unidentified to species, but are assumed to be in Theeae based on their geographic origin (Yang et al., 2004; Yakandawala & Gunathilake, 2008).

Seeds were observed with a light microscope then mounted and left uncoated for observation under SEM with a Jeol JSM 5600LV in a low vacuum mode (20–100 Pa) at 30KV, with a backscatter detector. A seed of *Gordonia* s.l. can be divided into two sections: the part that contains the embryo, and the flattened membranous extension of the seed coat that forms an apical wing (Fig. 1). We obtained SEM images at three locations on the seed: at the proximal end (over the embryo), midway (at the embryo – wing interface), and at the distal end (over the wing). Images were obtained at X50, X100 and X220.

In order to explore micromorphological variation, the following measurements were obtained: (1) Length of the seed (in mm), measured to be the longest dimension of the seed. (2) Width of

the seed (in mm), measured as the longest dimension perpendicular to the length. (3) Number of cells intersected by transects on images at X220 magnification (“transect cell number”). Two images were used for each seed, one at the proximal end over the embryo, and one at the distal end over the wing. For each image, two transects were drawn diagonal to the axes of the image and the number of cells intersecting each line was counted separately. Cells extending beyond the edge of the image were not counted. The higher number of the two counts was noted as the transect cell number. (4) The average length of five random cells intersected by each transect in (3) (“average cell length”), for a total of 20 cells per seed.

Results

Seeds of the 24 species of Theaceae examined vary from globose to compressed, ovoid, oblong, or reniform in profile (Fig. 1). Seeds of several specimens have thin, flattened regions that may be described as wings, although the size, shape, and location of these wings vary across taxa. All specimens examined of *Gordonia* s.l. have prominent apical wings. These wings are longer than the length of the embryo in all examined seeds of *Gordonia* s.l. in Theeae and in half of *Gordonia* s.l. of Gordonieae. Such well-developed wings were not observed in seeds from any other taxon of Theaceae.

Seed length and width were measured from 35 specimens representing two species of *Gordonia* s.l. in Gordonieae and 11 species in Theeae. Seed length of one sample in Gordonieae could not be obtained due to damage to the wing. Seeds of *Gordonia* s.l. assigned to Gordonieae (i.e., *G. lasianthus* and *G. brandegeei*) had lengths of 9.0–14.0 mm ($mean = 10.5$, $SD = 1.7$) and widths of 3.2–6.0 mm ($mean = 3.9$, $SD = 0.82$). All other species of *Gordonia* s.l., all assigned to Theeae here, had seed lengths of 10.3–25.3 mm ($mean = 18.05$, $SD = 4.5$) and widths of 3.0–8.7 mm ($mean = 5.4$, $SD = 1.4$). Two-tailed student T-tests indicate significant differences between Gordonieae and Theeae for both length ($p = 6.91E-05$) and width ($p = 0.0043$). Data on seed length and width, as well as transect cell number and cell length (see below), are available from the authors on request.

Diagonal transects drawn on images of X220 magnification correspond to 750 μ m on the seed. Transect cell number was obtained from 25 seeds

TABLE I
SOURCE OF SEEDS EXAMINED USING SEM.

