Original article

Are scale leaves essencial in temperate-cold climates? An evaluation in tree species from temperate rainforests of South America

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ABSTRACT

The development of scaly buds (= cataphylls) has been traditionally associated with seasonally cold climates, although only few species from the southern hemisphere were investigated in this regard. The present work focuses on apical and axillary buds of seven tree species native to the South-American Temperate Rainforests (STR). Due to differences in the lineages from which these species derived, high levels of inter-specific variation in bud structure were expected. Apical and axillary buds were dissected under stereomicroscope, and the sizes of their parent shoots were evaluated. Cataphylls and leaf primordia were counted, and the presence of colleters and/or trichomes registered. Both intra- and inter-specific variations in bud structure were found. The apical buds were scaly in two out of seven species, and naked in the other species. Axillary buds were scaly in all but one species. In general terms, larger shoots developed buds with more organs. The presence of colleters (in four species) was not restricted to those buds lacking an outer cover of cataphylls. Further studies should focus on the relevance at a broader scale of colleters and trichomes as protective structures in tree buds.

Keywords: Tree buds, South-American Temperate Rainforest, naked buds, scaly buds, preformation.

Introduction

The structure of vegetative buds, i.e. the precursors of leafy shoots, is fundamental for the understanding of meristem function (Powell 2008; Font Quer 2009). In woody plants, buds are usually developed at the distal end of each shoot (apical buds) and at each node (axillary buds; Barthélémy & Caraglio 2007). Buds have traditionally been classified into scaly buds and naked buds, based on the presence/absence of specialized, scale-like leaves on their outside (Koriba 1958; Hallé *et al.* 1978; Nitta & Ohsawa 1998). These covering leaves, known as cataphylls or scale leaves, lack a green blade and are tougher than the green leaves typical of the species concerned. For this reason, cataphylls have often been linked to the protection of the underlying primordia and meristem against extreme conditions such as desiccation, low or high temperatures and herbivory (Wiegand 1906; Vertucci & Stushnoff 1992;

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Nitta & Ohsawa 1998; Bell 2008; Magnin *et al.* 2012; Alla *et al.* 2013; Barykina & Churikova 2014; Kuprian *et al.* 2017). It has frequently been assumed that the presence of cataphylls in buds is an adaptation of woody plants to regions where the climate is drastically seasonal (Barthélémy & Caraglio 2007; Robitaille 2017). According to Koriba (1958), bud traits are related to growth traits. Shoot-extension dynamics, which depends on both genetic and environmental factors, determines variations in leaf size and shape along shoots (Hollender & Dardick 2015). Under this assumption, naked buds would relate to continuous leaf production, and scaly buds with periodical leaf production (i.e. rhythmic growth; van der Schoot *et al.* 2014). Naked buds would thus prevail in regions with low levels of within-year climatic seasonality, whereas scaly buds would prevail wherever the climate is definitely seasonal (Schimper *et al.* 1903; Dawson & Sneddon 1969; Fisher *et al.* 1990; Powell 2008; Ohsawa *et al.* 2011; Hirons & Thomas 2017). However, a recent study has shown that a significant proportion of the woody species from temperate regions have naked buds (Schoonderwoerd & Friedman 2021), which supports the idea that leaf primordia may have protective means other than cataphylls.

In some species, the presence of trichomes and/or secretory organs known as colleters may protect the underlying primordia against factors such as herbivores, pathogens, desiccation or freezing (Thomas 1991). Colleters are often found in shoot apices of many species, usually associated with leaves (Thomas 1991), and secrete mucilaginous or resinous substances that protect buds (Lersten 1974; Fahn 1979; William *et al.* 1980; Durkee *et al.* 1984; Mohan & Inamdar 1986; Thomas & Dave 1989; Mangalan *et al.* 1990; Rio *et al.* 2002; García *et al.* 2006; Leitão & Cortelazzo 2008; Miguel *et al.* 2009; Muravnik *et al.* 2014; Silva *et al.* 2012; 2019; Mayer *et al.* 2013; Judkevich *et al.* 2017). It may be hypothesized that the presence of colleters and/or trichomes could be one of the reasons why the development of scaly or naked buds is not as tightly linked to climate as initially expected.

Generalizations regarding bud structure derive mainly from observations and investigations made on tree species from the northern hemisphere (Kozlowski 1971; Remphrey & Powell 1984; Sabatier & Barthélémy 1995; Powell 2008). Few studies dealt with the bud structure of woody species native to temperate regions of the southern hemisphere (e.g. Barthélémy *et al.* 1999; Souza *et al.* 2000; García *et al.* 2006; Magnin *et al.* 2012). The South-American Temperate Rainforests (STR) occupy an area of about 70,000 km^2 in central-southern Chile and small areas of Argentina, and are the outcome of a rich history of biogeographical and geological events that shaped biodiversity (Donoso 1993; Jaramillo & Cárdenas 2013; Segovia & Armesto 2015). STR exhibit important levels of endemism (32% of plant genera) and have high conservation value (Ezcurra & Brion 2005). The low extension of the continent relative to the

seas at this latitude has a moderating effect on maximum and minimum temperatures compared with regions of the northern hemisphere with a similar latitude (Muñoz Schick 1980; Conti 1998; Peel *et al.* 2007; CONAMA 2008). Biogeographical studies have concluded that the STR separated from the subtropical rainforests of South America at least 10 MA ago (Villagrán & Hinojosa 1997; Blisniuk *et al.* 2005; Guillaume *et al.* 2009; Le Roux 2012), which may have favoured the evolution of distinctive morphological traits. The present study was aimed at evaluating the extent to which the evolution of trees from different lineages in the temperate rainforests of South America has resulted in inter-specific similarities regarding the structure of apical and axillary buds. We propose the hypothesis that coexisting plant species that belong to different lineages may differ regarding the morphological attributes they develop to favour the persistence of leaf primordia, namely bud scales, colleters and trichomes.

Materials and methods

The selected species for this study were: *Aextoxicon punctatum* Ruiz & Pav. (locally known as "tique"; Aextoxicaceae), *Caldcluvia paniculata* (Cav.) D. Don ("tiaca", Cunoniaceae), *Eucryphia cordifolia* Cav. ("ulmo", Cunoniaceae), *Luma apiculata* (DC.) Burret ("quetri"; Myrtaceae), *Myrceugenia exsucca* (DC.) O. Berg. ("pitra"; Myrtaceae), *Persea lingue* (Ruiz & Pav.) Nees ("tepa"; Lauraceae) and *Sophora cassioides* (Phil.) Sparre ("pelú"; Fabaceae). All but *S. cassioides* are considered native to Chile and Argentina. *Sophora cassioides* is an exclusively Chilean species, and is occasionally cultivated for ornament in nearby regions of Argentina.

