Morphology, reproduction and karyology in the rare Andean *Poa gymnantha*

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We report agamic reproduction in *Poa gymnantha*, an endemic species from the high Andes of Peru, Bolivia and Chile, known only from pistillate plants. This is the first description of apomixis in *Poa* from South America. The species is a decaploid ($2n = 10x = 70$) with a basic karyotype formula of 4 m + 3 sm chromosomes. Diagnostic characters, geographic distribution and taxonomic circumscription of the species are given, along with illustrations. The paper contributes to a better understanding of floral morphology and reproduction in this agamic taxon.

KEYWORDS: apomixis, chromosome number, karyotype, *Poa gymnantha*, Poaceae, Punapoa, reproduction, taxonomy

INTRODUCTION

Perfect flowers are the usual structure in the grass family. However, some grasses have imperfect flowers in various numbers and combinations. *Poa* L. is probably one of the most diverse genera in its floral morphology, since alongside with the perfect flowers, a number of deviations (dioecism in the strict sense and in the special form of gynodioecism, gynomonoecism, chasmogamy and cleistogamy, and apomixis) are documented (Anton & Connor, 1995). Stebbins (1950: 405) considered that the genus might be regarded as a single huge polyploid complex, which is in part purely sexual, in part facultatively apomictic and which contains in addition obligate apomicts.

Poa gymnantha Pilg. is one of the rare species of Andean *Poa* and exhibits an unusual mode of reproduction, producing only functionally pistillate flowers. Apomixis has been suspected, in at least seven species from the Andes known only from female plants (Anton & Connor, 1995). *Poa gymnantha* Pilg. belongs to the informal group "Punapoa" (Soreng & al., 2003), including plants either forming both perfect and pistillate flowers (gynomonoecious) or only pistillate flowers.

The species has been interpreted as including *P. ovata* Tovar (Soreng & al., 2003) and *P. perligulata* Pilg. (Foster, 1966), but we consider it distinct from both entities. It differs from *P. ovata* in its acute ligules, exserted inflorescences, longer glumes (covering three quarters of the adjacent lemma), and female spikelets. *Poa ovata* has truncate ligules, short pedicellate inflorescences sometimes included in the basal foliage, shorter glumes that cover half of the adjacent lemma or less, and is gynomonoecious. *Poa perligulata* is easily recognised by its dwarf habit, 2–8 cm high, with small inflorescences, less than 3 mm long and its spikelets consistently 2-flowered, and with obtuse glumes and lemmas.

Poa gymnantha has been reported from Argentina (Cabrera, 1957; Zuloaga & al., 1994; Soreng & al. 2003), northern Chile (Gillespie & Soreng, 2005), Bolivia (Renvoize, 1998) and Peru (Tovar, 1993), where it grows either in dry and humid puna habitats, at elevations ranging from 3,900 to 5,000 m.

Although the chromosome numbers of many species of *Poa* have been counted with $x = 7$ being the basic number of the genus (cf. Soreng, 1990; Watson & Dallwitz, 1992; various plant chromosome indexes), the members of Andean *Poa* remain karyologically unknown.

The purpose of this paper is to report the morphology, chromosome number and the mode of reproduction in *Poa gymnantha*.

