Braz. J. Bot (2016) 39(2):721–727
DOI 10.1007/s40415-015-0244-9

Seed micromorphology and its taxonomic significance to *Xyris* (Xyridaceae, Poales)

Kaire de Oliveira Nardi[1] · Aline Oriani[1] · Vera Lucia Scatena[1]

Received: 24 August 2015 / Accepted: 14 December 2015 / Published online: 19 January 2016
© Botanical Society of Sao Paulo 2016

Abstract The seed micromorphology was studied in eight species of *Xyris* (Xyridaceae) with taxonomic purposes. The results show that the presence of longitudinal endotegmic ridges in the seed coat is a pattern for the genus and that the shape of these ridges differentiates among the species. The following characteristics are also useful to identify the species: shape and size of the seed, number of cell rows between the ridges, and the striation pattern of the seed coat. Based on these characteristics, a standard terminology is proposed to describe the seed coat in species of the genus. An identification key for the studied species is also provided.

Keywords Morphology · Seed coat ornamentation · Taxonomy · Xyridoideae

Introduction

Xyridaceae include ca. 430 species and comprise two subfamilies: Xyridoideae (*Xyris* L.) and Abolbodoideae (*Abolboda* Humb., *Achlyphila* Maguire & Wurdack, *Aratitiyopea* Steyerm. & P.E.Berry and *Orectanthe* Maguire). *Xyris* is the largest genus, comprising about 90 % of the species of the family, with a pantropical distribution (Wanderley 1992; Campbell et al. 2009).

The anatomy of the seeds was studied for species of *Abolboda*, *Orectanthe* (Carlquist 1960; Oriani and Scatena 2014) and *Xyris* (Weinzieher 1914; Rudall and Sajo 1999; Nardi et al. 2015) and proved to be useful taxonomically. The importance of seed coat ornamentation for the taxonomy of *Xyris* has already been demonstrated in the literature (Wanderley 1992; Silva 2010; Mota et al. 2015). Mota et al. (2015), for example, analyzed the seed coat surface of 17 species of *Xyris* and used characteristics such as length and shape of seed, type of striae, and pattern of cross-lines to distinguish among species. It is worth pointing out that the knowledge of the ontogeny of the seed is very important for the analysis of micromorphology because it facilitates the understanding of the origin of the tissues responsible for seed coat ornamentation. Nardi et al. (2015) studied the ontogeny of seed coat of six species of *Xyris* and demonstrated that the mature seeds present exotesta, endotesta, and endotegmen, and that the endotegmen undergoes radial elongation, forming ridges in the seed surface.

In Poales, micromorphological studies of seeds with taxonomic purposes were also performed for species of Bromeliaceae (Gross 1988; Melcher et al. 2004), Eriocaulaceae (Giulietti et al. 1986; Nair 1987; Scatena and Bouman 2001; Melcher et al. 2004; Zona et al. 2012; Barreto et al. 2013), Poaceae (Melcher et al. 2004), Rapateaceae (Venturelli and Bouman 1988), and Juncaceae (Brooks and Kuhn 1986; Zech and Wujek 1990; Melcher et al. 2004; Khalik 2010). These studies have demonstrated that the micromorphological characteristics of seeds may be useful for infrageneric classification and may indicate relationships among taxonomic groups, emphasizing the importance of seed characteristics for taxonomy and phylogeny.

In light of the large number of species of *Xyris* and the difficulty in identification because of the morphological similarity (Wanderley 2011), this study aimed to identify

✉ Kaire de Oliveira Nardi
kairenardi@gmail.com

[1] Departamento de Botânica, Instituto de Biociências, Universidade Estadual Paulista (UNESP), Av. 24A, 1515, C. Postal 199, Rio Claro, SP 13506-900, Brazil

