

Seed morphology of Hamelieae with emphasis on the *Deppea* complex (Cinchonoideae, Rubiaceae)

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Background and aims – The neotropical tribe Hamelieae currently includes 16 genera mainly characterized by raphides, ebracteolate inflorescences, and 4-merous flowers with contorted corolla aestivation. Within this tribe, the circumscription of *Deppea* has been particularly controversial, as depending on the authors, several morphologically closely related genera are either treated as synonyms or accepted as such. This generic group, hereafter referred to as the *Deppea* complex, consists of up to 10 genera. Within Rubiaceae, seed morphology has proved to have taxonomic value for generic circumscriptions, however, it remained unexplored for Hamelieae and the *Deppea* complex.

Material and methods – We present a detailed study of the seed morphology of 37 species representing 15 out of the 16 genera recognized within Hamelieae, including all putative genera of the *Deppea* complex. Using scanning electron and light microscopy, we investigate 16 quantitative and qualitative seed characters that could have taxonomic value.

Key results – Our results show that the combination of some seed characters, such as shape and colour, dorsiventral compression, hilum position, and the periclinal microrelief, helps to distinguish some genera and most species, supporting or refuting the current taxonomic circumscription.

Conclusion – We conclude that the seed morphology within Hamelieae has taxonomic value but should be combined with other characters to achieve unequivocal delineation of the genera.

Keywords – Generic circumscription; LM; morphology; seed; SEM; systematics; testa.

INTRODUCTION

Hamelieae is a Neotropical tribe comprising 16 genera and approximately 225 species, notably diverse in Mexico where it has 14 genera and ca 100 species (Lorence & Dwyer 1988; Borhidi 2006, 2012). It is characterized by shrubs or treelets, presence of raphides, typically ebracteolate inflorescences, often yellow or yellowish 4-merous flowers, contorted corolla aestivation, and berries or capsular fruits (Bremer 1987; Borhidi et al. 2004a, 2004b; Manns & Bremer 2010; Stranzinger et al. 2014).

The tribe Hamelieae was traditionally classified in subfamily Rubioideae due to the presence of raphides (Bremekamp 1966). Nevertheless, molecular phylogenetic

analyses including relatively few species of Hamelieae have indicated that this tribe is more appropriately classified within Cinchonoideae (Bremer et al. 1995; Andersson & Rova 1999; Robbrecht & Manen 2006), even when using phylogenomic data, although this analysis only represented the tribe with one species (Antonelli et al. 2021).

Within the tribe, the generic circumscription has been controversial; the first classification systems for Rubiaceae (De Candolle 1830; Endlicher 1836) recognized eleven genera within Hamelieae: *Alibertia* A.Rich., *Axanthes* Blum, *Brignolia* DC., *Evosmia* Humb. & Bonpl., *Hamelia* Jacq., *Olostyla* DC., *Patima* Aubl., *Tepesia* Gartn., *Urophyllum* Jack ex Wall., *Sabicea* Aubl., and *Schradera* Vahl. Hooker (1873) considered only six genera based on the contorted

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and imbricate corolla aestivation plus fleshy fruits: *Bertiera* Aubl., *Bothriospora* Hook.f., *Gouldia* A.Gray, *Hamelia*, *Heinsia* DC., and *Hoffmannia* Sw. According to Schumann (1897), Hamelieae was considered a subtribe within Gardenieae, including five genera: *Bothriospora*, *Catesbaea* L., *Hamelia*, *Hoffmannia*, and *Phyllacantha* Hook.f. Later on, Verdcourt (1958) recognized in Hamelieae only *Bertiera*, *Hamelia*, and *Heinsia*, while Bremekamp (1966) restricted it even more, including only two genera, *Hamelia* and *Hoffmannia*, based on raphide presence plus fleshy fruits. Bremer (1987) recognized five genera within Hamelieae based on raphide presence and imbricate corolla aestivation: *Deppea* Schtdl. & Cham., *Hamelia*, *Hoffmannia*, *Omitelia* Standl., and *Pinarophyllon* Brandege. Lorence & Dwyer (1988) considered the genus *Schenckia* K.Schum. to be a synonym of *Deppea*. The genus *Editheia* Standl. was included within Hamelieae by Robbrecht (1988), but treated as *Omitelia* by Kirkbride (1984), as *Deppea* by Lorence & Dwyer (1988), and resurrected by Borhidi et al. (2004a). Robbrecht & Bridson (1993) added *Eizia* Standl. and *Plocaniophyllon* Brandege to the tribe, while McDowell (1996) included the monotypic genus *Syringantha* Standl. Finally, Borhidi et al. (2004b) segregated *Bellizinca* Borhidi and *Csapodya* Borhidi from *Deppea*, both genera included in Hamelieae by the same authors.

Based on molecular evidence, Manns & Bremer (2010) recognized seven genera within Hamelieae: *Cosmocalyx* Standl., *Deppea*, *Hamelia*, *Hoffmannia*, *Omitelia*, *Pinarophyllon*, and *Plocaniophyllon*, plus two tentatively included (*Eizia* and *Patima*), while the genera *Bellizinca*, *Csapodya*, *Editheia*, and *Schenckia* were suggested as synonyms of *Deppea*.

In total, nine genera are morphologically closely related to *Deppea*, even treated as synonyms by some authors (Lorence & Dwyer 1988; Manns & Bremer 2010; Lorence 2012). These genera will be hereafter referred to as the *Deppea* complex: *Bellizinca*, *Csapodya*, *Deppea*, *Deppeopsis*, *Editheia*, *Omitelia*, *Pinarophyllon*, *Plocaniophyllon*, *Pseudomitelia* Borhidi, and *Schenckia* (fig. 1). Hence, the *Deppea* complex constitutes the majority of the Hamelieae tribe, including 10 out of the 16 genera and ca 50 species.

Molecular phylogenetic analyses retrieved a monophyletic and highly supported Hamelieae but did not solve the *Deppea* taxonomic problem: Manns & Bremer (2010) used a set of six markers (nrITS, *atpB-rbcL*, *ndhF*, *rbcL*, *rps16*, and *trnL-F*), but only studied six genera of the *Deppea* complex with a few species each, leaving many questions related to their generic circumscription open. Stranczinger et al. (2014), on the other hand, included representatives of all genera in the *Deppea* complex, but they only used two markers (ITS and *trnL-F*) resulting in a consensus tree with many polytomies that made the taxonomic decisions untenable. Their phylogenetic analysis showed that none of the genera in the *Deppea* complex were supported as monophyletic and that the genus *Renistipula* Borhidi should be included as a member of Hamelieae despite its lack of the diagnostic raphides. A phylogenetic analysis by Torres-Montúfar (2018) however placed *Renistipula* within Guettardeae and treated it as a synonym of *Arachnothryx* Planch. All morphological evidence indicates that *Renistipula* belongs in Guettardeae

and so we favour the results by Torres-Montúfar (2018), suggesting that the results by Stranczinger et al. (2014) are most likely an artifact of a methodological error.

In the literature, some morphological characters have been used to differentiate among the genera within Hamelieae and more precisely within the *Deppea* complex, such as the position of the stamen insertion on the corolla, calyx lobe form, and corolla length and shape, as well as fruit form and dehiscence (Borhidi et al. 2004a, 2004b; Borhidi 2006, 2012; Borhidi & Stranczinger 2012). Nevertheless, these characters display a complex overlapping mosaic and none of them is useful to clearly distinguish genera when not used in combination with other characters.

Therefore, the morphological and molecular data gathered so far does not provide convincing evidence for the adequate generic circumscription within Hamelieae, particularly among genera in the *Deppea* complex. Consequently, the number of genera that should be recognized within Hamelieae remains unclear and new sources of evidence should be incorporated to solve this problem.

Even though seed morphology has not been commonly used in Rubiaceae systematics, some studies indicate its taxonomical potential (e.g. Hayden & Dwyer 1969; Terrell et al. 1986; Robbrecht 1988; Khalik et al. 2008). Several seed characters have thus potential to be good taxonomic markers, such as seed shape (Breedlove & Lorence 1987), testa ornamentation (Hayden & Dwyer 1969), general form and hilum position (Terrell et al. 1986), seed size and anticlinal and periclinal cell wall boundary (Khalik et al. 2008). This information has helped the delimitation of some taxa at species level (e.g. Khalik et al. 2008) or at generic level (e.g. Hayden & Dwyer 1969). For the tribe Hamelieae, Robbrecht (1988) mentioned that the exotestal outer cells walls are granulate or tuberculate, while Martínez-Cabrera et al. (2014) observed unitegmatic orthotropous ovules, horizontally oriented in the ovary locule. Other than that, information on the taxonomic value of the seed morphology within Hamelieae and among genera in the *Deppea* complex is scattered. It is important to mention that the seeds of species within the *Deppea* complex are in general very small, resembling dust, and this might be a reason why they have not been thoroughly studied. However, the seeds of *Plocaniophyllon* were described by Martínez-Cabrera et al. (2008) in a study of the genus including morphology and anatomy. In addition, studies by Borhidi et al. (2004a, 2004b) indicated the existence of seed differences in shape, hilum position, perpendicular wall thickness, and horizontal wall type and ornamentation among *Bellizinca*, *Csapodya*, *Deppea*, *Editheia*, *Omitelia*, and *Pseudomitelia*. Regardless of the evidence that seed morphology can provide taxonomically useful information within Hamelieae and the *Deppea* complex, there is no study using uniform terminology devoted to systematically comparing seed morphology among genera within the tribe.

Therefore, our aims in this work are to provide a thorough seed description for all genera of Hamelieae and to examine the potential utility of seed morphology for the generic circumscription in the tribe, mainly focusing on the genera within the *Deppea* complex.

