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GRIMMIA FUSCOLUTEA WITH GEMMAE AND OBSERVATIONS ON OTHER PROPAGULIFEROUS *GRIMMIA*[1]

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Abstract. Gemmae are reported for the first time in *Grimmia fuscolutea* Hook. The morphology, development and abscission mechanisms are described and contrasted with other gemmiferous *Grimmia* species in the *Rhabdogrimmia* clade. Light microscopy and cryo-scanning electron microscopy were used to make observations, the latter a novel technique that allows detection of any water soluble surface ornamentation and to distinguish between hydrophilic and hydrophobic surfaces. Smooth walled highly hydrophilic gemmae in *G. fuscolutea* occur on the dorsal and ventral laminal and costal cells usually medially but sometimes distally. Gemma ontogeny follows a similar pattern in all propaguliferous *Grimmia* with cells of either the lamina or costa or both giving rise to uniseriate or branched multicellular filaments which differentiate into gemmae. The parent filament never re-differentiates into a new initial from which further gemmae arise. Detachment of gemmae from the parent filament is usually by formation of specialized abscission cells whilst separation of individual gemmae is by the breakdown of the middle lamellae and either or both mechanisms may occur in one species. Similarly to *G. fuscolutea*, the position of gemmae in *G. trichophylla* Grev. is variable. Uppermost leaves that support distal gemmae result in highly modified leaves consisting of almost exclusively smooth-walled elongate cells. In all other gemmiferous *Grimmia* species, including *G. austrofunalis* Müll. Hal, the position of gemmae is much more stable; in *G. austrofunalis* gemmae occur almost exclusively proximally on the dorsal costa.

Key words: abscission cells, asexual reproduction, cryo-scanning electron microscopy, foliar gemmae, *Grimmia*, Grimmiaceae, *Rhabdogrimmia*

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INTRODUCTION

Asexual propagules are rare in the Grimmiaceae with foliar gemmae in *Grimmia* (Hedw.) Schimp. being the only known vegetative diaspores produced by the family (protonemal gemmae, rhizoidal gemmae and tubers are unknown) (Pressel and Duckett, personal observations).

Phylogenetic studies based on molecular and morphological characters recognized two main clades within the Grimmiaceae, the *Rhabdogrimmia* clade containing the species traditionally placed in the subgenus *Rhabdogrimmia* Limpr. and the *Grimmia* clade, containing the remaining *Grimmia* species (Streiff 2006). The presence or absence of foliar gemmae is considered a main morphological difference between the two clades (Streiff 2006). *Grimmia fuscolutea* Hook. has traditionally been placed either in the subgenus *Grimmia* (Hedw.) Schimp (Limpricht 1890) or in the subgenus *Guembelia* (Hampe) Schimp. (Brotherus 1924). More recently, *G. fuscolutea* has been placed in the subgenus *Rhabdogrimmia* (Nyholm 1998) and Maier (2010) has retained this position even though the capsule is faintly ribbed to occasionally smooth.

Correns (1899) described and illustrated gemma development, morphology and abscission mechanisms in *Grimmia*, and showed that gemma formation is either by modification of the leaf apex into large gemma clusters (*G. anomala* Hampe and *G. hartmanii* Schimp.) or by formation of filaments, either branched or not, from both laminal and costal cells and variably on both sides of the leaf, which then differentiate terminally into gemmae (e.g. *G. muehlenbeckii* Schimp. and *G. trichophylla* Grev.). Correns (1899) described how gemma liberation in the species included in

[1] This paper is dedicated to the late Dr. Marian Kuc.

his work is either by formation and subsequent rupture of a Tmema (abscission cell) with the remnants of the Tmema cell wall remaining on both parent cell and liberated gemma (lysogeny) or by breakdown of the middle lamella (schizolysis). More recently Muñoz (1999) and Ignatova and Muñoz (2004) provided extensive details of gemma development and morphology in *Grimmia* and stressed (Muñoz 1999) how 'features associated with the development of gemmae are critical to distinguish the closely related *G. austrofunalis* and *G. trichophylla*'. However, they did not discuss abscission mechanisms and gemmae were not reported in G. *fuscolutea* in their studies or any other studies, as far as we are aware.

The recent discovery by one of us (RDP) of a propaguliferous population of *G. fuscolutea* from Réunion Island prompted this work. Thus the aims of this study were to describe for the first time the occurrence, development, morphology and abscission mechanisms of gemmae in *G. fuscolutea* and compare these with the same in other *Grimmia* species, in particular the closely related *G. trichophylla* and *G. austrofunalis* Müll. Hal.

Materials and methods

Plant material

During a British Bryological Society Tropical Bryology Group visit to Réunion Island in 2008 a propaguliferous *Grimmia* was collected by RDP which was later determined as *G. fuscolutea* (Ah-Peng *et al.* 2010). Although there is a previous record of this species for Réunion Island (Muñoz & Pando 2000), this article describes for the first time the occurrence of gemmae in *G. fuscolutea*.

Five collections of *G. fuscolutea* were made, three from Col des Bouefs, on the western edge of Cirque de Salazie, and two from Route des Tamarins Nord, Ravine Divon, north-west of le Maïdo. The two localities are separated by the mountainous terrain of Cirque de Mafate, but are only about 10 km apart as the crow flies and are within a National Park and a proposed UNESCO World Heritage site. Plants with gemmae were found only at the latter site on boulders in an intermittently inundated ravine at an altitude of 1750 metres (Fig. 1). Propaguliferous plants can easily be overlooked in the field, particularly in the dry state and furthermore only a few shoots in a cushion are gemmiferous.

Plants in all five collections lacked sporophytes. Because in the absence of sporophytes *G. fuscolutea* can easily be confused with *G. trichophylla* (also given the similarities in gemma ontogeny and liberation described in this paper), our identification was based on careful examination of the gametophytic characters, in particular the costal architecture, summarised in Table 1 (see also Fig. 2).

Additional *Grimmia* species were studied from herbarium specimens; these include *G. anomala* Hampe, *G. austrofunalis* Müll. Hal, *G. hartmanii* Schimp., *G. lisae* De Not., *G. muehlenbeckii* Schimp., *G. torquata* Drumm., and *G. trichophylla* Grev. Nomenclature of *Grimmia* follows Maier (2010).

Microscopy

For light microscopy, rehydrated specimens were mounted in distilled water and photographed with a Zeiss Axioscop 2 microscope equipped with an AxioCam MRc

Table 1. Comparison of *Grimmia fuscolutea* Hook. and *G. trichophylla* Grev. gametophytes. Characters of the costal architecture are crucial to distinguish the two species. The other characters are subject to some variation and are therefore less reliable.

Character	*Grimmia fuscolutea*	*Grimmia trichophylla*
Number of guide cells in TS of costa in leaf base/at insertion	4, the outer two contiguous with the laminal cells (Fig. 2A)	4 with a second tier below of one or more slightly smaller guide cells (Fig. 2K)
Guide cells in TS of costa just above broadest part of leaf	Narrow-elliptical, obliquely arranged to leaf axis (Fig. 2G & H)	Rounded (Fig. 2P & Q)
Transitional cells	Strongly to scarcely sinuose, rectangular, arranged in strict perpendicular rows parallel to costa	Weakly to moderately sinuose, isodiametric to elongate-rectangular
Exterior walls of dorsal costal cells in TS	Mammillate bulging to smooth (Fig. 2A–H)	Smooth, occasionally bulging (Fig. 2K–Q)
Leaf base/insertion in TS	A plica present on one side near costa (Fig. 2E)	Occasionally a weak plica present on one side near costa

Fig. 1. Ravine Divon, below le Maïdo, Réunion, habitat of propaguliferous *Grimmia fuscolutea* Hook., on exposed boulders in dry stream bed at 1750 m.

digital camera. Specimens were also analysed by cryo-scanning electron microscopy (cryo-SEM), a technique that, in contrast to standard SEM, allows detection of any water soluble surface ornamentation and to distinguish between hydrophilic and hydrophobic surfaces. Rehydrated plants were mounted on an aluminium stub using Tissue-Tek (Sakura Finetek, Zoeterwoude, Netherlands) and plunged in liquid nitrogen slush to preserve their hydrated state in a frozen condition in a Gatan Alto 2500 (Abingdon, UK). Once frozen, they were vacuum-transferred to a high vacuum cryogenic preparation chamber to prevent contamination and the build–up of ice. Ice was sublimed off the surface by raising the temperature up to –90°C for 5 min. Samples were cooled to –130°C, AuPd sputter-coated with a cold magnetron sputter coater and then inserted directly into the SEM via an airlock to avoid ice build-up and to maintain their frozen state. Inside the SEM, the samples rested on a cold stage with the temperature maintained at –130°C. A FEI Quanta 3D FEG dual beam microscope with an Oxford Instruments energy dispersive spectroscopy (EDX) attachment was used to image the sample at 5kV and beam current of 53pA.

Results

Formation of foliar gemmae in *G. fuscolutea* begins with ventral and dorsal cells of upper leaves developing protruding tips at their ends (prorae) (Figs 3A and 5A–C). Mostly this is in the median part of the leaf (Fig. 5A, B) but occasionally extends distally (Figs 3C, D, 5D, 6A, B). The prorae continue to develop, forming uniseriate filaments, seldom branched, and usually comprising 3 or more cells, with each cell ranging from 15 µm to up to 50 µm in length (Figs 3B, 6C, D).

The distal filament cells swell and begin to divide forming more or less spherical gemmae

(Figs 4A, 6D) 30 to 40 μm in diameter which are arranged either in short chains or irregular clusters (in size close to *G. trichophylla*) (Figs 3E and 8I). Gemma liberation from the parent filament is usually by formation of a short Tmema cell between the proximal filament cell (stalk cell) and the gemma. After gemma detachment, scars with remnants of the Tmema cell wall remain clearly visible both under the light and scanning electron microscope, on both lamina and costa (Figs 4B–E, 7A) and on the detached gemmae (Figs 3E, 4H, 7D). Short Tmema cells also form between individual gemma cells, most often those arranged into short chains (Figs 3B, 4G), whilst detachment of individual gemma cells arranged into clusters is usually by breakdown of the middle lamella (schizolysis) (Fig. 7E, F), a process that leaves behind smooth scars (i.e. with no cell wall remnants; Figs 3E, 7G). Following gemma liberation from lamina cells the parent filament degenerates – we found no evidence of percurrent proliferation – and eventually shrinks leaving behind a wide smooth-walled depression in the leaf lamina (Fig. 7C). Gemma liberation from the costa is different in that the basal cell of the filaments does not degenerate but remains intact on the costa (Fig. 7A) as described previously by Muñoz (1999) in *G. austrofunalis* and by Ignatova & Muñoz (2004) in *G. torquata*. Gemmae are invariably smooth-walled with highly hydrophilic surfaces. Filaments and gemmae developing on the leaf lamina are most often thin-walled, whilst those forming on the costa exhibit more pronounced wall pigmentation (Fig. 4E, F).

