

The taxonomic significance of seed morphology in the *Passiflora* subgenus *Astrophea* (Passifloraceae)

Ana Carolina Mezzonato-Pires^{1*}, Cláudia Barbieri Ferreira Mendonça¹, Michaele Alvim Milward-De-Azevedo² and Vania Gonçalves-Esteves¹

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ABSTRACT

The center of diversity for *Passiflora* subg. *Astrophea* is in low altitude areas of northern South America. The majority of species of this group are difficult to find in nature. Seed morphology was described in detail for 25 species of *Passiflora* subgenus *Astrophea*, a subgenus that until now did not exist. For morphological analysis, 20 seeds per species were measured for length, width and thickness, and the arithmetic means calculated. The seeds varied in length, width and thickness. Eight types of ornamentation were found. The margins varied among crenate, dentate, parted, entire and parted-crenate. The seed apex can be distinguished by the shape and position of the apical appendage. Seed shape varied among obovate, lanceolate, cordiform, and oblong to elliptical. An identification key was developed and a PCA was performed both using the principal morphological characters. Morphological characters of seeds are a new source of data for delimiting taxa with quite conflicting morphological boundaries, such as seen here with the *Passiflora* subgenus *Astrophea*. Furthermore, seed morphology is especially useful for the identification of specimens with only fruits and, consequently, seeds available.

Keywords: *Astrophea*, fruits, morphological characters, seeds, systematics

Introduction

Passiflora subgenus *Astrophea* is one of five subgenera belonging to the genus *Passiflora*. It is comprised of about 60 species distributed in South and Central America, with only two species in the latter, *Passiflora pittieri* and *Passiflora tica* (Escobar 1994). The major center of diversity for *Passiflora* subg. *Astrophea* is in areas of low altitude in northern South America (Ulmer & MacDougal 2004). In Brazil, the subgenus is represented by approximately 25 species (BFG 2015) with the greatest concentration being in the north. According to Ulmer & MacDougal (2004), species of *Passiflora* subg. *Astrophea* are similar to typical *Passiflora* but with unique morphological

characteristics, and so are considered the most unusual of the genus. In addition to having a climbing habit, this subgenus also exhibits shrub or tree habits, and has tiny stipules, entire leaf blades, two glands on the petiole, tiny bracts, and campanulate lily flowers with a yellow corona or red tubular flowers with a reddish corona. The majority of species of this group are rare and difficult to find in nature. Species identification of specimens in the absence of flowers is usually impossible since most of the diagnostic characters are in the flowers, and so when specimens are only fruits, seeds can serve as a valuable identification tool.

The importance of describing seeds extends beyond taxonomy and systematics to archaeology, paleobotany and even to farming and gardening by helping identify

¹ Departamento de Botânica, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, São Cristóvão, 20940-040, Rio de Janeiro, RJ, Brazil

² Departamento de Ciências do Meio Ambiente, Instituto Três Rios, Universidade Federal Rural do Rio de Janeiro, Av. Prefeito Alberto da Silva Lavinhas 1847, 25802-100, Três Rios, RJ, Brazil

* Corresponding author: carolina.mezzonato@gmail.com

cultured species (Johri 1984). According to Haridasan & Mukherjee (1988), environmental conditions have little influence on the characteristics of the seed surface, and thus these characters should better reflect the underlying genetics and be more useful in analyses of phylogenetic relationships. In a morphological study of the seeds of 51 species of the genus *Passiflora*, Pérez-Cortéz *et al.* (2002) demonstrated the existence of great diversity. Ulmer & MacDougal (2004) claim that the size and, especially, the surface of seeds are often useful characters for taxonomy.

As an understudied subgenus with poorly known and defined species, pollen studies of *Passiflora* subg. *Astrophea* were conducted with the hope of finding characters useful in species delimitations. The species of *Passiflora* subg. *Astrophea* analyzed (Mezzonato-Pires *et al.* 2015) revealed various important characters.

Thus, the present study aims to describe the seed morphology of 25 species of the *Passiflora* subg. *Astrophea* in order to identify characters with taxonomic potential that can assist in classification and be used in future phylogenetic analyses.

Materials and methods

The seeds of 25 species of the *Passiflora* subgenus *Astrophea* (DC.) Mast. were studied (S1 in supplementary material). The material used was taken from exsiccates deposited in herbaria whose acronyms are in accordance with the Index Herbariorum (Thiers, continuously updated): IAN, INPA, MG, MO, NYBG, R, RB and UB.

For morphological analysis, 20 seeds per species (when possible) were measured for length, width and thickness (Fig. 1), and the arithmetic means calculated. Description of the base, apex, margin and ornamentation used the terminology of Pérez-Cortéz *et al.* (2002) with some modifications. The classification of seeds followed Pérez-Cortéz *et al.* (2002), except that the most ornamented region of the margin was considered, and the apex was more fully described. The measurements were performed using a digital caliper. The aryl was removed and the seeds were digitally photographed using a Leica M205C stereomicroscope with an attached DFC295 digital camera. Selected seeds were fixed to double-sided carbon tape and then metalized with a thin layer of palladium gold (ca. 150° thickness) for three minutes and analyzed with a Jeol Model JSM 6390 scanning electron microscope of the Departamento de Invertebrados do Museu Nacional, Universidade Federal do Rio de Janeiro.

The principal component analysis (PCA) was performed on 42 variables (power = 0.5, square root transformed) using the software PC-ORD 5.0 (McCune & Mefford 2006). The variance-covariance matrix and biplot of distance were selected. The results are presented in a two-dimensional graph of the first and second principal components.

An identification key was developed using the principal morphological characters of the seeds.

Results

The seeds examined in this study are illustrated in Figures 2-9 and their measurements and morphological characters are presented, in Tables 1 and 2, respectively. The species analyzed were: *Passiflora candida*, *Passiflora ceratocarpa*, *Passiflora costata*, *Passiflora elliptica*, *Passiflora engleriana*, *Passiflora faroana*, *Passiflora haematostigma*, *Passiflora hexagonocarpa*, *Passiflora longiracemosa*, *Passiflora macrophylla*, *Passiflora mansoi*, *Passiflora ovata*, *Passiflora pentagona*, *Passiflora pyrhantha*, *Passiflora rhamnifolia*, *Passiflora rusbyi*, *Passiflora securiclata*, *Passiflora skiantha*, *Passiflora sphaerocarpa*, *Passiflora spinosa*, *Passiflora tessmannii*, *Passiflora tica*, *Passiflora venosa*, *Passiflora* sp. 1 and *Passiflora* sp. 2.

The seeds varied in length (3.85-14.82 mm), width (2.68-8.62 mm) and thickness (1.03-4.33 mm) (Tab. 1). The species *Passiflora rusbyi* stood out as having the longest (14.82 mm), widest (8.62 mm) and thickest (4.33 mm) seeds. *Passiflora tica* had the shortest (3.85 mm) and thinnest seeds, while *Passiflora hexagonocarpa*, had the narrowest (2.68 mm) (Tab. 1).

Eight types of ornamentation were found: reticulate (Fig. 8A), coarsely reticulate (Fig. 8B), reticulate-foveolate (Fig. 8C), reticulate-foveolate with a single central foveola costate toward the margin (Fig. 8D), reticulate-alveolate (Fig. 8E) (with homogeneous or heterogeneous alveola), transversely costate (Fig. 8F), transversely costate toward the margin (Fig. 8G) and scrobiculate (Fig. 8H). The margins varied among crestate (Fig. 8I), dentate (Fig. 8J), parted (Fig. 8K), entire and parted-crestate (Fig. 8M).

The seed apex can be distinguished by the shape and position of the apical appendage (Tab. 2). Some species have the apical appendage positioned exactly on the apex of the seed, such as: *P. costata*, *P. faroana*, *P. haematostigma*, *P. pyrhantha*, *P. rhamnifolia*, *P. rusbyi*, *P. securiclata*, *P. skiantha*,

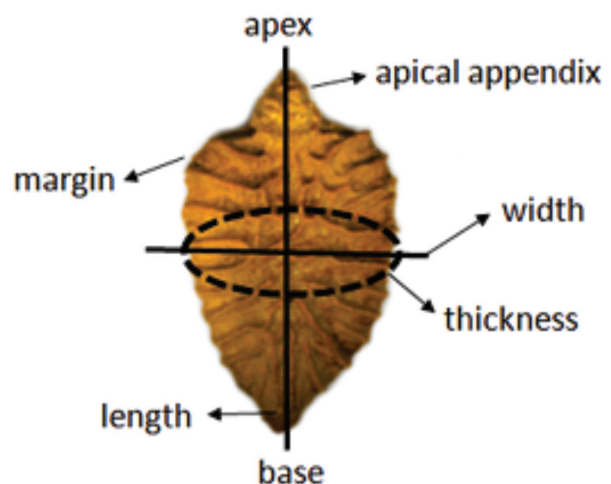


Figure 1. Photomicrographs of a seed showing the features and dimensions measured.



