Hoehnea 42(2): 233-237, 1 fig., 2015 http://dx.doi.org/10.1590/2236-8906-36/2014

Ploidy level and obligate apogamy in two populations of *Argyrochosma nivea* var. *tenera* (Pteridaceae)

Marcela A. Hernández[1], Aldo R. Andrada[1], Valeria de los A. Páez[1] and Olga G. Martínez[2,3]

Received: 22.07.2014; accepted: 17.12.2014

ABSTRACT - (Ploidy level and obligate apogamy in two populations of *Argyrochosma nivea* var. *tenera* (Pteridaceae)). *Argyrochosma nivea* var. *tenera* is a cheilanthoid fern from the American continent. We present herewith the study of meiotic behavior and gametic number as well as number of spores and reproduction mode (with observations from cultured gametophytes). The study material is from Northwestern Argentina. For the study of meiosis, sporangia were fixed in ethanol-acetic acid (3:1) and stained with 2% propionic haematoxylin. Micrographs were taken using light and scanning electron microscopes. Meiotic chromosome number is 2n = 3x = 81 and the presence of lagging chromosomes at telophase II is mentioned for the first time; three sets of chromosome occur at diakinesis. Sporangia have 32 trilete spores. Adult gametophytes are laminar, cordiform, asymetrical, glabrous, without glandular trichomes. In our samples, the adult gametophytes were neuter, without antheridia or archegonia, with obligate apogamous reproduction. Both populations studied might be of hybrid origin.
Keywords: apogamy, *Argyrochosma*, chromosome number, lagging chromosomes

RESUMEN - (Nivel de ploidía y apogamia abligada en dos poblaciones de *Argyrochosma nivea* var. *tenera* (Pteridaceae)). *Argyrochosma nivea* var. *tenera* es un helecho cheilantoideo del continente americano. Se presenta aquí el estudio del comportamiento meiótico y número gamético así como el número de esporas y el tipo de reproducción (con observaciones de gametofitos cultivados). El material de estudio proviene del Noroeste argentino. Para el estudio de la meiosis, los esporangios se fijaron en etanol-ácido acético (3:1) y se tiñeron con hematoxilina propiónica 2%. Se presentan fotografías tomadas con microscopio de luz y electrónico de barrido. El número cromosómico es n = 2n = 3x = 81. La presencia de cromosomas rezagados en telofase II se cita por primera vez para esta especie. Tres grupos de cromosomas se producen mostrando el carácter triploide de este taxón. Los esporangios contienen 32 esporas triletes. Los gametofitos adultos son laminados, cordiformes, asimétricos, glabros, sin tricomas glandulares. En nuestras muestras los gametofitos adultos son neutros, sin anteridios o arquegonios; la reproducción es apógama obligada. Ambas poblaciones estudiadas podrían tener origen híbrido.
Palabras claves: apogamia, *Argyrochosma*, cromosomas rezagados, número de cromosomas

Introduction

The genus *Argyrochosma* Windham (Pteridaceae subfamily Cheilanthoideae) is widespread in the New World and has homoplastic adaptations to seasonally dry environments, such as the presence of glandular trichomes abaxially on the leaves in the species which produce white or yellow farina. *Argyrochosma nivea* (Poir.) Windham var. *tenera* (Gillies *ex* Hook.) Ponce (figure 1a) grows in the Andean region, from Peru to Argentina, mainly in rock crevices in exposed sites.

Molecular studies performed by Gastony & Rollo (1998) determined the monophyly of the genus *Argyrochosma*. Recently, Sigel *et al.* (2011) presented a phylogenetic analysis of this genus, in which they described two large monophyletic groups: one exclusively non-farinose and the other primarily farinose, in which *A. nivea* var. *tenera* is included.

The basic chromosome number in *Argyrochosma* is x = 27, unlike other cheilanthoid ferns, which mostly have x = 29 or x = 30 (Brownlie 1957, Windham 1987, Moran & Yatskievych 1995, Windham & Yatskievych 2003). Windham & Yatskievych (2003) compared molecular results with chromosome numbers for the different genera and concluded that x = 27 observed in *Argyrochosma* might have been derived by aneuploidy

1. Fundación Miguel Lillo, Miguel Lillo 251, 4000 Tucumán, Argentina
2. Universidad Nacional de Salta, Facultad de Ciencias Naturales, IBIGEO, 4400 Salta, Argentina.
3. Corresponding author: martinezog@gmail.com

of x = 29 present in other taxa of cheilanthoid ferns, such as *Astrolepis*, *Pellaea*, and some *Cheilanthes*. According to Sigel *et al*. (2011), *A. nivea* var. *tenera* has two ploidy levels, 2x and 3x.