For the analysis of bud structure, shoots developed at the periphery of the crown of young-adult trees between 2 and 5 m in height located in three northwestern Patagonian areas of Argentina were sampled.

(1) *San Carlos de Bariloche*. The selected trees were at a native forest (Llao-Llao Nature Reserve) and in urban areas of San Carlos de Bariloche (41°08'10" S, 71°17'17" W, and 780 to 820 m a.s.l.).

(2) *Parque Nacional Lago Puelo*. In the native forest, two sampling areas were chosen (42°06'02" to 42°05'50"S, 71°42'28" to 71°41'35" W, and 224 and 360 m a.s.l., respectively); these areas are about 1800 m east from the international border between Argentina and Chile.

(3) *El Bolsón*. The selected trees had been planted in urban parks of El Bolsón (41°57'47" S, 71°32'16" W, and 290 to 330 m a.s.l.).

Some of the characteristics of each species and their sampling sites are indicated in Tab. 1.

In May 2016, 40 shoots were cut for each species. At the time of sampling, all shoots had ended their seasonal extension (2015-2016 growth season). One shoot per tree

Table 1. Green-leaf traits, length, basal diameter and number of nodes of the shoots sampled for the analysis of bud structure, and sampling sites for each of the species included in the present study. LP: Parque Nacional Lago Puelo, BRC: Bariloche, EB: El Bolsón.

Species	Leaf traits			Shoot traits			Areas
	Maximum leaf size (length x width, cm)	Blade division	Stipules	Length (mm) (mean±SE)	Diameter (mm) (mean±SE)	Leaves (number) (mean±SE)	
A. punctatum	11 x 4	Simple	No	117 ± 8.5	2.5 ± 0.12	6 ± 0.4	LP
C. paniculata	14 x 6	Simple	Yes	117 ± 13.1	2.1 ± 0.09	6 ± 0.4	LP
E. cordifolia	13 x 5	Simple	Yes	126 ± 18.3	2.9 ± 0.15	8 ± 0.5	LP
L. apiculata	3 x 2	Simple	No	185 ± 11.5	2.0 ± 0.09	28 ± 2.3	BRC, EB
M. exsucca	7 x 4	Simple	No	152 ± 9.7	2.2 ± 0.08	10 ± 0.4	BRC
P. lingue	11 x 6	Simple	No	192 ± 21.0	3.5 ± 0.17	11 ± 0.6	LP
S. cassioides	15 x 3	Compound	No	251 ± 30.5	2.6 ± 0.11	8 ± 0.7	BRC, EB

was cut except in the case of *S. cassioides*, for which all shoots derived from five trees. The proximal limit of each shoot was determined through morphological markers that were identified in a previous study on the same species: this limit is marked by short internodes in *E. cordifolia*, *L. apiculata*, *M. exsucca*, *P. lingue* and *S. cassioides*, and by the presence of axillary branches in *C. paniculata*, *E. cordifolia* and *L. apiculata* (Sosa 2019). The length, basal diameter and number of leaves of each sampled shoot were registered. The shoots were transferred to the laboratory in plastic bags; the distal end of each shoot, including its apical bud and at least one axillary bud, was submerged in 70% ethanol for two days before performing bud dissections. The apical bud and one of the distal axillary buds of each shoot were dissected under stereomicroscope (Olympus SZH10) with up to 70x. The preformed leaves of each bud were removed carefully with dissection needles until the undifferentiated shoot apex could be seen. According to previous studies, the magnification level that was employed allows the identification of primordia up to the stage when they are 0.1 mm long pegs on the side of the apical dome (see Williams 1975; Lyndon 1988). This technique imposes limitations since primordia <0.1 mm long may have been unaccounted for; even though it was considered adequate as a means of making inter-specific comparisons (e.g. Williams 1975; García *et al.* 2006). The numbers of cataphylls and leaf primordia were recorded for each bud. These two leaf types were distinguished based on their consistency and on previous observations of buds and shoots of each species. The number of leaf primordia could not be determined in some buds due to insect damage or bud breakage during the dissection process. The presence of stipules, colleters and trichomes in cataphylls and green-leaf primordia were registered. Photographic records were taken. None of the buds included preformed flowers.

Statistical analysis

For each species, the number of leaves per bud was compared between apical and axillary buds by means of Student's t-tests after proving the precondition of normality (Kolmogorov-Smirnov test). The relationships between the total numbers of organs (adding cataphylls and green-leaf primordia) in apical and axillary buds and the variables describing the size of their parent shoots (length, basal diameter and number of leaves), were evaluated for each species by means of Pearson's correlation coefficients (Sokal & Rohlf 1981). In all comparisons, a 0.05 significance level was adopted. Statistical analyses were performed with Minitab 14.

Results

Bud structure

Aextoxicon punctatum. Apical buds of *A. punctatum* consist of a partially extended stem with green-leaf primordia in all of its nodes and an apical meristem (Fig. 1A). They lack cataphylls. The blade of each leaf primordium is folded lengthwise along the mid-vein and towards the adaxial surface. Leaf primordia may be arranged following decussate-opposite or alternate phyllotaxis; in some cases, phyllotaxis changed from one type to the other along a bud. The epidermis of all bud components exhibits a continuous cover of peltate trichomes (lepidote pubescence). Colleters were not observed. The proximal internodes of a bud are rather long, so that most leaf primordia are clearly evident without dissection. The axillary buds of the most proximal leaf primordia of the apical buds may be seen. The number of leaf primordia per bud varied between 4 and 16 (Fig. 2).

Each axillary bud of *A. punctatum* has at least two very small (~1 mm long) and hemisphaerical cataphylls at its base (Fig. 3A). These cataphylls do not play a covering role at any stage after the axillary bud is set, and get detached during shoot extension, leaving tiny scars on the stem. Leaf primordia in these buds were, in general terms, smaller than those of the apical buds. We recorded between 2 and 4 cataphylls and between 2 and 8 green-leaf primordia in each axillary bud of this species. Apical and axillary buds of *A. punctatum* were similar in terms of total number of organs (i.e. adding cataphylls and green-leaf primordia; p=0.71, degrees of freedom, d.f.= 69; Fig. 2).

