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Sporangia and Spores in the Fern Genera *Spicantopsis* and *Struthiopteris* (Blechnaceae, Polypodiopsida)

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Abstract

Struthiopteris (Blechnaceae) has recently been classified on the basis of molecular and morphological evidence, and some of its species are now included in the sister genus *Spicantopsis*. However, the lack of studies on several important morphological features impedes a sound assessment of their congruence with this new systematic arrangement, as well as of their range of variation and taxonomic value in this group of ferns. Here we present a study on the spores and sporangia using both light and scanning electron microscopy in *Struthiopteris* and *Spicantopsis*, using samples of all their species, and almost all their varieties. We provide full morphological descriptions of the spores and sporangia of all these taxa. We point out that the perispore structure and ornamentation and the number and the thickness of stomium cells in the sporangium clearly distinguish both genera.

Keywords Annulus · *Blechnum* · Capsule · Ferns · Perispore · Rosette · SEM · Taxonomy

Introduction

The Blechnaceae is a lineage within the leptosporangiate ferns (Polypodiidae), which comprises 25 genera and ca. 250 species, according to the most recent classifications (PPG1 2016; Gasper et al. 2016; Molino et al. 2019b). Most of these genera were previously included in *Blechnum* (Kramer et al. 1990). One is *Struthiopteris* Scop., which consists mainly of small- to medium-sized plants, with pinnate, dimorphic fronds. Based on both morphological and molecular information, some species of *Struthiopteris* have been recently classified in *Spicantopsis* Nakai (Nakai 1933; Molino et al. 2019b).

Spicantopsis and *Struthiopteris* both contain few species and are restricted to the Northern Hemisphere. *Spicantopsis* has three species, all of them restricted to Asia (Chiou et al. 1994; Faguo et al. 2013): two are endemic to Japan, *Spicantopsis amabilis*

(Makino) Nakai, and *S. niponica* (Kunze) Nakai (with a variety named *S. niponica* var. *minima* Tawaga), and a third one, *S. hancockii* (Hance) Masam., occurs in both Japan and Taiwan. *Struthiopteris*, in its current circumscription, comprises 3 species. The first one is *Struthiopteris spicant* (L.) Weiss., which is widely distributed in two disjunct centers: one of them in Europe, where it is very common in the west and center of the continent, and less frequent in the eastern part, the Macaronesian archipelagos, and also in North Africa, and a second center in the Pacific coast area of the United States and Canada. This species is a quite variable entity for which several subspecies and varieties have been proposed; currently, two of them are accepted, *S. spicant* var. *homophyllum* (Merino) Gabriel y Galán & R. Pino and *S. spicant* var. *pradae* S. Molino & Gabriel y Galán (Molino et al. 2019a). The second species is *S. fallax* (Lange) S. Molino, Gabriel y Galán & Wasowicz, which is endemic to Iceland (Molino et al. 2019a); and the third species is *S. castanea* (Makino & Nemoto) Nakai, endemic to Japan (Fig. 1).

Reproductive structures in the sporophyte have long been known to render good characters in fern taxonomy. However, different authors have used different terminology to refer to the structures of the sporangia (Atkinson 1893; Bower 1928; Haider 1954; Wilson 1959; Lellinger 2002; Prada et al. 2016). To clarify this terminology, here we will use what is stated in Wilson (1959) and Lellinger (2002) with modifications from Prada et al. (2016), focusing on the following sporangial traits: number of cells of the pedicel and the presence of a rosette between the pedicel and the capsule, which is formed by the apical cells of the pedicels that are different in their sizes and wall composition, sensu Prada et al. (2016); the structure of the annulus sensu Wilson (1959), which is the sum of the mechanic cells (arcus), the structure of the stomial area (formed by the epistomium,

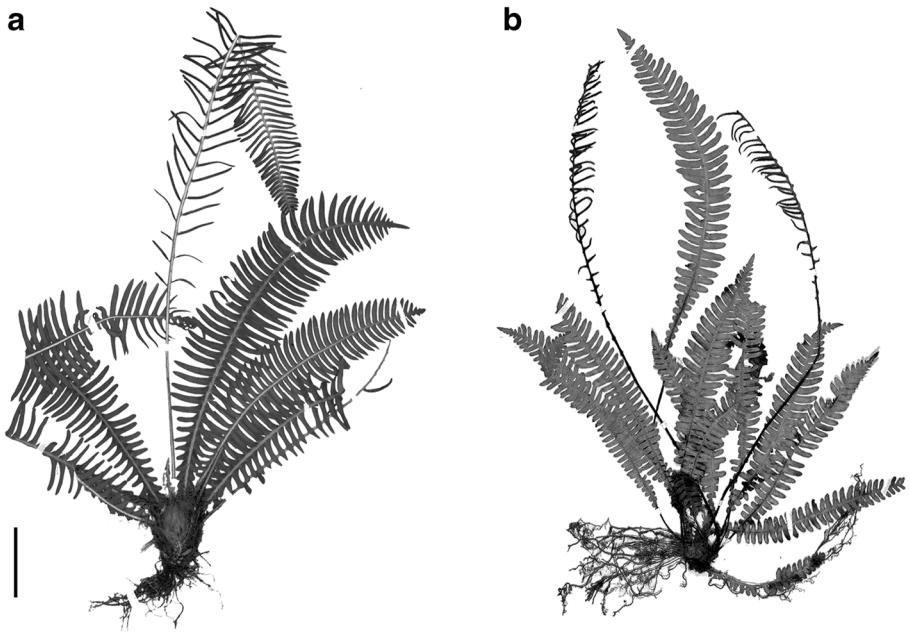

Fig. 1 Photographs of herbarium specimens of representative plants of the genera *Spicantopsis* and *Struthiopteris*: **a**) *Spicantopsis niponica* (*Umemura 37,* P 01406521). **b**) *Struthiopteris spicant* (*E. Blanco 1182,* MA 564805). Scale bar = 3 cm in both

the hypostomium and the lips sensu Prada et al. 2016) and the presence of posterior basal cells (between the arcus and the pedicel in the posterior part of the capsule, opposite to the stomium area, term introduced by us) (Fig. 2).

Spores have been used also to separate fern taxonomic groups, thus becoming an extremely important source of character traits with taxonomic relevance, mainly the spore dimensions, the model of laesura, and the perispore ornamentation and structure (Olsen & Gullvag 1974; Lugardon 1974; Barrington et al. 1986; Tryon & Lugardon 1991; Palacios-Rios et al. 2017).

