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Are stem nectaries common in Gentianaceae Juss.?

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ABSTRACT

Extrafloral nectaries (EFNs) are specialized structures that produce and release nectar and are located on leaves, cotyledons and, more seldomly, stems. Peculiar leaf nectaries have been described for thirty-three Neotropical species of Gentianaceae, while stem nectaries have been reported for only nine. The aim of this study was to verify the occurrence of stem EFNs within Gentianaceae and investigate the existence of a correlation between their occurrence and the geographical distribution of species. Samples of internodal regions from field and herbarium specimens were submitted to standard light microscopy techniques. Data regarding the geographical distribution of species were acquired from herbarium specimens and the literature. A total of 37 species were investigated, representing 25 genera distributed among five tribes. Nectaries, composed of modified epidermal cells, were observed in 16 species restricted to the Neotropical tribes Helieae, Saccifolieae, Potalieae and Coutoubeinae; exceptions were *Cicendia quadrangularis* and *Zygostigma australe*, which both occur in the Neotropics but do not possess EFNs. These results demonstrate that stem EFNs are common among Neotropical taxa of Gentianaceae, and are typically absent from taxa in temperate regions.

Keywords: Coutoubeinae, Helieae, Potalieae, Saccifolieae, secretory structures

Introduction

Nectaries are structures that release nectar, a solution composed mainly of mono- and disaccharides, amino acids, proteins and trace amounts of other compounds (Elias 1983; Nicolson & Thornburg 2007). Extrafloral nectaries (EFNs) play an important ecological role in plant protection, since nectar acts as a carbohydrate-rich liquid reward for ants in exchange for protection against herbivores (Heil 2015; Del-Claro *et al*. 2016).

EFNs occur in 110 families of vascular tracheophytes, but are unknown in gymnosperms, early angiosperms and magnoliids (Weber & Keeler 2013; Weber *et al.* 2015). Although EFNs occur in monocots, the bulk of their occurrence is widespread among eudicots, especially in the rosids clade (Weber & Keeler 2013). Evidence suggests that EFNs originated a remarkable number of times in the evolution of tracheophytes (Weber & Keeler 2013) and played a key evolutionary role in the diversification of certain plant clades (Marazzi & Sanderson 2010; Nogueira *et al.* 2012).

Although EFNs have been reported to be absent in Gentianaceae Juss. (Elias 1983) peculiar nectaries composed of only modified epidermal cells were described for 33 species of Gentianaceae, including 13 Neotropical genera (Delgado *et al.* 2011a; b; Dalvi *et al*. 2013; 2014; Guimarães *et al.* 2013). Such nectaries are microscopic structures and occur along the leaf blade in single units or aggregates (Dalvi *et al.* 2013). Anatomically similar nectaries were also observed on the stem of seven species of *Curtia*, in *Hockinia*

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montana (Dalvi *et al.* 2014), and in *Schultesia pachyphylla* (Guimarães *et al.* 2013). However, a profuse secretion and the presence of ants were observed only in the aggregated leaf nectaries of *Calolisianthus speciosus* (Delgado *et al.* 2011a) and *Chelonanthus viridiflorus* (Dalvi *et al.* 2013). In both species the nectaries occur at the base of the leaf blade are recognized as yellow areas. As usually described, the nectar of *Calolisianthus speciosus* is composed of glucose, fructose, and sucrose (Delgado *et al.* 2011a).

Gentianaceae is comprised of 1700 species and 91 genera organized in seven tribes, Chironieae, Exaceae, Gentianeae, Helieae, Potalieae, Saccifolieae and Voyrieae (Struwe *et al.* 2002; Merckx *et al.* 2013; Gentian Research Network 2016). Although this family is cosmopolitan, most of the species occur in temperate regions (Sousa & Lorenzi 2008), while the greatest diversity of genera lies on South and Central America (Albert & Struwe 2002).