Caldcluvia paniculata. The apical bud of *C. paniculata* lacks cataphylls and is surrounded externally by two pairs of foliaceous stipules that correspond to the two most distal leaves of the parent shoot (Fig. 1B). This bud consists of an apical meristem and a primordial shoot that includes a variable number of leaf primordia, whose stipules also contribute to the external cover of the bud. Leaf blade primordia are notably falcate in outline and are arranged in a decussate-opposite arrangement. Both primordia of each node do not come in contact with one another, so that they do not cover more distally positioned primordia. Trichomes and colleters are evident at the base of each leaf primordium (Fig. 4A). Each leaf blade primordia is folded lengthwise towards the adaxial side. Bud internodes are
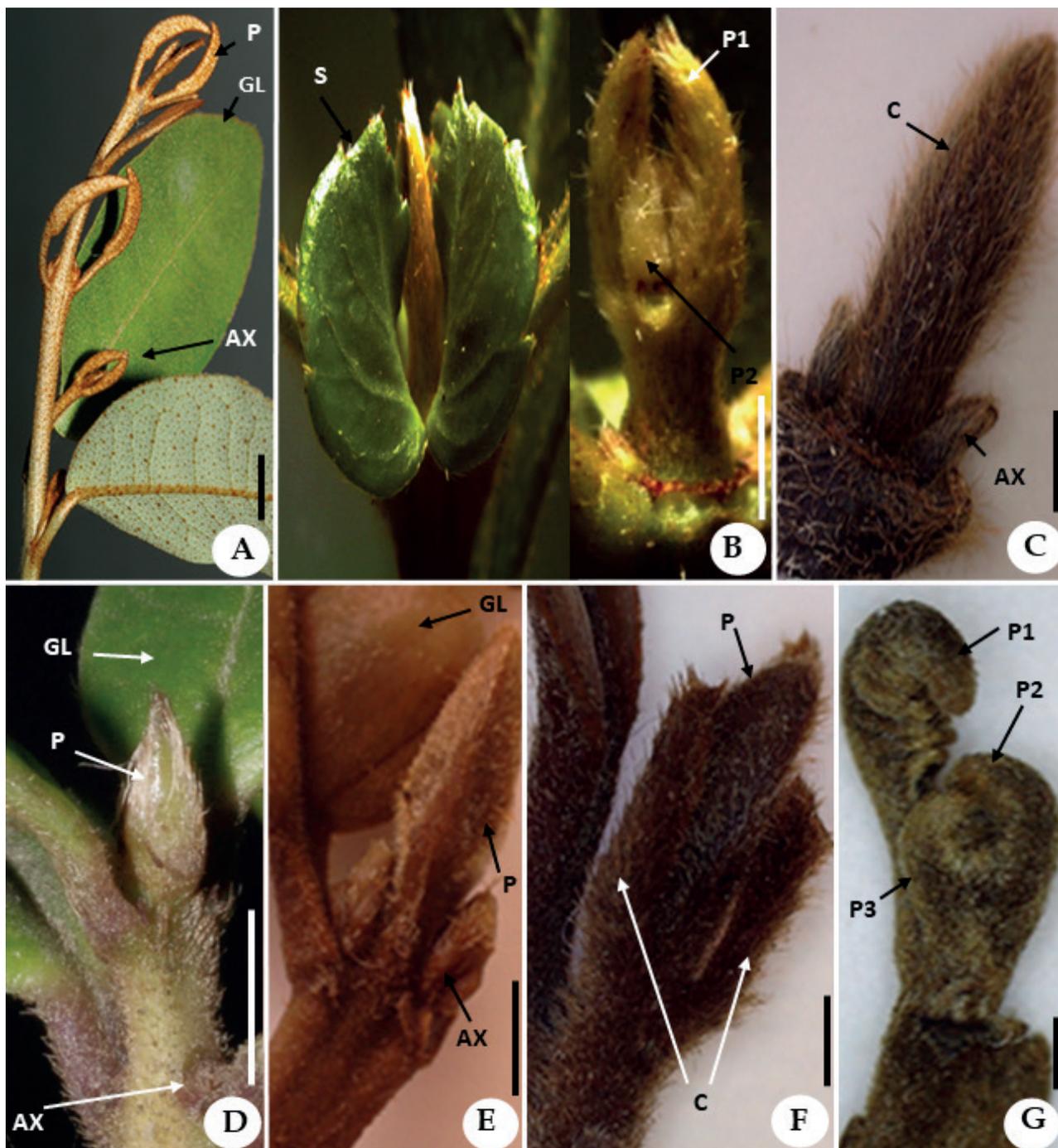
Figure 1. Distal ends of shoots sampled during the resting period: (**A**) *Aextoxicon punctatum*, (**B**) *Caldcluvia paniculata*, (**C**) *Eucryphia cordifolia*, (**D**) *Luma apiculata*, (**E**) *Myrceugenia exsucca*, (**F**) *Persea lingue*, (**G**) *Sophora cassioides*. GL: blade of the most distal green leaf, which marks the proximal limit of the apical bud. AX: axillary bud. S: stipule. P: green-leaf primordia. P1, P2 and P3: proximal, intermediate and distal primordia, respectively. C: cataphyll. Green leaves were removed in (B), (C), (D) and (E) so as to improve the visualization of the apical bud. In the detail of (B) the stipules that were covering green-leaf primordia of the apical bud were removed. Scale: 1 cm.

not extended, so that the only externally evident axillary buds are those of the most basal leaf primordia. The size of stipules decreases notably from the proximal to the distal end of each bud. The number of leaf primordia per apical bud varied between 4 and 16 (Fig. 2).

Each axillary bud of *C. paniculata* is loosely covered by the stipules of the subtending leaf. In addition, these buds have basal cataphylls that correspond to the stipules of the bladeless leaves of the bud's proximal node (Fig. 3B). The green-leaf primordia of the axillary buds are similar to those described for the apical buds of this species. The number of organs per axillary bud was lower than that in apical buds (Fig. 2). Each axillary bud included between 2 and 4 cataphylls and between 2 and 10 green-leaf primordia. Apical buds had a slightly higher mean number of leaves than axillary buds (p=0.04, d.f.= 73).

Eucryphia cordifolia. In *E. cordifolia*, an apical bud consists of an apical meristem and an embryonary shoot with green-leaf primordia arranged following a decussate-opposite phyllotaxis and an outer cover of cataphylls (Fig. 1C). The cataphylls correspond to the connate leaves of one or two nodes, thus forming a hood-like structure that hide the underlying green-leaf primordia of the bud. Cataphylls and green-leaf primordia are densely pubescent. Colleters are present between the base of each green-leaf primordia and its corresponding axillary bud (Fig. 4B). Prefoliation is

revolute. Each green-leaf primordium has a pair of stipules. The preformed internodes of the bud are not extended. Preformed axillary buds can be identified only for the lowermost pair of green-leaf primordia of an apical bud. Between 2 and 6 cataphylls and between 4 and 10 green-leaf primordia per bud were counted (Fig. 2).

Axillary buds resembled apical buds in structure, but included less organs on average (p<0.001, d.f.= 77; Figs. 2 and 3C). The numbers of cataphylls and green-leaf primordia per axillary bud ranged between 2 and 4, and between 2 and 10, respectively.

