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Ferns and Lycophytes as new challenges

Morphogenesis of the gametophyte of *Physematium montevidensis* (Woodsiaceae)

María Luz Martinenco[1,3], *Marcelo Daniel Arana*[2] & *María Luján Luna*[1,4.5]

Abstract
In vitro spore culture and gametophyte characteristics may contribute understanding the life cycle in different fern taxa and also to systematic and taxonomic studies. The family Woodsiaceae contains two genera: *Physematium* and *Woodsia*. *Physematium montevidensis* is the only species recognized for South America, and up to now, no information has been recorded on the gametophytic phase. The aim of this work is to study the development pattern, the morphology and sexual expression of the gametophyte of *Physematium montevidensis* to provide information about its reproductive biology. The germination pattern was of the *Vittaria*-type and the gametophyte development of the *Aspidium*-type. Gametangia appeared 30–40 days after spore germination. Both, cordiform bisexual and spatulate-elongated male gametophytes developed in the cultures. The sporophytes emerged near 3 months after spore sowing. The gametophyte development of *Physematium montevidensis* and its morphological features are broadly similar to those registered in other taxa of Woodsiaceae.
Key words: ferns, *in vitro* spore culture, *Physematium*, prothallus.

Resumo
O cultivo de esporos *in vitro* e as características dos gametófitos podem contribuir para a compreensão do ciclo de vida em diferentes táxons de samambaias e também para estudos sistemáticos e taxonômicos. Woodsiaceae contém dois gêneros, *Physematium* e *Woodsia*. *Physematium montevidensis* é a única espécie registrada para a América do Sul. Até o momento, a fase gametofítica desta espécie é desconhecida.. O objetivo deste trabalho é estudar o padrão de desenvolvimento, a morfologia e a expressão sexual do gametófito de *Physematium montevidensis* para fornecer informações sobre sua biologia reprodutiva. O padrão de germinação foi do tipo-Vittaria e o desenvolvimento do gametófito do tipo- Aspidium. Os gametângios surgiram 30–40 dias após a germinação dos esporos. Ambos, gametófito bisexual-cordiforme e masculino espatulado-alongado se desenvolveram nas culturas. Os esporófitos emergiram em aproximadamente três meses após a semeadura dos esporos. O desenvolvimento do gametófito de *Physematium montevidensis* e suas características morfológicas são amplamente semelhantes aos registrados em outros táxons de Woodsiaceae.
Palavras-chave: samambaias, cultura *in vitro* de esporos, *Physematium*, protalo.

Woodsiaceae Herter contains two genera predominantly lithophytic: *Physematium* Kaulf. with about 28 species distributed in America (mainly Central and South America), Asia and Africa including Madagascar, and *Woodsia* R. Br. with about 37 species distributed in East Asia and circumboreal regions (Lu *et al*. 2020).

The genus *Physematium* (as delimited by Lu *et al*. 2020) is characterized by the laminae 2- pinnate to 2- pinnate-pinnatifid with glandular hairs, indusia globose or with false indusia, and chromosome number x = 33, 37, 38 or 41. In South America, only one species of *Physematium* has been recognized, *P. montevidensis* (Spreng.)

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[1] Universidad Nacional de La Plata, Facultad de Ciencias Naturales y Museo, Laboratorio de Anatomía Comparada, Propagación y Conservación de Embriofitas, La Plata, Argentina.

[2] Universidad Nacional de Río Cuarto, Instituto de Ciencias de la Tierra, Biodiversidad y Ambiente (ICBIA), Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Depto. Ciencias Naturales, Grupo GIVE, Córdoba, Argentina. ORCID: <https://orcid.org/0000-0001-7921-6186>.

[3] ORCID: <https://orcid.org/0000-0001-5228-7128>. [4] ORCID: <https://orcid.org/0000-0001-7025-782X>.

[5] Author for correspondence: lujanluna@fcnym.unlp.edu.ar

Shmakov, ranging from Venezuela and Colombia south through the Andes to central and southern Argentina and southern Brazil (Mynssen & Arana 2020).