Despite the low number of species of both *Spicantopsis* and *Struthiopteris*, and the fact that their morphology, distribution and cytotaxonomy is comparatively well known (e.g. Nakai 1933; Löve and Löve 1968; Nakato 1987; Horjales et al. 1990), data on sporangia and spore traits are scarce and incomplete. A recent publication (Prada et al. 2016) dealt with the sporangia of 47 species of *Blechnum* L. s.l., and examined characteristics of pedicels, rosettes, annuli, arcus and stomial areas. However, this comprehensive work only considered one of the species of our interest, *S. spicant*, characterizing in the sporangia the length of the pedicel, the number of cells of the arcus, and the number of cells of the stomial area. Information about these characters are unknown in the other taxa of *Struthiopteris* and *Spicantopsis*.

The spores of both genera have received a little more attention in the past. By far, *S. spicant* is again the most investigated of all the taxa considered here, and its spores have been described in several papers, including details of their size, ornamentation, and structure (Lugardon 1965; Tryon and Lugardon 1991; Passarelli et al. 2010). However, this information deals only with the typical variety of this extremely diverse entity, and no other data are available for the rest of taxa. The spores of most of the Japanese species (*Struthiopteris castanea*, *Spicantopsis niponica* and *S. amabilis*) have been only very briefly described by Mitui (1979), who provided some SEM photographs. Moran et al. (2018), in its phylogenetic and evolutionary study, included also

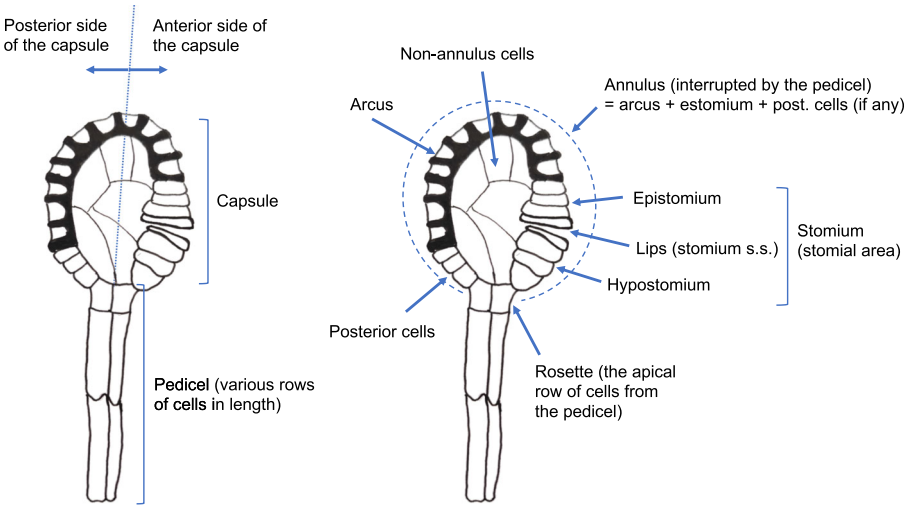

Fig. 2 Schematic of a leptosporangiate vertical sporangium, typical of the Polypodiales, with indication of the main traits considered in this work. Terminology is based on Lellinger (2002) and Wilson (1959), except for the terms "rosette" and "lips", from Prada et al. (2016), and "posterior cells of the annulus", proposed here.

some information about the spores of other taxa within the family Blechnaceae. As far as we know, *Struthiopteris fallax* and *Spicantopsis hancockii* lack any published data on their sporangia or spores.

The goal of this study is to provide new, comprehensive descriptions of the sporangia and the spores of *Spicantopsis* and *Struthiopteris*, and assess their value for distinguishing the two genera.

Materials and Methods

We used both plants from new field collections and preserved material of several herbaria. In the former case, a voucher specimen has been deposited in the herbarium MACB. We obtained samples of all the taxa of interest except *Spicantopsis niponica* var. *minima*, since the only material we had access to was an isotype, and we decided not to damage the specimen while extracting sporangia and spores. Appendix 1 shows basic voucher information regarding plant material.

Sporangia observations were obtained from at least five sporangia of at least two different individuals per taxon, depending on the available material, following standard procedures for sample extraction, staining and microscope preparation, widely used for these purposes (Ruzin 1999; Passarelli et al. 2010; Palacios-Rios et al. 2017). All the observations were made under an optical microscope (OM) *Nikon Labophot-2*. Exospore dimensions have been measured in 20–30 spores under the OM from at least two individuals per taxa. We measured and described the following traits: major and minor equatorial diameters of spores, perispore ornamentation, and perispore structure.

Fine details about the sporangia and about the ornamentation and structure of perispore and exospore of spores were obtained with scanning electron microscope (SEM) techniques. Sample preparation followed standard protocols (Nation 1983), making two samples from two different individuals per taxon. The observations were made with a SEM *Hitachi S-3000 N* operated at 20 kv, with a ESED (environmental secondary electron detector) coupled to an X-ray dispersive energy analyzer (INCAx-sight, *Oxford Instruments*). The SEM study was done in the facilities at Universidad Autónoma de Madrid (Servicio Interdepartamental de Investigación).

We adjusted the taxonomic terms of the structures observed to the standardized terminology in Wilson (1959) and Lellinger (2002) with modifications from Prada et al. (2016), as has been introduced before.

We described the sporangium by observing and checking traits from the pedicel (number of cell-rows in the pedicel, characteristics of the rosette), the capsule (length of the capsule), and the annulus (number of cells in the arcus, lips, epistomium, hypostomium and posterior basal area of the annulus) (Fig. 2).

Results

Sporangia

The sporangia in *Spicantopsis* and *Struthiopteris* are of the Polypodialean type, a capsule with a vertical annulus, sustained by a pedicel (Fig. 3). In all the species

studied, the sporangia present short pedicels, with a length of 2–3 rows of cells, which do not differ notably between taxa. All of the taxa present a rosette in the pedicel (Fig. 3a-d). The rosette differs from the rest of the pedicel cells in size and shape. This rosette shows no important differences in size between taxa. The pedicel in cross section is composed by three cells in all the species observed in this work (Fig. 4a).

The capsules are more or less spheroidal (Fig. 3a-d, Fig. 4b-d), the length for each species can be observed in Table 1. The annulus is vertical and incomplete, being interrupted at the pedicel.

In all the taxa, a complex stomial area has been observed in the anterior part of the capsule, between the pedicel and the end of the arcus. Within this area, the 2–5 central cells constitute the lip, with thickened walls, along which the sporangium will open. The lip is composed of 4–5 cells in the case of *Spicantopsis* (Fig. 3a, c) and (2)3 cells in the case of *Struthiopteris* (Fig. 3d; Fig. 4b-d). The cells of the lip are narrower in *Spicantopsis*, 5–10 (12.5) μm, than in *Struthiopteris*, 12.5–17.5(37.5) μm. More infrequently, there are only 2 cells in the lip. All the taxa studied present epistomium and hypostomium (Fig. 3a, c, d; Fig. 4b-d).