Luma apiculata. Apical buds of *L. apiculata* are devoid of scale leaves, and consist of an apical meristem and an embryonary shoot with green-leaf primordia arranged following a decussate-opposite phyllotaxis (Fig. 1D). By external observation of the apical bud only the outermost pair of leaf primordia is visible; these primordia are in contact by their borders, thus covering the inner primordia. Primordia are densely pubescent and thick; the most proximal primordia resemble fully developed leaves in outline, though smaller in size, whereas the most distal primordia have a lower level of differentiation. Colleters are present, aligned at both sides of each leaf primordia, flanking the corresponding axillary bud, whenever this is present (Fig. 4C). Axillary buds are evident for the most proximal primordia of an apical bud. Leaf primordia are slightly
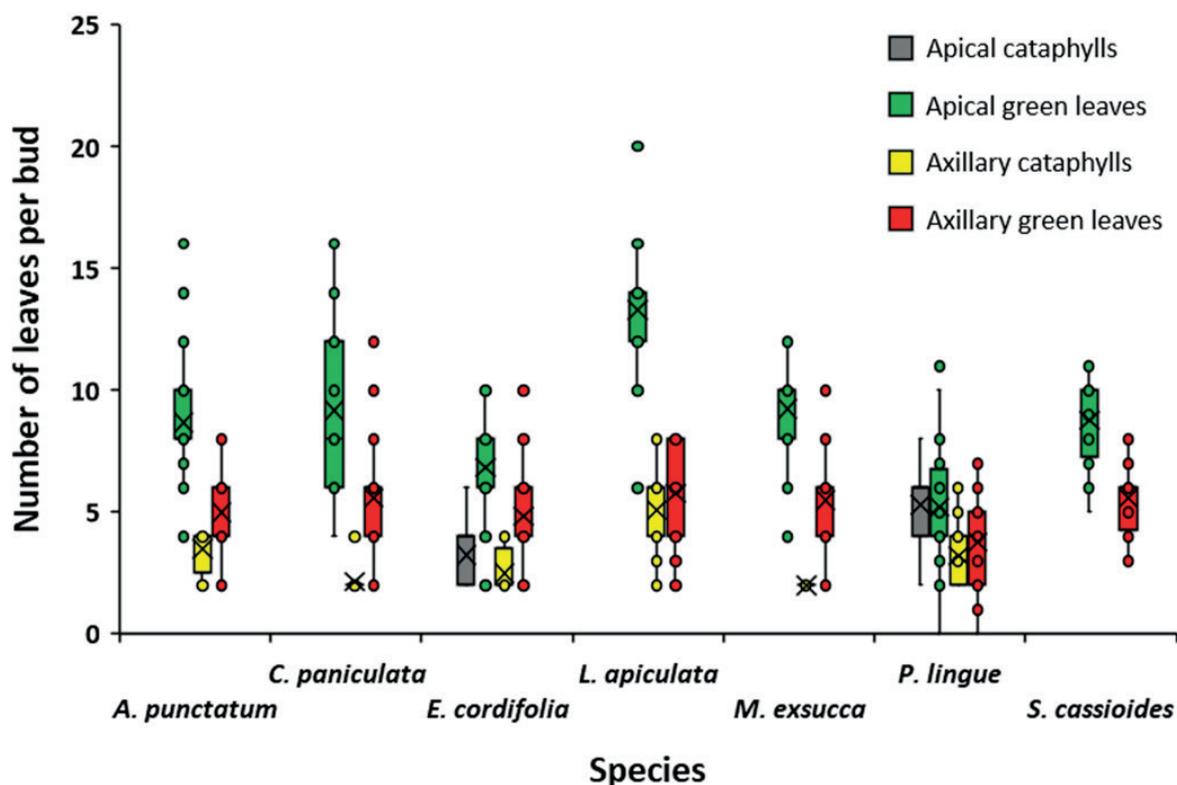
Figure 2. Boxplots of the numbers of cataphylls (gray/yellow) and green-leaf primordia (red/green) per apical and axillary bud of seven tree species native to the South American temperate rainforests.