Our observations of gemma formation in *G. trichophylla* (Figs 8 and 9) reveal a greater variability than previously described (Muñoz 1999). On the lamina, filaments develop from the larger, smooth-walled rectangular cells usually positioned proximally and not from the distal, smaller sinuose isodiametric cells (Figs 8A, 9A–C). However, in the uppermost propaguliferous leaves which consist almost exclusively of larger smooth-walled rectangular cells, gemmae develop distally and on both lamina and costa (Figs 8B, C, 9D–F). Here too, as described for *G. fuscolutea*, gemma liberation from the parent filament is by formation of a short Tmema cell, with scars clearly visible on both lamina (Fig. 8F, G) and costa cells (Figs 8H, 9E, F) following gemma detachment. In some instances, we observed on the lamina, but never on the costa, stalk cells following detachment that did not bear any apparent scars, suggesting a schistolytic mechanism, and the same was also occasionally true in *G. fuscolutea* (data not shown). As in *G. fuscolutea*, the basal cell of the parent filament remains intact following detachment from the costa but degenerates when on the lamina (not shown).

In *G. austrofunalis* (Fig. 10) gemmae develop almost exclusively on the proximal dorsal costa (Fig. 10A). Filaments and gemmae have highly pigmented walls (Fig. 10B–D) and following their detachment the basal stalk cells remain on the costa (Muñoz 1999) with scars clearly visible (Fig. 10D – see also Fig. 8 bottom right in Muñoz 1999, although Tmema cells are not mentioned in the text) – thus in this species gemma liberation is by lysogeny. Normally gemmae are released from the parent filament whilst still arranged into large clusters (Fig. 10E) and contain copious amounts of lipids as revealed by the numerous lipid droplets released from squashed gemmae (Fig. 10F). Because of their proximal position, detached gemmae are often retained within the leaves and may germinate *in situ* thus producing rhizoids (Fig. 10G, H). Non-propaguliferous leaves also sometimes produce rhizoids from their proximal dorsal costa (Fig. 10I).

Liberation of gemmae from parent filaments by formation and subsequent rupture of Tmema cells also occurs in all the other gemmiferous species we have analysed, including *G. hartmanii* (Fig. 11) and *G. anomala* (Fig. 12), where gemmae production is restricted to the leaf apex (and as described and illustrated previously by Correns 1899 and Ignatova & Muñoz 2004). In these species too gemma formation starts with the development of filaments, here exclusively from leaf apical cells (Fig. 11C, D) which then differentiate into large gemma clusters (Figs 11A, B, E, F, 12A–C, D, E). Detachment of these clusters leaves behind on the parent filaments prominent scars (Figs 11G, 12F–H). On the other hand, individual gemmae

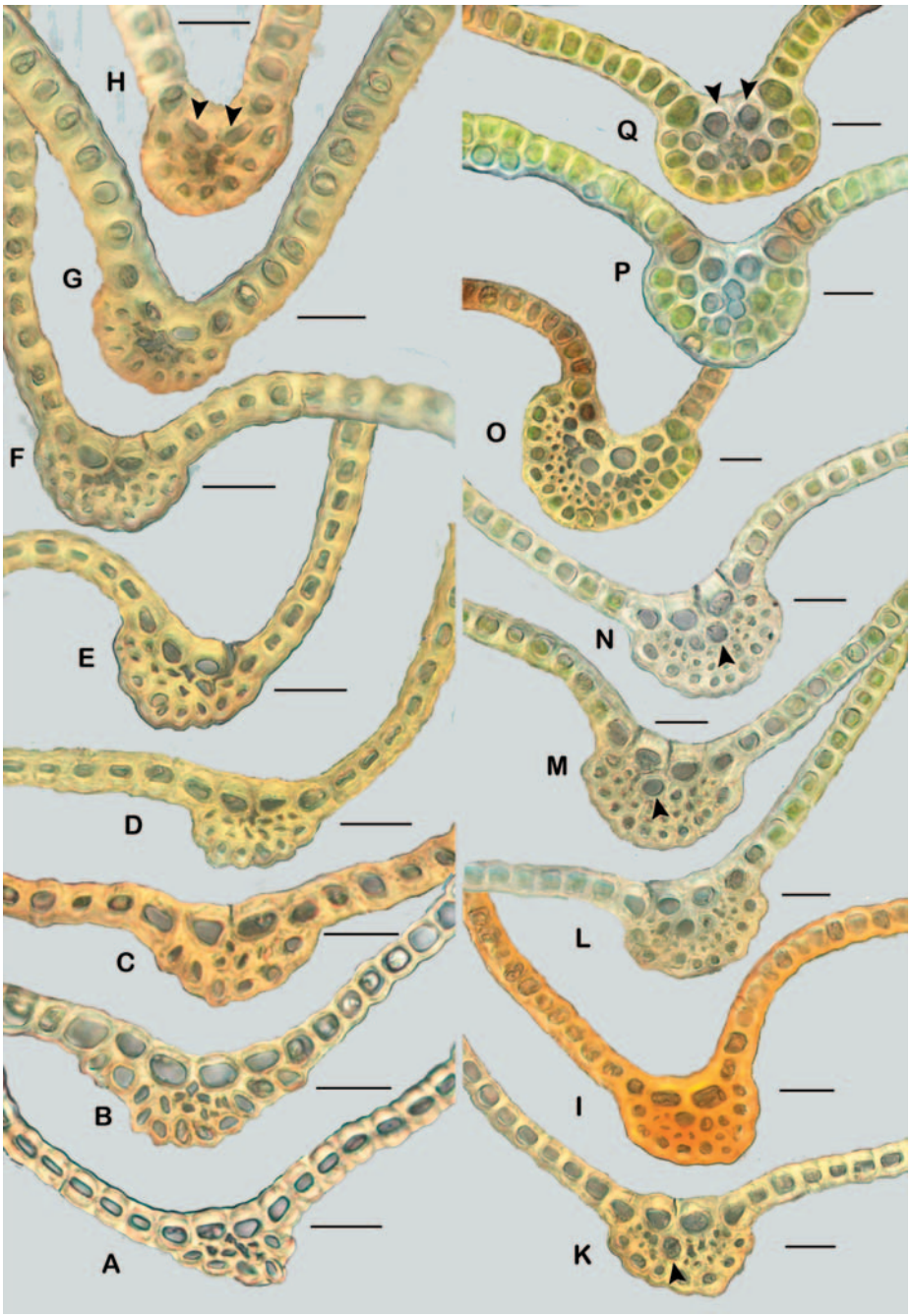

Fig. 2. Comparison of *Grimmia fuscolutea* Hook. (A–H) and *G. trichophylla* Grev. (K–Q) costal architecture. Transverse sections of leaves. In *G. fuscolutea* there are 4 guide cells only. In the proximal part of leaf (A), the outer two are contiguous with the laminal cells. In mid leaf, just above the broadest part of the leaf, the guide cells become narrow-elliptical and obliquely arranged (arrowed) to the leaf axis (F–H). In *G. trichophylla*, in the proximal part of leaf, there is a second tier of somewhat smaller guide cells (arrowed) below the 4 guide cells (K–O). At mid leaf, the guide cells are rounded (arrowed) (O–Q). Scale bars = 20 μm.

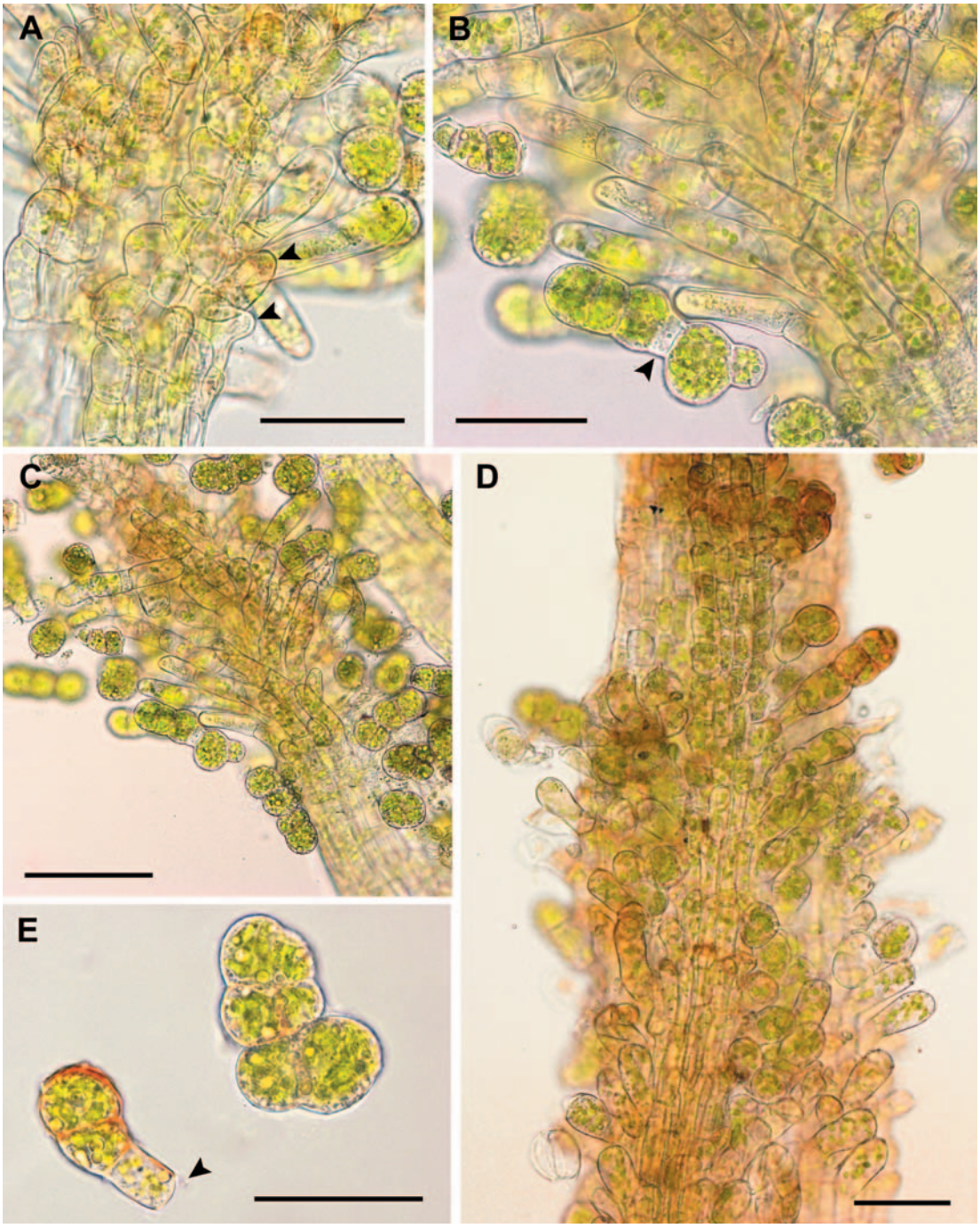

Fig. 3. *Grimmia fuscolutea* Hook. Light microscopy. A – Leaf cells developing protruding tips at their ends (prorae), arrowed. B – Prorae forming 3-celled or more uniseriate filaments (stalks) – arrow indicates Tmema cell. C & D – Prorae forming on distal part of leaf (C) on costa and lamina cells, including marginal cells (D). E – Foliar gemmae in short chains or larger clusters. In the former, a scar with remnants of Tmema cell wall is visible (arrowed). Scale bars: A, B, D, E = 50 µm; C = 100 µm.

