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The circumscription of problematic species of *Ophioglossum* (Ophioglossaceae) from Southern South America: a palynological approach

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Abstract

The genus *Ophioglossum* has around 25 species with a nearly cosmopolitan distribution. It has historically posed a number of taxonomic problems due to the lack of morphological characters upon which to base species delimitations. In order to address this, we analyzed the sculpture patterns of spores from ten critical taxa from southern South America. Palynological data was compared with the morphology of the sporophyte. The sculpturing pattern was the most valuable and consistent taxonomic character upon which to base species delimitation in *Ophioglossum*. Nomenclatural types of studied species were examined. The name *Ophioglossum melipillense* is reinstated and the other species recognized to the genus *Ophioglossum s.s*. in this region are the following: *O. crotalophoroides, O. nudicaule, O. opacum,* and *O. vulgatum*. *Ophioglossum fernandezianum* appears to be a doubtful species. *Ophioglossum lusitanicum* is excluded from the study area, but the lectotype of *O. lusitanicum* is designated, and a revised synonymy is presented.

Resumen

El género *Ophioglossum* cuenta con alrededor de 25 especies y presenta distribución casi cosmopolita. Históricamente se han planteado numerosos problemas taxonómicos debido a la falta de caracteres morfológicos sobre los cuales basar la delimitación de las especies. Para hacer frente a esto, se analizaron los patrones de escultura de esporas de diez taxones críticos en el género *Ophioglossum* del Cono Sur de América. Los datos palinológicos se compararon con la morfología de la esporofito. El patrón de la escultura fue un carácter taxonómico muy valioso y consistente sobre la cual basar la delimitación de las especies. Se examinaron los tipos nomenclaturales de las especies estudiadas. Se restablece el nombre *Ophioglossum melipillense* y para el área de estudio se reconocen las siguientes especies para género *Ophioglossum s.s.*: *O. crotalophoroides, O. nudicaule, O. opacum, O. vulgatum* y el nombre *O. lusitanicum* es excluido. *Ophioglossum fernandezianum* es considerada una especie dudosa. Se examinan todos los tipos nomenclaturales de las especies estudiadas, se designa el lectotipo de *Ophioglossum lusitanicum* y se presentan nuevas sinonimias.

Keywords: Linnaean species; ophioglossoid species; palynology, southern South America; spores; typification

Introduction

The genus *Ophioglossum* was described by Linnaeus (1754: 484), and probably has 20–25 species worldwide (Mickel & Smith 2004). It occurs mostly in disturbed, open and grassy habitats and is often overlooked because of superficial resemblance to seedlings of monocotyledonous plants (Wagner & Wagner 1993). This genus has historically posed a number of problems for taxonomists because of the lack of morphological characters upon which to base species delimitations. Its simple structure and presumed variation within a species has resulted in many distinct taxa being lumped in large 'traditional' species complexes due mainly to an insufficient characters with which to set them apart (Burrows, 1997). Burrows (1997) found that the spore morphology provided the most consistent taxonomic character upon which to delimit most species. In an effort to find other diagnostic characters, several taxonomists working in this family focused on the ornamentation of the spore walls to complement their systematic studies (e.g.: Burrows 1997, Burrows & Edwards 1993, 1995; Uehara & Kurita 1989).

A world-wide revision of *Ophioglossaceae* was conducted by Clausen (1938), and the species from southern

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South America were reviewed by Lichtenstein (1944). Later, Clausen (1949) made some changes to the infraspecific systematics of *Ophioglossum crotalophoroides* Walter (1788: 256).