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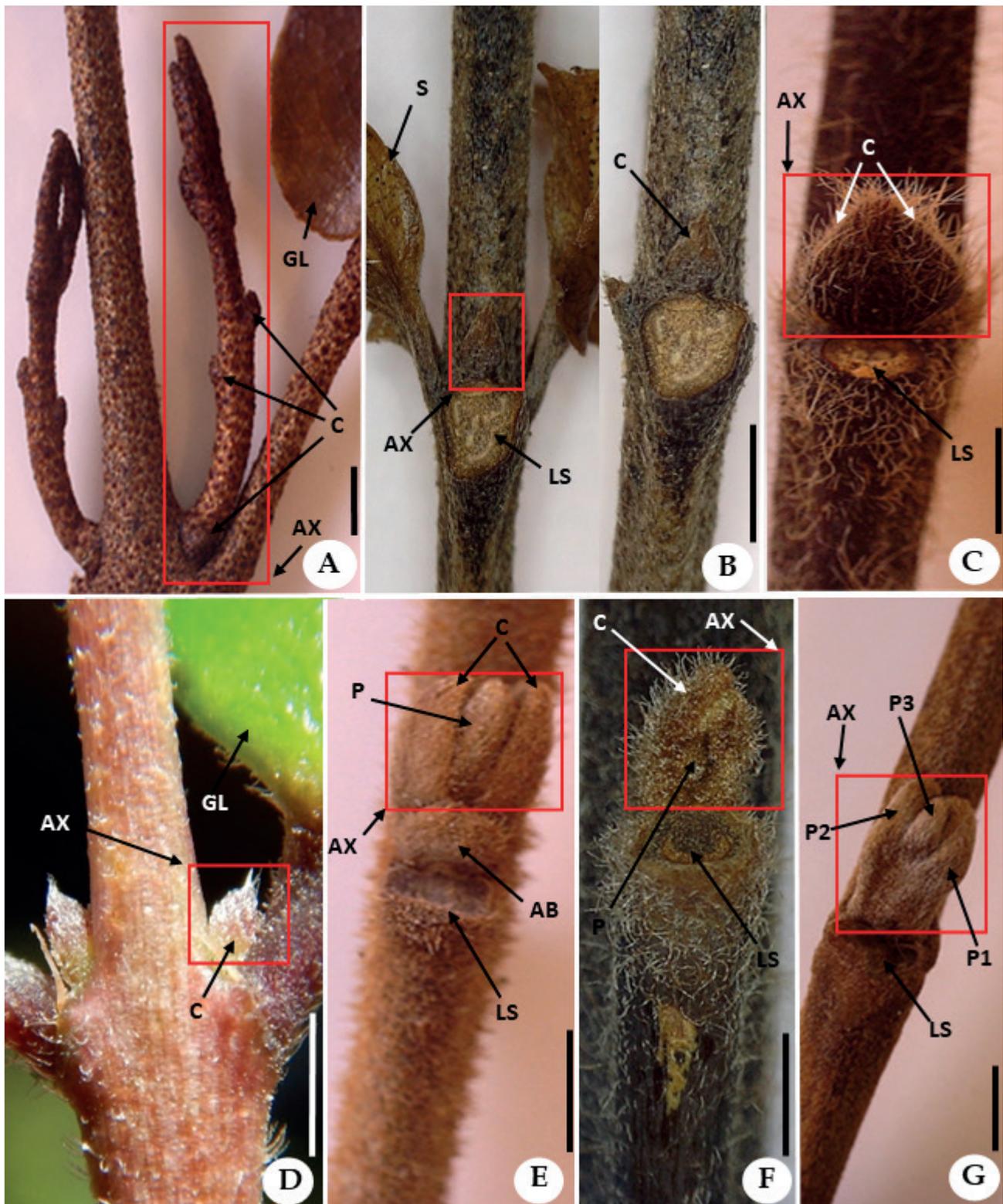
Figure 3. Intermediate portion of shoots sampled for the present study during the resting period: (**A**) *Aextoxicon punctatum*, (**B**) *Caldcluvia paniculata*, (**C**) *Eucryphia cordifolia*, (**D**) *Luma apiculata*, (**E**) *Myrceugenia exsucca*, (**F**) *Persea lingue*, (**G**) *Sophora cassioides*. GL: blade of the green leaf subtending an axillary bud (AX, red rectangle). LS: scar left by the subtending leaf after its removal. S: stipule. P: green-leaf primordia. P1, P2 and P3: proximal, intermediate and distal primordia, respectively. C: cataphyll. AB: accessory bud. Scale: 1 cm.
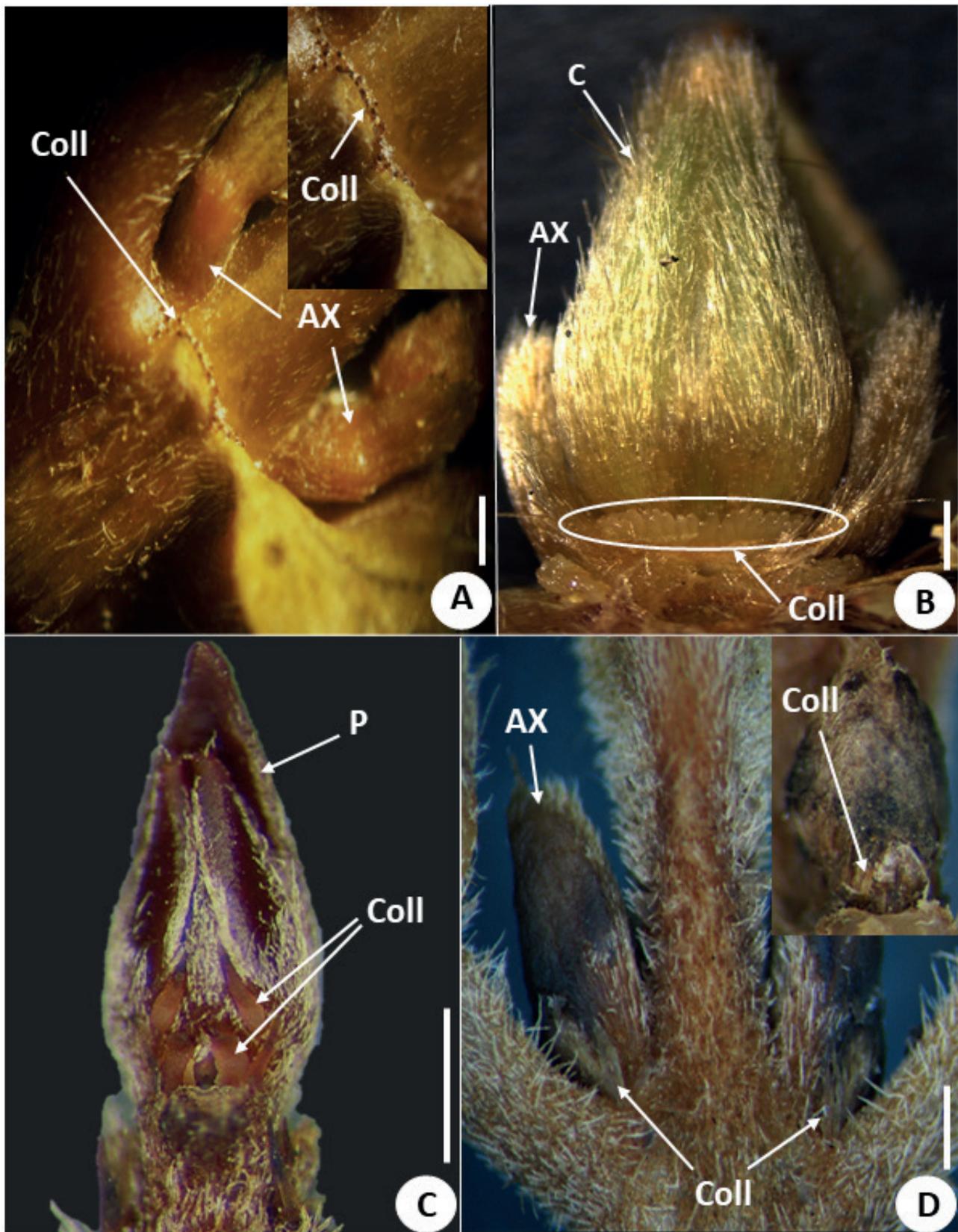
Figura 4. Colleters (Coll) in (**A**) *Caldcluvia paniculata*, (**B**) *Eucryphia cordifolia*, (**C**) *Luma apiculata* and (**D**) *Myrceugenia exsucca*. Inlaid details are shown in A and D. AX: axillary bud. C: cataphyll. P: green-leaf primordia. Scale: 1 mm.

folded to the adaxial surface. Primordial internodes are not extended in these buds. The number of leaf primordia in apical buds of *L. apiculata* varied between 6 and 20 (Fig. 2).

The first two leaves of the axillary buds of *L. apiculata* may vary notably in size. In many cases they are small, tough and scale like, and fall off during budbreak, so that they can be described as cataphylls. In other cases, these leaves are larger, green, delicate (not scale like), and reach the size of small green leaves after budbreak. Other features of the axillary buds of *L. apiculata* are similar to those of apical buds, although the latter consisted of a higher number of organs (Figs. 2 and 3D). The number of primordia per axillary bud varied between 4 and 14 (including cataphylls) and was, on average, lower than that of apical buds (p<0.001, d.f.= 70; Fig. 2). Each leaf of *L. apiculata* has an accessory axillary bud, smaller than the main axillary bud, and placed in a proximal position relative to this (shown for *M. exsucca* in Fig. 3E).