In general, ploidy level is closely related to spore size in *Argyrochosma* (Windham & Yatskievych 2003). Morbelli *et al.* (2001) found spores of variable size in *Argyroschosma*, with measures between 38 and more than 100 µm in equatorial diameter; for *A. nivea* var. *tenera*, such authors mentioned values ranging between 49 and 82 µm.

According to Knobloch (1966), the number spores per sporangium is related to the reproduction mode; plants with 64 spores generally have sexual reproduction, whereas those with 32 or 16 spores usually have apogamous reproduction. In *Argyroschosma nivea* var. *tenera*, Sigel *et al.* (2011) found very variable results regarding the number of spores per sporangium, reproductive type and presence of farina. Hence, here we studied meiotic behavior and gametic number as well as number of spores per sporangium and reproductive mode in two Argentine population of *A. nivea* var. *tenera*, aimed at enhancing knowledge that may help as a support for phylogenetic studies of this apparently monophyletic clade.

Material and methods

The study material was collected from Argentina: Salta province, Chicoana department, Quebrada de Escoipe; 25º09'24"S, 65º41'29"W; 20-V-2012, *Hernández 1701* (LIL); Tucumán province, Tafí department, Los Cardones; 26º41'00"S, 65º48'00"W, 12-IX-2011, *Hernández et al. 1950* (LIL).

Fifty capsules were observed under light microscope to count the number of spores per sporangium. For the cytogenetic studies sporangia were fixed in ethanol-acetic acid (3:1) for 48 hours and preserved in 70° ethanol at 4 ºC. They were hydrolyzed in 1 N HCl at 60 ºC for 20 min, immediately rinsed with distilled water and then stained with 2% propionic haematoxylin.

Spores were sterilized with 10% sodium hypochloride for 10 minutes before sowing. Spores were sown in 5 cm diameter Petri dishes with Dyer medium (Dyer 1979) and 10 g/L of agar with laminar flux camera. For each species, 10 Petri dishes were kept at room temperature with a light regime of 12 light-hours per day. For SEM studies, the gametophytes were fixed with 2% glutaraldehyde in phosphate buffer for 72 hours and dehydrated in a graded series of alcohols (10%-absolute ethanol). Once dehydrated, the gametophytes were critical point dried with carbon dioxide (Denton Vacuum DCP-1) and were fixed to aluminum stubs with double-sided adhesive graphite tape, sputter-coated with gold.

Micrographs were taken with a Nikon Eclipse E200 microscope equipped with a Moticam 1000 digital camera (1.3 Mp); the scanning electron microscope JEOL JSM 6480 LV SEM (Japan) belongs to the Laboratorio de Microscopía Electrónica de Barrido y Microanálisis (LASEM), Universidad Nacional de Salta, Argentina.

Results

Both studied populations of *Argyrochosma nivea* var. *tenera* showed similar results: immature sporangia presented eight spore mother cells. At diakinesis, 2n = 81 were observed (figure 1b); at a late stage of diakinesis, chromosomes were grouped in three sets (figure 1c). At metaphase, chromosomes lying outside the equatorial plate (figure 1d) were observed; lagging chromosomes were present at anaphase I (figures 1e, f). However, the development of telophase I was regular. Throughout the second division, lagging chromosomes were observed at anaphase II and telophase II. *Argyrochosma nivea* var. *tenera* were triploid with 2n = 3x = 81. The number of spores per sporangia in the studied specimens was 32, corresponding to the eight mother cells found in the immature sporangia.

The palynological analysis indicated that spores are trilete, globose, with slightly ridged perispore. Spore size is very variable, with equatorial diameter of 37(71)106 µm; a small number of spores (8%-10%) of small size, without cell content, of equatorial diameter of 43(47)49 µm, were also observed (figure 1g).