Life cycle consists of two alternating free-living and morphologically different growth forms, the sporophyte macroscopic phase (spore producing), and the gametophyte microscopic phase (gamete producing), the latter responsible for sexual reproduction and control of genetic diversity (Farrar *et al*. 2008). There are some studies on gametophyte development and ecology in different species of Woodsiaceae from diverse regions of the world (Brown 1964; Kaur & Chandra 1973; Peck 1980, 1985; Watson & Vazquez 1981; Yilun & Fuxiong 1986; Kromer *et al*. 2006; Aguraiuja 2011; Vivero-Barón *et al*. 2012), but in general terms data on their life cycle, morphology and sexuality are scarce. Particularly for *Physematium montevidensis*, there is no information about the gametophyte phase. Gametophyte features as presence/absence of trichomes, gametangia ontogeny, pattern of gametophyte development, among others, have been considered an important source of information to understand the phylogeny and population biology and dynamics in ferns (Atkinson & Stokey 1964; Nayar & Kaur 1971; Farrar *et al*. 2008; Farrar & Johnson 2022).

Gametophytes can be easily and quickly multiplied from *in vitro* spore culture, often without application of plant growth regulators (Fernández & Revilla 2003; Goller & Rybczyński 2007; Ballesteros & Pence 2018). As part of projects that address systematic, reproductive and conservation aspects of ferns of the Argentine flora, we have grown different taxa from spores in the laboratory (Ramos Giacosa *et al*. 2014, 2017; Luna *et al*. 2016; Gorrer *et al*. 2018; Bejar *et al*. 2019).

The goal of this work is to study the development pattern, the morphology and sexual expression of the gametophyte of *Physematium montevidensis*, to provide information about its reproductive biology.

Portions of fronds of *Physematium montevidensis* with closed mature sporangia were obtained from different populations growing in four localities of the Montane Chacoan district, Chaco biogeographic province, Neotropical region (Arana *et al* 2021): Las Jarillas (31°32’03.16”S, 64°32’14.98”W), Atos Pampa (31°57’49.34”S, 64°40’13.19”W), Las Albahacas (32°53’59.02”S, 64°50”17.97”W), and Achiras (33°09’07.38”S, 64°59’32.78”W). In this environment dominated by xerophytic species of shrublands and montane forests, *P. montevidensis* inhabits in microhabitats of deep cracks and rock crevices (Fig. 1a-b).

Materials were placed in paper envelopes and transported to the laboratory where they were maintained at room temperature (20–25°C) until spore release. The remains of sporangia were eliminated by a mesh with pores 88 µm in diameter. The spores were not sterilized before sowing. They were cultured in previously autoclaved (120 °C for 20 minutes) Petri dishes 9 cm in diameter, containing Dyer liquid medium (Dyer 1979). The dishes (10 repetitions) where sealed with Parafilm and placed in a growth chamber with a photoperiod of 12 hours under white fluorescent illumination 28 µmol m^{-2} s^{-1} at 20±2 °C. The cultures were

