Revista Brasileira de Farmacognosia 25 (2015) 328–343

Revista Brasileira de Farmacognosia
BRAZILIAN JOURNAL OF PHARMACOGNOSY
www.sbfgnosia.org.br/revista

Original Article

Comparative study of *Passiflora* taxa leaves: I. A morpho-anatomic profile

Luma Wosch [a], Daniela Cristina Imig [b], Armando Carlos Cervi [b,1], Bárbara Baêsso Moura [b], Jane Manfron Budel [c], Cid Aimbiré de Moraes Santos [a,*]

[a] *Programa de Pós-graduação em Ciências Farmacêuticas, Laboratório de Farmacognosia, Universidade Federal do Paraná, Curitiba, PR, Brazil*
[b] *Departamento de Botânica, Universidade Federal do Paraná, Curitiba, PR, Brazil*
[c] *Departamento de Ciências Farmacêuticas, Universidade Estadual de Ponta Grossa, Ponta Grossa, PR, Brazil*

ARTICLE INFO

Article history:
Received 14 May 2015
Accepted 26 June 2015
Available online 14 July 2015

Keywords:
Passiflora
Morpho-anatomy
Passion fruit
Pharmacobotanical study
Quality control

ABSTRACT

Determining the authenticity and quality of plant raw materials used in the formulation of herbal medicines, teas and cosmetics is essential to ensure their safety and efficacy for clinical use. Some *Passiflora* species are officially recognized in the pharmaceutical compendia of various countries and have therapeutic uses, particularly as sedatives and anxiolytics. However, the large number of *Passiflora* species, coupled with the fact that most species are popularly known as passion fruit, increases the misidentification problem. The purpose of this study is to make a pharmacognostic comparison between various *Passiflora* species to establish a morpho-anatomical profile that could contribute to the quality control of herbal drug products that contain passion fruit. This was conducted by collecting samples of leaves from twelve *Passiflora* taxa (ten species and two forms of *P. edulis*): *P. actinia*, *P. alata*, *P. amethystina*, *P. capsularis*, *P. cincinnata*, *P. edulis* f. *flavicarpa*, *P. edulis* f. *edulis*, *P. incarnata*, *P. morifolia*, *P. urnifolia*, *P. coccinea* and *P. setacea*, from different locations and their morpho-anatomical features were analyzed using optical microscopy and scanning electron microscopy. Microscopic analysis allowed to indicate a set of characters that can help to differentiate species. These include midrib and petiole shape, midrib and petiole vascular pattern, medium vein shape, presence of trichomes, presence of blade epidermal papillae and sclerenchymatic cells adjoining the vascular bundles. These characters could be used to assist in the determination of herbal drug quality and authenticity derived from a species of *Passiflora*.

Introduction

The origin of the term pharmacognosy (*pharmakon*: drug and *gnosis*: knowledge) clarifies the discipline of knowledge about drugs. According to the World Health Organization (WHO, 2000), a drug is a medicinal plant, or parts thereof, after collection processes, stabilization and drying, in its full form, fragmented or powdered. Considering that medicinal plants are chemically and microbiologically stable, they are acquired by phytotherapic industries as herbal drugs, which make their authentication difficult.

The use of *Passiflora* species as medicinal plants began in the seventeenth century in Europe due to their sedative property (Hoehne, 1939). Currently, *Passiflora* species are widely used in folk medicine in many countries, largely as sedatives and anxiolytics (Conrado et al., 2003).

P. incarnata L. is included in the official pharmaceutical codes of several countries (Gosmann et al., 2011) and is the most widely studied in this aspect. Consequently, in Brazil, most herbal medicines were prepared from this species. However, *P. incarnata* is native to the North America and cannot adapt to the Brazilian climate. For this reason, the Brazilian Pharmacopoeia 5th edition (Farmacopeia, 2010) chose to make *P. alata* Curtis and *P. edulis* Sims. the constituents of herbal medicines, with one of either species in its constitution.

These data, coupled with the large number of *Passiflora* species (approximately 600) and the same common name (passion fruit) given to several species, have increased the probability of mistakes in species identification, or even in the adulteration of herbal drugs. In order to minimize these problems, pharmacobotanical analysis can be used. This technique is used to identify morphological and anatomical characters for differentiation between similar species. Freitas (1985) employed pharmacobotanical analyses on a comparative study of four *Passiflora* species: *P. alata*, *P. edulis*, *P. incarnata* and *P. quadrangularis*, and identified some distinguishing features such as the presence, location and type of trichomes.

* Corresponding author.
E-mail: cid@ufpr.br (C.A.M. Santos).
[1] In memoriam.

http://dx.doi.org/10.1016/j.bjp.2015.06.004
0102-695X/

Considering the morphological similarities between different *Passiflora* species, the aim of this research was to examine, morpho-anatomically, the leaves from twelve *Passiflora* taxa. The revealed pharmacobotanical features can be used to identify these medicinal plants, as well as differentiate between *Passiflora* species, contributing to the quality control of herbal products containing passion fruit.

Material and methods

Botanical materials

Plant material (32 samples from twelve taxa) was collected from different areas of Brazil, specifically from the South (Paraná, Santa Catarina and Rio Grande do Sul), Distrito Federal and Rio Grande do Norte, in 2013 and 2014. The plant material was used to make voucher specimens that were identified by the taxonomist Daniela Cristina Imig, and a representative sample was stored in the Herbarium listed in Box 1.

Morpho-anatomical study

Passiflora taxa plant material was collected 15 cm from the apex of the plant. The leaves were fixed in FAA 70 solution (Johansen, 1940) and stored in 70% ethanol (Berlyn and Miksche, 1976). The remaining leaves were dried at 50 °C and ground in an analytical mill (Quimis® G298A21). The dried, ground samples were standardized by size to ≤300 μm. The obtained powder was analyzed by light microscopy.

For permanent slides, samples were taken from the middle region of the third leaf from the apex. Technical embedding in historesin (Leica Microsystems®) was used by employing the previously determined materials. The material was trans-sectioned in the transverse plane in a rotary microtome, which produced 5 μm sections. Toluidine blue was used for staining (Feder and O'Brien, 1968).

For the frontal view of the epidermis, the leaves were subjected to clearing technique (Kraus and Arduin, 1997). The samples were stained with safranin and the slides were set with glycerinated gelatine (Kaiser, 1880). Semi-permanent slides were also prepared for drug characterization in powder using chloral hydrate.

For the analysis of scanning electron microscopy (SEM), the samples were fixed in FAA 70, dehydrated in a graded ethanol series and CO_2 critical point drying apparatus (Bal-Tec CPD-030), coated with gold (Balzers SCD-030) and examined using a Jeol JSM-6360LV microscope. This procedure was performed at the Electron Microscopy Center at the Federal University of Paraná (UFPR). For the morpho-anatomical description, we followed the procedure of Radford et al. (1974).

Results

Morphological analysis

Morphological analysis of *Passiflora* leaves shows important characteristics for differentiating between the taxa (Fig. 1 and

Box 1
Data collection, vouchers and Herbarium of deposited *Passiflora* species.

Sample	Species	Locality of collection	Collection date	Voucher	Herbarium
1.1	*P. actinia* Hook.	C. Politécnico, UFPR, Curitiba-PR	30/08/2013	L. Wosch 01	HACC, UPCB
1.2		C. Politécnico, UFPR, Curitiba-PR	06/05/2014	D. C. Imig 332	HACC, MBM, UPCB
1.3		C. Botânico, UFPR, Curitiba-PR	06/05/2014	L. Wosch 03	HACC, UPCB
2.1	*P. alata* Curtis	Planaltina-DF	15/08/2013	D. C. Imig 52	UPCB, MBM
2.2		Ipira-SC	05/04/2014	D. C. Imig 336	HACC, UPCB
2.3		Piratuba-SC	05/04/2014	D. C. Imig 347	HACC, MBM
2.4		Foz do Iguaçu-PR	24/04/2014	C. L. Reichert 04	HACC
2.5		Largo da Ordem, Curitiba-PR	07/05/2014	L. Wosch 04	HACC
3.1	*P. amethystina* J.C. Mikan	Ipira-SC	05/04/2014	D. C. Imig 334	HACC, MBM
3.2		Piratuba-SC	05/04/2014	D. C. Imig 348	HACC, MBM
3.3		Ipira-SC	05/04/2014	D. C. Imig 335	HACC, MBM
4.1	*P. capsularis* L.	Ipira-SC	05/04/2014	D. C. Imig 337	HACC, MBM
4.2		Ipira-SC	05/04/2014	D. C. Imig 319	HACC, MBM
4.3		Ipira-SC	05/04/2014	D. C. Imig 320	HACC, MBM
4.4		Serra do mar – PR	29/05/2014	D. C. Imig et al., s.n.	HACC
5	*P. cincinnata* Mast.	Natal-RN	07/2014	L. Wosch 05	HACC, MBM
6.1	*P. edulis* f. *flavicarpa* O. Deg.	C. Politécnico, UFPR, Curitiba-PR	30/08/2013	L. Wosch 02	HACC, UPCB
6.2		Foz do Iguaçu-PR	24/04/2014	C. L. Reichert 02	HACC
6.3		Foz do Iguaçu-PR	24/04/2014	C. L. Reichert 03	HACC
6.4		Foz do Iguaçu-PR	24/04/2014	C. L. Reichert 05	HACC
7.1	*P. edulis* Sims f. *edulis*	Serra do mar – PR	29/05/2014	M. E. Engels et al., 2490	MBM
7.2		Capinzal – SC	21/07/2014	D.C. Imig 346	HACC, MBM
8.1	*P. incarnata* L.	Vera Cruz do Oeste-PR	25/04/2014	C. L. Reichert 06	HACC
8.2		Vera Cruz do Oeste-PR	02/06/2014	C. L. Reichert 06	HACC
9.1	*P. morifolia* Mast.	Ipira-SC	04/04/2014	D. C. Imig 338	HACC, UPCB, MBM
9.2		Peritiba-SC	04/04/2014	D. C. Imig 339	HACC, UPCB
9.3		Ipira-SC	04/04/2014	D. C. Imig 340	HACC, UPCB, MBM
10.1	*P. urnifolia* Rusby	Irani-SC	04/04/2014	D. C. Imig 343	HACC, UPCB
10.2		Concórdia-SC	04/04/2014	D. C. Imig 342	HACC, MBM
10.3		Peritiba-SC	04/04/2014	D. C. Imig 341	HACC, MBM
11	*P. coccinea* Aubl.	Planaltina-DF	15/08/2013	D. C. Imig et al. 293	MBM
12	*P. setacea* DC.	Planaltina-DF	15/08/2013	D. C. Imig et al. 284	HACC, UPCB, MBM

HACC, Armando Carlos Cervi Herbarium; UPCB, Herbarium of Botany Department of Federal University of Paraná; MBM, Municipal Botanical Museum, Curitiba, PR, Brasil.