Myrceugenia exsucca. In this species, apical buds are devoid of cataphylls and consist of an apical meristem and an embryonary shoot with a variable number of leaf primordia arranged in a decussate-opposite phyllotaxis (Fig. 1E). Leaf primordia have trichomes and colleters located at the base of each primordium, between this and the stem (in the position of stipules; Fig. 4D). The most proximal primordia are more densely pubescent, harder and larger than the distal ones, which are completely covered by the former. Prefoliation is flat or slightly curved to the adaxial surface of the primordium. These buds have short internodes. Axillary buds can be distinguished only for the most proximal nodes of these apical buds. The number of leaf primordia in the apical buds of *M. exsucca* varied between 4 and 12 (Fig. 2).

Axillary buds of *M. exsucca* are similar to apical buds in structure, except for the fact that the two most proximal leaves categorize as cataphylls because of their small size, dark color and hard consistency (Fig. 3E). The number of primordia per bud varied from 2 to 12 which was, on average, lower than the number of primordia in apical buds (p<0.001, d.f.= 75; Fig. 2). The majority of the leaves of the sampled shoots had an accessory axillary bud between the primary axillary bud and the base of the subtending leaf; the accessory bud was less developed than the primary bud (Fig. 3E).

Persea lingue. An apical bud of *P. lingue* consists of an apical meristem and an embryonary shoot with cataphylls and green-leaf primordia arranged following a spiral-alternate phyllotaxis (Fig. 1F). This bud is densely pubescent and lacks colleters and stipules. The most proximal cataphylls are rather fleshy, smaller than the distal ones and do not cover the underlying green-leaf primordia. Cataphylls and green-leaf primordia are difficult to tell apart due to the gradual change in the size of these leaves from the proximal to the distal end of each bud. The

leaves corresponding to the most proximal nodes of a bud may be described undoubtedly as cataphylls due to their size and consistency, and to the fact that they do not turn foliaceous or green after budbreak. Subsequent leaves, however, while showing cataphyll size and consistency in the bud, may increase in size and turn green and foliaceous during budbreak, which complicates their categorization. Their size after shoot extension is intermediate between those of cataphylls and green leaves. Prefoliation is slightly involute to flat. The primordial internodes of these buds are short and axillary buds are not evident at their nodes. Account taken of the unclear distinction between cataphylls and green-leaf primordia in this species, their numbers per bud may be estimated to be from 2 to 8 and from 2 to 11, respectively (Fig. 2).

Axillary buds of *P. lingue* included at least two cataphylls that covered completely the underlying primordia and fell off after budbreak (Fig. 3F). The number of cataphylls in these buds varied between 2 and 6, and that of green-leaf primordia between 1 and 7, adding a lower total number of organs than apical buds (p<0.001, d.f.= 72; Fig. 2).

Sophora cassioides. In *S. cassioides*, apical buds are devoid of cataphylls; they consist of an apical meristem and a set of green-leaf primordia organized following a spiral-alternate phyllotaxis (Fig. 1G). These buds are densely pubescent and lack stipules and colleters. Primordia decrease in size and degree of differentiation from proximal to distal positions, and axillary buds may be observed for the proximal primordia. Prefoliation is longitudinally involute. Internode primordia are very short, and leaf primordia are tightly packed over one another. The number of leaf primordia observed in these buds varied between 5 and 11 (Fig. 2).

Axillary buds of *S. cassioides* also lack cataphylls, colleters and stipules (Fig. 3G). The leaf primordia corresponding to the most proximal nodes complete their growth as small but foliaceous green-leaves after budbreak. The number of leaf primordia in these buds varied between 3 and 8 which was, on average, lower than the number of primordia of apical buds (p<0.001, d.f.= 75; Fig. 2).

Bud structure as related to parent-shoot size

The relationships between the total number of leaves integrating a bud (adding up cataphylls and green-leaf primordia) and each of the variables describing the size of their parent shoot (i.e. length, basal diameter and number of leaves) varied between species (Tab. 2; Fig. S1, S2, S3). The length of the parent shoot was positively correlated (p<0.05) with the numbers of leaves in apical and axillary buds of all species except *L. apiculata*. These correlations reached a more significant level in the case of *C. paniculata*. Parent-shoot diameter was significantly correlated with the numbers of leaves in apical buds in all species but *A. punctatum* and *C. paniculata*. The correlation between

parent-shoot diameter and the numbers of leaves in axillary buds was not significant only in the case of *A. punctatum*. The number of leaves of the parent shoot was positively correlated with the numbers of leaves in the apical and axillary buds of *E. cordifolia*, *L. apiculata*, *M. exsucca* and *P. lingue*, and was not correlated with the numbers of leaves in the buds of *A. punctatum*, *C. paniculata* and *S. cassioides*.

Table 2. Correlations between the numbers of leaves in apical and axillary buds and the size of their parent shoots for seven tree species from the temperate rainforests of South America. Pearson's correlation coefficients and their significance levels are provided. *** p<0.001, ** p<0.01, * p<0.05, ns p>0.05.

Species	Type of bud	Parent shoot size descriptor variable		
		Length	Diameter	Number of leaves
A. punctatum	Apical	0.550 **	0.052 ns	0.284 ns
	Axillary	0.323 *	0.196 ns	0.120 ns
C. paniculata	Apical	0.603 ***	0.205 ns	0.160 ns
	Axillary	0.588 ***	0.433 **	0.295 ns
E. cordifolia	Apical	0.388 *	0.426 **	0.231 *
	Axillary	0.399 *	0.529 **	0.372 *
L. apiculata	Apical	0.027 ns	0.360 *	0.421 *
	Axillary	0.270 ns	0.558 ***	0.725 ***
M. exsucca	Apical	0.353 *	0.368 *	0.377 *
	Axillary	0.566 ***	0.541 **	0.504 **
P. lingue	Apical	0.467 **	0.410 *	0.441 **
	Axillary	0.532 **	0.561 **	0.604 ***
S. cassioides	Apical	0.520 **	0.411 **	0.147 ns
	Axillary	0.391 *	0.408 **	0.218 ns

Discussion

Bud types in trees from the temperate Patagonian rainforests

For a long time, the presence of an external cover of cataphylls in the apical buds of woody plants has been considered a trait typical of woody species from temperate or cold regions, and their absence a typical trait of species from tropical or subtropical regions (Kozlowski 1971; Bell 2008). Challenges to this view are provided both by the presence of buds with cataphylls in the latter regions (e.g. Melo *et al.* 2021), and by the fact that the cataphylls of a scaly bud may not be covering the underlying primordia, so that labelling a bud as scaly (following the traditional definition) would not imply that its primordia are protected. For this reason, it has been proposed that the term "scaly bud" be applied only to those buds in which the green-leaf primordia are completely covered by cataphylls (Schoonderwoerd & Friedman 2021).