MATERIALS AND METHODS

To determine the identity of *Poa gymnantha*, herbarium specimens from AAU, BAA, CONC, CORD, F, K, LPB, MOL, S, SI, and USM were examined. Inflorescences, seeds, voucher specimens and living plants were collected during field trips to Bolivia (years 1997–2004) in the Valle del Zongo, near the peak of Mt. Huayna Potosí, Dpto. La Paz, Prov. Murillo (Appendix).
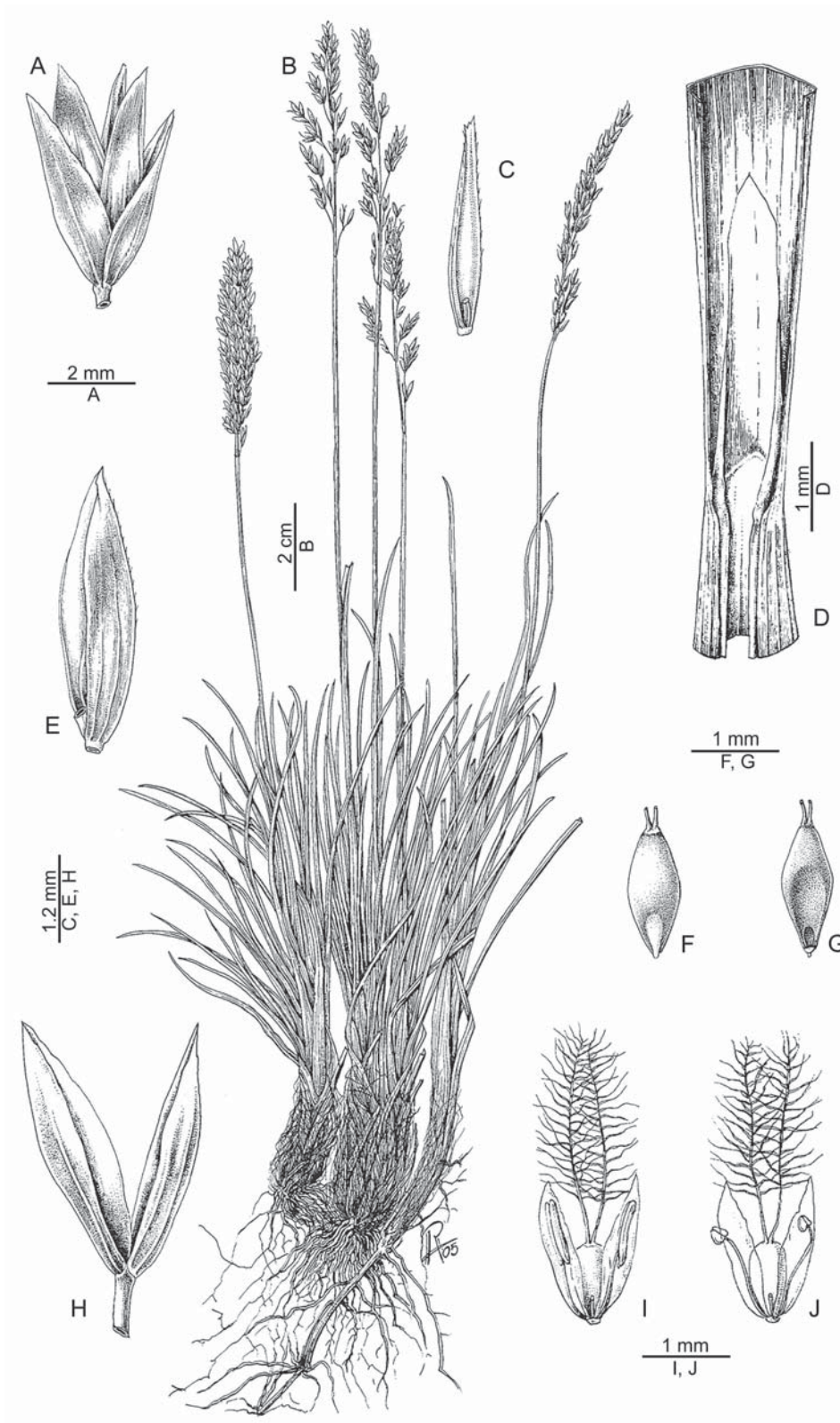
Fig. 1. *Poa gymnantha* Pilg. (*A.E. Cocucci & al. 911*). A, spikelet; B, habit; C, palea; D, ligule; E, lemma; F–G, caryopsis; H, glumes; I–J, female florets with small and diminutive staminodes respectively, the frontal one eliminated, and lodicules.

For embryological analysis, pistillate inflorescences at different stages of development were fixed and stored in FAA (70% ethanol : glacial acetic acid : formaldehyde, 18 : 1 : 1). The spikelets were dehydrated in ethanol alcohol series and embedded in paraffin. Samples were sectioned at 7–10 μm and stained with safranin, fast green and toluidine blue. Preparations were observed under light transmission microscope. Microscopic images were captured by a Nikon digital camera connected to a Zeiss Axiophot microscope; they were combined and processed with the Adobe Photoshop software. The specimens studied were: *Cocucci, Negritto & Romanutti 911* and *Negritto & Meneses 128*.

Somatic chromosomes were observed in squashed shoot meristems obtained from germinating seeds. The shoot apices were fixed in 1 : 3 acetic acid/ethanol mixture for 12 h after a pretreatment in 2 mM 8-hydroxyquinoline for 3 h at room temperature, and 3 h at 4°C. The material was kept at –20°C until examination. Shoot apices were macerated with a pectinase-cellulase solution and squashed in a drop of 45% acetic acid according to Schwarzacher & al. (1980). Preparations were air dried after removing the coverslip with cold CO_2 and then, stained with Giemsa 2% (Guerra, 1983) and mounted in Euparal. Chromosomes were observed and photographed in a Leica DMLB microscope equipped with a Leica DC 250 digital camera and the Leica IM 1000 image management system. Two individuals from different populations in Dpto. La Paz, Bolivia: *Loza de la Cruz 46* and *R. López s.n.* were analyzed. Somatic chromosome measurements (l = long arm length, s = short arm length, and c = chromosome length) were taken from three metaphase plates. The arm ratio (r = l/s) was used to classify the chromosomes as recognized by Levan & al. (1964) as metacentric or "m" (r = 1.00–1.69), and submetacentric or "sm" (r = 1.70–2.99). Karyograms and the idiogram were prepared by organizing the chromosomes in two groups according to their increasing arm ratio (metacentric to submetacentric), and then according to the decreasing length within each group.