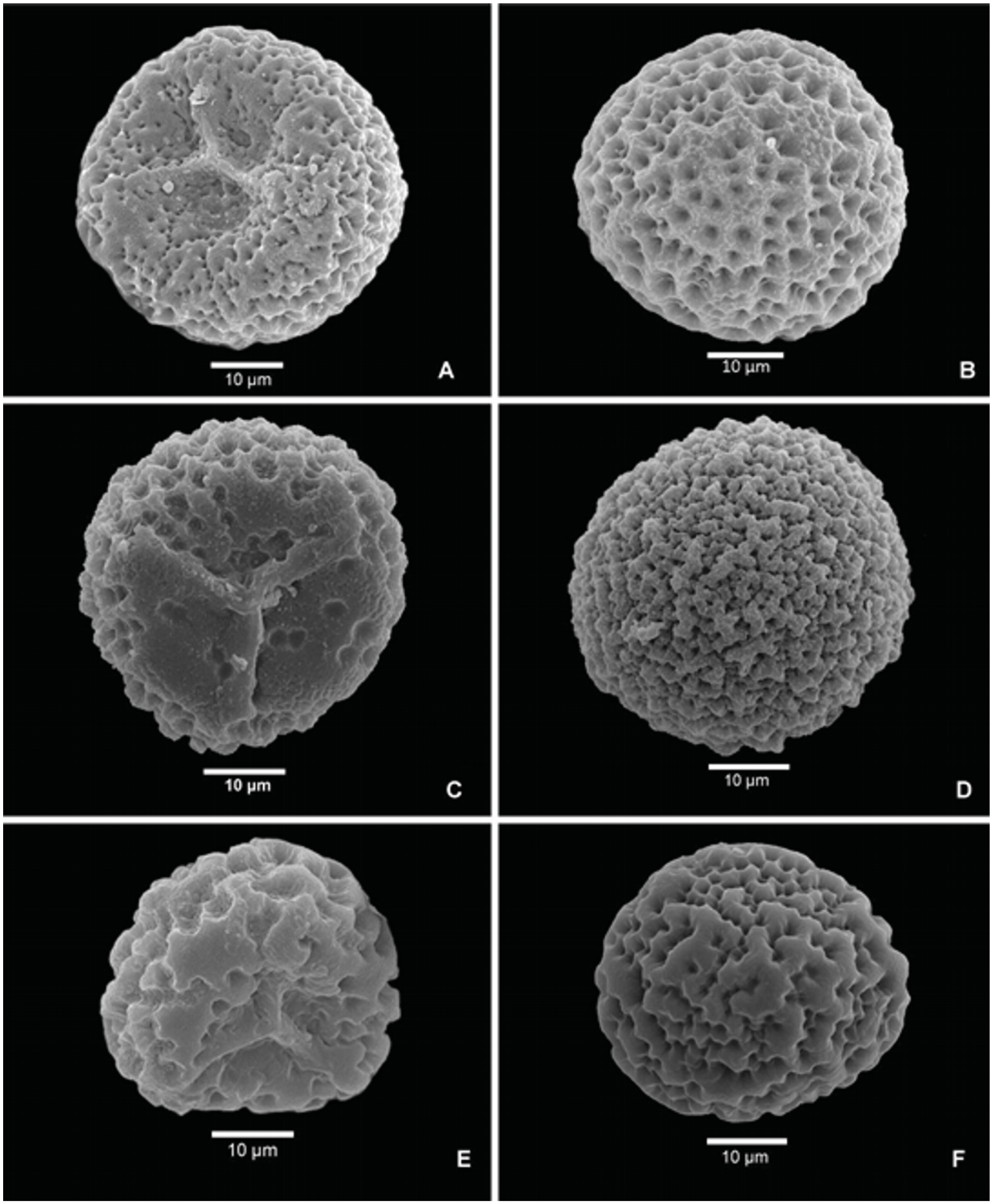

FIGURE 1. A, B: *Ophioglossum crotalophoroides* spore type. A, proximal face; B, distal face, [*Osten & Rojas 8707* (MVM)]. C, D: *Ophioglossum vulgatum* spore type from American specimens; C, proximal face; D: distal face [(v*on Rentzell 6215* (SI)]; E: *Ophioglossum vulgatum* type from European specimens [*Pedersen 7420* (CTES)]. F. *Ophioglossum coriaceum*, from New Zeland, distal face [*Large & Large 169* (CTES)]

In the Southern Cone (Argentina, Southern Brazil, Chile, Paraguay, and Uruguay), as defined by Zuloaga *et al.* (2008), several taxa were described including *Ophioglossum tuberosum* Hooker & Arnott (1832: 53), and *O. stipatum*

Colla (1836: 238), but both species were included in the synonymy of *O. crotalophoroides* by Clausen (1938). On the other hand, for the Chilean flora *O. melipillense* Gay (1853: 542) was described, a name that was placed in synonymy of *O. nudicaule* Linnaeus f. (1782: 443) var. *tenerum* (Mettenius in Prantl 1883: 352) Clausen (1938: 146), and later was transferred by Lichtenstein (1944) as a doubtful species under the name *O. lusitanicum* Linnaeus (1753: 1063) subsp. *coriaceum* (Cunningham 1837: 361) Clausen (1938). The name *O. melipillense* was not widely used, and this name was soon forgotten. Other names cited for this geographical area are the following taxa: *Ophioglossum crotalophoroides s.l.* [*O. crotalophoroides* var. *crotalophoroides,* from northern Argentina, Bolivia, Paraguay and Uruguay; *O. crotalophoroides* var. *nanum* Osten (in Lichtenstein 1944: 397–398), from central Argentina and Paraguay; and *O. crotalophoroides* subsp. *robustum* Clausen (1949: 183), from Southern Argentina and Chilean Patagonia]; *O. ellipticum* Hooker & Greville (1831: 23); *O. fernandezianum* Christensen (in Christensen & Skottsberg 1920: 44); *O. nudicaule* var. *nudicaule, O. nudicaule* var. *robustum* Lichtenstein (1944: 413–414), *O. opacum* Carmichael (1819: 509), *O. reticulatum* Linnaeus (1753: 1063), and *O. vulgatum* Linnaeus (1753: 1062), (see: de la Sota & Ponce 2008, Meza Torres *et al*. 2013a, 2013b). Another taxon cited for southern Chilean and Argentinean Patagonia is *Ophioglossum vulgatum* var. *valdivianum* (Philippi 1865: 306) Lichtenstein (1944: 405). Palynological studies on some of these taxa were reported by Heusser (1971), Morbelli (1978, 1980, 1983), Murillo & Bless (1974), Tryon & Lugardon (1991) and Stafford & Paul (2008).