Table 1 Micromorphological characteristics of seeds of *Xyris*

Species	Seed shape	Seed size (mm)	Endotegmic ridges shape	Number of cell rows between ridges	Longitudinal striations in the zones between ridges	Transversal striations in the zones between ridges	Hilum
X. asperula Mart.	Oblong	1.02 ± 0.040 × 0.54 ± 0048	Smooth	1–2	Conspicuous	Conspicuous	Acute
X. bialata Malme	Ovoid	1.72 ± 0316 × 0.50 ± 0050	Moniliform	2–3	Inconspicuous	Inconspicuous	Truncate
X. minarum Seub.	Ellipsoidal	0.40 ± 0020 × 0.21 ± 0018	Sulcate	1	Inconspicuous	Conspicuous	Acute
X. nubigena Kunth.	Ellipsoidal	0.42 ± 0026 × 0.21 ± 0016	Smooth	1–2	Inconspicuous	Conspicuous	Acute
X. obtusiuscula L.A.Nilsson	Fusiform	0.53 ± 0032 × 0.19 ± 0023	Spiral	2–3	Inconspicuous	Inconspicuous	Acute
X. pilosa Kunth.	Fusiform	0.72 ± 0031 × 0.23 ± 0019	Smooth	1–3	Conspicuous	Conspicuous	Acute
X. pterygoblephara Steud.	Ellipsoidal	0.45 ± 0018 × 0.25 ± 0020	Sulcate	1–3	Conspicuous	Conspicuous	Acute
X. subsetigera Malme	Ellipsoidal	0.46 ± 0022 × 0.21 ± 0018	Spiral	1–2	Absent	Inconspicuous	Truncate

Figs. 1–8 Scanning electron micrographs of seeds of *Xyris*. **1**, **2** *X. asperula*. **3**, **4** *X. bialata*. **5**, **6** *X. minarum*. **7**, **8** *X. nubigena*. *Scale bar* 100 µm (**1**, **3**, **5**, **7**); 25 µm (**2**, **4**, **6**, **8**). *8* hilum, *op* operculum ▶

seed micromorphological characteristics with taxonomic importance to identify species in the genus. Based on a previous study of the seed development in *Xyris* (Nardi et al. 2015), a standard terminology is proposed to describe the seed coat in species of this genus.

