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# Do leaves in Cyperoideae (Cyperaceae) have a multiple epidermis or a hypodermis?

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ABSTRACT

In Cyperaceae, leaf anatomical characters, in particular the presence of a hypodermis or of a multiple epidermis, have contributed in taxonomic and phylogenetic studies. In this family, the leaf epidermis is often described as uniseriate, and the cells of the subepidermal layers having no chloroplasts are treated as hypodermis. Both tissues have a different ontogenetic origin and hence are not homologous. The aim of the present work was to verify the origin of the subepidermal layers in eight species belonging to Cyperoideae. All species studied presented multiple epidermal layers that were confirmed by leaf ontogeny. In *Fimbristylis complanata*, *F. dichotoma*, *Pycreus flavescens* and *P. polystachyos* the mature leaves present multiple epidermal layers with cells of the distinct layers similar in shape and size; in the other species studied these cells are different. Especially in the latter case, a multiple epidermis is easily interpreted erroneously as a hypodermis, possibly leading to erroneous evolutionary conclusions. Making correctly distinction between a hypodermis and a multiple epidermis, and hence in case of doubt investigating the origin of the questioned tissue, is compulsory in order to use both characters in a phylogenetic context. Though in the past often called 'hypodermis', our leaf ontogenetical observations show that in all species studied, the subepidermical layers constitute a multiple epidermis, originating from the protodermis.


## Introduction

The family Cyperaceae has 109 genera and approximately 5500 species (Govaerts et al., 2007) and Cyperoideae is the larger of the two subfamilies of Cyperaceae. Anatomical characters are often employed in taxonomic (Alves et al., 2002; Holm, 1899; Kukkonen, 1967; Metcalfe, 1971; Plowman, 1906; Standley, 1990) and phylogenetical (Bruhl, 1995; Starr et al., 2004) studies of the family and among the leaf anatomical characteristics, those related to epidermis and hypodermis are of particular interest (Goetghebeur, 1998; Koyama, 1967; Sherferd, 1976).

The leaf epidermis is generally described as uniseriate in Cyperaceae (Bruhl, 1995; Denton, 1983; Metcalfe, 1971), and the principal epidermal characters used for taxonomy are the cell shape and the presence of trichomes, papillae and silica bodies (Honaine et al., 2009; Mehra and Sharma, 1965; Metcalfe, 1969; Starr et al., 2004). In the family, the subepidermal layers with cells having no chloroplasts are treated as hypodermis and present characters with taxonomic potential, involving the cell shape, cell wall thickening as well as the number and continuity of the layers (Bruhl, 1995; Govindarajalu, 1974; Metcalfe, 1971).

In a phylogenetic study, Bruhl (1995) used the term hypodermis for the subepidermal layers but commented that, in some groups, these layers can constitute a multiple epidermis. Sharma and Mehra (1972) in their taxonomic study of *Fimbristylis* (Cyperaceae) reported similarity between the epidermal and hypodermal layers of the leaf. These authors then indicated the presence of a multiple epidermis in *Fimbristylis* species but did not verify its origin. Correct usage of the terms hypodermis and multiple epidermis in Cyperaceae requires knowing the origin of these tissues, which are not homologous. The multiple epidermis originates from periclinal cell division of the protodermis different from the hypodermic that derivates from the ground meristem (Esau, 1965).

The use of homolog structures is fundamental in phylogenetical approach and ontogenetic studies have been important to solve problems of homology in vegetative (Martins and Scatena, 2011) and reproductive organs (Vrijdaghs et al., 2009, 2010) of Cyperaceae species.

The aim of the study was investigate the ontogeny of the subepidermal layers of leaves in species from different tribes and genera of Cyperoideae to verify their origin.