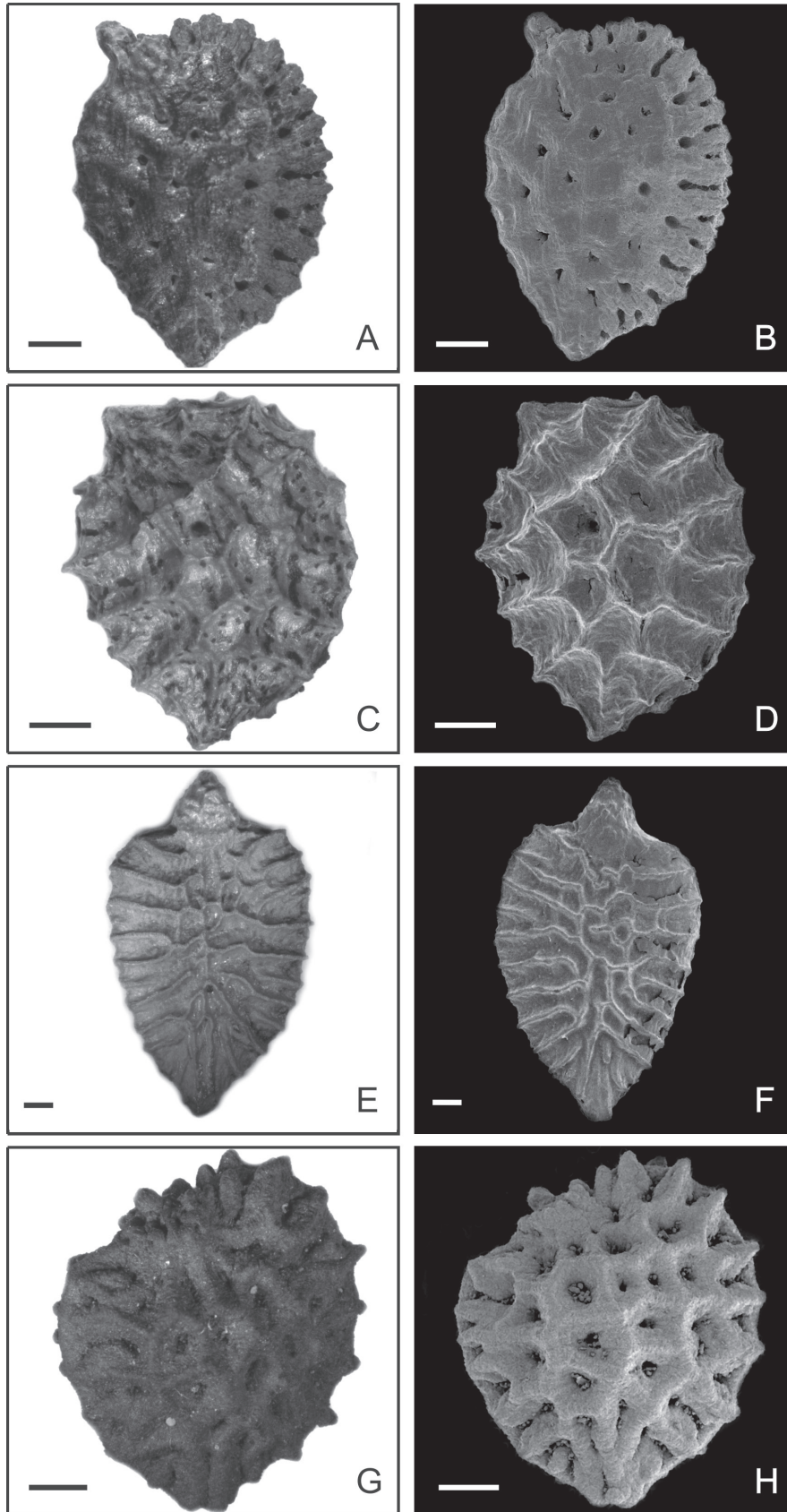


Figure 2. Photomicrographs and electron photomicrographs of seeds of the species of *Passiflora* subgenus *Astrophea*. A-B. *P. candida*. C-D. *P. ceratocarpa*. E-F. *P. costata* G-H. *P. elliptica*. Bar: 1 mm.



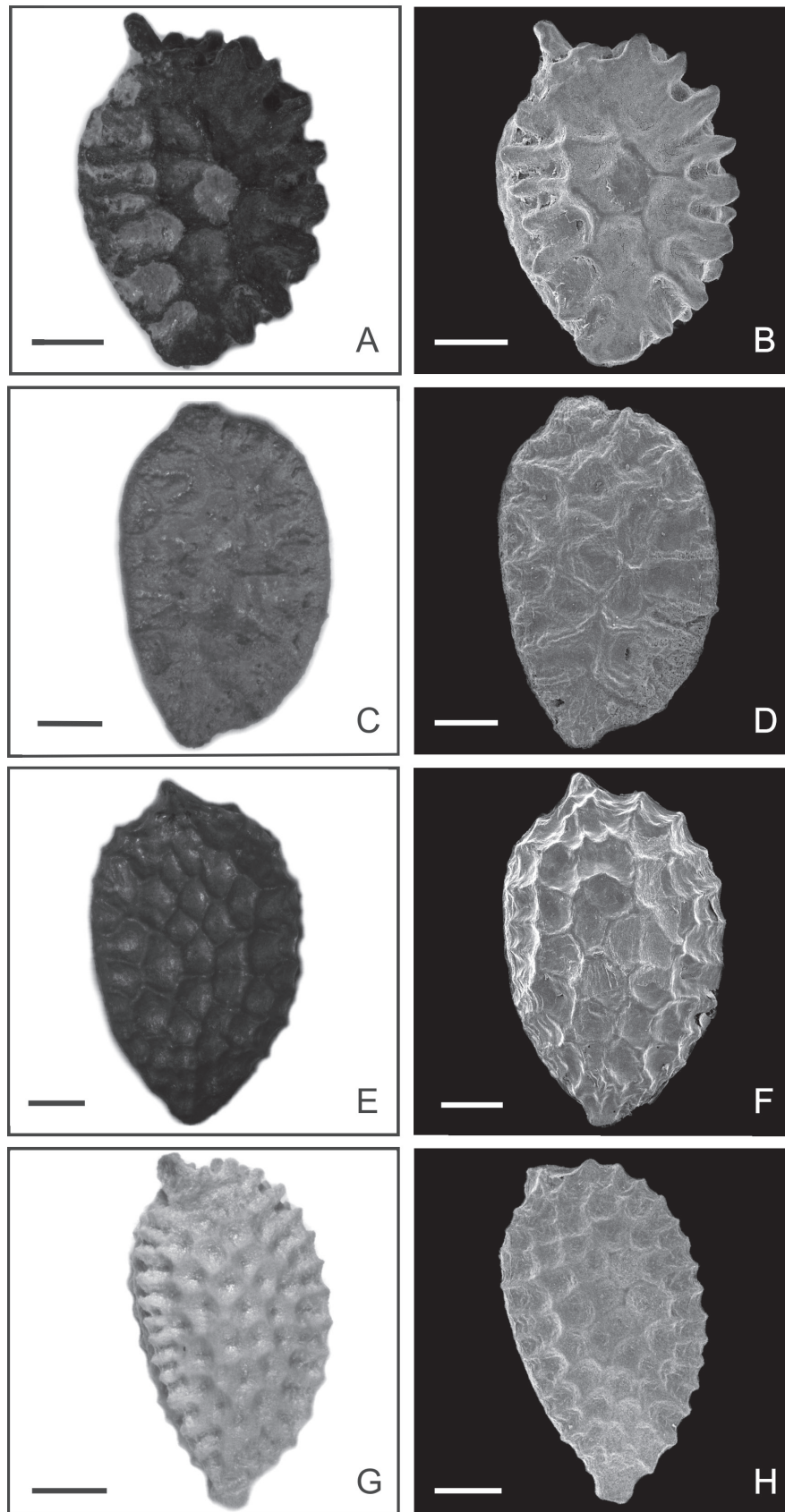


Figure 3. Photomicrographs and electron photomicrographs of seeds of the species of *Passiflora* subgenus *Astrophea*. A-B. *P. engleriana*. C-D. *P. faroana*. E-F. *P. haematostigma*. G-H. *P. hexagonocarpa*. Bar: 1 mm.