Spore germination occurs at 3-4 days after sowing; at the end of the first week, about 80% of spores have germinated; 30-45 days later, they reach their laminar, cordate, asymmetrical shape. These laminar gametophytes are sterile; under our cultural conditions they do not develop antheridia or archegonia during the mature phase. After 60 days a protuberance develops in the neck canal; first it is glabrous, then it enlarges and is covered with brown scales surrounding the incipient apogamous sporophyte that starts to develop (figure 1h).

Discussion

Our findings show a chromosome number n = 2n = 3x = 81 in both populations of *Argyrochosma*

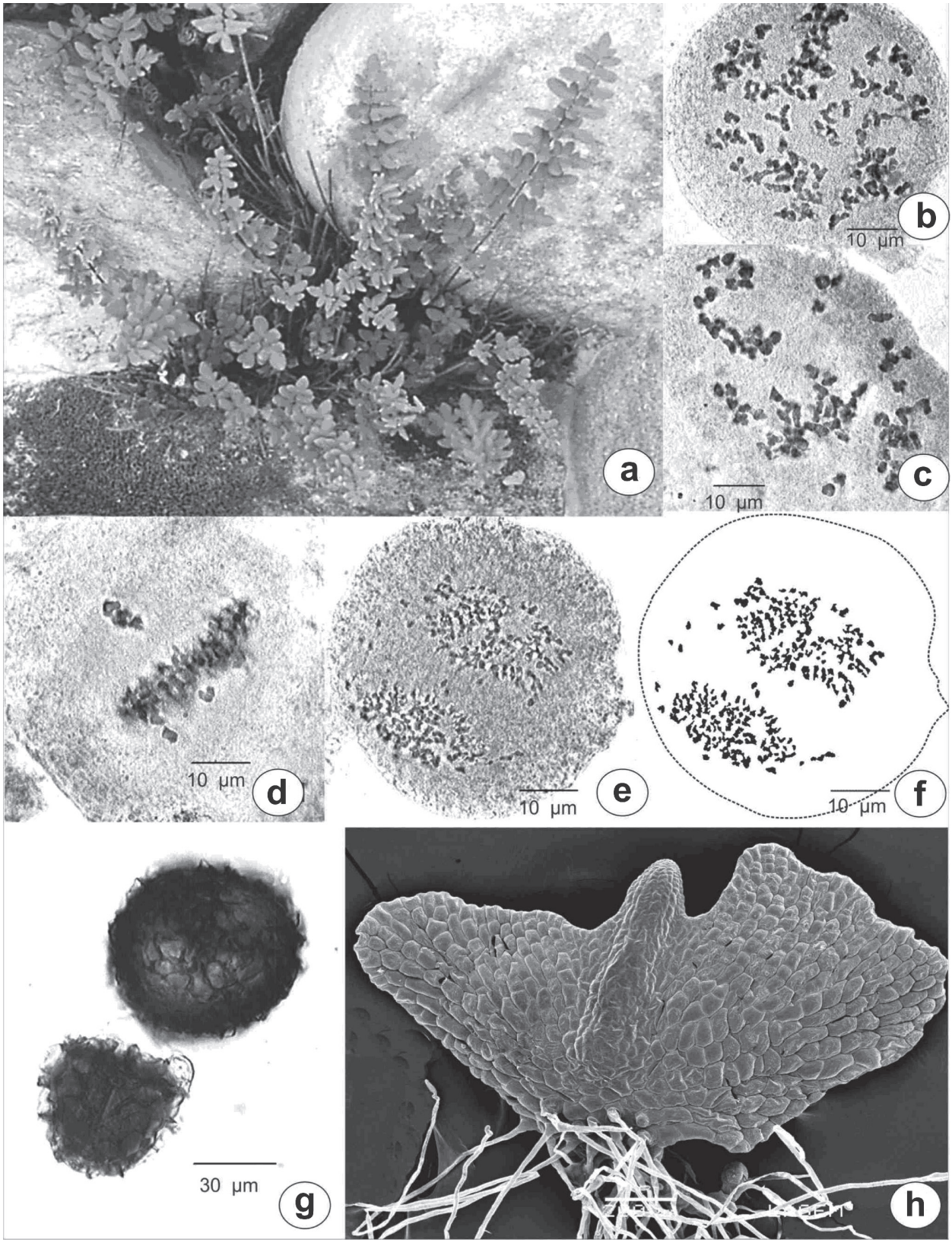

Figure 1. *Argyrochosma nivea* var. *tenera.* a. General view of the plant. b. Diakinesis, n = 81 chromosomes. c. Late stage of diakinesis, Chromosomes grouped in three set. d. metaphase I with chromosomes that lie outside the equatorial plate. e-f. Anaphase I with lagging chromosomes. g. globose trilete spores showing very variable size and slightly ridged perispore. h. Gametophyte with incipient apogamous sporophyte.