Species	Voucher	Collection locality
Gordoniaeae		
<i>Franklinia alatamaha</i> Marshall	<i>Atha 518</i> (NY)	U.S.A. (Florida)
<i>Gordonia brandegeei</i> H. Keng	<i>Brenes 5357</i> (NY)	Costa Rica
<i>G. brandegeei</i>	<i>von Hagen and von Hagen 2127</i> (NY)	Panama
<i>G. brandegeei</i>	<i>Schultes and Reko 798</i> (A)	Mexico
<i>G. lasianthus</i> (L.) J. Ellis	<i>Baker 16-5</i> (Harvard University Herbaria Fruit and Seed Collection)	U.S.A. (Florida)
<i>G. lasianthus</i>	<i>Sheridan and Telford 1116</i> (FTG)	U.S.A. (Florida)
<i>G. lasianthus</i>	<i>Craighead s.n.</i> (FTG)	U.S.A. (Florida)
<i>G. lasianthus</i>	<i>Godfrey 84777</i> (NY)	U.S.A. (Florida)
<i>G. lasianthus</i>	<i>Buckley s.n.</i> (NY)	U.S.A. (Florida)
<i>G. lasianthus</i>	<i>Hill 22659</i> (NY)	U.S.A. (S. Carolina)
<i>G. lasianthus</i>	<i>Britt 3066</i> (NY)	U.S.A. (N. Carolina)
<i>Schima argentea</i> E. Pritz. ex Diels	<i>Fang 5685</i> (NY)	China
<i>S. wallichii</i> (DC.) Korth	<i>Soepadmo and Suhaimi s76</i> (NY)	Malaysia
Theaceae		
<i>Camellia sinensis</i> (L.) Kuntze	<i>Brach and Palomino 1754</i> (NY)	Peru
<i>Gordonia axillaris</i> (Roxb. ex Ker) Endl. ^a	<i>Tsang 25604</i> (A)	China
<i>G. axillaris</i> ^a	<i>Rock 7299</i> (A)	China
<i>G. axillaris</i> ^a	<i>Henry 10398</i> (A)	China
<i>G. axillaris</i> ^a	<i>Sun and Chang 814</i> (A)	China
<i>G. axillaris</i> ^a	<i>Chen 1671</i> (A)	Taiwan
<i>G. balansae</i> Pit. ^a	<i>How 73182</i> (A)	China
<i>G. balansae</i> ^a	<i>Liang 62024</i> (A)	China
<i>G. balansae</i> A ^a	<i>Lau 91</i> (A)	China
<i>G. balansae</i> B ^a	<i>Lau 91</i> (A)	China
<i>G. ceylanica</i> Wight ^a	<i>Gunathilake S042</i> (field collection)	Sri Lanka
<i>G. ceylanica</i> ^a	<i>Gunathilake S043</i> (field collection)	Sri Lanka
<i>G. ceylanica</i> ^a	<i>Gunathilake S051</i> (field collection)	Sri Lanka
<i>G. ceylanica</i> ^a	<i>Gunathilake S080</i> (field collection)	Sri Lanka
<i>G. concentricatrix</i> Burkill ^c	<i>Youn 95078</i> (A)	Malaysia
<i>G. dalglieshiana</i> Craib ^c	<i>Nagamasu T49949</i> (A)	Thailand
<i>G. dasanayakei</i> Wadhwa & Weeras. ^a	<i>Gunathilake S012</i> (field collection)	Sri Lanka
<i>G. elliptica</i> Gardner ^a	<i>Gunathilake S013</i> (field collection)	Sri Lanka
<i>G. elliptica</i> ^a	<i>Gunathilake S009</i> (field collection)	Sri Lanka
<i>G. fruticosa</i> (Schrad.) H. Keng ^b	<i>Mexia 7460</i> (A)	Ecuador
<i>G. fruticosa</i> ^b	<i>Garcia-Barriga et al. 18587</i> (A)	Colombia
<i>G. fruticosa</i> ^b	<i>Fosberg 20068</i> (A)	Colombia
<i>G. haematoxylon</i> Sw. ^b	<i>Alain 264</i> (A)	Cuba
<i>G. haematoxylon</i> ^b	<i>Leon and Victorin 17185</i> (A)	Cuba
<i>G. havilandii</i> Burkill ^c	<i>Stevens et al. 242</i> (A)	Malaysia
<i>G. longicarpa</i> Hung T. Chang ^a	<i>Li Heng 11546</i> (A)	China
<i>G. penangensis</i> Ridl. ^c	<i>Henderson 32948</i> (A)	Malaysia
<i>Gordonia</i> sp. ^c	<i>Kostermans s.n.</i> (A)	Sri Lanka
<i>Gordonia</i> sp. ^c	<i>Li 2755</i> (A)	China
<i>Gordonia</i> sp. ^c	<i>Kouchummen FRI023160</i> (A)	Malaysia
<i>Tutcheria greeniae</i> Chun	<i>Steward and Cheo 1045</i> (NY)	China
<i>T. shinkoensis</i> (Hayata) Nakai	<i>Boufford and Bartholomew 25109</i> (NY)	Taiwan
Stewartieae		
<i>Stewartia malacodendron</i> Nakai	<i>Mackenzie 1698</i> (NY)	U.S.A. (Virginia)
<i>S. monadelpha</i> Siebold & Zucc.	<i>Ahles 35399</i> (NY)	U.S.A. (Florida)
<i>S. ovata</i> (Cav.) Weath.	<i>Kearney 548</i> (NY)	U.S.A. (Tennessee)
<i>S. pteropetiolata</i> W. C. Cheng	<i>Tsang 24021</i> (NY)	China

^a Species of *Gordonia* s.l. transferred to *Polyspora* (Yang et al., 2004; Orel et al., 2012).

^b Species of *Gordonia* s.l. hypothesized to be *Laplacea* (Prince, 2009).

^c Species hypothesized to be Theaceae, in either *Laplacea* or *Polyspora*.