Another observed differential trait refers to the number of posterior basal cells of the annulus, those situated between the posterior end of the arcus and the pedicel, opposite to the hypostomium. In *Spicantopsis niponica* this area is formed by 3–4 cells (Fig. 3c) whereas in the rest of the species of *Spicantopsis* and all the taxa within *Struthiopteris* the arcus emerges in direct contact with the pedicel, i.e. no area of posterior basal cells differentiates (Fig. 3b, d).

The arcus is manifest, with a number of cells ranging between 19 and 29 in *Spicantopsis* (Fig. 5a-c) and 12–21 in *Struthiopteris* (Fig. 5d-h). Table 1 shows the values measured for each taxa.

Spore Size and Ornamentation

The spores of all the taxa in *Spicantopsis* and *Struthiopteris* are monolete, plane-convex in equatorial view, and elliptic in polar view (Fig. 6).

Spores of *Spicantopsis amabilis* present a dark brown perispore, not folded, with a verrucate-granulate ornamentation; the laesura is slightly prominent, almost as long as the spore (Fig. 6a). *Spicantopsis hancockii* has spores with a pale brown perispore, not angular, covered by numerous irregular tabications with granulate surface, anastomosed into an irregularly reticulate ornamentation; in some parts of the spore the walls are covered by a thin, smooth or granulate, external layer; the laesura is subterminal and not prominent (Fig. 6b).

The spores of *Spicantopsis niponica* present the same appearance as in *S. hancockii* but they are larger and with the laesura slightly more prominent (Fig. 6c).

The spores of *Struthiopteris castanea* have a dark brown perispore, rugate, irregularly and obtusely angular, not reticulate; laesura slightly prominent, thick, almost as long as the spore (Fig. 6d), with the perispore sticking out above it. The spores of *Struthiopteris fallax* and *S. spicant* are quite similar, with a pale brown perispore which appears rugate, slightly angular, with irregular wrinkles not anastomosing into areolae; in *S. fallax* the perispore is more sharply angular and the laesura is almost as long as the spore (Fig. 6e, f). The spores of *S. spicant* are slightly smaller than those of *S. fallax*.

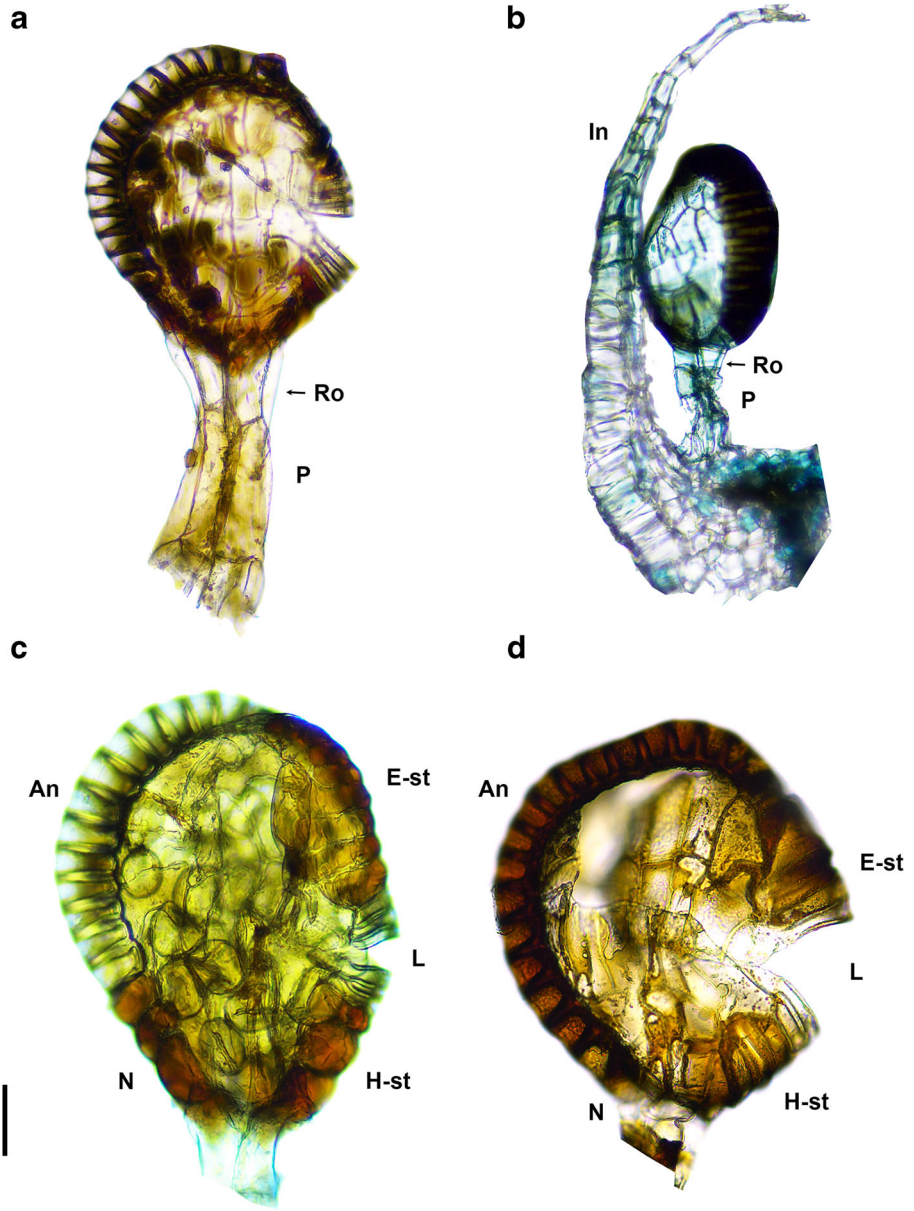

Fig. 3 Light microscope details of the sporangium pedicel and stomial area in taxa of *Spicantopsis* (**a**, **c**) and *Struthiopteris* (**b**, **d**). **a**) complete sporangium of *S. niponica* (*B. Estébanez s.n.,* MACB 110657). **b**) in situ complete sporangium of *S. spicant* (*Gabriel y Galán s.n.* MACB 109622). **c**) details of the capsule of *S. niponica*, showing the stomial area (*Estébanez s.n.,* MACB 110657). **d**) details of the capsule of *S. spicant*, showing the stomial area (*Gabriel y Galán s.n.,* MACB 109622). An = arcus; E-st = epistomium; H-st = hypostomium; In = indusium; L = lip; P = pedicel; N = posterior basal cells; Ro = rosette. Bar = 80 μm in **a**, 90 μm in**a b**, 65 μm in **c**, 50 μm in **d**.

