

Vegetative anatomy of some Brazilian *Zygopetalinae* (Orchidaceae)

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ABSTRACT - The anatomy of the vegetative organs of the Brazilian *Zygopetalinae* are poorly known. In the present paper we analyze the anatomy of roots, stems and leaves of twelve species, many of them of uncertain taxonomic position. We compare our results with those previously described for the same subtribe and some anatomical features, such as the number of velamen, root cortex and mesophyll layers, that can be useful in the systematic analysis of determined taxa, specially *Dichaea* Lindl., *Promenaea* Lindl. and *Zygopetalum* Hook. We also found features that can be interpreted as adaptations to drought, a situation that typically prevails in epiphytic environments where most of the species studied here live.

Keywords: *Epidendroideae*, *Maxillarieae*, orchid, vegetative habit

RESUMO - Anatomia vegetativa de algumas *Zygopetalinae* (Orchidaceae). A anatomia dos órgãos vegetativos de *Zygopetalinae* brasileiras é pouco conhecida. No presente estudo foram analisadas a anatomia de raízes, caules e folhas de doze espécies, muitas das quais apresentam posicionamento taxonômico incerto. Comparamos os resultados obtidos com os previamente descritos para a mesma subtribo e alguns caracteres anatômicos tais como: o número de camadas do velame, do córtex radicular e do mesófilo que podem ser úteis em análises sistemáticas de determinados *taxa*, especialmente em *Dichaea* Lindl., *Promenaea* Lindl. and *Zygopetalum* Hook. Também foram encontrados caracteres que podem ser interpretados como adaptações à escassez hídrica, uma situação que tipicamente prevalece em ambientes epifíticos onde a maioria das espécies estudadas habita.

Palavras-chave: *Epidendroideae*, *Maxillarieae*, orquídea, hábito vegetativo

INTRODUCTION

The *Orchidaceae*, with around 780 genera and 25,000 species (Soltis *et al.* 2005, Pridgeon *et al.* 2009), possesses a wide geographic distribution (Dressler 1981) with more than 2.000 species occurring in Brazil (Barros 1990, Dressler 2005, Campos 2008, Souza & Lorenzi 2012). It is an easily recognizable group with strongly zygomorphic flowers in which perianth and reproductive elements are highly modified (Barros 1990, Dressler & Chase 1995, Ruschi 1997, Pinheiro *et al.* 2004, Souza & Lorenzi 2012). Within the family plants can be epiphytic, terrestrial or saprophytic and have sympodial or monopodial growth. Those with sympodial growth usually have a rhizoma plus a straight axis with a single internode the heteroblastic type, or with more than one internode, the homoblastic type. Heteroblastic branches can be thickened forming pseudobulbs that store water and nutrients (Braga 1977, Williams 1979, Dressler 1981, Braga 1987, Campos 2008).

Leaves are simple, with or without distinction between blade and sheath, and displayed in a distichous or spiral phyllotaxy. They vary in shape and texture and are, in general dorsiventral although some representatives have

ensiform or cylindrical blades (Schultz 1991, Souza & Lorenzi 2012). Both epiphytic and terrestrial orchids possess specialized roots that absorb nutrients and store water. In general, the roots establish endomycorrhizal associations that improve their ability to absorb nutrients (Pridgeon 1986, Mauseth 2009, Souza & Lorenzi 2012).

Some authors recognize three families of orchids (Dahlgren *et al.* 1985) but molecular analyzes confirm the monophyly of the group and the occurrence of five subfamilies: *Apostasioideae*, *Vanilloideae*, *Cypripedioideae*, *Orchidoideae* and *Epidendroideae* (Chase *et al.* 2003).

The subtribe *Zygopetalinae* belongs to the subfamily *Epidendroideae* and comprises around 30 genera and 400 species of neotropical orchids with diverse vegetative and floral morphologies (Whitten *et al.* 2005). It was traditionally included in the tribe *Maxillarieae* (Whitten *et al.* 2005) but recent molecular analyses indicate that they form, together to *Cymbidiinae*, *Eulophiinae*, *Bromheadiinae* and *Catasetinae*, a monophyletic tribe: the *Cymbidieae* (Chase *et al.* 2003). The circumscriptions of *Zygopetalinae* have been a matter of discussion (see Senghas & Dietrich 1992; Dressler 1993; Szlachetko 1995) and the combined analysis of Whitten *et al.* (2005) recognized them as a

monophyletic group that also includes the morphologically distinct genera *Cryptarrhena* (4 species) and *Dichaea* (111 species). Most *Zygopetalinae* are epiphytic, possess pseudobulbs and establish mutualistic relationships with mycorrhizal fungi (Dressler 1981). The pseudobulbs usually possess a single internode and the leaves vary on shape. The flowers, growing on lateral inflorescences, may or may not be resupinate, have a column of a variable size and four superposed pollinia (Dressler 1993, Whitten *et al.* 2005).

In an extensive study of *Maxillarieae* representatives, Stern *et al.* (2004) described the anatomy of the vegetative organs of many *Zygopetalinae* although no Brazilian species were included. In the present paper we describe the roots, stems and leaves of twelve Brazilian *Zygopetalinae* and compare the results with those of Stern *et al.* (2004).

MATERIAL AND METHODS

The material was collected from the living collection at the Instituto de Botânica de São Paulo (CO), according to the following specification: *Dichaea pendula* (Aubl.) Cogn. CO: 10448, 14816, 13461, *Dichaea trulla* Rchb. f. CO: 12875, 14815, 18144, *Hoehneella gehrtiana* (Hoehne) Ruschi CO: A1, *Huntleya meleagris* Lindl. CO: 17951, 18136, *Koellensteinia tricolor* (Lindl.) Rchb.f. CO: A1, *Paradisanthus micranthus* (Barb. Rodr.) Schltr. CO: VC1, VC2, *Promenaea rollissonii* (Rchb. f.) Lindl. CO: 10804, PR1, PR2, *Promenaea xanthina* Lindl. CO: VT1, VT2, VT3, *Warczewiczella wailesiana* (Lindl) E. Morren CO: 13620, 3184, *Zygopetalum mackayi* Hook CO: 16557; AOSP, *Zygopetalum maxillare* Lodd. CO: 1 Squeegee, Squeegee 2 and *Zygopetalum pedicellatum* (Sw.) Garay. CO: 16562.

For the anatomical analyses, the vegetative organs were fixed in 50% FAA and preserved in 70% alcohol. The samples were free hand cut and the cross sections stained with 0.05 % astra blue and safranin (Bukatsh 1972) and mounted in glycerin. For starch identification we used Lugol's solution (Bücherl 1962); for ligninin, Floroglucin plus Chloridric Acid (Jansen 1962); for lipids, Sudan III (Johansen 1940) and for flavonoids, Potassium Hydroxide (Costa 1982). The results were recorded with a digital camera coupled to an Olympus microscope (BX51 model).

RESULTS

Root anatomy

All roots (Figs. 1A-D) are cylindrical and possess a multilayered epidermis (velamen), a parenchymatous cortex and a vascular cylinder, although the organ diameter varies according to the species (Tabs.1, 2).

In most of species, the tests indicated the presence of suberin and lignin in the velamen cells. The velamen is formed by 2-6 layers (Tab. 1) of elliptical or rectangular cells with varied secondary thickening in the walls, depending on the species. The cells of the velamen outer layer (epivelamen) are papilloses, in *Dichaea trulla* (Fig. 2A), and, in some other species, slightly smaller than

those of the inner layers (Figs. 2B, C, Tabs. 1, 2). In the inner velamen layer (endovelamen), the isodiametric cells possess spirally thickened walls (Figs. 2B, C). In both *Dichaea pendula* (Fig. 2D) and *Promenaea rollissonii* there are fungal hyphae in the velamen. In the root cortex, the isodiametric exodermal cells have thickened walls in the outer periclinal surface, except for the passage cells that are without thickening (Figs. 1, 2A-D, Tab. 2).

Internally to the exodermis, the parenchymatous cortex is formed by a variable number of layers, according to the species (Figs. 1A-D, Tab. 1). In this region, there are small intercellular spaces and the thin-walled cells are smaller close to the exodermis and to the endodermis layers (Figs. 1A- D).

Crystals of raphids are common in this region (Figs. 1A- D, Tab. 1) and fungal hyphae were observed in *Zygopetalum mackayi*, *Paradisanthus micranthus* and *Promenaea xanthina* (Figs. 1C, D). The endodermis is one layered and its cell walls have U-shaped thickening (anticlinal and inner periclinal walls) (Tab. 1), except for the passage cells, opposite the xylem poles, that are thin-walled (Fig. 2D).

