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The structure of leaf trichomes in Proteaceae: taxonomic value, ecology and development

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Summary: Plant trichomes have many functions including interactions with ecological factors such as solar radiance, drought and predators. Many taxa of Proteaceae are distributed in arid regions with prominent seasonality and have evolved certain adaptations to these types of habitats, resulting in special trichome structure and density. Proteaceae share a common plan of trichome structure, namely uniseriate trichomes composed of a basal cell, a stalk cell and terminal cells (usually one). Some minor deviations from this plan also occur. In some cases, the basal cell undergoes anticlinal divisions. The shape of the terminal cell may vary from unbranched (straight or curly) to having 2 equal or unequal branches with its longer axis perpendicular to the stalk cell. Shorter papilloid trichomes were not studied in detail, but these most likely appear due to premature arrest of normal trichome development. *Grevillea* is the only genus of Proteaceae, where multicellular glandular hairs are documented. Although trichome presence is the ancestral state within the family, several lineages evolved glabrous leaves independently.

Keywords: Proteaceae, trichomes, *Grevillea*, indumentum, evolution, morphology

Trichomes and their traits can be an important source of information for plant identification (including fossil records), their characters often have a taxonomic value. Nevertheless, precision of indumentum traits as a taxonomic tool is not always certain. Large taxonomic groups of plants usually have a common pattern of trichome structure. For example, peltate or scale-like hairs are characteristic of Eleagnaceae (Mishra 2009), chandelier-shaped trichomes with whorls of branches are common in Platanaceae (Carpenter et al. 2005), three-celled uniseriate hairs are typical for Proteaceae (Johnson & Briggs 1975). On the other hand, simple, branched and glandular hairs are found in many taxa, and their presence or absence may serve as a diagnostic feature for species or genera recognition. The importance of indumentum for taxonomy is recognized for Asteraceae (Adedeji & Jewoola 2008; Krak & Mráz 2008), Brassicaceae (Beilstein et al. 2006), Lamiaceae (Eiji & Salmaki 2016) and other families.

Recent studies of genetic regulation of trichome development proved that in *Arabidopsis thaliana* (L.) Heynh. some significant changes in trichome structure may be caused by single mutations (Schwab et al. 2000; Walker et al. 2000; Ilgenfritz et al. 2003). There are also certain mutations (*glabra1*, *trichomeless1* and several others) that result in glabrousness in normally pubescent species (Wang et al. 2007). Therefore, the presence of trichomes as a morphological trait could be evolutionary quite fluid, and the loss of hairs could occur independently in different groups within a taxon.

The indumentum of plants has many functions, some of them are directly involved in vital processes of the plant itself, while the others ensure its interaction with various biotic and abiotic external factors. Hairs protect plant organs from phytophages, mechanically blocking access to plant tissues (Rebelo 2001), or, in case of glandular hairs, producing substances that deter, immobilize and / or poison herbivores. Lapinjoki et al. (1991) found that in populations of *Betula pendula* Roth individual plants are capable of switching from one strategy of protection to another. Some seedlings of this species had mostly dispersed glandular indumentum that produced a protecting layer of sticky resin, while other seedlings had a very thick indumentum of simple hairs serving as a mechanical barrier, but their resin secretion was insignificant.

Insulating and light diffusing properties of indumentum provide protection from the excess of insolation, heat or cold (Skelton et al. 2012; Carpenter 2012). Glandular hairs may carry out bactericidal and fungicidal functions, secreting volatile oils that often contain terpenoids (Lange 2015). Glandular trichomes of many insectivorous plants have evolved towards massive secretion of lytic enzymes (chitinases, proteinases) and nectar that attracts their prey (Buvat 1989; Renner & Specht 2013). Same substances, produced by the same glandular hairs, may have various functions. Mucilages can serve as a lubricant that helps young leaves to unfold (Modenesi et al. 1984), favour the development of symbiotic bacteria in *Psychotria* (Lersten & Horner 1967) or facilitate seed dispersal (Werker 1997). Nielsen et al. (1991) supposed that plants may use trichomes to perform synthesis and excretion of potentially phytotoxic substances compartmentalizing them in cells that are situated outside the main tissues of the leaf. For instance, glandular hairs of *Phillyrea latifolia* L. growing in highly saline environments start functioning as salt glands, although it is not their main function in normal conditions (Gravano et al. 1998). Highly specialized trichomes with salt excretion as their main function were discovered in the halophyte *Atriplex halimus* L. (Smaoui 1975) and in the halotolerant mangrove plant *Avicennia marina* (Forssk.) Vierh. (Shimony et al. 1973; Fahn 2000).

In some cases non-glandular hairs on the same organ of a plant develop faster than glandular ones. According to Werker (1997), non-glandular trichomes in this case provide a mechanical protection for more fragile glandular trichomes that use more metabolic resources during their development. It is worth mentioning that metabolic activity in glandular hairs persists for prolonged periods of time. In particular, cells of glandular trichomes in certain plants retain chloroplasts, which are known to play the key role in terpenoid biosynthesis (Glas et al. 2012).