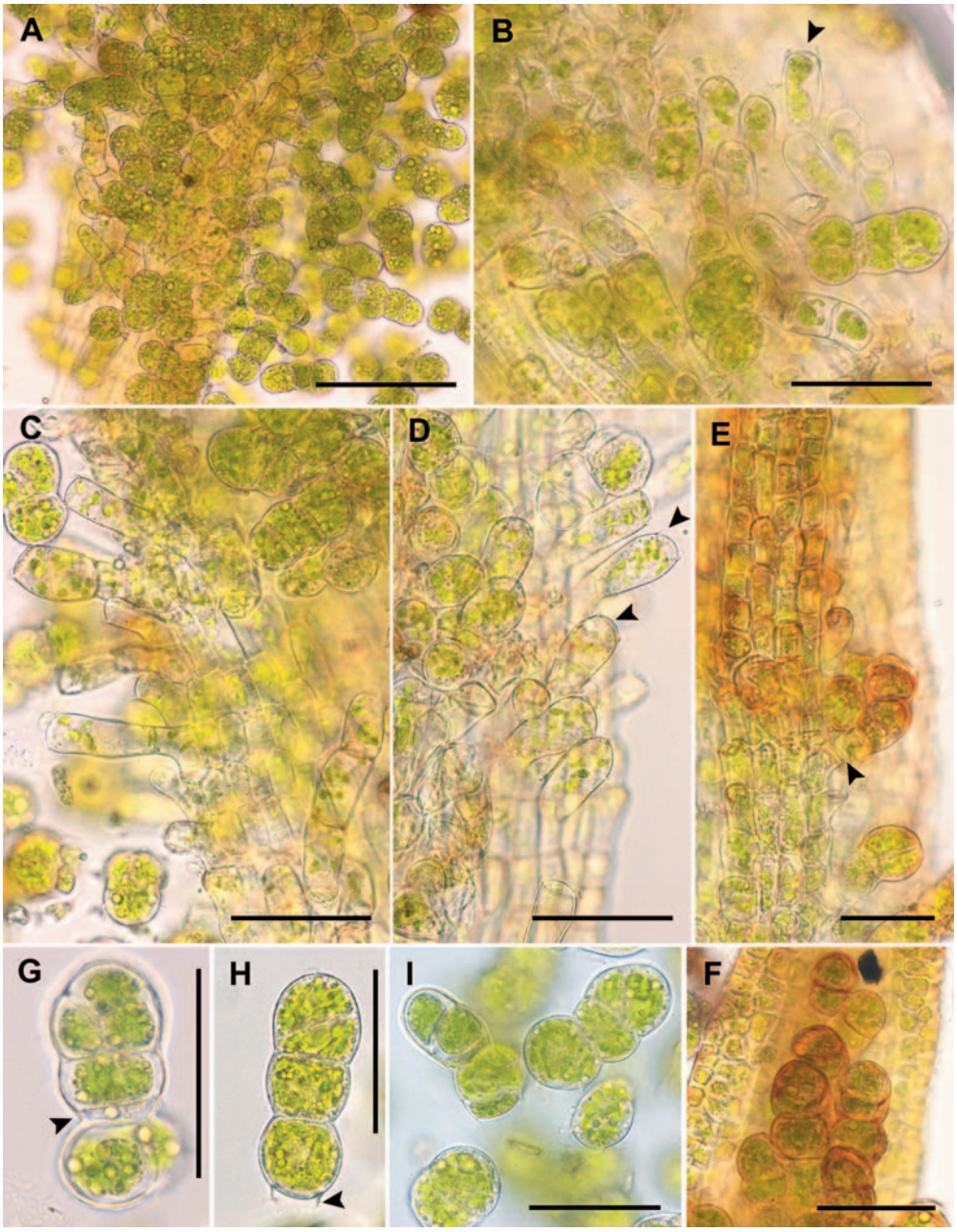

Fig. 4. *Grimmia fuscolutea* Hook. Light microscopy. A – Highly propaguliferous leaf with prorae and gemmae consisting mostly of short chains. B–E – Following gemma detachment, the basal cell of the filaments remains on both lamina (B–D) and costa (E). Whilst those on the lamina are generally thin-walled (B–D), those that remain on the costa have thicker, pigmented walls. Arrows indicate scars that remain on stalk cells following gemmae detachment. F–I – Gemmae after detachment. By and large these consist of short chains. Those produced on the costa (F) have thicker, pigmented walls as compared to those that form on the lamina (G–I). Tmema cells form between the stalks and the gemma proper and also within individual cells of gemmae (arrowed in G, see also Fig. 3B). Scars are clearly visible on the proximal cell of gemmae (arrowed in H). Scale bars: A = 100 μm; B–I = 50 μm.

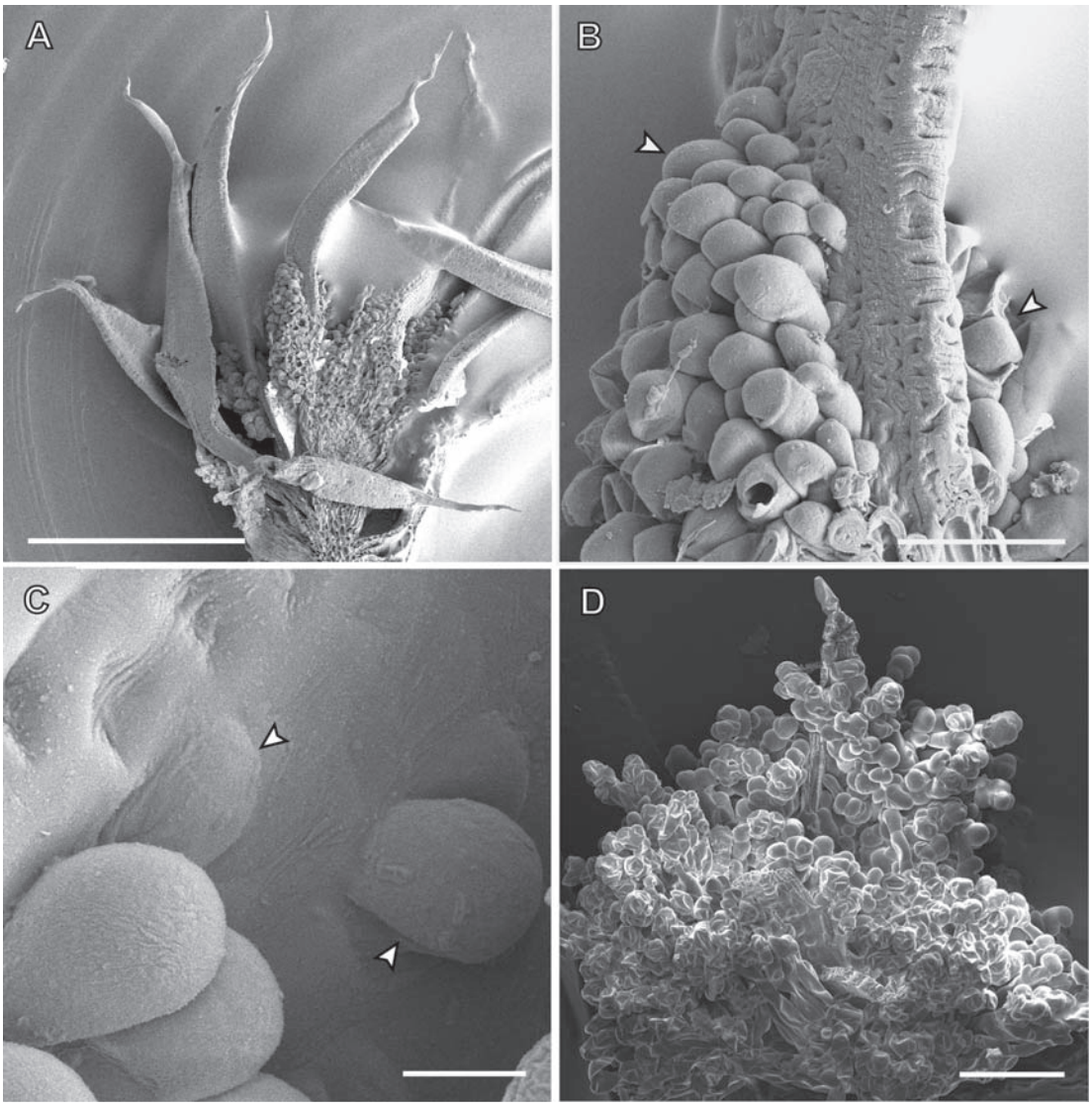

Fig. 5. *Grimmia fuscolutea* Hook. Cryo-scanning electron microscopy. A – Propaguliferous shoot with prorae on median dorsal and ventral costa and lamina, enlarged in B – arrows show prorae. C – Lamina cells developing protruding tips (prorae), arrowed. D – Highly propaguliferous shoot with gemmae on distal part of leaf. Scale bars: A = 500 μm; B = 50 μm; C = 10 μm; D = 100 μm.

separate by schizolysis (Fig. 11H). Although the two species are very similar in gemma development and morphology they can clearly be distinguished by the striolate lamina due to the presence of longitudinal ridges characteristic of *G. anomala* (Fig. 12I, J) but absent in *G. hartmanii* (Fig. 11I), as discussed previously by Ignatova and Muñoz (2004). In other species (e.g. *G. lisae* – Fig. 13A–C, *G. muehlenbeckii* – Fig. 13D–F), and *G. torquata* (Fig. 13G–J) where gemmae form on either the lamina or costa or both, the position of the gemmae appears much more stable than that reported here in *G. fuscolutea* and *G. trichophylla*. Thus in *G. lisae* (Fig. 13A, B) and *G. muehlen-*