EFNs have been considered more abundant in species from tropical communities than in those from temperate regions (Oliveira & Freitas 2004; Rico-Gray & Oliveira 2007). However, information about the possible influences of geographic regions over the occurrence of EFNs is still scarce (Pemberton 1998). In addition, data about stem EFNs are limited to just a few families, and detailed structural descriptions are lacking (Machado *et al.* 2008; Weber & Keeler 2013).

Considering the widespread distribution of Gentianaceae and the usual occurrence of leaf nectaries in Neotropical species, the aim of this study was to investigate how common is the occurrence of stem nectaries in Neotropical and temperate species of Gentianaceae.

Materials and methods

Samples from the third to fifth internodes were obtained from exsiccates of national and international herbaria (Tab. 1). The material was rehydrated by microwaving in distilled water for 5 min and left to rest overnight. Rehydrated samples were then treated with 2 % potassium hydroxide for 1-2 h, dehydrated in an ethanol series and stored in 70 % ethanol (Smith & Smith 1942). According to the occurrence of species, different locations in the states of Minas Gerais and Bahia (Brazil) were selected for field expeditions. In the same way, samples from the third to fifth internodes of field-collected material were fixed in FAA (formalin, acetic acid, 50 % ethanol, 1:1:18 by volume) and stored in 70 % ethanol (Johansen 1940). Voucher materials were deposited in the VIC Herbarium of Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil. Fragments of young branches were sampled only from *Potalia resinifera* Mart., the unique tree species analyzed here.

For the anatomical studies, samples stored in 70 % ethanol were submitted to an ethanol dehydration series and subsequently embedded in methacrylate (Historesin, Leica Microsystems Nussloch GmbH, Heidelberg, Germany).

Table 1. Sampled Gentianaceae species, organized by tribes, with their collectors (herbaria), collection sites and geographical distribution of tribes, subtribes and species.

Tribe/subtribe/species	Collector and herbarium	Local collection	Distribution – Tribe and species
CHIRONIEAE - Subtribe Canscorinae			PALEOTROPICAL (Struwe *et al.* 2002)
1 - Canscora diffusa (Vahl) R. Br. ex Roem. & Schult.	D.A.Nangoma and K.Kaunda 201 (NY)	Malawi, Africa	Asia, India, Africa, Madagascar and Australia (Thiv & Kadeiret 2002)
	NR (NY)	Cameroon, Africa	
	Koyama et al. s.n. (NY)	Doi Inthanon, Thailand	
CHIRONIEAE - Subtribe Chironiinae			PANTROPICAL TO TEMPERATE (Struwe *et al.* 2002)
2 - *Blackstonia perfoliata* (L.) Huds. subsp grandiflora	J.Lewalle 8763 (NY)	Tétouan, Maroc	Mediterranean (Mansion & Struwe 2004)
3 - *Centaurium erythraea* Rafn	I.Cordeiro et al. 2993 (SP)	Rio de Janeiro, Brazil	Mediterranean (Mansion & Struwe 2004)
	H.Luedrewaldt 51 (SP)	Rio de Janeiro, Brazil	
	R.Kral 75992 (SP)	São Paulo, Brazil	
4 - Centaurium maritimum (L.) Fritsch	Pajarón 53 (NY)	Los Barrios, Spain	Mediterranean (Mansion & Struwe 2004)
	Pajarón 53 (MBM)	Los Barrios, Spain	
5 - *Centauriumpulchellum* (Sw.) Druce	A.W.Cusick 1133 (NY)	Ohio, USA	Mediterranean (Mansion & Struwe 2004)
	A.W.Cusick 34481 (NY)	Ohio, USA	
	W.D.Longbotton 13784 (NY)	Maryland, USA	
6 - Chironia baccifera L.	H.J.T.Venter 10676 (NY)	South Africa, Africa	Southern Africa (Mansion & Struwe 2004); Africa and Madagascar (Gentian Research Network 2016)
	R.Brand et al. 175 (NY)	South Africa, Africa	
	NR (NY)	South Africa, Africa	
7 - *Cicendia filiformis* Delarbre	J.Stefani s.n. (US)	NR	Mediterranean and Western Europe (Struwe *et al.* 2002)
	P.Aellen s.n. (US)	Solenzara, France	
	J.Stefani s.n. (SP)	NR	

Table 1. Cont.

