CSIRO PUBLISHING

Australian Journal of Botany, 2014, **62**, 150–163
http://dx.doi.org/10.1071/BT13301

Functional groups in Lychnophorinae (Asteraceae: Vernonieae) based on morphological and anatomical traits

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Abstract. The Lychnophorinae subtribe (Asteraceae: Vernonieae) occurs mainly on the Central Brazilian Plateau, especially in areas characterised by intense solar radiation, large daily temperature fluctuations, water scarcity during autumn and winter, and frequent fire. However, a few species of Lychnophorinae, such as *Blanchetia heterotricha* DC., are not restricted to the Cerrado Domain, but can live in open and humid areas of borders of the Atlantic Forest. To understand the mechanisms of Lychnophorinae adaptation to these environmental conditions, habit, leaf and stem functional traits from 12 species were analysed. Leaf and stem samples were processed and stained using standard plant-anatomy methodology. A comparative analysis of functional groups was performed by principal coordinates analysis, which was applied to two matrices of 30 qualitative and five quantitative traits. The multivariate analysis based on qualitative traits highlighted the following four functional types that might be linked to ecological conditions: Group I adapted at open and humid areas of borders of the Atlantic Forest; Group II, with features mostly related to foggy habitats; Group III, exhibiting traits quite adapted to the seasonally dry and variable conditions of the *campos rupestres*; and Group IV, showing the most specialised morphological and anatomical traits, which might be related to the extreme conditions of the *campos rupestres*.

Additional keywords: Cerrado Domain, Compositae, plant adaptation, plant anatomy, plant functional types.

Received 18 December 2013, accepted 10 April 2014,

Introduction

The Lychnophorinae subtribe comprises 18 genera and 104 species, including shrubs, treelets and rarely herbs. It is largely restricted to Brazil, mainly in the Cerrado Domain in areas of *campos rupestres* (rupestrian fields) and savannas of the Central Plateau (Fiaschi and Pirani 2009; Batalha 2011). The most species-rich area is in the Espinhaço Range Mountains in the states of Minas Gerais and Bahia (Loeuille 2011).

Plant species that occupy the Cerrado Domain, especially areas of *campos rupestres*, are often subject to intense solar radiation, great fluctuations in daily temperature, water scarcity during autumn and winter, and the occurrence of fire (Goodland and Ferri 1979; Rizzini 1997; Ribeiro and Walter 2008). To survive in these extreme environmental conditions, plants have developed several structural adaptations, such as well developed underground systems, conspicuous indumenta, the presence of crypts and hypodermal tissue in leaves, and densely imbricated leaves (Vogelmann and Martin 1993; Jordan *et al.* 2005; Hassiotou *et al*. 2009; Appezzato-da-Glória and Cury 2011; Semir *et al*. 2011; Clarke *et al*. 2013). The peculiar ecological conditions of *campos rupestres* probably promoted intense speciation. This could be one reason for the endemism and isolation found in species and genera of this habitat (Giulietti *et al*. 1997; Mansanares *et al*. 2002, 2007; Rapini *et al*. 2008; Echternacht *et al*. 2011). The Espinhaço Range Region is characterised by a high number of micro- and macroendemic species of which 15 Lychnophorinae taxa are considered rare (Nakajima *et al*. 2009) and 18 are endangered (Fundação Biodiversitas 2005). Interestingly, whereas all late diverging taxa of Lychnophorinae are restricted to the savanna and *campos rupestres*, some of the early diverging taxa (such as *Albertinia brasiliensis* Spreng., *Blanchetia heterotricha* DC., and *Gorceixia decurrens* Baker) are not restricted to the Cerrado Domain, but also occur in very different habitats, namely, the open areas of the Atlantic Forest Domain (Loeuille 2011), where rains are frequent and fire absent (INMET 2013).

The principal aim of this research was to study plant-environment adaptations of species to the peculiar environmental conditions of *campos rupestres*. For this purpose, habit, leaf and stem functional traits were investigated in 12 Lychnophorinae species, 11 which live exclusively in *campos rupestres* and one which is also able to colonise the open areas of the Atlantic Forest. The inclusion of the latter species could provide important contributions to the understanding of the responses of Lychnophorinae species to different environments. It is well known that plant functional traits determine how plants respond to environmental conditions (e.g. Walker 1992; Noble and Gitay 1996; Díaz and Cabido 1997; Díaz *et al*. 2004; Pérez-Harguindeguy *et al*. 2013). In other words, plant functional types can be defined as groups of plant species sharing similar functioning at the organism level, similar responses to environmental factors, and/or similar roles in ecosystems or biomes (Cornelissen *et al*. 2003). In the present study, we explored the relationship between some Lychnophorinae species and the *campos rupestres* habitat, testing 35 morphological and anatomical functional traits as putative adaptations to environmental conditions. In particular, we attempted to answer the following questions: (1) which functional traits among the 35 selected in this research could be interpreted as an adaptive character to the *campos rupestres* and (2) are the plant functional types identified among the Lychnophorinae species related to environmental conditions?

Materials and methods

Environmental characterisation

The plants in the present study were collected from two different habitats in Brazil: the *campos rupestres* of the Cerrado Domain, along the Espinhaço Range of Mountains in the states of Minas Gerais and Bahia, considered the centre of diversity of Lychnophorinae (Loeuille 2011), and an open area of secondary forest in the Atlantic Forest Domain (Table 1, Fig. 1).

The Cerrado Domain, also known as the Brazilian savanna, is characterised by water scarcity during autumn and winter, when fires can occur. The vegetation of this area is a complex mosaic of different vegetation types such as forest, savannah, and field formations (Ribeiro and Walter 2008) including the *campos rupestres* with occasional rocky outcrops. The *campos rupestres* in this region experience a mesothermic to tropical climate (Peel *et al*. 2007) with average annual temperatures between 17.4°C and 19.8°C (Giulietti *et al*. 1997). The rainy season lasts 7–8 months (from mid spring to mid autumn), with precipitation of 850–1400 mm; whereas the dry period may last 3–4 months (corresponding to winter), with precipitation of 50–200 mm (Giulietti *et al*. 1997; Gontijo 2008; INMET 2013). The *campos rupestres* usually occur at altitudes above 900 m, in areas characterised by constant winds, extreme temperature variations, warm days and cold nights. In some places, fog occurs, mainly at the highest altitudes of the state of Minas Gerais. The soils are acidic and nutrient-poor, with low organic matter content, and originate from the decomposition of quartzite and arenite in the cracks of outcrops (Ribeiro and Walter 2008). They may have an influence, together with water availability or dispersal and recruitment limitations, about the occurrence of species in the communities of Cerrado Domain (Dantas and Batalha 2011). In general, the availability of water is limited since the rainwater seeps quickly into the rivers because of the shallowness and water retention of the soil (Ribeiro and Walter 2008).

The open areas bordering the secondary forests of the Atlantic Forest Domain of north-eastern Brazil experience a tropical humid climate (Peel *et al*. 2007). These areas occur at low altitudes and are characterised by temperatures between 20°C and 30°C, an annual average precipitation of 1350 mm, regular rain and the absence of fire (INMET 2013).

Plant material

Leaves and stems were sampled from adult plants belonging to 12 species of Lychnophorinae chosen to represent the major clades in the subtribe (Loeuille 2011). Voucher specimens were deposited in the Herbarium of Sao Paulo University (SPF) (Table 1).