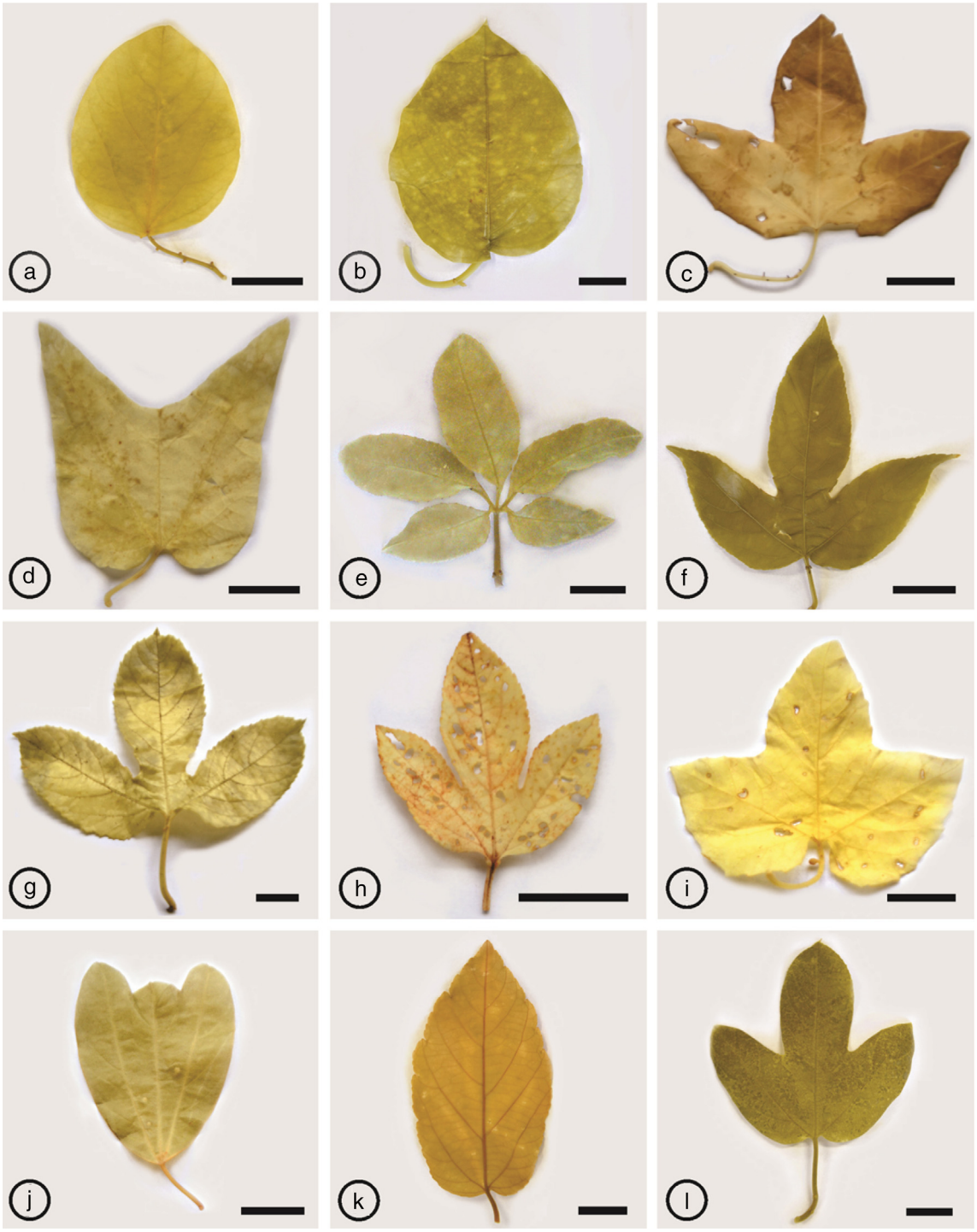

Fig. 1. *Passiflora* spp. – Morphological aspect of leaves – Adaxial side. a. *P. actinia*; b. *P. alata*; c. *P. amethystina*; d. *P. capsularis*; e. *P. cincinnata*; f. *P. edulis* f. *flavicarpa*; g. *P. edulis* f. *edulis*; h. *P. incarnata*; i. *P. morifolia*; j. *P. urnifolia*; k. *P. coccinea*; l. *P. setacea*. Ps.: the images were obtained after fixation with FAA. Bars = 2 cm.

Box 2). The leaves are simple and the blade shape ranges from entire to 2-, 3-, 5-lobed or 5-partite. The leaf margin characteristics may be serrate or crenulate. The apex ranges from acute, obtuse, to acuminate. The bases are rounded, cordate, subcordate or attenuate. The dimensions of the leaf blade and petiole vary significantly between the taxa. The presence and number of glands on the petiole also vary considerably.

Anatomical analysis

Epidermis

The surface view of the blade shows different epidermal cell anticlinal walls as observed in Box 3 and in Figs. 2 and 3 for the adaxial and abaxial side, respectively.

Regarding the presence of stomata, only *P. alata* and *P. incarnata* have amphistomatic leaves, although a few stomata can be

Box 2
Morphological characteristics of *Passiflora* species leaves.

	P. actinia	*P. alata*	*P. amethystina*	*P. capsularis*	*P. cincinnata*	*P. edulis* f. *flavicarpa*
Leaf blade						
Shape	Ovate	Ovate to elliptical	3-Oblong lobs	2–3 Ovate lobs	5-Lobed to 5-partite	3-Oblong lobs
Size						
Width (cm)	4–7	7–15	6–10	6–8.5	9	11–22.5
Length (cm)	5–9	9.5–17.5	5–8	7–9.5	6–7	9–17
Apex	Obtuse to acute	Acuminate	Obtuse	Acute	Acute to obtuse	Acute to acuminate
Base	Rounded	Rounded to subcordate	Cordate to subcordate	Cordate to subcordate	Truncate	Subcordate
Margin	Entire	Entire to sparsely glandular serrate	Entire with glands in the sinus	Entire	Glandular serrate	Serrate
Color	Light green and glossy on both sides	Light green on both sides	Dark green, glaucous on abaxial side	Dark green, glaucous on abaxial side	Dark green on both sides, slightly opaque on abaxial side	Dark green on both sides, glossy on the adaxial side and opaque on abaxial side
Color after drying	Clearer on abaxial side	Slightly clearer on abaxial side	Darkened, clearer on abaxial side	Clearer and gray on abaxial side	Clearer and uniform on abaxial side	Clearer and opaque on abaxial side
Venation	Pinnate	Pinnate	Palmate	Palmate	Palmate	Palmate
Petiole						
Size (cm)	2.5–3.5	3.5–5	2–4.5	2.5–4	3	2–6
Glands						
Frequency	~6	2–4	5–7	Absent	2	2
Location	Scattered	Above the median region	Scattered, near the leaf blade	–	About 1 cm from the base	Next to the leaf blade

	P. edulis f. *edulis*	*P. incarnata*	*P. morifolia*	*P. urnifolia*	*P. coccinea*	*P. setacea*
Leaf blade						
Shape	3-Oblong to lanceolate lobs	3-Oblong to lanceolate lobs	3-Lobed at the apex	2-Lanceolate lobs	Oblong to elliptical	3-Oblong lobs
Size						
Width (cm)	4.5–6	4.5–6	5.5–10.5	5.5–6	4–5	9.5
Length (cm)	4–6	4–6	5–9.5	7–8	11	10.5
Apex	Acuminate	Acute to acuminate	Acute to obtuse	Acute to obtuse	Acute	Acuminate to acute
Base	Subcordate to attenuate	Cordate to subcordate	Cordate	Attenuate	Rounded to subcordate	Cordate to subcordate
Margin	Serrate	Serrate	Serrate to crenulate	Entire	Serrate	Serrate
Color	Dark green on both sides, glossy on adaxial side and opaque on abaxial side	Green on both sides and glaucous on abaxial side	Light green and opaque on both sides	Green, sometimes variegated and opaque on abaxial side	Green on both sides	Green to light green on both sides and opaque on abaxial side
Color after drying	Clearer on abaxial face	Clearer in the abaxial face	Clearer in the abaxial face	Clearer in the abaxial face	Similar on both sides	Clearer in the abaxial face
Venation	Palmate	Palmate	Palmate	Palmate	Pinnate	Palmate
Petiole						
Size (cm)	5–6	1.5	2–4	2–2.5	2	4–5
Glands						
Frequency	2	2	2	Absent	Absent	4
Location	Next to the blade	Next to the blade	At the median region	–	–	At the median region

Box 3
Epidermal cell anticlinal walls on adaxial (AD) and abaxial (AB) sides of *Passiflora* species.

Epidermal cell anticlinal walls	*P. actinia*	*P. alata*	*P. amethystina*	*P. capsularis*	*P. cincinnata*	*P. edulis* f. *flavicarpa*	*P. edulis* f. *edulis* 7.1	*P. edulis* f. *edulis* 7.2	*P. incarnata*	*P. morifolia*	*P. urnifolia*	*P. coccinea*	*P. setacea*
Straight to slightly wavy		AD			AD		AD					AD	AD
Slightly wavy to wavy		AB			AB	AD	AB				AD		
Wavy to slightly sinuous	AD											AB	
Slightly sinuous to sinuous	AB		AD/AB	AD/AB		AB		AD/AB	AD/AB	AD/AB	AB		AB