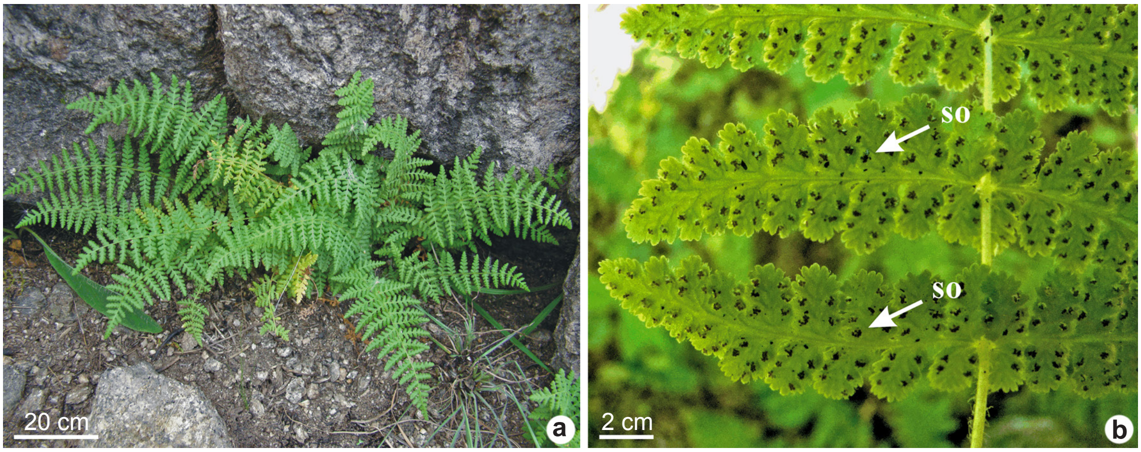

Figure 1 – a-b. Sporophytes of *Physematium montevidensis* – a. habit; b. detail of a fertile frond. so = sorus.

examined every 2–3 days under a stereoscopic microscope Nikon SMZ 1000. The different stages of gametophyte development were photographed employing a Nikon E200 light microscope. Samples were placed on microscopic slides and mounted in water.

When the gametophytes developed the gametangia, they were transferred to Petri dishes containing previously sterilised soil extracted from the sampling localities mentioned above. The terminology used to describe the patterns of spore germination and gametophyte development follows Nayar & Kaur (1971).

The spores of *Physematium montevidensis* are light brown, monolete, with a perispore cristate reticulate (Fig. 2a). Under controlled conditions spores begin to germinate 6–8 days after sowing. Germination is evidenced by the rupture of the spore wall at the laesura and the emergence of the first rhizoid. The first prothallial cell develops perpendicular to the rhizoid (Fig. 2b-c).

Within 7–10 days after germination, different stages of gametophyte development are observed. In some cases, the initial prothallial cell undergoes a series of transverse divisions and linear elongation, developing into filaments 4–5 cells long whose terminal cells produce a unicellular papilose trichome (Fig. 2d). In other instances, the formation of the trichome is delayed and the terminal cell divides longitudinally (Fig. 2e-f). When the terminal cell produces a trichome it becomes inactive mitotically, so the subapical cell divides longitudinally giving rise to the bidimensional laminar phase (Fig. 2g-i).

By successive mitotic divisions a broad asymmetrical plate with a differentiated notch is produced (10–20 days after spore germination) (Fig. 2j-l). As the gametophytes grow, new trichomes are formed on the margins and also on the prothallus surface. The trichomes are unicellular, have a thin cuticle and contain chloroplasts. At a later stage of development (25–30 days after spore germination), many gametophytes become cordiform and symmetrical, and as they mature a wide notch and wide wings are differentiated (Fig. 3a-b). At the same time, other gametophytes are spatulate-elongated, with a shallow notch, sometimes irregular in shape in a more mature stage (Fig. 3c-d).

Gametangia appear 30–40 days after spore germination. Antheridia develop first whereas archegonia emerge about 10 days later. The gametophytes show a dimorphism associated with sexual expression. The smaller, spatulate-elongated gametophytes are male (Fig. 3d). Antheridia develop over the entire surface, sometimes on the gametophyte margin (Fig. 3e). The antheridia consist of three cells: opercular, ring and basal cell. The larger, cordiform gametophytes are bisexual. In this case, archegonia develop near the notch whereas antheridia arise in the basal part of the prothalli, between the rhizoids (Fig. 3f-g). The archegonia possess a neck 4–5 cells long composed of four rows of cells (Fig. 3h). The mature gametophytes show hyaline rhizoids, which develop from the cells at the basal part of the gametophyte (Fig. 3b, d). The posterior region of the prothallus may remain uniseriate (2–3 cells long) to advanced stages of development, or became biseriate. The sporophytes appear about 3 months after spore sowing (Fig. 3i).