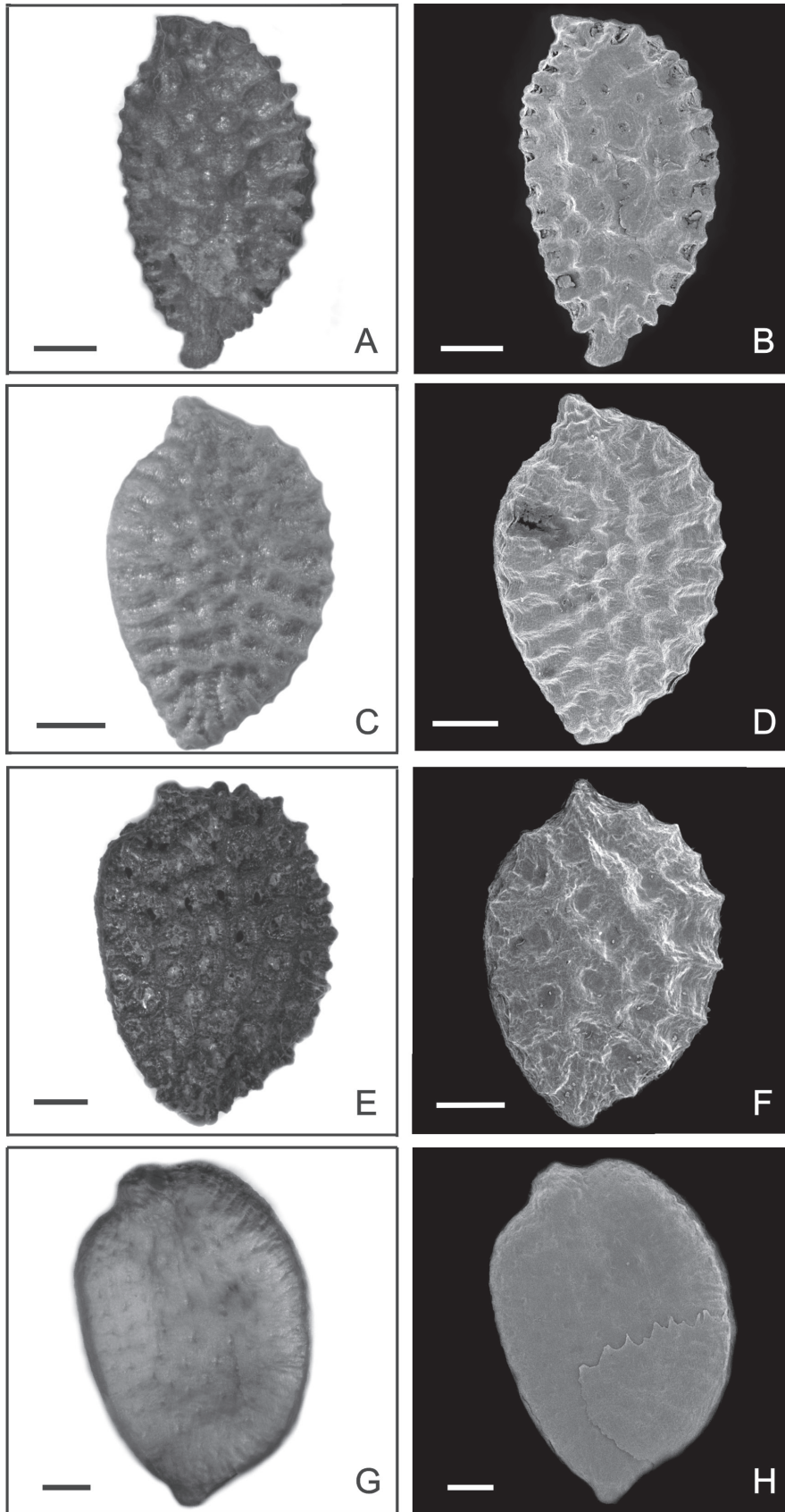


Figure 4. Photomicrographs and electron photomicrographs of seeds of the species of *Passiflora* subgenus *Astrophea*. A-B. *P. longiracemosa*. C-D. *P. macrophylla*. E-F. *P. mansoi*. G-H. *P. ovata* RB552847. Bar: 1 mm.



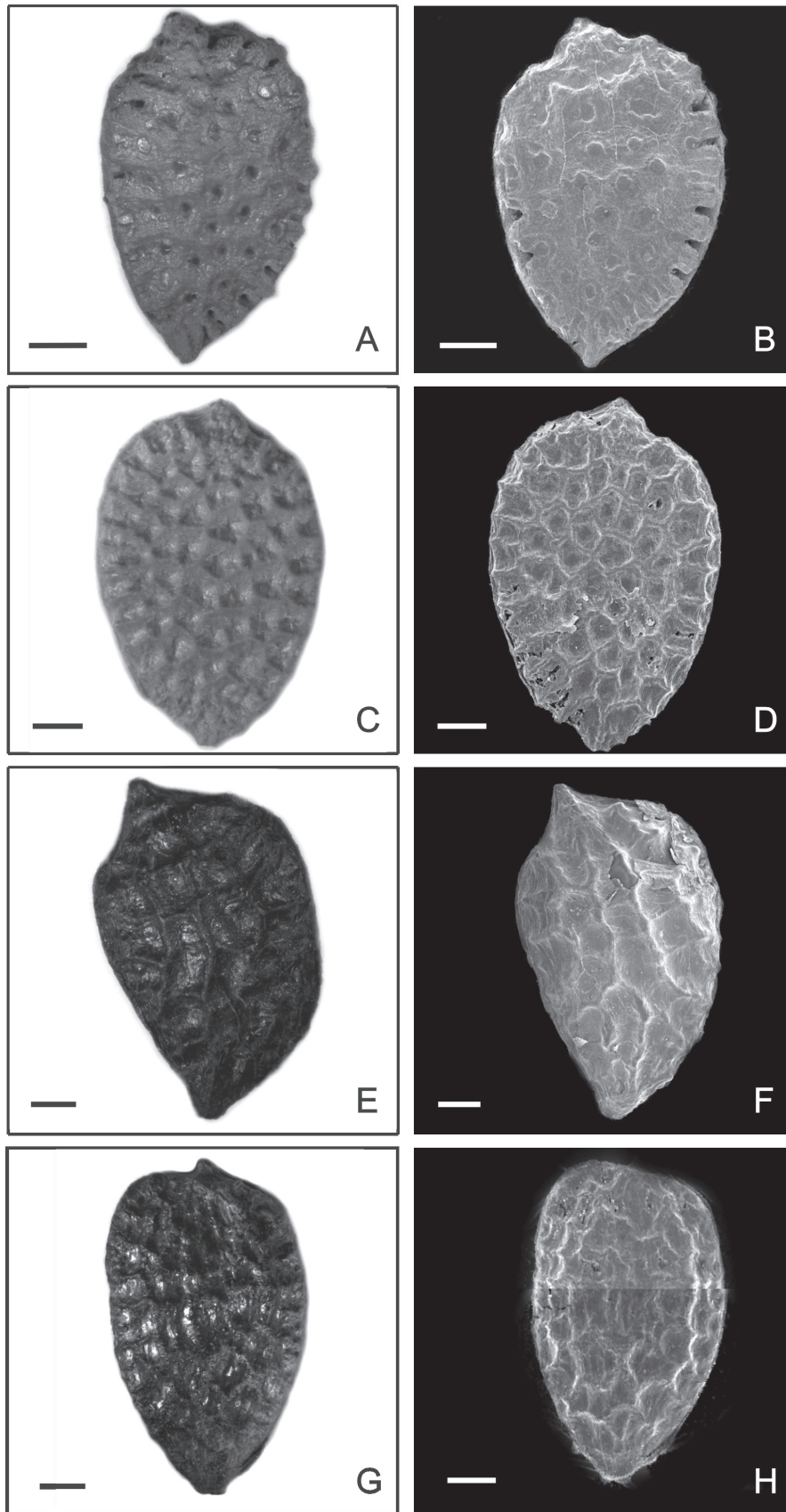


Figure 5. Photomicrographs and electron photomicrographs of seeds of the species of *Passiflora* subgenus *Astrophea*. A-B. *P. pentagona*. C-D. *P. pyrhantha*. E-F. *P. rhamnifolia*. G-H. *P. rusbyi*. Bar: 1 mm (A-E). 2 mm (G-H).