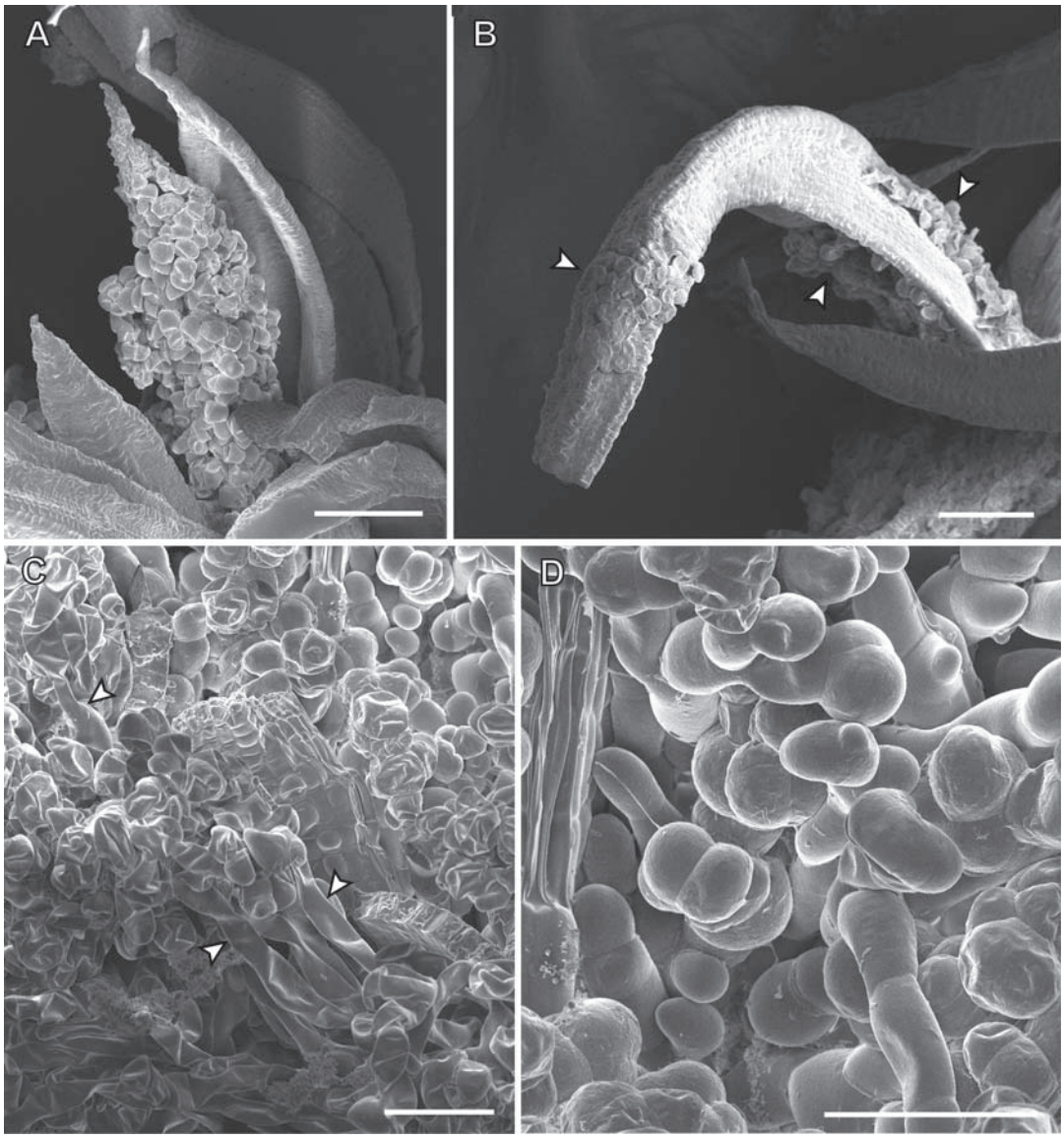

Fig. 6. *Grimmia fuscolutea* Hook. Cryo-scanning electron microscopy. A – Uppermost propaguliferous leaf covered in gemmae. B – A leaf with gemmae in median position, both dorsal and ventral, and distally on dorsal leaf – arrowed. C & D – Long filaments yet to differentiate into gemmae (arrowed in C) and those with gemmae – enlarged in D. Scale bars: A & B = 100 μm; C & D = 50 μm.

beckii (Fig. 13D, E) gemmae form at the tip of filaments from proximal laminal cells, whilst in *G. torquata* (Fig. 13G, H) they arise from filaments on the proximal dorsal costa, in a manner similar to that observed in *G. austrofunalis*. Another similarity between the latter two species is that, following detachment, gemmae remain arranged generally into large clusters (Fig. 13I) (larger in *G. austrofunalis*), have thick, pigmented walls (Fig. 13I) and a high lipid content (Fig. 13J) – also refer to Fig. 10 for details of *G. austrofunalis*.

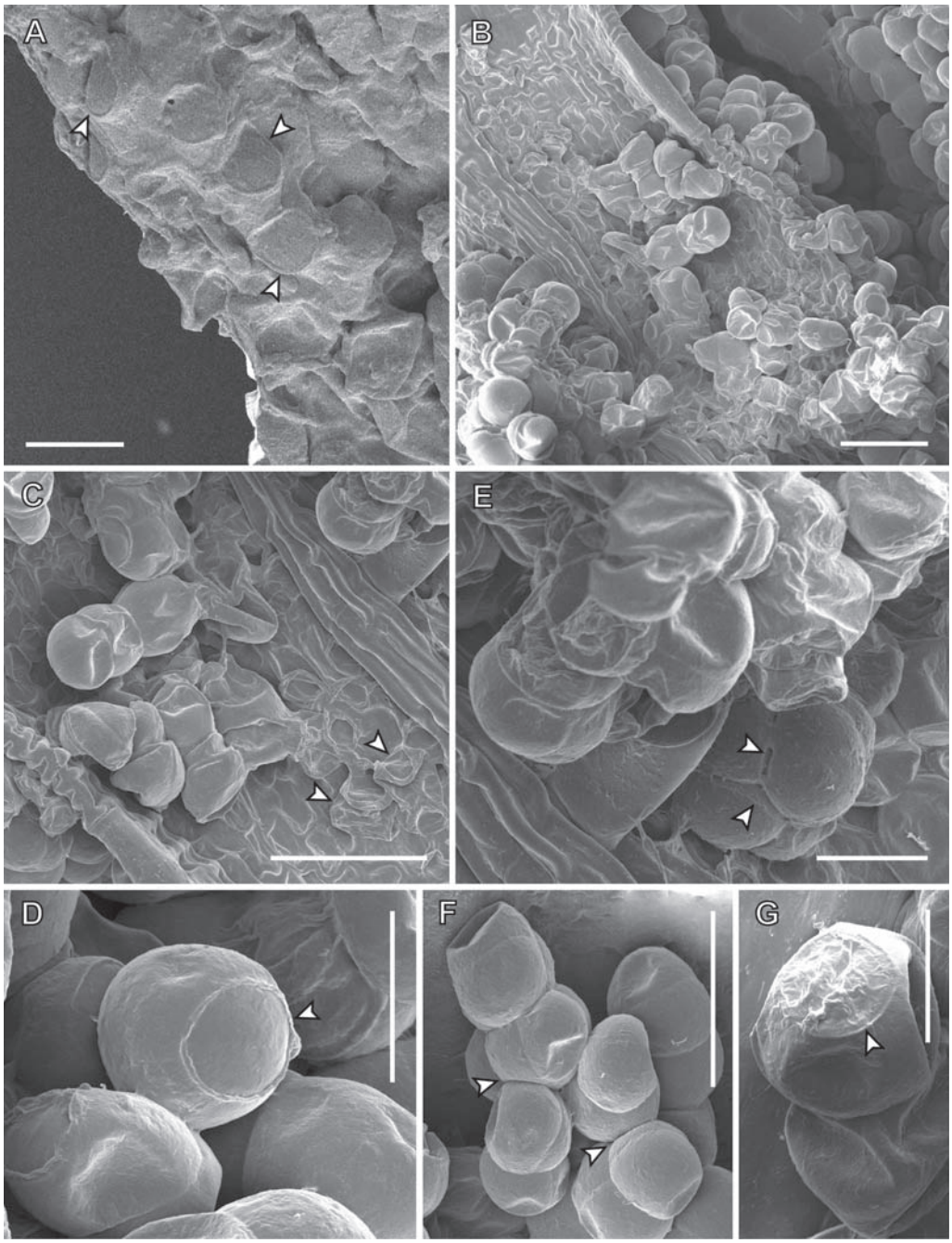

Fig. 7. *Grimmia fuscolutea* Hook. Cryo-scanning electron microscopy. A – Basal cell of stalks remaining on costa after gemma detachment, with scars (arrowed). B & C – Following gemma detachment from lamina cells, the basal cells of the stalks shrink leaving behind a wide-smooth walled depression in the leaf lamina, arrowed in C. D–G – Gemma liberation is either by formation of a Tmema cell, which, after rupturing, leaves behind a scar with remnants of its cell wall (arrowed in D) or by breakdown of the middle lamella (arrowed in E and F), this leaves behind a smooth scar as shown in G (arrowed). Scale bars: B, C, F = 50 μm; A, D, E, G = 20 μm.