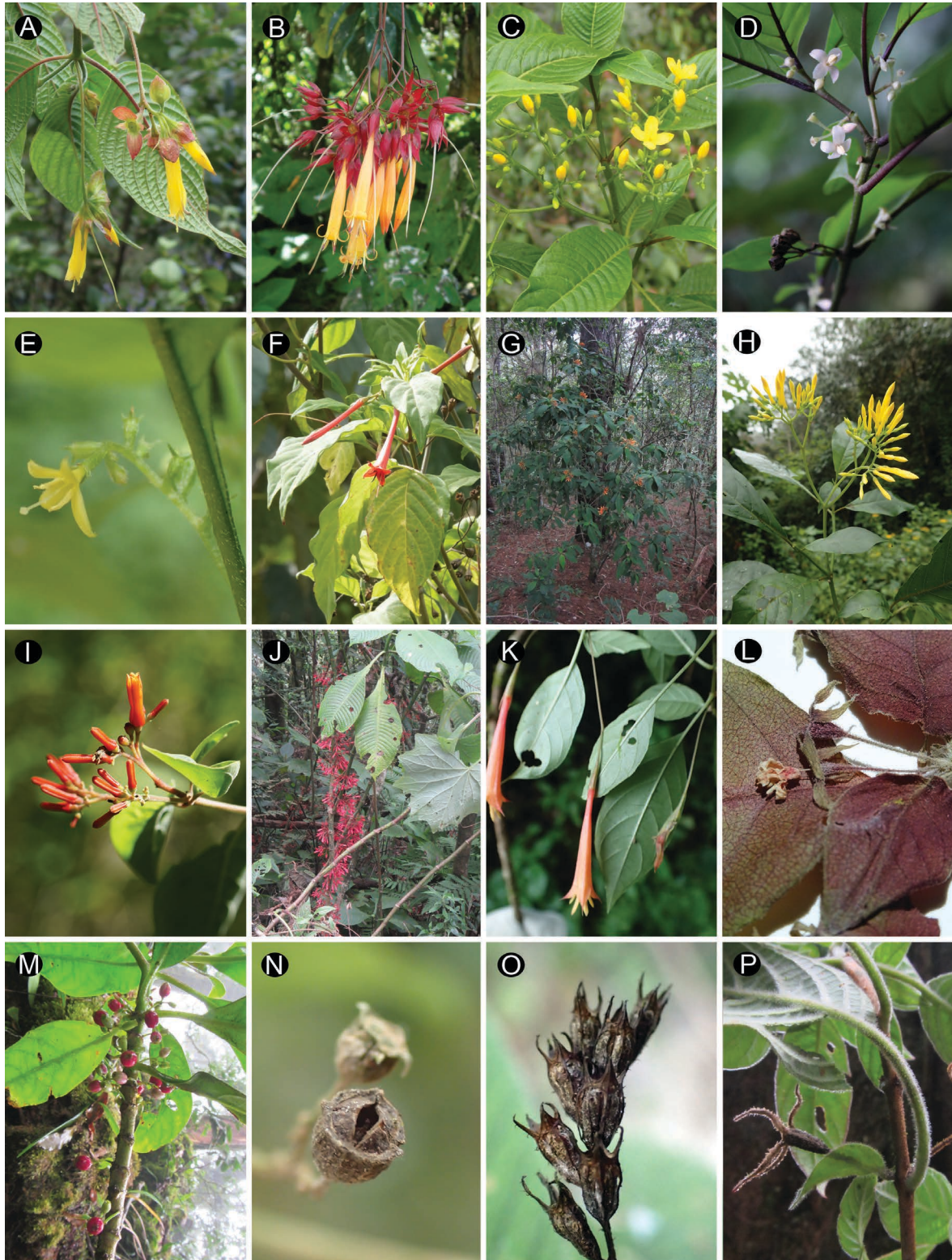


Figure 1 – Different species representing the genera within Hamelieae. **A–L.** Flowers. **A.** *Bellizinca scoti* (Ochoterena et al. 1119). **B.** *Csapodya splendens* (Ochoterena et al. 1147). **C.** *Deppea cornifolia* (Ochoterena et al. 1120). **D.** *Deppea purpurascens* (Ochoterena et al. 1175). **E.** *Deppeopsis anisophylla* (Ochoterena et al. 1102). **F.** *Eizia mexicana* (Ochoterena et al. 1126). **G.** *Editheia miahuatlanica* (Ochoterena et al. 1095). **H.** *Editheia schiblii* (Ochoterena et al. 1112). **I.** *Hamelia patens* (Ochoterena et al. 1074). **J.** *Hoffmannia* sp. (Ochoterena et al. 1166). **K.** *Omiltemia parvifolia* (Ochoterena et al. 1105). **L.** *Schenckia blumenaviensis*. **M–P.** Fruits. **M.** *Hoffmannia* sp. (Ochoterena et al. 1164) **N.** *Deppea grandiflora* (Ochoterena et al. 1142). **O.** *Pinarophyllon flavum* (Ochoterena et al. 1133). **P.** *Pseudomiltemia davidsonii* (Ochoterena et al. 1162). All photographs by Helga Ochoterena, except G by Alejandro Torres.

MATERIAL AND METHODS

Our study includes all 16 genera within Hamelieae except for the South American *Patima* (with two species) for which we could not obtain seeds. This study includes all 10 genera within the *Deppea* complex. Seeds of at least one species of all the genera of the *Deppea* complex and five other genera in Hamelieae were examined. The voucher specimens of the 37 species are listed in supplementary file 1. Seeds were obtained from herbarium specimens at the Herbario Nacional de México (MEXU) and the Copenhagen herbarium (C). All seeds were chosen from mature fruits, with several seeds randomly selected, and the seeds were not treated or cleaned before microscopy due to their small size. For each sample, two groups of seeds were formed to be subsequently processed with different microscope techniques at the Laboratorio de Microscopía y Fotografía de la Biodiversidad at Instituto de Biología, UNAM. For scanning electron microscopy (SEM), the sampled seeds were sputter-coated with gold-palladium for 2–3 minutes with a HUMMER V Sputter Coater and they were observed and photographed using a Hitachi S-4000. For light microscopy (LM), the seeds did not receive any pre-treatment and stereoscopic photographs were taken using a Leica microscope equipped with a Leica Z16 APO A camera.

Seed colours were defined by placing the eyedropper tool of Photoshop (we used Photoshop CS3) on top of the central portion of the seed on the captured image and once the RGB colour palette was displayed, the colours were assigned to the standardized nearest colour: black, around brown as brownish, around red as reddish, or around yellow as yellowish.

Measurements were taken using the software Leica Application Suite v.2.8.1; at least five seeds per specimens were measured for size, length, and width. All measurements are presented in mm. Boxplots were created in Microsoft Excel and boxes represent the interquartile range, the horizontal line inside the box represents the median, the “X” represents the mean, and the circles are outliers.

The terminology proposed by Koch et al. (2009) and Barthlott & Hunt (2000) was adopted to describe the seed coat micromorphology observed using SEM, the other characters were defined here by the authors or follow Harris & Harris (2001). The micropyle position was defined assuming the orthotropous ovule nature described for the tribe by Martínez-Cabrera et al. (2014) or the anatropous position described for most Rubiaceae (Robbrecht 1988). A character matrix was constructed using Winclada (Nixon 1999) to show parallel descriptions of the 15 characters in the studied species. An illustrated overview of the 15 characters and character states is presented in supplementary file 2. Full seed descriptions for the 37 studied species in 15 genera are presented in the supplementary file 3, including the seed size that is not considered in the supplementary file 2 because it is a continuous character.

The seed morphology of the 15 studied genera is graphically summarized in fig. 2; we also compared the 28 species within the *Deppea* complex (fig. 3). The boxplots comparing seed length and width of the 15 studied genera

are shown in fig. 4. The SEM micrographs are shown in figs 5–10 and the LM micrographs are shown in figs 11–13.

RESULTS

In total, seeds from 15 genera and 37 species within Hamelieae were studied, among which 10 genera and 28 species belonging in the *Deppea* complex. Our results show high morphological overlap among the studied genera (fig. 2). Seed length and width (fig. 4) only allow for the recognition of *Cosmocalyx*, which has the largest seeds ($7.9\text{--}10.7 \times 4.8\text{--}5.8$ mm) of linear form (fig. 11C) and has the straight anticlinal wall boundaries (fig. 5F–H). The only other genus among Hamelieae that can be easily recognized by its seeds is *Syringantha*, because of the winged margins (figs 10A, 13Q, R).

The other 13 genera show a diversity in general shape, dorsiventral shape (figs 5–10), colour, anticlinal wall boundary, periclinal microrelief, and micropyle position (figs 11–13). Only a combination of these characters allows for taxonomic grouping, which is not necessarily similar to the current generic circumscription.

Outside the *Deppea* complex, the genus *Eizia* can be distinguished by the combination of black seeds (fig. 12S, T) with evidently depressed dorsiventral shape of cells (fig. 8I), and a sub-central micropyle position. These character states are each present in at least one species within the *Deppea* complex, but never in this combination. The studied *Hamelia* species share a rhomboidal seed shape, straight anticlinal wall boundaries, and papillose periclinal microrelief (figs 8M–X, 12U–Z). The rhomboidal seed shape is also present in *Deppea erythrorhiza* (figs 5Y, 11M) and *Omiltemia longipes* (figs 9I, 13E), from which *Hamelia* is easily distinguished by the straight anticlinal wall boundaries and the evident hilum, in contrast to the U-undulate anticlinal wall boundaries and the inconspicuous hilum in the former two species (figs 5Z–AA, 9J–K, 11M, 13E). The genus *Hoffmannia* shows variation among the studied species and therefore is hard to characterize at generic level by its seeds (fig. 2). Among the evaluated species in this genus, *H. ghiesbreghtii* and *H. oaxacensis* are similar by the exotesta cells with verrucose periclinal microrelief (fig. 9D, H), a character state shared with *Bellizinca* (fig. 5C–D) and *Deppea grandiflora*. This is in contrast to the concave outlines with sunken profile of the periclinal microrelief in *H. gesnerioides* (fig. 8AB), which is a character state shared with many other species within the studied genera.

Within the *Deppea* complex, dorsiventrally flattened seeds is one of the main character states useful for taxon grouping. The genera *Csapodya*, *Deppeopsis*, *Omiltemia*, and *Pseudomiltemia* have flattened seeds, in contrast to the polygonal seeds in *Bellizinca*, *Deppea*, *Edithea*, *Pinarophyllon*, *Plocaniophyllon*, and *Schenckia* (fig. 2). Among the taxa with flattened seeds, *Csapodya* and *Pseudomiltemia* have concave outlines with a sunken profile periclinal microrelief (fig. 5L, P), while it is papillose in *Deppeopsis* (fig. 7L, P, T), *Omiltemia* (fig. 9L, P), *Pinarophyllon*, and *Plocaniophyllon*. Most of the character states of *Pseudomiltemia* seeds overlap with those of at least one of the *Csapodya* species (fig. 2), however, both genera