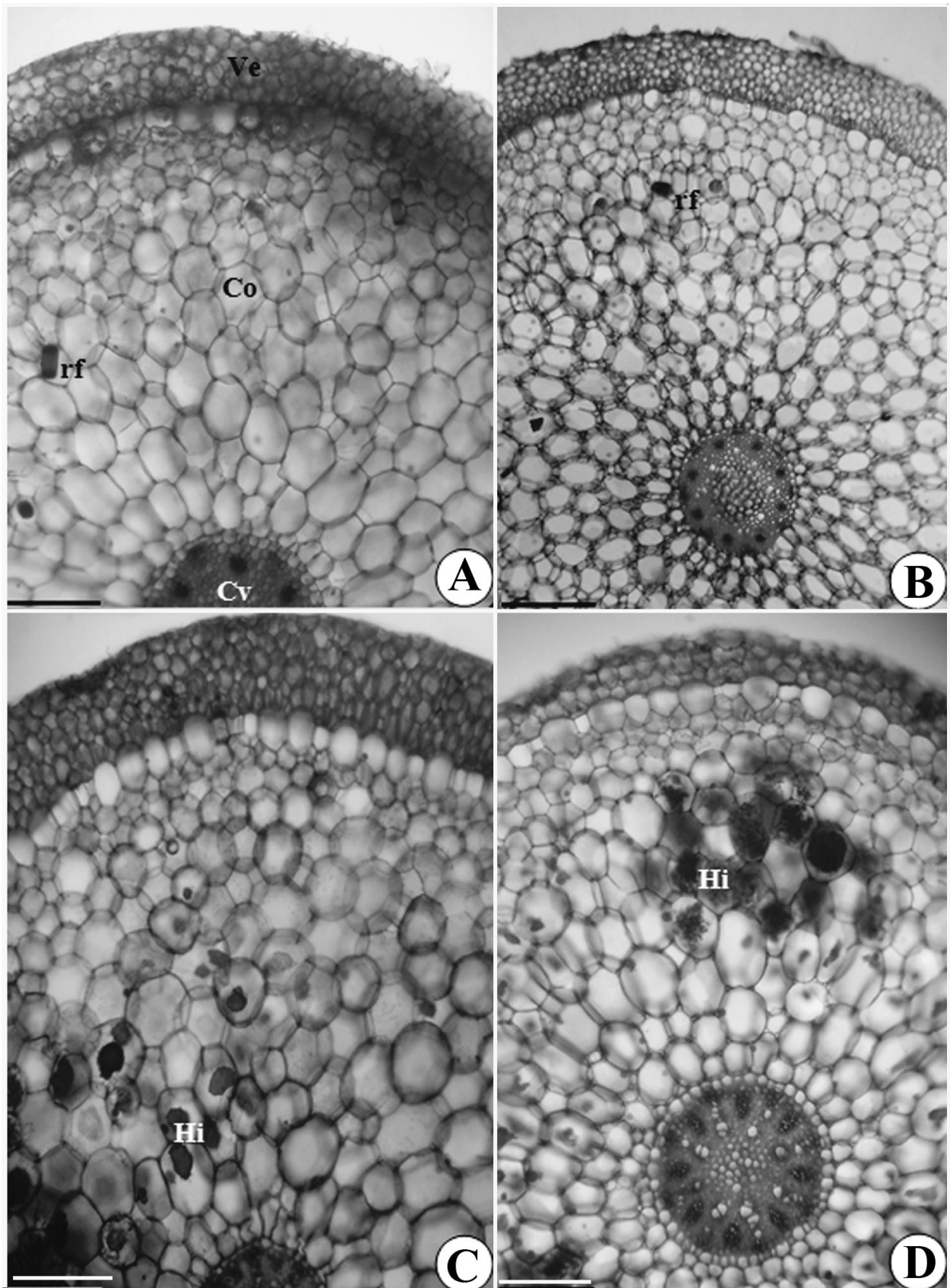
The central cylinder is polyarch (Figs. 1A, D) with the number of protoxylem poles varying according to the species (Tab. 1). In the root center, the cells have with thickened walls in *Hoehneella gehrtiana*, *Huntleya meleagris*, *Promenaea xanthina* (Fig. 2E) and *Warczewiczella wailesiana*.

Stem anatomy

Sympodial growth predominates in the studied species, although in *Zygopetalum pedicellatum* it is monopodial. *Dichaea pendula*, *Dichaea trulla* and *Z. pedicellatum* (Figs. 3A-C) possess an aerial stem and *Koellensteinia tricolor*, *Paradisanthus micranthus*, *Promenaea rollissonii*, *Promenaea xanthina*, *Zygopetalum mackayi* and *Zygopetalum maxillare* a developed rhizome and pseudobulbs (Figs. 4A, B). In *Hoehneella gehrtiana* there is a rhizome and a reduced pseudobulb (Fig. 3D), and in *Huntleya meleagris* and *Warczewiczella wailesiana* (Figs. 4C, D) there are only rhizomes, from which tufts of leaves arise.

Both the pseudobulbs and the aerial stems (Figs. 5, 6) are covered by a single-layered epidermis of thin walled cells, plus a cuticle (Figs. 5A-D, 6A). In the aerial stem of *D. trulla* (Fig. 6B) and in the pseudobulbs of *P. rollissonii*, *P. xanthina*, *Z. mackayi* and *Z. maxillare* (Figs. 5A-D) the cuticle is slightly thickened.

The parenchymatous cortex is narrow in the aerial stems and wide in the pseudobulbs (Figs. 5A-D, 6A, B). In the aerial stem of *D. pendula* there is not a clear morphological distinction between the cortical region and the vascular cylinder (Fig. 6A), but in those of *D. trulla* and *Z. pedicellatum* a ring of sclerified cells occurs in this limit. All pseudobulbs possess large parenchyma cells that store water and small parenchyma cells with amyloplasts (Figs. 5A, C, D).



Figs. 1A-D. Cross-sections of roots showing the general structure. **A.** *Hoehneela gehrtiana*; **B.** *Koellensteinia tricolor*; **C.** *Paradisanthus micranthus*; **D.** *Promenaea xanthina*. ve = velamen, co = cortex, cv = central cylinder, Hi = fungal hyphae, rf = raphids. Bars: **Figs. 1A, C, D** = 50 μ m; **Fig. 1B** = 200 μ m.

Table 1. Anatomical features of *Zygopetalinae* roots analyzed. PC = Papillary cell; RC = Reduced cell; TE = thickening in the external tangential wall; U = thickening U.

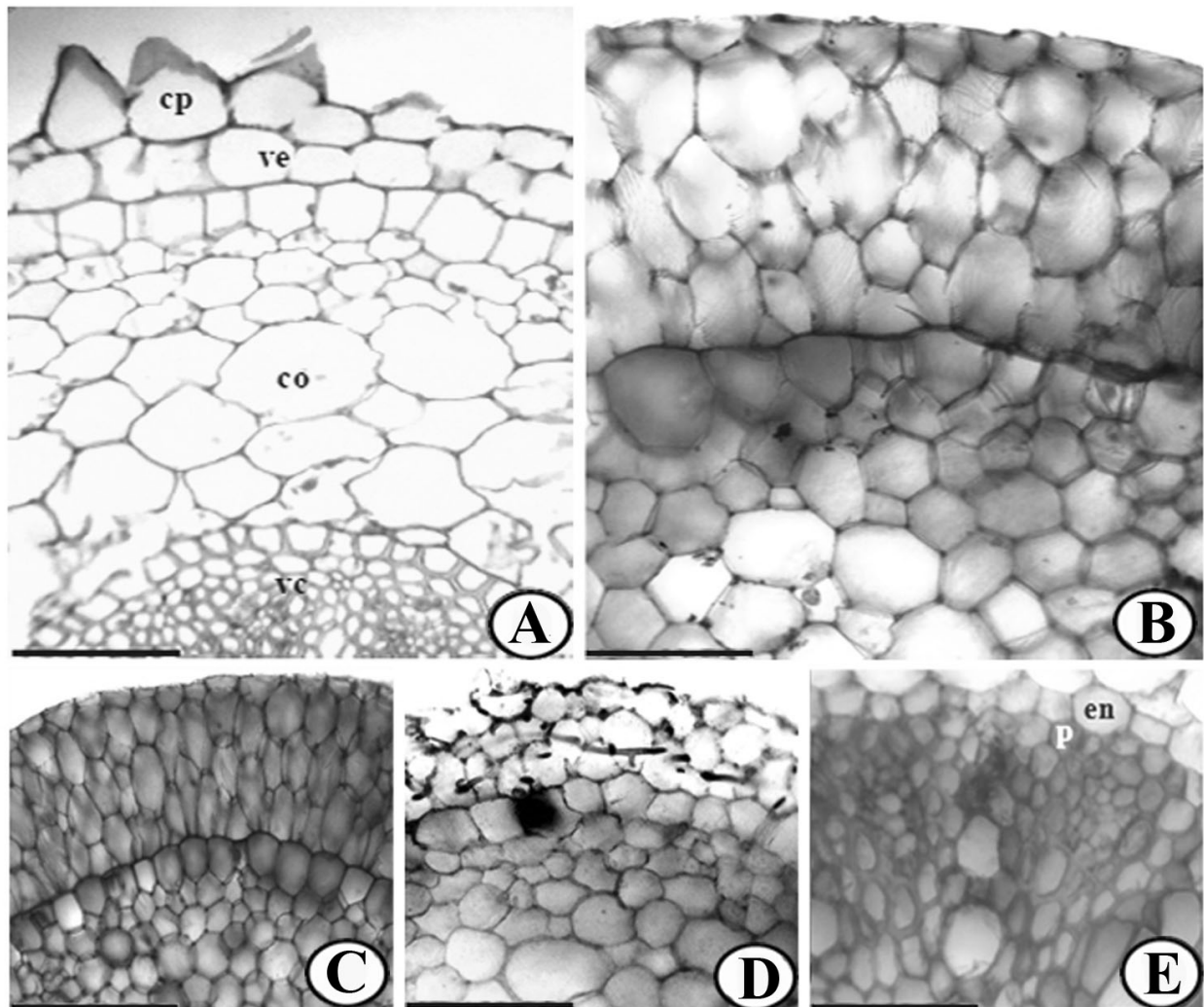
Species	Overall diameter (mm)	Velamen diameter (mm)	Cortex diameter (mm)	Vascular cylinder diameter (mm)	Velamen		Exoderm	Cortex	Endoderm	Protoxylem
					Cell layers number	Epivelamen cells type	Parietal thickening type	Cell layers number	Parietal thickening type	Poles number
<i>Dichaea pendula</i>	0,08	0,015	0,04	0,025	3	PC	TE	6-7	U	8
<i>Dichaea trulla</i>	0,12	0,025	0,08	0,015	3	RC	TE	9-10	U	7
<i>Hoeneella gehrtiana</i>	0,4	0,02	0,37	0,01	5	RC	TE	10-13	U	7
<i>Huntleya meleagris</i>	0,4	0,03	0,35	0,02	4	RC	TE	10-13	U	7
<i>Koellensteinia tricolor</i>	0,5	0,05	0,42	0,03	6	PC	TE	10-13	U	10
<i>Paradisanthus micranthus</i>	0,6	0,05	0,5	0,05	6	RC	TE	12-15	U	12
<i>Promenaea rollisonii</i>	0,18	0,025	0,22	0,02	4	RC	TE	7-9	U	7
<i>Promenaea. xanthina</i>	0,3	0,015	0,18	0,02	3	RC	TE	9-10	U	7
<i>Warczewiczella wailesiana</i>	0,6	0,02	0,4	0,18	4	RC	TE	6-7	U	7
<i>Zygopetalum mackayi</i>	0,4	0,03	0,35	0,02	4	RC	TE	10-13	U	10
<i>Zygopetalum. pedicellatum</i>	0,45	0,02	0,4	0,03	4	RC	TE	10-13	U	10