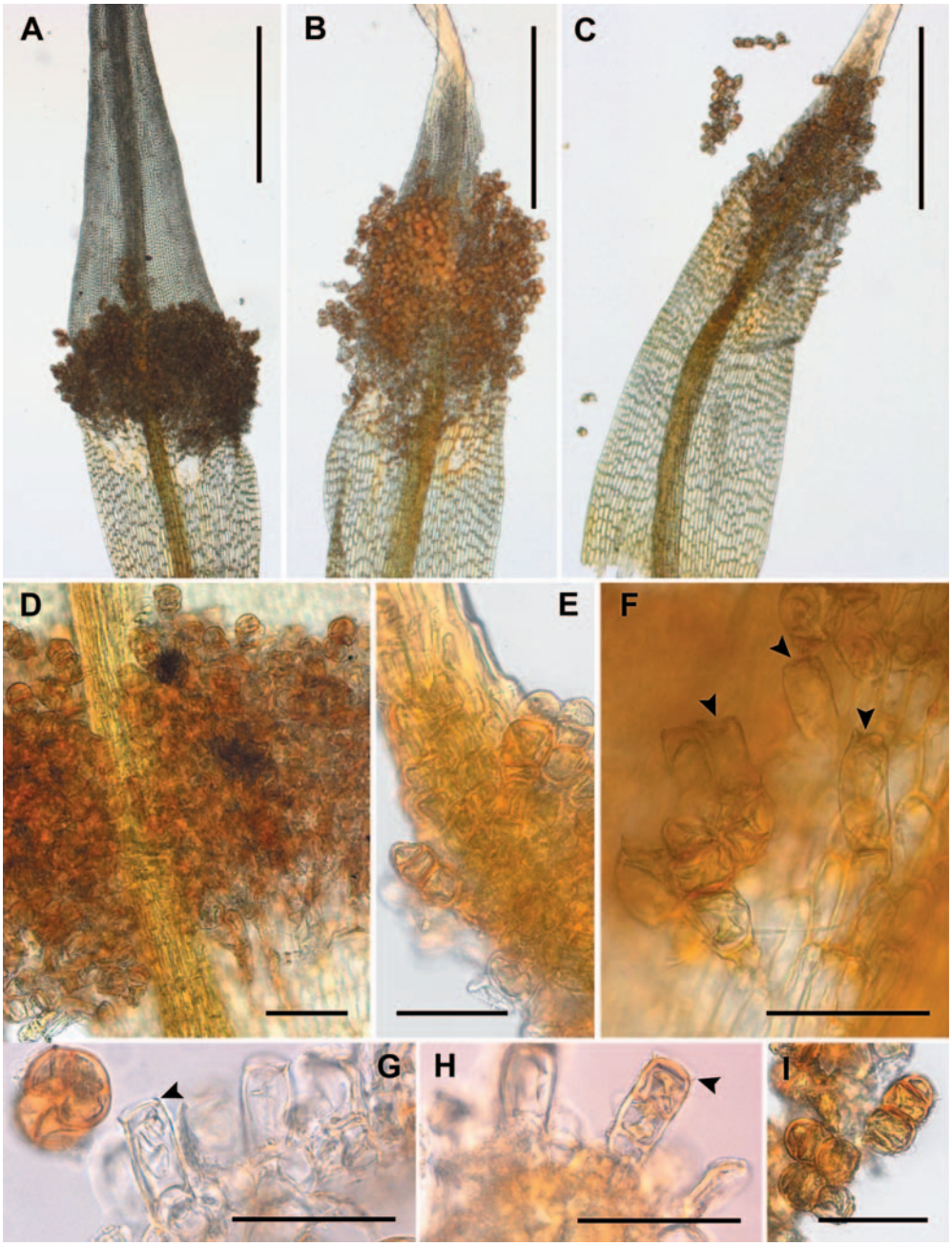

Fig. 8. *Grimmia trichophylla* Grev. Light microscopy. A–C − Position of gemmae on leaves. Gemmae usually develop on the proximal dorsal lamina (A). However, in the uppermost propaguliferous leaves, gemmae often occur distally (B), sometimes just below the hair point (C) and on both lamina and costa (B and C). These leaves consist mostly (B) or exclusively (C) of the larger, rectangular cells that, in most leaves, are confined proximally. D − Gemmae on proximal part of leaf – only lamina cells are involved in gemma production, the costa is unaffected. E − Gemmae on costa just below hair point. F–H − After gemma release, basal cells of stalks remain on both the lamina (F, G – arrowed) and the costa (H, arrowed). I − Short chains of gemmae after detachment. Scale bars: A–C = 500 µm; D = 100 µm; E–I – 50 µm.

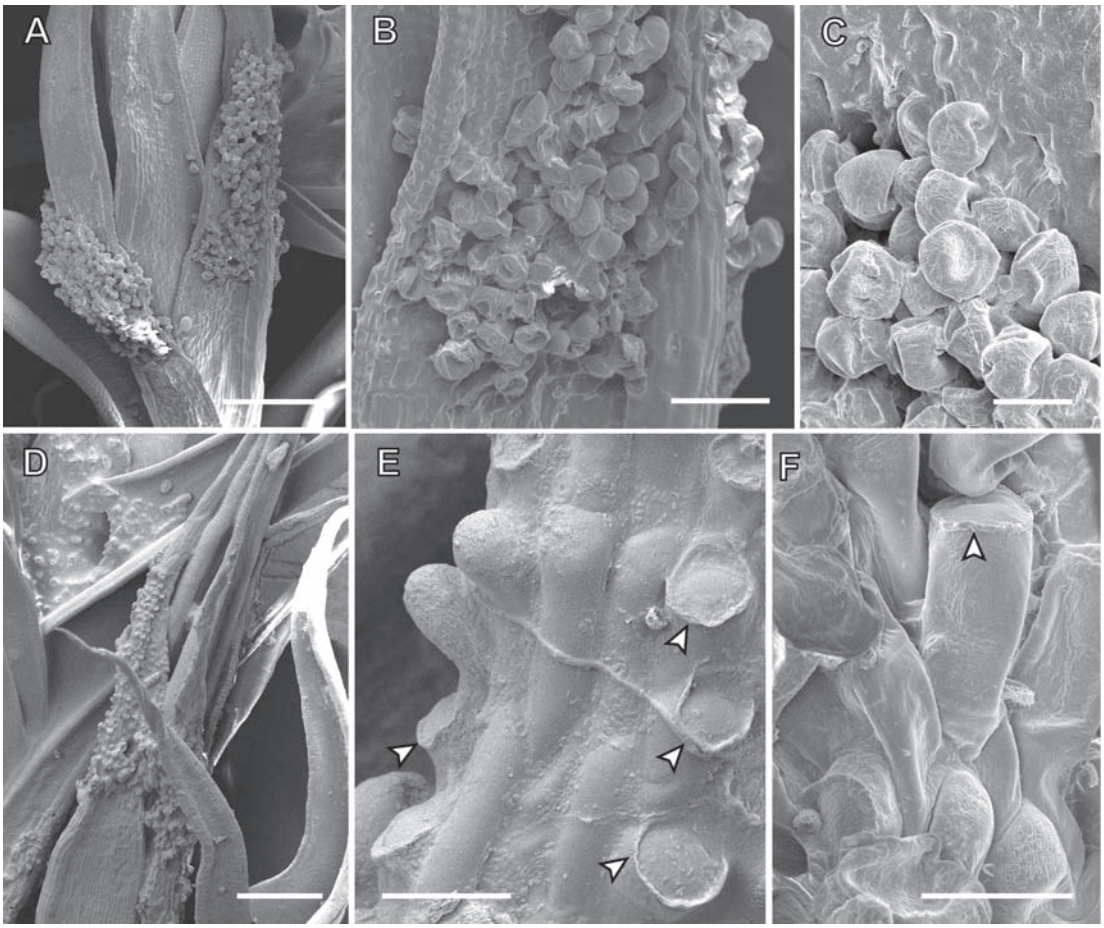

Fig. 9. *Grimmia trichophylla* Grev. Cryo-scanning electron microscopy. A & B – Gemmiferous leaves with gemmae on proximal dorsal lamina. C – Prorae forming on leaf lamina. D–F – Gemmae on distal part of leaf. After gemma detachment, basal cells of filaments remain on the costa, with clearly visible scars (arrowed in E and F). Scale bars: A & D = 200 μm; B = 50 μm; C, E, F = 20 μm.

Discussion

As far as we are aware this is the first report of foliar gemmae in *G. fuscolutea*. Several other *Grimmia* develop foliar gemmae (Table 2) including *G. trichophylla*, *G. torquata* and *G. muehlenbeckii* in which gemmae formation and detachment was described in the classic work by Correns (1899). Formation of gemmae in *G. fuscolutea* shows striking similarities to that seen in *G. trichophylla* and, albeit to a lesser extent, in its allies and in the southern hemisphere species *G. austrofunalis* (both illustrated in Muñoz 1999). All species in Table 2 (with the exception of *G. shastai* in which there is doubt over the position of this taxon in the genus, see Maier 2010) are members of the subgenera *Rhabdogrimmia* (*sensu* Hagen 1909) and furthermore all are in the segregate *Dryptodon* after Ochyra *et al*. (2003).

Generally, gemma ontogeny follows a similar pattern in all propaguliferous *Grimmia* with cells of either the lamina or costa or both giving rise to uniseriate or branched multicellular filaments which differentiate into gemmae. Gemma detachment from the parent filament is by formation of a usually short Tmema cell whilst separation of