The spore germination pattern observed in *Physematium montevidensis* corresponds to the *Vittaria*-type, while the gametophyte development pattern is of the *Aspidium*-type. As described Nayar & Kaur (1971), we registered two variations of prothalial development. In one of them, a terminal trichome is differentiated during the filamentous stage, whereas in other instances trichome formation is delayed until the beginning of the laminar phase. According to Nayar & Kaur (1971), variations in prothallial development may occur together in the same taxon. There is little information on the gametophyte phase in species of Woodsiaceae. Particularly for *P. montevidensis*, characteristics of gametophyte development, morphology and sexuality, they are reported here for the first time. Regarding the spore germination and gametophyte development patterns, few differences were found with that described for other Woodsiaceae. According to Nayar & Kaur (1971), prothallial development in *Woodsia* s.l. is nearly *Drynaria*-type, because trichome formation is delayed until the young gametophytes are cordiform. Kaur & Chandra (1973) found an *Aspidium*-type of gametophyte development in *Physematium obtusum* (Spreng.) Torr. with three variations within this type, both with or without the formation of a terminal trichome. Brown (1964) and Yilun & Fuxiong (1986) only observed terminal trichomes during the initial stages of laminar growth in different species of *Physematium* and *Woodsia* [*Physematium obtusum*, *Woodsia ilvensis* (L.) R. Br., *W. oregana* D.C. Eaton, *W. polystichoides* D.C. Eaton, *W. subcordata* Turcz. and *W. scopulina* D.C. Eaton].