Table 2. Root traits of *Zygopetalinae* genres analyzed in this study and the Stern *et al.* (2004). PC = Papillary cell; RC = Reduced cell; TE = thickening in the external tangential wall; U = thickening U; n/a = notanalyzed.

Genus	Overall diameter (mm)	Velamen diameter (mm)	Cortex diameter (mm)	Vascular cylinder Diameter (mm)	Velamen		Exoderm	Cortex	Endoderm	Protoxylem
					(Cells layers number)	(Epivelamen type)	(Parietal thickening type)	(Cells layers number)	(Parietal Thickening type)	(Poles number)
This study										
<i>Dichaea</i>	0,1	0,02	0,06	0,02	3	RC/PC	TE	4-9	U	7-8
<i>Hoeneella</i>	0,4	0,02	0,37	0,01	5	RC	TE	10-13	U	7
<i>Huntleya</i>	0,4	0,03	0,35	0,02	4	RC	TE	10 -13	U	7
<i>Koellensteinia</i>	0,5	0,05	0,42	0,03	6	PC	TE	10 -13	U	10
<i>Paradisanthus</i>	0,6	0,05	0,5	0,05	6	RC	TE	12-15	U	12
<i>Promenaea</i>	0,24	0,02	0,2	0,02	3-4	RC	TE	8-9	U	7
<i>Warczewiczella</i>	0,6	0,02	0,4	0,18	4	RC	TE	6-7	U	7
<i>Zygopetalum</i>	0,35	0,03	0,30	0,02	2-4	RC	TE	10-13	U	9-10
Stern <i>et al.</i> (2004)										
<i>Dichaea</i>	n/a	n/a	n/a	n/a	4	n/a	TE	5	U	6
<i>Hoeneella</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
<i>Huntleya</i>	n/a	n/a	n/a	n/a	4	n/a	TE	5	U	11
<i>Koellensteinia</i>	n/a	n/a	n/a	n/a	5	n/a	TE	6	U	11
<i>Paradisanthus</i>	n/a	n/a	n/a	n/a	5	n/a	TE	6	Thin	6
<i>Promenaea</i>	n/a	n/a	n/a	n/a	4	n/a	TE	5	U	9
<i>Warczewiczella</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
<i>Zygopetalum</i>	n/a	n/a	n/a	n/a	4	n/a	TE	5	U	10

The vascular bundles are dispersed in the central region of the aerial stems (Figs. 6A-B) and are scattered in the pseudobulbs (Fig. 5B). The bundles are collateral and are surrounded by sclerified cells, exhibit caps of sclerified cells adjacent to the phloem and xylem or have several

layers of sclerified cells surrounding the phloem (Figs. 5E-G). In the vascular bundles of the pseudobulbs of *H. meleagris*, *Z. mackayi* and *Z. maxillare* there are two layers of sclerified cells between the phloem and the xylem. Stegmata, sclerified cells bearing spherical silica bodies



Figs. 2A-E. Cross-sections of roots showing velamen, exodermis and endodermis. **A.** *Dichaeta trulla*.; **B.** *Huntleya meleagris*; **C.** *Zygopetalum mackayi*; **D.** *Dichaeta pendula*; **E.** *Promenaea xanthina*. cp = papillary cell, co = cortex, vc = vascular cylinder, en = endoderm, p = periciclo, ve = velamen. Bars: **Figs. A; D** = 50 μ m; **Figs. B; C; E** = 100 μ m.

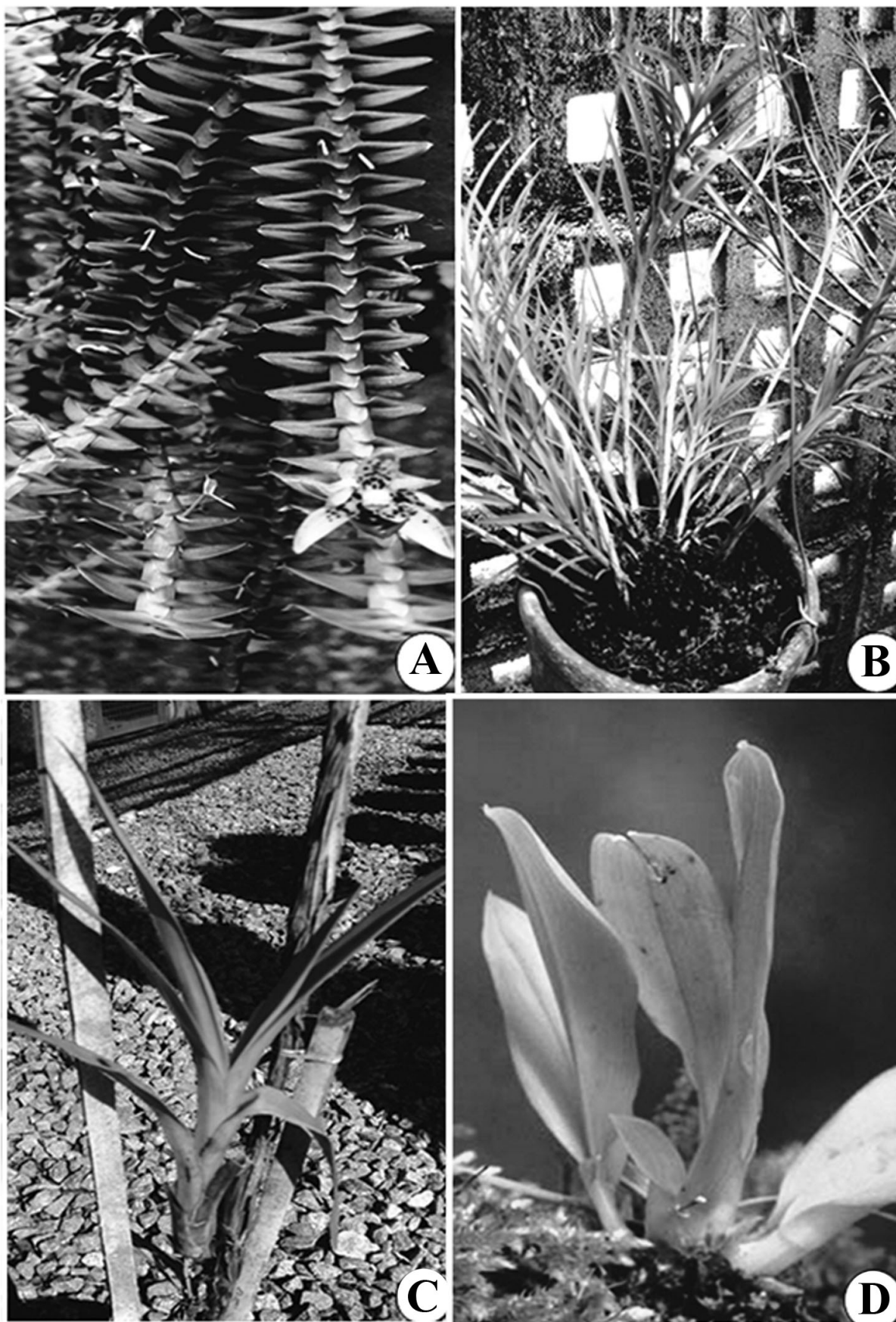
(Fig. 5E), are commonly found in the periphery of the pseudobulb bundles (Tabs. 3, 4). Flavonoidic crystals occur in several pseudobulbs and in the aerial stem of *D. trulla* (Tab. 3) that also possesses starch grains (Tabs. 3, 4). Crystals of oxalate of calcium occur near the periphery of all pseudobulbs (Tab. 3).