The aims of this study is to evaluate the diagnostic value of the sculpture of the spores and supplement this data with morphological characters of the sporophyte in the delimitation of conflicting species of *Ophioglossum.* In addition we intend to publish nomenclatural adjustments resulting from the revision of this genus for the species from southern South America.

Materials and methods

Taxonomic analysis

All nomenclatural types of the studied species were examined. These are detailed in the taxonomic treatment.

Palynological analysis

Qualitative characters of the sporoderm morphology were studied by using light microscopy (LM), and scanning electron microscopy (SEM). To preserve the morphology of the spores studied, sporangia were covered with an envelope in the field during the collection, and the spores were obtained thereby, only in the comparison materials the spores were obtained from herbarium specimens.

The spores were studied without chemical treatment, since the acetolyses frequently dissolves fine ornamentation and is not recommended as a standard technique for this family (Burrows 1997). The terms proposed by Tryon & Lugardon (1991) and Punt *et al.* (2007) were used for describing the spore surface and structure.

We analyzed the following specimens:

Ophioglossum coriaceum: COOK ISLANDS (New Zealand): Rarotonga, Papua Stream, 22 July 2010*, Large & Large 169* (CTES).

Ophioglossum crotalophoroides: ARGENTINA. Tucumán: Dpto. Tafí del Valle, La Quebradita, base del Cerro Pabellón, 18 February 2007, *Meza Torres 514* (CTES).

Ophioglossum crotalophoroides var. ***nanum***: ARGENTINA. Córdoba: Dpto. Punilla, Pampa de Achala, altas cumbres, 2174 m, 31°36’40.21”S 64°48’18.47”W, 6 February 2008, *Meza Torres et al. 680* (CTES). PARAGUAY. Dpto. Cordillera: San Bernardino, September 1916*, Osten & Rojas 8707* (isotype MVM).

Ophioglossum crotalophoroides subsp. ***robustum***: ARGENTINA. Tierra del Fuego: Dpto. Ushuaia, Ea. Harberton, campo Loma Larga Norte, c. 300 m, 54° 50’S, 67° 16,5’W, 22 June 1968, *Moore 1607* (BAB).

Ophioglossum lusitanicum: SPAIN. Canary Islands: El Hierro, Municipio Valverde, San Andrés, 10 February 1990, *Pedersen 15499* (CTES).

Ophioglossum lusitanicum subsp. ***coriaceum***: ARGENTINA. San Luis: Dpto. San Martín, a 12 km del empalme de RP 41 y RP 38, 1323 m, 32°42'14.08"S, 65°49'36.26"W, RP 41, 10 April 2008, *Meza Torres et al. 777* (CTES); Catamarca: Dpto. Ambato, RP 1, a 4,3 km aprox. de la RP 48, camino a Singuil, cerros, 27°38'51.47"S, 65°56'55.00"W, 12 April 2008, *Meza Torres et al. 790* (CTES).

Ophioglossum opacum: SOUTH GEORGIA (U.K.). Bom Valley, 40 m, 15 March 1960, *Bomer 166* (K); 23 March 1961, *Greene 3550* (K).

Ophioglossum vulgatum: GERMANY. In pratis paludosis inter rivum, July 1911, *Harz 5595* (LIL). DENMARK. Fredriksborg Amt, Lynge-Kromborg Hened, Karlebo sogn: Niva, ad litus, *Pedersen 7420* (CTES).

Ophioglossum vulgatum var. ***valdivianum***: ARGENTINA. Río Negro: Dpto. Bariloche, San Carlos de Bariloche, área municipal intangible Laguna Fantasma, 783 m, 41° 5'33.29"S, 71°27'1.22"W, 20 January 2008, *Meza Torres et al. 699* (CTES).