The spores of the varieties of *S. spicant*, var. *homophyllum* and var. *pradae*, show the same characteristics of the typical variety (Fig. 6g, h).

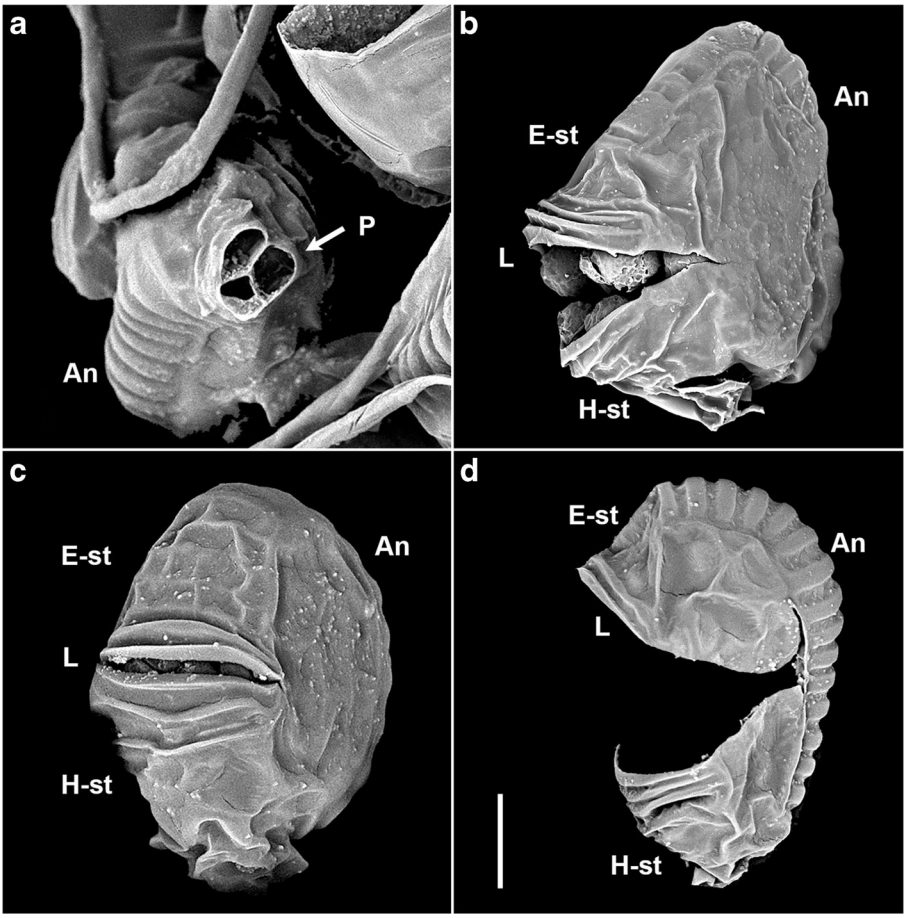

Fig. 4 SEM details of the sporangium pedicel and stomium in taxa of *Spicantopsis* (**a**) and *Struthiopteris* (**b**-**d**). **a**) cross-section of the sporangium pedicel, showing the three-celled configuration at the rosette, *S. hancockii* (*Knapp 4311,* P 02435597). **b**-**d**: mature opened sporangia with complex stomium showing the lips, the epistomium and the hypostomium. **b**) *S. castanea* (*Mizushima 13,853,* S 1982); **c**) *S. spicant* var. *spicant* (*Gabriel y Galán s.n.,* MACB 109622); **d**) *S. spicant* var. *homophyllum* (*Barrera s.n.,* MACB 32367). An = arcus; E-st = epistomium; H-st = hypostomium; L = lip; P = pedicel. Scale bar = 60 μm in **a**; 85 μm in **b**; 75 μm in **c**, **d**.

Table 1 summarizes the main information about spore size and ornamentation for all taxa.

Spore Structure

The spores of *Spicantopsis amabilis* (Fig. 7a, b) present a compact perispore, up to 5 μm thick, with a complex internal structure, composed of microlamellae with a smooth surface, anastomosed, forming an intermediate lacunar layer, covered by a thin external layer giving the exospore a smooth ornamentation. *Spicantopsis hancockii* and *S. niponica* show also a compact perispore, up to 5 μm thick, and with an exospore showing small, sparse bullae (Fig. 7c, d).

Table 1 Comparison table of sporangia and spore characters for *Spicantopsis* and *Struthiopteris*

Taxon	# arc cells	# cells lips	lip cells thickness (μm)	Capsule size (μm)	# cells epistomium	# cells hypostomium	pedicell lenght (μm)	Spore size (μm)	Perispore thickness (μm)	Perispore ornamentation	Perispore structure	Exospore
Spicantopsis												
S. amabilis	20–26	7	5–10	320–380	3–5	3–4	150–400	55.52 × 37.76	up to 5	verrugate–granulate	compact	smooth
S. hancockii	20–29	4–7	7.5–12.3	280–370	8	3	200–300	49.74 × 33.64	up to 5	irregulary reticulate	compact	small bullae
S. niponica	19–20	5–6	7.4–12.3	280–370	8–10	3	180–200	59.29 × 42.48	up to 5	irregulary reticulate	compact	small bullae
Struthiopteris												
S. castanea	16–20	2–3	27.5–37.5	340–460	5–8	4	150–250	56.26 × 41.87	up to 12	rugate	alveolate	smooth
S. fallax	13–21	3	12.5–17.5	210–250	3–8	3–5	120–300	43.33 × 31.83	up to 12	rugate	alveolate	smooth
S. spicant var. *spicant*	12–18	2–3	12.7–19	240–300	5–6	3–4	250–280	40.37 × 28.47	up to 12	rugate	alveolate	smooth
S. spicant var. *homophyllum*	12–19	2–3	12.5–17.5	230–280	4–7	3–6	170	38.01 × 26.63	up to 12	rugate	alveolate	smooth
S. spicant var. *pradae*	12–20	2–3	12.5–17.5	210–260	4–6	3–6	150–200	42.05 × 29.35	up to 12	rugate	alveolate	smooth