The diversity of trichome types within the Proteaceae is relatively low. Most characteristic of the family are uniseriate multicellular trichomes, consisting of a basal cell submerged in the epidermis, a relatively short stalk cell and, typically, a single elongated terminal cell. Mature trichomes often break off, leaving a fragment of stalk cell still visible on the surface of the leaf. This generalized plan of trichome morphology in Proteaceae is more or less consistent, although some minor variants of it occur (T-shaped hairs, glandular hairs, papilloid hairs, etc., see Fig. 1A–H)

The Platanaceae is another family in the order Proteales, closely related to Proteaceae (APG IV 2016). Leaf trichomes in *Platanus* also display a basal cell and a stalk cell in the proximal part of trichomes, but their distal structures are more complex, multicellular, with branches arranged in multi-tier whorls. Mature trichomes in Platanaceae (similar to those in Proteaceae) often break off, leaving a characteristic scar-like vestige (Carpenter 2005).

Although information regarding general structure plan of trichomes in Proteaceae and distribution of their certain types can be found in fundamental monographs, some problems of trichome biology remain unresolved. By the moment the taxa of the family are not covered sufficiently. Morphology and development of less common trichome variants in the family deserve more

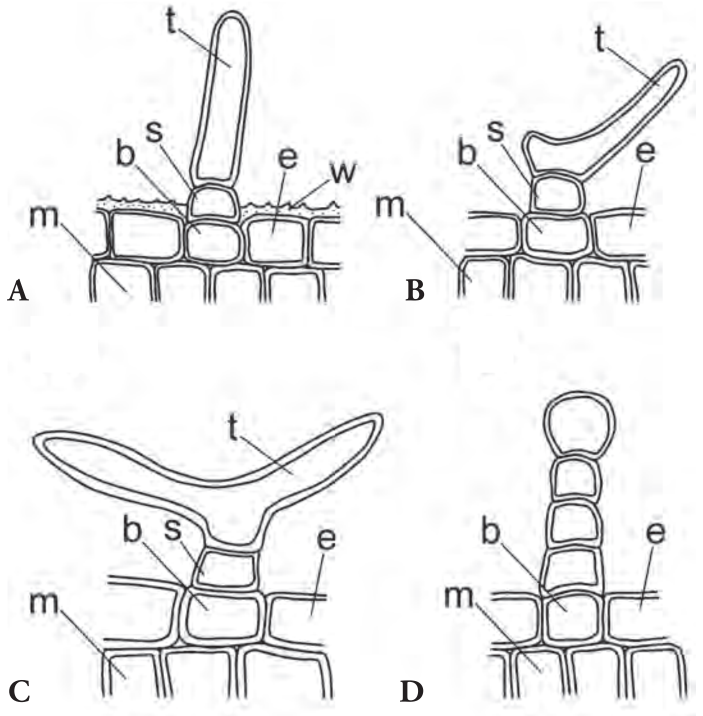

Figure 1. Trichome structure in Proteaceae. A – generalized (ancestral) plan of structure; B – transitional form with unequal branches; C – T-shaped trichome; D – glandular trichome. b = basal cell, e = endodermis, s = stalk cell, t = terminal cell, m = palisade cells of mesophyll, w = wax layer.

detailed investigation. Evolutionary trends of indumentum transformation and their ecological significance need further clarification.

History of trichome study in Proteaceae

One of the earliest descriptions of indumentum in Proteaceae was given in a chapter on Proteaceae by Engler (1888–1889) in 'Die natürlichen Pflanzenfamilien', a digest edited by Enler and Prantl.

"Die Haare sind 1-zellig, sonst aber recht verschieden. Sehr verbreitet sind steife Haare mit stark verdickter Membran und sehr engem Lumen, dagegen finden wir dünne, krause, einen dichten Filz bildende Haare hauptsächlich bei den Gattungen *Banksia* und *Driandra*, aber auch bei *Grevillea pinaster*. Sehr eigentümlich sind die nur bei *Grevillea* und *Hakea*, jedoch wie es scheint bei allen Arten dieser Gattungen an irgend einem Teile vorkommenden zweischenkeligen Haare: auf einer zwischen den Oberhautzellen eingekeilten und über dieselben sich manchmal bedeutend erhebenden, nach oben sich verbreiternden, aber fast vollständig lumenlosen Stielzelle befindet sich mit ihrer Mitte aufsitzend die dickwandige, nach beiden Enden zugespitzte Haarzelle."

["Hairs are unicellular, very diverse. Coarse hairs with greatly thickened walls and narrow lumina are common; on the other side, mainly in *Banksia* and *Dryandra*, but also in *Grevillea pinaster*, thin, undulate hairs forming a thick felt can be found. Two-horned hairs occur only in *Grevillea* and *Hakea*, but on all the species and on all parts of the plants. Their stalk cell, almost lacking lumina, with a wedge-shaped base situated between epidermal cells and sometimes towering over it, becomes wider further up and attaches to the centre of a thick-walled hair cell, narrowing to both of its ends."]

An illustration is given that depicts a hair of *Grevillea leucadendron* (currently *Grevillea pyramidalis* subsp. *leucadendron* (A. Cunn. ex R. Br.) Makinson.) with a T-shape that is characteristic for *Grevillea* and *Hakea*. Engler thought that trichomes of most Proteaceae are unicellular. The T-shaped trichome in the illustration has a narrowing base, submerged into epiderm and adjoining the parenchymal palisade cells. It is worth mentioning that the basal part of the trichome is depicted narrower than it should be, and, according to Engler's views, it is not divided into a stalk cell and a basal cell.