The rhizomes are covered by a one-layered epidermis and a cuticle. The cortex, poor developed in relation to the central cylinder, is formed by small isodiametric cells (Figs. 6C-F). Raphides of oxalate of calcium are common in this region in all analyzed plants (Tab. 3). The central cylinder is composed by many vascular bundles, which possess several layers of sclerified cells adjacent to the phloem (Figs. 6C-F). Although the vascular bundles are clustered in the central region of the rhizomes, there is not a clear boundary between this region and the cortex, except for *Z. maxillare* that has 1-3 pericyclics cell layers of sclerified walls in this position (Fig. 6G). As observed for the pseudobulbs and the aerial stems, stegmata associated

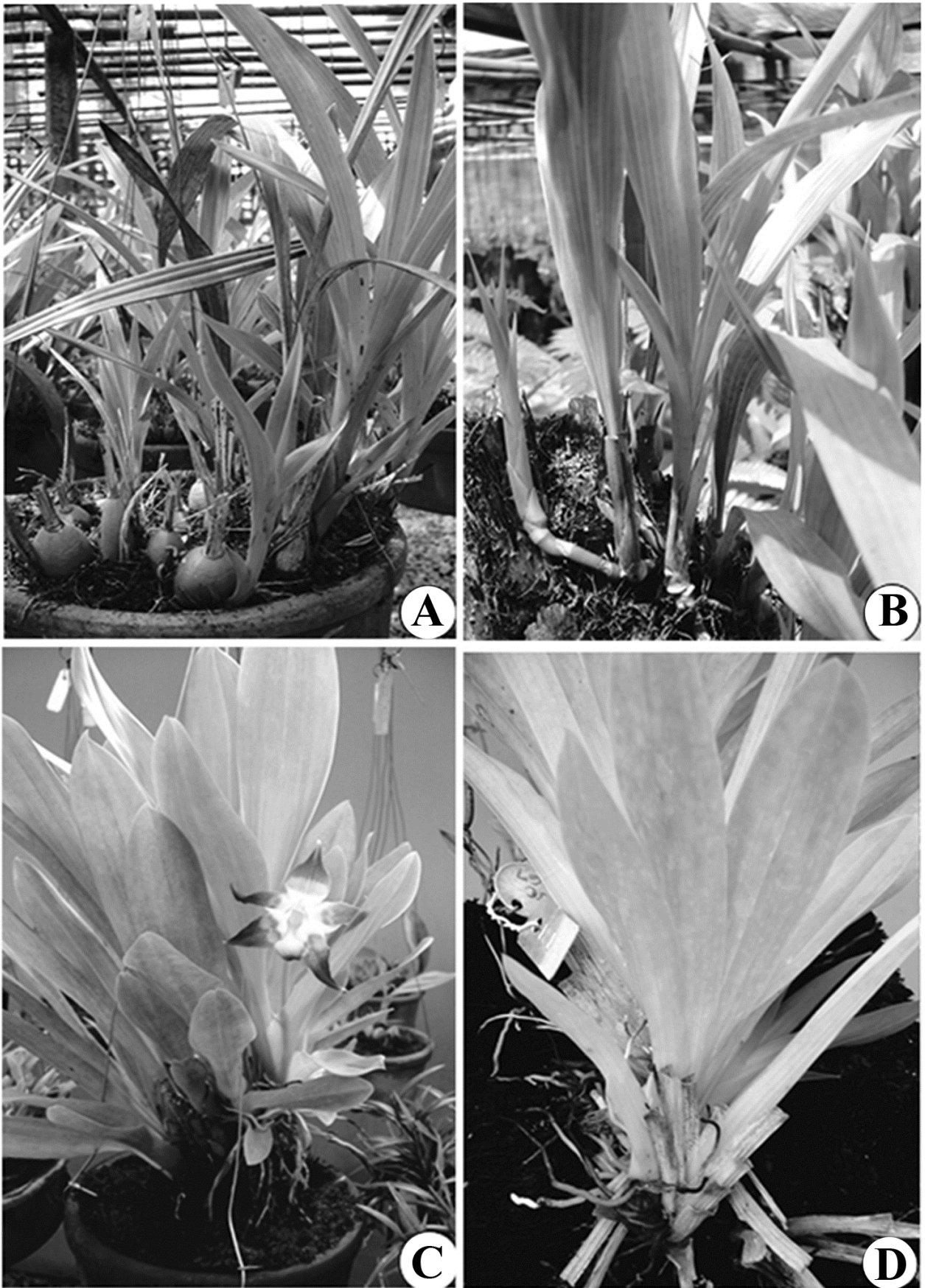
with vascular bundles are frequent in the rhizomes (Tab. 3). Crystals of flavonoids occur in the parenchymatous cells of most rhizomes (Tab. 3), and hyphae of fungus are frequent in the cortical region of *P. xanthina* and *P. rollissonii*.

Leaf anatomy

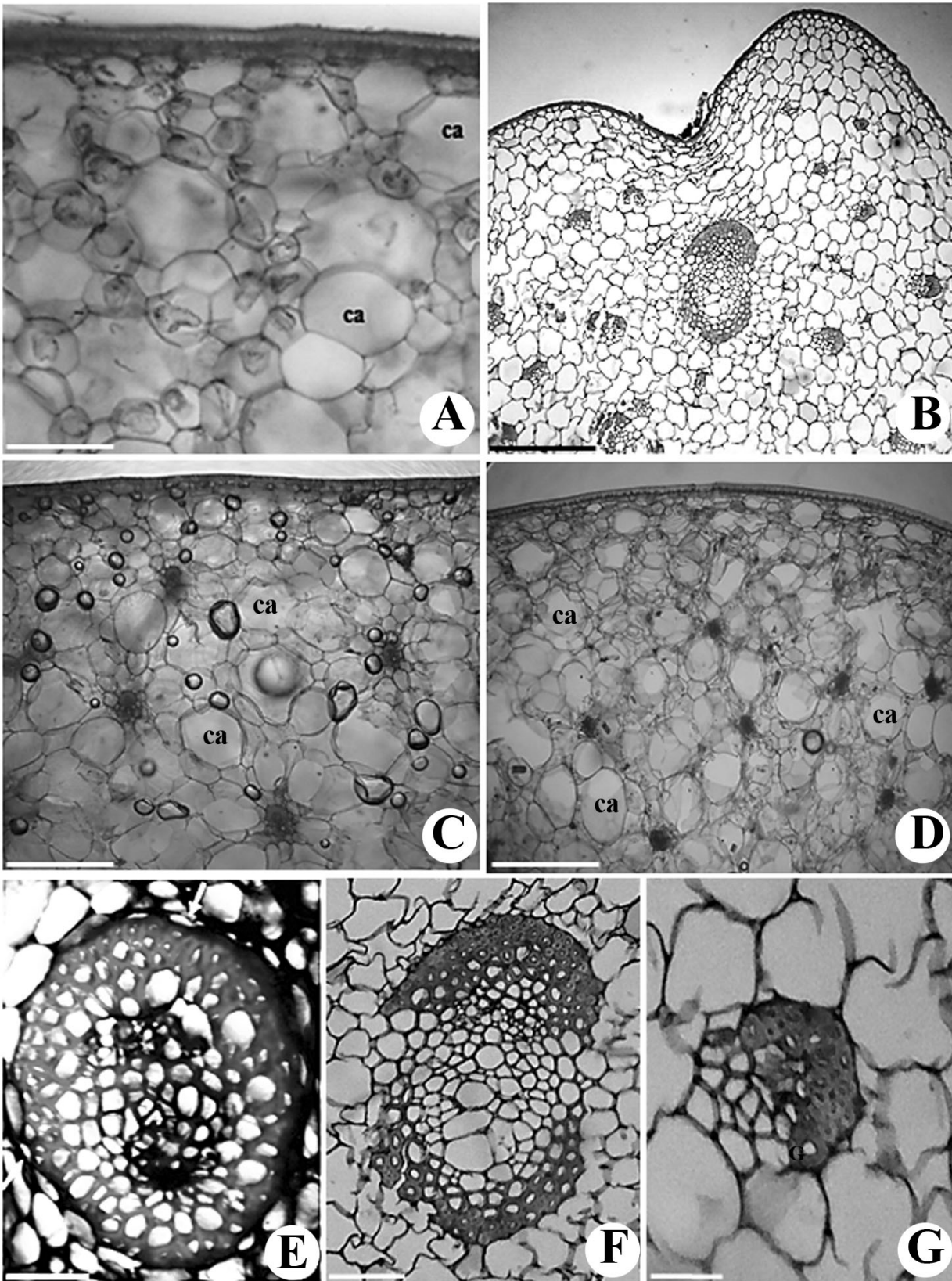
All the leaf mesophylls are narrow (Tab. 5) and irrigated by large and small vascular bundles (Figs. 7A-H). In *Dichaeta pendula* and *Hoehneella gehrtiana* all the bundles are the same size. The leaves are covered by a continuous cuticle (Figs. 7B -C), with a thickness that varies according to the species (Tab. 5). The epidermal cells, of similar size on both faces, are rounded, rectangular, elliptical or polygonal in cross section. In *Dichaeta trulla* the epidermal cells have a papillose appearance (Figs. 7B-C). All leaves are hypostomatic with the stomata situated on the same level as the other epidermal cells, or slightly projected, as in *D. trulla* (Fig. 7C). The stomata cells have thickened walls in the region between the guard cells, giving