Tribe/subtribe/species	Collector and herbarium	Local collection	Distribution – Tribe and species
8 - Cicendia quadrangularis (Lam.) Griseb.	H.W.Camp 3516 (NY)	Chimborazo, Ecuador	Mediterranean (Mansion & Struwe 2004); disjunct distribution in Southern and Western North America; and South America, from Ecuador to Argentina (Struwe *et al.* 2002)
	B.Ertter et al. s.n. (NY)	California, USA	
	NR (NY)	California, USA	
9 - Gyrandra tenuiflora (Martens & Galeotti) Mansion	A.Neil and B.R.Harriman s.n. (NY)	Ozaukee, USA	Mountains of Western Mexico (Mansion 2004)
	T.Gviniashvili et al. 464 (NY)	Georgia, USA	
10 - Sabatia angularis (L.) Pursh	J.B.Nelson and A.Aurich 16714 (NY)	South Carolina, USA	USA (Gentian Research Network 2016)
	F.W.H. 10462 (NY)	North Carolina, USA	
	R.Dale Thomas 124348 (NY)	Louisiana, USA	
11 - *Sabatia campestris* Nutt.	G.L.Webster and R.L.Wilbur 3261 (NY)	Louisiana, USA	USA (Gentian Research Network 2016)
	R.Dale Thomas 100115 (NY)	Louisiana, USA	
	R.Dale Thomas and C. Amazon (NY)	Louisiana, USA	
12 - Schenkia spicata (L.) Mansion	J.Risler and R.A.Kerrigan 403 (NY)	Alroy Downs, Australia	Mediterranean (Mansion & Struwe 2004); Western Europe to Eastern Asia and North Africa; introduced in North America (Mansion 2004)
	D.E.Symon 15270 (NY)	South Australia, Australia	
	H.P. Vonow 911 (NY)	South Australia, Australia	
13 - *Zeltnera stricta* (Schiede) Mansion	C.R.Broome and R.M. Lloyd 634 (NY)	Mexico	Endemic to South and Central Mexico (Mansion 2004)
	C.R.Broome 746 (NY)	Mexico	
	C.R.Broome and R.M. Lloyd 620 (NY)	Mexico	
14 - *Zygostigma australe* (Cham. & Schltdl.) Griseb.	G.Hatschbach et al. 71812 (MBM)	Paraná, Brazil	Brazil, Argentina and Uruguay (Struwe *et al.* 2002)
	A.Usteri s.n. (SP)	São Paulo, Brazil	
	F.C.Hoehne s.n. (SP)	São Paulo, Brazil	
CHIRONIEAE - Subtribe Coutoubeinae			NEOTROPICAL (Struwe *et al.* 2002)
15 - Symphyllophyton caprifolioides Gilg	J.A.Ratter et al. 6742 (INPA)	Maranhão, Brazil	South America (Mansion & Struwe 2004); and Brazil (Guimarães & Saavedra 2013)
	I.Gottesberg and G.Gottesberg s.n. (SP)	Goiás, Brazil	
	J.A.Ratter et al. s.n. (MBM)	Maranhão, Brazil	
16 - Xestaea lisianthoides Griseb.	P.H.Gentle 9052 (US)	Cayo, Honduras	Central and South America (Struwe *et al.* 2002)
	P.C.Standley 30379 (US)	France Field, Panama	
	E.P.Killip 3362 (US)	Panama	
GENTIANEAE - Subtribe Gentianinae			TEMPERATE-ALPINE (Struwe *et al.* 2002)
17 - Gentiana sedifolia H. B. K.	L.B.Holm-Nielsen and J. Jamarilho s.n. (US)	Tungurahua, Ecuador	*Asia, Europe, North and South America, Northwest Africa, and East Australia (Struwe *et al.* 2002)
	P. Acevedo-Rodzg s.n. (US)	Cochabamba, Bolivia	
	John L. Clark 719 (US)	Tungurahua, Ecuador	
GENTIANEAE - Subtribe Swertiinae			TEMPERATE-ALPINE (Struwe *et al.* 2002)
18 - Bartoniapaniculata (Michx.) Muhl.	L.K. Magrath 17310 (NY)	Oklahoma, USA	*North America, from Texas and Florida to Newfoundland (Struwe *et al.* 2002)
	H.E. Ahles 36920 (NY)	North Carolina, USA	
19 - Bartonia virginica (L.) Britton, Sterns & Poggenb	M.L. Fernald s.n. (NY)	Nova Scotia, Canada	*North America, from Texas and Florida to Newfoundland (Struwe *et al.* 2002)
	R.C.Bean and D. White s.n. (NY)	Nova Scotia, Canada	
	M.L. Fernald and B. Long s.n. (NY)	Nova Scotia, Canada	
20 - Gentianella amarella (L.) Börner	C.G. Alm s.n. (NYBG)	Torneträsk, Sweden	Europe and North America (Gentian Research Network 2016)
	N.Jacobsen and J. Suedsen s.n. (NY)	NR	
	Harry Smith s.n. (NY)	Sweden	
21 - Halenia corniculata (L.) Cornaz	H.H.Iltis et al. 636 (NY)	Siberia	Asia (Hagen 2003)
	N.Naruhashi s.n. (NY)	Hokkaido, Japan	
	H.H.Iltis et al. 873 (NY)	Lake Baikal, Siberia	