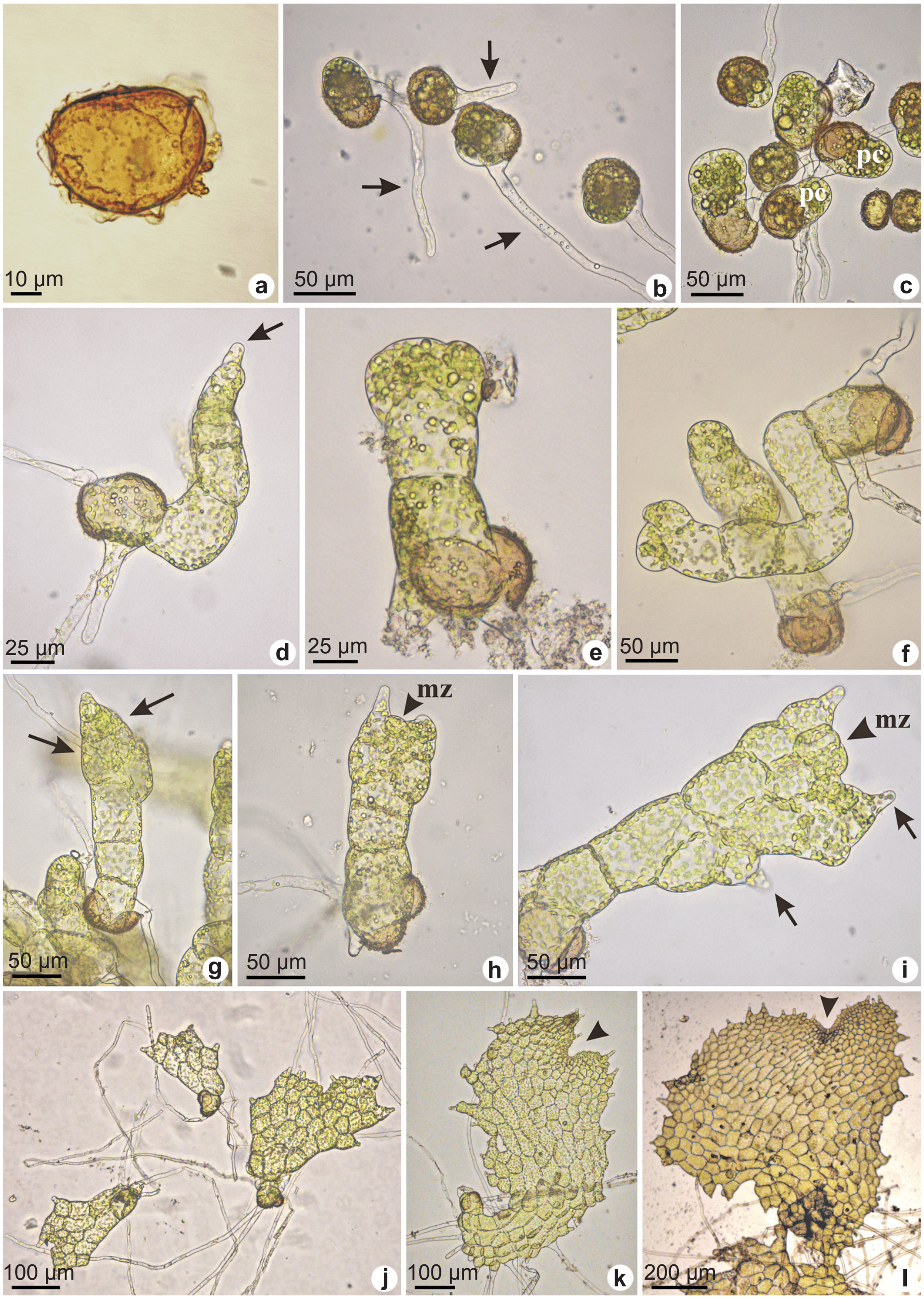

Figure 2 – a-l. Spore and early stages of gametophyte development in *Physematium montevidensis* – a. spore under light microscope; b. first stage of spore germination showing emerging rhizoids (arrows); c. emergence of the first prothallial cell perpendicular to the rhizoid; d. early filamentous phase with terminal trichome (arrow); e-f. early filamentous phase without a terminal trichome; g. mitotic divisions in subapical cells giving rise to the bidimensional laminar phase; h-i. early laminar gametophytes showing development of new trichomes (arrows) and the meristematic zone; j. gametophytes in a more advanced laminar phase; k-l. asymmetrical cordiform gametophytes with a distinguishable notch (arrowheads). pc = prothallial cell; mz = meristematic zone.