Materials and methods

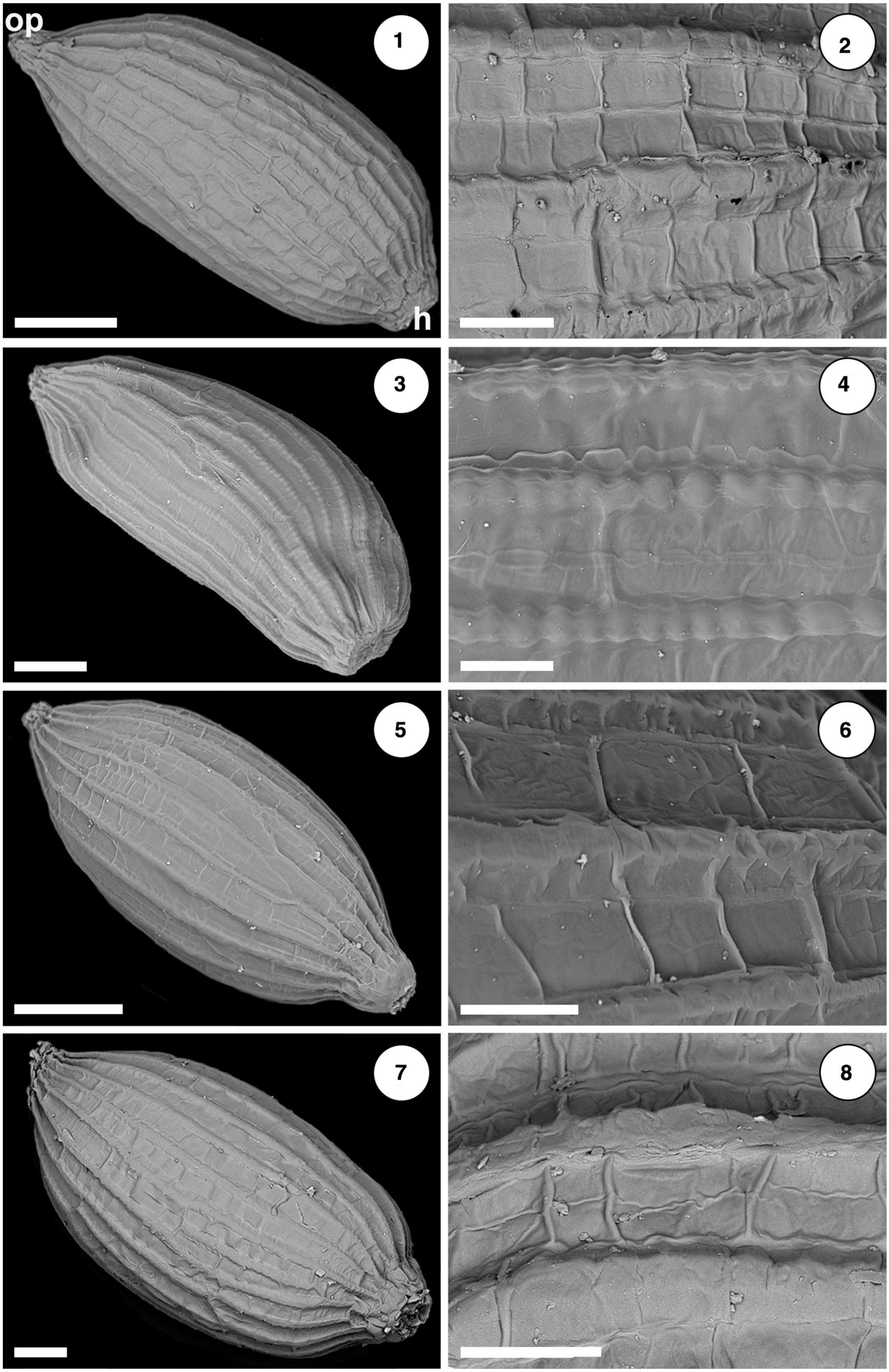
Mature seeds of eight species of *Xyris* were collected in the Serra do Cipó in Minas Gerais (Brazil) (19°–20° S; 43°–44 W) and vouchers were deposited in the Herbarium Rioclarense (HRCB) at Universidade Estadual Paulista. All of the species studied belong to section *Nematopus*: *X. asperula* Mart. (*Scatena* et al. *446*), *X. bialata* Malme (*Nardi* et al. *7*), *X. minarum* Seub. (*Nardi* et al. *9*), *X. nubigena* Kunth. (*Scatena* et al. *448, 451; Nardi* et al. *8*), *X. obtusiuscula* L.A.Nilsson (*Nardi* et al. *13*), *X. pilosa* Kunth. (*Scatena* et al. *455; Nardi* et al. *4*), *X. pterygoblephara* Steud. (*Scatena* et al. *453; Nardi* et al. *11, 12, 16*), and *X. subsetigera* Malme (*Scatena* et al. *447; Nardi* et al. *14, 15, 17*). These species were chosen because they have a sympatric distribution, similar morphology and flower simultaneously.

For the scanning electron microscopy (SEM) study, mature seeds from at least ten individuals of each species fixed in FAA 50 were dehydrated in an acetone series, critical point dried (Balzers, CPD 030) and coated with gold (Bal-Tec, SCD 050). The results were recorded using the "TM 3000 Application Program." Measurements of length and width of the seeds were analyzed in 30 samples of each species with an image-capturing device (Leica, DFC 290) coupled to a microscope (Leica, DMLB) using the LAS (Leica Application Suite V3.6.0) digital imaging system.

Results

The micromorphological characteristics of seeds are summarized in Table 1 and Figs. 1–8 and 9–16. The seed shape varies from oblong in *Xyris asperula* (Fig. 1), to ovoid in *X. bialata* (Fig. 3), ellipsoidal in *X. minarum* (Fig. 5), *X. nubigena* (Fig. 7), *X. pterygoblephara* (Fig. 13), *X. subsetigera* (Fig. 15), and fusiform in *X. obtusiuscula* (Fig. 9) and *X. pilosa* (Fig. 11).