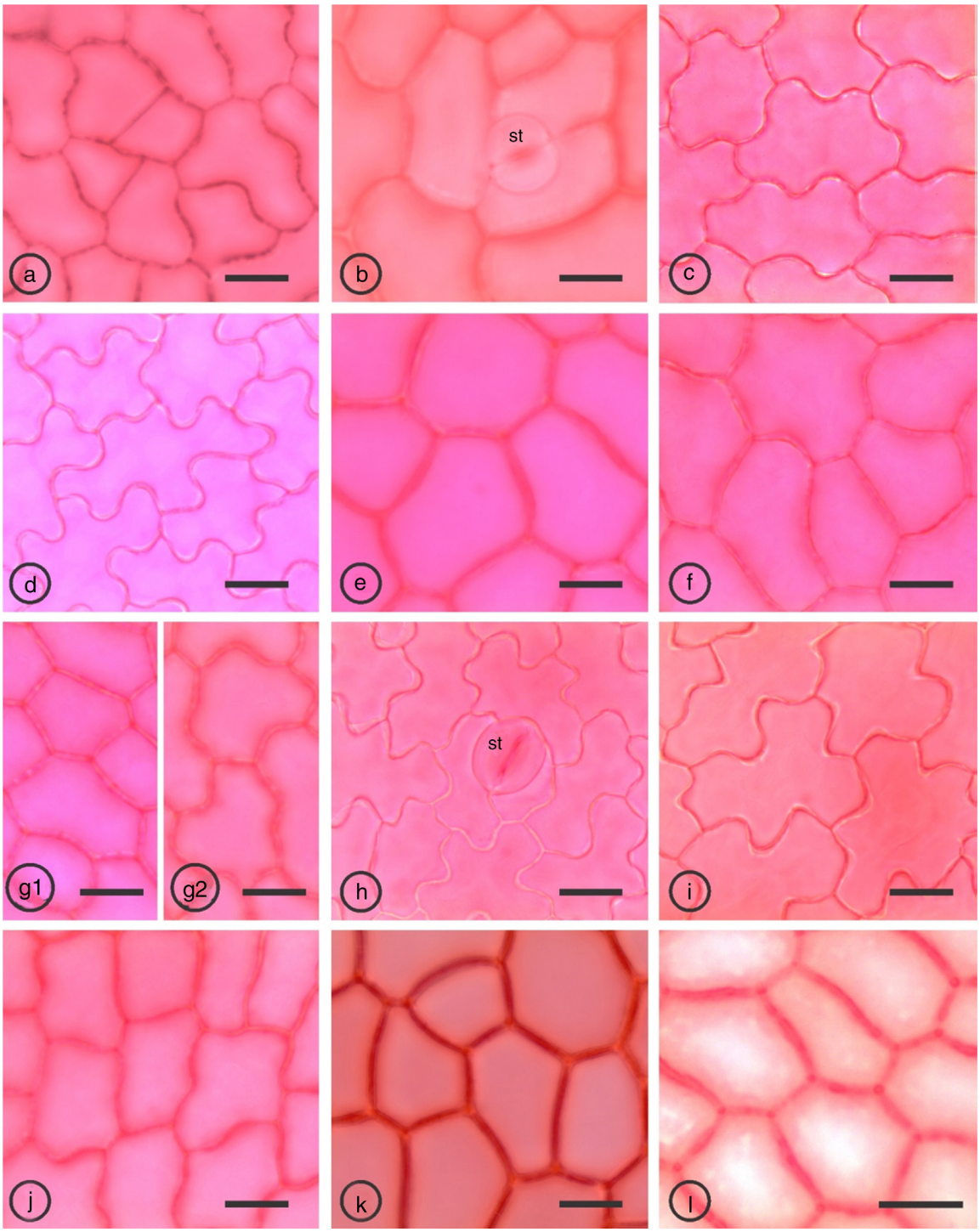

Fig. 2. *Passiflora* spp. – View of the leaf surface (epidermis) – Adaxial side. a. *P. actinia*; b. *P. alata*; c. *P. amethystina*; d. *P. capsularis*; e. *P. cincinnata*; f. *P. edulis* f. *flavicarpa*; g1. *P. edulis* f. *edulis* sample 7. 1; g2. *P. edulis* f. *edulis* sample 7.2; h. *P. incarnata*; i. *P. morifolia*; j. *P. urnifolia*; k. *P. coccinea;* l. *P. setacea*. st: stomata. Bars = 20 μm.

observed on the adaxial side (Figs. 2b and 2h). All other taxa have hypostomatic leaves (Figs. 2 and 3). The stomata are located at the same level of other epidermal cells in *P. actinia*, *P. alata*, *P. cincinnata* and *P. coccinea*; at the same level or in a slight depression in *P. amethystina*, *P. morifolia* and *P. urnifolia*; at the same level or slightly above in *P. capsularis*, *P. edulis* f. *flavicarpa*, *P. edulis* f. *edulis* and *P. setacea*, and slightly above in *P. incarnata*.

P. actinia shows anomocytic stomata (Fig. 3a). *P. alata*, *P. amethystina*, *P. capsularis*, *P. cincinnata*, *P. edulis* f. *flavicarpa*, *P. edulis* f. *edulis* and on the abaxial side of *P. incarnata* shows anomocytic stomata (Fig. 3h), anisocytic stomata (Fig. 3c) and paracytic stomata (Fig. 3f). Anisocytic stomata can be observed on the adaxial side of *P. incarnata*. Although *P. morifolia* and *P. urnifolia* show anomocytic stomata (Fig. 3i, j), a few anisocytic stomata can also be observed. In *P. coccinea*, anomocytic (Fig. 3k), anisocytic and paracytic stomata are observed but anisocytic stomata are rare. In *P. setacea* (Fig. 3l), all three types are observed but paracytic stomata are uncommon.

P. actinia (Fig. 4a, b), *P. alata* (Fig. 4c, d) and *P. amethystina* (Fig. 4e, f) are glabrous on both sides of the blade. Other taxa show non-glandular trichomes (Fig. 5). *P. cincinnata* has evidence of

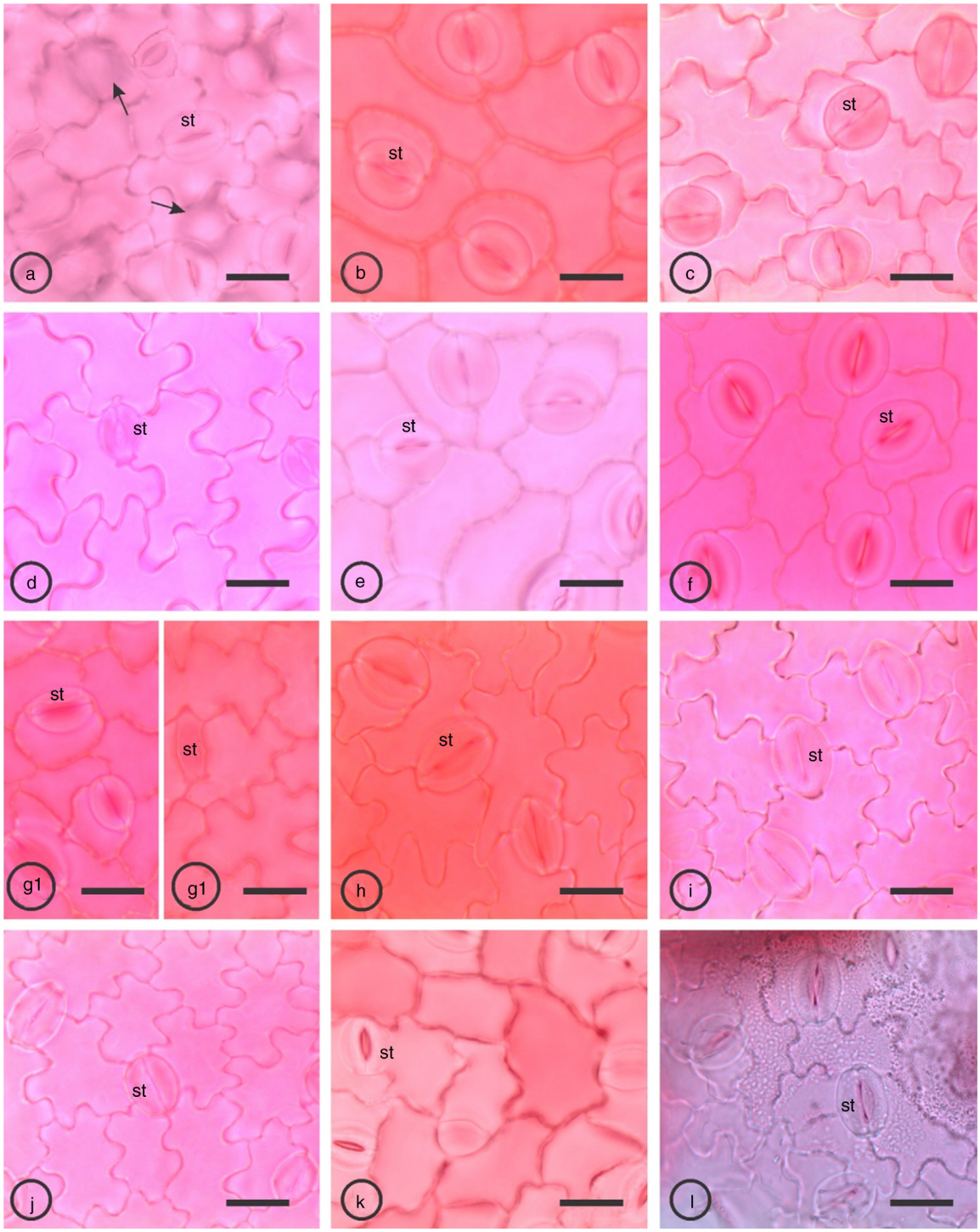

Fig. 3. *Passiflora* spp. – View of the leaf surface (epidermis) – Abaxial side. a. *P. actinia*; b. *P. alata*; c. *P. amethystina*; d. *P. capsularis*; e. *P. cincinnata*; f. *P. edulis* f. *flavicarpa*; g1. *P. edulis* f. *edulis* sample 7.1; g2. *P. edulis* f. *edulis* sample 7.2; h. *P. incarnata*; i. *P. morifolia*; j. *P. urnifolia*; k. *P. coccinea*; l. *P. setacea*. st: stomata; arrows indicate papillae. Bars = 20 μm.

non-glandular trichomes (Fig. 5b) only on the adaxial side. *P. capsularis* (Fig. 5a), *P. edulis* f. *flavicarpa* (Fig. 5c), *P. edulis* f. *edulis* (Fig. 5d, e), *P. incarnata* (Fig. 5f), *P. morifolia* (Fig. 5g), *P. urnifolia* (Fig. 5h), *P. coccinea* (Fig. 5i) and *P. setacea* (Figs. 4g, h and 5j) are pubescent on both sides.

P. capsularis presents two types of non-glandular trichomes distributed throughout the leaf blade; however, there are more trichomes on the abaxial side. The first type is conical, straight (Fig. 5a, right side) and commonly bicellular, but it can be unicellular or multicellular; the base cell is wider and shows a region in half sphere; the cuticle is striate. The second type is cylindrical (Fig. 5a, left side) and unicellular; the cell wall is thin and the apex is rounded; the width is constant and covered by a striate cuticle; they are smaller than the other trichome previously described.

P. cincinnata shows conical, slightly curved (Fig. 5b) and unicellular non-glandular trichomes. They are short, the cell wall is thickened and the cuticle is striate. These trichomes are located only on the larger veins of the adaxial side (Fig. 5b).