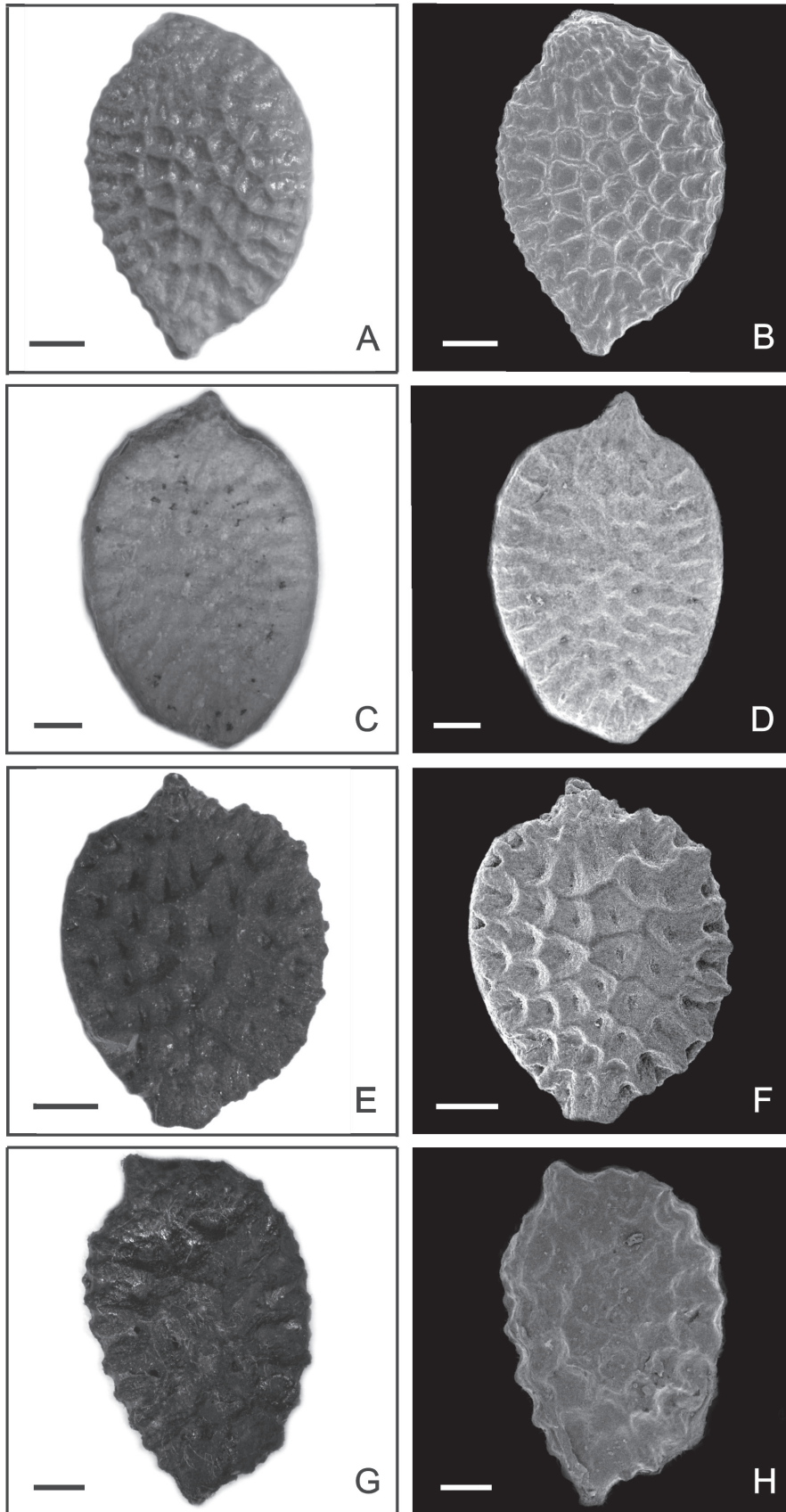


Figure 6. Photomicrographs and electron photomicrographs of seeds of the species of *Passiflora* subgenus *Astrophea*. A-B. *P. securiclata*. C-D. *P. skiantha*. E-F. *P. sphaerocarpa*. G-H. *P. spinosa*. Bar: 1 mm.



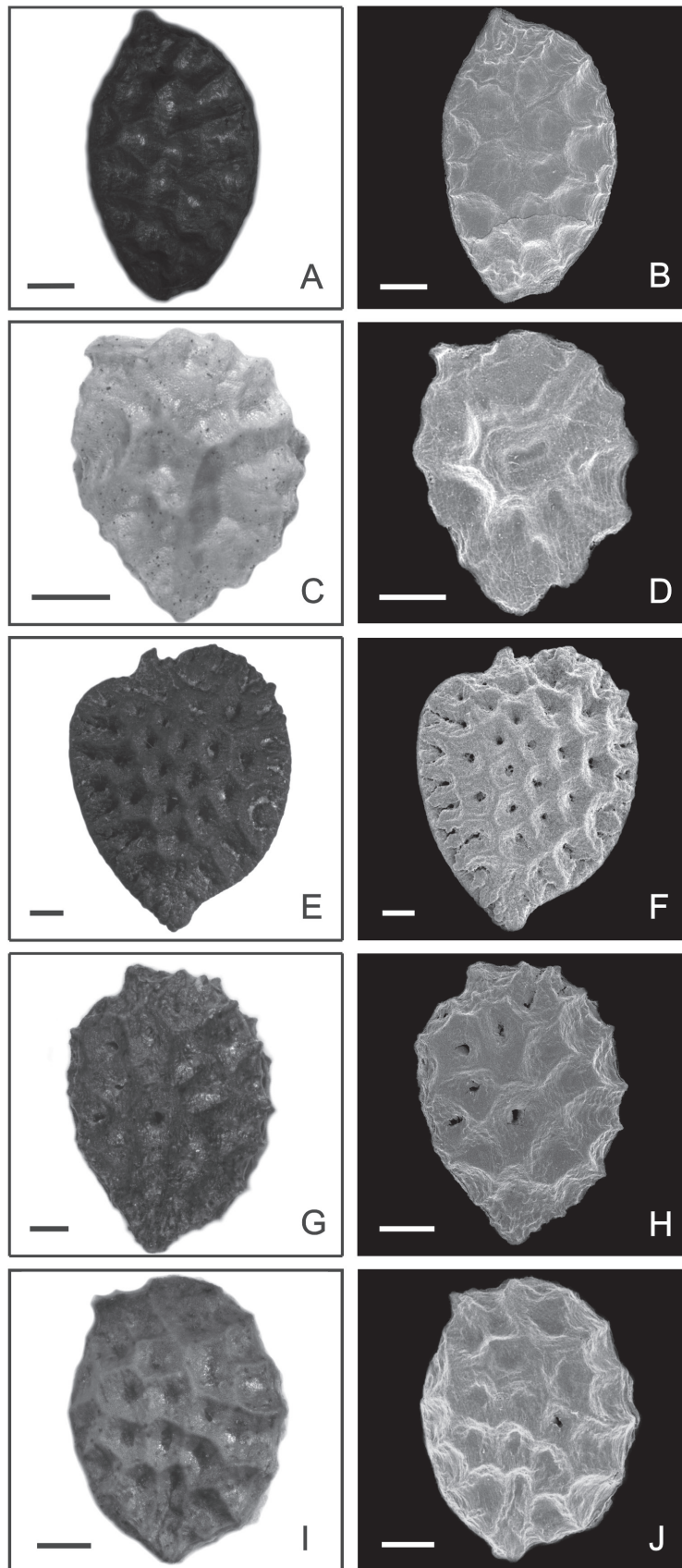


Figure 7. Photomicrographs and electron photomicrographs of seeds of the species of *Passiflora* subgenus *Astrophea*. A-B. *P. tessmannii*. C-D. *P. tica*. E-F. *P. venosa*. G-H. *P. sp.1*. I-J. *P. sp. 2*. Bar: 1 mm.