The seed length ranges from 0.40 to 0.72 mm, and the seed width ranges from 0.19 to 0.25 mm, except in *X. asperula* and *X. bialata*, which have larger seeds with mean


op
1
h
2
3
4
5
6
7
8

dimensions of 1.02 mm × 0.54 mm and 1.72 mm × 0.50 mm, respectively.

All of the studied species have longitudinal endotegmic ridges in their seed coat surfaces, which can be smooth (Figs. 1, 2, 7, 8 and 11, 12), moniliform (Figs. 3, 4), sulcate (Figs. 5, 6, 13, 14), or spiral (Figs. 9, 10, 15, 16).

The number of cell rows between the ridges also exhibits interspecific variation and occur as 1 row (Figs. 5, 6), 1–2 rows (Figs. 1, 2, 7, 8, 15, 16), 1–3 rows (Figs. 11, 14), or 2–3 rows (Figs. 3, 4, 9, 10). Longitudinal and/or transversal striations are observed in the zones between ridges and may be conspicuous or inconspicuous (Figs. 2, 4, 6, 8, 10, 12, 14, 16).

The hilum (h) and micropyle appear at opposite ends of the seed (Fig. 1). The micropyle is protected by the operculum (op) (Fig. 1). The hilar region can be acute (Figs. 1, 5, 7, 9, 11, 13) or truncate (Figs. 3, 15).

Based on these characteristics, an identification key for the studied species is provided.

Key to *Xyris* species based on seed characteristics

1. Smooth endotegmic ridges
    - 2. Cell rows between ridges, 1–3………*X. pilosa*
    - 2′. Cell rows between ridges, 1–2
        - 3. Conspicuous longitudinal striations between ridges………*X. asperula*
        - 3′. Inconspicuous longitudinal striations between ridges………*X. nubigena*

1′. Moniliform, sulcate, or spiral endotegmic ridges
- 4. Moniliform ridges………*X. bialata*
- 4′. Sulcate or spiral ridges
    - 5. Sulcate ridges
        - 6. Cell row between ridges, 1; inconspicuous longitudinal striations between ridges………*X. minarum*
        - 6′. Cell rows between ridges, 1–3; conspicuous longitudinal striations between ridge…*X. pterygoblephara*
    - 5′. Spiral ridges
        - 7. Cell rows between ridges, 2–3; inconspicuous longitudinal striations between ridges….*X. obtusiuscula*
        - 7′. Cell rows between ridges, 1–2; absent longitudinal striations between ridges…..…..….*X. subsetigera*