Analyses of three adult individuals for each species were conducted. Samples of leaves and stems were collected for structural analysis and for histochemical tests. A part of each sample was fixed in Karnovsky solution (Karnovsky 1965; modified by using phosphate buffer, pH 7.2) and placed into a vacuum pump to remove air from the tissue. Fixed samples were

Table 1. A list of Lychnophorinae species used in the study

Blanchetia heterotricha is more frequently distributed at the borders of secondary forests of the Atlantic Forest; otherwise, the other 11 species of Lychnophorinae are endemic from *campos rupestres* in areas characterised by constant winds, extreme temperature variations, warm days and cold nights

Lychnophorinae species	Species code	Geographic coordinates	Altitude (m)	Voucher
Anteremanthus hatschbachii H.Rob.	Anthat	16°35′37.6″S, 42°54′07.7″W	673	Loeuille *et al*. 533 (SPF)
Blanchetia heterotricha DC.	Blahet	12°54′11.1″S, 39°12′06.6″W	193	Loeuille *et al*. 680 (SPF)
Chronopappus bifrons (DC. ex Pers.) DC.	Chrbif	18°23′49.9″S, 43°21′21.0″W	1819	Lusa *et al*. 63 (SPF)
Eremanthus polycephalus (DC.) MacLeish	Erepol	18°11′52.0″S, 43°37′33.0″W	1141	Loeuille *et al*. 531 (SPF)
Heterocoma gracilis Loeuille, J.N.Nakaj. & Semir.	Hetgra	18°07′42.1″S, 43°22′40.5″W	1039	Lusa *et al*. 61 (SPF)
Lychnophora tomentosa (Mart. ex DC.) Sch. Bip.	Lyctom	18°12′52.9″S, 43°35′44.1″W	1362	Loeuille *et al*. 528 (SPF)
Lychnophora diamantinana Coile & S.B.Jones	Lycdia	18°11′52.5″S, 43°37′33.1″W	1141	Loeuille *et al*. 530 (SPF)
Lychnophora triflora (Mattf.) H.Rob.	Lyctri	13°31′33.4″S, 41°57′55.5″W	1849	Loeuille *et al*. 673 (SPF)
Minasia scapigera H.Rob.	Minsca	18°12′52.9″, 43°35′44.1″	1362	Loeuille *et al*. 529 (SPF)
Paralychnophora bicolor (DC.) MacLeish	Parbic	13°31′33.7″S, 41°57′56.7″W	1876	Loeuille *et al*. 672 (SPF)
Piptolepis monticola Loeuille	Pipmon	18°23′53,8″S, 43°19′33,0″W	1630	Lusa *et al*. 62 (SPF)
Prestelia eriopus Sch. Bip.	Preeri	19°17′28.1″S, 43°36′01.5″W	1131	Loeuille *et al*. 524 (SPF)

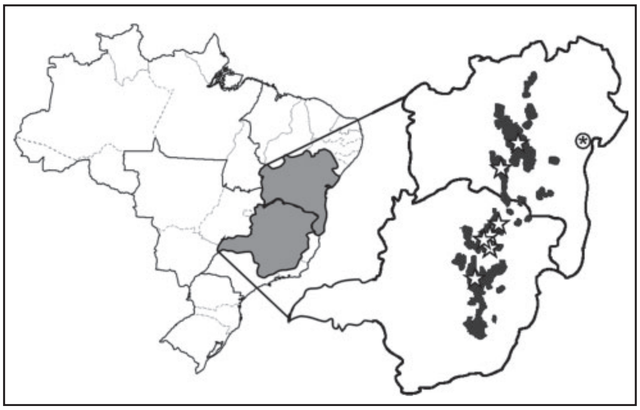

Fig. 1. The location of Lychnophorinae collection sites (white stars) in the states of Minas Gerais and Bahia, Brazil, along the Espinhaço Mountain Range (shaded) and in an open area of the Atlantic Forest (asterisk in white circle).

dehydrated in an ethanol series, embedded in hydroxyethyl methacrylate Leica Historesin (Heraeus-Kulzer, Hanau, Hesse, Germany) according to the manufacturer's instructions, and sectioned with steel knives at 5–7 µm thickness on a Leica rotary microtome. For structural analysis, the sections were stained with toluidine blue 0.05% in citrate-phosphate buffer, pH 4.5 (Sakai 1973), and mounted in synthetic resin Entellan Merck (Darmstadt, Hesse, Germany).

For the histochemical analyses, fresh material was tested for terpenoids using NADI reagent (David and Carde 1964) and for flavonoids using aluminium chloride to induce fluorescence under UV light (Charrière-Ladreix 1976). Additionally, the embedded material – as described above – was tested for pectic carbohydrates using ruthenium red 0.01% (Chamberlain 1932) and for general phenolic compounds using ferric chloride 3% (Johansen 1940). Observations and records were made using a Leica DM LB microscope equipped with Dialux epifluorescence (Leica Microsystems, Heerbrugg, Switzerland) coupled to a Leica DC 300F video camera. To visualise cellulosic thickening of cell walls, histological slides were observed under polarised light using a Zeiss Axioplan Microscope equipped with crossed polarisers (Carl Zeiss Jena GmbH, Jena, Germany) coupled to a Canon EOS 60D (Tokyo, Japan) digital camera.

Trait measurements

Thirty qualitative and five quantitative functional traits regarding habit, leaf and stem morpho-anatomy, and presence or absence of metabolic classes were assessed for 12 species (Table 2). These traits were selected because they were considered good correlates of plant function; i.e. responsible for responses or effects at the ecosystem or biome scale (Cornelissen *et al*. 2003). Anatomic features were obtained from fully expanded leaves, which were analysed in the median region of the blade and stems (from apical to median regions of the axis or of the branches). The number of evaluated nodes varied among the species (see Appendix 1), depending on length of internodes, differentiation of secondary structure, and thickening of branches. Because indumentum varied with leaf age, partly expanded and fully expanded leaves were considered for qualitative traits related to indumentum form and histochemistry. Partly expanded leaves were sampled at an intermediate stage between primordia and fully expanded leaves. Quantitative measurements were obtained from three fully expanded leaves from three individuals of each species. The leaf thickness was measured from adaxial to abaxial epidermis on leaf cross-sections. To assess midrib thickness, measurements were obtained from sections of the middle part of the leaf blade (nine measurements for each species). To assess interneval thickness, measurements were obtained from three sectors of the middle part of the leaf blade without vascular bundles, where only the parenchymal tissues were present (27 measurements for each species). Furthermore, epidermal cuticle and external cell wall thickness were measured on the same interneval regions. Functional traits were assessed using a Leitz DIAPLAN light microscope (Wetzlar, Germany) coupled to a DFC 420 Leica video camera (Leica Microsystems); the Leica Application Suite Version 3.4.0 (Leica Microsystems) was used.

Statistical analyses

To identify major trends in the grouping of the Lychnophorinae according to their morphological and anatomical similarities, Principal Coordinates Analysis (PCoA) using GINKGO software (a statistical module of the VEGANA package, De Cáceres *et al*. 2003) was applied to two matrices: a matrix of 30 qualitative traits by 12 taxa (Appendix 2), and a matrix of five quantitative traits by 12 groups (Appendix 3). For the two matrices, we used the Bray–Curtis index to obtain similarity matrices. The quantitative matrix was square-root transformed before the Bray–Curtis measure was applied. PCoA is an ordination method that enables visualisation of the position of the samples in relation to each other in reduced numbers of dimensions (Podani 2007).

A preliminary analysis indicated which traits were linearly dependent; subsequent analyses therefore used matrices that were reduced to independent traits only. This ordination identified functional groups among the species analysed.