A major input into understanding the morphology of Proteaceae was made by Venkata Rao (1961, 1967a, 1967b, 1971). His focus of interest was mostly concentrated on generative organs, and indumentum traits drew his attention to much lesser extent, yet, speaking of gynoecium in Macadamiae, he mentions simple hairs on the ovary that are unicellular according to his opinion. These hairs are eventually shed in *Panopsis*, *Macadamia* and *Helicia*, but are retained in *Brabejum* and *Roupala* (Venkata Rao 1971). He notices that the extent of hairiness of flowers varies greatly in *Grevillea* and believes that stiff hairs, forming a thick indumentum on flowers of *Grevillea oxystigma* Meisn., are also single-celled (Venkata Rao 1967a). All the genera of Proteeae (roughly corresponding to Proteoideae in modern taxonomy of the family) have multicellular hairs, covering the ovary from the outside (Venkata Rao 1967b). Describing the development of the ovary in *Conospermum taxifolium* C.F. Gaertn., he mentioned that by the time the primary parietal cell is cut off in the ovary, the cells of the ovary epiderm become papillate, and at the tetrade stage multicellular hairs develop, having isodiametric stalk cells rich in cytoplasm and an elongated terminal cell filled with tannins. This is by and large the first account of trichome morphogenesis in Proteaceae (Venkata Rao 1961). On *Placospermum* stigmas he found multiple glandular hairs, on which pollen started to germinate (Venkata Rao 1961). Glandular, papilloid or elongated simple hairs on the stigma surface, aiding in pollen trapping, are also common in Australian genera of Proteoideae (Venkata Rao 1967b).

Johnson & Briggs' (1975) monograph 'On the Proteaceae: the evolution and classification of a southern family' was undoubtedly the most influential work on the evolution, biogeography and taxonomy of the family for several decades. It was first presented at a meeting of the Linnaean Society on 6th December 1973 at the 200 years anniversary of Robert Brown. The taxonomy of the family, as given in the monograph with just a few minor modifications, has been most widely accepted for more than 30 years, until the work of Weston & Barker (2006), who used molecular data for the first time to clarify the relations between the major taxa within the family.

The hypothetical ancestors of the Proteaceae, according to Johnson and Briggs' conclusion, looked like as follows:
"Trees, evergreen, leptocaul, of rainforest type […]. Hairs uniseriate, 3-celled (commonly incorrectly termed 'unicellular hairs'), basal cell embedded in the epidermis, stalk cell short, apical cell elongated and unbranched; glandular hairs absent. Stomates paracytic."

Speaking of trichomes and cuticles of extant Proteaceae, Johnson and Briggs describe the general structure plan of a trichome and present at length their special types:

"The characteristic Proteaceous trichomes are found in all genera, though sometimes only on the ovary, or on very young vegetative, inflorescence or floral buds, where they are clearly protective.

They have mostly been misdescribed as 'unicellular hairs' but are in fact always uniseriate and 3-celled, […] including the basal cell in the epidermis. Above this basal cell is a stalk cell (very short in many cases, somewhat elongated in a few Grevilleeae) and the trichome ends in a slightly to very elongated acute terminal cell. As a rule, both stalk and terminal cells are thick-walled and have contracted contents in the mature stage."

T-shaped trichomes and taxa within which they occur were specially mentioned:

"In most genera the terminal cell is unbranched, but in *Sphalmium* (Sphalmioideae) and a number of Grevilleoideae (some species only of *Stenocarpus*, *Strangea* and *Helicia* s. str., universally in *Hollandaea*, *Floydia*, *Grevillea*, *Finschia* and *Hakea*) the terminal cell consists of two unequal or equal branches, and appears more or less transverse to the stalk cell. This hair type must have arisen more than once in the Grevilleeae. Some rather transitional types are found in *Dilobeia* (Proteoideae), Stenocarpinae and Helicieae."

Finally the functionally distinct glandular hairs were discussed:

"A few species, especially of *Grevillea*, also develop trichomes of an additional and distinct type. These are uniseriate and multicellular, with thin walls and abundant cytoplasm. They are secretory and are usually described as glandular hairs."

In a book edited by Kubitzki (2006), a special attention was given to characteristic structures that are left after trichome shedding. These trichome remnants or 'scars', as termed by the Australian botanist Carpenter (Carpenter 1994; Carpenter et al. 2005), are shared by all Proteaceae and prove their close relation with Platanaceae. Because of the similarities of trichome base structures, fossil samples of Platanaceae and Proteaceae sometimes can be discriminated, only by using stomatal traits (Carpenter et al. 2005, 2015).

Several monographs and papers on certain taxonomic, geographical and ecological groups in the family add more details to the diversity of trichomes and related epidermal structures.

In his 'Field guide to proteas of Southern Africa', Rebelo (2001) describes a distinct type of spirally coiled trichomes in *Leucospermum*, forming a thick, tangled, felt-like indumentum. He also mentions 'denticles' which are present only in one section of type genus *Protea* – trichome-related structures that give a rough, sandpapery appearance to the surface of the leaves.

Three Argentinian researchers, Gonzalez, Gandolfo & Cuneo (2004), considering morphology of epidermal structures in Argentinian Proteaceae, mentioned multiseriate hairs occurring in *Gevuina avellana* Molina, and some peculiar-shaped structures on leaf glands of *Lomatia hirsuta* (Lam.) Diels of unknown origin. Regrettably, no photographs or drawings of the abovementioned multiseriate hairs are present in the paper.