Gordonieae

Theeae

Stewartieae

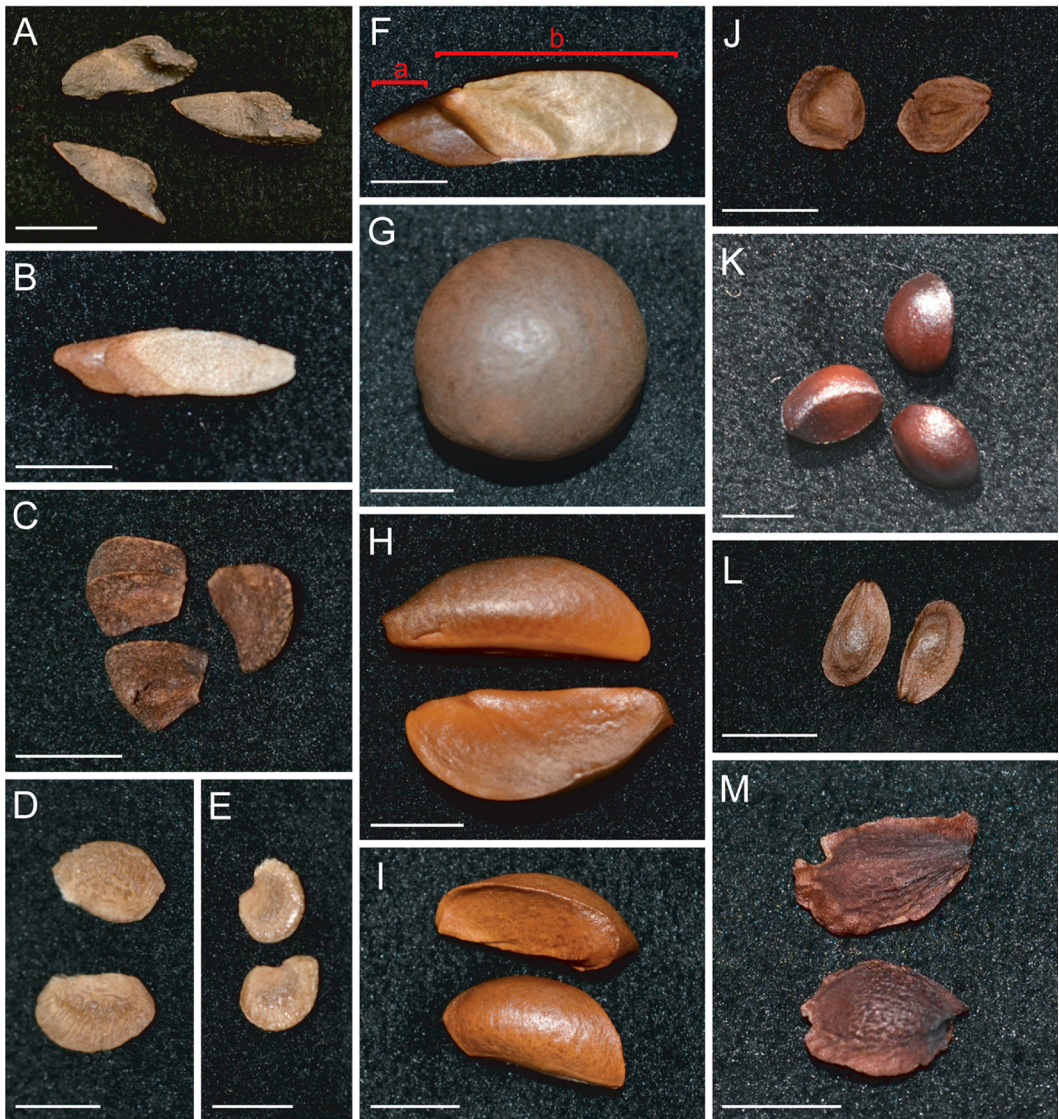


FIG. 1. Seeds of Theaceae. A. *Gordonia lasianthus* (Sheridan & Telford 1116). B. *Gordonia brandegeei* (Schultes & Reko 798). C. *Franklinia alatamaha* (Atha 518). D. *Schima wallichii* (Soepadmo & Suhaimi s76). E. *Schima argentea* (Fang 5685). F. *Gordonia* (= *Polyspora*) *axillaris*, a: embryo, b: wing (Rock 7299). G. *Camellia sinensis* (Brach & Palomino 1754). H. *Tutcheria greeniae* (Steward & Cheo 1045). I. *Tutcheria shinkoensis* (Boufford and Bartholemew 25109). J. *Stewartia pteropetalata* (Tsang 24021). K. *Stewartia malacodendron* (Mackenzie 1698). L. *Stewartia monadelpha* (Ahles 35399). M. *Stewartia ovata* (Kearney 548). See Table 1 for voucher information. Scale bar = 5 mm.

of 11 species of *Gordonia* s.l. and three samples not identified to species. Due to the ridged and irregular surface of the seed coat, we obtained measurements for transect cell number and cell length from only four of the nine samples of Gordonieae included in Table 1. Statistical

analyses on relationships among seed length, transect cell number, and transect cell length were thus only performed on samples from Theeae.

A multiple regression analysis to examine the relationship between total seed length and transect cell number in Theeae indicates that transect cell

number of the wing is significantly and negatively related to total seed length ($b_{wing} = -0.26$, $SE = 0.1$, $t_{(18)} = -2.65$, $p = 0.01$). The relationship between transect cell number over the embryo and the total seed length was not significant ($b_{embryo} = 0.002$, $SE = 0.14$, $t_{(18)} = 0.02$, $p = 0.99$). The overall model used for the analysis was significant ($F_{(2,18)} = 4.19$, $p = 0.03$, adjusted $R^2 = 0.24$). A multiple regression analysis also indicates a significant correlation between the cell length in the wing and total seed length in Theeae ($b_{wing} = 78.842$, $SE = 21.64$, $t_{(18)} = 3.644$, $p = 0.002$), but no significant correlation between cell length over the embryo and seed length ($b_{embryo} = -2.92526$, $SE = 19.74$, $t_{(18)} = -0.14$, $p = 0.884$). The overall model was significant ($F_{(2,18)} = 10.85561$, $p = 0.0008$, adjusted $R^2 = 0.49636$).