Results

Multivariate analyses using qualitative and quantitative data enabled the distinction of functional groups among Lychnophorinae species (Figs 2, 3, Tables 3, 4). The PCoA of qualitative data indicated the presence of four clearly distinguished functional groups (Fig. 2, Table 3). The first PCoA axis (PCoA1) explained ~65% of total variation, whereas the second axis (PCoA2) accounted for ~17% of total variation. Six traits were strongly correlated with the first two axes (Pearson's coefficient >0.8): habit type, overlapping leaves, the presence of sclereids and crypts in leaves, the presence of chloroplasts in the leaf epidermis and the distribution of stomata in leaves. *Blanchetia heterotricha* (Group I) was at the lower end of the first axis (Fig. 2), because of its subshrub habit and absence of overlapping leaves, absence of crypts and sclereids in leaves (Table 3); *Minasia scapigera* and *Prestelia eriopus* (Group IV) were at the opposite end (Fig. 2) with rosulate habit, where all leaves were overlapped and with the presence of sclereids in leaves (Table 3). Species of Group II were distinguished from the other groups by leaves without crypts

Table 2. Traits measured in Lychnophorinae species

Scales of measurement were originally binary (bin), categorical (cat) or continuous (cont). AB, abaxial leaf face; AD, adaxial leaf face

Code	Trait	Type of variable	Description of classes in matrix	Ecological correlate
1	Leaf phenology	cat	1 – evergreen; 2 – semi-deciduous; 3 – deciduous	Nutrient- and water-use strategy (Pérez-Harguindeguy *et al*. 2013)
2	Leaf rolling	cat	1 – absent; 2 – present	Drought resistance (Navarro *et al*. 2006)
3	Overlapping leaf	cat	0 – absent; 1 – present in apex; 2 – present in adult stem	Protection against fire (Givnish *et al*. 1986; Smith and Young 1987); increase the volume of impounded rainwater (Givnish *et al*. 1986)
4	Crypts in leaf	bin	0 – absent; 1 – present	Leaf transpiration and water loss reduction (Turner 1994; Bosabalidis and Kofidis 2002; Rotondi *et al*. 2003; Jordan *et al*. 2008); carbon dioxide diffusion from abaxial to adaxial surface (Hassiotou *et al*. 2009)
5a	Indumentum in partly expanded leaf	cat	0 – absent; 1 – low; 2 – high	Leaf transpiration and water loss reduction (Turner 1994; Bosabalidis and Kofidis 2002; Rotondi *et al*. 2003; Jordan *et al*. 2008)
5b	Indumentum in fully expanded leaf	cat	0 – absent; 1 – low AB; 2 – high AB; 3 – low AB/AD; 4 – high AB/AD	Leaf transpiration and water loss reduction (Turner 1994; Bosabalidis and Kofidis 2002; Rotondi *et al*. 2003; Jordan *et al*. 2008)
5c	Coating stem	cat	0 – remaining leaf bases; 1 – short trichomes; 2 – high trichomes	Protection against fire (Givnish *et al*. 1986; Smith and Young 1987); water loss reduction (Turner 1994; Rotondi *et al*. 2003; Jordan *et al*. 2008)
6	Habit	cat	0 – subshrub; 1 – shrub; 2 – treelet; 3 – rosette	Several ecophysiological adaptations to the environment (Givnish *et al*. 1986; Smith and Young 1987; Pérez-Harguindeguy *et al*. 2013)
7a	Stomata in leaf	bin	0 – present in AD/AB; 1 – present in AB	High luminosity and water loss reduction (Turner 1994; Scatena *et al*. 2005)
7b	Stomata position in leaf	cat	1 – sunken; 2 – same level epidermis; 3 – elevated	Transpiration and water loss reduction (Turner 1994; Bosabalidis and Kofidis 2002; Rotondi *et al*. 2003; Jordan *et al*. 2008)
7c	Stomata position in stem	cat	0 – absent; 1 – same level epidermis; 2 – elevated	Transpiration and water loss reduction (Turner 1994; Bosabalidis and Kofidis 2002; Rotondi *et al*. 2003; Jordan *et al*. 2008)
8a	Chloroplasts in leaf epidermis	bin	0 – absent; 1 – present	Photosynthetic efficiency (Cox *et al*. 1987; Sakurai *et al*. 2005; Fujinami *et al*. 2011)
8b	Chloroplasts in stem epidermis	bin	0 – absent; 1 – present	Photosynthetic efficiency (Cox *et al*. 1987; Sakurai *et al*. 2005; Fujinami *et al*. 2011)
9	Hypodermis in leaf	bin	0 – absent; 1 – present	Leaf thickening; water store (Turner 1994)
10a	Bundle-sheath extension in leaf	bin	0 – absent; 1 – present	Strength, stiffness and toughness of plant tissues (Turner 1994)
10b	Bundle-sheath extension – cell-wall thickness	cat	1 – cellulosic; 2 – pectic; 3 – both cellulosic and pectic	Water conservation (Bosabalidis and Kofidis 2002); increasing sclerenchyma fraction: strength, stiffness and toughness (Turner 1994)
11	Aquifer parenchyma in leaf	bin	0 – absent; 1 – present	Resistance to drought stress (Fahn and Cutler 1992)
12a	Carbohydrates in leaf	bin	0 – absent; 1 – present	Water conservation (Bosabalidis and Kofidis 2002)
12b	Carbohydrates in stem	bin	0 – absent; 1 – present	Water conservation (Bosabalidis and Kofidis 2002)
13a	Terpenoids in leaf	bin	0 – absent; 1 – present	Thermotolerance (Singsaas *et al*. 1997; Sharkey *et al*. 2001)
13b	Terpenoids in stem	bin	0 – absent; 1 – present	Thermotolerance (Singsaas *et al*. 1997; Sharkey *et al*. 2001)
14a	Flavonoids in leaf	bin	0 – absent; 1 – present	Protection against UV-B radiation (Kofidis *et al*. 2003)
14b	Flavonoids in stem	bin	0 – absent; 1 – present	Protection against UV-B radiation (Kofidis *et al*. 2003)
15a	Phenolic compounds in leaf	bin	0 – absent; 1 – present	Protection against UV-B radiation; water conservation (Bosabalidis and Kofidis 2002; Kofidis *et al*. 2003)
15b	Phenolic compounds in stem	bin	0 – absent; 1 – present	Protection against UV-B radiation; water conservation (Bosabalidis and Kofidis 2002; Kofidis *et al*. 2003)

(*continued next page*)

Table 2. (*continued*)

Code	Trait	Type of variable	Description of classes in matrix	Ecological correlate
16a	Crystals in leaf	bin	0 – absent; 1 – present	Plant protection, ion balance, tissue support/plant rigidity, light gathering and reflection (Franceschi and Nakata 2005)
16b	Crystals in stem	bin	0 – absent; 1 – present	Plant protection, ion balance, tissue support/plant rigidity, light gathering and reflection (Franceschi and Nakata 2005)
17a	Sclereids in leaf	bin	0 – absent; 1 – present	Increasing sclerenchyma fraction: protection, strength, stiffness and toughness of plant tissues (Turner 1994); light distribution within the mesophyll (Karabourniotis 1998; Bosabalidis and Kofidis 2002)
17b	Sclereids in stem	bin	0 – absent; 1 – present	Increasing sclerenchyma fraction: protection, strength, stiffness and toughness of plant tissues (Turner 1994); light distribution within the chlorophilian tissue (Karabourniotis 1998; Bosabalidis and Kofidis 2002)
18	Spaces in cork of stem	bin	0 – absent; 1 – present	Protection against fire; rainwater retention (Givnish *et al.* 1986)
CT	Cuticle thickness (internerval region)	cont	µm	UV-B protection; water conservation (Turner 1994; Bosabalidis and Kofidis 2002)
OW	Epidermic outer cell-wall thickness (internerval region)	cont	µm	UV-B protection; water conservation (Turner 1994; Bosabalidis and Kofidis 2002)
ET	Epidermal thickness (internerval region)	cont	µm	UV-B protection; prevention of insect mouth plant insertion; water and nutrient conservation (Turner 1994)
LT	Leaf thickness (midrib)	cont	µm	Prevention of the cracking; insect mouth plant insertion and reduction of their digestibility; water and nutrient conservation (Turner 1994)
IT	Leaf thickness (internerval region)	cont	µm	Prevention of the cracking; insect mouth plant insertion and reduction of their digestibility; water and nutrient conservation (Turner 1994)