Ophioglossum nudicaule var. ***robustum***: CHILE, XI Region: Territorio Aisen, Región del Lago Buenos Aires, Valle Leones, fin del valle Leones, 11 February 1939, *von Rentzell 6215* (holotype SI).

Results

The level of resolution as observed through the LM cannot contribute with significant palynological characters for interspecific delimitation. For this reason, the specimens analyzed were grouped in five spore types, considering similar patterns sculpture observed in the SEM only.

1. *Ophioglossum crotalophoroides* spore type (Fig. 1 A, B): The spores of the specimens known under the names of *Ophioglossum crotalophoroides* and *O. crotalophoroides* var. *nanum* were characterized by conspicuously depressed areas in the proximal face outside the laesurae and sculpture formed by broad ridges in the proximal face (Fig. 1 A, B).

2. *Ophioglossum vulgatum* spore type (Fig. 1 C–E): The group of specimens mentioned as *Ophioglossum coriaceum*, *O. nudicaule var. robustum, O. vulgatum*, and *O vulgatum* var. *valdivianum* have spores with a sculpture formed by thick ridges in the equatorial region and are coarsely reticulate in the distal face, with variable thickness of muri (predominantly thick) in this region (Fig. 1 D). All spores examined of *O. coriaceum* (Fig. 1 F) have presented a surface that is collapsed in the proximal face, so its ornamentation in this face could not be observed in detail. The other specimens of this group showed broad ridges on the proximal face.

3. *Ophioglossum opacum* spore type: The spores of *Ophioglossum crotalophoroides* var. *robustum* and *O. opacum* were characterized by sculpture formed by thin ridges in the proximal face outside the thin laesurae and the distal face was foveolate-reticulate (Fig. 2 A, B).

4. *Ophioglossum lusitanicum* spore type: The spores of *Ophioglossum lusitanicum* showed straight and moderately broad laesural arms in the proximal face, that become slightly raised and extending approximately to the equator. In the distal face, sculpture was finely rugulate to finely reticulate (Fig. 2 C, D).

5. *Ophioglossum melipillense* spore type: The spores of the specimen mentioned above as *Ophioglossum lusitanicum* subsp. *coriaceum* was included here, and showed in equatorial and proximal views a coarsely microrugulate-microreticulate sculpture (Fig. 2 E, F).

Discussion

Similar to Burrows (1997), we found that the spore sculpture is the single most taxonomically significant morphological feature in the delimitation of species of *Ophioglossum*. The dimensions of the spores are not valid characters for differentiating species, as already expressed by Morbelli (1980).