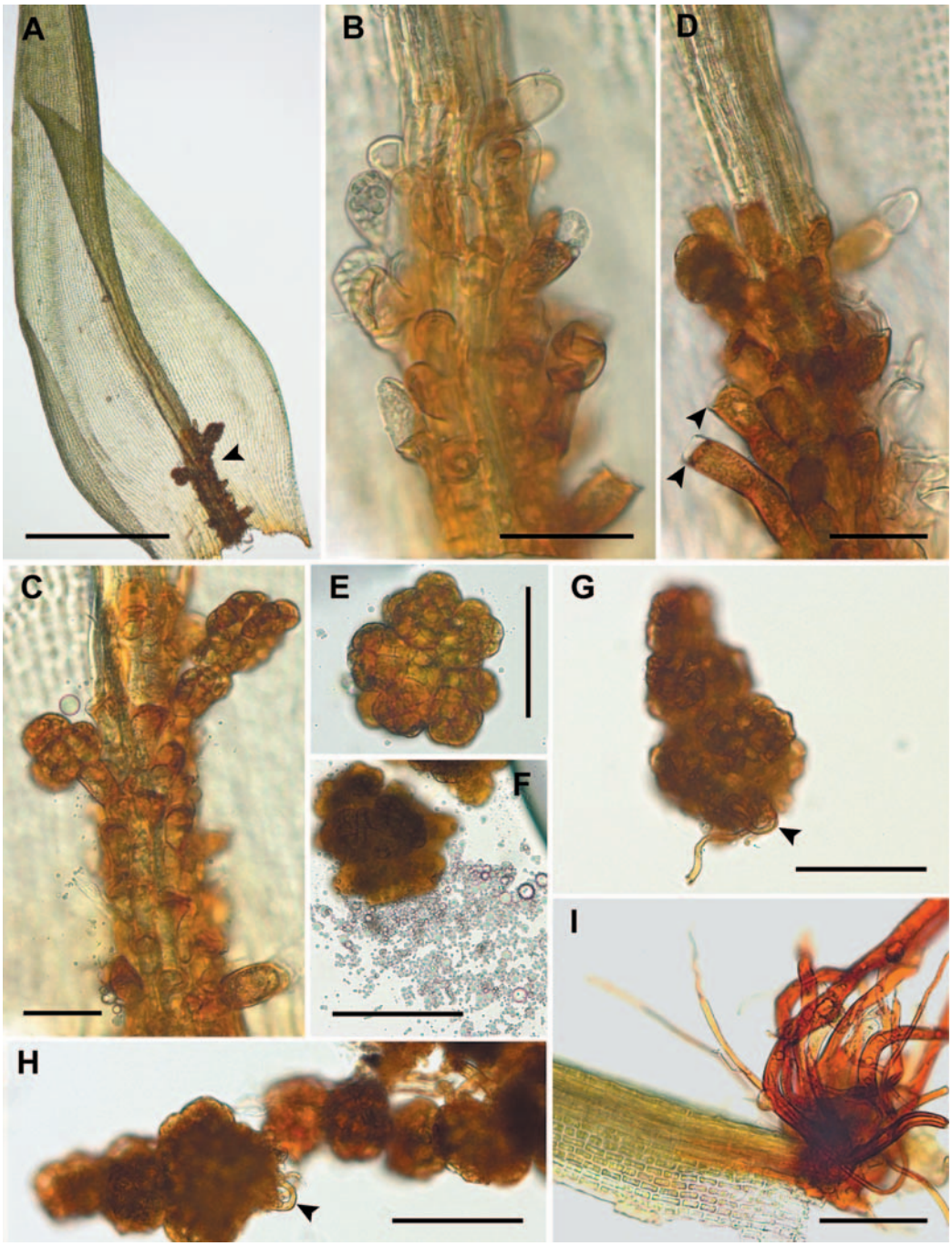

Fig. 10. *Grimmia austrofunalis* Müll. Hal. Light microscopy. A – Typical position of gemmae, on proximal dorsal costa. B – Filaments forming on dorsal costa. C – Filaments bearing gemmae on proximal dorsal costa – note the highly pigmented walls. D – Following gemma detachment, basal cells of stalks persist – scars arrowed. E – Gemma cluster with pigmented walls. F – Squashed gemma cluster with abundant lipid droplets. G & H – Gemmae producing rhizoids. I – Rhizoids generating from basal leaf. Scale bars: A = 500 μm; B–D = 50 μm; E–I = 100 μm.

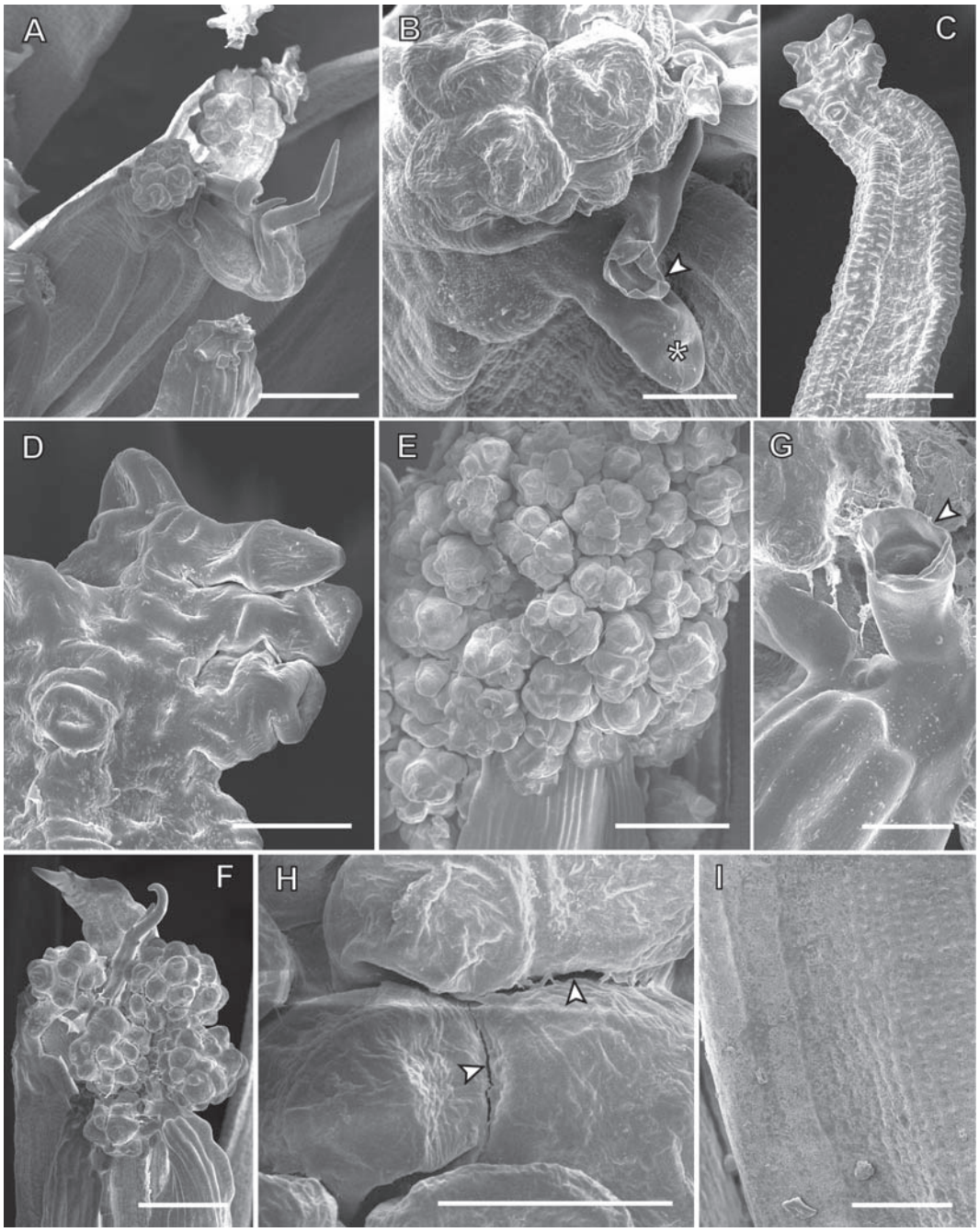

Fig. 11. *Grimmia hartmanii* Schimp. Cryo-scanning electron microscopy. A – Propaguliferous leaves. B – Gemma cluster at leaf apex, next to basal stalk cell with scar (arrowed) remaining after gemma detachment and developing stalk (*). C – Leaf apex turning propaguliferous, enlarged in D. E – Gemmae clusters at leaf apex. F – Following detachment of a gemma cluster, a basal stalk cell with remnants of Tmema cell walls remains, enlarged and arrowed in G. In F also note the ice (water) covering the gemma clusters. H – Separation of individual gemmae arranged in clusters is by breakdown of the middle lamella (arrowed). I – Leaf surface. Scale bars: A, E, F = 100 μm; B, D, G, H = 20 μm; C & I = 50 μm.

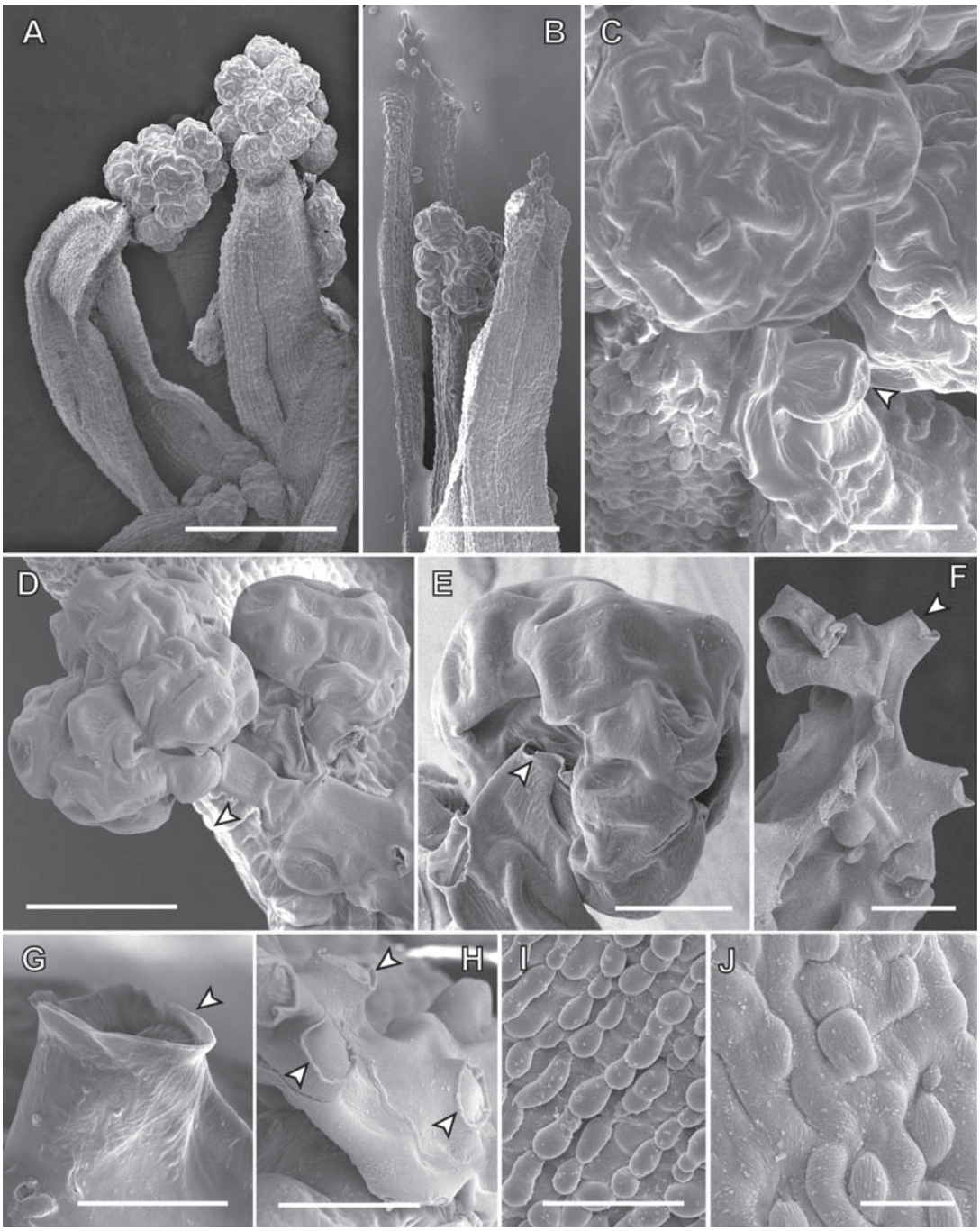

Fig. 12. *Grimmia anomala* Hempe. Cryo-scanning electron microscopy. A & B – Leaves with gemmae at apex and after gemma detachment. C – Cluster of gemmae next to a nascent stalk cell (arrowed). D – Gemma cluster with subtending stalk (arrowed). E – Gemma cluster detaching from leaf apex. F – Leaf apex following detachment of all its gemmae – arrow indicates scar. G & H – Scars (arrowed) remain following gemma detachment. I & J – Details of leaf surface. Scale bars: A & B = 200 μm; C, E, F, H, I = 20 μm; D = 50 μm; G & J = 10 μm.

individual cells arranged into clusters is by the breakdown of the middle lamella (detachment along cell walls). The position of gemmae on the leaf and whether they develop from cells of the lamina or costa is often a useful taxonomic character; *Grimmia anomala* and *G. hartmanii* can be readily distinguished from the *trichophylla* group by the occurrence of gemmae exclusively at the leaf apex. However, as this study has shown the position of gemmae on the leaf is more variable than hitherto described.