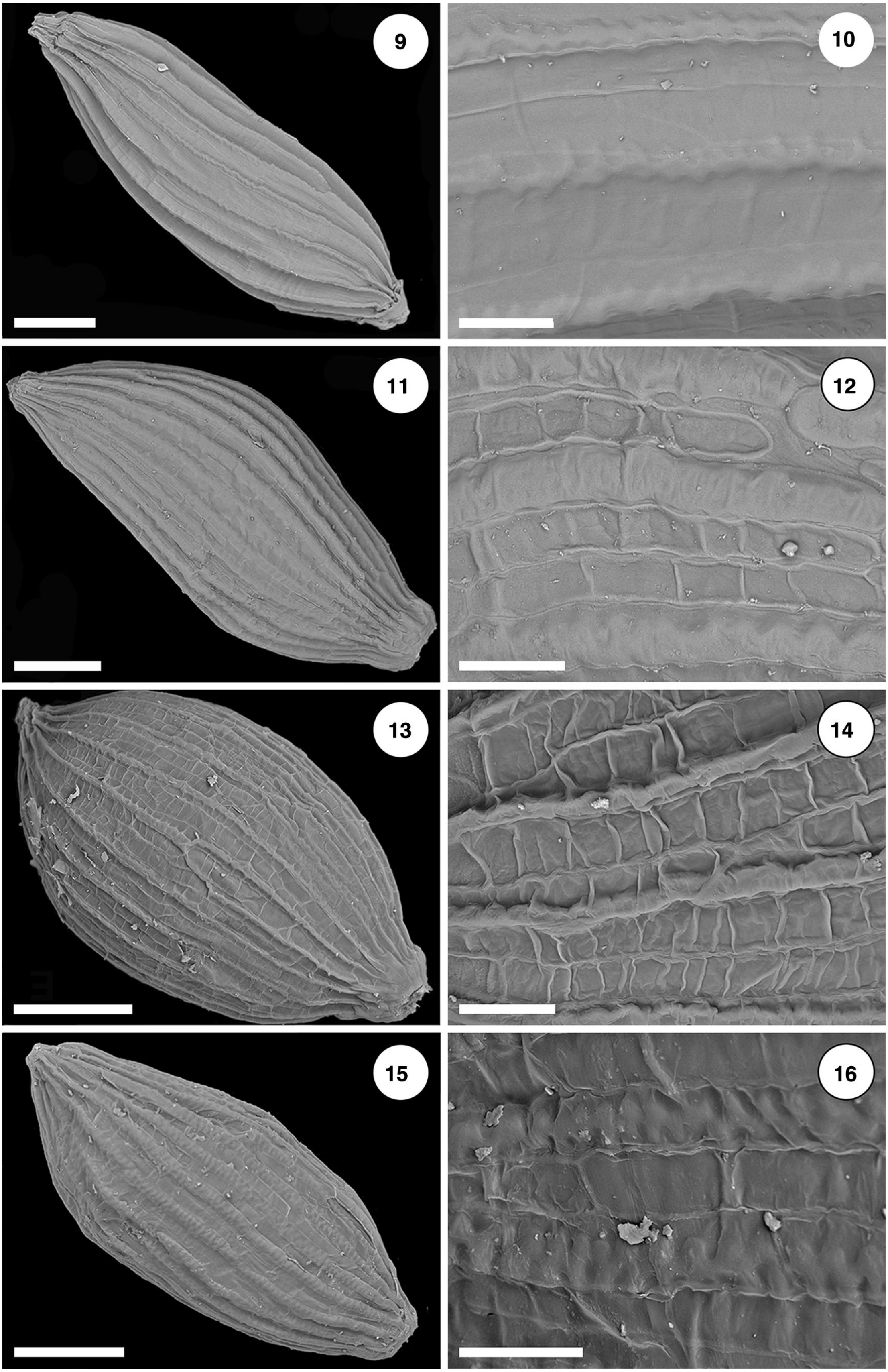
Figs. 9–16 Scanning electron micrographs of seeds of *Xyris*. **9, 10** *X. obtusiuscula*. **11, 12** *X. pilosa*. **13, 14** *X. pterygoblephara*. **15, 16** *X. subsetigera*. *Scale bar* 100 µm (**9, 11, 13, 15**); 25 µm (**10, 12, 14, 16**) ▶

Discussion

Based on the obtained results, the following seed coat characteristics have taxonomic importance to identify species of *Xyris*: the shape and the size of the seed, the shape of the endotegmic ridges, the number of cell rows between the ridges, and the striation pattern of the seed coat. These characteristics vary among the species studied, allowing their identification, and are therefore of great importance to the genus considering that numerous species of *Xyris* occur sympatrically and are difficult to identify because of their high morphological similarity. Although we have not observed any intraspecific variation in relation to these characteristics, there are reports in the literature that seed shape may vary within the species, for example, in *Xyris asperula*, whose seed shape may be fusiform or ellipsoidal according to Wanderley (2011). Considering this variation, we used only the most consistent characteristics in the species identification key such as shape of the endotegmic ridges, the number of cell rows between the ridges, and the striation pattern of the seed coat. The importance of seed micromorphology for taxonomy has been previously reported for the closely related family Eriocaulaceae, whose species have also seed coat characteristics with interspecific variation (Giulietti et al. 1986; Nair 1987; Zona et al. 2012).