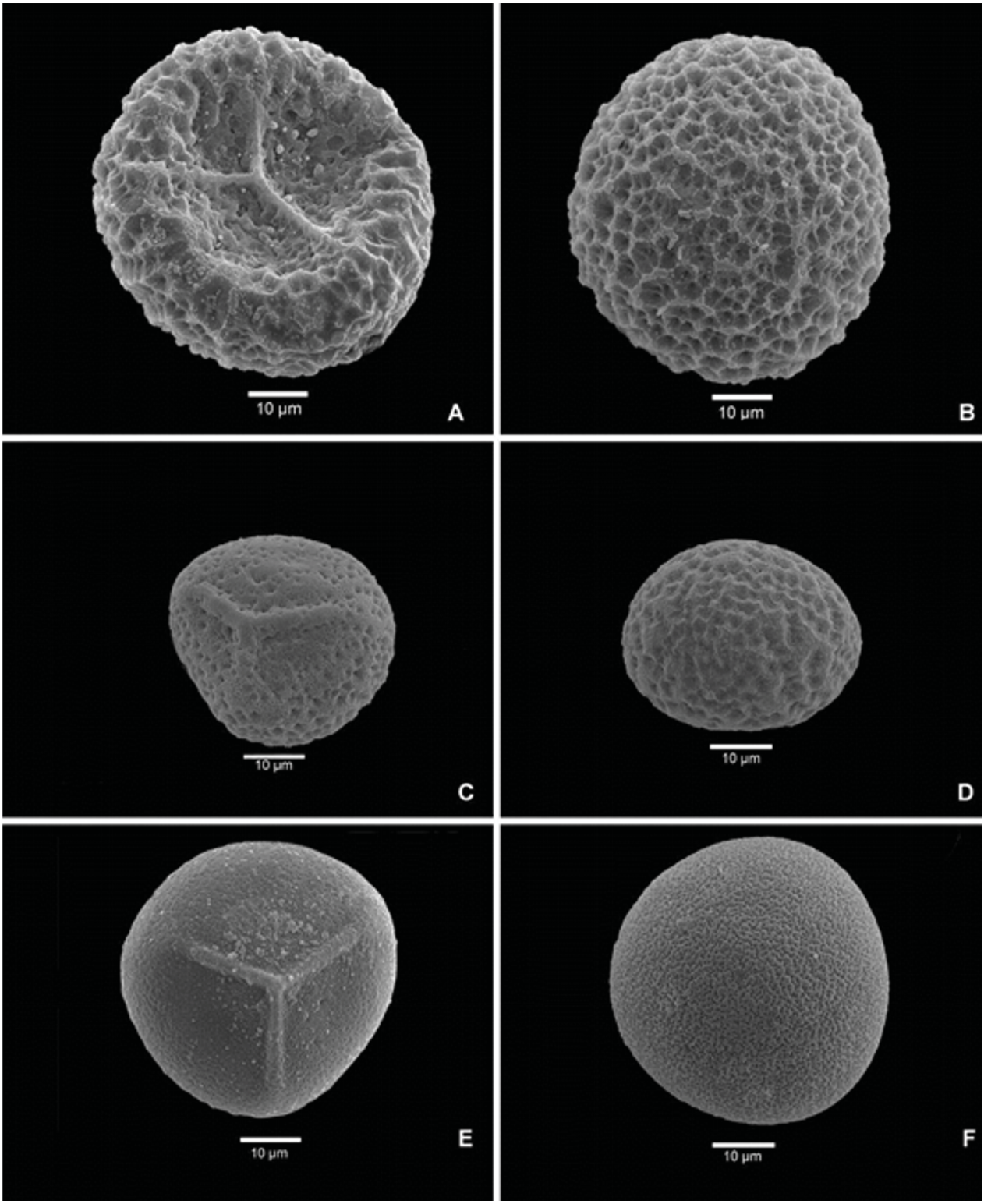

FIGURE 2. A, B: *Ophioglossum robustum* type. A, proximal face; B, distal face. A, B: *Bomer 166* (K). C, D: *Ophioglossum lusitanicum* type. C, proximal face; D, distal face [*Pedersen 15499* (CTES)]. E, F: *Ophioglossum melipillense* type. E, proximal face; F, distal face. [*Meza Torres et al. 777* (CTES)]

The spore surface details observed here in specimens of *Ophioglossum crotalophoroides* are in agreement with those reported by Tryon & Lugardon (1991) for the same species. Morbelli (1980, 1983) described the spores of *O. crotalophoroides s.l.*, and reported differences between specimens from northern Argentina and the specimens from Chile and southern Argentina. The characters descripted by Morbelli (1983) on specimen from northern Argentina, correspond here to the *O. crotalophoroides* spore type, whereas the characters of specimens from Chile and southern

Argentina descripted by Heusser (1971) and Morbelli (1980, 1983), correspond to the *O. opacum* spore type from the southern Atlantic Islands. Our observations of the spore ornamentation patterns of *O. crotalophoroides* var. *nanum* and *O. crotalophoroides* subsp. *robustum* are in agreement with the conclusions of Clausen (1949) on observations of the sporophytes. Clausen (1949) recognized only two taxa in the *Ophioglossum crotalophoroides s.l.* group, considering *O. crotalophoroides* var. *nanum* as a synonym of the typical variety, but described subsp. *robustum* from the Falkland Islands as a new taxon. The latter corresponds to the *O. opacum* spore type, whereas var. *nanum* matches the *O. crotalophoroides* spore type.

The characters of the *O. vulgatum* spore type are in agreement with the description given for *O. vulgatum* by Tryon and Lugardon (1991), and Stafford and Paul (2008). The characters of the spore surface observed in *Ophioglossum lusitanicum* type are in agreement with the description of Stafford & Paul (2008).

The coarsely microrugulate-microreticulate sculpture exhibited by the *O. melipillense* spore type, revealed unique characters that cannot be compared with published palynological descriptions of this genus.

Taxonomy

Taking into account the palynological observations and supported by the study of type specimens, we propose the following list of species and synonymy for the Southern Cone with their respective synonyms and typification.

Ophioglossum Linnaeus (1754: 484). Lectotype: *Ophioglossum vulgatum* Linnaeus (1754: 484), designated by Smith (1875: 367).

Ophioglossum crotalophoroides Walter (1788: 256) ≡ *Ophioglossum vulgatum* L. *var. crotalophoroides* (Walter) Eaton (1860: 599).

Holotype:—U.S.A: *Walter s.n*. (BM [presumably lost]). Neotype designate by Ward (2008): USA: South Carolina. Hampton County, along Simons Road, 1/2 mile SW of SW of SC 68, just NW of Yemassee, 12 March 1991, *Douglass s.n.* (USCH; Foto CTES!)