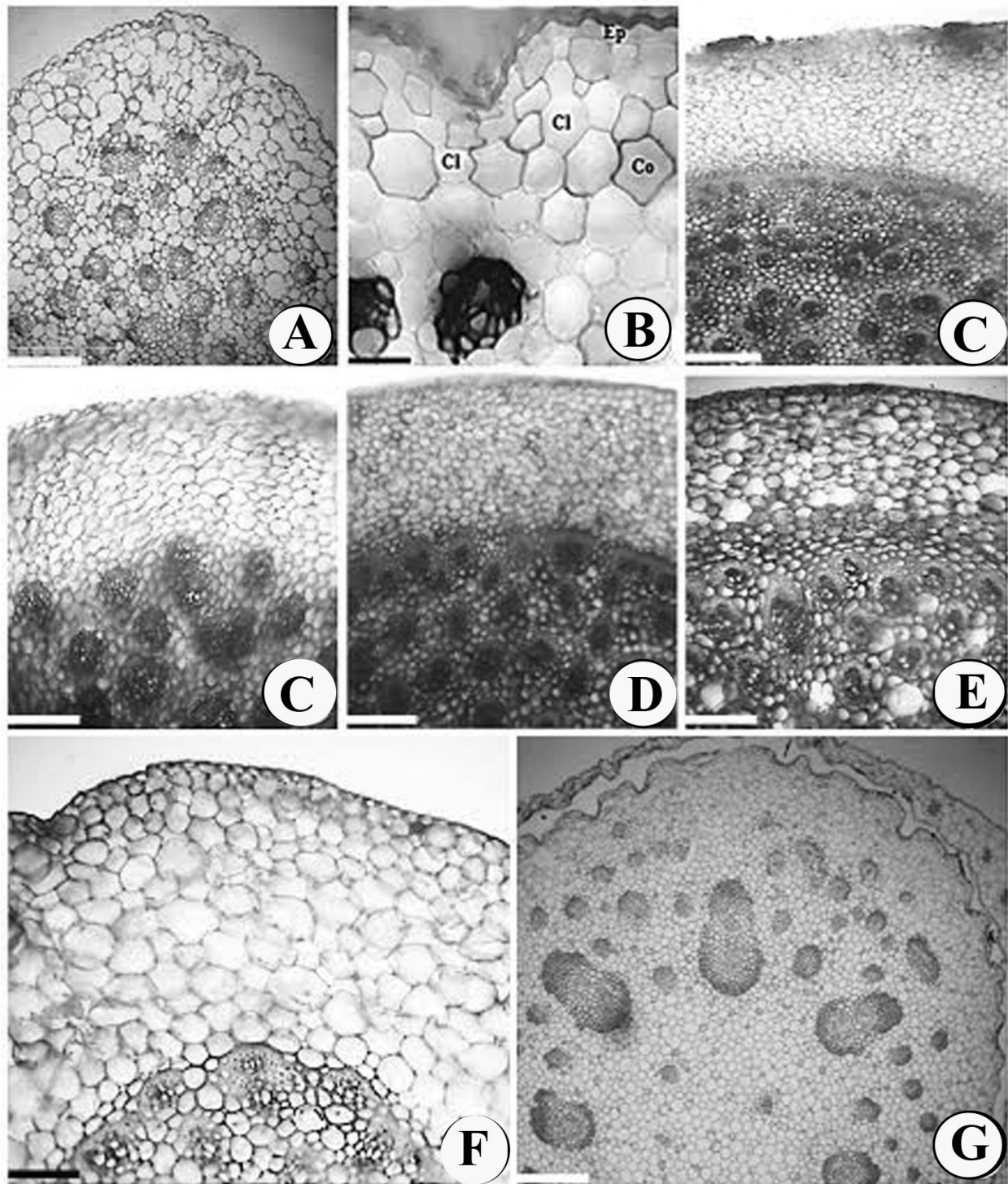
Figs. 3A-D. Growth habits. A. *Dichaea pendula*; B. *Dichaea trulla*; C. *Zygopetalum pedicelatum*; D. *Hoehneella gehrtiana*.



Figs. 4A-D. Growth habits. A. *Zygopetalum mackayi*; B. *Zygopetalum maxillare*; C. *Huntleya meleagris*; D. *Warczewiczella walesiana*.



Figs. 5A-G. General cross-sections of pseudobulbs and disclosure of vascular bundles. **A.** *Promenaea rollisonii*; **B.** *Promenaea xanthine*; **C.** *Zygopetalum mackayi*; **D.** *Zygopetalum maxillare*; **E.** *Huntleya meleagris*; **F.** *Promenaea xanthine*; **G.** *Paradisanthus micranthus*. ca = water storage cells, arrow = stegmata. Bars: **Figs. A-G** = 100 μ m.



Figs 6A-G. Cross sections of aerial stems and rhizomes. **A.** *Dichaea pendula*; **B.** *Dichaea trulla*; **C.** *Huntleya meleagris*; **D.** *Hoehneella gehrtiana*; **E.** *Promenaea rollissoniis*; **F.** *Zygopetalum mackayi*; **G.** *Zygopetalum maxillare*. cl = claviform cells, co = cortex, p = epidermis. Bars: **Figs. A, B:** = 50 μ m; **Figs. C-H** = 100 μ m.

a triangular appearance to the cell lumen. There are cuticular projections above the stomata opening forming a supra-stomatal chamber. In some representatives (Tab. 5) there are 1-2 layers of large achlorophyllous subepidermal cells adaxially positioned forming a hypodermis (Figs. 7B, 8A). The mesophyll is not differentiated into palisade and spongy tissue and its cells are rounded or elliptical (Figs. 7I, 8A-F).

The vascular bundles are collateral and possess a cap of sclerified cells adjacent to the xylem and the phloem (Figs. 7A-B, D-H); the larger bundles may be surrounded by sclerified cells. The midrib possesses a single collateral bundle that, in most of species, is abaxially displaced and surrounded by sclerified cells (Figs. 7I, 8A-C). The midrib bundle occupies almost the entire leaf thickness in *Koellensteinia*

Table 3. Features of analyzed stems in *Zygopetalinae* species. Epidermis: - = no evident thickening; + = thickened. Perivascular fibers: + = around the vascular bundle; Ph = adjacent to the phloem. Crystals: Fl = flavonoidics; Co = Calcium oxalate.

Genus	<i>Epidermis</i>	<i>Cortex</i>	Limitation	Perivascular fibers	Starch grain	Crystals	Stegmatas
	Thickening	Water storage cells					
Aerial stems							
<i>Dichaea pendula</i>	-	-	-	+	+	Co	-
<i>Dichaea trulla</i>	+	+	+	+	+	Co, Fl	-
<i>Zygopetalum pedicellatum</i>	+	+	+	+	+	Co	-
Pseudobulbs							
<i>Hoeneella gehrtiana</i>	-	+	-	+	+	Co	+
<i>Huntleya meleagris</i>	-	+	-	+	+	-	+
<i>Koellensteinia tricolor</i>	-	+	-	-	+	Co	-
<i>Paradisanthus micranthus</i>	-	+	-	Ph	+	Co	-
<i>Promenaea rollisonii</i>	+	+	-	-	+	Co, Fl	+
<i>Promenaea xanthina</i>	+	+	-	-	+	Co, Fl	+
<i>Zygopetalum mackayi</i>	+	+	+	+	+	Co	+
<i>Zygopetalum maxillare</i>	+	+	+	+	+	Co, Fl	+
Rhizome							
<i>Hoeneella gehrtiana</i>	-	-	-	+	+	Co	+
<i>Huntleya meleagris</i>	-	-	+	+	+	Co, Fl	+
<i>Koellensteinia tricolor</i>	-	-	-	+	+	Co, Fl	+
<i>Paradisanthus micranthus</i>	-	-	-	+	+	Co, Fl	+
<i>Promenaea rollisonii</i>	-	-	-	+	+	Co	+
<i>Promenaea xanthina</i>	-	-	-	+	+	Co, Fl	+
<i>Warczewiczella wailesiana</i>	-	-	-	+	+	Co, Fl	+
<i>Zygopetalum mackayi</i>	-	-	-	+	+	Co	+
<i>Zygopetalum maxillare</i>	-	-	-	+	+	Co	+

tricolor, *Paradisanthus micranthus*, *Zygopetalum mackayi*, *Zygopetalum maxillare* and *Zygopetalum pedicellatum* (Figs. 8D-F). Stegmata were observed in all leaves. Groups of fibers not associated with the vascular bundles, lying close to the abaxial surface, occur in the mesophyll of *Huntleya meleagris* and *K. tricolor* (Fig. 7H). Hyphae of fungus were observed close to the adaxial surface in several species (Tab. 5). In the mesophyll of *D. trulla*, *H. gehrtiana* and *K. tricolor* there are idioblasts with raphides and grains of starch are frequent in the mesophyll of *Dichaea pendula*, *Hoehneella gehrtian*, *Warczewiczella wailesiana* and *Zygopetalum mackayi* (Tab. 5).