SEED COAT MICROMORPHOLOGY

Gordoniae.—Selected images of seeds from the five species sampled from *Gordoniae* are shown in Fig. 2. These seeds include the laterally flattened and winged seeds of *Gordonia* s.s., including *G. lasianthus* and *G. brandegeei*, and angular, reniform seeds of *Franklinia* W. Bartram ex Marshall and *Schima* Reinw. ex Blume (Fig. 1C-E).

When observed under SEM, seeds of all species examined of *Gordoniae* show conspicuous protruding groups of testa cells that form ridges distributed through the length of the seed. The distribution, density, size and shape of these ridges varied from seed to seed. In *G. brandegeei* and *G. lasianthus*, ridges are more common in the seed coat covering the embryo than in the part forming the wing. In three out of the seven seeds observed for *G. lasianthus*, we did not detect any protrusions in the wing. In *G. brandegeei*, the protruding ridges are more regularly sized and spaced, and may be responsible for the lepidote appearance of the seed visible to the naked eye; the non-protruding testa cells of *G. brandegeei* appear collapsed, revealing reticulate sculpting in the inner periclinal walls (Fig. 2A, B). Protruding groups of testa cells were not observed on the seed coats of any species of Theeae or Stewartiae, including all other species of *Gordonia* s.l. sampled. In one specimen of *Tutcheria shinkoensis* (Hayata) Nakai of Theeae, slightly elevated cells were observed (Fig. 3G); however, these seem very different from what was observed in seeds of *Gordoniae*, with only a few single scattered testa cells appearing elevated and the degree of elevation is very slight.

Theeae.—Selected images of seeds from the 15+ species sampled from Theeae are shown in Figs. 3 and 4. These include the globose seeds of *Camellia sinensis* (L.) Kuntze, the angular oblong seeds of *Tutcheria* Dunn, and the conspicuously winged seeds of *Gordonia* s.l. that likely should all be transferred to *Polyspora* or *Laplacea*. Seeds observed from all species of Theeae have relatively smooth surfaces (Fig. 3) and lack any protrusions seen in species of *Gordoniae*. The shape of cells varies, ranging from isodiametric to elongate (Fig. 4). In some instances both cell shapes were observed on the same seed (e.g., Fig. 4C, E).

Stewartiae.—Selected images of seeds from the four species sampled from Stewartiae are shown in Fig. 5. Seeds of species examined were ovoid to oblong, angular or compressed, some with a small wing surrounding the entire embryo (e.g., *Stewartia monadelpha* Siebold & Zucc.; Fig. 1L). In all seeds observed, testa cells appear consistently small and isodiametric (Fig. 5). In two of the four species (*S. pteropetiolata* W.C. Cheng (= *Hartia sinensis* Dunn) and *S. ovata* (Cav.) Weath.; Fig. 5A, B), testa cells show sculpting on the periclinal surface giving them a plicate appearance. A third species, *S. monadelpha*, has less prominent sculpting of even bumps on the periclinal surface. In contrast, testa cells of *S. malacodendron* L. appear exceptionally smooth.

Discussion

The variation in seed coat micromorphology observed here supports the polyphyly of *Gordonia* s.l. and the recognition of Theeae, *Gordoniae*, and Stewartiae as identified in recent molecular phylogenetic analyses of Theaceae (Prince & Parks, 2001). Despite variation in macroscopic seed morphology and seed size, all of the seeds examined from *Gordoniae*, including *G. lasianthus* and *G. brandegeei*, share irregular ridges formed by groups of protruding testa cells. Seeds of Theeae, including all other species of *Gordonia* s.l. sampled, have a smooth appearance, without ridges or visible sculpting, and have both isodiametric and linear testa cells. Seeds examined from Stewartiae also lack protruding ridges, but appear to have solely isodiametric testa cells, with sculpting visible in three of the four species examined. These three species form a clade sister to the fourth species, *S. malacodendron* (Prince, 2002), suggesting