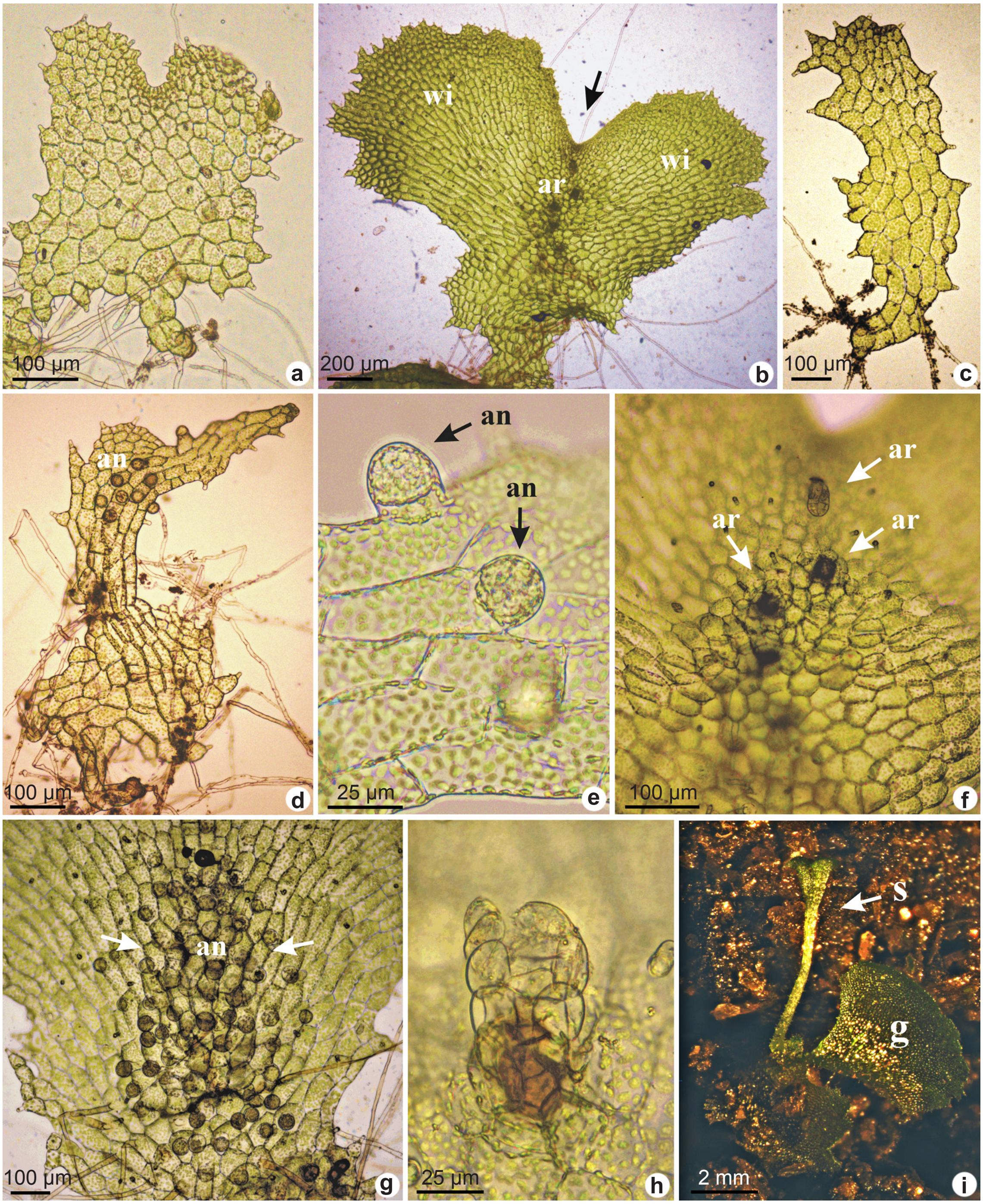

Figure 3 – a-i. Different morphologies of *Physematium montevidensis* gametophytes associated with sexual expression – a. young symmetrical cordiform gametophyte; b. bisexual symmetrical cordiform gametophyte with a deep notch, wide wings and gametangia; c. young spatulate-elongated gametophyte; d. irregularly shaped male gametophyte; e. detail of antheridia on the margin and the surface of a male gametophyte; f. archegonia near the notch in a bisexual gametophyte; g. antheridia in the basal portion of a bisexual gametophyte; h. an archegonium in detail; i. young sporophyte attached to the gametophyte. an = anteridium; ar = archegonium; g = gametophyte; s = sporophyte; wi = wing.

Vivero-Baron *et al*. (2012) reported a *Drynaria*-type prothalial development in *Physematium molle* Kaulf.

Spore germination and gametophyte development were relatively fast in *P. montevidensis*, with antheridia and archegonia appearing between 30–40 days after spore sowing. Brown (1964) and Kaur & Chandra (1973) observed in different taxa of Woodsiaceae that spores required from 6 to 15 days to germinate under laboratory conditions, employing different substrates. These results are quite similar to those found in the present study. Vivero-Baron *et al*. (2012) reported that the germination of *Physematium molle* spores occurs between 32 to 63 days from spore sown using various substrates. In *Woodsia alpina* (Bolton) Gray and *W. ilvensis*, spore germination and filament formation occurred between 46 and 96 days in MS medium, or a mix of sand and soil (Kromer *et al*. 2006; Aguraiuja 2011). In laboratory cultures, the development and the sexual expression of the fern prothallus are influenced by nutrients, luminosity, pH, physical state of the medium and gametophyte density (Menéndez *et al*. 2011).