nivea var. *tenera* analyzed. During some diakinesis, chromosome grouping into three sets provides additional evidence that corrobarates the triploid character of the taxon. Triploid ferns may have originated from the crossing of a diploid and a tetraploid fern, in which both (diploid and tetraploid) can be cytotypes of the same or a different taxon in which there has been interspecific crossing (Knobloch *et al.* 1975). The presence of lagging chromosomes has not been reported for *Argyrochosma* or any other cheilanthoid genus, because meiotic studies are scarce. According to Windham & Yatskievych (2003), this is because sporangia are not aggregated into sori and are protected by trichomes, scales and/or the indusia. The irregular behavior of such meiosis in the germ line (e.g., the presence of secondary associations and lagging chromosomes) suggests that such taxa might be of hybrid origin. This is consistent with the previous cytogenetic reports of Zhang et al. (2008) for *Osmunda japonica* Houtt.

In sporangia, the 32 spores correspond to the eight mother cells of the spores observed. The number of mature spores (32) is similar to that indicated by Sigel *et al.* (2011) for 50% of the reported specimens; in the remaining 50%, these authors found some plants producing 64-spored sporangia and others with both 32 and 64 spores. This characteristic was also described by Whittier (1965) for apogamous *Cheilanthes* specimens, which exhibited 32- and 16-spored sporangia in a single specimen.

In cheilanthoid ferns, spores are usually trilete; however, Morbelli *et al.* (2001) observed monolete spores as well. In this study of *Argyrochosma nivea* var. *tenera*, only trilete spores have been observed. Morphological variability of spores is present in other Pteridaceae, which exhibit polyploidy and apogamous reproduction, such as *Pteris cretica*, *P. denticulata*, and *P. tristicula* (Martínez & Morbelli 2009, Martínez 2011).

Spore ornamentation of *Argyrochosma nivea* var. *tenera* agrees with the information provided by Morbelli *et al.* (2001) and Tryon & Lugardon (1991). The mean equatorial diameter of the spores studied in this work is within the range of 58-82 µm mentioned by Morbelli *et al.* (2001), and of 49.13 ± 1.79 to 76 ± 3.67 µm found by Sigel *et al.* (2011). Nevertheless the extreme values observed here, 37 and 106 µm are not frequent dimensions for *A. nivea* var. *tenera*, but are frequent for other varieties of the same species, such as *A. nivea* var. *nivea*, with equatorial diameter values which can reach 106.5 µm (Morbelli *et al.* 2001).

Spore germination time is similar to that observed by Gabriel y Galán (2011); however, the irregular structure of adult gametophytes mentioned by this author does not agree with our findings for the studied variety of *Argyrochosma*. The apogamous or agamosporic type of reproduction is frequent in cheilanthoid ferns (Nayar & Kaur 1971); it has been described for several species, mainly of genus such as *Cheilanthes*, *Notholaena*, and *Pellaea* (Hayes 1924, Knoblock 1966, Tryon 1968, Gastony & Rollo 1998).

Woronin (1907) and Nayar & Bajpai (1964) mentioned apogamous reproduction for some *Argyrochosma* species, despite the formation of male and female gametangia. However, in the present study one can observe that the gametophyte has obligate apogamy because it does not exhibit the development of antheridia or archegonia. Gabriel y Galán (2011) reported similar results in *Argyrochosma nivea*.

The results of this work confirm the hypothesis formulated by Knobloch (1967), who argued that in a xeric environment where water for fertilization is at a premium, it is reasonable to assume that natural selection favors apogamous reproduction, and by Lovis (1977), who considered that most of the species with this mode of reproduction are triploid. Furthermore, the results contribute to the cytological knowledge of xeric-adapted ferns, considering that chromosomal counts are still unknown for most South American taxa (Windham & Yatskievych 2003).

This study might give support to the phylogenetic analysis of the cheilanthoid ferns and show intraspecific variation in the chromosome number and ploidy level in this species**.**

Literature cited

Brownlie, G. 1957. Cytotaxonomic Studies on New Zealand Pteridaceae. New Phytologist 56: 207-209.