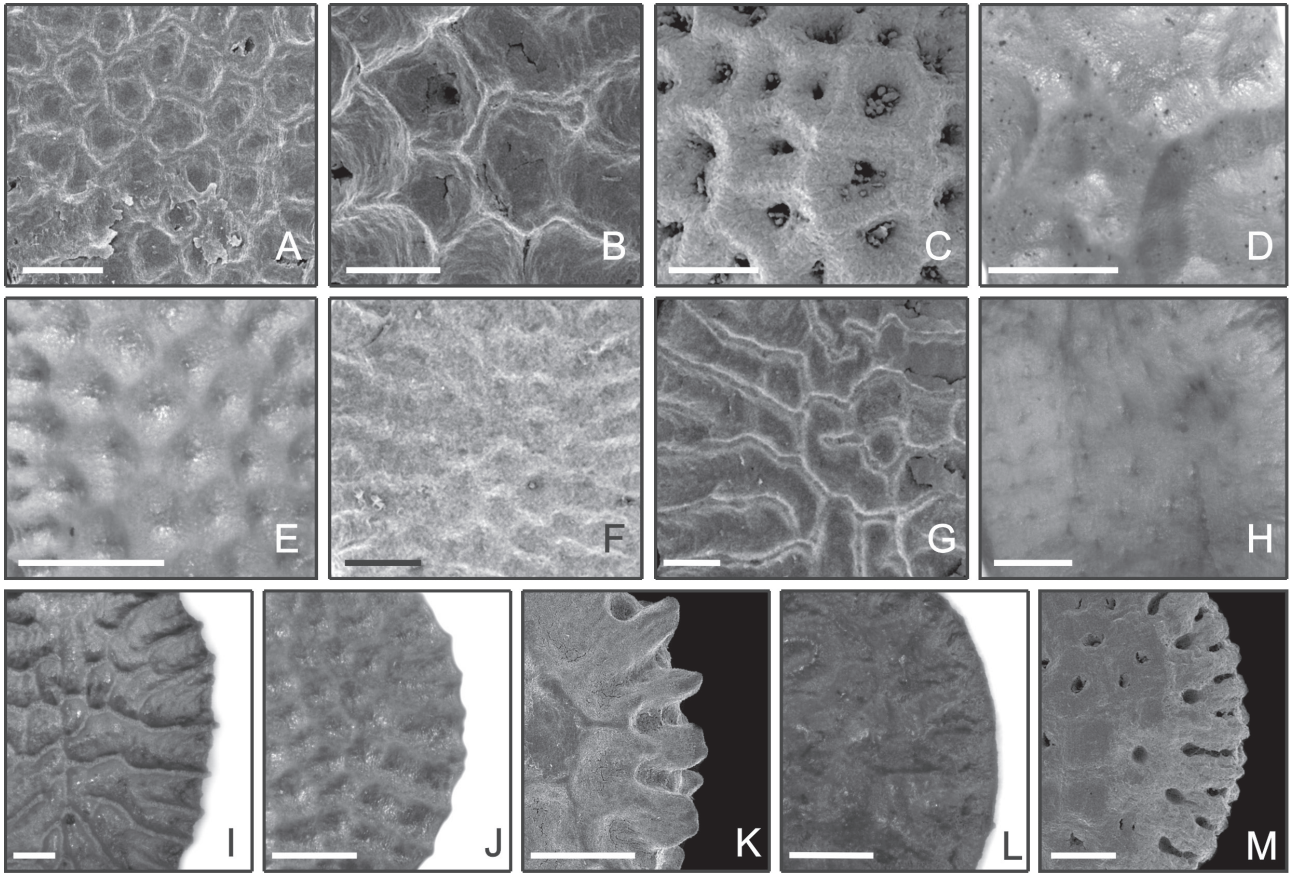


Figure 8. Photomicrographs and electron photomicrographs of ornamentation and margin of the seeds of the species of *Passiflora* subgenus *Astrophea*. Ornamentation: A. reticulate (*P. pyrrhantha*). B. coarsely reticulate (*P. ceratocarpa*). C. reticulate-foveolate (*P. elliptica*). D. reticulate-foveolate with a single central foveola costate toward the margin (*P. tica*). E. reticulate-alveolate (*P. hexagonocarpa*). F. transversely costate (*P. skiantha*). G. transversely costate toward the margin (*P. costata*). H. scrobiculate (*P. ovata*). Margin: I. crestate (*P. costata*). J. dentate (*P. macrophylla*). K. parted (*P. engleriana*). L. entire (*P. faroana*). M. parted-crestate (*P. pentagona*). Bar: 1mm

P. sphaerocarpa, *P. spinosa* and *P. venosa*. The appendage of other species are positioned 10° to 40° from the apex and their shapes vary among: acute (Fig. 9A), acute with an evident elevated region (Fig. 9B), acute prominent (Fig. 9C), acute slightly prominent (Fig. 9D), acute-truncate (Fig. 9E), obtuse slightly prominent (Fig. 9F), truncated slightly prominent (Fig. 9G), fused (Fig. 9H) and inconspicuous (Fig. 9I) (Tab. 2). The seeds of the majority of species have an acute base (Fig. 9J), but others are rounded (Fig. 9K), cordate (Fig. 9L), truncate (Fig. 9M), obtuse (Fig. 9N) and truncate to slightly acute (Fig. 9O).

The shape of the analyzed seeds (Tab. 2) varied among obovate, lanceolate, cordiform, and oblong to elliptical. The predominant shape in the majority of species was obovate, with the variation of narrowly obovate in only two species.

Considering only seed characters, it is apparent that there is great morphological variety within the *Passiflora* subg. *Astrophea*. The characteristics that varied most were surface ornamentation, shape of the apical appendage and size and condition of the margin. Thus, the description of

seeds is very useful in the delimitation of species of this group, thereby assisting in the identification of specimens with only fruit.

All of the quantitative and qualitative characters were used in a PCA. The results of the PCA explained 32.64 % of the accumulative variance (Tab. 3). The first principal component explained 19.52 % of the total variance, with the most significant characters being those of appendage displacement and the shape of the obtuse apex. Only species with seeds with a displaced appendage occurred on the positive side of Axis 1, whereas species with the appendage on the apex were on the negative side. Species with an obtuse seed apex were also grouped on the positive side of Axis 1. The second principal component explained 13.11 % of the total variance, with the most significant character being the shape of the acute prominent appendage. All reticulate species were positioned on the negative side of Axis 2. The third principal component explained 9.71 % of the total variance, mainly through the character of obovate seed shape.



The taxonomic significance of seed morphology in the *Passiflora* subgenus *Astrophea* (Passifloraceae)

Table 1. Measurements (in mm) of the seeds of the species of *Passiflora* subgenus *Astrophea*.

Species	Length	Width	Thickness
<i>P. candida</i> (Figs. 2A, B)	6.74	4.78	2.30
<i>P. ceratocarpa</i> (Figs. 2C, D, 8B, 9A)	5.87	4.46	2.37
<i>P. costata</i> (Figs. 2E, F, 8I, 9C)	11.00	6.90	1.84
<i>P. elliptica</i> (Figs. 2G, H, 8C, 9H)	6.21	5.45	3.17
<i>P. engleriana</i> (Figs. 3A, B, 8K)	4.97	3.39	1.55
<i>P. faroana</i> (Figs. 3C, D, 8L, 9G)	5.20	3.51	1.60
<i>P. haematostigma</i> (Figs. 3E, F, 9O)	6.12	3.71	1.68
<i>P. hexagonocarpa</i> (Figs. 3G, H, 8E, 9E)	4.84	2.68	1.18
<i>P. longiracemosa</i> (Figs. 4A, B, 9M)	5.68	2.99	1.39
<i>P. macrophylla</i> (Figs. 4C, D, 8J)	5.11	3.32	1.07
<i>P. mansoi</i> (Figs. 4E, F, 9N)	6.84	4.74	2.44
<i>P. ovata</i> (Figs. 4G, H, 8H)	7.56	4.85	1.14
<i>P. pentagona</i> (Figs. 5A, B, 8M, 9D)	5.84	3.25	1.91
<i>P. pyrrantha</i> (Figs. 5C, D, 8A)	7.29	4.58	1.12
<i>P. rhamnifolia</i> (Figs. 5E, F)	7.73	4.49	2.07
<i>P. rusbyi</i> (Figs. 5G, H)	14.82	8.62	4.33
<i>P. securiclata</i> (Figs. 6A, B, 9F)	6.16	3.96	1.24
<i>P. skiantha</i> (Figs. 6C, D, 8F, 9K)	7.70	4.84	1.31
<i>P. sphaerocarpa</i> (Figs. 6E, F, 9L)	5.34	4.09	1.82
<i>P. spinosa</i> (Figs. 6G, H, 9J)	6.82	4.41	1.87
<i>P. tessmannii</i> (Figs. 7A, B)	6.21	3.76	1.24
<i>P. tica</i> (Figs. 7C, D, 8D)	3.85	3.01	1.03
<i>P. venosa</i> (Figs. 7E, F, 9B)	8.18	6.44	2.93
<i>P. sp. 1</i> (Figs. 7G, H)	5.49	3.81	1.80
<i>P. sp. 2</i> (Figs. 7I, J, 9I)	5.43	3.95	1.11

Table 2. Morphological characterization of seeds of species of *Passiflora* subgenus *Astrophea*

Species	Shape	Margin	Apex	Base	Ornamentation
<i>P. candida</i> (Figs. 2A, B)	obovate	parted-crestate	obtuse. with appendage acute prominent. positioned to 40° from the apex	acute	reticulate-foveolate
<i>P. ceratocarpa</i> (Figs. 2C, D, 8B, 9A)	obovate	crestate	truncate. with appendage acute. positioned to 30° from the apex	acute	coarsely reticulate
<i>P. costata</i> (Figs. 2E, F, 8I, 9C)	lanceolate	crestate	with appendage acute prominent	acute	transversely costate toward the margin
<i>P. elliptica</i> (Figs. 2G, H, 8C, 9H)	cordiform	crestate	acute. with appendage fused	cordate	reticulate-foveolate
<i>P. engleriana</i> (Figs. 3A, B, 8K)	obovate	parted	obtuse. with appendage acute prominent, positioned o 20° from the apex	truncate	transversely costate toward the margin
<i>P. faroana</i> (Figs. 3C, D, 8L, 9G)	oblong	entire	with appendage truncate slightly prominent	obtuse	reticulate
<i>P. haematostigma</i> (Figs. 3E, F, 9O)	obovate	dentate	wth appendage acute slightly prominent	truncate to slightly acute	reticulate
<i>P. hexagonocarpa</i> (Figs. 3G, H, 8E, 9E)	narrowly obovate	parted-crestate	acute. with appendage acute-truncate positioned to 30° from the apex	truncate	reticulate-alveolate. alveola homogeneous
<i>P. longiracemosa</i> (Figs. 4A, B, 9M)	narrowly obovate	parted-crestate	obtuse. with appendage acute slightly prominent. positioned to 20° from the apex	truncate	reticulate-alveolate. alveola heterogeneous
<i>P. macrophylla</i> (Figs. 4C, D, 8J)	obovate	dentate	obtuse. with appendage acute slightly prominent. positioned to 20° from the apex	obtuse	reticulate



Table 2. Cont.