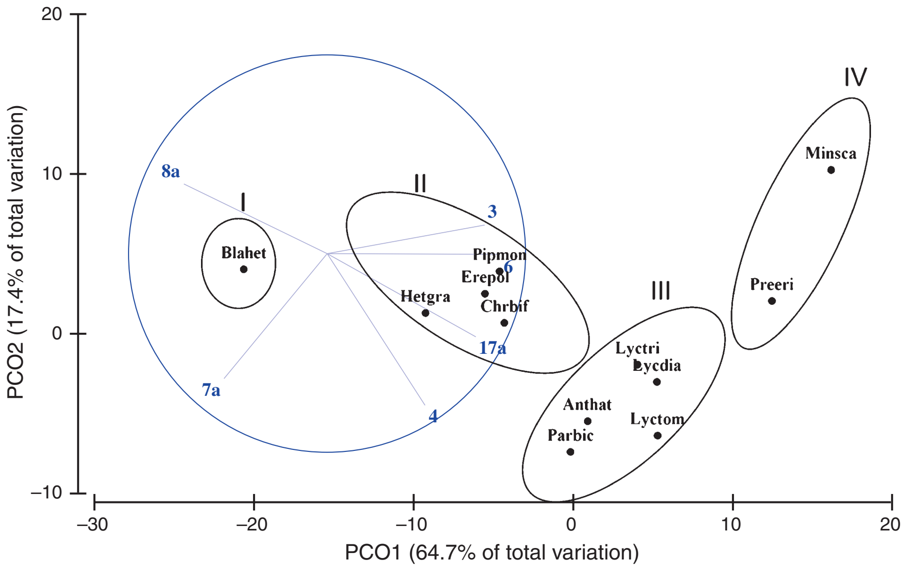

Fig. 2. Principal coordinates analyses (PCoA) of qualitative traits, based on morphological and anatomical characteristics in Lychnophorinae. Traits highly correlated with the first two axes (Pearson's coefficient >0.8) were habit type (6), overlapping leaves (3), sclereids in the leaf (17a), chloroplasts in the leaf epidermis (8a), crypts in the leaf (4) and the distribution of stomata in the leaf (7a). The four groups of plants (I, II, III, IV) distinguished by the PCoA are indicated by ellipses.

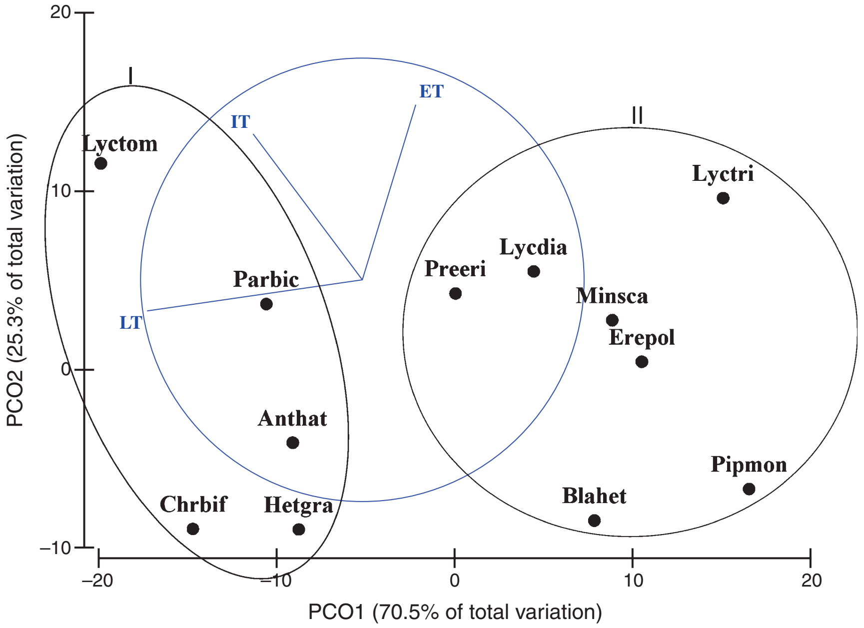

Fig. 3. Principal coordinates analyses (PCoA) of quantitative traits, based on anatomical characteristics in Lychnophorinae. Traits highly correlated with the first two axes (Pearson's coefficient >0.8) were leaf thickness in midrib (LT), in internerval region (IT), and in epidermis (ET). The two groups of plants (I, II) distinguished by the PCoA are indicated by ellipses.

Table 3. Functional groups of Lychnophorinae species distinguished according to principal coordinates analysis, on the basis of qualitative traits

The name of species is abbreviated according to Table 1. (+) = presence; (–) = absence

Trait	Group I Blahet	Group II Chrbif, Erepol, Hetgra, Pipmon	Group III Anthat, Lycdia, Lyctom, Lyctri, Parbic,	Group IV Minsca, Preeri
Habit	Subshrub	Treelet or shrub	Treelet	Rosette
Overlapping leaves	(–)	(+/–) Apex	(+/–) Apex and/or adult stem	(+) Apex and adult stem
Sclereids in leaf	(–)	(+/–)	(+)	(+)
Chloroplasts in leaf epidermis	(+)	(+/–)	(–)	(–)
Crypts in leaf	(–)	(–)	(+)	(+/–)
Distribution of stomata in leaf	Abaxial face	Abaxial face	Abaxial face	Abaxial and/or adaxial face

Table 4. Functional groups of Lychnophorinae species distinguished according to principal coordinates analysis, on the basis of quantitative traits

For each trait, mean value ± s.d. are reported. Trait abbreviations are explained in Table 2. Species names are abbreviated according to Table 1

Trait	Group I Anthat, Chrbif, Hetgra, Lyctom, Parbic	Group II Blahet, Erepol, Lycdia, Lyctri, Minsca, Pipmon, Preeri
LT	2424.8 ± 129.0	832.1 ± 64.4
IT	429.2 ± 38.8	219.7 ± 23.8
ET	31.6 ± 3.7	50.8 ± 5.6

and the presence of chloroplasts in leaf epidermis (except *Eremanthus polycephalus*), while all species of the Group III showed crypts in leaves and no chloroplasts in leaf epidermis (Table 3).

Regarding habit, Group I comprised subshrub plants (Fig. 4*a*), Group II shrubs (Fig. 4*b, c*) or treelets, whereas Group III comprised only treelets (Fig. 4*d, e*), and Group IV rosulate plants (Fig. 4*f, g*). Overlapping leaves were absent in Group I (Fig. 5*a*) and in Group II (except *Chronopappus bifrons*, Fig. 5*b*), whereas they were present in Group III (except *Paralychnophora bicolor*, Fig. 5*c*), and in Group IV (Fig. 4*f, g*). In Group II, if present, overlapping leaves occurred only at the apex (Fig. 5*b*), whereas in several species of Group III (*Lychnophora diamantinana*, *L. tomentosa*, *L. triflora*) they occurred along the adult branches (Fig. 5*c*); and, finally, in Group IV the overlapping leaves always enrobed completely the apex and stems (Fig. 4*f, g*). Sclereids were observed in the stems of almost all groups. In addition, this character was absent in leaves of Group I (Fig. 6*a*) and Group II (except *E. polycephalus*), whereas it always occurred in Group III (Fig. 6*b*) and in Group IV. If present, sclereids occurred mainly in aquiferous parenchyma, including the aquiferous hypodermis (Fig. 6*c*). Leaf crypts were absent in Group I (Fig. 6*d*) and in Group II, whereas they were present in Group III (Fig. 6*c*) and only in *P. eriopus* of Group IV. Chloroplasts were observed in the leaf epidermis in Group I (Fig. 6*d*) and in