One of the largest and most rapidly diverging genera in Proteaceae is *Grevillea*, a type genus of Grevilleoideae. There are several monographs on this genus, mostly by Australian botanists. In regards to trichomes, it is worth to mention the work by McGillivray & Makinson (1993), in which different types of trichomes characteristic of *Grevillea* and their distribution are considered in detail. According to McGillivray & Makinson (1993), it is not quite clear where the borders between trichome cells must be drawn; although the authors agree that T-shaped hairs, most typical of *Grevillea*, are three-celled, they believe that simple straight hairs are quite likely unicellular. Rhomboid trichomes (a modification of T-shaped trichomes) present in *G. plurijuga*

F. Muell. and related species, lack the stalk cell in his opinion and thus are a distinct type of trichomes. They suggested that short papilloid trichomes, occurring mostly on various parts of the gynoecium, are also unicellular. They provided one of the first and most complete descriptions of the glandular, evidently multicellular, hairs in *Grevillea*. It is notable that they believed that the T-shaped trichomes with equal arms are basic for the genus, while all transitions to trichomes with a straight, unbranched terminal cell are their later derivatives. These 'grevilleocentric' views of McGillivray & Makinson (1993) may be probably attributed to their insufficient attention to the evolution of hairs in the family in general.

In a later and more comprehensive monograph on the same genus, Olde & Marriott (1994) give a thorough description of leaf epidermal structures, indicating up to five types of trichomes in *Grevillea*, often simultaneously occurring on different organs of plants or during different developmental stages:

- T-shaped (on most organs)
- glandular multicellular (usually on generative parts, but in some species also all over the leaf surface; sometimes intermixed with the T-shaped ones)
- simple (typical of all the other Proteaceae: simple 3-celled hairs, usually on the ovary)
- rhombic (in *G. plurijuga* and related species), a distinct type according to McGillivray & Makinson (1993).
- papilloid.

A major breakthrough into understanding of epidermal structures in the family was made by Carpenter (1994, 2012) and Carpenter et al. (2005), who designed a new method of obtaining cuticular envelopes: mesophyll tissues were dissolved with 10% aqueous chromium trioxide, sometimes with gentle heating, then the cleared cuticle envelopes were rinsed, soaked briefly in 5% aqueous ammonia and stained with safranin O. If persistent trichomes obscured the leaf surface, they were physically removed with adhesive tape prior to dissolving. This method makes it possible to study the structure of both inner and outer epidermal cell surface; ensuring that the borders between epidermal cells are distinctly seen.

Due to Carpenter's research, it became known that trichomes of Proteaceae, morphogenetically three-celled, do not always remain. Their basal cell quite often undergoes one or more anticlinal divisions, and after that the stalk cell becomes attached to a group of cells in the epidermis (up to 16) (Fig. 2). Carpenter postulated that the basal cell will remain unicellular, if the terminal cell persists, and it divides in taxa, where mature trichomes are shed.

Carpenter believed that a more detailed study of trichome development and morphology would provide more data to establish taxonomic interpretations, as trichomes are known to possess features of phylogenetic significance (Carpenter 1994). He and his coauthors obtained comprehensive data on epidermal characteristics for most groups of extant and fossil Australian Proteaceae; they were planning a study of the cuticles in the whole family, including extra-Australian taxa, but this project had never been carried out.

Carpenter's studies in paleontology make a considerable contribution to understanding the history of the origin and evolution of xerophyllous, fire-prone plant communities of the southern hemisphere with a prominent role of Proteaceae (Carpenter 2012; Carpenter et al. 2015).

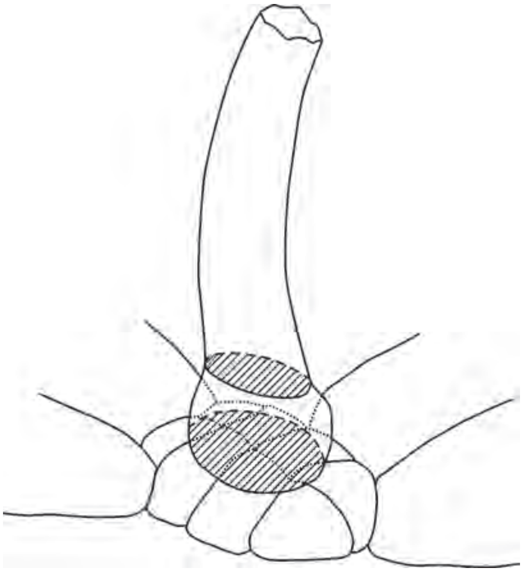

Figure 2. Basal cell division.

These studies proved that morphology of epidermal structures of fossil samples, including trichomes, can serve as a powerful diagnostic tool.

Types of trichomes and their distribution within different taxonomic groups of Proteaceae

1. Simple straight hairs (a generalized / ancestral type), with a single or divided basal cell.

Occur in almost all taxa on leaves, stems, floral parts and fruits. Some of the so called 'glabrous' species still have a few sporadic early shed hairs occurring only on the leaf margin or at the petiole. Simple hairs are quite often shed when the leaf reaches its maturity. The shedding trichome typically breaks at the level of the cell wall between stalk cell and terminal cell: in some cases the stalk cell also collapses (Fig. 3A).