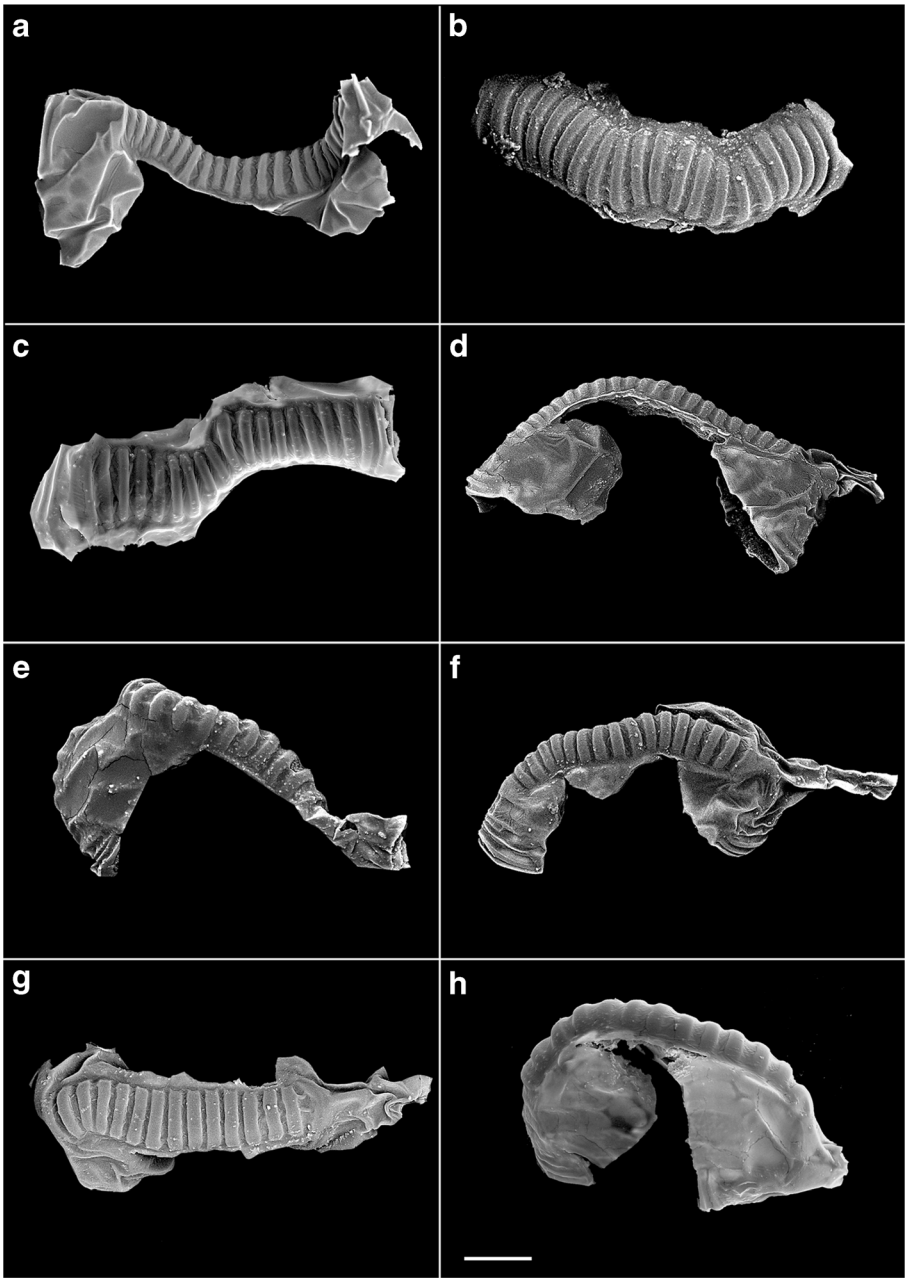

Fig. 5 Details of the annulus in *Spicantopsis* (**a**-**c**) and *Struthiopteris*, (**d**-**h**) showing the thickened cells of the arcus. **a**) *S. amabilis* (*Faurié 5612,* P 01406648), 20 cells. **b**) *S. hancockii* (*Knapp 4311,* P 02435597), 20 cells. **c**) *S. niponica* (*Tagawa & Iwatsuki 3014,* P 01575937), 19 cells. **d**) *S. castanea* (*Mizushima 13,853,* S 1982), 19 cells. **e**) *S. fallax*, 18 cells (*Gabriel y Galán & Wasowicz s.n.,* MACB 109359). **f**) *S spicant* var. *spicant* (*Gabriel y Galán s.n.,* MACB 109622), 16 cells. **g**) *S. spicant* var. *homophyllum* (*Barrera s.n.,* MACB 32367), 13 cells. **h**) *S. spicant* var. *pradae* (*Molino* et al. *s.n.,* MACB 110654), 12 cells. Bar = 75 μm in **b**, **e** and **f**; 80 μm in **c**; 85 μm in **d**; 90 μm in **a**, **g** and **h**.