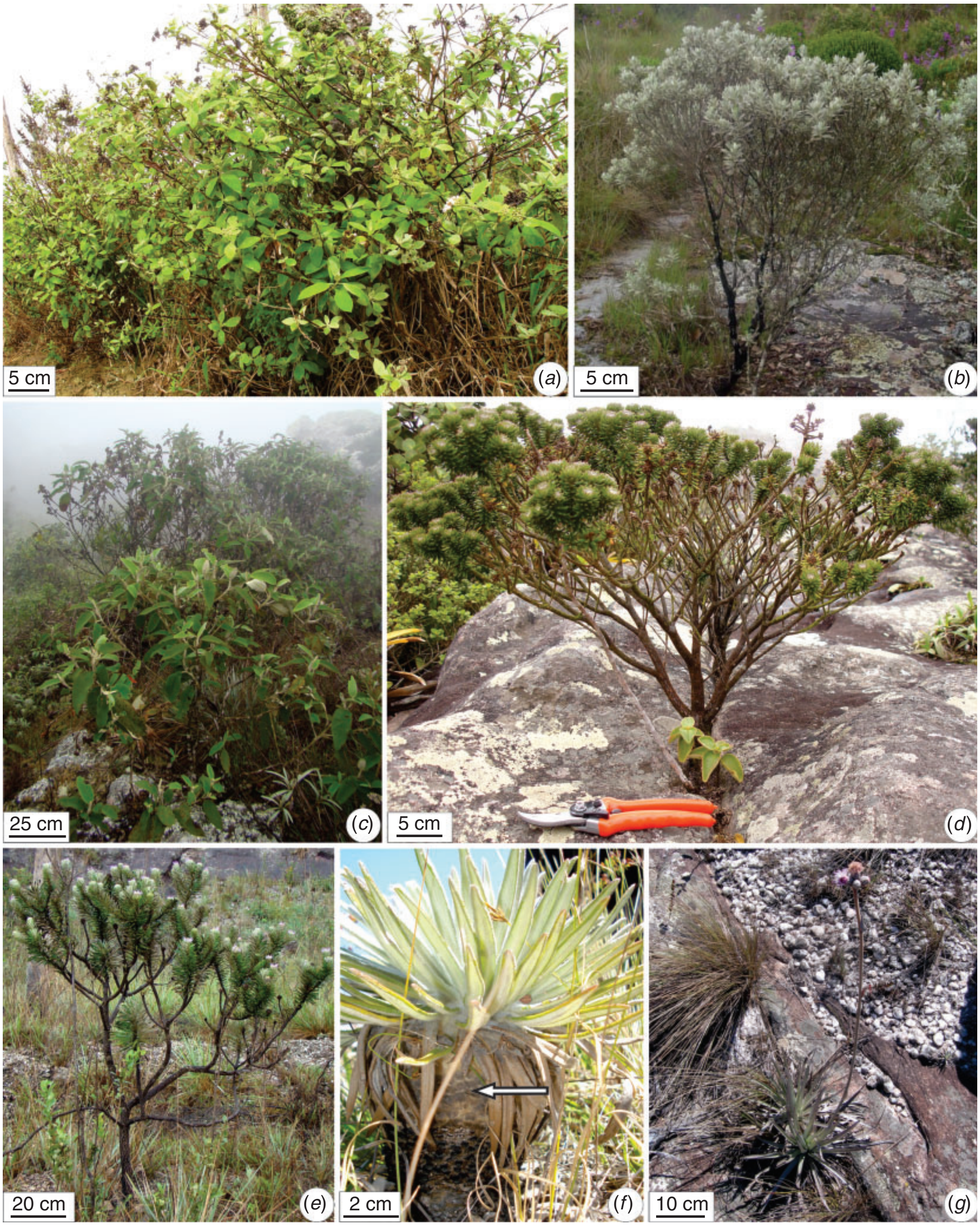

Fig. 4. Distinctive morphological characteristics of Lychnophorinae: habit (a qualitative trait). (*a*) *Blanchetia heterotricha*. (*b*) *Piptolepis monticola*. (*c*) *Chronopappus bifrons*. (*d*) *Lychnophora triflora*. (*e*) *L. diamantinana*. (*f*) *Prestelia eriopus*. (*g*) *Minasia scapigera.* (*a*) Subshrub habit. (*b*, *c*) Shrub. (*d*, *e*) Treelet. (*f*, *g*) Rosette. Note the remaining leaf bases on stems in *f* (arrow).

Group II (Fig. 6*e, f*) (except *E. polycephalus*, Fig. 6*g*), whereas they were absent in Group III and in Group IV. The leaves of all species except *Minasia scapigera* (Group III) were hypostomatic (i.e. the stomata were restricted to the abaxial surface). *Minasia scapigera* (Fig. 6*h*) was amphistomatic.

The PCoA performed on quantitative traits identified two groups of species (Fig. 3, Table 4). The first PCoA axis explained ~70% of total variation, whereas the second axis accounted for ~25%. Three traits were highly correlated with the first two axes (Pearson's coefficient >0.8): midrib leaf thickness (LT), internerval leaf thickness (IT), and epidermal leaf thickness (ET). PCoA1 was mostly correlated with LT, in fact Group I (see Table 4 for species composition) was located at the left end of PCoA1 and exhibited higher values of LT (Fig. 7*a*). In contrast, Group II (see Table 4 for species composition) occurred at the right end of axis 1 (Fig. 3), and were characterised by lower values of LT (Fig. 7*b*). Conversely, IT and ET were correlated with PCoA2. In fact, species of both

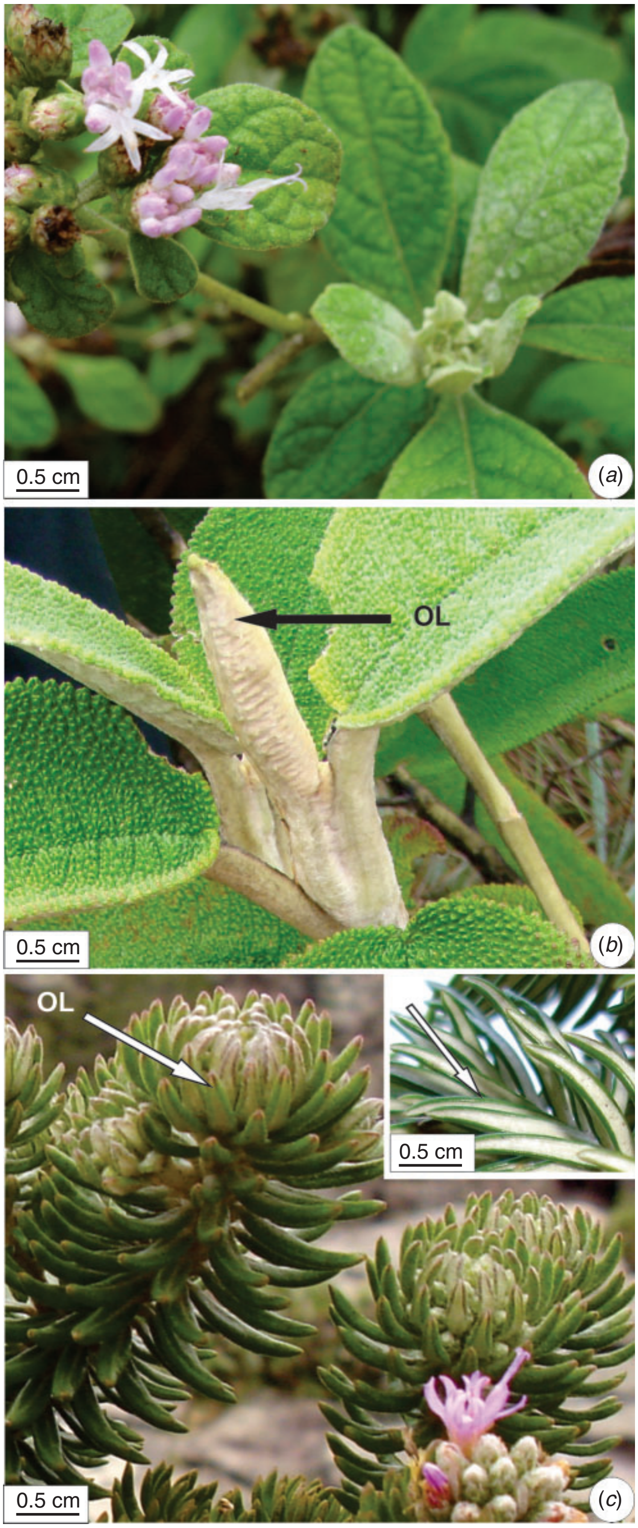

Fig. 5. Distinctive morphological characteristics of Lychnophorinae: overlapping leaves (OL). (*a*) Stem apex without overlapping leaves in *Blanchetia heterotricha*. (*b*) OL only on the stem apex (black arrow) in *Chronopappus bifrons*. (*c*) OL on the stem apex and along adult branches (white arrow) in *Lychnophora triflora*. Inset: detail of the revolute margin (abaxially curled) of the overlapping leaves (white arrow) of *L. triflora*.

groups with positive values of PCoA2 were characterised by the highest values for IT and ET (Fig. 7*a*, *c*), whereas species with negative values of PCoA2 exhibited the lowest values of IT and ET (Figs 3, 7*b*, *d*, Appendix 3). The functional groups obtained from the quantitative analysis were not equivalent of the four groups from the analysis of qualitative traits. In fact, species of Groups II and III of qualitative analysis were distributed into both groups of quantitative analysis. On the other hand, species of Groups I and IV from the analysis of qualitative traits were clustered in Group II of quantitative analysis (Figs 2, 3).