Species	Shape	Margin	Apex	Base	Ornamentation
<i>P. mansoi</i> (Figs. 4E, F, 9N)	obovate	crestate	obtuse. with appendage acute slightly prominent. positioned to 20° from the apex	obtuse	reticulate-foveolate
<i>P. ovata</i> (Figs. 4G, H, 8H)	oblong	entire	obtuse. with appendage truncate slightly prominent. positioned to 30° from the apex	acute	scrobiculate
<i>P. pentagona</i> (Figs. 5A, B, 8M, 9D)	obovate	parted	obtuse. with appendage acute slightly prominent. positioned to 20° from the apex	acute	reticulate-foveolate
<i>P. pyrrantha</i> (Figs. 5C, D, 8A)	obovate	entire	with appendage obtuse slightly prominent	obtuse	reticulate
<i>P. rhamnifolia</i> (Figs. 5E, F)	obovate	entire	with appendage acute	obtuse	reticulate
<i>P. rusbyi</i> (Figs. 5G, H)	obovate	entire	with appendage acute slightly prominent	truncate	reticulate
<i>P. securiclata</i> (Figs. 6A, B, 9F)	obovate	dentate	with appendage obtuse slightly prominent	truncate	reticulate
<i>P. skiantha</i> (Figs. 6C, D, 8E, 9K)	oblong	entire	with appendage acute prominent	rounded	transversely costate
<i>P. sphaerocarpa</i> (Figs. 6E, F, 9L)	cordiform	parted	with appendage acute prominent	cordate	reticulate-alveolate
<i>P. spinosa</i> (Figs. 6G, H, 9J)	obovate	dentate	with appendage acute slightly prominent	acute	reticulate-foveolate
<i>P. tessmannii</i> (Figs. 7A, B)	elliptical	entire	obtuse. with appendage acute slightly prominent. positioned to 20° from the apex	lightly cordate	reticulate
<i>P. tica</i> (Figs. 7C, D, 8D)	cordiform	dentate	obtuse. with appendage acute prominent. positioned to 40° from the apex	acute	reticulate-foveolate with a single central foveola costate toward the margin
<i>P. venosa</i> (Figs. 7E, F, 9B)	cordiform	entire	with appendage acute with an evident elevated region	acute	reticulate-alveolate
<i>P. sp. 1</i> (Figs. 7G, H)	obovate	dentate	obtuse. with appendage acute slightly prominent. positioned to 10° from the apex	acute	reticulate
<i>P. sp. 2</i> (Figs. 7I, J, 9I)	elliptical	slightly dentate	obtuse. with appendage inconspicuous. positioned to 40° from the apex	acute	reticulate-alveolate

Table 3. Cumulative variance and vectors values of principal component analysis (PCA) using palinological characters of 25 táxons of *Passiflora* subgenus *Astrophea*.

Axis of the principal component	1	2	3
Cumulative variance (%)	32.647	19.528	13.119
Characters	Axis		
Length	-0.226	0.0156	0.1798
Width	-0.1561	0.0816	0.2012
Thickness	-0.0581	0.0447	0.1843
Shape obovate	-0.0153	-0.4270	0.3568
Shape narrowly obovate	0.0818	0.0448	-0.2482
Shape lanceolate	-0.0303	0.0803	0.0977
Shape cordiform	0.0133	0.2701	0.0208
Shape oblong	-0.0970	0.0911	-0.1282
Shape elliptical	0.0476	-0.0593	-0.0990
Margin crestate	0.0445	0.1460	0.2725
Margin dentate	0.0785	-0.2670	0.0798
Margin parted	0.0673	0.0712	0.0376



Table 3. Cont.

Axis of the principal component	1	2	3
Cumulative variance (%)	32.647	19.528	13.119
Characters	Axis		
Margin entire	-0.3153	0.0134	-0.1933
Margin parted-crestate	0.1250	0.0364	-0.1965
Appendage on the apex	-0.4695	0.0340	0.0873
Appendage displaced	0.4695	-0.0340	-0.0873
Shape of apex obtuse	0.3928	-0.1881	-0.0674
Shape of apex truncate	0.0226	0.0408	0.0934
Shape of apex acute	0.0541	0.1133	-0.1133
Shape of appendage acute	-0.0184	-0.0462	0.0476
Shape of appendage acute prominent	0.0066	0.3254	0.1507
Shape of appendage acute slightly prominent	0.1082	-0.3766	0.0687
Shape of appendage acute with an evident elevated region	-0.0387	0.0730	0.0096
Shape of appendage acute-truncate	0.0335	0.0444	-0.1376
Shape of appendage obtuse slightly prominent	-0.1018	-0.0894	-0.0333
Shape of appendage truncate slightly prominent	-0.0448	0.0277	-0.1055
Shape of appendage fused	0.0206	0.0689	0.0243
Shape of appendage inconspicuous	0.0347	-0.0273	-0.0243
Base acute	0.1861	0.1313	0.3784
Base cordate	0.0149	0.1247	-0.0700
Base truncate	0.0115	-0.0299	-0.2470
Base obtuse	-0.1291	-0.2215	-0.0554
Base truncate to slightly acute	-0.0312	-0.0680	0.0167
Base rounded	-0.0522	0.0634	-0.0227
Reticulate	-0.2500	-0.3921	-0.1891
Coarsely reticulate	0.0226	0.0408	0.0934
Reticulate-foveolate	0.1375	-0.0455	0.2883
Reticulate-foveolate with a single central foveola costate toward the margin	0.0499	0.0404	0.0065
Reticulate-alveolate	0.0727	0.1692	-0.2361
Transversely costate	-0.522	0.0634	-0.0227
Transversely costate toward the margin	0.0049	0.0930	0.0930
Scrobiculate	0.0146	0.0309	-0.0333

In the two-dimensional graph of the PCA (Fig. 10), it can be seen that the species *P. pentagona*, *P. tica* and *P. longiracemosa* have the highest values on the first principal component and *P. sphaerocarpa*, *P. costata* and *P. venosa*, on

the second. *Passiflora rusbyi*, *P. pyrhantha* and *P. rhamnifolia* have the lowest values on the first principal component and *P. macrophylla*, *P. haematostigma* and *P. securiclata* on the second.

Key based on seed characters for species identification of the *Passiflora* subg. *Astrophea*

1. Seeds with surface transversely costate
2. Shape lanceolate, margin crestate (Figs. 2E, F, 8I, 9C) *P. costata*
- 2'. Shape obovate, oblong, margin parted or entire
3. Appendage in the seed apex, rounded base (Figs. 6C, D, 8F, 9K) *P. skiantha*
- 3'. Appendage positioned 20° from the seed apex, truncate base (Figs. 3A, B, 8K) *P. engleriana*
- 1'. Seeds with surface no transversely costate
4. Surface scrobiculate (Figs. 4G, H, 8H) *P. ovata*
- 4'. Surface reticulate, coarsely reticulate, reticulate-foveolate, reticulate-foveolate with a single central foveola costate toward the margin or reticulate-alveolate
5. Seed base cordate to slightly cordate
6. Shape elliptical (Figs. 7A, B) *P. tessmannii*
- 6'. Shape cordiform
7. Surface reticulate-alveolate, apex with acute prominent appendage (Figs. 6E, F, 9L) *P. sphaerocarpa*



- 7'. Surface reticulate-foveolate, apex acute with fused appendage (Figs. 2G, H, 8C, 9H) *P. elliptica*
5'. Seed base acute, truncate or obtuse
8. Margin entire
9. Appendage truncate (Figs. 3C, D, 8L, 9G) *P. faroana*
9'. Appendage no truncate
10. Shape cordiforme (Figs. 7E, F, 9B) *P. venosa*
10'. Shape obovate, narrowly obovate or elliptical
11. Base truncate (Figs. 5G, H) *P. rusbyi*
11'. Base obtuse
12. Appendage acute (Figs. 5E, F) *P. rhamnifolia*
12'. Appendage obtuse (Figs. 5C, D, 8A) *P. pyrhantha*
8'. Margin crestate, parted-crestate, parted or dentate
13. Surface reticulate or coarsely reticulate
14. Margin crestate (Figs. 2C, D, 8B, 9A) *P. ceratocarpa*
14'. Margin dentate
15. Shape elliptical (Figs. 7I, J, 9I) *P. sp. 2*
15'. Shape obovate
16. Base obtuse, appendage displaced (Figs. 4C, D, 8J) *P. macrophylla*
16'. Base truncate to slightly acute, appendage at the apex
17. Appendage acute (Figs. 3E, F, 9O) *P. haematostigma*
17'. Appendage obtuse (Figs. 6A, B, 9F) *P. securiclata*
13'. Surface reticulate-foveolate or reticulate-alveolate
18. Base truncate, shape narrowly obovate
19. Appendage acute-truncate, alveola homogeneous (Figs. 3G, H, 8E, 9E) *P. hexagonocarpa*
19'. Appendage acute slightly prominent, alveola heterogeneous (Figs. 4A, B, 9M) *P. longiracemosa*
18'. Base acute or obtuse, shape obovate or cordiform
20. Appendage acute prominent
21. Shape cordiform, dimensions 3,85x30,1x1,03mm (Figs. 7C, D, 8D) *P. tica*
21'. Shape obovate, dimensions 6,74x4,78x2,30mm (Figs. 2A, B) *P. candida*
20'. Appendage acute slightly prominent
22. Margin dentate
23. Surface reticulate-foveolate (Figs. 6G, H, 9J) *P. spinosa*
23'. Surface reticulate-alveolate (Figs. 7G, H) *P. sp. 1*
22'. Margin partite or crestate
24. Base obtuse, dimensions 6,84x4,74x2,44 mm (Figs. 4E, F, 9N) *P. mansoi*
24'. Base acute, dimensions 5,84x3,25x1,91 mm (Figs. 5A, B, 8M, 9D) *P. pentagona*