2. Simple wavy / spirally twisted hairs.

In contrast to straight hairs, the fibrils of the terminal cell of these hairs are spirally arranged to some extent, resulting in wavy (Fig. 3B) or spirally twisted hairs, often forming a thick felt layer. This restructuring of fibrils is most likely a result of cortical cytoskeleton rearrangement during hair development.

In some genera of the so called Cape Clade (*Leucospermum*, *Mimetes*, *Diastella*), hairs that are coiled or wavy to various degrees are typical. This feature has likely evolved independently in different groups of taxa, as it is observed in South African genus *Paranomus* (Fig. 3C), from another clade within Proteoideae (according to Weston & Barker 2006) and some Australian genera from Grevilleoideae (e.g. *Banksia*). Wavy hairs, matting into a felt layer, also occur in some sections of the type genus *Protea*. Wavy hairs are usually considerably long and dense, and these are likely an adaptation for protecting leaf tissues from the excess of insolation, heat and desiccation (insulating layer). In some species matting wavy or spirally coiled hairs coexist in indumentum together with straight hairs; in these cases, straight trichomes are usually noticeably

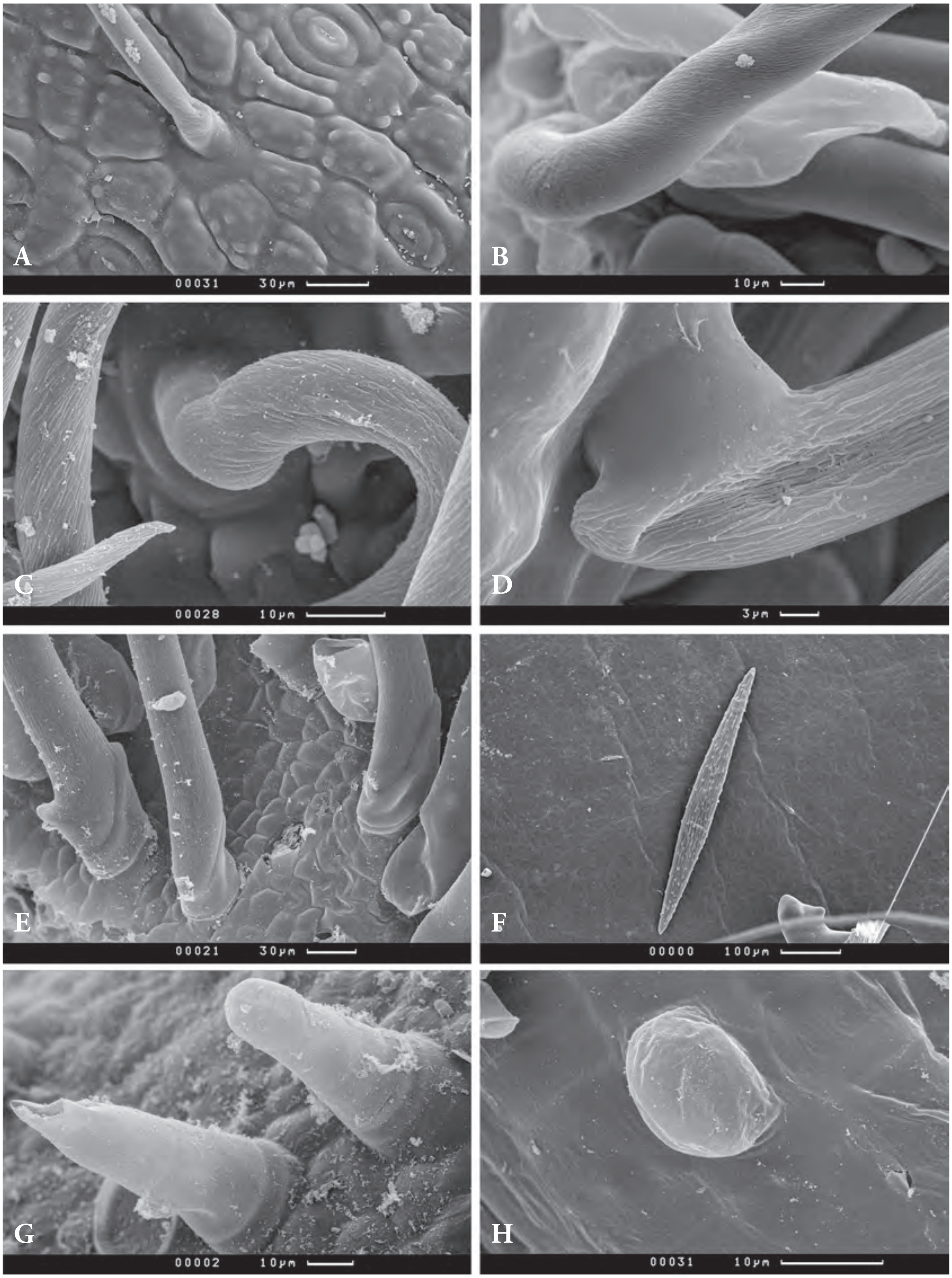

Figure 3. SEM photographs of different trichome types in Proteaceae. A – simple straight trichome of *Serruria elongata* R. Br., surrounded by epidermal pavement cells; B – wavy trichome of *Leucadendron teretifolium* (Andrews) I. Williams; C – spirally coiled trichome of *Paranomus bracteolaris* Salisb. ex Knight; D – horizontally spread trichome with a terminal cell protrusion of *Lambertia inermis* R. Br.; E – trichome of transitional shape on ovary of *Grevillea lanigera* A. Cunn. ex R. Br.; F – rhombic (anvil-shaped) trichome of *Grevillea quercifolia* R. Br.; G – glandular trichomes with intact (right) and broken (left) secretory cells of *Grevillea* sp. (sample WA587, Sokoloff 2015); H – papilloid trichome on ovary of *Grevillea rosmarinifolia* A. Cunn.

larger than coiled ones, which is most evident, when comparing the relative size of their stalk cells (our preliminary data).