Heterotypic synonyms:

Ophioglossum bulbosum Michaux (1803: 276). Holotype:—U.S.A., North Carolina. '*in sabularis Caroline'. Michaux s.n*. (P-MICH!), isotypes: E!, G!, P barcode 01653492!

Ophioglossum tuberosum Hooker & Arnott (1832: 53). Holotype:—CHILE: XII Región, Prov. Concepción, *'Hab. Concepcion', Collie and Lay s.n.* (E!), isotype: K!

Ophioglossum stipatum Miers *ex* Colla (1836: 238). Holotype:—CHILE: V Región, Valparaíso. Prov. Valparaíso, Quillota, '*in pascuis declivibus humidis'*, August 1829–1830? *Bertero 1193* (TO!), possible isotypes: BM!, GH barcode 00021728 [photo!] NY!, P barcode 01653509!, P barcode 01653511!, P barcode 01653512!

Ophioglossum crotalophoroides Walter var. *nanum* Osten in Lichtenstein (1944: 397), ***syn. nov.*** Holotype:—PARAGUAY: San Bernardino, *'in ripa, locis humidis muscosis, inter caespitis Paspali et Eryngium'*, September 1916, *Osten & Rojas 8707* (SI!), isotypes: BM!, MVM!, S!.

Notes:—The holotype (TO) of *O. stipatum* has in handwriting on its label the year of 1930, and the number of *Bertero 1193*. The possible isotypes of BM, GH and P have printed labels with the same year, but the number of *Bertero 1195*. One isotype in P (barcode 01653511) has two labels, one handwriten with the number 1193, another printed with number 1195. Probably an error was made when labels for the duplicates were printed and specimens were distributed, although it is of course possible that not all specimens are of the same gathering.

Ophioglossum lusitanicum Linnaeus (1753: 1063). Lectotype ***(designated here by Meza Torres):*** Barrelier (1714: t. 252, f. 2.). Epitype ***(designated here by Meza Torres):*** PORTUGAL?. *"Ophioglossum pumilum autumnale" A.L. de Jussieu 1574A* (P-JU!).

Notes:—The protologue gives two elements. The first is a quote by Grisley (*Viridid. Grisley lusit*.) "*Ophioglossum pumilum autumnale. Grisl. lusit*", which is not associated with any illustrations or specimens. The other element cites Barrelier (1714: 117, t. 252, f. 2.), and this element has a plate that includes three plants (Fig. 3). *Jussieu 1574A* from

the historical Jussieu herbarium in Paris consists of three complete plants (only one fertile) plus a rhizome without leaves. This specimen also includes a handwritten label stating "*Ophioglossum pumilum autumnale*...", similar to Grisley's quote, and a glued copy of Barrelier's figure. Possibly these plants were the specimens used for Barrelier's plate. For this reason, the specimen of *Jussieu 1574A* is chosen as epitype to assist in the interpretation of Barrelier's plate, which could allow us to study the spores of typical material of this species.

FIGURE 3. Original plate of Barrelier (1714). The central plant in the plate (II) corresponds to *Ophioglossum lusitanicum.*

Ophioglossum melipillense Gay (1853: 542). Type: CHILE: se halla en los prados montanos de Melipilla, *Gay s.n.* (not found). Neotype (**designated here by Meza Torres**): Chile: Valparaíso. V Region, Marga Marga Province, Limache, La Cruz Mountain, 150 m a.s.l., estepas de hierbas, 1XI.1935, *Looser 2565* (BM barcode 001078153!), duplicate specimens: BM barcode 001078152!, BM barcode 001078154!, GH?, SGO?)

Notes:—The type specimen of Gay could not be located by the respective curators in any of the relevant herbaria (CONC, GH, NY, PC, SGO), nor in herbaria reviewed by the first author (K, M, P). As the name *O. melipillense* was validly published and the protologue description agrees with specimens examined, here is a necessity to apply this name appropriately, which hereby results in selection of a neotype. The specimen of Looser is selected here, because in addition to the very well-preserved specimen, there is a letter from Weatherby to Christensen, in which it is commented that this specimen was collected in the general area from where the type of *O. melipillense* originated.

The specimens identified here as *O. melipillense* were often placed under the name *O. lusitanicum* subsp. *coriaceum* in the past. On the basis of comparisons of spores of *O. lusitanicum* from European specimens and other specimens from Pacific localities identified as *O. coriaceum* (a species described from New Zealand), it should be noted that the three taxa have very different spore wall sculptures and should be treated as separate species. This demonstrates that the South American taxon that was known under the name *O. lusitanicum* subsp. *coriaceum*, is different from either typical *O. lusitanicum* or *O. coriaceum*. For this reason we can be certain that *O lusitanicum* does not occur in the studied area.