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**Table 1**
Species of Cyperoideae studied and characteristics of the epidermis.

| Species | Voucher | Cell size among the epidermal layers | Number of the epidermal layers |
|---|---|---|---|
| **Abildgaardieae** | | | |
| *Fimbristylis complanata* | V.L. Scatena 344 | Similar | 3–6 |
| *F. dichotoma* | S. Martins 398 | Similar | 1–2 |
| **Cypereae** | | | |
| *Cyperus ligularis* | S. Martins 330 | Different | 3–8 |
| *Pycreus flavescens* | S. Martins 327 | Similar | 1–3 |
| *P. niger* | AGR 16 | Different | 3–7 |
| *P. polystachyos* | N. Guarise 24 | Similar | 1–3 |
| **Rhynchosporeae** | | | |
| *Rhynchospora globosa* | S. Martins 305 | Different | 6–10 |
| *R. terminalis* | S. Martins 302 | Different | 2–5 |

## Materials and methods

*Cyperus ligularis* L., *Fimbristylis complanata* (Retz.) Link, *F. dichotoma* (L.) Vahl, *Pycreus flavescens* (L.) Rchb., *P. niger* (Ruiz & Pav.) Cufod., *P. polystachyos* (Rottb.) P. Beauv., *Rhynchospora globosa* Lindl. and *R. terminalis* Kunth were studied (Table 1). Species were chosen based on the degree of similarity of the cells that constitute the subepidermal layers. Voucher materials were deposited at the Herbarium of the Department of Botany, Universidade Estadual Paulista (HRCB), and the Herbarium of the Faculdad de Ciencias Agrarias, Universidad Nacional del Litoral (SF).

For the ontogenetic study, young rhizomes with vegetative apices were collected, fixed in FAA 50 (Johansen, 1940) and stored in 70% ethanol. Portions of the rhizome apices with developing leaves were dehydrated in an ethyl alcohol series and then, as described by Feder and O'Brien (1968), embedded in Historesin (Leica Historesin Embedding Kit, Nussloch, Germany). Longitudinal and transverse sections of the young leaves located in a rhizome apex were made using a microtome, stained with periodic acid-Schiff's reagent and toluidine blue (Feder and O'Brien, 1968) and mounted in Entellan (Merck). Transverse sections of the median region of mature leaves were made, stained with basic fuchsin and Astra blue (Roeser, 1972) and mounted in glycerin jelly.

## Results

The mature leaves of the species studied possess a multiple epidermis only on the adaxial surface (Fig. 1A–L – asterisk) with cells of similar shapes and sizes in *Fimbristylis complanata* (Fig. 1A), *F. dichotoma* (Fig. 1B), *Pycreus flavescens* (Fig. 1C and D) and *P. polystachyos* (Fig. 1E and F) and different shapes and sizes in *Cyperus ligularis* (Fig. 1G and H), *Pycreus niger* (Fig. 1I and J), *Rhynchospora globosa* (Fig. 1K) and *R. terminalis* (Fig. 1L). These differences in the epidermis are outlined in a diagrammatic representation (Fig. 3A–D). The number of cell epidermal layers in the multiple epidermis varies among the species studied and also intraspecifically (Table 1 and Fig. 1A–L).

In young leaves of the species studied, at the stage in which the tissues are undifferentiated, the outmost layer (protodermis) is uniseriate on both surfaces (Fig. 2A). After the tissues begin differentiation, the protodermal cells divide periclinally giving rise to their derivatives, as in *Fimbristylis dichotoma* (Fig. 2B and C, arrows), *Pycreus flavescens* (Fig. 2E and F, arrows), *Cyperus ligularis* (Fig. 2H and I, arrows) and *Rhynchospora globosa* (Fig. 2K and L, arrows). In the derivative cells of the protodermis, anticlinal division can also occur (Fig. 2I and M, arrowheads), but this type of division is less frequent than periclinal division.

In the species that possess a multiple epidermis constituted by similar cells (Table 1), generally one (Fig. 2C) or two (Fig. 2F) periclinal divisions occur that result in two to three cell epidermal layers (Fig. 2D and G – asterisk). During the stage in which the tissues are totally differentiated, all the cells of the multiple epidermis increase in size (Figs. 2D and G and 3B). In the species that have a multiple epidermis constituted by different cells (Table 1), in general more than two periclinal divisions occur (Fig. 2I, L and M, arrows), resulting in several cell epidermal layers (Fig. 2J and M – asterisk), in addition to the anticlinal divisions (Fig. 2I and M, arrowheads). In these species, at the stage in which the tissues are already differentiated, the epidermal cells increase in size, but not evenly, and the cells of the outmost epidermal layer become larger than the cells of the innermost layers (Fig. 3C), as seen in *R. terminalis* (Fig. 1L), or smaller (Fig. 3D), as seen in *Cyperus ligularis* (Figs. 1G and H and 2J), *Pycreus niger* (Fig. 1I and J) and *Rhynchospora globosa* (Figs. 1K and 2M).