Within the *trichophylla* group gemma formation clearly distinguishes *G. austrofunalis* from *G. trichophylla* (Muñoz 1999) and indeed from *G. fuscolutea*, as described here for the first time. Our analyses show that in *G. austrofunalis*, gemmae form exclusively from filaments arising from the proximal dorsal costa, although Muñoz (1999) reported that in one highly gemmiferous collection of this species filaments also occurred on laminar cells on both sides of the costa. Thus, in terms of gemma development and morphology, *G. austrofunalis* is clearly different from *G. trichophylla* and *G. fuscolutea* but rather resembles *G. torquata*. Gemmae in both species are arranged into large clusters (generally larger in *G. austrofunalis*), with thick, pigmented walls and very high lipid content, a characteristic usually associated with long-lived, desiccation-tolerant, slow-germinating propagules (Longton & Schuster 1983; Duckett & Pressel 2003) such as rhizoidal tubers. On the other hand, our light microscopy and cryo-SEM observations reveal that gemma formation is very similar in *G. trichophylla* and *G. fuscolutea* and thus is probably not a reliable character to separate these two taxa.

It is interesting to note that phylogenetic relationships within *Grimmia* reported by a recent study based on both DNA sequences and morphological characters (Streiff 2006) do not always agree with those put forward by previous classifications based on morphology alone and referred to in this paper so far. Thus, in Streiff's study (2006) *G. fuscolutea* was found close to *G. decipiens* and *G. muehlenbeckii* with *G. trichophylla* close to *G. ramondii*. *G. austrofunalis* was found in a basal position with respect to the subclade *Rhabdogrimmia* whilst the position of *G. torquata* in the *Rhabdogrimmia* clade (based on molecular data only) was either unresolved or basal and close to *G. austrofunalis* (in the combined analysis).

Previous studies (Muñoz 1999; Maier 2010) have reported that in all gemmiferous *Grimmia* species (excluding of course *G. anomala* and *G. hartmanii* and with the exception of *G. shasti* but including *G. trichophylla*) gemmae form on the proximal (base or lower) part of the leaf (see Table 2 and also Fig. 10 and Fig. 13 in this paper). Whilst our observations on *G. austrofunalis*, *G. lisae*, *G. muehlenbeckii* and *G. torquata* are in line with these previous reports, we found much more variability in the position of gemmae in *G. trichophylla* than hitherto described (Muñoz 1999; Maier 2010), and the same appears to be true also in *G. fuscolutea*. Thus, in this species gemmae not only occur proximally on the dorsal and ventral lamina but, in the uppermost propaguliferous leaves, they also develop distally and from both costal and laminal cells (Fig. 8A–C). These leaves are highly modified in that they consist almost exclusively of the larger, smooth-walled rectangular cells usually positioned proximally. In fact, filaments appear to originate only from these larger smooth-walled rectangular cells, whatever their position on the leaf, and not from the distal, smaller sinuose isodiametric cells. These observations appear consistent with those of Correns' (1899), describing the filaments in *G. trichophylla* as positioned on the lamina at the point where the square shaped cells of the upper part of the leaf change into the elongated cells of the leaf base. However, further ultrastructural studies, such as that describing the development of foliar gemmae in *Tortula* by Ligrone *et al.* (1996) are now needed to confirm these observations.

As originally described by Correns (1899) and later discussed extensively by Duckett and Ligrone (1992) two fundamentally different mechanisms of diaspore liberation exist in mosses: a schizolytic mechanism involving detachment along cell walls and a lysigenic one i.e. the formation and rupture of a specialized Tmema or abscission cell. Differences in liberation mechanisms can often be diagnostic of a group; thus gemma detachment in

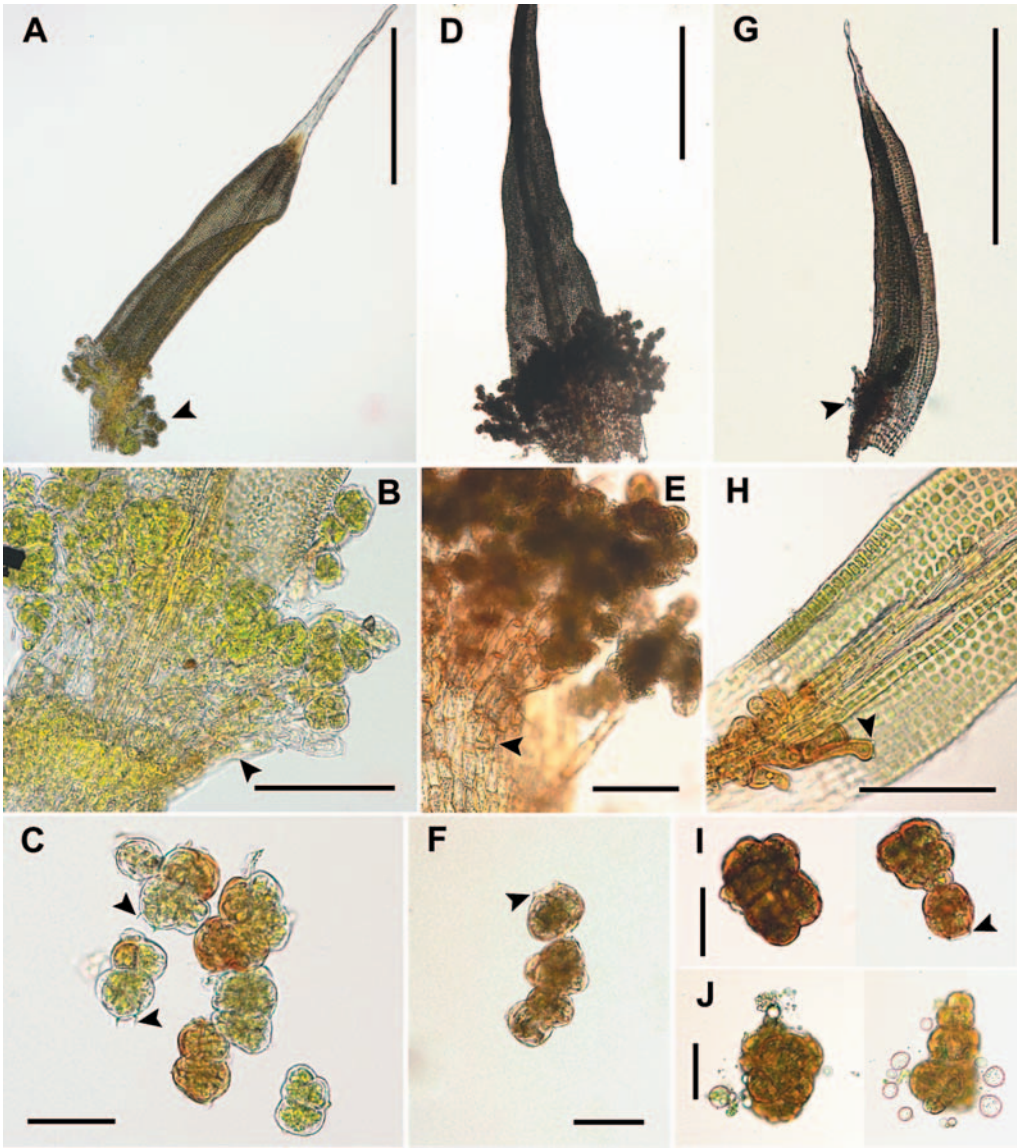

Fig. 13. *Grimmia lisae* De Not. (A–C); *G. muehlenbeckii* Schimp. (D–F); *G. torquata* Drumm. (G–J). Light microscopy. A – Position of gemmae on proximal dorsal leaf base (arrowed). B – Gemmae forming on uniseriate filaments (arrowed). C – Scars (arrowed) remaining on gemmae after their detachment. D – Position of gemmae on proximal dorsal leaf base. E – Gemmae forming on long uniseriate filaments (arrowed). F – Gemma after detachment, arrow indicates scar. G – Position of gemmae on proximal dorsal costa. H – Following gemma detachment, the costa is preserved and filaments remain on the costa, arrow indicates scar. I – Gemma clusters with pigmented walls – arrow indicates scar. J – Lipid droplets released from squashed gemmae. Scale bars: A, D, G = 500 μm; B, E, H = 100 μm; C, F, I, J = 50 μm.

the Pottiales is typically by schizolysis and clearly sets apart the Pottiales from the Dicranales where lysogenic mechanisms are the norm (Duckett *et al*. 2004) whilst in the Bryaceae schizolysis may well be a synapomorphy for the Mniaceae (Pressel *et al*. 2007). However, this does not appear to be the case for *Grimmia*. Our present work confirms previous observations by Correns (1899) that both

Table 2. Comparison of foliar gemmae characters in selected *Grimmia* species.