Table 1. Cont.

Tribe/subtribe/species	Collector and herbarium	Local collection	Distribution – Tribe and species
22 - Halenia palmeri A. Gray	Rogers McVaugh 21741 (NY)	Durango, Mexico	Mexico (Hagen 2003))
	N/H. Holmgren and T. K. Lowrey 8073 (NY)	Durango, Mexico	
	F.W.Pennell s.n. (NY)	Durango, Mexico	
23 - Lomatogonium carinthiacum (Wulf.) Rchb.	V.Zuer (NY)	Altai, Russia	*North America, temperate Asia, and Europe (*Struwe et al.* 2002)
	Otar Abdalazed et al. 686 (NY)	Kazbegi, Georgia	
	G.Nakahutsrishvili and O.Abdalazed 160 (NY)	Kazbegi, Georgia	
HELIEAE			NEOTROPICAL (Struwe *et al.* 2002)
24 - Adenolisianthus arboreus Gilg	J.J.Wurdack and L.S.Adderley s.n. (NY)	Amazonas, Brazil	South America (Lepis 2009)
	B.G.S.Ribeiro 1060 (RB)	Amazonas, Brazil	
25 - Calolisianthuspedunculatus (Cham. & Schltdl.) Gilg	V.C.Dalvi et al. 98 (VIC)	Minas Gerais, Brazil	Brazil (Calió 2009)
	V.C.Dalvi et al. 102 (VIC)	Minas Gerais, Brazil	
	V.C.Dalvi et al. 109 (VIC)	Minas Gerais, Brazil	
26 - Calolisianthus speciosus Gilg	G.Valente et al. 1969(VIC)	Minas Gerais, Brazil	Brazil (Calió 2009)
	G.Valente et al. 1941 (VIC)	Minas Gerais, Brazil	
	G.Valente et al. 2237 (VIC)	Minas Gerais, Brazil	
27 - Chelonanthus alatus (Aubl.) Pulle	C.Todzia et al. 2213(INPA)	Amazonas, Brazil	Central and South America (Lepis 2009)
	E.Brocki 14 (INPA)	Amazonas, Brazil	
	M.Groppo Jr. et al. 956 (INPA)	Amazonas, Brazil	
28 - Chelonanthus albus (Spruce ex Progel) Badillo	F.E.Miranda and M.C.C. Miranda 829 (INPA)	Amazonas, Brazil	Central and South America (Lepis 2009)
	J.Chagas s.n. (INPA)	Amazonas, Brazil	
	W.Rodrigues and J. Chagas 4503 (INPA)	Amazonas, Brazil	
	P.J.Maas et al s.n. (NY)	Amazonas, Brazil	
29 - Chelonanthus grandiflorus (Aubl.) Chodat & E. Hassl.	M.F.Silva et al. 618 (INPA)	Amazonas, Brazil	Central and South America (Lepis 2009)
	L.A.Maia et al 403 (INPA)	Amazonas, Brazil	
	D.W.Stevenson et al. s./n. (INPA)	Amazonas, Brazil	
30 - Chelonanthus purpurascens (Aubl.) Struwe, S. Nilsson & V.A. Albert	V.C.Dalvi et al. 34 (VIC)	Bahia, Brazil	South America (Lepis 2009)
	V.C.Dalvi et al. 52 (VIC)	Bahia, Brazil	
	V.C.Dalvi et al. 61 (VIC)	Bahia, Brazil	
31 - Chelonanthus viridiflorus (Mart.) Gilg	J. A. Ratter et al. s.n. (INPA)	Roraima, Brazil	South America (Lepis 2009)
	V.CDalvi and D,M,T,F.Francino 03 (VIC)	Minas Gerais, Brazil	
	V.C.Dalvi and D.M.T.F.Francino 12 (VIC)	Minas Gerais, Brazil	
32- Helia oblongifolia Mart.	M.M.K. Carra and P. J .M. Maas (SP)	NR	Brazil and Paraguay (Calió 2009)
	M.F. Calió 205 et al. (SPF)	Minas Gerais, Brazil	
	A.C.Brade s.n. (SP)	São Paulo, Brazil	
33 - Irlbachia nemorosa (Willd. ex Roem. & Schult.) Merr.	W.Montovani and D.M.S.Rocha s.n. (SPF)	Amazonas, Brazil	Brazil (Guimarães & Saavedra 2013)
	Fábio de Barros 947 (SP)	Amazonas, Brazil	
34 - Tetrapollinia caerulescens (Aubl.) Maguire & B.M. Boom	R.M.Harley et al 25990 (SP)	Bahia, Brazil	Central and South America (Gentian Research Network 2016)
	M.F.Calió et al 154 (SPF)	Minas Gerais, Brazil	
	Fábio de Barros 862 (SP)	Mato Grosso do Sul, Brazil	
POTALIEAE - Subtribe Faroinae			PANTROPICAL (Struwe *et al.* 2002)