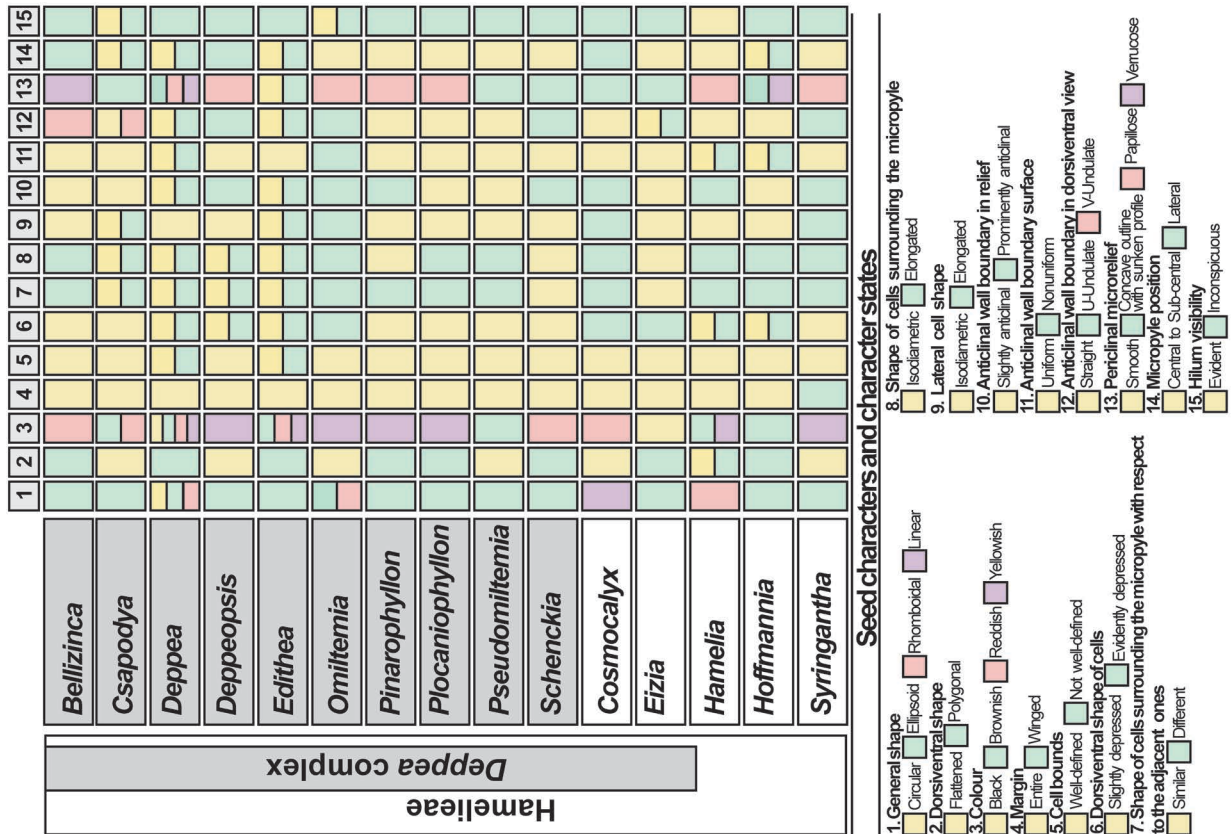


Figure 2 – Graphical representation of the variation within 15 seed characters and their character states for 15 Hamelieae genera.

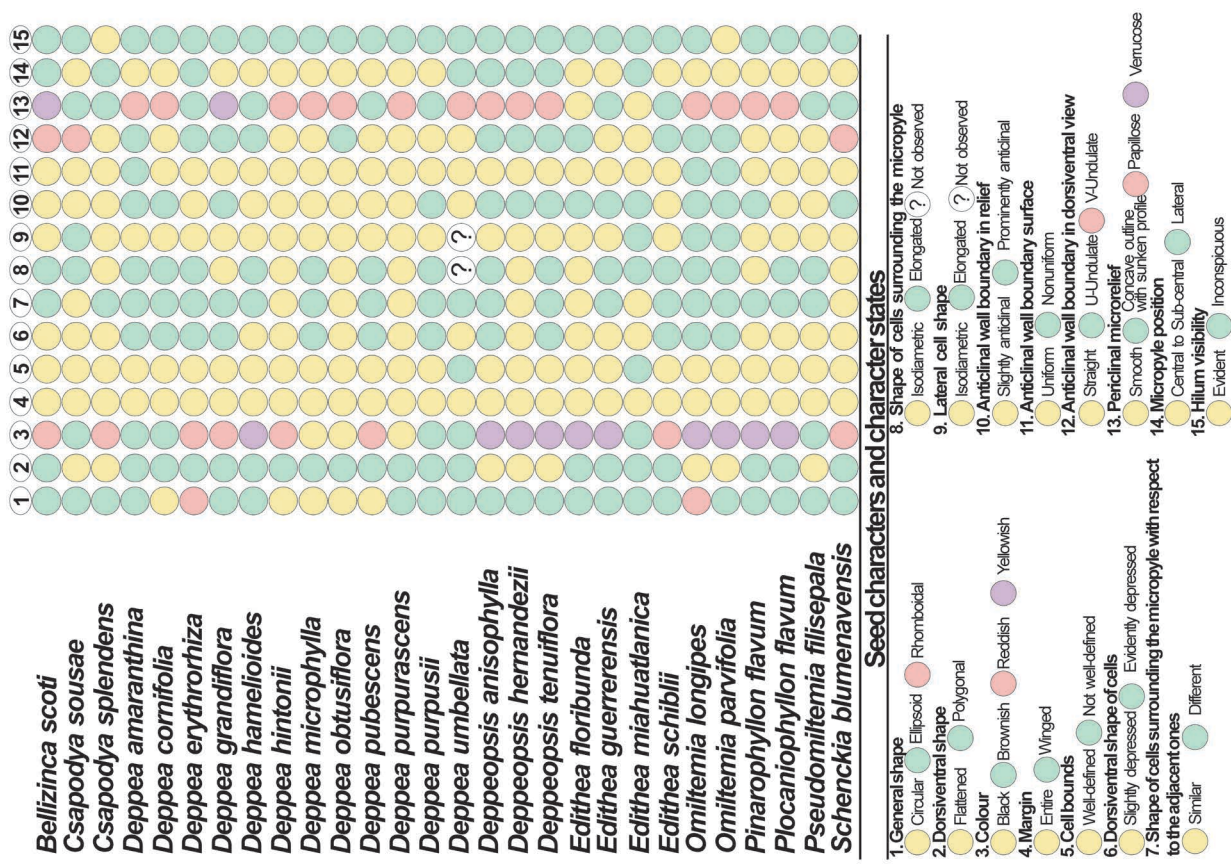


Figure 3 – Graphical representation of the variation within the 15 seed characters and their character states of representative species within the *Deppea* complex.

can be distinguished by the shape of the cells next to the micropyle that differ from the shape of the lateral cells, in combination with the elongated lateral cells, and the straight anticlinal wall boundary in *Pseudomiltemia*. The seeds differ between *Deppeopsis* and *Omittemia* in that the latter has elongated lateral cells, and the micropyle in central/sub-central position (fig. 9I–P), isodiametric lateral cells, and lateral micropyle position of *Deppeopsis* (fig. 7I–T).

Among the genera which have polygonal seeds, differences in the periclinal microrelief are useful for grouping them. The genus *Bellizinca* is easily recognizable by the verrucose microrelief (fig. 5D), which is only shared with *Deppea grandiflora* (fig. 6D), but *Bellizinca* is distinguishable by the V-undulate anticlinal wall boundary and the elongated cells surrounding the micropyle (fig. 5A–C), in contrast to the U-undulate and isodiametric cells surrounding the micropyle in *D. grandiflora* (fig. 6A–C).

The genera *Pinarophyllon* and *Plocaniophyllon* share a papillose microrelief (fig. 9T, X), also found in several *Deppea* species (fig. 3). The genera can be distinguished by the elongated cells surrounding the micropyle in *Plocaniophyllon* (fig. 9U–V), in contrast to the isodiametric cells surrounding the micropyle in *Pinarophyllon* (fig. 9Q–R). The combination of seed colour, anticlinal wall boundary, and micropyle position is useful to differentiate both genera from *Deppea*, even though they share a papillose microrelief (fig. 3). The genus *Schenckia* shares the concave outline with a sunken profile microrelief (fig. 10E–H) with species of *Edithea* and *Deppea* (fig. 3), however, *Schenckia* is characterized by the V-undulate anticlinal wall boundary (fig. 10G), while the *Deppea* and *Edithea* species have straight anticlinal wall boundaries (figs 5Q–AB, 8A–H).

The genus *Deppea* is morphologically heterogeneous and shows overlap among the character states with all other genera: its seed shape, colour, and periclinal microrelief are variable. At species level, the rhomboidal seeds allow

for the recognition of *Deppea erythrorhiza* (figs 5Y, 11M) among the other studied species in the genus, since the other species have circular or ellipsoidal seeds. Among the species with ellipsoid seeds, *D. grandiflora* can be distinguished by the verrucose periclinal microrelief (fig. 6D) instead of the papillose or concave outlines with a sunken profile in the other species. The rest of the species with ellipsoid seeds can be distinguished by the colour: *D. amaranthina* has brown seeds (fig. 11I–J), *D. hamelioides* yellowish (fig. 11Q–R), *D. purpurascens* black (fig. 11AA–AB), and *D. purpusii* and *D. umbellata* have reddish seeds (fig. 12A–D). The latter two differ from each other by their testa cells: well-defined in *D. purpusii* (fig. 7B) but not well-defined in *D. umbellata* (fig. 7F). It is worth mentioning that for *D. umbellata*, the cells surrounding the micropyle could not be observed and therefore these character states were coded in the matrix with a question mark “?”; this is the only taxon that has this state.

Among the *Deppea* species with circular seeds (fig. 3), *D. obtusiflora* is distinguishable by the U-undulate anticlinal wall boundaries (fig. 6R–S), while the rest of the species in the genus have straight boundaries; among these, the seeds of *D. hintonii* have isodiametric lateral cells (figs 6I, 11S–T), while *D. cornifolia* and *D. microphylla* have elongated lateral cells (figs 5U, 6M, 11K, U). The differences between *D. cornifolia* and *D. microphylla* are the prominent anticlinal wall boundaries in the former in contrast to the only slightly prominent anticlinal wall boundaries in the latter (figs 5V–W, 6N–O).

For the genera sampled with more than one species, the examined *Csapodya* species are very different (figs 5I–P, 11E–H) from each other: brown seeds in *C. sousae* and reddish in *C. splendens*; elongated lateral cells in *C. sousae* and isodiametric in *C. splendens*; V-undulate anticlinal wall boundaries in *C. sousae* and straight in *C. splendens*; sub-central micropyle position in *C. sousae* and lateral in *C.*

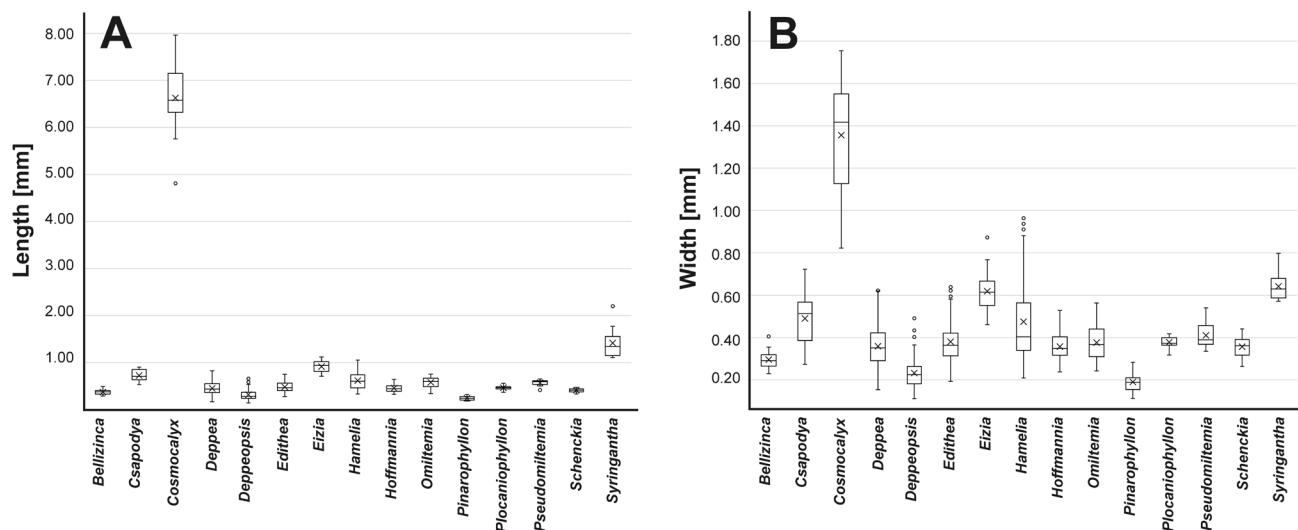


Figure 4 – Boxplots showing the variation in seed length (A) and width (B) per genera. Boxes represent the interquartile range; whiskers are drawn within the 1.5 IQR value; the “X” represents the mean; circles are outliers..