Taxon	Nature of gemmae	Reference
G. austrofunalis Müll. Hal	On uniseriate sometimes branched filaments on proximal dorsal costa (rarely on lamina); costa preserved on release of gemma with filaments remaining attached	Muñoz 1999; this paper
G. consobrina Kunze *ex* Müll. Hal.	On dorsal lamina in transitional part, degeneration of laminal cells on release	Maier 2010
G. dissimulata E. Maier	On proximal dorsal leaf base; laminal cells degenerate but costa preserved on release of propagula	Maier 2002
G. fuscolutea Hook.	On uniseriate sometimes branched filaments on median dorsal and ventral costa and lamina; mid-leaf laminal cells degenerate, costa preserved	This paper
G. lisae De Not.	On uniseriate branched filaments on proximal dorsal leaf base	Maier 2002; Muñoz 1999; this paper
G. meridionalis (Müll. Hal.) E. Maier	On proximal dorsal leaf base	Maier 2002
G. muehlenbeckii Schimp.	On uniseriate branched filaments on proximal ventral and dorsal leaf base	Ignatova & Muñoz 2004; Maier 2009; this paper
G. shastai Greven (Californian endemic)	On distal ventral lamina and costa	Greven 2003 (but see Maier 2010)
G. torquata Drumm.	On uniseriate filaments on proximal dorsal costa; costa preserved on release of gemma with filaments remaining attached	Ignatova & Muñoz 2004; Maier 2009; this paper
G. trichophylla Grev.	Sessile on proximal dorsal (and ventral, Maier 2009) lamina; degeneration of lamina cells on release of gemma lead to leaf breakdown. Sometimes also on distal dorsal lamina and costa. Costa preserved after gemma detachment.	Maier 2002; Maier 2009; Muñoz 1999 This paper

schyzolitic and lysogenic mechanisms are involved in gemma liberation in *Grimmia* and suggest that this may well be the case in all gemmiferous taxa, with both mechanisms often occurring in the same species (e.g. *G. fuscolutea* and *G. hartmanii*).

In all the *Grimmia* we have analysed we found no evidence of percurrent growth (Hughes, 1971), a common phenomenon in mosses e.g. *Tortula* (Ligrone *et al*. 1996), *Orthotrichum* and *Zygodon* (Duckett & Ligrone 1992). In *Aulocomnium* (cauline gemmae) (Ligrone *et al*. 1996) and in *Calymperes* (foliar gemmae) (Ligrone *et al*., 1992), also lacking percurrent growth, the stalk cells invariably degenerate following gemma detachment, whilst in other members of the Calymperaceae e.g. *Syrropodon armatus* (Pressel, personal observation) the stalks persist after liberation and re-differentiate into new initials from which additional gemmae arise. In *Grimmia* not only the filaments that degenerate (when on the lamina – not surprisingly given that these filaments are thin walled like the cells from which they arise) but also those that persist (when originated from costal cells) never re-differentiate so that gemmae are always produced from new initials arising from cells nearby. As is almost always the case in mosses, minus few notable exceptions e.g. the synchronous development of *Calymperes* foliar gemmae (Ligrone *et al*. 1992), gemma formation and liberation is a continuous process.

The importance of vegetative reproduction in mosses is well documented: the loss of capacity to produce sporophytes is a common and widespread tendency in bryophytes often due to regression in sexual reproduction (Longton & Schuster 1983) and/or spatial separation of male and female plants (Odu 1987). The inability to produce sporophytes has been linked in particular with the dioicous habit although substantial numbers of dioicous species 'fruit' freely (Longton 1997). The majority of

Grimmia species known to produce foliar gemmae are dioicous (all the gemmiferous samples we have examined lacked sporophytes) with the exception of *G. fuscolutea*, which is autoicous and is generally an abundantly fruiting species. However, sporophytes were not seen on Réunion Island. It should be noted however that the generally held assumption that abundantly fertile species lack vegetative propagules has been contradicted recently (Duckett & Matcham 1995; Duckett *et al*. 2001; Pressel *et al*. 2007). Pressel *et al*. (2007) found that in the genus *Bryum* protonemal gemmae are equally distributed between species with different sexualities, including taxa which produce abundant sporophytes. There are various advantages for sexual and asexual reproduction to occur in parallel. Whilst spore production and liberation is seasonal and subject to numerous physiological and ecological constraints (Longton & Shuster 1983; Mishler 1988; Longton 1997), the production and release of asexual propagules by the gametophyte is most often continuous and thus is extremely important for population maintenance and spread in numerous bryophyte taxa (Longton & Shuster 1983; Mishler 1988). It has also been suggested that spores and vegetative diasporas may well differ in their longevity and conditions required for germination, although this remains to be tested (Duckett & Matcham 1995).

Systematic considerations aside, the position of gemmae on the gametophyte has important implications for their dispersal. Overall, there are essentially two strategies: gemmae are held high and well above the vegetative leaves so that they remain exposed even when the plants are dry and thus can be dispersed under both dry and wet conditions – extreme examples are the gemmae borne terminally on long pseudopodial axes in *Aulacomnium androgynum* (Ligrone *et al*. 1996), and those produced by highly modified leaves or stenophylls in some Calymperaceae (Reese 2000). Alternatively, gemmae are produced at various positions along the leaf length and become "trapped" (enclosed) as the leaves curl and contort when the plant dries out so that gemma release occurs exclusively under wet conditions. Looking at the gemmiferous *Grimmia*, whilst *G. anomala* and *G. hartmanii* clearly fit the first strategy with their large apical gemma clusters which remain exposed even when the leaves become curled and twisted; in all the remaining species, including *G. fuscolutea*, the position of the gemmae coupled to their highly hydrophilic nature, makes them perfectly adapted for wet dispersal only. This would certainly be advantageous in the intermittently inundated environment where the gemmiferous population of *G. fuscolutea* occurs in Réunion.

Populations of *G. fuscolutea* with gemmae should now be looked for elsewhere. Réunion is a relatively young tropical oceanic island, generated from an 'oceanic hotspot' under the sea a mere three million years ago. Located in the Indian Ocean, 800 kilometres from Madagascar, Réunion presents enormous opportunities for investigating island colonisation and is still a potential source of new bryological discoveries. The distribution of *G. fuscolutea* with gemmae may show parallels to the pan-south temperate species *G. austrofunalis* in which there are geographically disjunct propaguliferous populations; gemmae in this species are occasionally present in South America but are mostly lacking in Australian, New Zealand (R. Ochyra, personal communication) and Réunion populations (Ah-Peng *et al*. 2010). The nearest known populations of *G. fuscolutea* are the mountains of South Africa and East Africa (Kenya, Tanzania and Uganda) (O'Shea 2006) and gemmae may play an important role in local if not long distance dispersal of *G. fuscolutea*. The relatively high lipid content of these asexual diaspores, albeit not to the extent observed in other species e.g. *G. austrofunalis* (Fig. 9F), G. *hartmanii* and *G. anomala* (data not shown), certainly suggest that they probably are long-lived propagules, although their longevity remains to be tested.

SPECIMENS EXAMINED: ***Grimmia fuscolutea***: RÉUNION, Ravine Divon, below le Maïdo, by Route des Tamarins Nord, 21°03′04″S, 55°22′13″E, alt. 1750 m, on exposed boulders in intermittent stream, 17 Sept. 2008, *Porley REU9823d*, det. Maier, *Porley REU9823a*, det. Porley (hb. R. D. Porley); Col des Boeufs, Cirque de Salazie, 21°02′54″S, 55°27′24″E, alt. 1850 m, on thin soil overlying rock on steep cliff below road, 16 Sept. 2008, *Porley REU9801*, det. Porley (hb. R. D.

Porley); Col des Boeufs, Cirque de Salazie, 21°02′54″S, 55°27′24″E, alt. 1850 m, on concrete capstone of low retaining wall on edge of raod/track above steep bank, 16 Sept. 2008, *Porley REU9800*, det. Porley (hb. R. D. Porley). ***G. anomala***: ITALY, Lac Falin, Valle de Via, alt. 1620 m, on shaded rock outcrops, 27 July 1997, *Porley*, det. Porley (hb. R. D. Porley); Hochgang, S. of ridge, Partschins, alt. 1940 m, on rock outcrops, 2 July 1995, *Porley*, det. Porley (hb. R. D. Porley). *G. austrofunalis*: CHILE, Region VIII, Prov. Bio-Bio. Los Cervos Farm (Endesa) at Polcura River, 37°14′S, 71°27′W, alt. 840 m, 22 Nov. 2002, *Ireland & Bellolio 35327*, det. Ireland (BM 000977653). ***G. hartmanii***: UNITED KINGDOM, Carn Dearg, Skye, N. Ebudes, VC 104, NG 59781364, on boulders on cliff top, 6 July 2006, *Porley 2942*, det. Porley (hb. R. D. Porley); Snowdonia, Caernarvonshire VC 49, Nant Gwynant, on top of shaded boulder in stream in ravine, 11 Sept. 2005, *Porley 2719*, det. Porley (hb. R. D. Porley). ***G. lisae***: South Devon VC3, SX 766358, Gammon Head, South Hams, 8 Sept. 2004, *Porley 2537*, det. Porley (hb. R. D. Porley); South Devon, VC 3, SX 8188136773, Lannacombe Bay, Start Point, 8 Sept. 2004, *Porley 2538*, det. Porley (hb. R. D. Porley). ***G. muehlenbeckii***: SWITZERLAND, 690.8/112.2, Palagnedra, TI, alt. 560 m, full sun on silicate, 26 June 1992, *Maier* (ex herbarium Hermann et Eva Maier); Switzerland 802.50/186.40, Alp d'Immez, Lavin, GR, alt. 1930 m on stones, silicate, 24 Aug. 1995, *Maier* (ex herbarium Hermann et Eva Maier); Switzerland 572.1/110.05, Jeur Brûlée, VS, alt. 1620 m, on blocs in *Picea* forest, 16 April 1991, *Maier* (ex herbarium Hermann et Eva Maier). SLOVAKIA, Vajskovská dolina, Nízke Tatry Mountains, alt. 1580 m, on rocks on flushed montane slope, 17 Aug. 1995, *Porley*, det. H. C. Greven (hb. R. D. Porley). ***G. torquata***: UNITED KINGDOM, North Ebudes VC104, NG 3132800244, Bloodstone Hill, Rhum, on north facing crags, 1 July 2004, *Porley 2623*, det. Porley (hb. R. D. Porley); Brecon VC42, SN 963218, Craig Cerrig-gleisiad NNR, alt. 90m, on old red sandstone crags, 17 April 1999, *Porley 1485*, det. Porley (herb. R. D. Porley). ***G. trichophylla***: UNITED KINGDOM, West Perthshire VC87, NN 555162, Strathyre, Lock Lubnaig, alt. 150 m, 19 Aug. 1991, *Matcham 64.17a*, det. Matcham (hb. H. W. Matcham); East Perthshire VC 89, NN 991622, Glen Brerachen, on acid boulder in open conifer plantation, 12 July 2003, *Porley 2491*, det. E. Maier (hb. R. D. Porley); North West Yorkshire VC 65, NY 85792939, Cronkley Fell, on stone wall in farmland, 24 June 2006, *Porley 2899*, det. Porley (hb. R. D. Porley); Herefordshire VC36, 50278340, Black Hill, Olchon Valley, alt. 580 m, 18 June 2012, *M. Lawley*, det. Porley (hb. R. D. Porley).

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