Table 1. Cont.

Tribe/subtribe/species	Collector and herbarium	Local collection	Distribution – Tribe and species
35 - Neurotheca loeselioides (Spruce ex Progel) Baill.	M.N.Silva et al. 182 (INPA)	Pará, Brazil	Northern South America, tropical Africa, and Western Madagascar (Struwe *et al.* 2002)
	B.W.Nelson et al. 1483 (INPA)	Amazonas, Brazil	
	O.P.Monteiro and J.F.Ramos 832 (INPA)	Amazonas, Brazil	
	A.Janssen 131 (RB)	Amazonas, Brazil	
POTALIEAE - Subtribe Potaliinae			PANTROPICAL (Struwe *et al.* 2002)
36 - Potalia resinifera Mart.	N.A.Rosa et al. 5487 (SPF)	Amazonas, Brazil	Brazil (Amazonas) and Andes (Struwe & Albert 2004)
	M.Goppo et al. 882 (SPF)	Amazonas, Brazil	
	A.Henderson and F.G.Padilha 2034 (NY)	Loreto, Peru	
SACCIFOLIEAE			NEOTROPICAL (Struwe *et al.* 2002)
37 - Voyriella parviflora Miq.	M.J.Pires and N.T.Silva 1641 (NY)	Pará, Brazil	South America and Panama (Struwe *et al.* 2002)
	E.A.Chritenson and S.R.George 1796 (NY)	French Guiana	
	Bassett Maguire et al. s.n. (NY)	Suriname	

(*) Distribution pattern of the genus. NR= Not reported. Acronyms of herbaria according to Index Herbariorum.