In the present study inter- and intra-specific variations in bud structure were found for seven trees species of the Patagonian rainforests. Two of them, *E. cordifolia* and *P. lingue*, develop apical buds that, due to the presence of cataphylls, may be labelled as scaly buds following the traditional classification. But according to the new perspective, only those of *E. cordifolia* may qualify as scaly buds since the apical buds of *P. lingue* have very small, unwrapping cataphylls, so that they may be better described as naked buds.

Due to their lack of cataphylls, the apical buds of *A. punctatum*, *L. apiculata*, *M. exsucca*, *S. cassioides* and *C. paniculata* may be labelled as naked buds (Koriba 1958; Hallé *et al.* 1978; Nitta & Ohsawa 1998; Font Quer 2009; Schoonderwoerd & Friedman 2021). However, several structural differences among them are evident. In *L. apiculata*, *M. exsucca* and *S. cassioides*, the proximal leaf primordia play a bud-covering function during the resting period and an assimilation function after budbreak, although they reach a smaller size than more distal leaves derived from the same buds. The underlying primordia, on the other hand, are never exposed before budbreak. It would be interesting to determine whether those green leaves that have played a covering role prior to extension differ anatomically or physiologically from those that were never exposed to unfavourable environmental conditions. In the apical buds of *C. paniculata* each pair of leaf primordia is surrounded by stipules corresponding to the preceding pair of primordia. Bud protection by means of stipules of green-leaf primordia has also been observed in Nothofagaceae and Betulaceae (Barthélémy *et al.* 1999; Souza *et al.* 2000). This kind of bud has been described as "unexposed naked bud"; they do not have cataphylls *sensu stricto*, but the primordia are completely covered by appendages derived from the green-leaf primordia (Schoonderwoerd & Friedman 2021). The apical buds of *A. punctatum* are different from all others observed in this study since the majority of their constituent primordia are fully exposed. These buds have all the attributes of naked buds in its strictest sense, as the extension of their internodes determines that the proximal primordia do not come into contact with each other. In contrast, the axillary buds of *A. punctatum* should be considered naked buds with unwrapping scales (Schoonderwoerd & Friedman 2021) since their cataphylls have no covering role.

Comparison of structure and composition between apical and axillary buds

Intra-specific structural differences between apical and axillary buds have been little investigated so far. Axillary buds have a major relevance in the annual growth of tree species with a predominantly sympodial construction (e.g. Macdonald *et al.* 1984; Barthélémy *et al.* 1999; Sabatier & Barthélémy 2001). This study showed clear qualitative differences between the two types of buds for most of the

species analysed, especially due to the scaly nature of the proximal leaves of the axillary buds, i.e. the prophylls. The common occurrence of wrapping scaly prophylls could increase the tolerance of axillary buds to unfavourable conditions, and be one of the reasons why axillary buds are less prone to death than the apical bud of the same shoot (Macdonald *et al.* 1984, Nicolini 1998; Puntieri *et al.* 1998; Stecconi *et al.* 2000; Souza *et al.* 2000). Intra-specific structural contrasts between apical and axillary buds could be relevant for the persistence of a species in a given environment by making resprouting more likely (Clarke *et al.* 2013). *Sophora cassioides* seems to be exceptional in this regard, as even the prophylls are green leaf primordia.

The number of bud constituents (i.e. its preformation) provides an idea of the eventual size that the shoot derived from a bud may reach, and the comparison of preformations between apical and axillary buds in close proximity indicates the degree of apical dominance of a plant's axis (Guédon *et al.* 2006). The higher numbers of preformed leaves in apical than in axillary buds in all but one of the species under survey indicate that, at a young-adult stage, these species exhibit a clearly hierarchical architecture, with the growth prevalence of major axes (derived from apical buds) over smaller axes (derived from axillary buds). This may not seem to hold true for *A. punctatum*, in which the mean numbers of organs in apical and axillary buds were alike. However, account must be taken of their differences in terms of organ morphology: all constituting organs of apical buds in this species were green-leaf primordia, whereas some cataphylls were present in its axillary buds. It may be proposed that the higher number of preformed organs in *L. apiculata* than in the other species could be related to differences in leaf size (Tab. 1). This view is supported by previous studies on Patagonian species: several species of *Nothofagus* with leaves similar in size to those of *L. apiculata* also resembled this species in terms of preformation (Puntieri *et al.* 2000; 2002a; b; Souza *et al.* 2000; Guédon *et al.* 2006), while several Proteaceae species with larger leaves had lower numbers of preformed leaves in their apical buds (Magnin *et al.* 2012). At a broader geographical scale, it has been shown that leaf size and leaf number per unit of stem length are, in general terms, negatively related (Yang *et al.* 2008; Huang *et al.* 2016).

The quantitative differences between the preformations of apical and axillary buds that were recorded here are in contrast with the similarities that, in this regard, were found previously for three Patagonian *Nothofagus* spp. (Puntieri *et al.* 2000; 2002a; Souza *et al.* 2000; Stecconi *et al.* 2000). This divergence could have an architectural basis: *Nothofagus* species have a clear tendency towards the building of a trunk by the superposition of sympodial units (Troll's model), with high frequencies of apical deaths and the subsequent development of relay shoots from axillary buds. In contrast, most of the species included in the present study seem to follow architectural patterns in which the main axes are monopodial (J. Puntieri, unpubl. data). In contrast, *S. cassioides* follows, like *Nothofagus* spp., Troll's architectural model (Tomlinson 1978).

Bud composition and parent-shoot size

The results of the present study indicate that, in general terms, the number of organs composing both apical and axillary buds are positively related to the size of the shoot on which these buds developed, which confirms the results of studies on other species (Puntieri *et al.* 2002b). The length and basal diameter of a shoot's stem were more frequently related to bud preformation than the number of nodes of that shoot. This may be explained by considering that stem volume, which is mostly dependent on its diameter and length, would have a strong influence on shoot mass and, therefore, on the amount of storage tissues (Sun *et al.* 2006). The extent of preformation that was not explained by parent-shoot size could be related to inter- and intra-specific variations in terms of resource allocation to each developing bud from shoots other than the bud's parent shoot. An alternative but not exclusive explanation for the variations in bud preformation may involve the ontogenetic stage of the axes from which parent shoots were sampled. Even though the trees selected for the present study were not notably different in terms of size, there may have been differences among them in the ontogenetic stages they were expressing, which are known to have significant effects on preformation (Puntieri *et al.* 2000; 2002b).