Ophioglossum californicum Prantl (1883: 351), described from western North America, is very similar in its morphology to *O. melipillense*, and both names may be applicable to the same species, with *O. melipillense* having nomenclatural priority. However, until palynological evidence for this statement is found, and in light with the above finding, and because of the biogeographical disjunction, we take a cautious approach and suggest that both species should be accepted for the time being.

FIGURE 4. *Ophioglossum melipillense*. A, B. Environments where this species was collected. A. Grazing grassland, 270 m, Dep. Andalgalá, Catamarca, Argentina. B. Montane grassland, 1323 m, Dep. San Martín, San Luis, Argentina. C. Aspect of the population from Dep. Andalgalá. D. General view of the plant. A, C: *Meza Torres et al. 790* (CTES). B, D: *Meza Torres et al. 777* (CTES).

Ophioglossum melipillense occurs in montane grasslands of Argentina and Chile (fig. 4 A, B), where it grows in dense colonies that can occupy several hectares (Fig. 4 C, D). In Argentina it was recorded for the provinces of San Luis, Catamarca, Jujuy and Salta. In Chile is present from the V to the VII Region. Owing to the unavailability of images of this species, an illustration is provided here (Fig. 5 A–D).

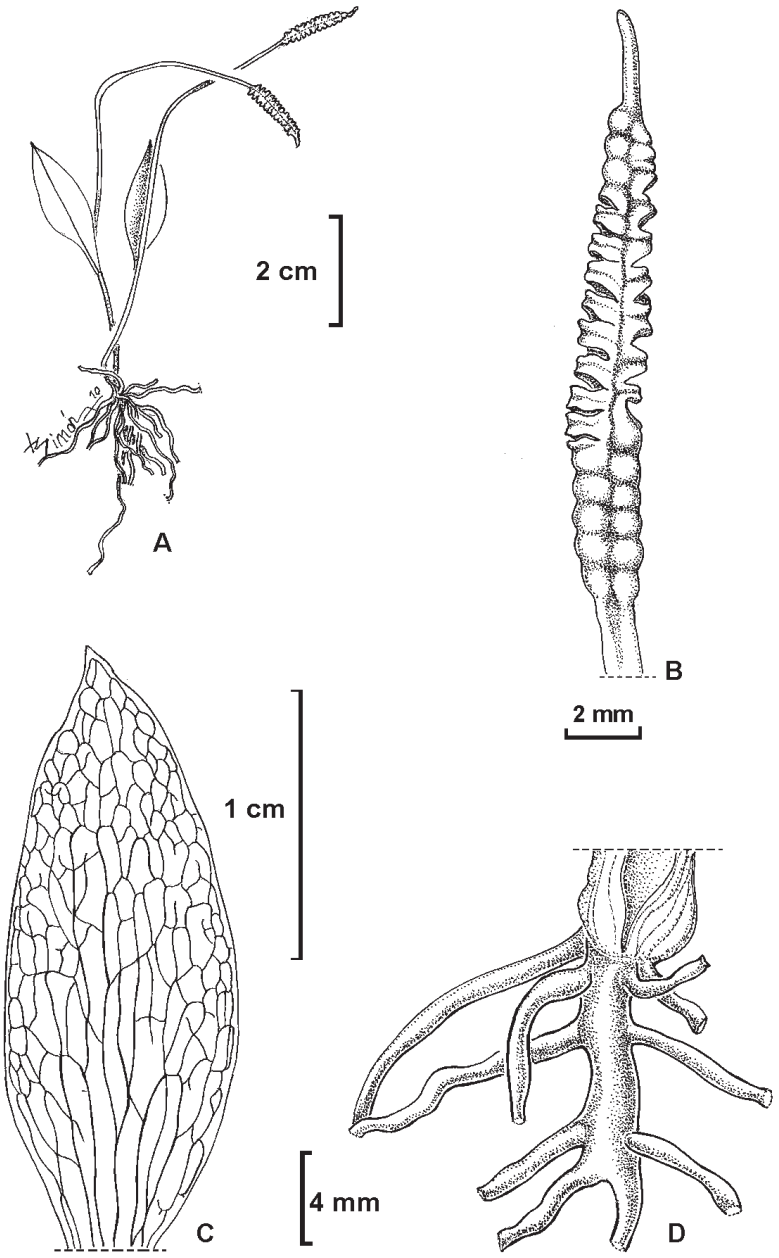

FIGURE 5. *Ophioglossum melipillense.* A. General view of the plant. B: Sporophore showing the distal peduncle with sporangia. C: Detail of the venation. D: Rhizome. A–D: *Meza Torres et al. 790* (CTES).

Ophioglossum opacum Carmichael (1819: 509). ≡ *Ophioglossum crotalophoroides* Walter var. *opacum* (Carmich.) J. S. Licht. (1944: 396–397).