It should be emphasized that the longitudinal ridges observed in the seeds of the studied species were also reported for all species of *Xyris* analyzed in previous studies (Rudall and Sajo 1999; Silva 2010) and are thus a pattern for the genus. An anatomical study of the ovule and seed development of *Xyris asperula* Mart., *X. cipoensis* L.B.Sm and Downs, *X. nubigena* Kunth, *X. pterygoblephara* Steud., *X. pilosa* Kunth., and *X. subsetigera* Malme (Nardi et al. 2015) has demonstrated that the cells of the exotegmen degenerate, while some cells of the inner layer of inner integument (endotegmen) elongate radially, forming ridges in the seeds, termed here as endotegmic ridges. In *Abolboda*, the seed coat ornamentation is given by the cells of the exotegmen, which do not degenerate, forming exotegmic ridges (Carlquist 1960; Oriani and Scatena 2014).

In Eriocaulaceae, the sister-group of Xyridaceae (Bouchenak-Khelladi et al. 2014), the development of the seed coat is similar in all the genera studied, including the


9
10
11
12
13
14
15
16

degeneration of the exostesta cells and of the outer periclinal walls of the endotesta cells (Scatena and Bouman 2001; Coan et al. 2010). In this family, the anticlinal walls of the endotesta cells thicken during the seed coat development and are responsible for the ornamentation of the seed surface, forming endotestal ribs (Scatena and Bouman 2001; Coan et al. 2010). Silva (2010) also used the term "ribs" to describe the seed coat ornamentation of seeds of *Xyris*, whereas Mota et al. (2015) used the term "striae." However, since the cell layer responsible for the seed coat ornamentation in Eriocaulaceae and Xyridaceae is not homologous, we believe that the term "endotegmic ridges" is more appropriate to describe the seed coat ornamentation of *Xyris*.

The term "reticules" used by Silva (2010) and "cross-lines" used by Mota et al. (2015) correspond to the "longitudinal and transversal striations" reported in the present study and the "reduced or evident intercostal spaces" (Silva 2010) are herein described by the number of cell rows between the ridges. According to our results, the terminology proposed here is the most suitable since it is based on the ontogeny of the seed structures (Nardi et al. 2015) and refers to characteristics that are easily observed.

In contrast to the ontogeny and anatomical structure of the seed, which are uniform across the genus (Nardi et al. 2015), the micromorphological characteristics of the seeds vary among the species of *Xyris* and, beyond their taxonomic significance, have ecological importance because these characteristics may be related to different dispersal strategies. To date there is no study on seed dispersal of Xyridaceae; however, based on our results we can suggest the following hypotheses: (I) Air bubbles may be retained between the ridges and striations favoring wind dispersal, as suggested for species of *Syngonanthus* Ruhland and *Paepalanthus* Kunth (Eriocaulaceae) (Melcher et al. 2004); (II) Ridges and striations can increase air resistance and air buoyancy, decreasing the sink velocity of the seed (Johri 1984) and facilitating dispersal for long distances from the mother-plant (Niklas 1994); (III) Ridges and striations can be hygroscopic, similar to the ribs of Eriocaulaceae seeds, and are most likely adaptations for the dispersal and the germination of species that mainly inhabit regions with dry periods (Giulietti et al. 1986); (IV) Water drops can be stored between the ridges and striations, facilitating their imbibition and promoting germination. Because the species of *Xyris* usually occur in regions with low water availability, the hypothesis III and IV are more plausible. However, further studies on seed dispersal are necessary to adequately test these hypotheses. We suggest that the micromorphological variations observed in the seeds of *Xyris* have arisen to provide effective seed dispersal, allowing seeds to reach areas suitable for germination and seedling establishment.

Acknowledgments We thank CAPES—Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (MSc fellowship to K.O.N.) and FAPESP—Fundação de Amparo à Pesquisa do Estado de São Paulo (grant 2011/11536-3 to A.O.) for financial support and the Laboratório de Microscopia Eletrônica da Universidade Estadual Paulista/Rio Claro for the use of the scanning electron microscope. We also thank Dr Maria das Graças Lapa Wanderley for identifying the species, and the anonymous reviewers for their valuable comments to improved the paper.