3. T-shaped hairs and related transitional forms.

In many taxa, the proximal part of the terminal cell forms a protrusion, pronounced to various degrees. Often simultaneously, the terminal cell becomes tilted away from the vertical axis of the stalk cell (Fig. 3D). The next evolutionary step is a biramous hair with two unequal branches, the smaller one could be assumed as a further development of a protrusion (Fig. 3E). Finally, the trichome branches become equal, and trichomes acquire the typical T-shape with horizontal position of the terminal cell.

Clearly T-shaped trichomes with equal branches of the terminal cell are characteristic of certain genera of different distantly related tribes of Grevilleoideae (see Johnson & Briggs 1975; taxonomy according to Weston & Barker 2006). This may imply that this character has evolved independently several times in the Proteaceae. This hypothesis is also supported by numerous transitional forms that are even more widely distributed within unrelated clades of the family. The branches of T-shaped trichomes vary in size, shape and length. In some cases, they are short and the terminal cell is flattened, anvil-shaped; the density of these hairs is usually lower (Fig. 3F). Rhombic trichomes observed by McGillivray & Makinson (1993) in *G. plurijuga* and related species belong to this variety. In other cases, T-shaped hairs form a thick insulating layer, completely hiding the stomata; long branches of their terminal cells may be wavy or curly, they tangle together, forming a felt-like structure with a protective function.

4. Glandular (multicellular) hairs.

In species from certain sections of genus *Grevillea*, glandular hairs consisting of more than three cells can be observed on generative and vegetative parts. The basal cells of these trichomes are submerged into epidermis, the stalk is formed by a series of several isodiametric cells roughly of the same size. The last cell in the row is secretory, its cell walls often collapse, letting its constituents flow out (Fig. 3G). Although the properties and chemical constituents of the exudate are largely unknown, it most likely provides some protection from phytophages. Glandular hairs usually grow in combination with other types of hairs, they are less prone to shedding than T-shaped trichomes and thus often become more prominent when the leaf matures. Werker (1997) supposed that glandular hairs develop slower than simple hairs because of the much more active processes of synthesis ongoing in them. Regrettably, we have no data on *Grevillea* that would confirm or contradict this hypothesis.

It is interesting to note that glandular hairs are found also on stigmas in many genera of Proteaceae (Venkata Rao 1961, 1967b). Their main function is mechanical capture of pollen and providing conditions favoring its germination. It is quite likely that these hairs are not homologous to protective glandular hairs on vegetative parts of some *Grevillea* species. Their exact cellular structure needs further clarification.

5. Papilloid (undeveloped) hairs.

First discussed by McGillivray & Makinson (1993) and Olde & Marriott (1994) in their monographies on *Grevillea*, papilloid hairs are simple protrusions of epidermis, occurring usually on different generative organs, mostly on the gynoecium (Fig. 3H). Venkata Rao mentions papilloid trichomes on ovary (Venkata Rao 1961) and on stigma (Venkata Rao 1967a).

We still poorly understand the cellular structure of papilloid hairs and how they differ from true papillae. On the one hand, they can be just protrusions of epidermal pavement cells; on the other hand, developing hairs, both straight and T-shaped, on a certain early stage closely resemble papilloid hairs (Venkata Rao 1961; our preliminary data). As the early development of trichomes is also poorly studied, there is a possibility that at this stage the cell walls between the basal cell and the future stalk cell are not formed yet.

6. Glabrous (hairless) taxa.

It is worth mentioning that leaves and other vegetative organs may be either primarily or secondarily glabrous. Juvenile leaves of Proteaceae are often covered with hairs that consequently break off along distal or proximal borders of the stalk cell, leaving characteristic remnants, ‘stumps’ or ‘scars’ (Carpenter 1994). In the meantime, the leaf surface in some species is completely glabrous already at primordial stage, or hairs are very sporadic and present only along leaf margins or near the petiole. Usually, this primary glabrous condition correlates with a considerable thickness of the cuticle and, especially, the surface wax layer of a mature leaf. A thick wax layer with deeply submerged stomata versus thick indumentum may present two alternative adaptations to xerophily, both reducing evaporation and reflecting the excess of insolation. In some cases of primarily glabrous leaves, characteristic cell ensembles are still observed. These allow a guess that some epidermal cells in these cases, although they never develop into trichomes, still differentiate as trichoblasts, forming around themselves a group of biochemically and morphologically distinct cells.

7. Distribution of trichomes on various plant organs and combinations of different trichome types.

Within the Proteaceae, hairs occur almost on all vegetative and generative organs. Simple straight hairs are the most widely distributed, regarding both different taxa in the family and different organs. Wavy, coiled or T-shaped trichomes, most likely deriving from the basal type, are more characteristic of leaves and stems and less frequently occur on generative organs. On the contrary, papilloid trichomes are usually present only on the ovary and very rarely appear on vegetative organs.