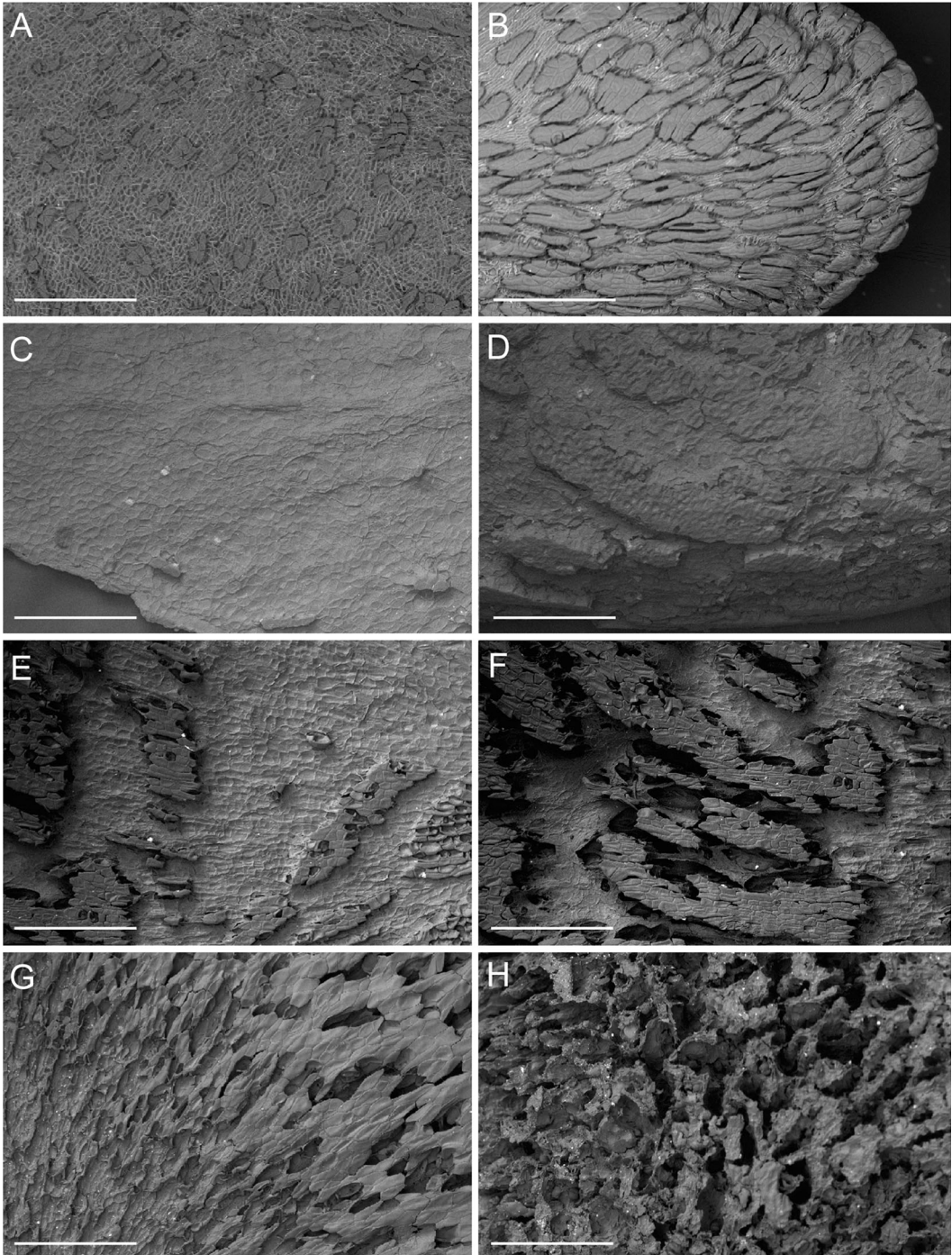


FIG. 2. Scanning electron micrographs of seed coats of Gordonieae. **A.** *Gordonia brandegeei*, wing (Schultes & Reko 798). **B.** *Gordonia brandegeei*, over embryo (Schultes & Reko 798). **C.** *Gordonia lasianthus*, wing showing few protrusions (Sheridan & Telford 1116). **D.** *Gordonia lasianthus*, over embryo (Sheridan and Telford 1116). **E, F.** *Schima wallichii* (Soepadmo & Suhaimi s76); **G.** *Schima argentea* (Fang 5685). **H.** *Franklinia alatamaha* (Atha 518). See Table 1 for voucher information. Scale bar = 500 μm .