The material was sectioned using a rotary microtome (model RM2155, Leica Microsystems Inc., Deerfield, USA). Cross and paradermal sections (7 µm thick) were stained with toluidine blue, pH 4.7 (O' Brien *et al.* 1964), and permanent slides were mounted with synthetic resin (Permount, Fisher Scientific, New Jersey, USA). To observe epidermal cells from frontal view, paradermal hand-sections were obtained, clarified in sodium hypochlorite (20 %), stained with 0.001 % basic alcoholic fuchsin and mounted in gelatin (Johansen 1940).

Analyses and image captures were conducted using an Olympus Optical AX70TRF (Tokyo, Japan) with a U-Photo system and coupled digital camera (AxioCam HRc; Zeiss, Göttingen, Germany) in the Laboratory of Plant Anatomy of UFV.

The data for the geographical distribution of species and tribes/subtribes/genera/species were obtained from herbarium data and literature (Tab.1).

Results

We analyzed 37 species (25 genera), comprising representatives of the tribes Chironieae, Gentianeae, Helieae, Potalieae and Saccifolieae (Tab. 1). The stem EFNs were not visible to the naked eye neither in materials collected in the field nor in those from herbaria. Conversely, secretion or other macro-morphological evidence of the presence of these nectaries was not detected as well. However nectaries were observed under light microscopy on internodes of the stems in 16 species of 11 genera comprising almost 50 % of taxa studied (Tab. 1).

The stem nectaries are non-vascularized glands comprised of a single central epidermal cell surrounded by concentrically arranged secretory cells, as shown in *Chelonanthus purpurascens* (Fig. 1A, B) and *Tetrapollinia caerulescens* (Fig. 1C, D). The region of contact between secretory cells and the central cell stained intensely with toluidine blue (Fig. 1E, F). Stem nectaries have a scattered distribution and are interspersed among stomata, except in *Potalia resinifera,* where stomata were not detected.

In the tribe Helieae, stem nectaries were observed in all analyzed species (Tab. 1). *Voyriella parviflora* was the only species of tribe Saccifolieae analyzed that bore stem nectaries. The presence of stem nectaries was ubiquitous in Potalieae. On the other hand, stem nectaries were absent in seven analyzed taxa of the tribe Gentianeae. In Chironieae, stem nectaries were present in three species from different genera. In Coutoubeinae, Canscorinae and Chironiinae the pattern of presence or absence of stem nectaries remained constant. Two species of Coutoubeinae, *Symphyllophytton caprifolioides* (Fig. 1E) and *Xestaea lisianthoides* (Fig. 1F), bore stem nectaries, while they were not detected in Canscorinae (*Canscora diffusa*) and in Chironiinae (Tab. 1).

Regarding the correlation between presence/absence of stem nectaries and pattern of geographic distribution of species, tribes or subtribes, the presence of these nectaries was constant in species restricted to the Neotropics, including all species of tribes Helieae, Saccifolieae, subtribe Coutoubeinae (Chironieae) and *Potalia resinifera* (Potalieae - Potaliinae) (Tab. 1). Besides occurring in the Neotropics, *Neurotheca loeselioides* (Potalieae-Faroinae) is also found in Africa and bears stem nectaries. Exceptions could be seen in Chironieae, in which *Cicendia quadrangularis*