Concerning sexual expression, the gametophytes of *P. montevidensis* are bisexual and unisexual male, the latter smaller in size. Gametophytes of homosporous ferns are potentially bisexual, but antheridiogens or environmental factors may influence sex expression (Sharpe & Mehltreter 2010). The presence of gametophytes that develop only antheridia leads to an increase in cross fertilization (Schneller *et al*. 1990). Peck (1980) reported bisexual and unisexual male gametophytes in cultures of *Physematium obtusum*, and demonstrated that this species produces an antheridiogenic substance. Other authors found mostly bisexual gametophytes in different species of *Physematium* (Brown 1964; Kaur & Chandra 1973; Peck 1985; Vivero-Barón *et al*. 2012). Although the presence of antheridiogens has been reported in various species of *Physematium*, it was not confirmed in the present study for *P. montevidensis*.

Regardless of the culture conditions, the gametophyte development of *Physematium montevidensis* and its morphological features are broadly similar to those reported in other taxa of Woodsiaceae. So, we can conclude that gametophyte characteristics do not provide, at the moment, reliable diagnostic information to differentiate both recognized genera of Woodsiaceae, nor the species of *Physematium* analysed to date.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

References

Aguraiuja R (2011) Reintroduction of the endangered fern species *Woodsia ilvensis* to Estonia: a long-term pilot study. Biodiversity and Conservation 20: 391-400.

Arana MD, Natale E, Oggero A, Ferreti N, Romano G, Martínez G, Posadas P & Morrone JJ (2021) Esquema biogeográfico de la República Argentina. Opera Lilloana 56: 1-240.

Atkinson LR & Stokey AG (1964) Comparative morphology of the gametophyte of homosporous ferns. Phytomorphology 14: 51-70.

Ballesteros D & Pence VC (2018) Fern conservation: spore, gametophyte, and sporophyte *ex situ* storage, *in vitro* culture, and cryopreservation. *In*: Fernández H (ed.) Current advances in fern research. Springer International, Oviedo. Pp. 227-249.

Bejar J, Luna ML, Murace MA & Saparrat MCN (2019) Effects of *Pestalotiopsis maculans* (Ascomycota, Amphisphaeriales) on the gametophyte development of *Lygodium venustum* (Lygodiaceae). Revista de Biología Tropical 67: 1520-1530.

Brown DF (1964) A monographic study of the fern genus *Woodsia*. Nova Hedwigia 16: 1-154.

Dyer AF (1979) The culture of fern gametophytes for experimental investigation. *In*: Dyer AF (ed.) The experimental biology of ferns. Academic Press, London. Pp. 253-305.

Farrar DR & Johnson CL (2022) Methodologies for soil extraction and conservation analysis of ferns and lycophytes with belowground gametophytes. Applications in Plant Sciences 10: e11469. DOI: 10.1002/aps3.11469

Farrar DR, Dassler C, Watkins Jr. JE & Skelton C (2008) Gametophyte ecology. *In*: Ranker TA & Haufler JH (eds.) Biology and evolution of ferns and lycophytes. Cambridge University Press, Cambridge. Pp. 222-251.

Fernández H & Revilla MA (2003) *In vitro* culture of ornamental ferns. Plant Cell Tissue and Organ Culture 73: 1-13.

Goller K & Rybczyński JJ (2007) Gametophyte and sporophyte of tree ferns *in vitro* culture. Acta Societatis Botanicorum Poloniae 76: 193-199.

Gorrer DA, Berrueta PC, Ramos Giacosa, JP, Giudice GE & Luna ML (2018) Morfogénesis de la fase sexual de los helechos epífitos *Microgramma mortoniana* y *Pleopeltis macrocarpa* (Polypodiaceae) de la Reserva Natural Punta Lara, Buenos Aires, Argentina. Revista de Biología Tropical 66: 1078-1089.

Kaur S & Chandra S (1973) Morphology of the prothallus of *Woodsia obtusa*. Phytomorphology (Delhi) 23: 171-175.