Discussion

Among the species of the *Passiflora* subgenus *Astrophea* described by Killip (1938), the seeds were briefly described for seven: *P. ceratocarpa*, *P. elliptica*, *P. faroana*, *P. hexagonocarpa*, *P. longiracemosa*, *P. pentagona* and *P. skiantha*. The results presented by Killip (1938) were corroborated here for the species *P. ceratocarpa* with regard to seed shape, while for *P. skiantha* and *P. pentagona*, the shape described here was oblong and obovate, respectively. *Passiflora elliptica* was described by Killip (1938) as widely ovate and coarsely reticulate while the present study found it to be cordiform and reticulate-foveolate. For the species *P. faroana*, *P. hexagonocarpa* and *P. longiracemosa*, the results found here differ from those of Killip (1938) with regard to seed ornamentation. These differences seems to be due to an error in species identifications since the ornamentation is clearly reticulate or reticulate-alveolate.

The seeds of *P. costata*, *P. lindeniana*, *P. mansoi* and *P. spinosa* were studied by Pérez-Cortéz *et al.* (2002), in a work that only addressed morphological characters of seeds. Among these species only *P. lindeniana* was not included in the present study because of the lack of available material. Here *P. costata* was found to be longer and thinner than that presented by Pérez-Cortéz *et al.* (2002), whereas the results for *P. mansoi* and *P. spinosa* were very similar for those of Pérez-Cortéz *et al.* (2002). There was disagreement between the present study and Pérez-Cortéz *et al.* (2002), in the seed shape described for *P. costata*. Pérez-Cortéz *et al.* (2002) described the seeds of *P. costata* as elliptical with an obtuse base and reticulate-crestate ornamentation, whereas the present study found the seeds to be lanceolate with an acute base and transversely costate ornamentation towards the margin. We believe that this variation is a result of the wide geographic distribution of this species, which may have allowed morphological divergence in seeds due to varying



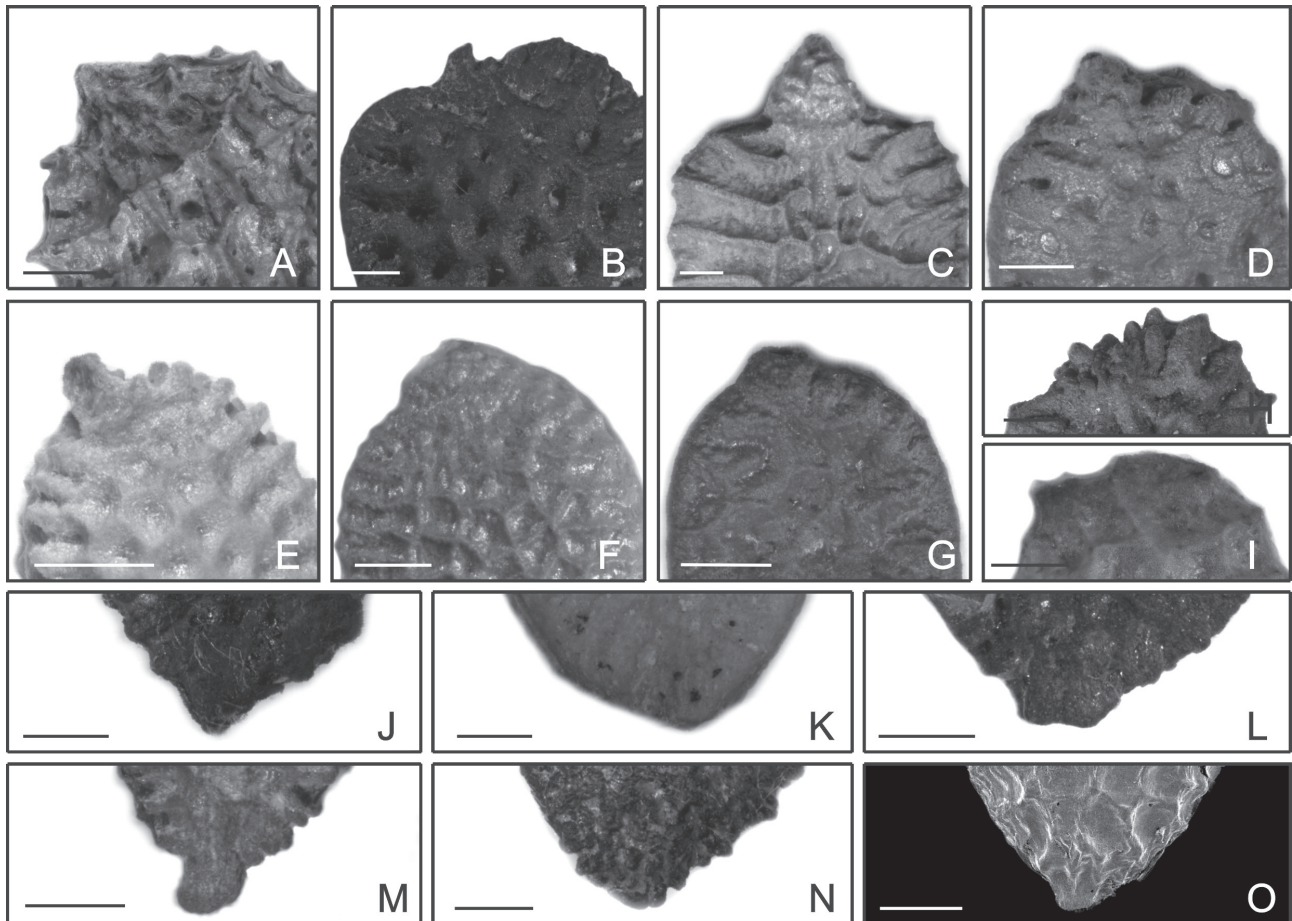


Figure 9. Photomicrographs and electron photomicrographs of apical appendage and base of the seeds of the species of *Passiflora* subgenus *Astrophea*. Apical appendage: A. acute (*P. ceratocarpa*). B. acute with an evident elevated region (*P. venosa*). C. acute prominent (*P. costata*). D. acute slightly prominent (*P. pentagona*). E. acute-truncate (*P. hexagonocarpa*). F. obtuse slightly prominent (*P. securiclata*). G. truncate slightly prominent (*P. faroana*). H. fused (*P. elliptica*). I. inconspicuous (*P. sp. 2*). Base: J. acute (*P. spinosa*). K. rounded (*P. skiantha*). L. cordate (*P. sphaerocarpa*). M. truncate (*P. longiracemosa*). N. obtuse (*P. mansoi*). O. truncated to slightly acute (*P. haematostigma*). Bar: 1mm

environments. The seed of *P. mansoi* was described by this same author as elliptical and with an entire margin; only the obtuse base and reticulate-alveolate ornamentation are corroborated by our results. The shape of the seed of *P. spinosa* was described as elliptical, and the margin crenate and the ornamentation reticulate-alveolate, thus, only the acute base was consistent with our findings. The apex was described in a different manner in our study, which we think is more detailed and understandable than that by Pérez-Cortéz *et al.* (2002), who described the seeds of these three species as simply with a “single horn”. These differences may be explained by the fact that *P. spinosa* is part of a taxonomic complex, and the variation seen in the seeds may be because the material analyzed by these authors may not have been *P. spinosa*, but another species of the complex.

Pérez-Cortéz *et al.* (2005; 2009) studied the anatomy of the seed cover for a total of 15 species of the *Passiflora* subgenus *Passiflora*. Eight species were analyzed in 2005 and seven in 2009, but only in the 2009 publication did

they describe the type of surface ornamentation, including reticulate, reticulate-foveolate and scrobiculate. These types of surface ornamentation were also found in the seeds of the *P.* subg. *Astrophea*, thereby demonstrating a degree of similarity between the two subgenera.