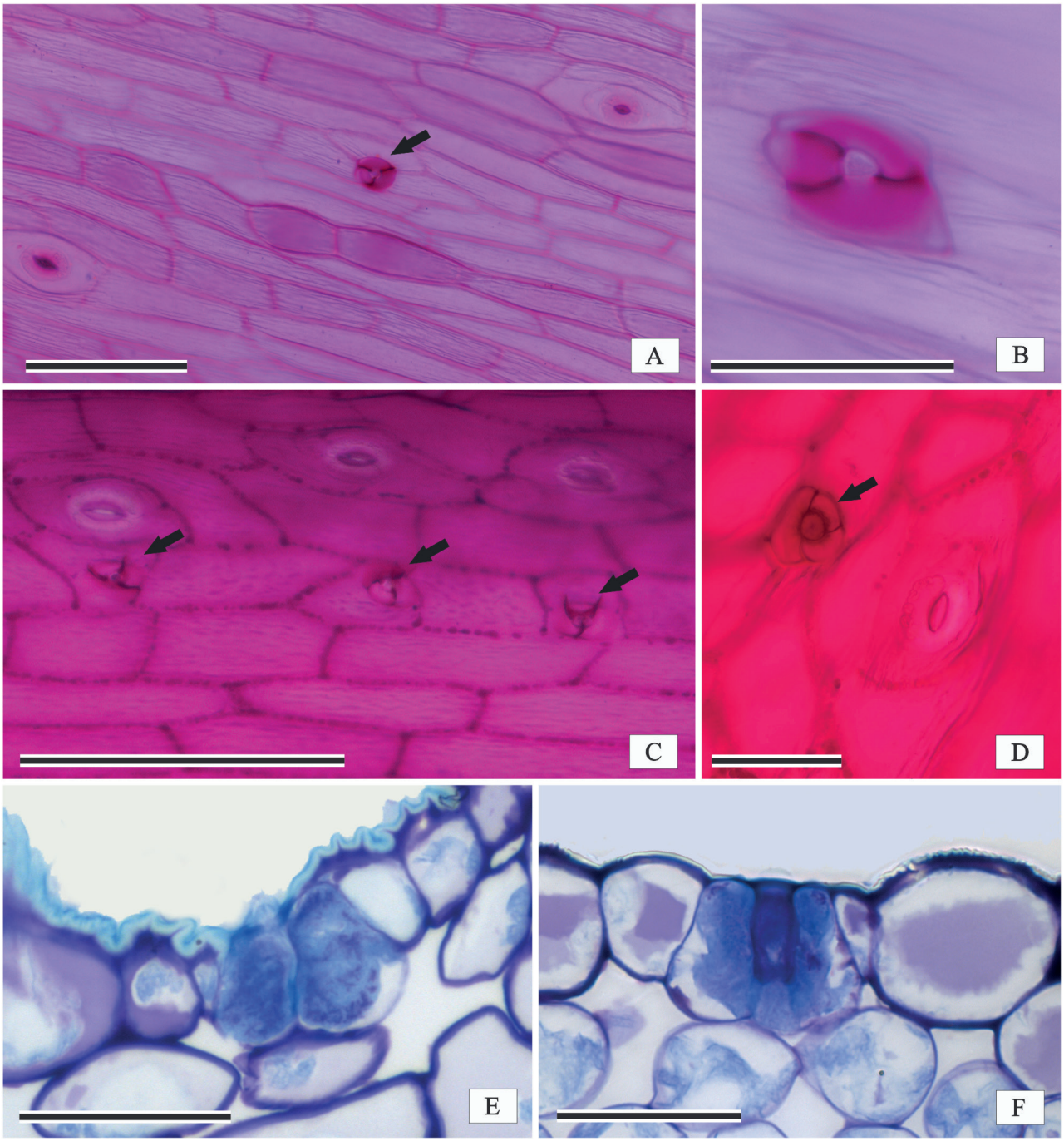

Figure 1. Stem nectaries of species of Gentianaceae in paradermal (A-D) and cross sections (E, F). (A) and (B) *Chelonanthus purpurascens*. (C) and (D) *Tetrapollinia caerulescens*. (E) *Symphyllophytton caprifolioides*. (F) *Xestaea lisianthoides*. Black arrows indicate EFNs. Bars = 50 µ.

and *Zygostigma australe* (Chironieae - Chironiinae), both occurring in South America, do not possess stem nectaries. In contrast, nectaries are absent in all species restricted to temperate-alpine regions (Gentianinae), as well as in species restricted to the Paleotropics (Chironieae - Canscorinae) and in those distributed in the Pantropics to temperate regions (Chironieae - Chironiinae) (Tab. 1).