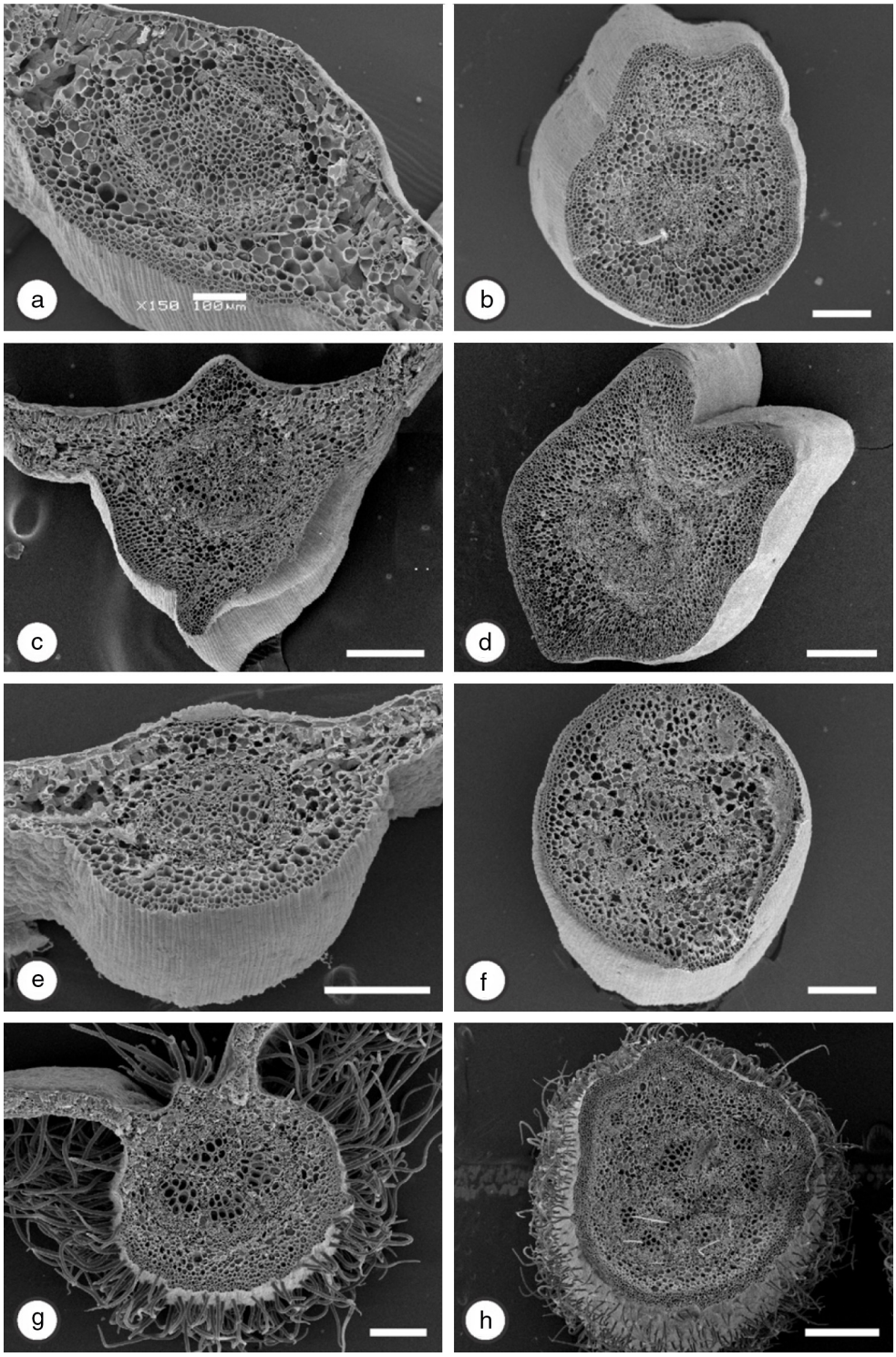

Fig. 4. *Passiflora* spp. Cross-sections of midrib and petiole of glabrous species *P. actinia*, a–b; *P. alata*, c–d; *P. amethystina*, e–f; and densely pubescent *P. setacea*, g–h. Bars (μm) = 100 (a), 200 (b, c, e–g), 500 (d, h).

In *P. edulis* f. *flavicarpa*, a few non-glandular trichomes can be seen on both sides of the midrib. They are conical and straight (Fig. 5c), short or medium in length and the cuticle is striate.

P. edulis f. *edulis* (sample 7.1) has cylindrical (Fig. 5d) and unicellular or bicellular non-glandular trichomes with a acute apex. These trichomes are encountered on both sides, particularly only on the larger vein on the adaxial side and more uniformly scattered on the abaxial surface. In sample 7.2, conical non-glandular trichomes (Fig. 5e) were encountered, positioned on the veins on both sides.

P. incarnata presents cylindrical, slightly curved, non-glandular trichomes (Fig. 5f) on both leaf sides. On the adaxial face they are unicellular or bicellular, wide and with a thick cell wall. They have different lengths and a tapering apical cell. Similar trichomes can be observed on the abaxial side, but they are multicellular and longer. On both leaf sides, these trichomes are observed mainly on the larger vein.

P. morifolia presents two types of non-glandular trichomes spread uniformly on the leaf blade. The first is unicellular and

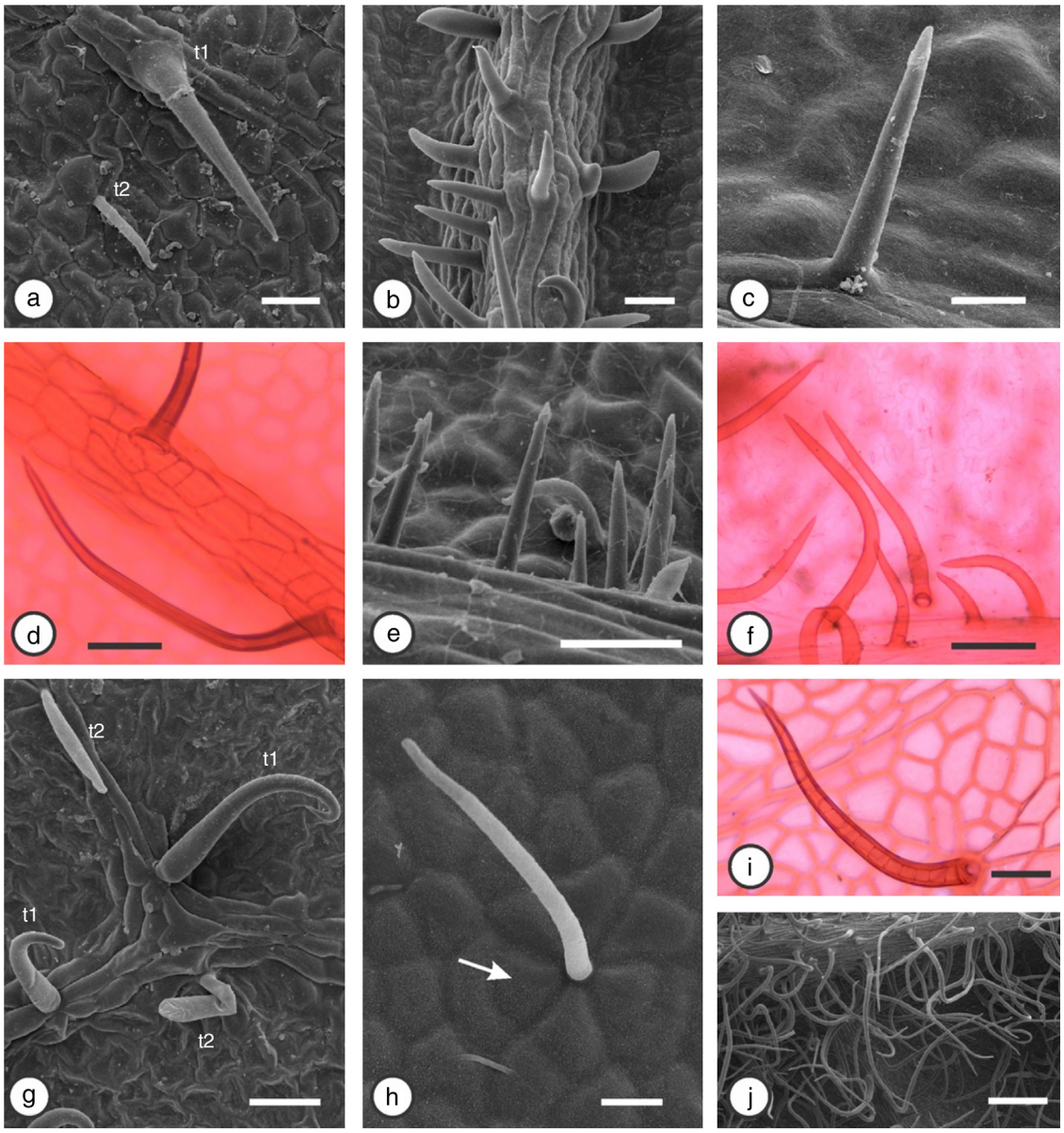

Fig. 5. *Passiflora* spp. – Non-glandular trichomes. a. P. capsularis; b. *P. cincinnata*; c. *P. edulis* f. *flavicarpa*; d. *P. edulis* f. *edulis* sample 7.1; e. *P. edulis* f. *edulis* sample 7.2; f. *P. incarnata*; g. *P. morifolia*; h. *P. urnifolia*; i. *P. coccinea*; j. *P. setacea*. t1: trichome type 1; t2: trichome type 2; arrow indicate cells organized in a rosette. Bars (μm) = 20 (c, h), 50 (a, b, d, e, g, i), 100 (f), 200 (j).

uncinated (Fig. 5g). It has a thick cell wall and a striate cuticle. The second type is unicellular and cylindrical with a rounded apex (Fig. 5g). It has a thin cell wall and a striate cuticle. The uncinated, non-glandular trichome is longer than the cylindrical trichome and on the abaxial side, the cylindrical type is less common.

P. urnifolia presents cylindrical, non-glandular trichomes (Fig. 5h) with a rounded apex, thick cell wall and a striate cuticle. They are distributed throughout the leaf blade although more frequent on the larger vein.

P. coccinea presents cylindrical (Fig. 5i) and unicellular, bicellular or multicellular non-glandular trichomes. They show a tapering apex, wide cell wall and the base cell has a region in half sphere. These trichomes are distributed uniformly on the leaf blade, more frequently on the abaxial side.

P. setacea presents uniserate, filiform, non-glandular trichomes. They are bicellular, formed by a base cell with a region in half sphere, or multicellular, formed by ten or more cells. On the adaxial side, these trichomes are only dispersed on the larger vein. On the abaxial side, they are more homogeneously distributed on the leaf blade (Fig. 5j).

The adjacent epidermal cells of the trichomes may be similar to other cells or grouped in a rosette. They vary according to taxa, trichome type or blade side. In *P. capsularis*, the basal portion of the trichome is surrounded by cells organized in a rosette or arranged slightly above the other epidermal cells (Fig. 5a). In *P. incarnata*, *P. morifolia*, *P. urnifolia* (Fig. 5h) and *P. coccinea*, the basal portion of the trichome is surrounded by cells organized in a rosette on both sides. In *P. setacea*, the adjacent cells are arranged slightly above the other epidermal cells surrounding the trichomes on the adaxial side. In *P. edulis* f. *edulis* (sample 7.1), the adjacent epidermal cells of the trichomes are similar to the other cells.