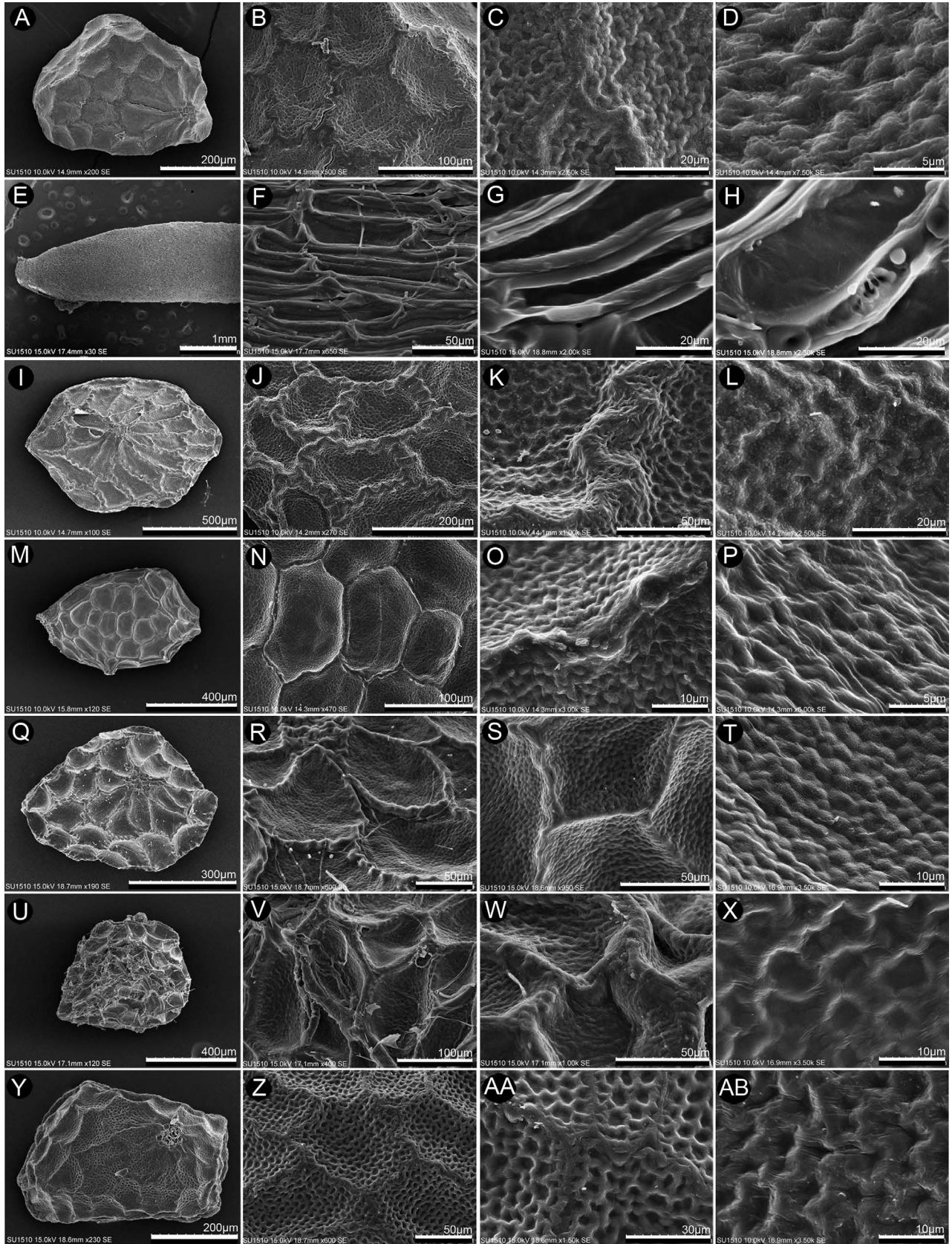


Figure 5 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Bellizincia scotti*. **E–H.** *Cosmocalyx spectabilis*. **I–L.** *Csapodya sousae*. **M–P.** *Csapodya splendens*. **Q–T.** *Deppea amaranthina*. **U–X.** *Deppea cornifolia*. **Y–AB.** *Deppea erythrorhiza*.

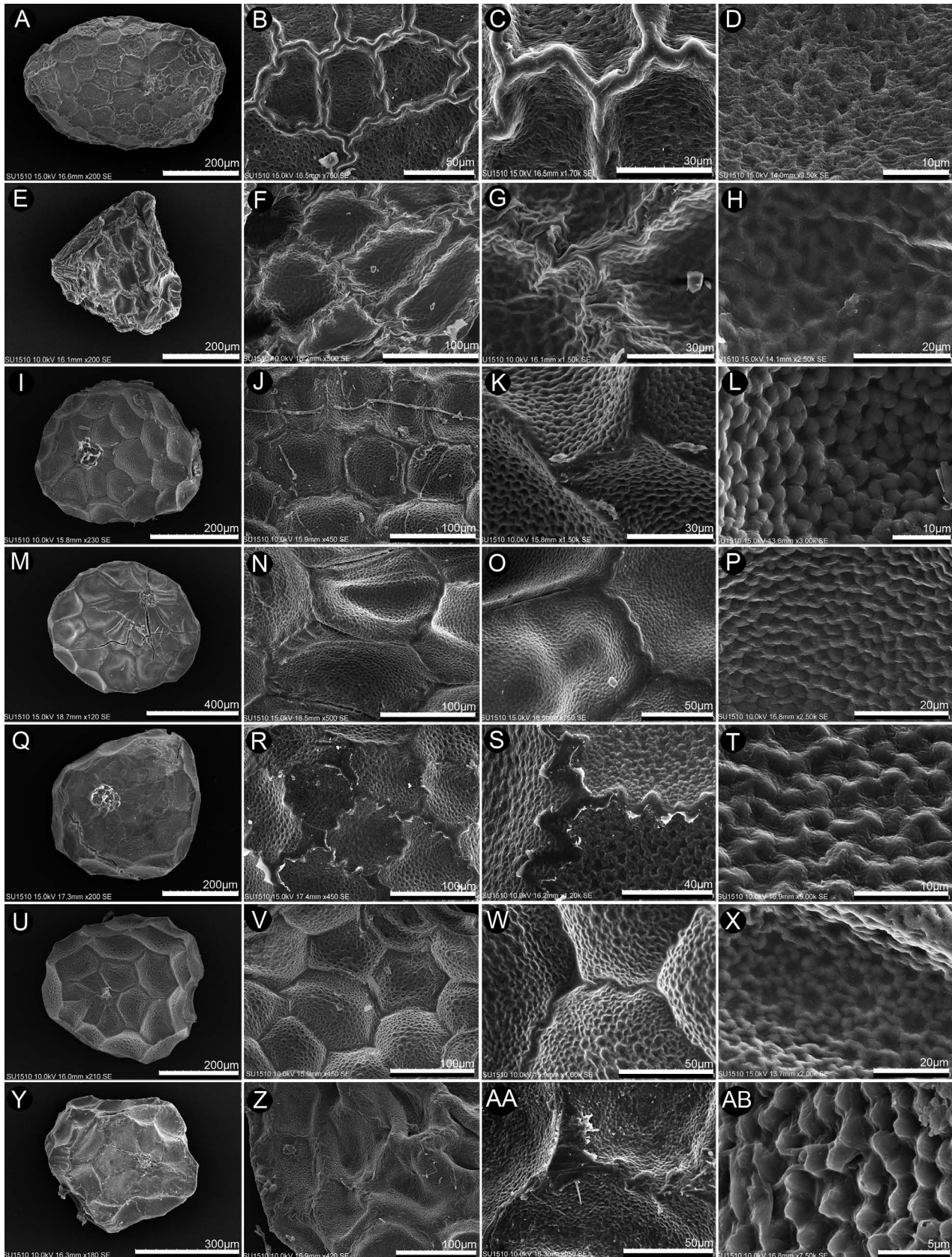


Figure 6 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Deppea grandiflora*. **E–H.** *Deppea hamelioides*. **I–L.** *Deppea hintonii*. **M–P.** *Deppea microphylla*. **Q–T.** *Deppea obtusiflora*. **U–X.** *Deppea pubescens*. **Y–AB.** *Deppea purpurascens*.