Holotype: TRISTAN DA CUNHA (U.K.): Grows high up on the dome, *Carmichael s.n.* (holotype BM no. 001038205!)

Heterotypic synonym: *Ophioglossum crotalophoroides* Walter subsp. *robustum* Clausen (1949: 183). Holotype: FALKLAND ISLANDS (U.K.). West Falkland Island: 1853, *Snyder s.n.* (CU, incorporated in BH; photo published by Clausen 1949) ***syn. nov.***

Notes:—The type specimen of *O. opacum* is a young plant with a short sporophore and is very similar to *O. crotalophoroides*, but it differs in the subcoriaceous and opaque leaf, a character that refers to the specific epithet. However, other specimens from Tristan da Cunha deposited in BM herbarium show the more robust habit of this taxon. Apart from the Mid-Atlantic Island of Tristan da Cunha, this species is also found in the Andean region of southern Argentina and Chile, and on the South Atlantic Islands.

Ophioglossum vulgatum Linnaeus (1753: 1062). Holotype: '*Habitat in Europae pratis sylvaticis' anon.* (LINN 1243-1!).

Heterotypic synonyms:

Ophioglossum valdivianum Philippi (1865: 306) ≡ *Ophioglossum vulgatum* L. var. *valdivianum* (Phil.) Lichtenstein (1944: 405). Holoype*:* CHILE: X Region, Los Lagos. *'Prope Tramalhue Januaris, unicum specimen invenit', F. Philippi* (SGO; Photo CTES!).

Ophioglossum nudicaule L.f. var. *robustum* Lichtenstein (1944: 413). Holotype: CHILE: XI Región, Territorio Aisen, Región del Lago Buenos Aires, Valle Leones, fin del valle Leones, a 550 m, plantita que crece en lugares pantanosos, 11 February 1939, *von Rentzell 6215* (SI!), isotypes NY!, SGO!, ***syn. nov.***

Notes:—*Ophioglossum nudicaule* var. *robustum* was described by Lichtenstein (1944) based on a single specimen collected by *von Rentzel*. It was believed that this taxon was endemic to the area of "Lago Buenos Aires", but the specimen shows no significant qualitative differences with respect to specimens of *Ophioglossum vulgatum*, characterized only by is smaller size, probably caused by the poor calcareous soil type in the area from where it was collected. We also studied spores of the type specimen, which compared with specimens of *O. vulgatum* from Argentinean Patagonia and Europe. It was found that all specimens showed identical characteristics in spore sculpture and this pattern was consistent with the description provided by Tryon & Lugardon (1991) and Stafford & Paul (2008). These similarities in the spore surface and in the morphology of the sporophyte, justify recognition of a single taxon.

This species is broadly distributed across the temperate northern hemisphere. Mickel & Smith (2004) list this species as occurring in the United States, Mexico, Europe and Asia. Although, in the area here studied this species occurs in the Subantarctic province, more precisely in the Valdivianum District, so it would be a case of disjunct distribution. In Argentina this species is reported from western provinces of Chubut, Neuquén and Río Negro, whereas in Chile is limited to the X Region of Los Lagos. In both countries it is present in the strip bounded approximately between 39°–43° latitude, but specimens are also known from the mountains in East Africa, Madagascar and eastern Australia (www.gbif.org), so the species is of broad occurrence in cooler climates on both hemispheres.

Conclusion

The spore sculpture pattern is the most valuable and consistent morphological character upon which to base taxonomic species delimitation in *Ophioglossum* and the best results were obtained by studying spores using SEM to evaluate types of spore sculpture. The different faces can take add valuable characters placing the specimen among the group of species that are analyzed, for example the conspicuously depressed areas in the proximal face of the spores in *O. crotalophoroides* and the thick ridges in equatorial area of *O. vulgatum.*

In southern South America there are only five species of *Ophioglossum s.s* (*O. crotalophoroides*, *O. melipillense, O. nudicaule, O. reticulatum*, and *O. opacum*), whereas *O. lusitanicum* is based on erroneous identifications in the past and does not occur there. *Ophioglossum fernandezianum* from the Juan Fernandez Islands is only known from the type specimen and is doubtfully distinct from *O. melipillense* with which it shares similarities of the sporophyte. Similarly *O. californicum* from North America is similar in sporophytic characters, but the spores of neither species have been investigated here and hence the status of these taxa is yet uncertain.

Comparisons between the spore morphology from southern South American specimens compared with material from Europe served to support a revised taxonomy and new synonymy for this area. Future studies of spores from

closely related taxa Africa, Asia, Australasia, North and South America would be required to revise species delimitation in the genus *Ophioglossum.*

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