Cross section of leaf blade

In cross-section, all taxa show a uniserate epidermis covered with a thin, smooth cuticle (Fig. 6). *P. actinia* shows papillae

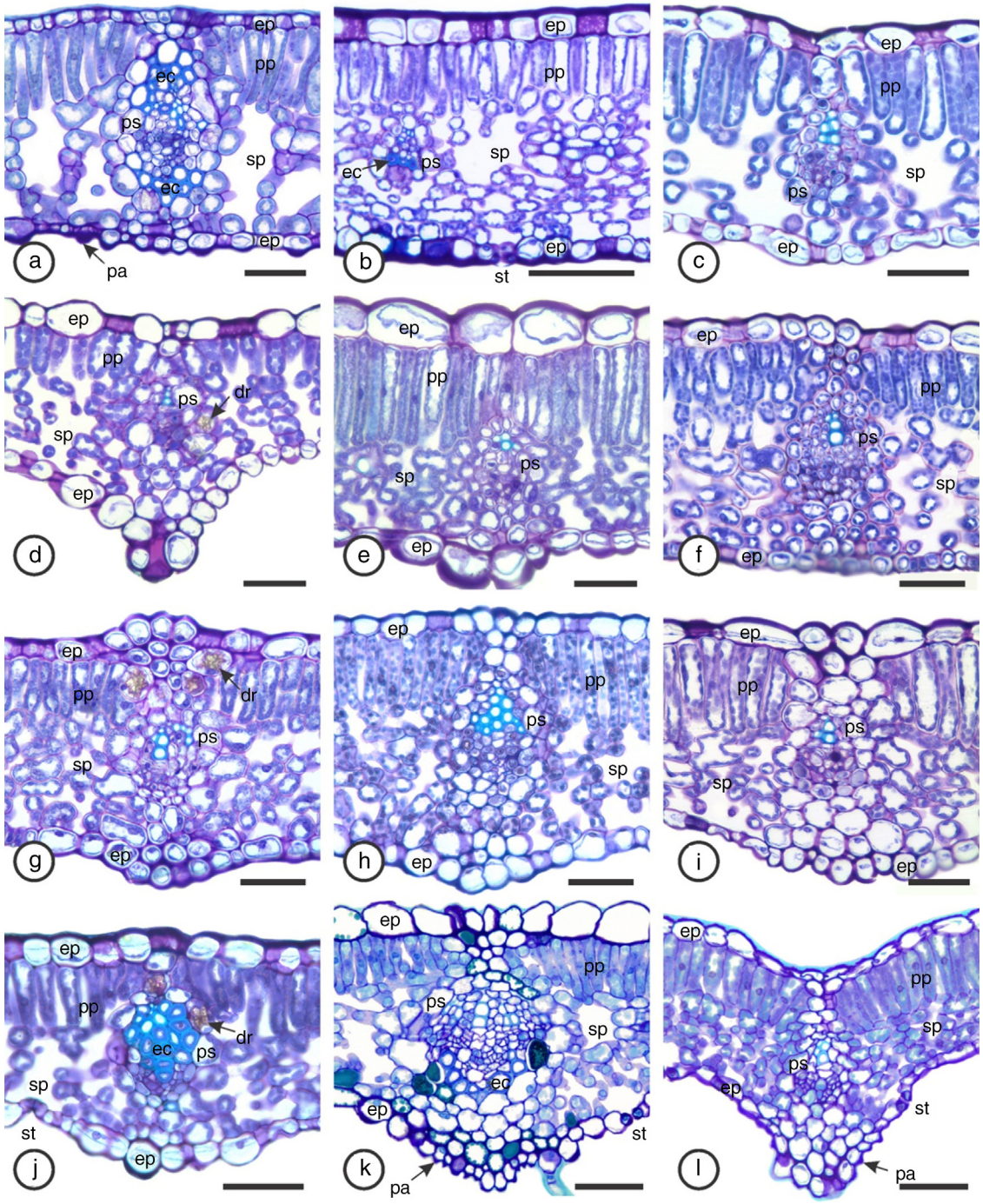

Fig. 6. *Passiflora* spp. – Leaf blade in cross-section at medium vein. a. *P. actinia*; b. *P. alata*; c. *P. amethystina*; d. *P. capsularis*; e. *P. cincinnata*; f. *P. edulis* f. *flavicarpa*; g. *P. edulis* f. *edulis*; h. *P. incarnata*; i. *P. morifolia*; j. *P. urnifolia*; k. *P. coccinea*; l. *P. setacea*. ep: epidermis; pp: palisade parenchyma; sp: spongy parenchyma; st: stomata; ps: parenchymatic sheath; ec: sclerenchyma; dr: druse; pa: papillae. Bars (μm) = 50 (a, c, d–l), 100 (b).

distributed on the abaxial side (Fig. 6a) and *P. coccinea* and *P. setacea* show some papillae on a minor vein on the abaxial side (Fig. 6k and l).

The mesophyll is dorsiventral and a stratum of palisade parenchyma appears in all taxa (Fig. 6). The layers of spongy parenchyma vary by species; 3–6 for *P. amethystina* (Fig. 6c), 3–4 for *P. capsularis* (Fig. 6d), 3–5 for *P. morifolia* (Fig. 6i) and *P. setacea* (Fig. 6l), and 7–10 for *P. alata* (Fig. 6b), for example. The samples of *P. capsularis* differed in leaf blade thickness. The sample 4.4 presented palisade parenchyma narrower than the rest of the samples of this species (Fig. 7).

Small and medium collateral vascular bundles can be detected immersed in the mesophyll and surrounded by the parenchymatic sheath (Fig. 6). *P. actinia*, *P. alata*, *P. amethystina*, *P. capsularis*, *P. cincinnata*, *P. edulis* f. *flavicarpa*, *P. edulis* f. *edulis*, *P. incarnata*, *P. urnifolia* and *P. setacea* show druses in the parenchymatic sheath, however, *P. coccinea* presents druses only in the palisade parenchyma. No druses appeared in the leaf blade of *P. morifolia*.

In *P. actinia* sclerenchymatic fibers caps are adjoined to the phloem and xylem (Fig. 6a), however, they do not occur in all bundles. *P. alata* (Fig. 6b) and *P. coccinea* (Fig. 6k) showed fiber caps adjoined only to the phloem, but they are not found in all bundles.

Box 4
Midrib shape in cross-section of *Passiflora* species.

Species	Midrib shape
P. actinia	Biconvex with prominent and rounded convexity on the abaxial side.
P. alata	Biconvex with slight convexity on the adaxial side and prominent and angular convexity on the abaxial side.
P. amethystina	Biconvex with prominent and rounded convexity on the abaxial side.
P. capsularis	Biconvex with prominent and truncate convexity on the abaxial side, irregular projections on both sides.
P. cincinnata	Biconvex, with an acute projection to the adaxial side and prominent and rounded convexity on the abaxial side.
P. edulis f. *flavicarpa*	Biconvex with cylindrical projection on the adaxial side and prominent and rounded convexity on the abaxial side, forming an ovate shape.
P. edulis f. *edulis*	Biconvex with cylindrical projection on the adaxial side and prominent and rounded convexity on the abaxial side, ovate in outline.
P. incarnata	Biconvex with acute projection on the adaxial side and prominent and truncate convexity on the abaxial side, triangular in outline.
P. morifolia	Biconvex with acute projection on the adaxial side and prominent convexity with small projections on the abaxial side.
P. urnifolia	Flat-convex shape, with a minor projection on the adaxial side and prominent convexity on the abaxial side.
P. coccinea	Biconvex with prominent and rounded convexity on the abaxial side.
P. setacea	Biconvex with acute projection on the adaxial side and prominent and rounded convexity on the abaxial side, with small projections.

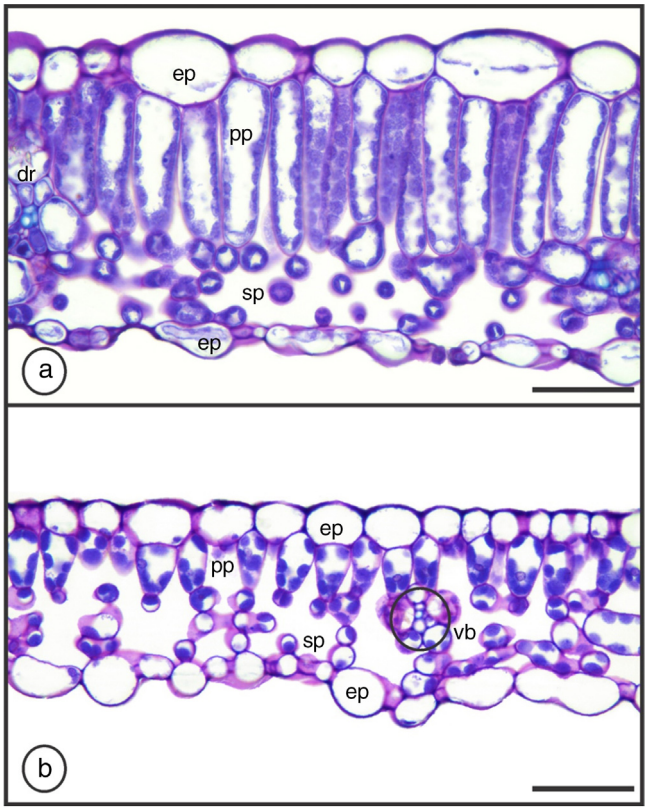

Fig. 7. *Passiflora capsularis*. Leaf blade in cross-section - Thickness differences of the leaf blade. a. sample 4.2 (similar as all other samples of this species); b. sample 4.4. ep: epidermis; pp: palisade parenchyma; sp: spongy parenchyma; dr: druse; vb: vascular bundle. Bars = 50 μm.

Box 5
Midrib vascular patterns of *Passiflora* species.

Species	Midrib vascular pattern
P. actinia	Three free traces in a flat arc and one dorsal trace.
P. alata	One flat arc with one dorsal trace.
P. amethystina	Three free traces in a flat arc and one dorsal trace, organizing in a central ring.
P. capsularis	One flat arc with one minor dorsal trace, organizing in a central ring with a medullar region.
P. cincinnata	Three free traces in flat arc, one dorsal and one lateral trace.
P. edulis f. *flavicarpa*	Four traces in a central ring.
P. edulis f. *edulis*	Four traces in a central ring.
P. incarnata	Three free traces in a flat arc and one dorsal trace.
P. morifolia	One flat arc with one dorsal trace, organizing in a central ring with a small medullar region.
P. urnifolia	Medullated cylinder.
P. coccinea	Five free traces in a flat arc and one dorsal trace.
P. setacea	Four free traces in ring.

P. urnifolia showed sclerenchymatic cells at different stages of lignification located in the center of the vascular bundle (Fig. 6j). No sclerenchyma appeared in the rest of the taxa.

In some taxa, the medium vein makes projections or depressions on both sides of the leaf blades. A convexity is observed on the abaxial side of *P. capsularis* (Fig. 6d), *P. cincinnata* (Fig. 6e), *P. incarnata* (Fig. 6h), *P. morifolia* (Fig. 6i), *P. urnifolia* (Fig. 6j), *P. coccinea* (Fig. 6k), and on both sides of *P. edulis* f. *edulis* (Fig. 6g). Only *P. setacea* presents a convexity on the abaxial side and a concavity on the adaxial side on the medium vein (Fig. 6l).

Cross section of midrib

In the cross-section, the midrib shape shows a different standard for *Passiflora* taxa as observed in Fig. 8 and summarized in Box 4.

Box 6
Petiole shape of *Passiflora* species.