DISCUSSION

Roots

As in other monocotyledons (Dahlgren & Clifford 1982), a multilayered velamen of thin walled cells covers all the studied roots. The velamen is a specialized tissue that protects the root against heating and water loss (Pridgeon 1987) and that can also avoid the excessive water storage inside the organ (Gonzaga & Gonzaga 1996). The occurrence of a velamen is associated with the epiphytic habit (Engard 1944, Dycus & Knudson 1957), although this tissue can also occur in terrestrial orchids (Porembski & Barthlott 1988, Stern *et al.* 1993, Kurzweil

et al. 1995) and be absent in some representatives of the family (Singh 1986). Velamen with two, three and four layers as observed here (Tab.I) was also reported for the roots of most *Zygopetalinae* studied by Stern *et al.* (2004).

The velamen comprises an endovelamen and an epivelamen (Sanford & Adanlawo 1973) that have the same origin as the intermediate layers and are formed by cells with no protoplast at maturity (Shushan 1974, Pridgeon 1987). In the roots of *Hoehneella gerhtiana*, *Huntleya meleagris*, *Paradisanthus micranthus*, *Promenaea rollisonii*, *Warczewiczella wailesiana* and *Zygopetalum mackayi*, the epivelamen cells are smaller than those of the endovelamen while in *Dichaea pendula* and *Koellensteinia tricolor* the epivelamen cells are papillose, a feature that possibly improves water absorption (Sanford & Adanlawo 1973, Pridgeon 1987). The endovelamen cells are isodiametric and possess banded or striped thickening in the walls, depending on the species, as in others *Zygopetalinae* (Stern *et al.* 2004). According to Benzing *et al.* (1983), the velamen cell walls are cellulosic with lignin and suberin impregnation that provides mechanical support and prevents cell collapsing during dry periods (Claus 1954). In most of roots studied here, the velamen cell walls are impregnated with suberin and lignin. However, as described for *Dichaea bryophila* (Oliveira & Sajo 1999a) the lignin is absent in the root velamen of this genus (*D. pendula* and *D. trulla*).

Table 4. Stem traits of *Zygopetalinae* genres analyzed in this study and the Stern et al. (2004). Epidermis: - = no evident thickening; + = thickened. Perivascular fibres: + = around the vascular bundle; Ph = adjacent to the phloem; n/a = not analyzed. 1-3 = Occurrence in *Dichaea trulla*; 4 = Stegmata not occur in *Promenaea xanthina*.

Genus	Epidermis	Cortex	Limitation	Perivascular fibres	Starch grain	Stegmatas
	Thickening	Water storage cells				
This study						
Aerial stems						
<i>Dichaea</i>	- / + ¹	- / + ²	- / + ³	+	+	-
<i>Zygopetalum</i>	+	+	+	+	+	-
Pseudobulbs						
<i>Hoeneella</i>	-	+	-	+	+	+
<i>Huntleya</i>	-	+	-	+	+	+
<i>Koellensteinia</i>	-	+	-	-	+	-
<i>Paradisanthus</i>	-	+	-	Ph	+	-
<i>Promenaea</i>	+	+	-	-	+	+
<i>Zygopetalum</i>	+	+	+	+	+	+
Rhizome						
<i>Hoeneella</i>	-	-	-	+	+	+
<i>Huntleya</i>	-	-	+	+	+	+
<i>Koellensteinia</i>	-	-	-	+	+	+
<i>Paradisanthus</i>	-	-	-	+	+	+
<i>Promenaea</i>	-	-	-	+	+	+
<i>Warczewiczella</i>	-	-	-	+	+	+
<i>Zygopetalum</i>	-	-	-	+	+	+
Stern et al. (2004)						
Aerial stems						
<i>Dichaea</i>	-	-	-	+	+	-
<i>Zygopetalum</i>	+	+	+	+	+	-
Pseudobulbs						
<i>Hoeneella</i>	n/a	n/a	n/a	n/a	n/a	n/a
<i>Huntleya</i>	-	+	-	+	+	-
<i>Koellensteinia</i>	-	+	-	-	+	-
<i>Paradisanthus</i>	-	+	-	Ph	+	-
<i>Promenaea</i>	+	+	-	-	+	- ⁴ / +
<i>Zygopetalum</i>	+	+	+	+	+	+
Rhizome						
<i>Hoeneella</i>	n/a	n/a	n/a	n/a	n/a	n/a
<i>Huntleya</i>	-	-	+	+	+	+
<i>Koellensteinia</i>	-	-	-	+	+	+
<i>Paradisanthus</i>	-	-	-	+	+	+
<i>Promenaea</i>	-	-	-	+	+	+
<i>Warczewiczella</i>	n/a	n/a	n/a	n/a	n/a	n/a
<i>Zygopetalum</i>	-	-	-	+	+	+

The outer layer of the cortex root, the exodermis, is formed by cells of tangentially thickened walls, as described for *Dichaea bryophila* (Oliveira & Sajo 1999a). According to Haberlandt (1914), the velamen-exodermis set works as a system with the long suberized/lignified cells of the exodermis protecting the root cortex against desiccation and the thin-walled cells leading nutritive substances from the velamen to the cortical tissue. Specialized exodermis cells, named tillossomas or coverage cells that assist in the water condensation, frequently occur in the orchids roots (Pridgeon 1987). However, they seem to be absent in

Zygopetalinae as we did not find them in the species studied here, nor did Stern *et al.* (2004) in those they investigated.

Internally to the exodermis, the root parenchyma has many intercellular spaces and idioblasts containing raphides are common in this region, as in other *Zygopetalinae* (Stern *et al.* 2004). Raphides are common in different organs of the *Orchidaceae* (Metcalf 1963) and occur in many other monocots (Prychid & Rudall 1999). The inner layer of the root cortex, the endodermis, is one-layered and formed by isodiametric cells with U thickened walls, as described for other *Zygopetalinae* (Stern *et al.* 2004).

Table 5. Environment, habit and anatomical features in analyzed *Zygopetalinae*. + = presence, - = absent, ct = cuticle thickness (μm), ae = abaxial stomata with protected pore, cs = group of cells sclerified, wsc = water storage cells, hyp = hypodermis, steg = sstegmata.

Genus	Water storage features				Resistance to desiccation features				Special features		
	Mesophyll		wsc	ct (μm)	ae	cs	Steg	Fibers	Mesophyll		
	Epidermis	Number of layers							Hyp	Hyphae	Starch
<i>Dichaea pendula</i>	-	5	+	+	3.6	+	-	+	+	-	+
<i>Dichaea trulla</i>	-	5	+	+	7.12	+	-	+	+	+	-
<i>Hoeneella gehrtiana</i>	-	8	+	+	3.6	+	-	+	+	-	+
<i>Huntleya meleagris</i>	-	10	+	+	3.6	+	-	+	+	-	-
<i>Koellensteinia tricolor</i>	-	5	-	+	3.6	+	-	+	+	+	-
<i>Paradisanthus micranthus</i>	-	8	-	+	3.6	+	+	+	+	-	-
<i>Promenaea rollissonii</i>	-	5	-	+	3.6	+	+	+	+	-	-
<i>Promenaea xanthina</i>	-	5	+	+	3.6	+	+	+	+	+	-
<i>Warczewiczella wailesiana</i>	-	12	-	+	7.12	+	-	+	+	-	+
<i>Zygopetalum mackayi</i>	-	5	-	+	3.6	+	-	+	+	+	+
<i>Zygopetalum maxillare</i>	-	7	-	+	7.12	+	-	+	+	+	-
<i>Zygopetalum pedicellatum</i>	-	7	-	+	3.6	+	+	+	+	-	-

Fungal hyphae were found in the root velamen of *Dichaea trulla* and *Promenaea rollissonii* and in the root cortex of *Paradisanthus micranthus*, *Promenaea xanthina*, *Zygopetalum mackayi* and *Zygopetalum maxillare*. Orchid roots frequently possess endomicorrhizic hyphae that are usually established during seed germination and support development by assisting in the absorption of nutrients (Arditti 1967). Many orchids maintain this association throughout their lives while others become independent as adults (Arditti 1967, Sanford 1974). According to Sanford (1974), orchids depending on this association are partially saprophytes since the organic compounds are provided by the fungi either by diffusion or active transport.

Orchid roots have been classified into 12 types according to the presence or absence of an epivelamen, to the number of the velamen layers, to the shape of the cell wall thickening in the velamen and exodermis and to the organization of the cortex (Porembski & Barthlott 1988). All the *Zygopetalinae* studied here correspond to the *Cymbidium* type as they possess an epivelamen, an exodermis of outer thickened cell walls and a cortex of up eight layers. This root uniformity coincides with that described by Stern *et al.* (2004) for other *Zygopetalinae*.