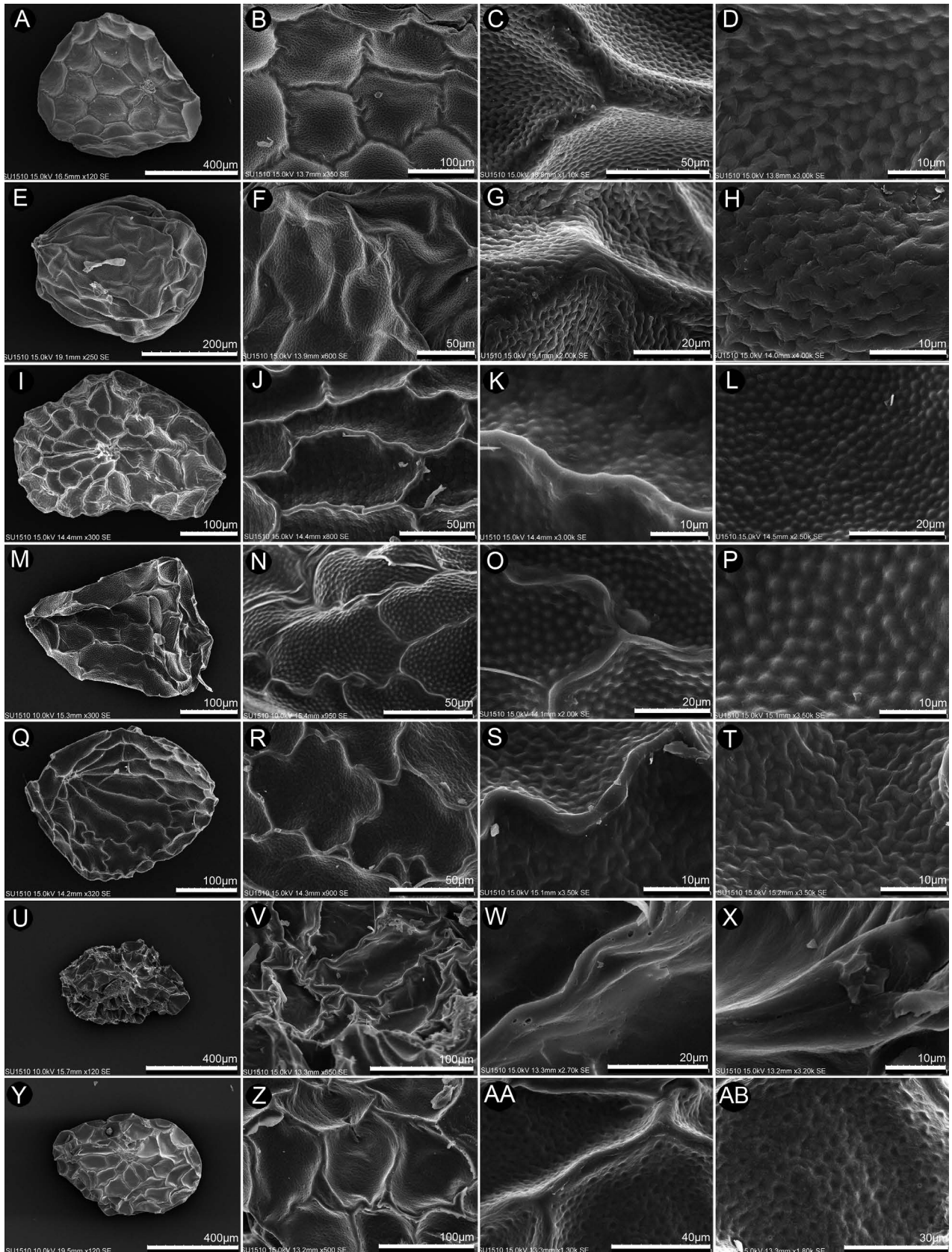


Figure 7 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Deppea purpusii*. **E–H.** *Deppea umbellata*. **I–L.** *Deppeopsis anisophylla*. **M–P.** *Deppeopsis hernandezii*. **Q–T.** *Deppeopsis tenuiflora*. **U–X.** *Editheia floribunda*. **Y–AB.** *Editheia guerrerensis*.

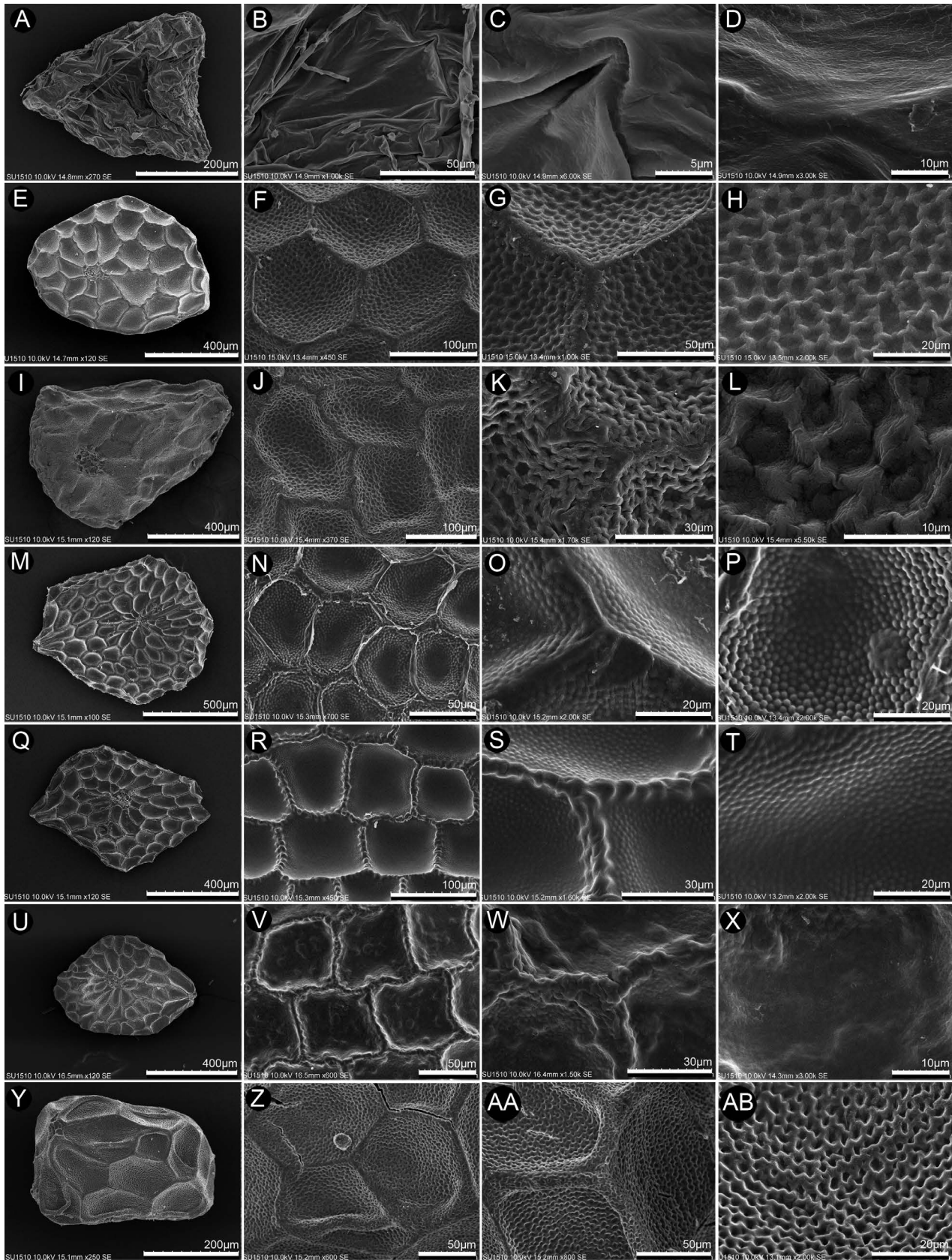


Figure 8 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Editheia miahuatlanica*. **E–H.** *Editheia schiblii*. **I–L.** *Eizia mexicana*. **M–P.** *Hamelia axillaris*. **Q–T.** *Hamelia patens*. **U–X.** *Hamelia xorullensis*. **Y–AB.** *Hoffmannia gesnerioides*.

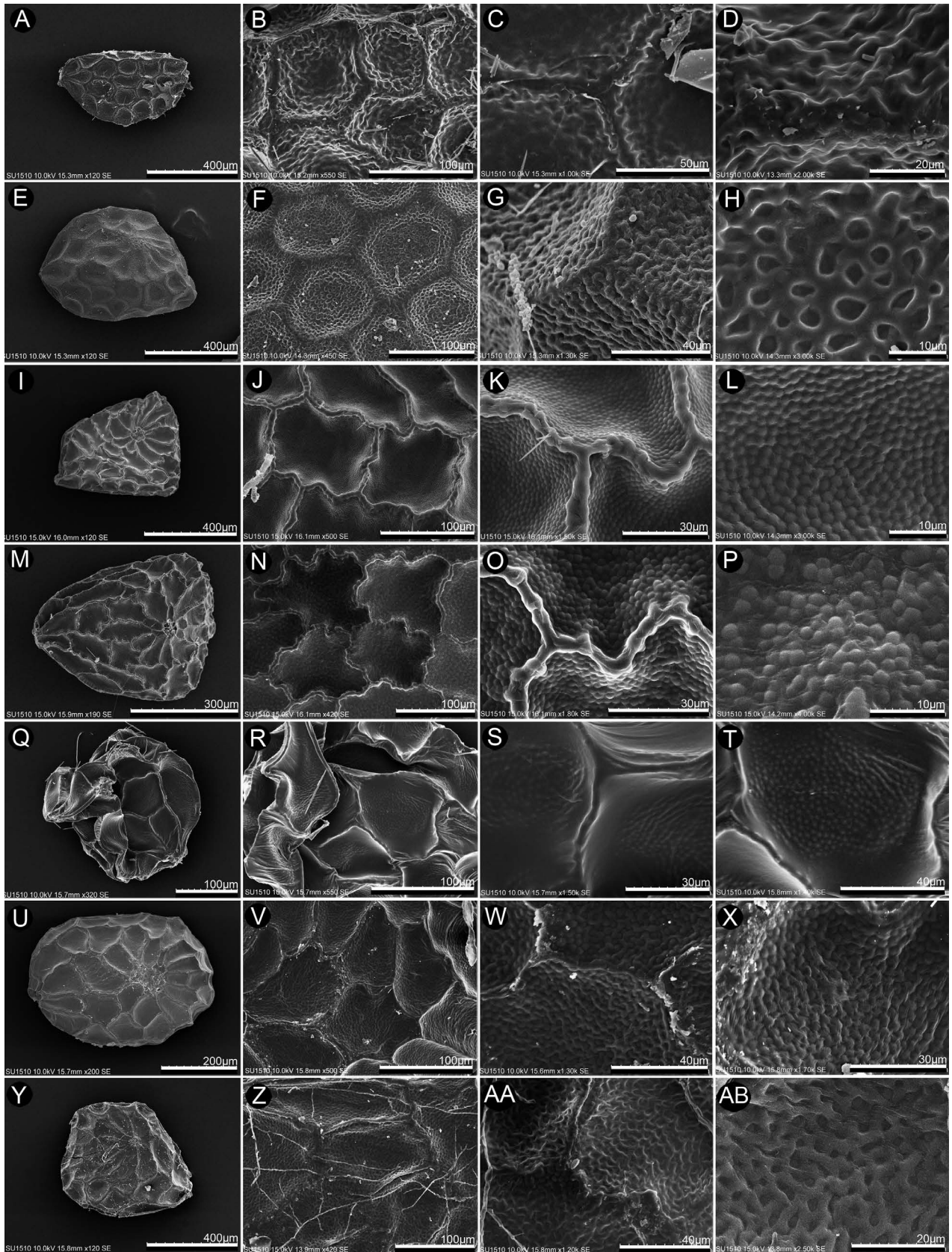


Figure 9 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. **A–D.** *Hoffmannia ghiesbreghtii*. **E–H.** *Hoffmannia oaxacensis*. **I–L.** *Omiletemia longipes*. **M–P.** *Omiletemia parvifolia*. **Q–T.** *Pinarophyllon flavum*. **U–X.** *Plocaniophyllon flavum*. **Y–AB.** *Pseudomiletemia filisepala*.

splendens; inconspicuous hilum in *C. sousae* and evident in *C. splendens*.

Within *Deppeopsis* (figs 7I–T, 12G–J), *D. hernandezii* can be distinguished from the other species in the genus by the isodiametric cells surrounding the micropyle (figs 7M–O), in contrast to the elongated cells surrounding the micropyle of the other two species (figs 7I–L, Q–T), for which no seed characters were found to differentiate between them.

The *Omitelia* species can be distinguished by the general seed shape and hilum visibility: *O. longipes* has rhomboidal seeds and an inconspicuous hilum (figs 9I, 13E–F), while *O. parvifolia* has ellipsoidal seeds and an evident hilum (figs 9M, 13G–H).