Species	Shape	Description
P. actinia	Flat-convex to concave-convex	Flat or slightly concave on the adaxial side and rounded convexity on the abaxial side.
P. alata	Concave-convex	Adaxial side with two ribs at the end and acute and prominent on the abaxial side.
P. amethystina	Flat-convex	Almost circular, but truncate on the adaxial side.
P. capsularis	Flat-convex	Almost circular, but truncate on the adaxial side.
P. cincinnata	Concave-convex	Adaxial side with two small ribs at the end and acute and prominent on the abaxial side, pentagonal in outline.
P. edulis f. *flavicarpa*	Concave-convex	Adaxial side with two conspicuous ribs at the end and prominent convexity sharper in the central region on the abaxial side.
P. edulis f. *edulis*	Concave-convex	Adaxial side with two conspicuous ribs at the end and prominent convexity sharper in the central region on the abaxial surface.
P. incarnata	Concave-convex	Adaxial side with two conspicuous ribs at the end and rounded and prominent convexity on the abaxial surface.
P. morifolia	Flat-convex	Almost circular, but truncate on the adaxial side.
P. urnifolia	Flat-convex to concave-convex	Flat or slightly concave on the adaxial side and rounded convexity on the abaxial side.
P. coccinea	Concave-convex	Adaxial side with two conspicuous ribs at the end and rounded and prominent convexity on the abaxial side.
P. setacea	Flat-convex	Narrow and truncate adaxial side and rounded and prominent convexity on the abaxial side, oval in outline.

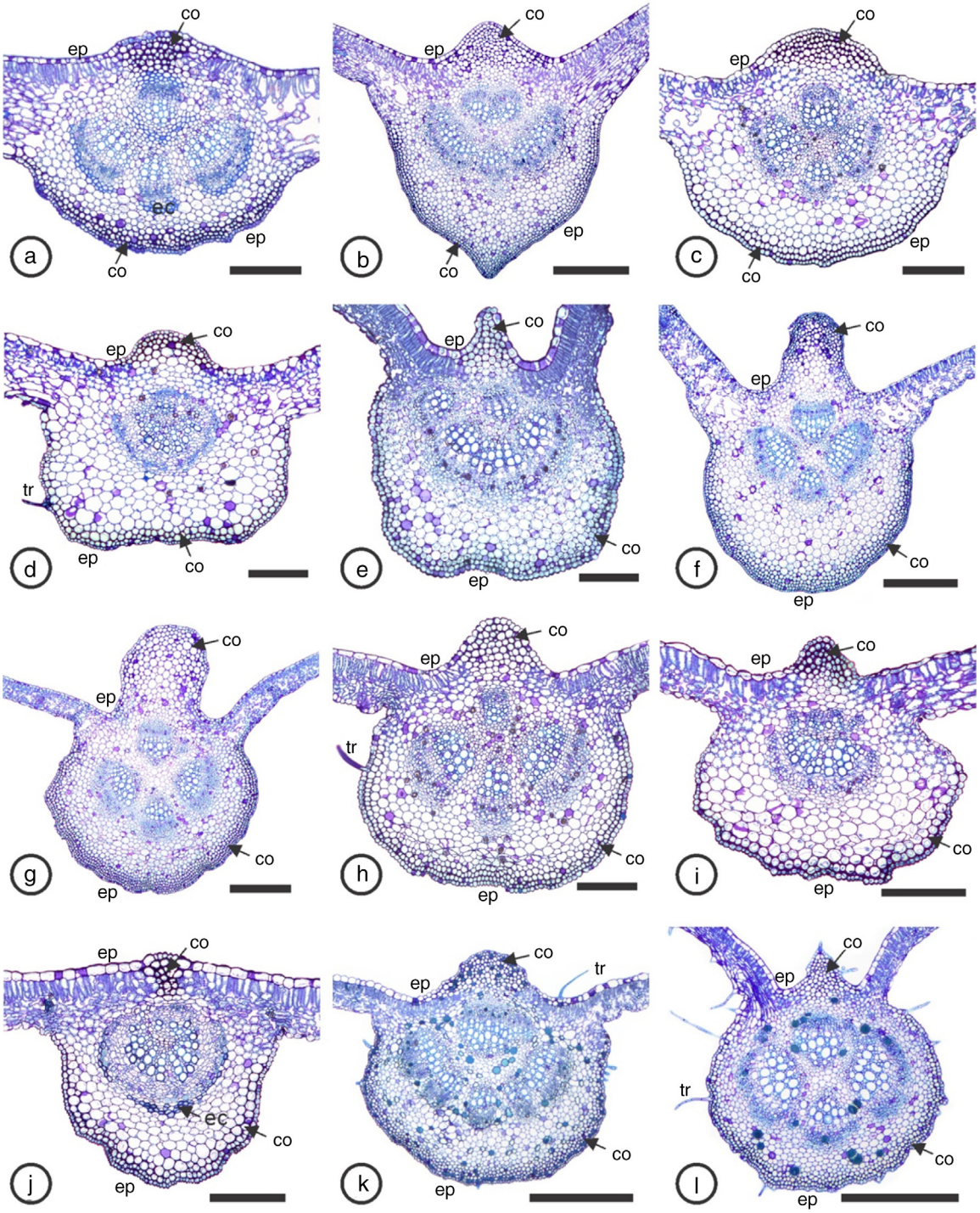

Fig. 8. Midrib in cross-section. a. *P. actinia*; b. *P. alata*; c. *P. amethystina*; d. *P. capsularis*; e. *P. cincinnata*; f. *P. edulis* f. *flavicarpa*; g. *P. edulis* f. *edulis*; h. *P. incarnata*; i. *P. morifolia*; j. *P. urnifolia*; k. *P. coccinea*; l. *P. setacea*. ep: epidermis; co: chollenchyma; ec: sclerenchyma; tr: non-glandular trichome. Bars (μm) = 200 (a, c, d, e, h, i, j), 500 (b, f, g, k, l).

Beneath the epidermis of the adaxial side, the number of angular collenchyma strata vary from 7 to 8 in *P. morifolia* (Fig. 8i), 7 to 9 in *P. cincinnata* (Fig. 8e) and *P. edulis* f. *flavicarpa* (Fig. 8f), 7 to 14 in *P. edulis* f. *edulis* (Fig. 8g) and *P. incarnata* (Fig. 8h). On the abaxial side, 2–5 angular collenchyma strata are present in all the taxa. Druses are dispersed in the ground parenchyma in all taxa (Fig. 8).

Sclerenchymatic tissue is represented by cells or caps. Perivascular caps adjoin the phloem and free cells near the xylem in *P. actinia* (Fig. 8a). *P. alata* shows sclerenchymatic cells adjoining the phloem (Fig. 8b). *P. urnifolia* shows perivascular caps adjoining the phloem (Fig. 8j). *P. coccinea* shows perivascular caps adjacent to the phloem of the dorsal sheath bundle (Fig. 8k).

The vascular system is represented by collateral vascular bundles; midrib vascular patterns are summarized in Box 5.

Cross section of petiole

The petiole of the *Passiflora* taxa has different shapes in cross-section. They vary from flat-convex to concave-convex, as observed in Fig. 9 and summarized in Box 6.

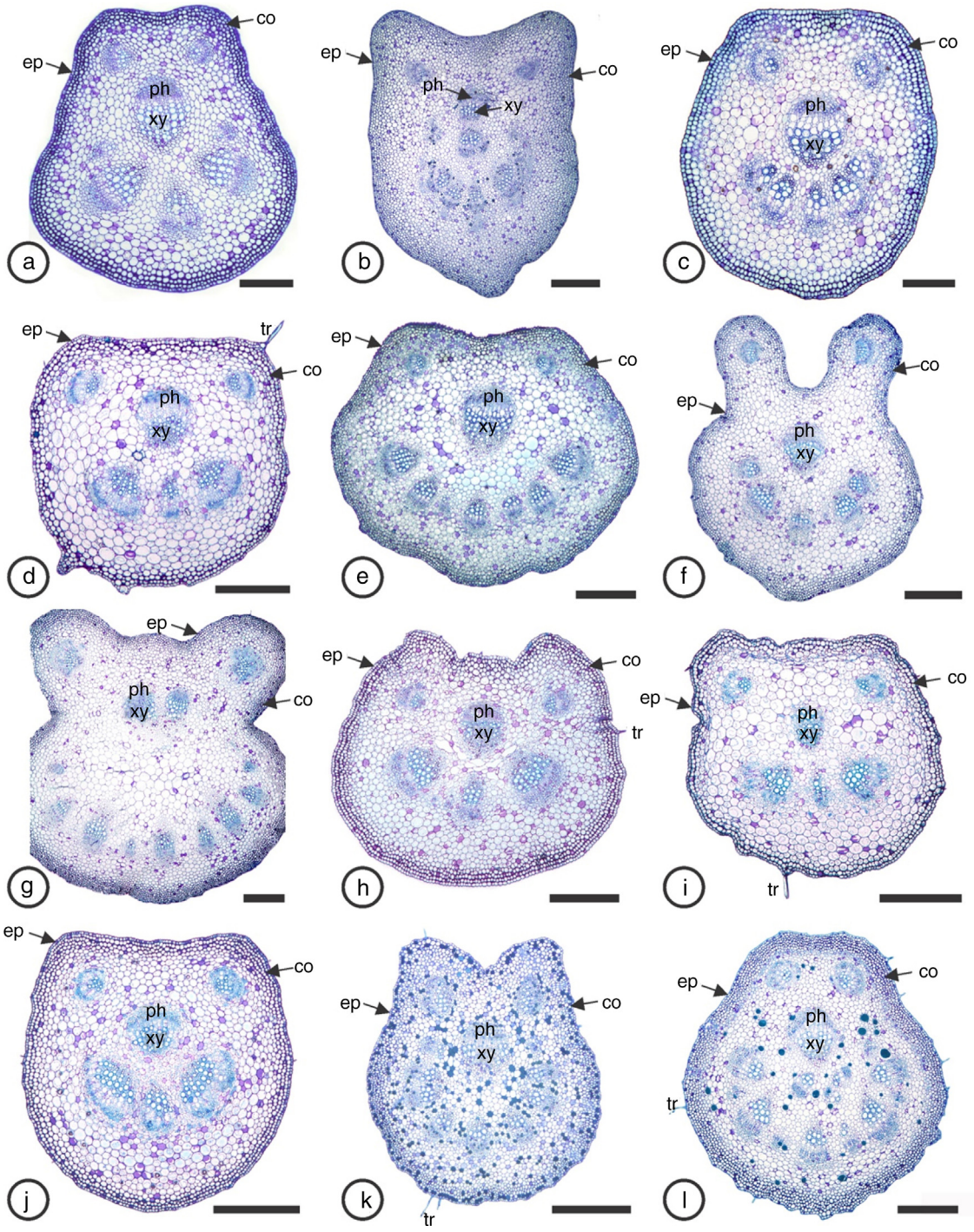

Fig. 9. Petiole in cross-section. a. *P. actinia*; b. *P. alata*; c. *P. amethystina*; d. *P. capsularis*; e. *P. cincinnata*; f. *P. edulis* f. *flavicarpa*; g. *P. edulis* f. *edulis*; h. *P. incarnata*; i. *P. morifolia*; j. *P. urnifolia*; k. *P. coccinea*; l. *P. setacea*. ep: epidermis; co: chollenchyma; ph: phloem; xy: xylem; tr: trichome. Bars (μm) = 200 (a, c), 500 (b, d–l).