Discussion

The multivariate analysis performed on 35 functional traits of 12 Lychnophorinae taxa showed that six qualitative and three quantitative traits were significant in grouping the species. The majority of qualitative traits (habit type, overlapping leaves, the presence of sclereids and crypts in the leaf and the distribution of stomata in the leaf) might be seen as putative ecological adaptations to the peculiar environmental conditions of the Cerrado Domain, especially of *campos rupestres.* Other traits, such as subshrub habit and chloroplasts in the leaf epidermis, could be correlated with environments subject to low irradiance owing to the higher frequency of cloudiness. These environmental features are most common in borders of secondary forests of the Atlantic Forest and in higher altitudes of the Espinhaço Range of Mountains, especially in Minas Gerais State.

The Lychnophorinae species analysed exhibited a variable habit from subshrubs, shrubs or treelets to rosulate plants. Rosulate habit was observed only in Group IV, suggesting the highest degree of specialisation in Lychnophorinae for protection of the apical meristem and the stem axis from biotic and abiotic damage. Givnish *et al*. (1986) observed that rosulate plants may have three functions, namely, protecting buds and young leaves from UV radiation and leaching, insulating terminal buds from fire and increasing the volume of impounded rainwater, which would also protect the buds against fire and grazing. All these abiotic factors are important in *campos rupestres*, and it appears clear that rosulate plants are highly specialised in overcoming these adversities.

The trait of overlapping leaves was observed on the apex of stems in some species, protecting the apical buds; however, in other species, this trait also occurred on adult stems and presumably also protected axillary meristems. Overlapping leaves and remaining leaf sheath or bases and the woolly indumentum (found in rosulate species of Group IV) are the principal forms of protection of apical buds against fire on apical sprouting in rosulate plants, according to Clarke *et al*. (2013).

Sclereids were present in the parenchymatic tissues of stems of almost all Lychnophorinae species analysed, but occurred only in some species in the leaf, mainly in plants with thick leaves. The increasing sclerenchyma fraction enhances the protection, strength, stiffness and toughness of plant tissues; consequently, these tissues are less liable to be damaged by a given agent (Turner 1994). Moreover, sclereids were considered lightly distributed within the mesophyll, especially under drought stress conditions (Karabourniotis 1998; Bosabalidis and Kofidis 2002). In Lychnophorinae species, sclereids occur

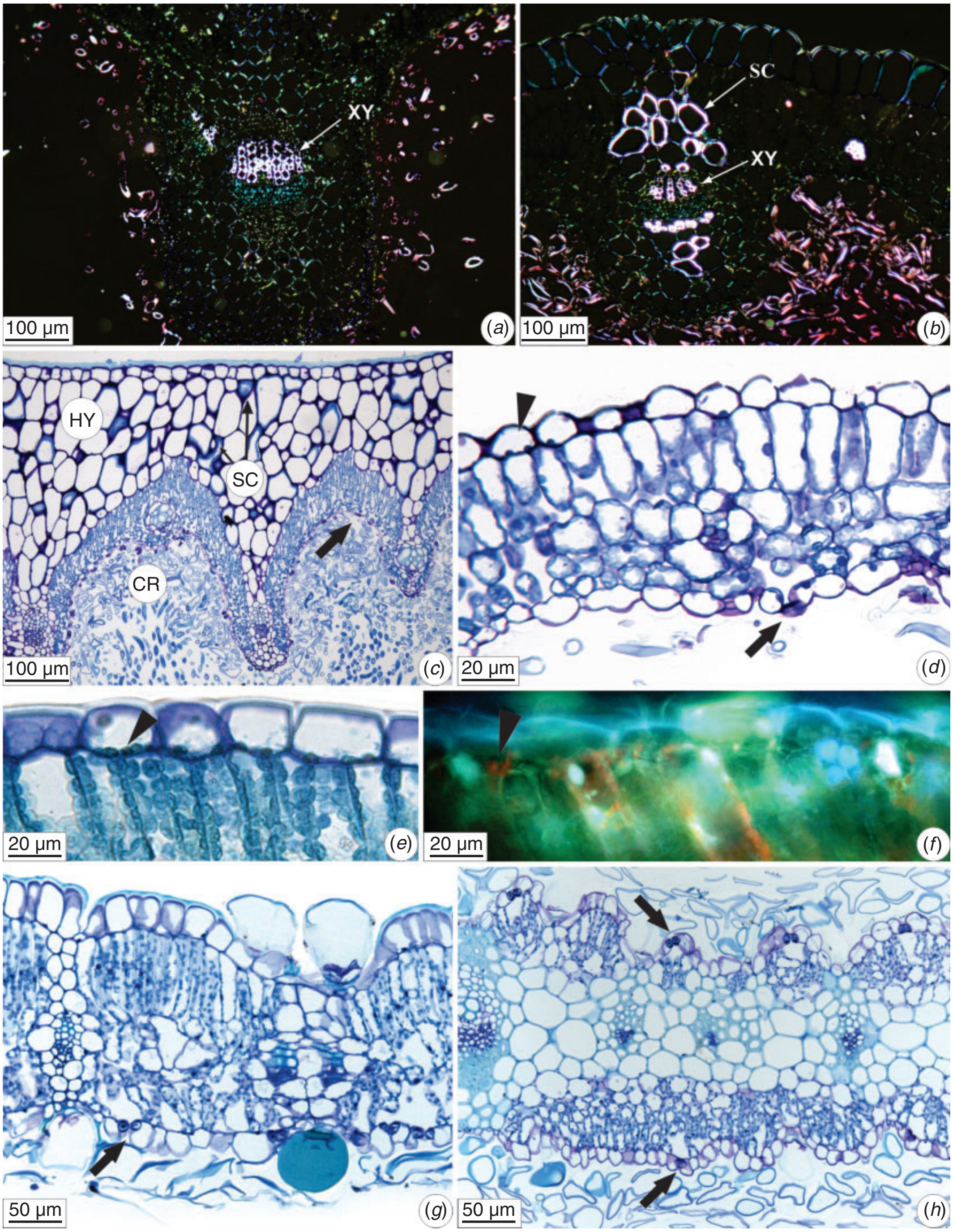

Fig. 6. Distinctive anatomical characteristics (qualitative traits) of Lychnophorinae: transverse sections of leaves. (*a*, *b*) Midrib under polarised light, showing (*a*) the absence of sclereids in *Blanchetia heterotricha* and (*b*) their presence in *Lychnophora triflora.* (*c*, *d*) Mesophyll with crypts and hypodermis (*c*) with sclereids in *L. tomentosa*, and (*d*) their absence in *B. heterotricha*. Note stomata (arrow) in (*c*) *L. tomentosa* and in (*d*) *B. heterotricha*, and (*d*) chloroplasts in the epidermis (arrowhead) in *B. heterotricha*. (*e*, *f*) Epidermis with chloroplasts (arrowhead) in *Chronopappus bifrons* (*e*) under light microscopy and (*f*) natural fluorescence under fluorescence microscopy. (*g*) *Eremanthus polycephalus*, showing epidermis without chloroplasts and with stomata (arrow) on the abaxial surface. (*h*) *Minasia scapigera* evidencing epidermis with stomata (arrow) on the adaxial and abaxial surfaces. CR = crypts; HY = hypodermis; SC = sclereids; XY = xylem.

mainly in aquiferous parenchyma including the hypodermis and could play a role in light reflection in chlorophyllian parenchyma, as argued by Jordan *et al.* (2005). However, sclereids could also play a structural role in supporting the parenchymatic tissue and preventing tissue collapse after variations in hydric state under drought stress conditions, as occurs in the water storage tissues of other species (Oliveira and Sajo 1999; Stern *et al*. 2004; Versieux *et al*. 2010).