## Discussion

The presence of a multiple epidermis in angiosperms in general is characterized by periclinal divisions of the outermost layer of the meristem (protodermis) (Beck, 2005; Esau, 1965), as observed in the Cyperaceae species studied in the present work and in other angiosperms taxa of the families Apocynaceae (*Nerium*: Beck, 2005), Moraceae (*Ficus*: Beardsell and Norden, 2004), and Piperaceae (*Peperomia*: Kaul, 1977). To the monocotyledons it is the first indication of leaves with multiple epidermis, detaching the importance of this character to Cyperaceae in future taxonomical and phylogenetical studies, both inter and intra-family.

The same shape and parallel position of the cell walls of the outmost epidermal layer with those of subepidermal cells (Fig. 3B) led Sharma and Mehra (1972) to indicate a multiple epidermis in *Fimbristylis*. A similar situation was observed in *Fimbristylis complanata*, *F. dichotoma*, *Pycreus flavescens* and *P. polystachyus* and it is ontogenetically demonstrated that these species have a multiple epidermis. However, in *Cyperus ligularis*, *Pycreus niger*, *Rhynchospora globosa* and *R. terminalis*, the cells of the outmost epidermal layer differ in shape and size from the cells of the other epidermal layers located internally, and their cell walls are not in a parallel position in the mature leaf (Fig. 3C and D). This non-aligned arrangement of the cells, when observed in the mature leaf, complicates the interpretation of a multiple epidermis. Such an arrangement of cells probably led many authors to call the subepidermal layers hypodermis (Govindarajalu, 1974, 1982; Li and Jones, 1994; Martins et al., 2008; Metcalfe, 1971), while in fact they are part of a multiple epidermis, as demonstrated here.

The multiple epidermis in each of the species studied vary in the shape of the cells and in number of the layers, and can constitute useful characters for taxonomic and phylogenetic investigations in Cyperaceae. A similar set of characters was used referring to the so-called hypodermis in taxonomic studies in Cyperaceae (Alves et al., 2002; Bruhl, 1995; Govindarajalu, 1974; Hefler and Longhi-Wagner, 2010).