The epidermis shows the same characteristics as described for the leaf blade. Trichomes can be observed in all taxa, except in *P. actinia*, *P. alata* and *P. amethystina*.

The vascular system is represented by collateral vascular bundles and the petiole vascular patterns are summarized in Box 7. Beneath the epidermis, 2–4 continuous angular collenchyma strata and druses are present scattered in the ground parenchyma (Fig. 9).

The main anatomical characteristics are summarized in Box 8.

Microscopic analysis of powder

The microscopic analysis of powder identified some characteristics previously described in the anatomical analyses (Fig. 10).

Passiflora taxa powder show druses, leaf blade fragments of the uniserate epidermis on both sides, a palisade parenchyma stratum, some strata of spongy parenchyma, collenchyma (Fig. 10c), epidermal cell walls as described for each species (Fig. 10b and g), non-glandular trichomes (Fig. 10d–f and h–i), stomata (Fig. 10b) and vascular tissue fragments.

Box 7
Petiole vascular patterns of *Passiflora* species.

Species	Petiole vascular pattern
P. actinia	Five free traces in a flat arc, a larger dorsal trace and two lateral rib traces.
P. alata	Eight free traces forming a central ring with a medullar trace and two lateral rib traces.
P. amethystina	Five free traces in a flat arc, a larger dorsal trace and two lateral rib traces.
P. capsularis	Three free traces in a flat arc, a larger dorsal trace and two lateral rib traces.
P. cincinnata	Seven free traces in a flat arc, a larger dorsal trace and two rib traces.
P. edulis f. *flavicarpa*	Seven free traces in a flat arc, a dorsal trace and two rib traces.
P. edulis f. *edulis*	Nine free traces in a flat arc and one or two dorsal traces and two rib traces.
P. incarnata	Five u-shaped free bundles and a dorsal trace.
P. morifolia	Three free traces in a flat arc, a dorsal trace and two lateral rib traces.
P. urnifolia	Three free traces in a flat arc, a dorsal trace and two lateral rib traces.
P. coccinea	Six u-shaped free bundles, a dorsal trace and two rib traces.
P. setacea	Six free traces forming a central ring with two or three traces in the middle and two lateral rib traces.

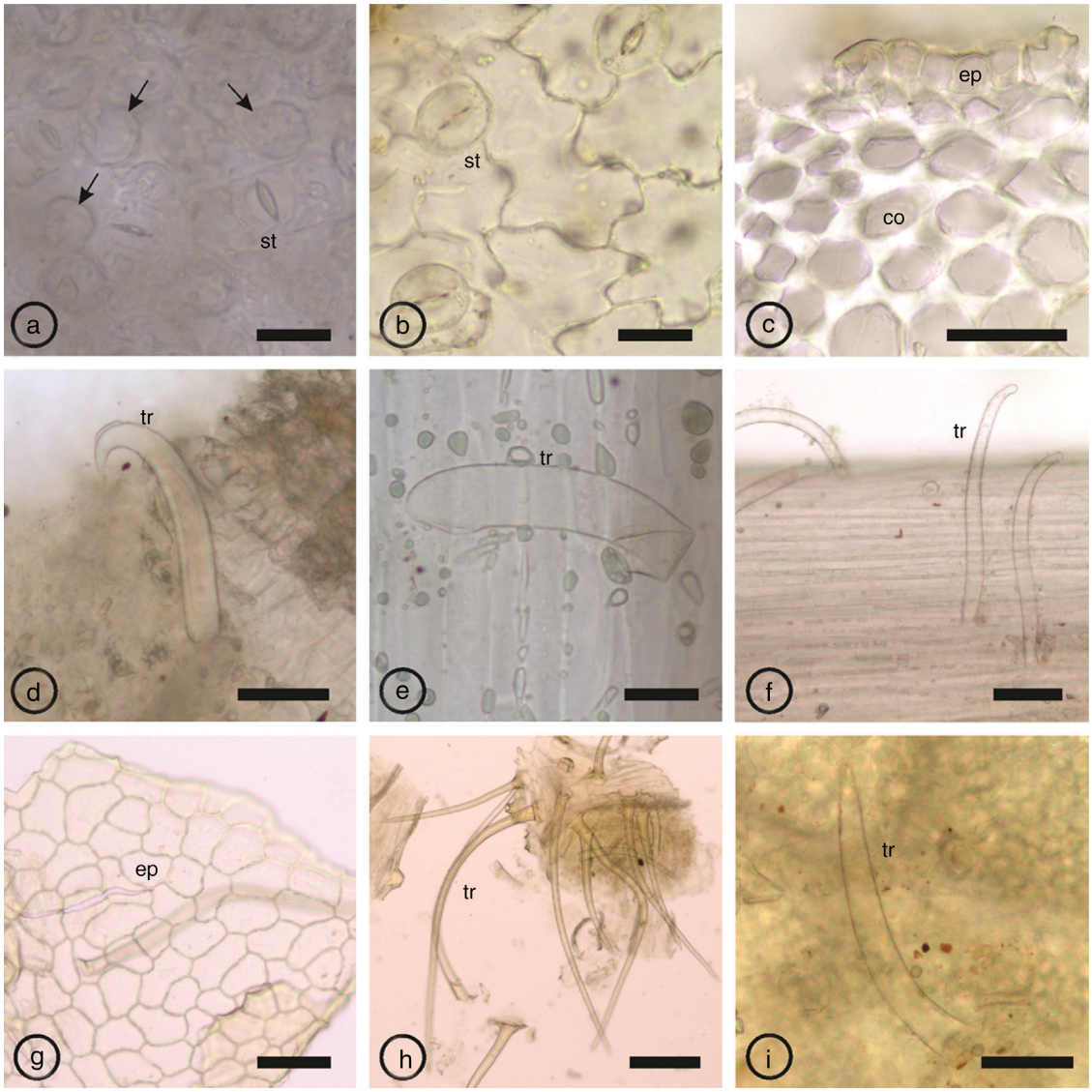

Fig. 10. Microscopic analysis of powder. a. *P. actinia*, papillae in frontal view of epidermis abaxial side; b. *P. amethystina*, sinuous anticlinal epidermal cell wall; c. *P. edulis* f. *edulis*, epidermis and angular chollenchyma; d. *P. morifolia*, uncinate non-glandular trichome; e. *P. morifolia*, cylindrical non-glandular trichome with rounded apex; f. *P. urnifolia*, cylindrical non-glandular trichome with rounded apex; g. *P. setacea*, slightly wavy anticlinal epidermal cell wall; h. *P. setacea*, filiform non-glandular trichome on the vein; i. *P. incarnata*, cylindrical non-glandular trichome with acute apex. ep: epidermis; st: stomata; co: chollenchyma; tr: non-glandular trichome; arrow: papillae. Bars (μm) = 20 (a, b, e), 50 (c), 100 (d, f, g, i), 200 (h).

In non-glabrous taxa, non-glandular trichomes are observed, except for *P. edulis* f. *flavicarpa* that has few trichomes. In addition, *P. actinia* show not only stomata but also numerous papillae on the abaxial side (Fig. 10a).

Discussion

Determining the authenticity and quality of plant raw materials used in the formulation of herbal medicines, teas

Box 8
Anatomical features of differentiation between *Passiflora* species.

	P. actinia	*P. alata*	*P. amethystina*	*P. capsularis*	*P. cincinnata*	*P. edulis* f. *flavicarpa*
Epidermal cell anticlinal walls						
Adaxial	Wavy to slightly sinuous	Straight to slightly wavy	Slightly sinuous to sinuous	Slightly sinuous to sinuous	Straight to slightly wavy	Slightly wavy to wavy
Abaxial	Slightly sinuous to sinuous	Slightly wavy to wavy	Slightly sinuous to sinuous	Slightly sinuous to sinuous	Slightly wavy to wavy	Slightly sinuous to sinuous
Non-glandular trichomes						
Adaxial	Glabrous	Glabrous	Glabrous	(1) Conical (2) cylindrical (rounded apex)	Glabrous	
Abaxial					Conical	Conical
Stomata location	Hypo	Amphi	Hypo	Hypo	Hypo	Hypo
Presence of papillae	Internerval region on abaxial face	–	–	–	–	–
Sclerenchymatic cells	Caps adjoin to phloem and xylem	Cells adjoin to phloem	–	–	–	–
Medium vein shape	Flat/flat	Flat/flat	Flat/flat	Flat/convex	Flat/slightly convex	Flat/flat
Midrib shape						
Midrib vascularization pattern	3 Free traces in flat arc and 1 dorsal trace	1 Flat arc with 1 dorsal trace	3 Free traces in flat arc and 1 dorsal trace, organizing in a central ring	1 Flat arc with one minor dorsal trace, organizing in a central ring with a medullar region	3 Free traces in flat arc, 1 dorsal and 1 lateral trace	4 Traces in a central ring
Petiole shape	Flat/convex to concave-convex	Concave/convex	Flat/convex	Flat/convex	Concave/convex	Concave/convex
Petiole vascularization pattern	5 Free traces in flat arc, a larger dorsal trace and 2 lateral rib traces	8 Free traces forming a central ring with a medullar trace and 2 lateral rib traces	5 Free traces in flat arc, a larger dorsal trace and 2 lateral rib traces	3 Free traces in flat arc, a larger dorsal trace and 2 lateral rib traces	7 Free traces in flat arc, a larger dorsal trace and 2 rib traces	7 Free traces in flat arc, a dorsal trace and 2 rib traces

	P. edulis f. *edulis*		*P. incarnata*	*P. morifolia*	*P. urnifolia*	*P. coccinea*	*P. setacea*
	7.1	7.2					
Epidermal cell anticlinal walls							
Adaxial	Straight to slightly wavy	Slightly sinuous to sinuous	Slightly sinuous to sinuous	Slightly sinuous to sinuous	Slightly wavy to wavy	Straight to slightly wavy	Straight to slightly wavy
Abaxial	Slightly wavy to wavy	Slightly sinuous to sinuous	Slightly sinuous to sinuous	Slightly sinuous to sinuous	Slightly sinuous to sinuous	Wavy to slightly sinuous	Slightly sinuous to sinuous
Non-glandular trichomes							
Adaxial	Cylindrical (pointed apex)	Conical	Cylindrical (pointed apex)	(1) Uncinate (2) cylindrical (rounded apex)	Cylindrical (rounded apex)	Cylindrical (pointed apex)	Filiform
Abaxial							
Stomata location	Hypo		Amphi	Hypo	Hypo	Hypo	Hypo
Presence of papillae	–		–	–	–	Medium venation	Medium venation
Sclerenchymatic cells	–		–	–	Cells in the center of the vascular bundle	Cells adjoined to phloem	–
Medium vein shape	Slightly convex/slightly convex		Flat/slightly convex	Flat/slightly convex	Flat/slightly convex	Flat/slightly convex	Concave/convex
Midrib shape							
Midrib vascularization pattern	4 Traces in a central ring		3 Free traces in flat arc and 1 dorsal trace	1 Flat arc with 1 dorsal trace, organizing in a central ring with a small medullar region	Medullated cylinder	5 Free traces in flat arc and 1 dorsal trace	4 Free traces in ring
Petiole shape	Concave/convex		Concave/convex	Flat/convex	Flat/convex to concave/convex	Concave/convex	Flat/convex
Petiole vascularization pattern	9 Free traces in flat arc and 1 or 2 dorsal traces and 2 rib traces		U-shaped 5 free bundles and a dorsal trace	3 Free traces in flat arc a dorsal trace and 2 lateral rib traces	About 3 free traces in flat arc, a dorsal trace and 2 lateral rib traces	U-shaped 6 free bundles, a dorsal trace and 2 rib traces	About 6 free traces forming a central ring with 2 or 3 traces in the middle and 2 lateral rib traces

Amphi, amphistomatic, Hypo, hypostomatic.

and cosmetics are essential to ensure its security and efficacy for clinical use. Misidentification and tampering increases the probability of adverse events, such as the hepatotoxicity cases reported by Pittler and Ernst (2003). Several raw materials are intentionally adulterated by other species that are morphologically similar, cheaper in price, or more readily available (Ma et al., 2002; Zhao et al., 2006; Zhang et al., 2007).