The *Edithea* species are variable (figs 7U–AB, 8A–H): *E. mihuantlanica* can be distinguished from other species by the not well-defined testa cells, elongated lateral cells, and the lateral micropyle position (figs 8A–B, 12O–P). The species *E. guerrerensis* and *E. schiblii* share the cells surrounding the micropyle different from the lateral ones, the elongated cells surrounding the micropyle plus the concave outline with a sunken profile microrelief (figs 7Y–AA, 8E–G); they differ by the evidently flattened seeds and the slightly anticlinal wall boundary in relief of *E. schiblii* (fig. 8F–G). The species *E. floribunda* and *E. guerrerensis* differ mainly by the smooth periclinal microrelief of the former (fig. 7W–X) in contrast to the concave outline with a sunken profile of the latter (fig. 7AA–AB).

DISCUSSION

This study includes species from all genera in Hamelieae, except for the South American genus *Patima*, and it demonstrates the usefulness of seed morphology for the circumscription of genera and species, at least in most cases, but almost always using combinations of seed character states. Our sampling even included the monotypic genus *Eizia*, which was presumed extinct until it was rediscovered a few years ago (Martínez-Camilo et al. 2015).

Seed morphology in Hamelieae

Some Hamelieae genera are easily recognizable by unique character states such as the winged seeds in the monotypic *Syringantha* or the seed size and anticlinal wall boundaries in *Cosmocalyx*. Other genera have a unique combination of character states: e.g. *Hamelia* (with ca 16 spp) has rhomboidal seeds, straight anticlinal wall boundaries, and papillose microrelief. The genus *Hoffmannia*, the largest in the tribe (ca 100 spp.), requires more sampling to firmly make conclusions about the homogeneity or variability of its seed characters, given that only three species were included in our study. Nevertheless, there are several constant characters in the randomly sampled species (see supplementary file 3) and they might be constant at genus level as well. *Hamelia* and *Hoffmannia* have fleshy fruits, an uncommon character state in the tribe since all other genera have capsular fruits, although more species of *Hoffmannia* should be investigated to confirm if these are constant characters related to fruit texture.

For the genus *Patima* (not studied here), Delprete (1998) described the seeds as minute (ca 0.3–0.5 mm), globose, deeply reticulate, with regular shaped cells, with (4)5 or 6 sides per cell. Based on this description, we consider that the seeds of *Patima* are different from those of any other genus in Hamelieae.

Winged seeds are associated with some lineages in Rubiaceae, and this character state is generally constant at tribal or generic level. For example, Cinchoneae is identified by this character state (Andersson 1995), as well as *Bouvardia* Salisb. and *Manettia* Mutis ex L. (Terrell & Robinson 2004). The wings can be highly diverse: they can be equal and present all around the margin of the seed (e.g. *Bouvardia*), or unequal with edges at both poles of the seeds (e.g. *Cinchona*), or unilateral with only one pole developed (e.g. *Simira*) (Bremer & Eriksson 1992). Within Hamelieae, a winged margin is not a constant character state and, although it is only well-defined in one genus (i.e. *Syringantha*), in some species it is possible to see a more translucent or thinly

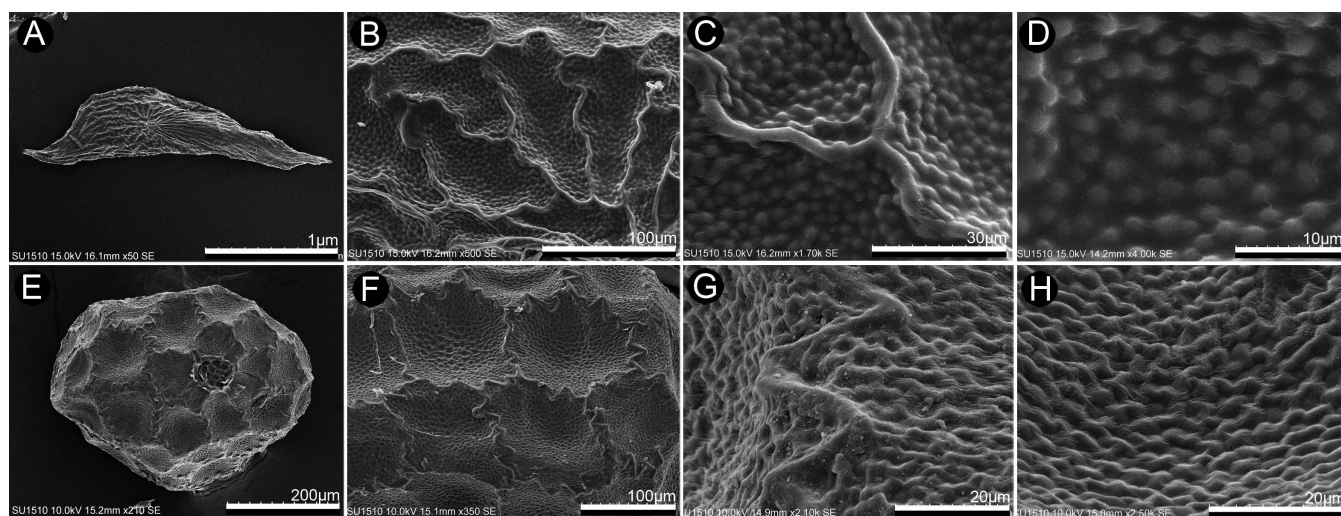


Figure 10 – SEM photographs showing, left to right, a general view of a seed, lateral cells, a detail of the anticlinal wall boundaries, and a detail of the periclinal microrelief. A–D. *Syringantha coulteri*. E–H. *Schenckia blumenaviensis*.

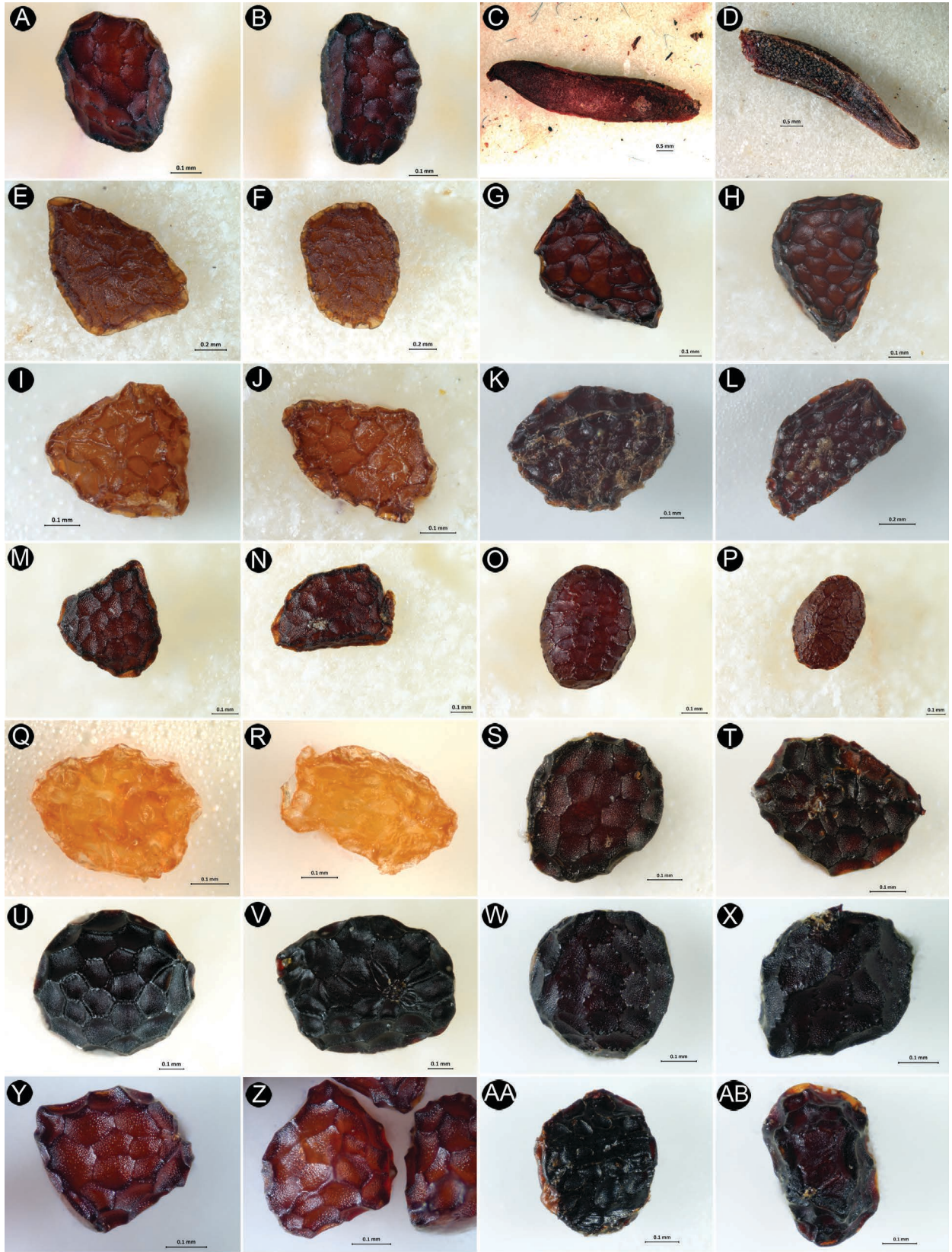


Figure 11 – LM photographs showing a dorsal (left) and a ventral (right) seed view. **A–B.** *Bellizinca scoti*. **C–D.** *Cosmocalyx spectabilis*. **E–F.** *Csapodya sousae*. **G–H.** *Csapodya splendens*. **I–J.** *Deppea amaranthina*. **K–L.** *Deppea cornifolia*. **M–N.** *Deppea erythrorhiza*. **O–P.** *Deppea grandiflora*. **Q–R.** *Deppea hamelioides*. **S–T.** *Deppea hintonii*. **U–V.** *Deppea microphylla*. **W–X.** *Deppea obtusiflora*. **Y–Z.** *Deppea pubescens*. **AA–AB.** *Deppea purpurascens*.