Combinations of straight and coiled hairs on the leaf surface occur in *Banksia*, *Paranomus*, *Protea* (our preliminary data). For *Grevillea* different combinations of various types of trichomes (glandular, simple straight, T-shaped) on the same plant organ (leaf, stem, ovary) have been reported (Olde & Marriott 1994).

Morphogenetic links between trichome types in Proteaceae.

Straight three-cellular hairs occurring in all subfamilies are characteristic of subfamilies considered as basal/primitive, and it is reasonable to regard them as a basal type and T-shaped trichomes as derived from them, according to Johnson & Briggs (1995) and opposed to the opinion of McGillivray & Makinson (1993).

T-shaped hairs are quite common in other families; their functions, cell structure details and morphogenesis may vary. Both uniseriate multicellular T-shaped trichomes with horizontally arranged terminal cell (Bhande et al. 2010) and biseriate T-shaped trichomes with each branch formed by a separate cell (Krak & Mráz 2008) are known in Asteraceae. T-shaped (anvil-shaped) structures in hops (*Humulus lupulus* L.), an adaptation to climbing support, are not purely epidermal and should be considered as emergences (Franz 1935). Finally, certain Malpighiaceae have unicellular T-shaped trichomes (Souto & Oliveira 2012) with a structure very much alike to that of Engler's drawing of a *Grevillea* trichome.

Several mutations in *Arabidopsis* result in biramous hairs (Schwab et al. 2000; Ilgenfritz et al. 2003). The position of nucleus in such hairs may vary – in *zwischel* mutants it is situated at the bifurcation point, while in *stichel* mutants it stays in a more proximal position. Cytoskeleton stabilization on the hair' end is an important stage of development, after which the branching would occur.

It is reasonable to suggest that wavy and spirally coiled hairs are also derivates of straight hairs. They most likely result from some rearrangement of cytoskeleton orientation.

Multicellular glandular hairs are found only in *Grevillea*, a genus in a process of rapid adaptive radiation. It can be assumed that they first emerged in certain clades of this genus and are an evolutionarily novel type of indumentum for Proteaceae with a distinct function. Morphogenetically, they are most likely formed by multiple longitudinal divisions of the trichome stalk cell.

Papilloid trichomes can also be regarded as derivatives of the basal type, but in this case, the cell divisions cease very early at the stage of a single cell or sometimes, probably, two cells. Details of their actual morphogenesis still need clarification. An absence of a clear borderline between terms 'papillae' and 'trichomes' has been mentioned many times (e.g. Werker 2000). It is probably reasonable to define papillae as outgrowths of ordinary pavement epidermal cells and papilloid trichomes as not fully developed trichoblasts.

Finally, glabrous leaves, at least in some cases, may be a result of arrest of trichoblast development at a yet earlier stage, when they can be told from surrounding cells only by their distinct position in the arrangement of epidermal cell groups. Studies of cuticular envelopes and structure of characteristic cell ensembles linked to hairs may provide a more precise answer of this question.

It is worth mentioning that glabrousness in Proteaceae is quite common and supposedly develops in relatively short terms from an evolutionary viewpoint, considering its evidently parallel development in extant taxa of the family, e.g. in different sections within the same genus: thus the lack of characteristic remnants of broken off hairs or 'scars' (Carpenter 2005) on fossil samples cannot be solely sufficient for excluding samples from Proteaceae family.

Lack of systematically arranged data makes it impossible to discuss, how the division of the trichome basal cell, demonstrated by Carpenter for Australian Proteaceae, had appeared first and evolved. Our preliminary unpublished data show that the division of trichome basal cell occurs also in South African genera. Carpenter's idea that basal cell division is directly linked to shedding off the terminal cell of a mature trichome needs to be tested on all larger groups of extant Proteaceae, paying special attention to morphogenesis of this process.

Anticlinal divisions of hair cells occur in *siamese* mutant of *Arabidopsis* (Walker et al. 2000) and in many Asteraceae (Krak & Mráz 2008). They can be limited only to apical cells as in *Solanum* (Bergau et al. 2015), middle cells as in *Hibiscus* (Werker 2000) or the stalk cell as in *Diplopterys* (Souto & Oliveira 2012).