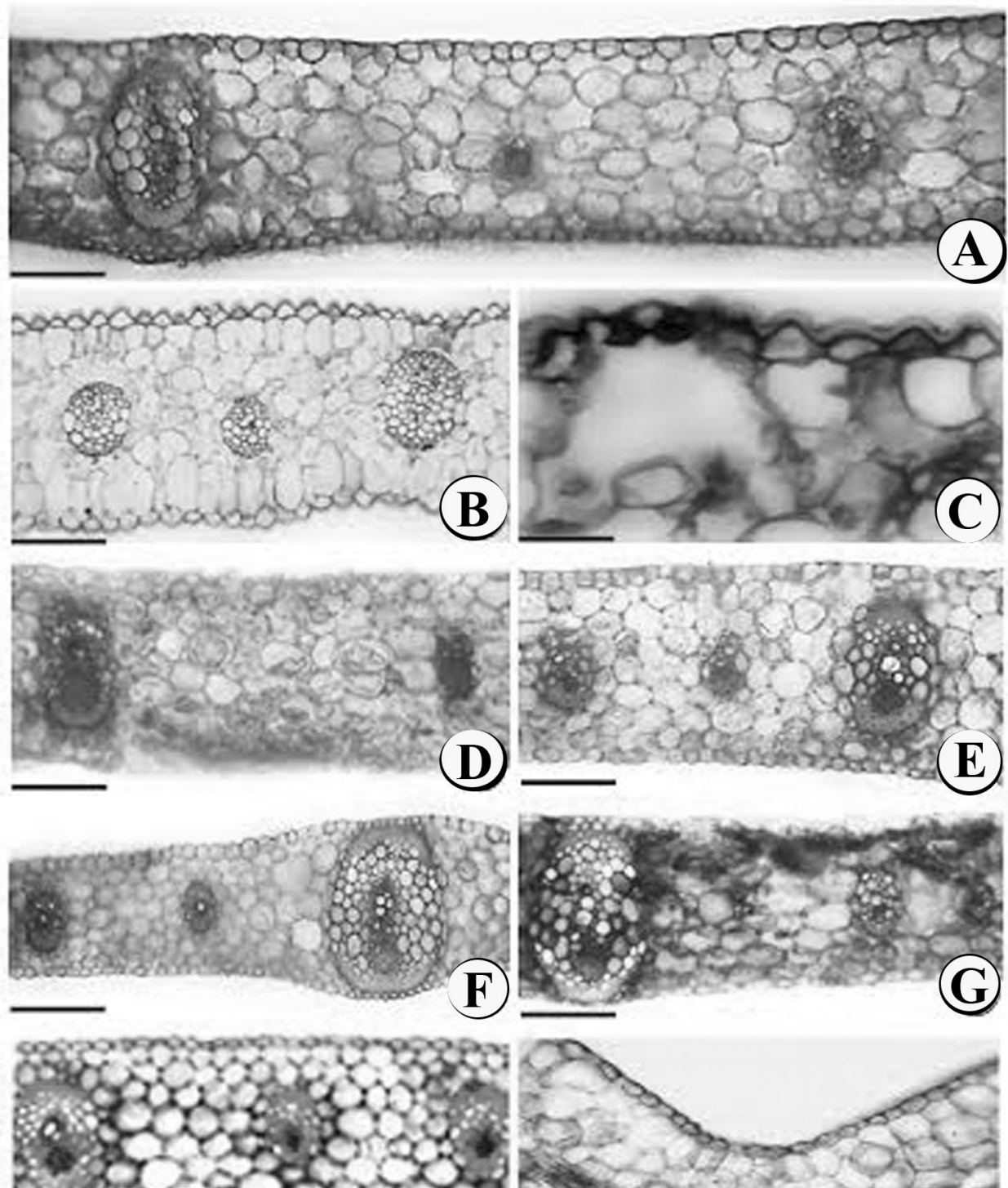
Xylem and phloem are radially arranged. The xylem is polyarch and the root with the highest number of protoxylem poles (*Paradisanthus micranthus*, with 12) is of a larger diameter, while that with few poles (*Dichaea pendula*, with eight) has a reduced diameter (Tab. 1). Rütter & Stern (1992) and Rosso (1966) described a close relationship between root diameter and the number of protoxylem poles, since this number varies in different roots of the same species, and in the same root, at different levels. The medulla is formed by thick-walled parenchyma cells, as in other *Zygopetalinae* (Stern *et al.* 2004).

Aerial stem, rhizome and pseudobulb

In many monocots, including the species studied here, growth originates in the rhizome which periodically gives rise to new stem branches and /or roots and pseudobulbs (enlarged stem segments where the leaves emerge). Rhizomes are thought to be restricted to plants with sympodial growth that can cover a large area of the substrate spreading indefinitely to achieve new sources of water and nutrients (Holtum 1955). All species studied here are of sympodial growth: *Dichaea pendula* and *Dichaea trulla* possess rhizomes and aerial stems and in *Koellensteinia tricolor*, *Paradisanthus micranthus*, *Promenaea rollissonii*, *Promenaea xanthina*, *Zygopetalum mackayi*, *Hoeneella gehrtiana* and *Zygopetalum maxillare* there are rhizomes and pseudobulbs. *Warczewiczella wailesiana* e *Huntleya meleagris* possess rhizomes from which spaced tufts of leaves arise.

Usually, the rhizomes and aerial stems are anatomically very similar being covered by a one-layered epidermis and possessing a parenchymatous cortex and many vascular bundles distributed in more than one ring in the central cylinder. This arrangement is common in the rhizomes of epiphytic *Orchidaceae* (Withner *et al.* 1974), although in the terrestrial ones (*Koellensteinia tricolor*, *Paradisanthus micranthus* and *Zygopetalum mackayi*) the old part of the rhizomes epidermis is destroyed by friction with the substrate.

In both in the rhizomes and aerial stems there is not a clear morphological distinction between the cortex and the central cylinder, except for the aerial stem of *Dichaea trulla* and the rhizome of *Zygopetalum maxillare*, that have a pericyclic ring of sclerified cells in this region, as reported for some *Pleurothallidinae* (Pridgeon & Williams 1979, Pridgeon 1982, Scatena & Nunes 1996) and for representatives of the subfamily *Cypripedioideae* (Rosso 1966). In general, at the boundary zone there is a higher concentration of vascular bundles without the presence of a differentiated endodermis.

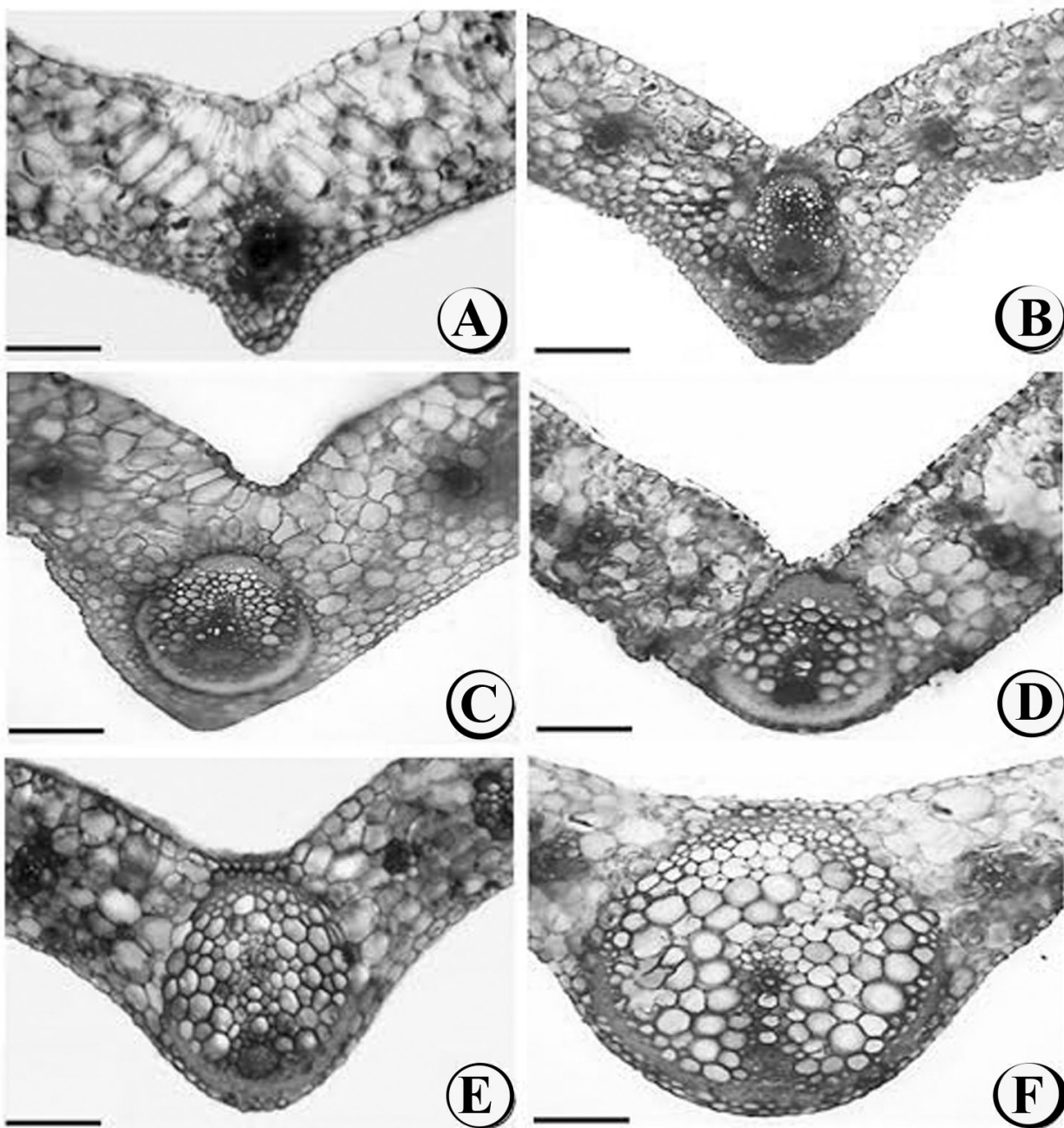


Figs 7A-G. Leaf cross-sections. **A.** *Huntleya meleagris*; **B.** *Dichaea trulla*; **C.** estomata of *Dichaea trulla* in superior position; **D.** *Promenaea rollisonii*; **E.** *Paradisanthus micranthus*; **F.** *Zygopetalum maxillarie*; **G.** *Zygopetalum pedicellatum*; **H.** *Koellensteinia tricolor*; **I.** *Warczewiczella wailesiana*. Bars: **Figs. A, B; D-I** = 100 μ m; **Fig. C** = 50 μ m.