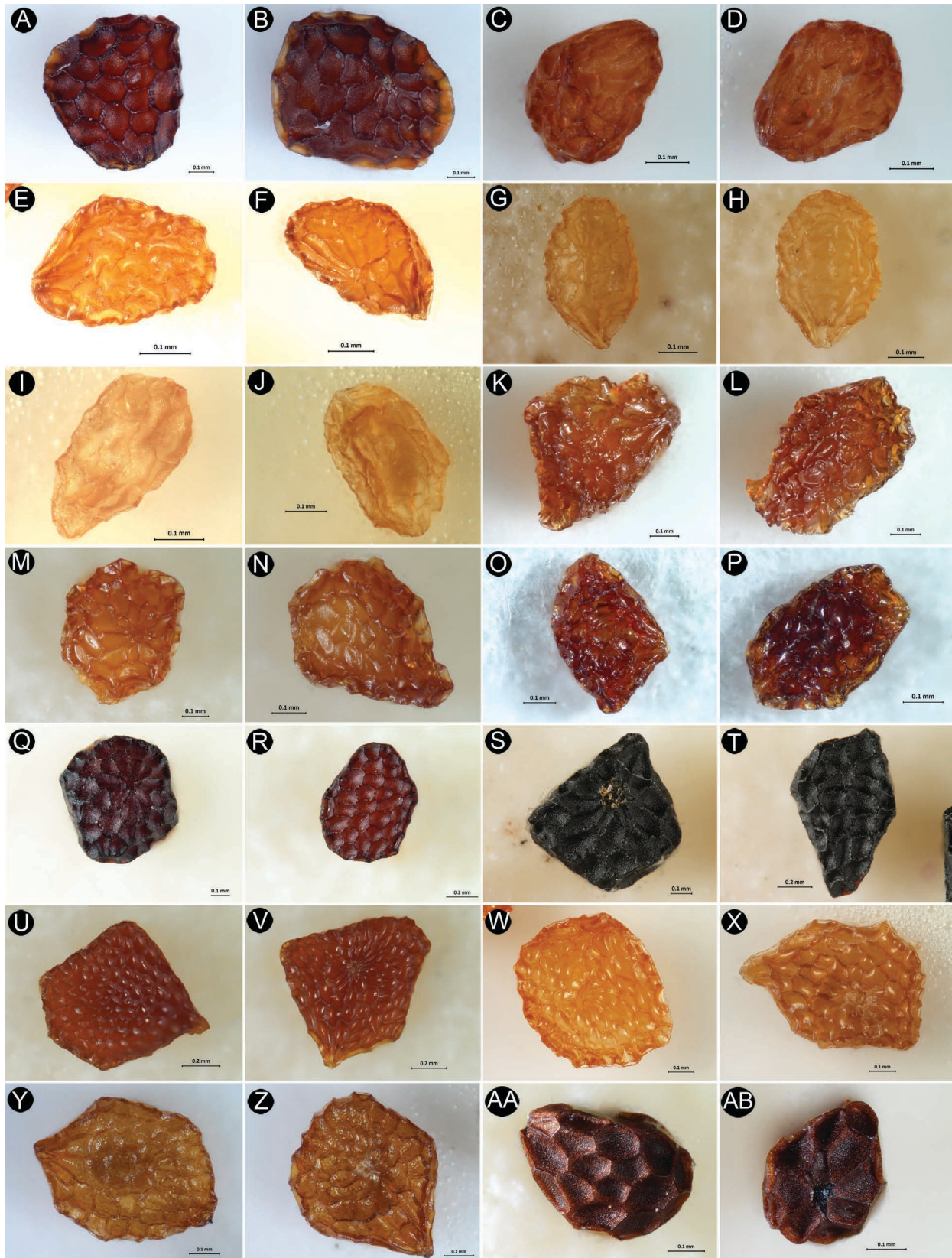


Figure 12 – LM photographs showing a dorsal (left) and a ventral (right) seed view. A–B. *Deppea purpusii*. C–D. *Deppea umbellata*. E–F. *Deppeopsis anisophylla*. G–H. *Deppeopsis hernandezii*. I–J. *Deppeopsis tenuiflora*. K–L. *Editheia floribunda*. M–N. *Editheia guerrerensis*. O–P. *Editheia miahuatlanica*. Q–R. *Editheia schiblii*. S–T. *Eizia mexicana*. U–V. *Hamelia axillaris*. W–X. *Hamelia patens*. Y–Z. *Hamelia xorullensis*. AA–AB. *Hoffmannia gesnerioides*.

slimmed edge (e.g. *Deppeopsis*; fig. 12I–J), but because it is very narrow we did not consider it as a true wing.

Seed shape could be related to the number of seeds per fruit or per locule, as discussed by Robbrecht (1988), who mentioned that seed shape can be dependent on available space, and small seeds with a convex abaxial side and angular radial sides are common in many-seeded fruits. However, this hypothesis is not corroborated by our observations, since the capsular fruits of the genera in the *Deppea* complex are many-seeded, and among them there are taxa with flattened seeds (e.g. *Deppeopsis*) and with polygonal seeds (e.g. *Deppea*), independent of fruit and seed size. Among

Hamelieae, there are two types of mature fruit according to texture: fleshy berries in *Hamelia*, *Hoffmannia*, and *Patima* (Delprete et al. 2005; Lorence 2012) and dry capsular fruits in the rest of the genera (Borhidi 2012; Lorence 2012). Nevertheless, there is no correlation between seed size or shape with fruit type as, for example, capsular fruits have seeds with different sizes and shapes. Typically, fleshy fruits are associated with zoochory (Bremer & Eriksson 1992) and in these cases, the seeds are sometimes covered by sclerified testa walls that protect the embryo from the digestive tract acids of the animals (e.g. Schaumann & Heinken 2002). We did not observe major differences between the testa walls

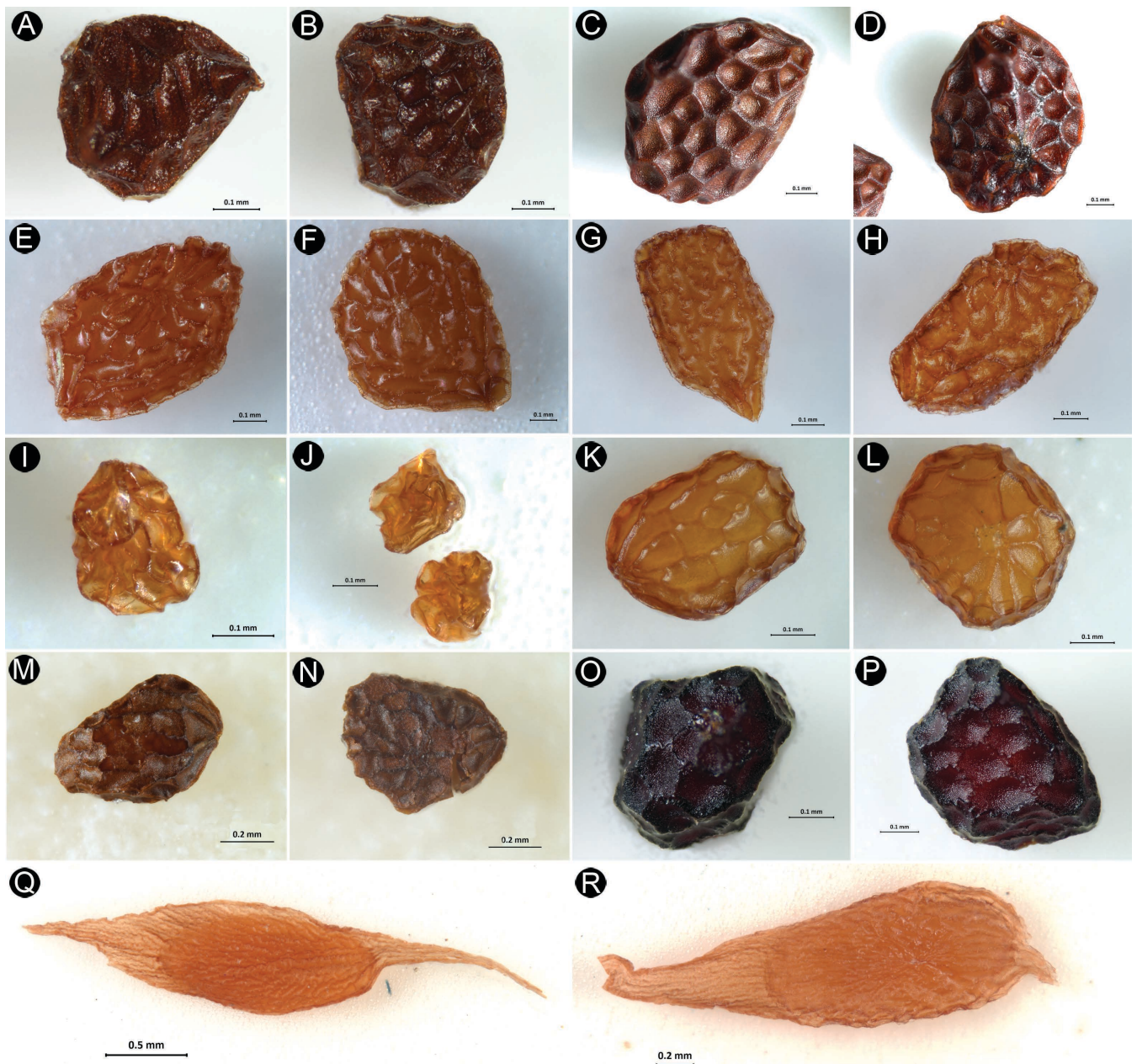


Figure 13 – LM photographs showing a dorsal (left) and a ventral (right) seed view. A–B. *Hoffmannia ghiesbreghtii*. C–D. *Hoffmannia oaxacensis*. E–F. *Omiletemia longipes*. G–H. *Omiletemia parvifolia*. I–J. *Pinarophyllon flavum*. K–L. *Plocantiophyllon flavum*. M–N. *Pseudomiletemia filisepala*. O–P. *Schenckia blumenaviensis*. Q–R. *Syringantha coulteri*.

from seeds in fleshy fruits (e.g. in *Hamelia*) as compared to those of seeds in dry fruits (e.g. in *Deppea*).

Systematics of the *Deppea* complex

The great generic diversity of Hamelieae is directly related to the *Deppea* complex, for which a phylogenetic framework is not yet conclusive. In the most complete phylogeny for the Hamelieae tribe, Stranczinger et al. (2014) included 13 genera and 33 species; their results showed that among the non-monotypic genera only *Hamelia* was retrieved as monophyletic, while all the genera in the *Deppea* complex were placed inside an unsolved clade or retrieved as paraphyletic. These results could reflect an artificial classification of the tribe, but they could also be an artifact of the few characters used to reconstruct the phylogenetic hypothesis (two molecular markers only). The seed morphological mosaic among the sampled taxa in this study does not solve the conflictive circumscription of *Deppea*, which depending on the author is synonymized with *Bellizinca*, *Csapodya*, *Deppeopsis*, *Edithea*, and *Schenckia* (Lorence & Dwyer 1988; Manns & Bremer 2010; Lorence 2012) or accepted as separate generic entities (Borhidi et al. 2004a, 2004b; Borhidi 2006, 2012). Either these genera are not monophyletic or the seed characters used here are in many cases highly homoplastic; only a robust phylogeny constructed with a diverse set of data will reveal which of these two explanations, if any, is more supported.

The phylogenetic hypothesis presented by Stranczinger et al. (2014) retrieved a clade without resolution in which *Bellizinca*, *Csapodya*, *Edithea*, and *Schenckia* are paraphyletic with respect to *Deppea*. This could support the recognition of a single large genus *Deppea*. Nevertheless, the flower morphology is very different in *Deppea* with respect to the other genera (fig. 1), not only regarding the colour of the petals, but also the corolla tube size. Morphologically, *Bellizinca*, *Csapodya*, and *Edithea* are different from *Deppea* by the corolla tube length being larger than 1 cm (Borhidi et al. 2004a). In addition, there are other differences in flower morphology among the genera: *Bellizinca* is recognized by the tubular corolla of 1.4 to 2.6 cm long, the foliaceous calyx lobes up to 1.8 cm long and the stamens attached at the base of the corolla tube (Borhidi et al. 2004a); *Csapodya* is characterized by the 5 to 5.5 cm tubular or salverform corolla, the foliaceous calyx lobes, 8 to 25 mm long, and the stamens attached at the middle portion of the corolla tube (Borhidi et al. 2004a; Borhidi & Reyes-García 2007), while *Edithea* is well characterized by the salverform corolla with the stamens attached near the corolla throat (Borhidi et al. 2004a). The corolla length and colour differences are interpreted as shifts in the pollination mechanism, with a bee pollination syndrome in *Deppea* and a hummingbird pollination syndrome in the other genera (Lorence & Dwyer 1988; Lorence 2012).