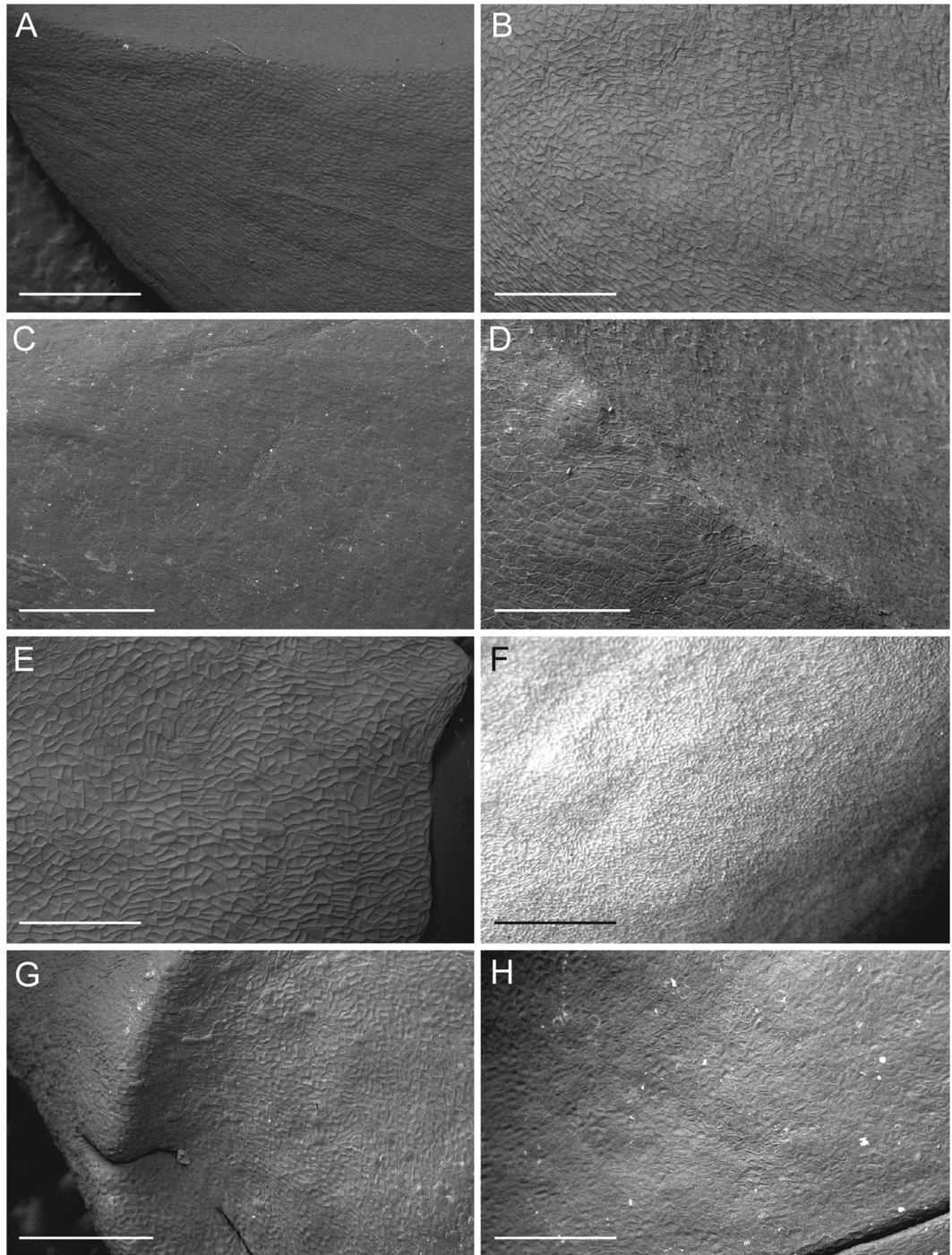


FIG. 3. Scanning electron micrographs of seed coats of Theaceae. **A.** *Gordonia* (=Polyspora?) *balansae*, middle of seed (How 73182). **B.** *Gordonia* (=Polyspora?) sp., over the embryo (Kostermans s.n.). **C.** *Gordonia* (=Polyspora?) *longicarpa*, over embryo (Li Heng 11546). **D.** *Gordonia* (=Polyspora?) *axillaris*, middle of seed (Tsang 25604). **E.** *Gordonia* (=Laplaced?) *fruticosa*, wing (García-Barriga et al. 18587). **F.** *Camellia sinensis* (Brach and Palomino 1754). **G.** *Tutcheria shinkoensis* (Boufford & Bartholomew 25109). **H.** *Tutcheria greeniae* (Steward & Cheo 1045). See Table 1 for voucher information. Scale bar = 500 μ m.

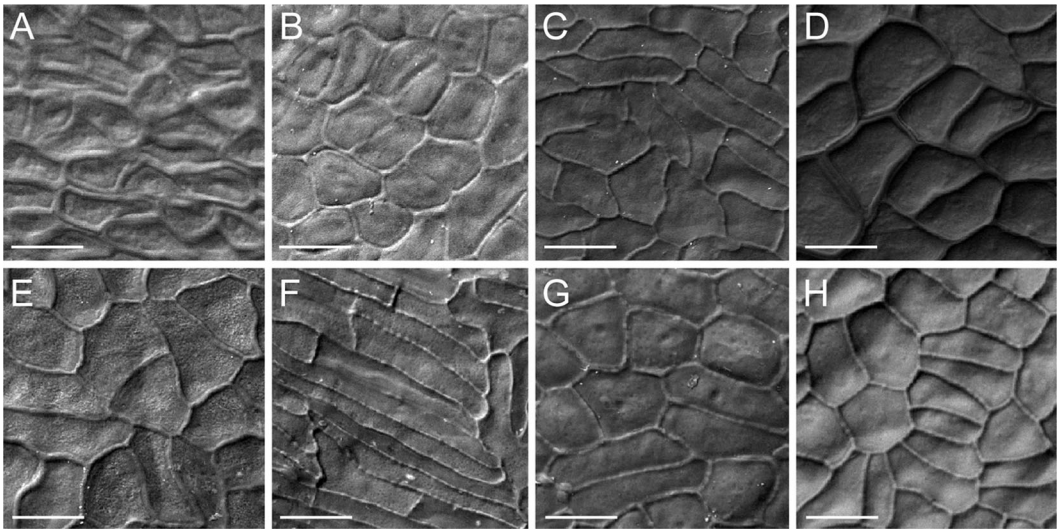


FIG. 4. Scanning electron micrographs showing isodiametric and elongate testa cells in Theaceae. **A.** *Tutcheria shinkoensis* (Boufford & Bartholomew 25109). **B.** *Tutcheria greeniae* (Steward & Cheo 1045); **C:** *Gordonia* (=Polyspora?) sp. (Li 2755) wing. **D.** *Gordonia* (=Laplacea) *fruticosa* (García-Barriga et al. 18587) wing, in middle of the seed. **E.** *Gordonia* (=Polyspora) *axillaris* (Rock 7299) wing, in middle of the seed. **F.** *Gordonia havilandii* (Stevens et al. 242) wing, in the middle of the seed. **G.** *Gordonia* (=Laplacea) *haematoxylon* (Leon & Victorin 17185) over the embryo. **H.** *Gordonia* (=Laplacea) *haematoxylon* (Alain 264) wing, in the middle of the seed. Scale bar = 50 μm .