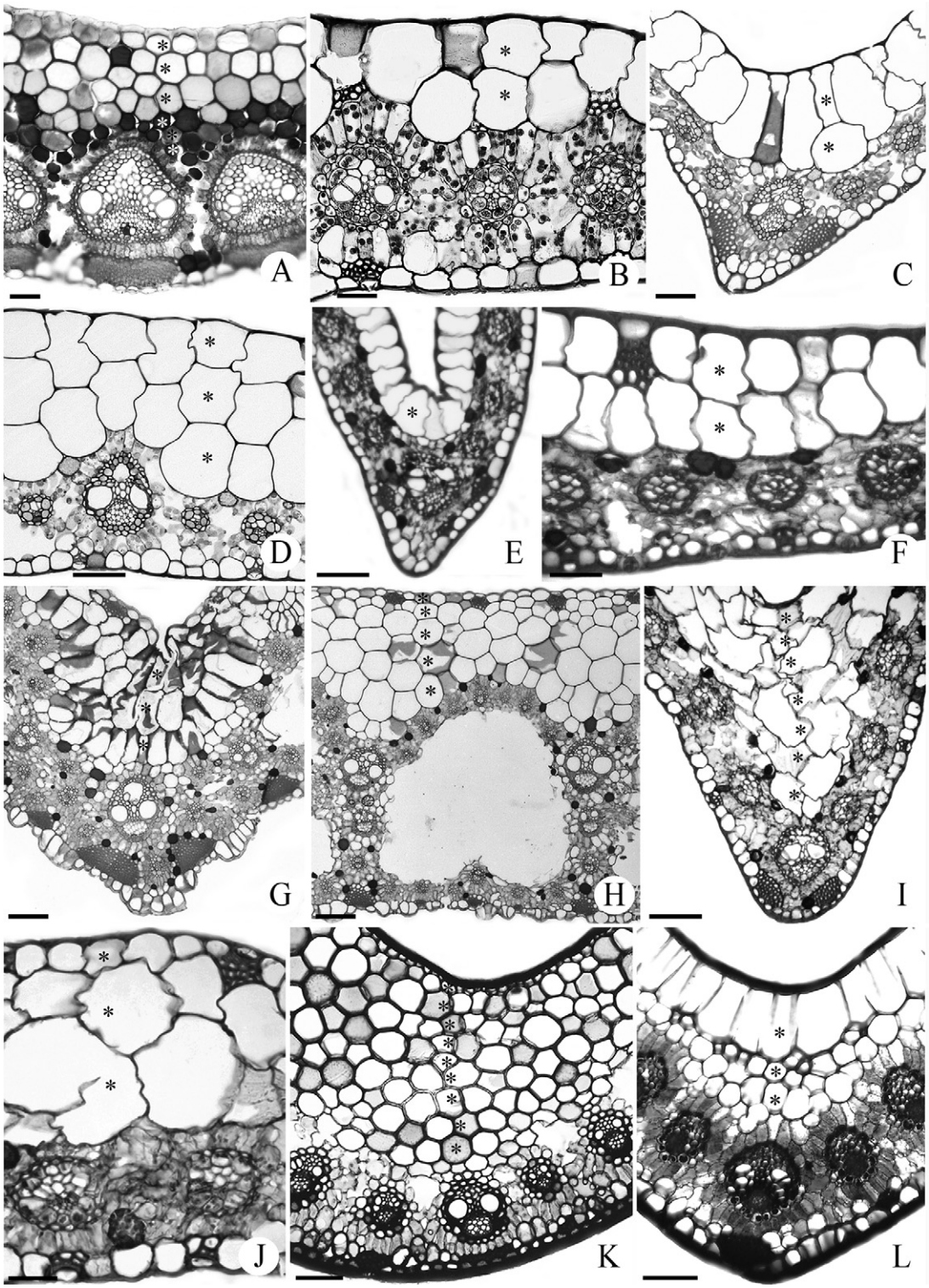


**Fig. 1.** Anatomical aspects of mature leaves of Cyperaceae in transverse section. Note adaxial multiple epidermis (asterisk). (A) *Fimbristylis complanata*, (B) *Fimbristylis dichotoma*, (C–D) *Pycreus flavescens*, (E–F) *Pycreus polystachyos*, (G–H) *Cyperus ligularis*, (I–J) *Pycreus niger*, (K) *Rhynchospora globosa*, (L) *Rhynchospora terminalis*. Bars = 30 μm (A and J); 50 μm (B–F, I and L); 100 μm (G–H and K).

The species studied here are included in the subfamily Cyperoideae and belong to three distinct tribes: Abildgaardieae (*Fimbristylis complanata* and *F. dichotoma*); Cypereae (*Cyperus ligularis*, *Pycreus flavescens*, *P. niger* and *P. polystachyus*) and Rhynchosporeae (*Rhynchospora globosa* and *R. terminalis*) (sensu Muasya et al., 2009). Consequently, the character of the multiple epidermis occurs in different taxonomic categories of Cyperaceae and probably can be found also in other genera of the family.