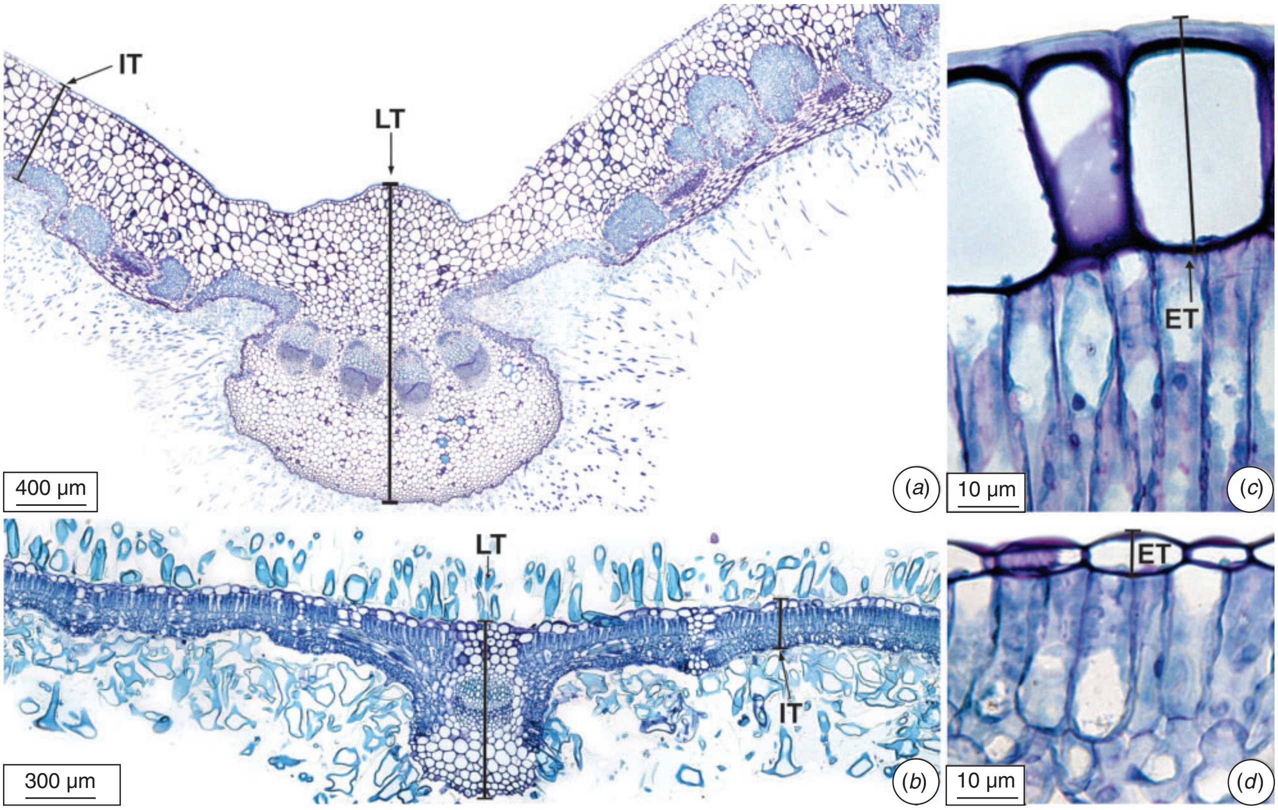

Fig. 7. Distinctive anatomical characters (quantitative traits) of Lychnophorinae. Transverse sections of leaves, showing the position of the measurements of leaf thickness in midrib region (LT) and in internerval region (IT) in (*a*) *Lychnophora tomentosa* and in (*b*) *Piptolepis monticola*; and epidermal thickness (ET) in (*c*) *L. triflora* and in (*d*) *Blanchetia heterotricha*.

The presence of crypts protected by trichomes is a known xerophytic feature involved in the reduction of leaf transpiration and water loss (Turner 1994; Rotondi *et al*. 2003; Jordan *et al*. 2008; Bartoli *et al*. 2013, 2014). Stomatal crypts can also facilitate the diffusion of carbon dioxide from the abaxial surface to adaxial palisade cells in thick leaves (Hassiotou *et al*. 2009). In the Lychnophorinae subtribe, abaxial crypts were observed in the thickest leaves, where this trait not only might be related to the diffusion of carbon dioxide, but also might play a role in water economy regulation.

In the analysed species, the stomata in the leaves are predominantly distributed on the abaxial face, except in *M. scapigera*. The hypostomy is a known feature of species that inhabit *campos rupestres* and the Cerrado Domain (Marques *et al.* 2000; Scatena *et al*. 2005; Bieras and Sajo 2009). The occurrence of stomata on both leaf surfaces of *M. scapigera* (Group IV) may be related to the angle of inclination of the leaves, which are organised in an apical rosette. The isobilateral and amphistomatous leaf of *M. scapigera* can intercept sunlight on both surfaces, as well as reduce light interception under high irradiance through a more vertical leaf orientation, thereby protecting the leaves against damage caused by high irradiance, as observed by James and Bell (2000) and Falster and Westoby (2003). Furthermore, amphistomy is most likely related to efficient use of vascular tissues (increasing the leaf conductance to CO_2) and allows leaves to be thick by minimising the distance from stomata to mesophyll (see Jordan *et al.* 2014; and references cited therein). Last, amphistomy is a common feature in plants of xeric habitats (Fahn and Cutler 1992) as observed in *M. scapigera*.

The presence of chloroplasts in leaf epidermis was a qualitative trait that characterised species of Groups I and II (except *E. polycephalus*). This feature can occur in aquatic plants as a way to improve photosynthetic efficiency underwater (Hough and Wetzel 1977; Sakurai *et al*. 2005; Fujinami *et al*. 2011; Lusa *et al*. 2011), whereas it is an unusual feature in terrestrial plants, occurring mostly in ferns (Cox *et al*. 1987) that inhabit shaded and wet places. In the Lychnophorinae, this trait was observed in *B. heterotricha* (Group I), which occurs in areas of borders of secondary forests of the Atlantic Forest, and in *C. bifrons*, *Piptolepis monticola* and *Heterocoma gracilis* (species of Group II) that occur in close geographic locations. The natural habitats of the species of Group II are frequently foggy, especially in cold days during winter and spring, and the presence of chloroplasts in epidermis could improve light use for photosynthesis under this climatic condition. In plants with epidermal chloroplasts, these chloroplasts can be protected under strong light by changes in their orientation along anticlinal cell walls, whereas the chloroplasts move towards the periclinal cell wall under weak light conditions (Takagi 2003). The leaf epidermal cells of Group I and II species present chloroplasts along the periclinal cell walls, most likely because we collected the botanical material on rainy (Group I) and foggy (Group II) days.