The seeds of the Brazilian species of the *Passiflora* subgenus *Decaloba* were described by Milward-de-Azevedo *et al.* (2012) and, when compared to the seeds of the *Passiflora* subgenus *Astrophea* analyzed here, are shorter and narrower (thickness was not measured). The shape varied from elliptical to obovate in most of the seeds, with the surface ornamentation having transverse striations with the number of striations varying from 5 to 11 in the majority of the species. This type of ornamentation was not found in any species of the *Passiflora* subg. *Astrophea*. Only two species of the *Passiflora* subgenus *Decaloba* did not have surface ornamentation with transverse striations, in which case they were described as foveolate and irregularly sulcate, the latter not found among the species studied in the present work.



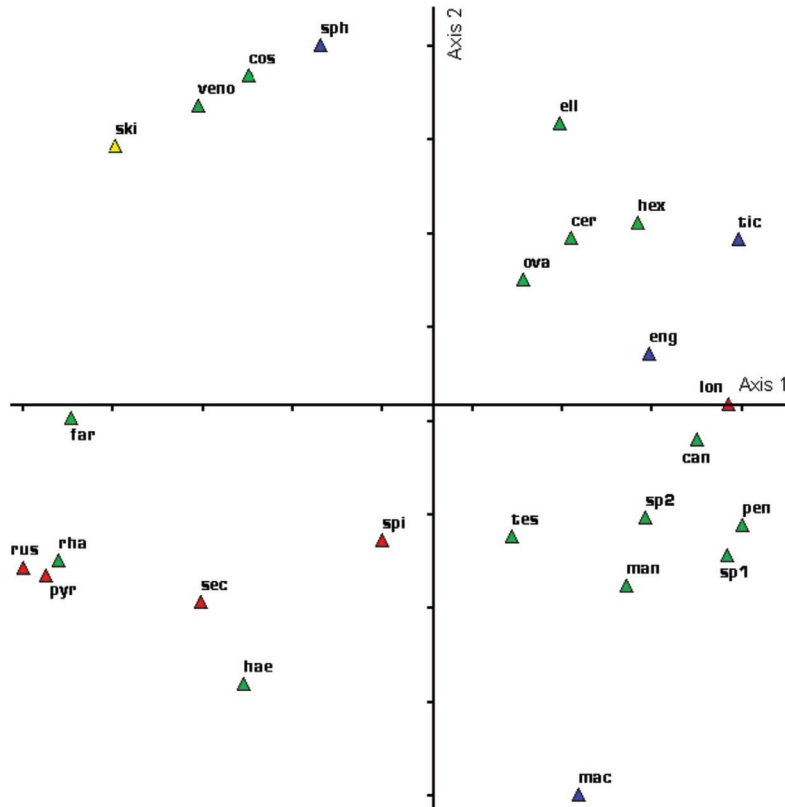


Figure 10. Principal component analysis performed with the seeds variables from *Passiflora* subgenus *Astrophea*. Species names are abbreviated with the first three letters of the epithet. The colors represent the sections of subgenus *Astrophea*. blue. section *Astrophea*; yellow. section *Capreolata*; green. section *Pseudoastrophea*; red. section *Botryastrophea*. Abbreviations: can. *candida*; cer. *ceratocarpa*; cos. *costata*; ell. *elliptica*; eng. *engleriana*; far. *faroana*; hae. *haematostigma*; hex. *hexagonocarpa*; lon. *longiracemosa*; mac. *macrophylla*; man. *mansoi*; ova. *ovata*; pen. *pentagona*; pyr. *pyrrhantha*; rha. *rhamnifolia*; rus. *rusbyi*; sec. *securiclata*; ski. *skiantha*; sph. *sphaerocarpa*; spi. *spinosa*; tes. *tessmannii*; tic. *tica*; ven. *venosa*. Please see the PDF version for color reference.

Passiflora subgenus *Passiflora* was studied by Cervi (1997) and among the 50 species treated, 36 had their seeds described, and most of those had their shape, size (length and width) and surface ornamentation mentioned. The shape of the seeds of this group presented greater variation than the subgenera *Astrophea* and *Decaloba*: obcordate, suborbiculate, “cordate”, “cordate”-oblong, ovate, obovate and oblong-obcordate. The seeds were smaller than for the *Passiflora* subg. *Astrophea* and most species had foveolate and non-reticulate ornamentation, like most species of this work.

The PCA analysis revealed an evident delimitation of the taxa of the *Passiflora* subgenus *Astrophea* pointing to important characters for their separation, such as the location of the appendage on the apex or displaced from the apex. Species that have often been difficult to delimit because of very tenuous characters, such as *P. rhamnifolia* and *P. pentagona*, were positioned individually on the graph, showing the separation of these species. Furthermore, the poorly known and collected species *P. elliptica*, of which there are few fertile specimens, has often been confused with *P. rhamnifolia* and *P. pentagona*, however, the PCA analysis demonstrates that it is spatially distanced from

them, thereby confirming its identity.

Despite these results, the seed characters do not support the current taxonomic classification of Feuillet & MacDougal (2003), as can easily be seen by color-coding the symbols for each species in the PCA plot. The section *Astrophea*, which was the only section of the genus *Passiflora* that had tree species, was represented by seeds of four species (*P. engleriana*, *P. macrophylla*, *P. sphaerocarpa* and *P. tica*), and each one of these had a different surface ornamentation. The section *Capreolata* was represented by one species (*P. skiantha*). Fifteen species of the section *Pseudoastrophea* were studied (*P. candida*, *P. ceratocarpa*, *P. costata*, *P. elliptica*, *P. faroana*, *P. haematostigma*, *P. hexagonocarpa*, *P. mansoi*, *P. ovata*, *P. pentagona*, *P. rhamnifolia*, *P. tessmannii*, *P. venosa*, and *P. sp. 1* and *P. sp. 2*), making it the most representative section and, not surprisingly, having most types of surface ornamentation being represented. Five species (*P. longiracemosa*, *P. pyrrhantha*, *P. rusbyi*, *P. securiclata* and *P. spinosa*) belong to the section *Botryastrophea* and exhibited three different types of surface ornamentation. These results demonstrate clearly that the great morphological diversity among the seeds within the genus *Passiflora* do not support its current infrageneric taxonomic classification.

Conclusion

Morphological characters of seeds are a new tool for delimiting taxa with quite conflicting morphological boundaries, as seen here with the *Passiflora* subgenus *Astrophea* and especially for the identification of specimens with only fruits and, consequently, seeds. In addition to the great taxonomic importance of this work, it will likely provide valuable contributions studies of phylogeny, archaeology, paleobotany, agriculture and horticulture as well.

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References

- BFG. 2015. Growing knowledge: an overview of seed plant diversity in Brazil. *Rodriguésia* 66: 1085-1113.
- Cervi AC. 1997. Passifloraceae do Brasil. Estudo do gênero *Passiflora* L. subgênero *Passiflora*. *Fontqueria* 45: 1-92.
- Escobar LK. 1994. Two new species and a key to the *Passiflora* subgenus *Astrophea*. *American Society of Plant Taxonomies. Systematic Botany* 19: 203-210.
- Feuillet C, MacDougal J. 2003. A new infrageneric classification of *Passiflora* L. (Passifloraceae). *Passiflora: The journal & Newsletter of Passiflora Society International* 13: 34-38.
- Haridasan VK, Mukherjee PK. 1988. Seed surface features of some members of the Indian Campanulaceae. *Phytomorphology* 37: 277-285.
- Johri BM. 1984. *Embryology of Angiosperms*. Berlin, Springer Verlag.
- Killip EP. 1938. The American species of Passifloraceae. *Publication Field Museum of Natural History, Botanical Series* 19: 1-613.
- Mccune B, Mefford MJ. 2006. PC-ORD, version 5.0, Multivariate analysis of ecological data. Glaneden Beach, MjM Software Desing.
- Mezzonato-Pires AC, Milward-de-Azevedo MA, Mendonça CBE, Gonçalves-Esteves V. 2015. Pollen morphology and detailed sexine of *Passiflora* L. subgenus *Astrophea* (DC.) Masters (Passifloraceae). *Plant Systematic and Evolution* 301: 2189-2202.
- Milward-de-Azevedo MA, Baumgratz JFA, Gonçalves-Esteves V. 2012. A taxonomic revision of *Passiflora* subgenus *Decaloba* (Passifloraceae) in Brazil. *Phytotaxa* 53: 1-68.
- Pérez-Cortéz S, Tillett S, Escala M. 2002. Estudio morfológico de la semilla de 51 especies del género *Passiflora* L. *Acta Botanica Venezuelica* 25: 67-96.
- Pérez-Cortéz S, Escala M, Tillett S. 2005. Anatomía de la cubierta seminal en ocho especies del género *Passiflora* L., subgênero *Passiflora*. *Acta Botanica Venezuelica* 28: 337-348.
- Pérez-Cortéz S, Escala M, Tillett S. 2009. Morfoanatomía de la cubierta seminal en siete especies de *Passiflora* L., subgênero *Passiflora* (Passifloraceae). *Hoehnea* 36: 131-137.
- Thiers B. [continuously updated]. *Index Herbariorum*: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/> 15 Feb.16.
- Ulmer T, MacDougal JM. 2004. *Passiflora*: Passionflowers of the world. Cambridge, Timber Press.