Discussion

The present work demonstrates that in Gentianaceae stem nectaries are common, occurring in 50 % of the studied species, and mainly in Neotropical taxa. These results are interesting since the presence of stem nectaries is considered

an uncommon character in eudicotyledon families (Elias 1983; Machado *et al.* 2008). Nectaries of the Gentianaceae were described by Vogel (1998) as unusual nectaries on the sepals of species of *Irlbachia* and were termed nectarioles by the author. Dalvi *et al.* (2013) named such structures present on leaves as epidermal nectaries, following the classification proposed by Bernadello (2007).

The lack of evidences regarding the occurrence of stem nectaries in Gentianaceae is due to the absence of microscopic analyses, as also stated by Dalvi *et al.* (2013) for leaf nectaries. Additionally, anatomical studies of the stem in the species of Gentianaceae are even scarcer than those performed with leaves, and are restricted to a few species of *Deianira*, *Schultesia* (Delgado *et al.* 2009; Guimarães *et al.* 2013), *Curtia* and *Hockinia* (Dalvi *et al.* 2014). In these genera, stem nectaries were reported for only seven species of *Curtia*, *Hockinia montana* and *Schultesia pachyphylla*. Besides demonstrating that stem nectaries are common in Gentianaceae, the present study highlights the importance of anatomical analysis to confirm the presence of these structures in this family.

Anatomically, the stem nectaries of Gentianaceae are similar to most of the leaf nectaries found in the family (Delgado *et al.* 2011a; b; Dalvi *et al*. 2013; 2014; Guimarães *et al*. 2013), with the exception of those on the leaf base of *Calolisianthus speciosus*, which are vascularized (Delgado *et al.* 2011a). Stem nectaries are randomly distributed and do not form aggregates, as observed for leaf nectaries of certain species of Gentianaceae (Dalvi *et al*. 2013). This pattern of distribution may explain why the exudate is not observed in the field. Abundant nectar was reported only for leaf nectaries of species of *Calolisianthus*, which occur aggregated at the leaf base (Delgado *et al.* 2011a). In other species of Gentianaceae, which have isolated nectaries dispersed along the leaf blade, an apparent secretion was also not detected (Delgado *et al.* 2011a; Dalvi *et al.* 2013; 2014).

The presence of visible secretion in the field may also be related to the stage of development of the organ, to the season and to the time of day in which the plant was collected. Delgado *et al.* (2011a) noted that in species of *Calolisianthus*, it is impossible to collect nectar during the dry season because the secretory cells are not active. Therefore, field studies are necessary to investigate the patterns of nectar secretion related to the environmental factors and to clarify the role played by both leaf and stem nectaries.

In the present study, a correlation between the presence of stem nectaries and geographic distribution of species, subtribes or tribes were indeed observed in Gentianaceae, since among all studied species only those restricted to the Neotropics bore stem nectaries. The Neotropics is a center of diversification of Gentianaceae and comprises the lineages of the most basal nodes of the clade, represented by Saccifolieae (Albert & Struwe 2002). The presence of units of stem nectaries could represent an ancestral condition in Gentianaceae. On the other hand, the non-occurrence of stem nectaries in the studied species of temperate and paleotropical regions suggests the loss of this structure in the species of these regions. However, futures studies focusing on combinations of morphoanatomical and molecular data are necessary to elucidated how nectaries evolved in Gentianaceae.

Zygostigma australis and *Cicendia quadrangularis*, both of the subtribe Chironiinae (Chironieae), represent the exceptions to the correlation described above since they occur in Neotropical regions but do not bear stem nectaries. *Cicendia quadrangularis* is pantropical, while *Zygostigma australis* is restricted to the South Region of Brazil. This variation represents an interesting model to test the hypothesis proposed by Pemberton (1998) that the incidence of plants bearing EFNs increases along the latitudinal gradient (cold temperate to warm subtropical regions).

The presence of stem nectaries corresponds with the presence of leaf nectaries in Gentianaceae, as reported by Dalvi *et al.* (2013). The stem nectaries in Gentianaceae are peculiar and originate exclusively by protoderm activity. The underlying premise that the presence of stem nectaries is common in Neotropical, and their absence typical among the temperate, Gentianaceae taxa is confirmed here.

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