Taxonomic implications of seed morphology in the *Deppea* complex

Seed morphology for the *Deppea* complex was briefly studied by Borhidi et al. (2004a), who compared seed differences among *Bellizinca*, *Csapodya*, *Edithea*, and

Deppea mentioning shape, hilum position, and testa ornamentation differences. Our results partially agree with those of Borhidi et al. (2004a) in that the seed shape among these genera is different. However, we found discrepancies in certain characters, particularly regarding the hilum position: we treat as the micropyle what Borhidi and collaborators considered to be the hilum, based on our SEM microphotographs and on the orthotropous nature of the ovule in most of the studied genera (Martínez-Cabrera et al. 2014). Orthotropic ovules were described using anatomical methods for many Hamelieae members by Martínez-Cabrera et al. (2014) including *Deppea*, *Hamelia*, *Hoffmannia*, *Omitelia*, *Pinarophyllon*, *Plocaniophyllon*, and *Syringantha*. Nevertheless, for *Csapodya splendens*, the hilum and micropyle positions indicate a more likely anatropous ovule nature, a condition common in most Rubiaceae genera (Robbrecht 1988; Martínez-Cabrera et al. 2014). Further anatomical studies are however needed to corroborate the ovule nature in *Csapodya*.

Csapodya* and *Bellizinca – The seeds of *Csapodya* are easily recognized from those of *Deppea* by being flattened, a character state shared with *Deppeopsis*, *Omitelia*, and *Pseudomitelia*. *Csapodya* differs by the combination of the concave outline with a sunken profile microrelief plus V-undulate anticlinal wall boundaries and the evident hilum. It should be noted that in the phylogenetic framework presented by Stranczinger et al. (2014), *Csapodya* is placed in a different lineage with respect to *Deppeopsis*, *Omitelia*, and *Pseudomitelia*, which share a recent common ancestor. The seeds in the genus *Bellizinca* can be distinguished by the V-undulate anticlinal wall boundary, in contrast to the U-undulate or straight anticlinal wall boundary in *Deppea*. This character state, in addition to the corolla characters, could support the recognition of *Bellizinca* and *Csapodya* as separate generic entities. The genus *Csapodya* was segregated from *Deppea* based on *D. splendens* (Borhidi et al. 2004a) and currently could include two or three species (Borhidi & Reyes-García 2007). However, in the Rubiaceae treatment of the Mesoamerican Flora (Lorence 2012), *Csapodya* is treated as a synonym of *Deppea*, and only one species potentially belonging in *Csapodya* (i.e. *D. splendens*) is recognized, arguing that the other species were described based on immature inflorescences. Despite several attempts to collect *C. challengerii*, we failed as there appear to be errors in the type locality (the provided coordinates do not coincide with the vegetation or elevation description), however, our results support that *C. sousae* and *C. splendens* are different species, since they have several contrasting characters, including the colour, exotesta cell shape, anticlinal wall boundaries, micropyle position, and hilum visibility.

Edithea – The seed morphology of the genus *Edithea* is variable and overlaps with most of the other genera; some species are easily recognizable by the smooth periclinal microrelief (*E. floribunda* and *E. miahuatlanica*), a unique character state among all the Hamelieae taxa studied. However, the other species in the genus have concave outlines with a sunken profile (*E. guerrerensis* and *E. schiblii*), a common character state among the species in the *Deppea* complex. In contrast to *Csapodya* and *Bellizinca*, which in the Stranczinger et al. (2014) phylogeny formed

a clade within *Deppea* in an unsolved position, the *Edithea* species are placed inside a clade with other *Deppea* species, highlighting the possibility of a polyphyletic *Deppea*. Seed morphology may support the previously suggested synonymy of *Edithea* and *Deppea* by using other morphological characters and molecular evidence (Lorence & Dwyer 1988; Manns & Bremer 2010; Lorence 2012). However, further studies with other sources of evidence and broader taxon sampling are needed to reliably take decisions upon the synonymy of the names or the acceptance of these taxa.

Deppeopsis – Another genus sometimes synonymized with *Deppea* is *Deppeopsis*, which was primarily segregated by its cylindrical fruit shape (Borhidi & Stranzinger 2012). Currently, it includes five species from Mexico and Guatemala (Borhidi 2012; Borhidi & Stranzinger 2012). In the Mesoamerican Rubiaceae treatment (Lorence 2012), only two species of this genus are included, and they are treated under *Deppea*. Using seed morphology, *Deppeopsis* is easily distinguishable from *Deppea* by the flattened seeds, but other character states, such as the yellowish testa colour (only shared with *Deppea hamelioides*), U-undulate anticlinal wall boundaries, papillose periclinal microrelief, and the lateral position of the micropyle are shared with at least one species of *Deppea*. Based on our seed character observations, we support the separation of *Deppeopsis* from *Deppea*, as it was also suggested by the Stranzinger et al. (2014) phylogenetic hypothesis, in which *Deppeopsis* is clearly a separate lineage from *Deppea*, more closely related to other taxa with flattened seeds such as *Omitelia* and *Pseudomitelia*.

Pseudomitelia* and *Omitelia – The Mexican genus *Pseudomitelia* was segregated from *Omitelia* (Borhidi et al. 2004b) and has two species characterized by the stamen insertion near the corolla throat (vs near the base in *Omitelia*) and the funnellform corolla (vs campanulate in *Omitelia*) (Martínez-Camilo et al. 2011; Borhidi 2012). The Mexican genus *Omitelia* was described by Standley (1918) based on the red and solitary flowers and has three species (Borhidi & Velasco-Gutiérrez 2010; Borhidi 2012; Lozada-Pérez & Rojas-Gutiérrez 2016). Both share flattened seeds, while the main difference is the periclinal microrelief, papillose in *Omitelia* and concave outlines with a sunken profile in *Pseudomitelia*. Borhidi et al. (2004b) also used seed morphology in conjunction with other evidence to justify the transfer of *Omitelia filisepala* to *Pseudomitelia* using differences in the hilum position (lateral in *Omitelia* and central *P. filisepala*); perpendicular walls surface (slightly undulate in *Omitelia* and slightly tuberculated in *P. filisepala*), and horizontal wall microrelief (smooth in *Omitelia* and densely tuberculated in *P. filisepala*). Our study differs in the description of the micropyle position, presumably treated as hilum in Borhidi et al. (2004b), considered to be for both species as sub-central, as well as in the microrelief that was considered to be smooth in *Omitelia*, while our results evidently show a papillose microrelief. Based on the seed morphology, both genera, *Pseudomitelia* and *Omitelia*, should be recognized.

Pinarophyllon* and *Plocaniophyllon – The other genera of the *Deppea* complex are *Pinarophyllon* and *Plocaniophyllon*. The genus *Pinarophyllon* was recognized by the herbaceous habit and the turbinate fruits (Brandegge 1914; Lorence

2012) and has two species in Mexico and Guatemala. The monotypic Mexican genus *Plocaniophyllon* was described based on the lineolate leaf venation (Brandegge 1914; Lorence 2012). In both genera, the seeds are polygonal and ellipsoid with straight anticlinal wall boundaries. These are the same character states as in *Hoffmannia*, but they differ in that *Pinarophyllon* and *Plocaniophyllon* have smooth or papillose periclinal wall microrelief, while *Hoffmannia* has verrucose or concave outlines with a sunken profile. The seeds of *Plocaniophyllon* were described by Martínez-Cabrera et al. (2008) as ovoid, “irregular”, 0.4–0.6 mm long, with large polygonal shaped cells and foveolate multicellular sculpture, anticlinal walls boundaries slightly undulated, and outer periclinal walls concave with tuberculate microornamentation. Our description agreed in the seed shape, size, and cell shape; however, Martínez-Cabrera et al. (2008) defined the cells boundaries as slightly undulated while we consider them to be straight, as well as the microrelief to be papillose instead of tuberculate. The seed character state to distinguish *Pinarophyllon* and *Plocaniophyllon* is the shape of the cells surrounding the micropyle: isodiametric in *Pinarophyllon* and elongated in *Plocaniophyllon*.

Schenckia – In the literature, there is consensus to treat *Schenckia* as a synonym of *Deppea* (Lorence & Dwyer 1988; Lorence 2012). Its inclusion gives the distribution of *Deppea* to a disjunct pattern since *Schenckia* is only known from Blumenau in Brazil and from the north of Argentina (Lorence & Dwyer 1988), whereas *Deppea* is practically restricted to the Mesoamerican region. The seed morphology, as the molecular evidence (Manns & Bremer 2010), supports its synonymy as there is no unique combination of characters to distinguish *Schenckia* from *Deppea*.

CONCLUSION

Our study reveals why the *Deppea* complex is so problematic from a taxonomic point of view and reflects the fact that a comprehensive approach with different lines of evidence, such as morphological, molecular, and phylogenetic characters, is needed to evaluate the generic circumscriptions within the *Deppea* complex in the Hamelieae tribe. Despite this, we addressed the importance of seed morphology as a source of characters in Hamelieae systematics and taxonomy and we highlight the broad diversity of seed morphological character states in this tribe.

Although the characters evaluated here show high overlap across the genera, there is some evidence that seed morphology can contribute to taking taxonomic decisions within the *Deppea* complex. Based on our study, we consider that there is support for the recognition of *Bellizinca*, *Csapodya*, and *Deppeopsis*, currently considered to be synonyms of *Deppea* by some authors. Also, seed morphology supports the synonymy of *Edithea* and *Schenckia* with *Deppea*, and provided additional morphological evidence for the recognition of *Omitelia*, *Pinarophyllon*, *Plocaniophyllon*, and *Pseudomitelia*. However, for these taxonomic decisions to be definitive, an integrative approach using a robust phylogenetic context is still necessary. In particular to establish the generic limits within the *Deppea* complex inside the Hamelieae tribe, a full

perspective that includes micro- and macromorphology plus molecular evidence is desirable to unravel this conflictive group.

SUPPLEMENTARY FILES

Supplementary file 1 – Voucher information of the studied specimens, which are all deposited at MEXU except for *Schenckia blumenaviensis*, which is deposited at C.

<https://doi.org/10.5091/plecevo.84486.suppl1>

Supplementary file 2 – Illustrated overview of the seed characters and character states.

<https://doi.org/10.5091/plecevo.84486.suppl2>

Supplementary file 3 – Full seed descriptions of the 37 studied species in 15 genera of the Hamelieae.

<https://doi.org/10.5091/plecevo.84486.suppl3>

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