Colleters and trichomes in buds

The degree of protection provided to the leaf primordia constituting a bud may be determined not only by the presence or not of scaly leaves in that bud, but also by their anatomical and/or chemical features (Korth *et al.* 2006). Among these features are the development of trichomes or colleters capable of providing insulation to the fragile organs from negative environmental influences (Silva *et al.* 2019). In four of the species investigated here, *L. apiculata*, *M. exsucca*, *C. paniculata* and *E. cordifolia*, colleters were observed (Fig. 4). It may be hypothesized that their secretions may insulate bud primordia from moisture and temperature extremes, or provide defense against herbivory, as indicated for other species (Levin 1973; Lersten 1974; Williams *et al.* 1980; Thomas & Dave 1989; Thomas 1991; Dalin *et al.* 2008; Beck 2010; Judkevich *et al.* 2017). For the species studied here no information is available on the protective function of their colleters which, whenever present, were close to the leaf bases and/or on the inner side of the stipules. It has been proposed that colleters could be more frequent in species with buds lacking the protection provided by specialized leaves (García *et al.* 2006), but our current data do not fully support this hypothesis, as the buds of *E. cordifolia* have both a tight cover of cataphylls and a notable stock of colleters. Moreover, the apical buds

of *Nothofagus* spp. have resinous colleters and wrapping scaly stipules (Barthélémy *et al.* 1999).

Leaf and stem primordia in buds of *A. punctatum* and *S. cassioides* are densely covered with non-glandular trichomes. In the case of *A. punctatum*, whose leaf primordia are completely exposed and separated by rather long primordial internodes, the presence of peltate trichomes (lepidote pubescence) all over the buds could mean an essential barrier against winter conditions and herbivory (Bell 2008; Dalin *et al.* 2008). A similar type of pubescence is present in *Elaeagnus pungens* (Elaeagnaceae) and in *Atamisquea emarginata* (Capparaceae), neither of them related to Aextoxicaceae (Angiosperm Phylogeny Group *et al.* 2016), both of which have naked apical buds *sensu stricto* with rather long primordial internodes (J. Puntieri, unpubl. data). On the other hand, the dense pubescence of simple trichomes in *S. cassioides* buds resembles that observed for Patagonian species of Proteaceae that are also devoid of cataphylls (Magnin *et al.* 2012). The protective role of trichomes during the unfavourable period of the year might then be inferred. This mechanism could be more relevant in those species that develop low numbers of large leaves per shoot: in these species the structural specialization of one or several leaves to develop a protective cover for the buds might entail a large loss of assimilation tissues (Magnin *et al.* 2012).

Bud structure, scales and plant evolution

The plant organs described as apical and axillary buds are little considered in plant descriptions, partly because of their small size. However, their structure is complex and fundamental to the survival of woody plants during the unfavourable period of the year. The idea that scale leaves (= cataphylls) could be essential for the survival of plants living in seasonal climates may be challenged on two grounds. On the one hand, defining what attributes a leaf must have in order to qualify or not as a scale leaf is not simple: some leaves may be partially scaly, partially assimilative (as in *Nothofagus* and *Betula*; Barthélémy *et al.* 1999), and others may be scaly but not have a covering function (as in *P. lingue*, this study). It is likely that the term "scale leaf" embraces too many leaf types for it to be useful in bud description. On the other hand, the persistence of a plant species in a particular habitat does not mean that the species is facing the selective pressures that favoured the evolution of its current morphological features: some characteristics of a plant's buds may have been critical to its survival under conditions that are not those the plant faces at present. The flora at a particular region reflects the interaction of biogeographical, geological and historical factors that result in the coexistence of species with contrasting traits. It becomes clear that the traditional classification of buds into scaly and naked buds is inadequate and that much more information on bud structure needs to be gathered to understand variations in bud structure at a worldwide scale (see Schoonderwoerd & Friedman 2021).

Conclusions and perspectives

Buds may include one or several means of meristem protection, depending on the evolutionary lineage of the species. The presence of colleters is not restricted to those buds lacking an outer cover of cataphylls. The variations in bud structure found here for seven tree species typical of the temperate rainforests of southern South America provide little support to the idea of adaptive convergences among these species. It has been suggested that the development of scaly buds may be related to resistance to seasonal periods of drought rather than to low temperatures (Schoonderwoerd & Friedman 2021). Following this idea, the high frequency of apical buds devoid of cataphylls among the Patagonian tree species could be related to their evolution in environments unconstrained by low humidity (see Barreda & Palazzesi 2007; Le Roux 2012; Barreda & Palazzesi 2014), and imply that these species could suffer high rates of apical bud deaths in case the predicted low precipitation levels in spring and summer for the coming decades were fulfilled (IPCC 2007; Barros *et al.* 2015; Cabré *et al.* 2016; Ruscica *et al.* 2016). Nonetheless, the high frequency of scaly axillary buds could set the possibility of plant recovery after apical deaths.

Supplementary material

The following online material is available for this article:
Figure S1 - Relationship between parent-shoot length and total number of organs (including cataphylls and green-leaf primordia) for apical and axillary buds of (A) *Aextoxicon punctatum*, (B) *Caldcluvia paniculata*, (C) *Eucryphia cordifolia*, (D) *Luma apiculata*, (E) *Myrceugenia exsucca*, (F) *Persea lingue* and (G) *Sophora cassioides*. Regression lines are shown wherever Pearson's correlation coefficients were significant. *** p<0.001, ** p<0.01, * p<0.05.
Figure S2 - Relationship between the basal diameter of the parent shoot and the total number of organs (including cataphylls and green-leaf primordia) for apical and axillary buds of (A) *Aextoxicon punctatum*, (B) *Caldcluvia paniculata*, (C) *Eucryphia cordifolia*, (D) *Luma apiculata*, (E) *Myrceugenia exsucca*, (F) *Persea lingue* and (G) *Sophora cassioides*. Regression lines are shown wherever the Pearson's correlation coefficient was significant. *** p<0.001, ** p<0.01, * p<0.05.
Figure S3 - Relationship between the number of leaves of the parent shoot and the total number of organs (including cataphylls and green-leaf primordia) for apical and axillary buds of (A) *Aextoxicon punctatum*, (B) *Caldcluvia paniculata*, (C) *Eucryphia cordifolia*, (D) *Luma apiculata*, (E) *Myrceugenia exsucca*, (F) *Persea lingue* and (G) *Sophora cassioides*. Regression lines are shown wherever the Pearson's correlation coefficient was significant. *** p<0.001, ** p<0.01, * p<0.05.

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