Kromer K, Marszał-Jagacka J, Kempińska K, Nowak T, Żołnierz L, Poturała D & Świerkosz K (2006) *In vitro* propagation and *ex situ* preservation of endangered ferns from lower Silesia. Botanical Guidebooks 29: 143-155.

Lu NT, Zhou X-M, Zhang L, Knapp R, Li C-X, Fan X-P, Zhou L, Wei H-J, Lu J-M, Xu B, Peng Y-L, Gao X-F & Zhang L-B (2020) A global plastid phylogeny of the cliff fern family Woodsiaceae and a two-genus classification of Woodsiaceae with the description of ×*Woodsimatium* nothogen. nov. Taxon 68: 1149-1172. DOI: https://doi.org/10.1002/tax.12180

Luna ML, Yáñez A, Ramos Giacosa JP, Gorrer D, Berrueta P & Giudice GE (2016) *In vitro* culture of the annual fern *Anogramma chaerophylla* (Pteridaceae): strategy for its conservation in Buenos Aires Province, Argentina. Boletín de la Sociedad Argentina de Botánica 51: 675-682.

Menéndez V, Arbesú R, Somer M, Revilla A & Fernández H (2011) From spore to sporophyte: how to proceed *in vitro*. *In*: Fernández H, Kumar A & Revilla MA (eds.) Working with ferns: issues and applications. Springer Science+Business Media Press, New York. Pp. 97-110. DOI: 10.1007/978-1-4419-7162-3

Mynssen CM & Arana MD (2020) Woodsiaceae in Flora e Funga do Brasil (continuously updated). Jardim Botânico do Rio de Janeiro. Available at <https://floradobrasil.jbrj.gov.br/FB92305>. Access on 23 August 2023.

Nayar BK & Kaur S (1971) Gametophytes of homosporous ferns. The Botanical Review 37: 295-396.

Peck JH (1980) Life history and reproductive biology of the ferns of Woodman Hollow, Webster County, Iowa. Retrospective Theses and Dissertations: 6799. Iowa State University, Ames. 553p. DOI: 10.31274/rtd-180813-4602

Peck CJ (1985) Reproductive biology of isolated fern gametophytes. Retrospective Theses and Dissertations: 8762. Iowa State University. Available at <https://doi.org/10.31274/rtd-180813-11765>. Access on 11 November 2022.

Ramos Giacosa JP, Giudice GE, Pipo L & Luna ML (2014) Morfología de las esporas, desarrollo gametofítico y conservación de *Thelypteris abbiattii* y *T. hispidula* (Thelypteridaceae) de la Reserva Natural Punta Lara, Provincia de Buenos Aires, Argentina. Boletín de la Sociedad Argentina de Botánica 49: 217-226.

Ramos Giacosa JP, Gorrer DA, Giudice GE & Luna ML (2017) Gametophyte development and conservation of *Ctenitis submarginalis* (Dryopteridaceae) in Buenos Aires Province, Argentina. Boletín de la Sociedad Argentina de Botánica 52: 697-703.

Schneller JJ, Haufler CH & Ranker TA (1990) Antheridiogen and natural gametophyte populations. American Fern Journal 80: 143-152.

Sharpe JM & Mehltreter K (2010) Ecological insights from ferns population dinamics. *In*: Mehltreter K, Walker LR & Sharper JM (eds.) Fern ecology. Cambridge University Press, Cambridge. Pp. 61-110.

Vivero-Barón J, Arreguín-Sánchez MDLL, Quiroz-García DL, Fernández-Nava R & Acosta-Castellanos S (2012) Seguimiento del ciclo de vida de *Woodsia mollis* (Kaulf.) J.Sm. (Athyriaceae-Pteridophyta) en tres sustratos naturales. Polibotánica 33: 75-83.

Watson PJ & Vazquez M (1981) Comparative ecology of *Woodsia scopulina* sporophytes and gametophytes. American Fern Journal 71: 3-9.

Yilun M & Fuxiong W (1986) A brief report of development of gametophytes in *Woodsia*. Acta Botanica Yunnanica 8: 133-140.

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