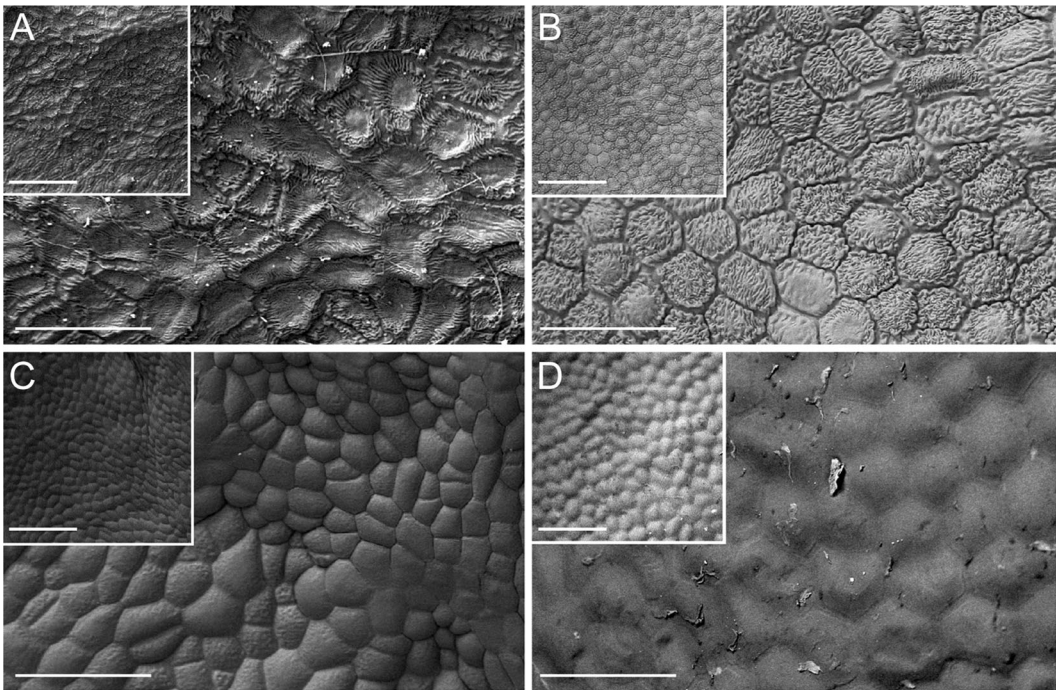


FIG. 5. Scanning electron micrographs of seed coats of Stewartieae at two magnifications. **A.** *Stewartia pteropetalata* (Tsang 24021). **B.** *Stewartia ovata* (Kearney 548). **C.** *Stewartia monadelphica* (Ahles 35399). **D.** *Stewartia malacodendron* (Mackenzie 1698). See Table 1 for voucher information. Scale bars = 100 μm for large images and = 250 μm for smaller inlays.

that periclinal sculpting may be derived within Stewartieae. The generality of our findings needs to be tested with additional sampling of species and genera within Theaceae, especially *Apterosperma* Hung T. Chang, and *Pyrenaria* Blume of Theaeae, and species of *Gordonia* s.l. from tropical Asia.

While there is wide variation in seed morphology across Theaceae, seeds of *Gordonia* s.l. are all flattened and oblong with a conspicuous apical wing (Fig. 1). This morphology appears unique within the family. Indeed, similarity in fruit and seeds was cited by Sealy (1958) and Keng (1980) to justify combining older generic concepts of *Laplacea* and *Polyspora* with *Gordonia*, resulting in the more recent concept of *Gordonia* s.l. Other taxa of Theaceae have seeds described as winged but these wings are not apical or as prominent as in *Gordonia* s.l. (e.g., Wang et al., 2006) and may have different ontogenies (Tsou, 1997).

The variation that we observed using SEM lends further support to the results of molecular phylogenetic analyses indicating that *Gordonia* s.l. is polyphyletic, with two American species in Gordonieae and all other species sampled in Theaeae. Thus, SEM can provide evidence to test the hypothesis of homology of seed wings in *Gordonia* s.l. Although morphological and anatomical variation has been described that distinguishes taxa in Theaeae and Gordonieae (Airy-Shaw, 1936; Tsou, 1997; Tsou, 1998; Wang et al., 2006), the apically winged seeds appeared uniform across lineages of *Gordonia* s.l. (Keng, 1980; Keng, 1984). The micromorphological variation among taxa of *Gordonia* s.l. is therefore significant. The prominent apical wings in separate lineages of *Gordonia* s.l. may thus be an example of convergent evolution as was concluded by morphological cladistic analyses (Wang et al., 2006), presumably as adaptations for wind dispersal. Alternatively, the prominent apical wings of seeds may be plesiomorphic within the combined Gordonieae/Theaeae clade of Theaceae. More densely sampled and resolved phylogenetic analyses are needed to test these alternatives.