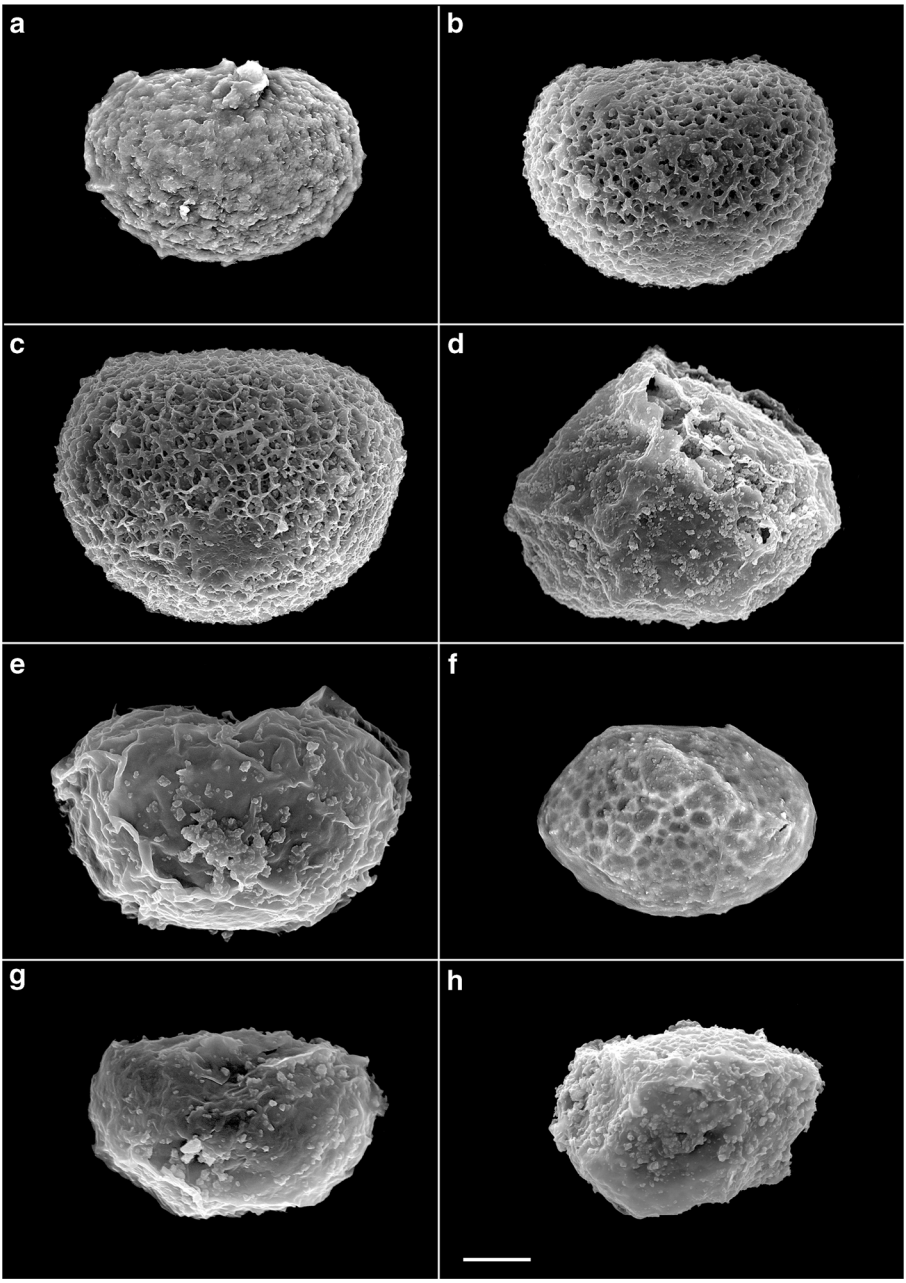

Fig. 6 Spores of *Spicantopsis*: **a**) *S. amabilis* (*Faurié 5612,* P 01406648). **b**) *S. hancockii* (*Knapp 4311,* P 02435597). **c**) *S. niponica* var. *niponica* (*Tagawa & Iwatsuki 3014,*P 01575937). Spores of *Struthiopteris*: **d**) *S. castanea* (*Saito s.n.,* TNS 792168). **e**) *S. fallax* (*Gabriel y Galán & Wasowicz s.n.,* MACB 109359). **f**) *S. spicant* var. *spicant* (*Gabriel y Galán s.n.,* MACB 110658). **g**) *S. spicant* var. *homophyllum* (*Barrera s.n.,* MACB 32367). **h**) *S. spicant* var. *pradae* (*Molino* et al. *s.n.,* MACB 110654). Bar =15 μm in all, except **a**, **e** and **g** = 10 μm

The three species of *Struthiopteris* (*S. castanea, S. spicant* including all its varieties, and *S. fallax*) have spores with perispore up to 10–12 μm thick; the structure is alveolate, with an inner layer of spongy appearance, an intermediate layer with alveoli, and an external smooth layer covering the alveoli; the exospore shows no external ornamentation (Fig. 7e-h).

Table 1 shows the basic information regarding spore structure for all taxa.

In addition, it has been observed that in all the taxa studied the perispore comes off easily, so is frequent to observe spores lacking half of the perispore (Fig. 7).

Discussion

In this work we contribute with new and updated detailed morphological observations about the sporophytic reproductive structures of *Spicantopsis* and *Struthiopteris*. We have been able to study almost all of the infrageneric taxa currently accepted for both genera. Due to the very recent resurrection of the genus *Spicantopsis* (Molino et al. 2019b), this information is particularly relevant from a taxonomical perspective at the generic level. Part of the information is new for all or some of the taxa within each genus, which thus aid in the fine and comprehensive descriptions of these taxonomic entities.

Our observations of the structure of the sporangia agree with some general ideas for the leptosporangiate ferns. For example, it has been traditionally reported that the normal number row cells in the pedicel is 1–4 (Wilson 1959). However, in a previous work about Blechnaceae, Prada et al. (2016) observed that it varies from 2 (i.e. *Blechnum australe* L.) to 6 (i.e. *Telmatoblechnum serrulatum* (Rich.) Perrie, D.J.Ohlsen & Brownsey), but normally with a number of 3 rows.

The data obtained here fall within this range established for the family.

Very few previous observations have been made regarding the number of cells in the arcus for Blechnaceae, although it seems to be an important taxonomical character. It is the case, for example, detected in this work, since this character allow us to distinguish between the two genera treated, being the largest average number for *Spicantopsis*. Also, at a more specific level, in the case of *Struthiopteris spicant* var. *spicant*, the data obtained here (12–18 cells) loosely agrees with previous reports (Prada et al. 2016), in which a number of 15–19 cells is given, with a normal value of 16. The case of *Spicantopsis* is different: Nakai (1933), in the original description of the genus, gave a number of 30–36 cells in the arcus, but in the current study none of the specimens of any species have shown sporangia with more than 29 cells.

We also noticed the presence of posterior basal cells in *Spicantopsis niponica*, which differs from the rest of the species within *Spicantopsis* and *Struthiopteris*. This character has been used in fern taxonomy to discriminate species, for example in the case of European *Polypodium cambricum* L., *Polypodium interjectum* Shivas and *Polypodium vulgare* L. (Muñoz Garmendia 1986), so it could be an interesting specific character for *S. niponica*.

The size of the capsule is an interesting character too. In general, the capsules of the species within *Spicantopsis* are larger than the ones within *Struthiopteris*, except in the case of *S. castanea*, which has the biggest capsules of all of the taxa studied here. However, this is probably due to its ploidy level, since it is a decaploid species, with the