The PCoA of qualitative traits provided evidence for distinguishing functional types. *Blanchetia heterotrichia* (Group I) seems to be adapted at open and humid areas of borders of the Atlantic Forest, exhibiting a subshrub habit and an absence of sclereids, leaf crypts and overlapping leaves. These findings might be explained by the location of *B. heterotricha*, which is characterised by regular rainfall, absence of fire, and higher water soil retention (INMET 2013). Almost all species belonging to Group II are characterised by leaves without crypts and with chloroplasts in epidermal cells. The species in Group II are typical of foggy habitats. Group III is only formed by species with crypts and sclereids in leaves, and seems to be quite adapted to the stressful conditions of *campos rupestres*. Group IV is constituted by rosulate plants showing overlapping leaves with crypts and sclereids. Interestingly, these species share some traits (i.e. pseudo-trunk covered with the leaf-sheaths remains, hairiness, tightly furled leaf rosettes, water-storage strategies, epidermal cell wall and cuticle thickenings, sclerified elements) with other taxa adapted to live in *campos rupestres* of the Espinhaço Mountain Range (Chueiri-Chiaretto 1984; Mello-Silva 1990; Sajo *et al*. 1995; Giulietti *et al*. 1997; Scatena *et al*. 2005) or in high altitude habitats (Baruch 1979; Baruch and Smith 1979; Smith and Young 1987). All these traits can strengthen the adaptive ability of plant species of Group IV. Consequently, with respect to the other groups, the Group IV seems to be the most adapted type to the prohibitive environmental conditions of *campos rupestres*.

Similarly to the qualitative characters, the three quantitative traits – LT, IT and ET – could also be seen as putative ecological adaptations to the *campos rupestres*. The general thickness or toughness of a leaf is related to sclerophylly, which clearly depends on leaf anatomy, i.e. thick cuticle, thick epidermal cells and sclerification (Turner 1994). These characteristics are frequent in evergreen plants and provide protection against UV-B penetration, prevent cracking, defend against herbivores, prevent insect mouth insertion and reduce plant digestibility, but can also play a role in terms of water and/or nutrient conservation (Turner 1994; Bosabalidis and Kofidis 2002; Kofidis *et al*. 2003). The PCoA based on quantitative data evidenced two main functional types that were distinguished mainly by LT: Group I (*Anteremanthus hatschbachii*, *C. bifrons*, *H. gracilis*, *Lychnophora tomentosa*, and *P. bicolor*), more specialised, with higher values of LT; and group II (*B. heterotrica*, *E. polycephalus*, *L. diamantinana*, *L. triflora*, *M. scapigera*, *P. monticola*, and *P. eriopus*), less adapted, with lower values of LT. These functional types were more heterogeneous and without a strong link to the environmental conditions or to a particular habitat, so they did not appear useful for the aims of the present research. For future analyses, enlarging sampling effort and considering more quantitative traits would be taken into account.

In the present study, the qualitative traits gave more convincing results than did quantitative characters. In fact, they provided statistically significant differences that enabled the identification of different functional groups in Lychnophorinae species that can be interpreted as morphological and anatomical adaptations to the *campos rupestres* of the Cerrado Domain (where drought and fire are important limiting factors during the dry season), and to the open areas of the Atlantic Forest Domain (characterised by regular rainfall, absence of fire and higher water soil retention).

In conclusion, we can answer the questions posed at the beginning of this paper.

(1) The most significant features in Lychnophorinae species in terms of circumscribing the identified functional groups were habit type, the presence of overlapping leaves, the presence of sclereids and crypts in the leaf, the presence of chloroplasts in the leaf epidermis, and the distribution of stomata in the leaf. These traits could be seen as plant adaptations to the environment where the species occur, and the absence of a trait or the occurrence of a less adaptive character reflects less drastic environmental conditions (i.e. *B. heterotricha*).

(2) The multivariate analysis, especially on the basis of qualitative traits, provided evidence that four functional types are likely to be related to the ecological conditions, as follows: Group I, adapted at open and humid areas of borders of the Atlantic Forest; Group II, with features mostly related to foggy habitats; Group III, exhibiting traits quite adapted to *campos rupestres*, and Group IV, presenting the most specialised morphological and anatomical traits, which might be related to the extreme conditions of the *campos rupestres*.

Acknowledgements

The authors thank Professor Laura M. C. Forino (University of Pisa, Italy) for her useful suggestions, Dr Monica Ruffini Castiglione (University of Pisa, Italy) for her technical and scientific support, and Professor Massimo D'Orazio (University of Pisa, Italy) for his valuable assistance with polarised light microscopy. We thank the IEF-MG for their logistical support with the plant collection and FAPESP (São Paulo Research Foundation) for providing financial support (Thematic Project Proc. no. 2010/51454–3) and grants for the first (2010/02085–5 and 2012/16071–1) and second (302776/2010–9) author. The paper is part of the doctoral thesis of M. G. Lusa.

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Appendix 1. Details on how stem nodes have been evaluated in each Lychnophorinae species

Lychnophorinae species	Stem nodes evaluated
Anteremanthus hatschbachii H.Rob.	1–20°
Blanchetia heterotricha DC.	1–15°
Chronopappus bifrons (DC. ex Pers.) DC.	1–10°
Eremanthus polycephalus (DC.) MacLeish	1–30°
Heterocoma gracilis Loeuille, J.N.Nakaj. & Semir.	1–15°
Lychnophora tomentosa (Mart. ex DC.) Sch. Bip.	1–10°
Lychnophora diamantinana Coile & S.B.Jones	1–50°
Lychnophora triflora (Mattf.) H.Rob.	1–50°
Minasia scapigera H.Rob.	[A]
Paralychnophora bicolor (DC.) MacLeish	1–20°
Piptolepis monticola Loeuille	1–30°
Prestelia eriopus Sch. Bip.	[A]

[A] Species with rosette habit. All the internodes have been analysed.

Appendix 2. Matrix of qualitative data used in principal coordinates analysis
For species code see Table 1. For variable numbers see Table 2

Species code	Variable																													
	1	2	3	4	5a	5b	5c	6	7a	7b	7c	8a	8b	9	10a	10b	11	12a	12b	13a	13b	14a	14b	15a	15b	16a	16b	17a	17b	18
Anthat	1	1	1	1	2	2	2	2	1	2	2	0	0	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Blahet	2	1	0	0	2	1	1	0	1	2	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
Chrbif	1	1	1	0	2	2	2	1	1	2	1	1	1	0	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Erepol	3	1	0	0	2	1	1	2	1	2	2	0	0	0	1	3	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Hetgra	2	1	0	0	2	4	2	2	1	2	2	1	0	0	1	2	1	1	1	1	1	1	1	1	1	1	1	0	1	0
Lycdia	1	1	1	1	2	2	2	2	1	2	2	0	0	1	1	3	1	1	1	1	0	1	0	1	1	1	1	1	1	1
Lyctom	1	2	2	1	2	2	2	2	1	3	1	0	0	0	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Lyctri	1	2	2	1	2	2	2	2	1	2	1	0	0	0	0	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Minsca	1	1	2	0	1	3	0	3	0	1	0	0	0	0	1	3	1	1	1	1	1	1	0	1	1	1	1	1	1	1
Parbic	1	1	0	1	2	2	2	2	1	3	1	0	0	1	1	3	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Pipmon	1	1	1	0	2	3	2	1	1	2	2	1	1	0	1	3	1	1	1	1	1	1	1	1	1	1	1	0	1	1
Preeri	1	2	2	1	2	2	0	3	1	2	0	0	0	0	1	3	1	1	1	1	1	1	0	1	1	1	1	1	1	1

Appendix 3. Matrix of quantitative data used in principal coordinates analysis
For species code see Table 1. For variable abbreviations see Table 2

Species code	Variable				
	CT	OW	ET	LT	IT
Anthat	5.15	2.03	20.85	2187.99	173.10
Blahet	1.75	2.55	22.28	922.45	115.81
Chrbif	2.83	1.35	25.37	3153.68	125.59
Erepol	3.76	2.19	34.71	725.55	217.86
Hetgra	0.00	1.60	18.19	2019.25	133.08
Lyctom	7.93	5.42	56.97	2629.06	1309.34
Lycdia	9.52	3.86	78.38	1057.18	241.55
Lyctri	5.36	5.32	94.47	509.90	249.20
Minsca	0.00	1.73	26.78	776.38	343.17
Parbic	16.34	1.54	36.70	2133.89	399.81
Pipmon	2.69	2.14	31.62	486.90	130.15
Preeri	7.65	3.48	67.68	1346.44	240.00