References

Barreto QC, Echternacht L, Garcia QS (2013) Seed coat sculpture in *Comanthera* (Eriocaulaceae) and its implications on taxonomy and phylogenetics. Plant Syst Evol 299:1461–1469

Bouchenak-Khelladi Y, Muaya AM, Linder HP (2014) A revised evolutionary history of Poales: origins and diversification. Bot J Linn Soc 175:4–16

Brooks RE, Kuhn C (1986) Seed morphology under SEM and light microscopy in Kansas *Juncus* (Juncaceae). Brittonia 38:201–209

Campbell LM, Wanderley MGL, Silva GO (2009) Neotropical Xyridaceae. In: Milliken W, Klitgard B, Baracat A. (2009 onwards). Neotropikey—Interactive key and information resources for flowering plants of the Neotropics. http://www.kew.org/science/tropamerica/neotropikey/families/Xyridaceae.htm. Accessed 10 Nov 2015

Carlquist S (1960) Anatomy of Guayana Xyridaceae: *Abolboda*, *Orectanthe* and *Achlyphila*. Mem N Y Bot Gard 10:65–117

Coan AI, Stutzel T, Scatena VL (2010) Comparative embryology and taxonomic considerations in Eriocaulaceae (Poales). Feddes Repert 122:1–17

Giulietti AM, Monteiro WR, Mayo SJ, Stephens J (1986) A preliminary survey of testa sculpture in Eriocaulaceae. Beitr Biol Pflanzen 62:189–209

Gross E (1988) Bromelienstudien. IV. Zur Morphologie der Bromeliaceen-Samen unter Berucksichtigung systematisch-taxonomischer Aspekte. Trop Subtrop Pflanzenwelt 64:415–625

Johri BM (1984) Embryology of angiosperms. Springer, Berlin

Khalik KNA (2010) Seed coat morphology and its systematic significance in *Juncus* L. (Juncaceae) in Egypt. J Syst Evol 48:215–223

Melcher IM, Bouman F, Cleef AM (2004) Seed atlas of the monocotyledonous genera of the páramo. Flora 199:286–308

Mota NFO, Campbell LM, Viana PL, Wanderley MGL (2015) Xyridaceae of Viruá National Park, Roraima state, Brazil. Rodriguésia 66:523–553

Nair RV (1987) Taxonomic significance of seed coat morphology in *Eriocaulon* Linn. (Eriocaulaceae). Seed Sci Technol 15:297–310

Nardi KO, Scatena VL, Oriani A (2015) Development of ovule, fruit and seed of *Xyris* (Xyridaceae, Poales) and taxonomic considerations. Bot J Linn Soc 177:619–628

Niklas KJ (1994) Plant allometry: the scaling of form and process. The University of Chicago Press, Chicago

Oriani A, Scatena VL (2014) Ovule, fruit, and seed development in *Abolboda* (Xyridaceae, Poales): implications for taxonomy and phylogeny. Bot J Linn Soc 175:144–154

Rudall PJ, Sajo MG (1999) Systematic position of *Xyris*: flower and seed anatomy. Int J Plant Sci 160:795–808

Scatena VL, Bouman F (2001) Embryology and seed development of *Paepalanthus* sect. *Actinocephalus* (Koern.) Ruhland (Eriocaulaceae). Plant Biol 3:341–350

Silva GO (2010) Flora de Mucugê, Bahia, Brasil: Xyridaceae. Dissertação de Mestrado, Instituto de Biociências, Universidade de São Paulo, São Paulo

Venturelli M, Bouman F (1988) Development of ovule and seed in Rapateaceae. Bot J Linn Soc 97:267–294

Wanderley MGL (1992) Estudos taxonômicos no gênero *Xyris* L. Tese de Doutorado. Universidade de São Paulo, São Paulo

Wanderley MGL (2011) Flora da Serra do Cipó, Minas Gerais: Xyridaceae. Boletim de Botânica da Universidade de São Paulo 29:69–134

Weinzieher S (1914) Beitrage sur Entwicklungsgeschichte von *Xyris indica*. Flora 106:393–432

Zech JC, Wujek DE (1990) Scanning electron microscopy of seeds in the taxonomy of Michigan *Juncus*. Michigan Bot 29:3–18

Zona S, Davis P, Gunathilake LAAH, Prince J, Horn JW (2012) Seeds of Eriocaulaceae of the United States and Canada. Castanea 77:37–45