Dyer, A. 1979. The culture of fern gametophytes for experimental investigation. *In*: A. Dyer (ed.). The experimental biology of ferns. Academic Press, London, pp. 254-305.

Gabriel y Galán, J.M. 2011. Gametophyte development and reproduction of *Argyrochosma nivea* (Pteridaceae). Biologia 66: 50-54.

Gastony, G.J. & Rollo, D.R. 1998. Cheilanthoid ferns (Pteridaceae: Cheilanthoideae) in the southwestern United States and adjacent Mexico- a molecular phylogenetic reassessment of generic lines. Aliso 17: 131-144.

Hayes, D.W. 1924. Some Studies of Apogamy in *Pellaea atropurpurea*. (L.) Link. Transactions of the American Microscopical Society 43: 119-135.

Knobloch, I.W. 1966. A Preliminary Review of Spore Number and Apogamy within the Genus *Cheilanthes*. American Fern Journal 56: 163-167.

Knobloch, I.W. 1967. Chromosomes numbers in *Cheilanthes*, *Notholaena*, *Llavea* and *Polypodium*. American Journal of Botany 54: 461-464.

Knobloch, I.W., Tai, W. & Adangappuram, T. N. 1975. Chromosome counts in *Cheilantes* and *Aspidotis* with a conspectus of the cytology of the Sinopteridaceae. American Journal of Botany 62: 649-654.

Lovis, J.D. 1977. Evolutionary patterns and processes in ferns. Advances in Botanical Research 4: 229-415.

Martínez, O.G. 2011. Morfología y distribución del complejo *Pteris cretica* (Pteridaceae). Candollea 66: 159-180.

Martínez, O.G. & Morbelli, M.A. 2009. The spores of *Pteris cretica* Complex (Pteridaceae-Pteridophyta) in America. Grana 48: 193-214.

Moran, R.C. & Yatskievych, G. 1995. Pteridaceae. *In*: G. Davidse, M. Sousa & S. Knapp (eds.). Flora Mesoamericana. *In*: R.C. Moran & R. Riba (eds.). Psilotaceae a Salviniaceae. Universidad Nacional Autónoma de México, Ciudad de México, v. 1, pp. 104-145.

Morbelli, A., Ponce, M.M., Macluf, C.C. & Piñeiro, M.R. 2001. Palynology of South American *Argyrochosma* and *Notholaena* (Pteridaceae) species. Grana 40: 280-291.

Nayar, B.K. & Bajpai, N. 1964. Morphology of the gametophytes of some species of *Pellaea* and *Notholaena*. Journal Linnean Society (Bot.) 59: 63-76.

Nayar, B.K. & Kaur, S. 1971. Gametophytes of Homosporous Ferns. Botanical Review 37: 295-396.

Sigel, E.M., Windham, M.D, Huiet, L., Yatskievych, G. & Pryer, K.M. 2011. Species relationships and farina evolution in the cheilanthoid fern genus *Argyrochosma* (Pteridaceae). Systematic Botany 36: 554-564.

Tryon, A.F. 1968. Comparisons of the sexual and apogamous races in the fern genus *Pellaea*. Rhodora 70: 1-24.

Tryon, A.F. & Lugardon, B. 1991. Spores of the Pteridophyta. Surface, wall structure and diversity based on electron Microscope Studies. Springer-Verlag, New York.

Whittier, D.P. 1965. Obligate Apogamy in *Cheilanthes tomentosa* and *C. alabamensis* Botanical Gazette 126: 275-281.

Windham, M.D. 1987. *Argyrochosma*, a new genus of cheilanthoid ferns. American Fern Journal 73: 37-41.

Windham, M.D. & Yatskievych, G. 2003. Chromosome studies of Cheilanthoid ferns (Pteridaceae: Cheilanthoideae) from the Western United States and Mexico. American Journal of Botany 90: 1788-1800.

Woronin, H. 1907. Apogamie und Aposporie bei einigen Farnen. Berichte der Deutschen Botanischen Gesellschaft 25: 115-135.

Zhang, S.Z, He, Z.C, Fan, C.R. & B. Yan. 2008. A cytogenetic study of five species in the genus Osmunda. Journal of Systematics and Evolution 46: 490-498.