P. edulis has been widely cultivated in Brazil and Moraes et al. (1997) reported that leaves and stems have been used as an adulterant for *P. alata*, which was included in the first three editions of the Brazilian Pharmacopoeia and was official in the country at that time. Moreover, in Brazil, several *Passiflora* species are popularly known as 'maracujá', which reinforces the importance of identification and differentiating between them (Beraldo and Kato, 2010).

Morphological characteristics are very important for correct species identification. However, several medicinal species are sold fragmented or powdered. Thus, the anatomical features can greatly contribute, plus to morphology, by indicating the characteristics for botanical identification.

In this research, similar morpho-anatomical characteristics were encountered in all *Passiflora* taxa, such as dorsiventral mesophyll, collateral vascular bundles and the presence of druses. However, the following structures might be emphasized as useful to distinguish species: midrib and petiole shape in cross-section, midrib and petiole vascular patterns, outline of medium veins in cross-section, sclerenchymatic cells adjoining the vascular bundle, presence and type of non-glandular trichomes and presence of papillae in the epidermis of the leaf blade.

The importance of standardizing medicinal plant cultivation is highlighted by a difference found between the samples of *P. capsularis*. The mesophyll thickness of sample 4.4 was narrower than the one of other samples, due to environmental influences. The sample 4.4 was collected from an Atlantic Forest where it had grown in the shadow of trees. The other samples were collected from an open space with direct sun exposure. Pires (2008) studied the behavior of some *Passiflora* species in relation to shading and found that *P. morifolia*, growing in full sun with 25% of shade, showed a thicker mesophyll formed by longer palisade cells and bigger intercellular spaces when compared with others growing in 50–75% of shade.

Crochemore et al. (2003) studied the diversity of *Passiflora* species in relation to morphological characteristics. They reported a great variability between the analyzed samples of *P. edulis* f. *edulis* in coincidence with the morpho-anatomical variability found in this study for the same form. Amorim et al. (2014) interpreted that the intra and interspecific variability within the *Passiflora* genus was associated with an ability to adapt to different environments. In addition, Viana et al. (2003) suggested that another reason was the easiness of *Passiflora* species sexual reproduction.

It is important to highlight the variability found in this study in *P. edulis* f. *flavicarpa* and *P. edulis* f. *edulis*, especially regarding to the presence and type of trichomes. However, the literature discusses this topic only at the species level and not at the form level. Besides, the reports are inconsistents (Pereira et al., 2009; Beraldo and Kato, 2010; Farmacopeia, 2010; Barbosa et al., 2013; Leite et al., 2013; Farias, 2014; Cervi, 1997).

In this study, only *P. alata* and *P. incarnata* showed amphistomatic leaves. However, they are considered as hypostomatic by Farmacopeia (2010), Pereira et al. (2009), Farias (2014) and WHO (2007). This probably happened because of the low density of stomata present on the adaxial side for both species.

Conclusion

The morphological characteristics of *Passiflora* spp. leaves support the differentiation of species; however, when used in herbal drugs, fragmented or powdered, anatomical characteristics provide additional useful data for differentiating them.

Authors' contributions

LW (MSc student) was responsible for collecting plant material, identification, confection of herbarium, running the laboratory work, analysis of the data and drafting the paper; DCI contributed to plant collecting, identification and herbarium confection; BBM contributed in making the slides and analysis of the data; ACC, JMB and CAMS designed the study, supervised the laboratory work and read the manuscript critically. All of the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

The authors thank Vera L. P. dos Santos and Celia R. C. Franco for their valuable work on the electronic microscopy slides preparation; an acknowledgment is also due to Dr. Fabio G. Faleiro, Embrapa Cerrados, Planaltina-DF for collecting some *Passiflora* species. CAMS thanks CNPq for the research schorlarship.

References

Amorim, J.S., Souza, M.M., Viana, A.J.C., Corrêa, R.X., Araújo, I.S., Ahnert, D., 2014. Cytogenetic, molecular and morphological characterization of *Passiflora capsularis* L. and *Passiflora rubra* L. Plant Syst. Evol. 300, 1147–1162.

Barbosa, N.C.S., Barbosa, C.J., Leite, K.R.B., da Silva, L.B., 2013. Anatomia das superfícies foliares de espécies de *Passiflora* (Passifloraceae) como subsídio para estudos agronômicos. 64 (Congresso Nacional de Botânica. Belo Horizonte, Brasil).

Beraldo, J., Kato, E.T.M., 2010. Morfoanatomia de folhas e caules de *Passiflora edulis* Sims, Passifloraceae. Rev. Bras. Farmacogn. 20, 233–239.

Berlyn, G.P., Miksche, J.P., 1976. Botanical Microtechnique and Cytochemistry. Iowa State University, Ames.

Cervi, A.C., 1997. Passifloraceae do Brasil. Estudo do gênero *Passiflora* L., subgênero *Passiflora*. Fontqueria 45, 1–92.

Conrado, D.J., Fronza, T., Paiva, R.M., Dresch, A.P., Geremias, D., Fenner, R., Viana, A.F., Rates, S.M.K., 2003. Aspectos químicos, farmacológicos e emprego terapêutico do gênero *Passiflora* (Maracujá). Rev. Afargs 15, 14–19.

Crochemore, M.L., Molinari, H.B., Stenzel, N.M.C., 2003. Caracterização agromorfológica do maracujazeiro (*Passiflora* spp). Rev. Bras. Frutic. 25, 5–10.

Farias, V., (Dissertação de mestrado) 2014. Anatomia foliar de *Passiflora* L. (Passifloraceae): aspectos taxonômicos e evolutivos. Curitiba. Programa de pós-graduação em Botânica, Universidade Federal do Paraná, 63 pp.

Farmacopeia Brasileira, 2010. 5th ed. Agência Nacional de Vigilância Sanitária, Brasília.

Feder, N., O'Brien, T.P., 1968. Plant microtechnique: some principles and new methods. Am. J. Bot. 55, 123–142.

Freitas, P.C.D., Dissertação de Mestrado, 1985. Estudo farmacognóstico comparativo de espécies brasileiras do gênero *Passiflora* L. São Paulo. Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, 133 pp.

Gosmann, G., Provensi, G., Comunello, L.N., Rates, S.M.K., 2011. Composição química e aspectos farmacológicos de espécies de *Passiflora* L. (Passifloraceae). Rev. Bras. Bioci. 9, 88–99.

Hoehne, F.C., 1939. Plantas e substâncias vegetais tóxicas e medicinais: coletânea de 114 aulas primeiramente publicadas em O Estado de S. Paulo de 1934-38. Departamento de Botânica do Estado, São Paulo.

Johansen, D.A., 1940. Plant Microtechnique. Mc Graw Hill Book, New York.

Kaiser, E., 1880. Verfahren zur Herstellung einer tadellosen Glycerin-Gelatine. Bot. Zentral. 1880, 25–26.

Kraus, J.E., Arduin, M., 1997. Manual básico de métodos em morfologia vegetal. EDUR, Rio de Janeiro.

Leite, S.S.M.S., França, E.S., Coffani-Nunes, J.V., 2013. Morfoanatomia de folha e pecíolo e sua aplicação taxonômica em *Passiflora* (Passifloraceae). Congresso Nacional de Botânica. Belo Horizonte, Brasil, 64 pp.

Ma, X.Q., Shi, Q., Duan, J.A., Dong, T.T.X., Tsim, K.W.K., 2002. Chemical analysis of *Radix Astragali* (Huangqi) in China: a comparison with its adulterants and seasonal variations. J. Agric. Food Chem. 50, 4861–4866.

Moraes, M.L.L., Vilegas, J.H.Y., Lanças, F.M., 1997. Supercritical fluid extraction of glycosylated flavonoids from *Passiflora* leaves. Phytochem. Anal. 8, 257–260.
Pereira, C.S., Kurita, H., Veja, R., Jiménez, M., Molinas, C., Benítez, F., 2009. Evaluación de la morfo-anatomía foliar de *Passiflora alata* Curtis y *Passiflora edulis* Sims. Steviana 1, 38–45.
Pires, M.V., (Dissertação de Mestrado) 2008. Respostas morfo-fisiológicas de espécies ornamentais de *Passiflora* ao sombreamento. Ilhéus. Programa de Pós-Graduação em Produção Vegetal, Universidade Estadual de Santa Cruz, 99 pp.
Pittler, M.H., Ernst, E., 2003. Hepatotoxic events associated with herbal medicinal products. Aliment. Pharm. Therap. 18, 451–471.
Radford, A.E., Dickison, W.C., Massey, J.R., Bell, C.R., 1974. Vascular Plant Systematics. Harper & Row Publishers, New York.
Viana, A.P., Pereira, T.N.S., Pereira, M.G., Souza, M.M., Maldonado, J.F.M., do Amaral Júnior, A.T., 2003. Diversidade genética entre genótipos comerciais de maracujazeiro amarelo (*Passiflora edulis* f. *flavicarpa*) e entre espécies de passifloras nativas determinada por marcadores RAPD. Rev. Bras. Frutic. 25, 489–493.
WHO, 2000. General Guidelines for Methodologies on Research and Evaluation of Tradicional Medicine. World Health Organization, 74 pp. http://whqlibdoc.who.int/hq/2000/WHO_EDM_TRM_2000.1.pdf?ua=1
WHO, 2007. WHO monographs on selected medicinal plants, vol. 3. WHO Pres., Spain.
Zhang, Y.B., Shaw, P.C., Sze, C.W., Wang, Z.T., Tong, Y., 2007. Molecular authentication of Chinese herbal materials. J. Food Drug Anal. 15, 1–9.
Zhao, Z., Hu, Y., Liang, Z., Yuen, J.P., Jiang, Z., Leung, K.S., 2006. Authentication is fundamental for standardization of Chinese medicines. Planta Med. 72, 865–874.