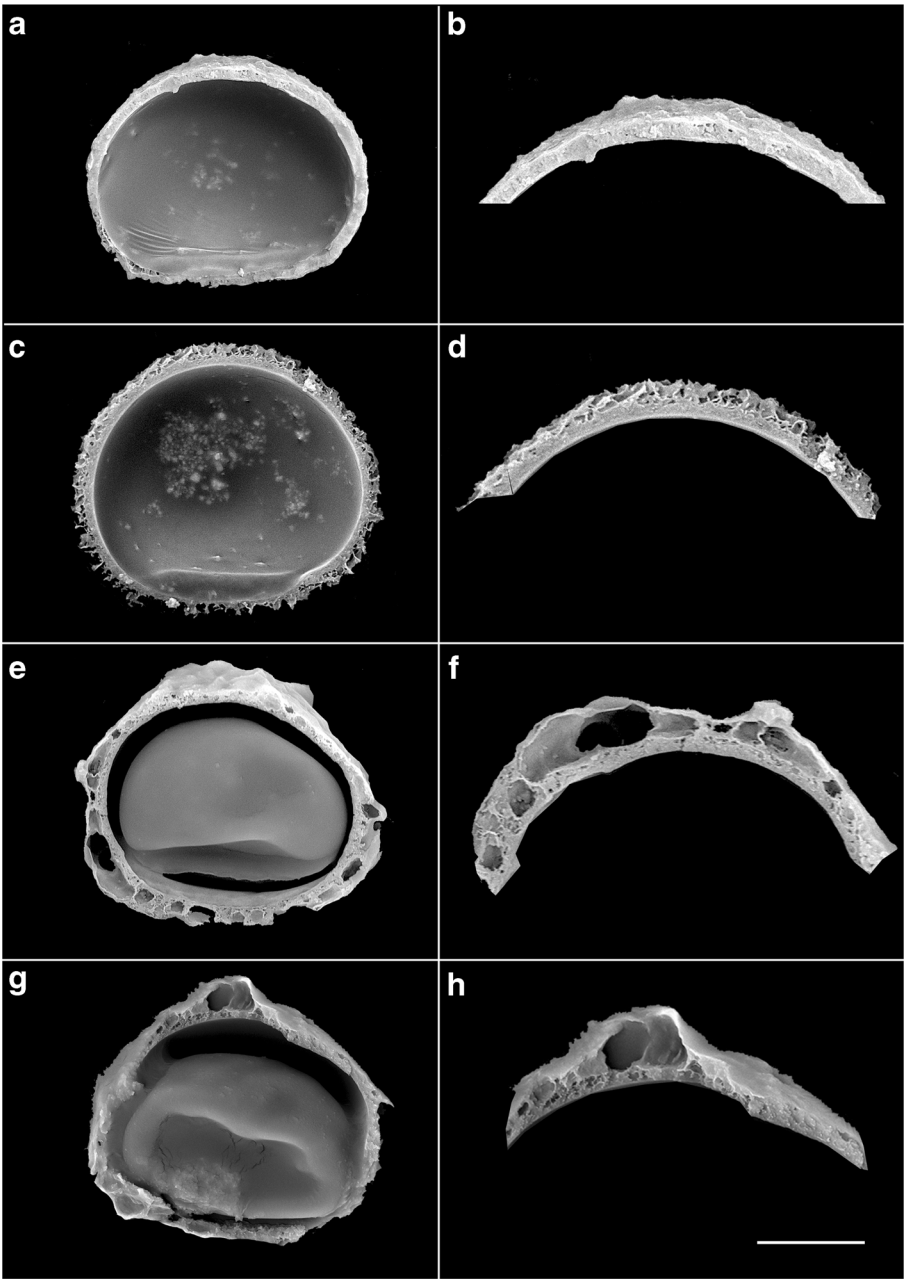

Fig. 7 Fragmented perispore of spores from *Spicantopsis* species (left) with details (right): **a**, **b**) *S. amabilis* (*Faurié 5612,* P 01406648). **c**, **d**) *S. hancockii* (*Knapp 4311,* P 02435597). Fragmented perispore of spores from *Struthiopteris* species (left) with details (right): **e**, **f**) *S. castanea* (*Saito s.n.,* TNS 792168). **g**, **h**) *S. spicant* (*Gabriel y Galán s.n.,* MACB 110658). Bar = 20 μm for the complete spores (left) and 10 μm for the details (right).

highest chromosome number reported in Blechnaceae (Nakato 1987), whereas the rest of taxa are diploid (Löve & Löve 1968; Nakato 1987; Horjales et al. 1990).

The best sporangial characters to separate the two genera seem to be the number and size of the cells in the lips (Table 1). These cells are more numerous and narrower (<12.5 μm) in *Spicantopsis*, and fewer and thicker (>12.5 μm) in *Struthiopteris*. The number of cells in the lips has been already reported for *S. spicant* in Prada et al. (2016) and agrees with what has been observed here.

Spores have been widely used to separate species and genera of the leptosporangiate ferns (Tryon & Tryon 1982; Tryon and Lugardon 1991). In particular, the size, ornamentation and wall structure of the spores are of high taxonomic value: size, ornamentation, wall structure, within a reasonable biometric range, these characters are constant for each species, although there is a considerable variation between species and upper taxonomic levels (Lugardon 1974; Tryon & Lugardon 1991).

In *Spicantopsis*, Mitui (1979) provided brief descriptions of some of the species (*S. niponica* and *S. amabilis*), which are expanded here with concise details of ornamentation and structure of perispore and exospore. The size of the spores reported by Mitui in his work is slightly smaller than reported here. This may be due to the different ways in which the samples have been measured: Mitui used cross sections after a fixation treatment, whereas in this work we measured the spores directly through the optical microscope, without any previous treatment.

Furthermore, the only information available about these traits in *S. hancockii* so far is the size of the spores mentioned in some floras (Chiou et al. 1994; Faguo et al. 2013), which agrees with the measurements reported here.

Our results indicate that *S. amabilis* has noticeable differences in its spores compared to those of *S. niponica* and *S. hancockii*. This could contribute to morphologically support the phylogenetic differences found between *S. amabilis* and a clade formed by the rest of species (Molino et al. 2019b).

Focusing in *Struthiopteris*, several previous works are known to have described some aspects of the spores of *S. spicant* (Tryon & Lugardon 1991; Passarelli et al. 2010; Passarelli 2007; Moran et al. 2018; Lugardon 1965), which is the only taxon whose spores have been described. The findings of those previous works agree in general with the observations made here. The varieties within *Struthiopteris spicant* (*S. spicant* var. *homophyllum* and *S. spicant* var. *pradae*) lacked any spore description until now. Only the size was reported in a recent study of the complex *S. spicant* (Molino et al. 2019a). Our results show the absence of relevant differences in spore traits between all these taxa, which indeed contribute to the idea that the rank of variety is appropriate for the plants within this complex (Molino et al. 2019a). We also report that the spore of *S. fallax* shows important differences in size and ornamentation from the spores of *S. spicant*. This supports the idea that this plant, which was considered as a variety of *S. spicant* until recently (Wasowicz et al. 2017), deserves a different status at the species level (Molino et al. 2019a). *Struthiopteris castanea* spores were briefly described by Mitui (1979), and his description agrees with what we have observed here in more detail. The spores of this species are the biggest in *Struthiopteris*, resembling the spore size of *Spicantopsis*, but again this could reflect the high ploidy level of this taxon.

Finally, it is remarkable the frequency in which we observed the perispore of the spores of all the taxa coming off so easily, fact already mentioned in the study by Mitui (1979). Perhaps this could be the reason why some other authors have erroneously reported that some Blechnaceae do not have perispore (Ching 1940; Tagawa 1959).