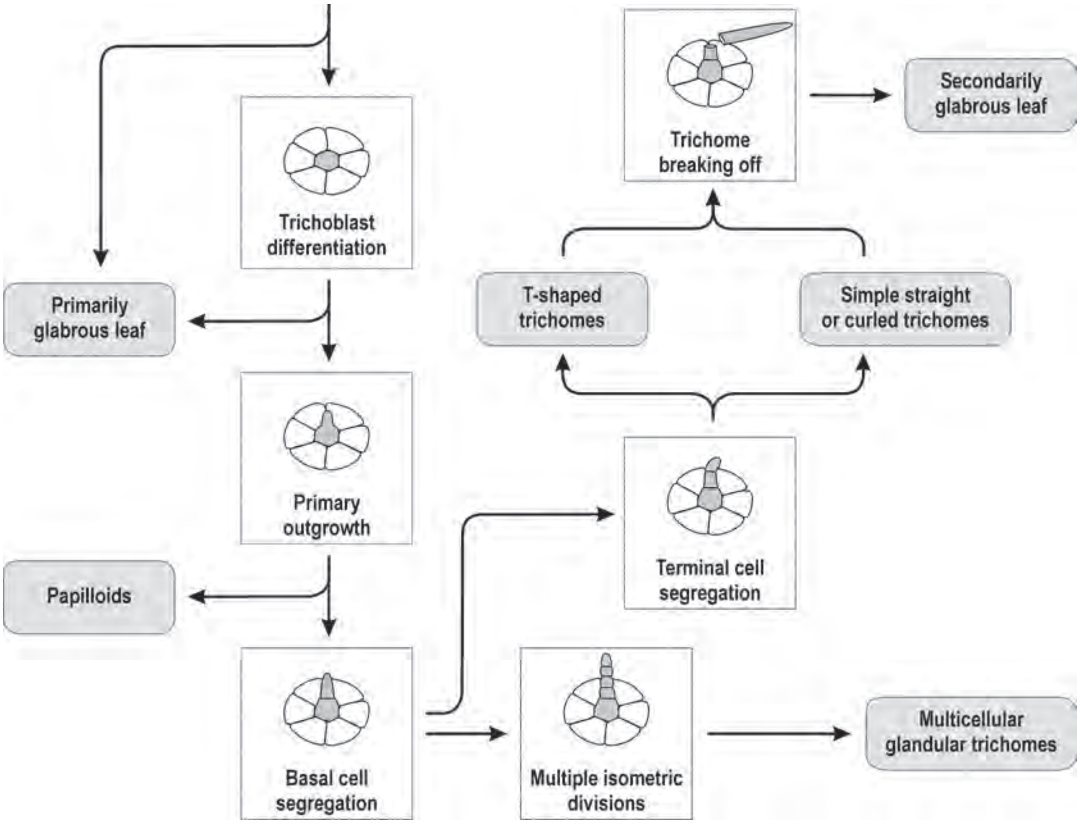

Figure 4. Morphogenesis of trichome types in Proteaceae.

Salt glands of *Avicennia* also have anticlinally divided basal cells; a single stalk cell is attached to this group of basal cells (Fahn 2000). This type of structure closely resembles the division of basal trichome cells in Proteaceae.

Thus, the interrelationship of different trichome types in Proteaceae could be presented as a general developmental chart with a few bifurcations (Fig. 4).

Different types of indumentum in the Proteaceae are inseparably linked to their functions and ecological adaptations they provide. Carpenter reasonably believed that the earlier Proteaceae of seasonally humid tropical and subtropical forests, being mostly the canopy trees, already had to adapt to the excess of insolation and to ambient air humidity almost to the same extent as those that colonized open xeric habitats later. Thus, this protecting function of indumentum is likely to be primary. At the same time, hairs also can serve as a mechanical protection from phytophages.

An alternative way of solving the same problems, which has likely evolved many times within the Proteaceae, is losing the indumentum altogether with a compensatory cuticle thickening and developing a considerable layer of wax over it. There is possibly a trade-off between spending resources on either of the two ways of protection from excess insolation and aridity.

Glandular trichomes have developed in Proteaceae relatively recently from evolutionary point of view, only in certain sections of rapidly diverging genus *Grevillea.* In theory they, are capable of providing the same protective functions in another way yet. Substances which they secrete can deter the phytophages physically and chemically, and they create a sticky layer over the leaf surface that offers some insulation, just like felt-like mats of tangled hairs or thick wax-covered cuticles mentioned above. Neither function has been confirmed for glandular hairs of *Grevillea* yet. A thorough qualitative and quantitative analysis of their exudate may shed more light on their actual function.

Future prospects

In conclusion, we would like to summarize and list the aspects of trichome structure and morphogenesis requiring further investigation, mentioned above in relevant sections.

1. There is a need to clearly identify the position of cell borders in different types of trichomes, the exact timing of their emergence in morphogenesis, the order of cell divisions, the arrangement of nuclei during different stages of morphogenesis in different hair types. Data regarding density of indumentum in various taxa of the family, expressed as cell distances and absolute metric measurements, are still missing.

2. A detailed knowledge of distribution of different types of indumentum in taxa of Proteaceae is required to understand the evolution of different trichome types in the family in general and in certain taxonomic groups. A comparison of ever growing bulk of molecular data with morphological and anatomical indumentum features could yield some fruitful results. In particular, it would be interesting to investigate the convergent loss of leaf trichomes and evolving of 'glabrousness' in different sections of *Protea* and possible evolution of glandular indumentum in some groups of *Grevillea*. Carpenter's works on epidermal structures, mentioning the division of the basal trichome cell, certainly deserve a follow-up, considering the evolution history of this trait in Proteaceae.

It is worth mentioning that studies of trichome morphogenesis in Proteaceae face some objective difficulties that are linked primarily to the availability of materials. The vast majority of the taxa in the family are found in habitats with pronounced seasonality. Consequently material for studying the early stages of leaf development may be obtained only during a rather short period of active vegetative growth, often lasting only a few weeks in a year. Many xerophytic taxa in the family are characterized by slow growth, and materials suitable for studying trichome morphogenesis are present only in terminal buds of shoots that are usually only a few on a plant; removing them causes noticeable damage to the plant and cannot be recommended in case of wild specimens of the protected species. As a method to overcome this problem, we suggest to create specialized living collections in botanical gardens.

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