As in others *Zygopetalinae* (Stern *et al.* 2004), raphides are common in the cortex of the aerial stems and rhizomes. Flavonoidic crystals were found in the rhizomes of *D. trulla*, *Huntleya meleagris*, *K. tricolor*, *P. micranthus*, *Promenaea xanthina* and *Warczewiczella wailesiana* (Tab. 3). Starch grains are common in the rhizomes and aerial stems,

as reported for other *Orchidaceae* (Rosso 1966, Scatena & Nunes 1996).

All pseudobulbs are similar in organization, with a one-layered epidermis devoid of trichomes and stomata and covered by a thickened cuticle. The epidermal cell walls are, in general, straight and narrow, except for *Zygopetalum*



Figs. 8A-F. Leaf cross-sections showing central vascular bundles. **A.** *Promenaea rollisonii*; **B.** *Promenaea xanthine*; **C.** *Huntleya meleagris*; **D.** *Zygopetalum mackayi*; **E.** *Zygopetalum pedicellatum*; **F.** *Zygopetalum maxillarie*. Bars: **Figs. 8A-F** = 100 μ m.

mackayi with waved thickened walls, as observed for *Eria braccata* from the Epidendroideae subfamily (Khasim Mohana & Rao 1990). Internally there is a parenchymatous tissue with scattered vascular bundles protected by layers of sclerified cells adjacent to the phloem (smaller bundles) and to the xylem and phloem (larger bundles). Like in the aerial stems and rhizomes, there is not a defined limit between the cortex and the central cylinder and in all pseudobulbs there is a higher concentration of smaller bundles near the organ surface (Fig. 5). This arrangement coincides with

that described for the pseudobulbs of other *Zygopetalinae* (Stern *et al.* 2004).

The parenchymatous region possesses large and small cells which commonly contain starch, confirming the storage function of the organs (Haberlandt 1914, Pridgeon 1986, Stern & Morris 1992). The large cells may or may not possess secondary thickened walls and are like the water storage cells found in leaves and pseudobulbs of the African epiphytic orchids. These cells are named tracheoid elements (Olatunji & Nengim 1980). Pridgeon (1982)

Table 6. Environment, habit and anatomical traits of *Zygopetalinae* genres analyzed in this study and the Stern et al. (2004). + = presence; - = absent, ct = cuticle thickness (μm); ae = abaxial stomata with protected pore; cs = group of cells sclerified; wsc = water storage cells; hyp = hypodermis; steg = sstegmata; n/a = not analyzed. 1 = absent in *Promenaea rollisonii*; 2 = presence only in *Zygopetalum pedicellatum*; 3 = absent in *Promenaea xanthina*; ? = there are no references as to character analysis.

Genus	Water storage features				Resistance to desiccation features				
	Mesophyll				Epidermis		Mesophyll		Fibers
	Epidermis	Number of layers	Hyp	wsc	ct (μm)	ae	cs	steg	Pericyclics
<u>This study</u>									
<i>Dichaea</i>	-	5	+	+	7-12	+	-	+	+
<i>Hoeneella</i>	-	8	+	+	3-6	+	-	+	+
<i>Huntleya</i>	-	10	+	+	3-6	+	-	+	+
<i>Koellensteinia</i>	-	5	-	+	3-6	+	-	+	+
<i>Paradisanthus</i>	-	8	-	+	3-6	+	+	+	+
<i>Promenaea</i>	-	5	+/- ¹	+	3-6	+	+	+	+
<i>Warczewiczella</i>	-	12	-	+	7-12	+	-	+	+
<i>Zygopetalum</i>	-	6	-	+	7-12	+	+ ² /-	+	+
<u>Stern et al. (2004)</u>									
<i>Dichaea</i>	-	5	+	+	7-7,5	+	-	+	+
<i>Hoeneella</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
<i>Huntleya</i>	-	10	+	+	7-7,5	+	-	+	+
<i>Koellensteinia</i>	-	5	+	+	5-6	+	-	+	+
<i>Paradisanthus</i>	-	8	+	?	3-5	+	-	+	+
<i>Promenaea</i>	-	6	+	+	3-5	+	-	+ / - ³	+
<i>Warczewiczella</i>	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
<i>Zygopetalum</i>	-	6	+	+	5-7,5	+	-	+	+

refers to these cells as “idioblasts with spiral thickening” and describes its presence in the leaf hypodermis and mesophyll of certain *Pleurothallidinae*.

Comparing the pseudobulbs with the rhizomes, the first possess a smaller amount of collateral vascular bundles. Withner *et al.* (1974) observed that the rhizomes contain about four times more vascular bundles than the pseudobulbs and interpreted this feature as a reason for the woody appearance of rhizomes, compared to the relative softness of the pseudobulbs.

Leaves

Like most of *Zygopetalinae* studied by Stern *et al.* (2004), the leaves are hypostomatic and possess a mesophyll of a few cell layers not differentiated into palisade and spongy parenchyma (Figs. 7-8). The epidermis is one-layered and is covered by a cuticle of variable thickness (3-6 to 7-12 micrometers). The cuticle thickness was used as a taxonomic character by Rosso (1966) who recognized within the *Cypripedioideae* (Orchidaceae) genera with plicate leaves and thin cuticle and genera with conduplicated leaves and thick cuticle. However, the cuticle thickness seems to be determined by the degree of sun exposure, as leaves exposed to intense solar radiation tend to display a thicker cuticle on both sides (Withner *et al.* 1974). In the case of the species studied here there seems not to be a direct relationship between cuticle thickness and the environment

occupied by plants. For example, in the two species of *Promenaea*, which are typical of shady places and grow as epiphytes on lower stems, the leaves are covered by a cuticle of the same thickness as in *Koellensteinia tricolor*, a species characteristic of open areas such as rock fields. Likewise, the leaves of *Zygopetalum mackayi*, which lives in open countryside possess cuticle of similar thickness to that of *Zygopetalum maxillare*, which grows primarily in shaded environments inside forests.

In all leaves, the stomata occupy the same level of the epidermal cells or are slightly projected. The stomatal guard cells are thickened walled and possess a small cuticular projections forming a supra-stomatal chamber, as in other *Zygopetalinae* (Stern *et al.* 2004). Supra-stomatal chambers, that keep a small compartment of moist air reducing the leaf transpiration, are common in epiphytic orchids living under high temperatures and low water availability (Bonates 1993, Rasmussen 1987), as many the species studied here.

Although not reported for any *Zygopetalinae* investigated by Stern *et al.* (2004), we observed in some representatives the occurrence of an adaxial hypodermis of large cells without chlorophyll, as described for other *Orchidaceae* (Ayensu & Williams 1972, Mohana Rao & Khasim 1987, Pridgeon & Williams 1979, Pridgeon & Stern 1982, Khasim & Mohana Rao 1990), including some *Zygopetalinae* (Oliveira & Sajo 1999b). This layer, interpreted as a structure for water

storage, seems to be frequent in epiphytic representatives appearing in *Gesneriaceae*, *Ericaceae*, *Clusiaceae*, *Araliaceae* and epiphytic bromeliads (Madison 1977). According to Haberlandt (1914) and Madison (1977), besides storing water, the hypodermis helps to avoid heating, especially in the epiphytic plants with Crassulacean acid metabolism (CAM).

In the mesophyll of all species there are some water storage cells like those described by Stern *et al.* (2004) (Tab. 6) suggesting that this feature is widespread amongst the *Zygopetalinae*.

In general, the species studied here possess anatomical features that can be interpreted as adaptations to drought, a situation that typically prevails in epiphytic environments where most of them live. Besides the occurrence of a velamen, which protects the root from heating and the subsequent loss of water (Pridgeon 1987) the leaves of all species are hypostomatic and possess stomata protected by cuticular projections, which are considered to reduce the rate of water loss (Rasmussen 1987, Bonates 1993). In the leaves there are also water storage cells and the vascular bundles are surrounded by fibers, which provide mechanical support during extreme dehydration. In some representatives, the leaves also possess a hypodermal layer to store water and/or groups of fibers not related to the vascular bundles (Tab. 5), which increase mechanical support during dehydrating periods. Moreover, several species possess pseudobulbs (Tab. 3), an enlarged stem axis which stores water and nutrients (Betchel *et al.* 1981).

Adjacent to the vascular bundles sclerified cells with silica bodies (the stegmata) appear in all organs, being more frequent in the pseudobulbs (Tab. 3). Although the role of the silica is not yet well established, stegmata are common in xerophytic, epiphytic orchids, and absent in terrestrial mesophytic orchids, indicating some relationship between xerophytism and silica (Møller & Rasmussen 1984). Also, the presence of flavonoid compounds in most of species (Tabs. 3, 5), may be related to their resistance/tolerance to possible water stress, since they are associated with the free radicals commonly produced in plants subjected to environmental pressures (Yamasaki *et al.* 1997). On the other hand, some features, such as the number of velamen, root cortex and mesophyll layers can be useful in the systematic analysis of determined taxa specially *Dichaea*, *Promenaea* and *Zygopetalum*.

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