In addition to differences in micromorphology, seed size may also distinguish species of *Gordonia* s.l. in Gordonieae from those in Theaeae (that should be reassigned to *Polyspora* or *Laplacea*). Despite substantial variation, seeds sampled from *Gordonia* s.l. of Theaeae were significantly larger in length and width than seeds sampled from *G. lasianthus* and *G. brandegeei* of

Gordonieae. Since most seeds included here were obtained from capsules that had already dehisced, we expect that all were mature and that observed differences in size are not due to developmental variation. Sampling of seeds from multiple conspecific plants of the same species is needed to determine the extent of phenotypic plasticity. Our observations do appear consistent with reports from the literature. For example, measurements of seed length of *G. lasianthus* and *G. brandegeei* are reported as less than 20 mm (Kobuski, 1950; Grote & Dilcher, 1992; Prince, 2009) while seeds of *Gordonia* s.l. from Theaeae are usually reported as 20 mm or greater (Keng, 1984; Ming & Bartholomew, 2007; Orel et al., 2012).

An increase in seed size could be the result of an increase in the number of testa cells, an increase in the size of testa cells, or both. Our measurements of testa cell number and size are indirect and likely imperfect at capturing variation among these seeds. However, results of multiple regression analyses suggest that larger seeds in *Gordonia* s.l. of Theaeae have larger testa cells in the seed wings. The shape and arrangement of testa cells may also play a role. One question for future investigation is if seed wings in Theaeae have more elongate testa cells that are arranged along the axis of the seed.

Our observations support the hypothesis of Prince and Parks (2001) that *Gordonia lasianthus* and *G. brandegeei* are the only species in Gordonieae, and the only species that should be retained in *Gordonia*. The seeds of all remaining species examined here have a micromorphology more consistent with Theaeae. It is important to note that many of these species have not yet been included in phylogenetic analyses and so their relationships are still uncertain. For example, the four species of *Gordonia* s.l. endemic to Sri Lanka have not been subject to phylogenetic analysis; however, seeds from all four species have smooth seed coats, lacking protrusions, with isodiametric and elongate cells (Fig. 4), consistent with placement in Theaeae and supporting the recent transfer to *Polyspora* (Orel et al., 2012).

Two well-supported subclades of *Gordonia* s.l. have been recovered within Theaeae, that have been recognized provisionally as *Polyspora* and *Laplacea*, but relationships between them are uncertain. We included seeds from two taxa, *G. fruticosa* (Schrad.) H.Keng and *G. haematoxylon* Sw., proposed to be the only species of *Laplacea*

from the Americas (Weitzman cited in Prince, 2009). All other *Gordonia* s.l. of Theaceae included here were from Asia and include some species that have been transferred to *Polyspora* and some of unknown affinity. We did not detect any seed characters that distinguished the putative species of *Laplacea* from the other sampled *Gordonia* s.l. from Asia.

The variation in seed coat micromorphology that we observed may help to infer relationships of fossil taxa. Theaceae have a rich fossil record, with fossils from the Tertiary widespread in the northern hemisphere, including Europe where there are no extant species (reviewed by Grote & Dilcher, 1989, 1992). Because many fossils are fruits and seeds, and because fruit and seed characters are important in delimiting genera, it has often been possible to infer relationships to extant genera. However, micromorphological details of the seed coat are generally not used. The nonmonophyly of genera such as *Gordonia* also has the potential to mislead some of these identifications.

Of particular interest are several fossil seeds and fruits recovered from the mid-Eocene Claiborne formation of Kentucky and Tennessee, which includes the earliest well-documented fossil fruit and seeds assigned to *Gordonia* (Grote & Dilcher, 1989, 1992). Seeds of *G. warmanensis* were described by Grote and Dilcher (1992) as intermediate between *G. lasianthus* (Gordonieae) and extant Asian species of *Gordonia* s.l. (now presumably in Theaceae). However, the authors noted that seeds of *G. warmanensis* lack the “warted surface” seen in *G. lasianthus*. If the presence or absence of protruding ridges on seed coats is a key character separating tribes of Theaceae, as our data suggest, then *G. warmanensis* would fit better in Theaceae than Gordonieae. *Gordoniopsis* was described and suggested as the sister group of *Gordonia* s.l., although the isodiametric- to elongate-shaped cells on its outer seed surface are more consistent with a close relationship to lineages in Theaceae rather than Gordonieae. Seeds of *Andrewsiocarpon*, also from the Claiborne formation, exhibit “ridges and tubercles” on the outer surface of the seed coat, consistent with a close relationship to *Franklinia* suggested by Grote and Dilcher (1989). Clarification of relationships of fossils, especially European taxa, will have implications for understanding the history of diversification and biogeography of Theaceae.

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