In conclusion, from a taxonomic perspective, we show here that *Struthiopteris* and *Spicantopsis* differ in sporangia and spores, particularly in the number and thickness of the cells in the lips, sporangial size, and structure and ornamentation of perispore. These characters are easy to study and could be very useful in the separation of both genera. Furthermore, they add to some other meaningful morphological characters already discovered by Molino et al. (2019b) –epidermal hairs, structure of indusia, etc.–, emphasizing the monophyly of both genera, *Struthiopteris* and *Spicantopsis*. Other characters of the sporangia and spores show differences that could be useful to identify species, subspecies, and varieties. Finally, traits such as the perispore ornamentation, thickness and internal structure, are shared between *Struthiopteris* and *Blechnidium* T. Moore (Moran et al. 2018), pointing at a closer phylogenetic relationship of those than between *Struthiopteris* and *Spicantopsis*, fact that coincides with what is stated in Molino et al. (2019b). The rest of characters studied here remain unknown for *Blechnidium*, whose phylogenetic position sister to *Struthiopteris* / *Spicantopsis* is striking and deserves further investigation.

To sum up the main characters that distinguish the taxa studied here, we propose the following identification key regarding sporangium and spore traits. This key is especially interesting for separating pairs of species in two cases, *Spicantopsis niponica* - *S. hancockii* and *Struthiopteris castanea* - *S. spicant*, which are difficult to distinguish by macromorphological characters.

The varieties of *S. spicant* could not be included in this key since we did not find any differential traits regarding the sporangia or the spores.

Identification Key

- Sporangia lips formed by 4–7 cells which are <12.5 μm thick; spores with a compact perispore which is <5 μm thick……...…….*Spicantopsis*
- Sporangia lips formed by 2–3 cells which are >12.5 μm thick; spores with an alveolate perispore which is >5 μm thick.……*Struthiopteris*

Spicantopsis

- Spores with verrucate-granulate perispore……*S. amabilis*
- Spores with irregularly reticulate perispore……2
- With posterior basal cells in the sporangia; 19–20 arcus cells; spores around 59 × 43 μm……*S. niponica*
- Without posterior basal cells in the sporangia; 20–29 arcus cells; spores around 50 × 35 μm……*S. hancockii*

Struthiopteris

- Sporangium capsule >300 μm; spores >50 μm in length……*S. castanea*
- Sporangium capsule <300 μm; spores <50 μm in length……...2

- Spores around 44 × 32 μm; perispore rugate, sharply angular; laesura almost as long as the spore…….*S. fallax*
- Spores around 38-41 × 26–29 μm (this variation includes all the varieties); perispore slightly rugate; laesura not as long as the spore…….*S. spicant*

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Appendix 1

List of material used, with basic voucher information, ordered by taxon:

Spicantopsis amabilis (Makino) Nakai. JAPAN: *Faurié 5612* 07/1904, (P01406648); Honshū: Echigo, Tsugawa, *Togashi 1576* 16/09/1957, (P01406637); Mie, Utobi, *Seto 6672* 23/11/1956, (P01608624); Nara, Shôfuken-dake, *Tawaga & Iwatsuki 5129* 4/08/1962, (P01575933); Tochigi, *Tagawa & Iwatsuki 1913* 13/09/1958, (P01608623).

Spicantopsis hancockii (Hance) Masam. TAIWAN: Chiayi: Alishan, Arisan, *Iwasaki s.n.* 22/07/1970, (TNS832514); Taipei: Beitou, *Knapp 3338* 18/01/2014, (P02439073); Taitung: Taiwu, *Knapp 3430* 2/06/2014, (P02439164); Yilan: Nan'ao Township, Taipingshan, *Knapp 4311* 3/09/2016, (P02435597); *ibidimen, Knapp & Huang 239* 5/12/2005, (P02436496).

Spicantopsis niponica (Kunze) Nakai. JAPAN: *without collector, locality and date*, (TNS01147387); Honshū: Hyôgo, Myôkô-san, Hikami-gun, *Tagawa & Iwatsuki 3014* 23/09/1960, (P01575937); Kanawaga, Kantō, Yokohama, *Maximowicz s.n.* 1862, (P01406592); Wakayama, Tanabe-shi, Nakahechi-cho, Mizukami, *B. Estébanez s.n.*, 21/08/2017 (MACB110657).

Struthiopteris castanea (Makino & Nemoto) Nakai. JAPAN: Honshū: Fukui, Imadate-gun, Ikeda-cho, Kanmuri-yama, *Saito s.n.* no date (TNS792168); Hyogo, Mikana-gun, Onsen-cho, Ueyama-kkogen, *Tobayashi s.n.* 25/07/1999, (TNS700642); Shinano, Shumominochi-gun, Minochi-mura, *Mizushima 13,853* 28/06/1956, (S1982).

Struthiopteris fallax (Lange) S. Molino, Gabriel y Galán & Wasowicz. ICELAND: Deildartunguhver, *Wasowicz & Gabriel y Galán s.n.*, jul 2016, (MACB109359) (3 individuals).

Struthiopteris spicant (L.) Weiss var. ***spicant***. SPAIN: Canary Islands:Anaga, Tenerife, *Gabriel y Galán s.n.* 14/09/2017, (MACB110658); Cantabria: Camaleño, Cosgaya, *Gabriel y Galán s.n.* 8/10/2016, (MACB109622); Galicia: Monte Aloya, Tuy, *Pajarón & Pangua s.n.* 15/07/1993, (MACB59142).

Struthiopteris spicant var. ***homophyllum*** (Merino) Gabriel y Galán & R. Pino. PORTUGAL: Braga, Vieira do Minho, *Prada s.n* 1/10/2004, (MACB109621). SPAIN: Galicia: between Tabagón y Tomiño, *Gabriel y Galán s.n.* 19/03/2016, (MACB109617); Santiago, Cantaleta, *Barrera s.n.* 29/07/1967, (MACB32367); Santiago, *no collector* 29/07/2007, (MA824045). Salamanca: Batuecas, *Gabriel y Galán s.n.* 15/05/2016, (MACB109626).

Struthiopteris spicant var. ***pradae*** S.Molino & Gabriel y Galán. SPAIN: Asturias: Valdés, Paladeperre, *Gabriel y Galán s.n.* 22/03/2016, (MACB109613); ibidem, 3/08/2016,

(MACB109615); Burgos: Sierra de San Millán, *Fuentes s.n.* 27/09/1975. (MACB5994), Zamora: Aciberos, *Molino, Seral, Gabriel y Galán & de la Fuente s.n.* 23/09/2017, (MACB110654).

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