In the giant genus *Cyperus*, the subepidermal layers of several species of *Cyperus* subg. *Cyperus* have been described and termed hypodermis (Hefler and Longhi-Wagner, 2010; Metcalfe, 1971). *Cyperus ligularis* studied here is included in *Cyperus* subg. *Cyperus* (Goetghebeur, 1998), but previously, this species belonged to

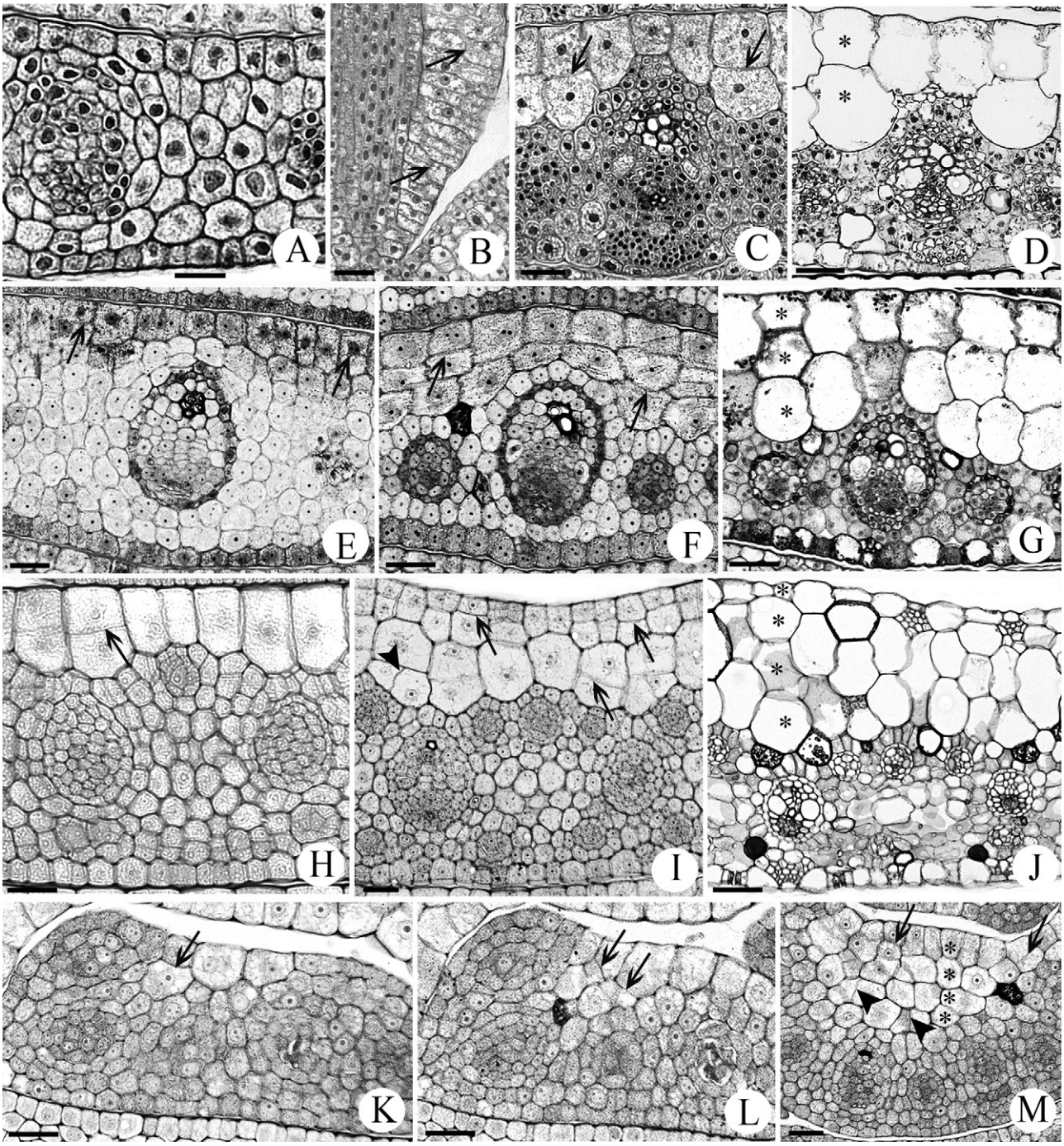


**Fig. 2.** Anatomical aspects of leaves of Cyperaceae in longitudinal (B) and transverse (A and C–N). Note different stages of development of the adaxial multiple epidermis. (A–D) *Fimbristylis dichotoma*, (E–G) *Pycreus flavescens*, (H–J) *Cyperus ligularis*, (K–M) *Rhynchospora globosa.* Arrowhead = anticlinal division of the cell; arrow = periclinal division of the cell; asterisk = multiple epidermis. Bars = 10 μm (E and H); 20 μm (F–G and I); 30 μm (A–B and K–M); 50 μm (C–D and J).

*Cyperus* subg. *Mariscus* according to Kükenthal (1935, 1936). Many species that belonged to this old taxonomic group have leaves with subepidermal layers on the adaxial surface (Hefler and Longhi-Wagner, 2010; Metcalfe, 1971) with similar characteristics to those of *Cyperus ligularis*, and probably are homologous with a multiple epidermis. We hypothesize that also in other species with subepidermal layers of the genus, treated as 'hypodermis', this is, in fact, a multiple epidermis developed.

In *Pycreus*, all species that have been anatomically described possess leaves with subepidermal layers that have also been termed as hypodermis (Bruhl, 1995; Leite and Scatena, 2009; Metcalfe, 1971) rather than as multiple epidermis, as presented here for *Pycreus flavescens*, *P. niger* and *P. polystachyos*. Most likely, the same character occurs in other species of *Pycreus* and is common to the genus.

*Rhynchospora globosa* and *R. terminalis*, which present a multiple epidermis, belong to the subgenus *Haplostylis* section *Pluriflorae* (Thomas et al., 2009). The other 11 species from this section also have subepidermal layers (A.C. Araújo, unpublished data) that must constitute a multiple epidermis. In *Rhynchospora fascicularis* var. *distans*, which belongs to the subgenus *Rhynchospora* section *Rhynchospora*, Metcalfe (1971) referred to the subepidermal layers as a

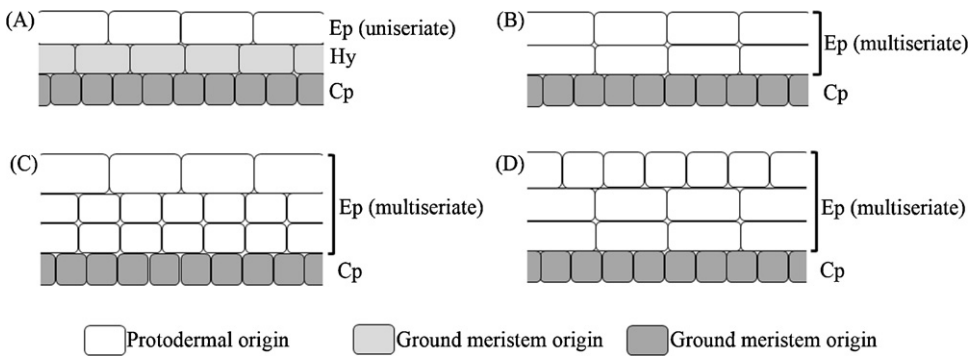


**Fig. 3.** Diagrams of leaf transverse sections showing differences in the epidermis of Cyperaceae species. (A) uniseriate epidermis, (B) multiseriate epidermis with cell walls of the different layers aligned, (C and D) multiseriate epidermis with cell walls of the different layers non-aligned, (C) cells of the outmost layer larger than the inner layers, (D) cells of the outmost layer smaller than the inner layers. Ep, epidermis; Hy, hypodermis; Cp, chlorophyll parenchyma cells.

hypodermis but stated that the boundary between the adaxial epidermis and the hypodermis is not very clear. The character 'multiple epidermis' may have appeared more than once in *Rhynchospora* because it occurs in species from distinct subgenera (Thomas et al., 2009).

## Conclusion

In all studied species, the subepidermal cell layers having no chloroplasts originate from the protodermis and hence are homologous with a multiple epidermis and not with a hypodermis. Treating the hypodermis and multiple epidermis as similar tissues is erroneous because these tissues have different origins and hence do not constitute homologous structures. Therefore, knowledge about the leaf ontogeny is necessary in all species with a putative multiple epidermis or putative hypodermis, in order to distinguish both characters and their respective character states and sub-characters.

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