■ RESULTS

Poa gymnantha Pilg. in Bot. Jahrb. Syst. 56 (Beibl. 123): 28. 1920 – Type: Perú, *Weberbauer 6905* (lectotype: S!; isolectotypes: USM!, fragment BAA!, US). Fig. 1.

Description. — Plants tufted, perennials, 12–35 (45) cm tall, with thin rhizomes. Leaves mainly basal, culms smooth with only one node exposed. Sheaths papery, glabrous, smooth, fibrous when older, in the uppermost the margins fused 1/3 the length. Ligules acute to obtuse, margin erose, (2)3–4(7) mm long. Blades conduplicate to involute, scabrous on the margins; apex acute. Inflorescence exserted well beyond the basal leaves, peduncle 8–17.5 (21) cm, congested, spiciform, elliptic, 3–7.8 cm long, 0.8–1.2 cm wide, the main axis 7–15-noded, with 1–3 scabrous branches at the lower nodes, spikelets congregated towards the distal end of the branches.

Spikelets oblong, 4.5–6.5 mm long, 2–2.5 mm wide, 2–3-flowered, all florets functionally pistillate. Glumes subequal, smooth or slightly scabrous at the apex, margins membranous, 2/3–3/4 as long as the spikelet, the lower 3.3–4 mm long, 1 mm wide from keel to margin, 1–3-nerved, the upper 3.5–4.8 mm long, 1–1.2 mm wide from keel to margin, 3-nerved. Callus glabrous. Lemmas ovate-lanceolate, 3.5–4.5 (5) mm long, 1–1.2 mm wide from keel to margin, 5-nerved, the upper portion of the body scabrid, shortly hairy on the keel, bronzed and/or purplish between veins, margins membranous. Paleas shorter than the lemmas, 4–4.3 mm long, translucent, smooth, shortly hairy on the keels. Rachilla glabrous, with the basal internode 0.4–0.5 mm. Flowers functionally pistillate, abortive androecium with 3 staminodes, the anthers ca. 0.2 mm long. Caryopsis ca. 1.7 mm long. Staminate flowers unknown.

Distribution and habitat. — *Poa gymnantha* occurs in Peru, Bolivia and Chile, along the Cordillera de Los Andes (Fig. 2). The species forms dense tufts in grassland and grassy slopes with rock outcrops and intermittent swamps and pools, at elevations between 3,900–5,000 m. It is part of the grass community formed mainly by species of *Deyeuxia*, *Calamagrostis*, *Dissanthelium*, *Stipa*, *Festuca* and *Agrostis*.

The species has also been reported from Argentina (Cabrera, 1957). We have examined most of the specimens cited by Cabrera (*Cabrera 8272, 8355, 8673, 8674, 9082, 9202* and *Cabrera & Schwabe 132, 148*) and found them belonging to *P. calchalquensis* Hack. We consider the species therefore to be absent, or not known from Argentina.

Fig. 2. Geographic distribution of *Poa gymnantha*.

Karyology. — The somatic chromosome number $2n = 10x = 70$ was counted in *Poa gymnantha*. A metaphase plate, a karyogram and the idiogram are shown in Fig. 3. The basic karyotype of this species comprises 4 m (numbers 1 to 4), and 3 sm (numbers 5 to 7) chromosomes. Satellites were not seen. The total length of the basic chromosome set (x) is 28.22 µm.

Caryopsis formation. — Caryopsis formation starts with the development of a unicellular (Fig. 4A) or bicellular (Figs. 4B; 5,0) archesporium. In many ovules degenerating tetrads of megaspores indicate that meiotic divisions had occurred (Figs. 4C; 5,1) but because of a failure of chromosome segregation, all the megaspores (or gynospores) are abortive; thus, embryo sac initials differentiate from another nucellar cells (Fig. 5,0). Subsequently, two different developmental patterns may occur:

Pattern 1. When vacuolization precedes nuclear division (Fig. 5,2–7), the nucleus is pulled out towards the micropylar end; nuclear divisions result in a 2-nucleate unipolar embryo sac (Figs. 5,4; 6A–B). At this stage, cytokinesis occurs giving rise to an embryo sac (gynophyte) with one egg cell and one endospermogenetic cell (Fig. 5,5). The egg cell develops a proembryo by parthenogenesis and the endospermogenetic nucleus, bearing the same genome of the egg cell, generates the endosperm (Figs. 5, 6; 6C–D). We have observed an 8-celled proembryo with a multicellular endosperm, cellular around the embryo and coenocytic elsewhere (Figs. 5,7; 6E–F).

Fig. 3. A, somatic metaphase of *Poa gymnantha* ($2n = 70$); ▶ **B**, karyogram of the metaphase in A showing seven chromosome groups and the respective idiogram. Scale bar = 10 µm.
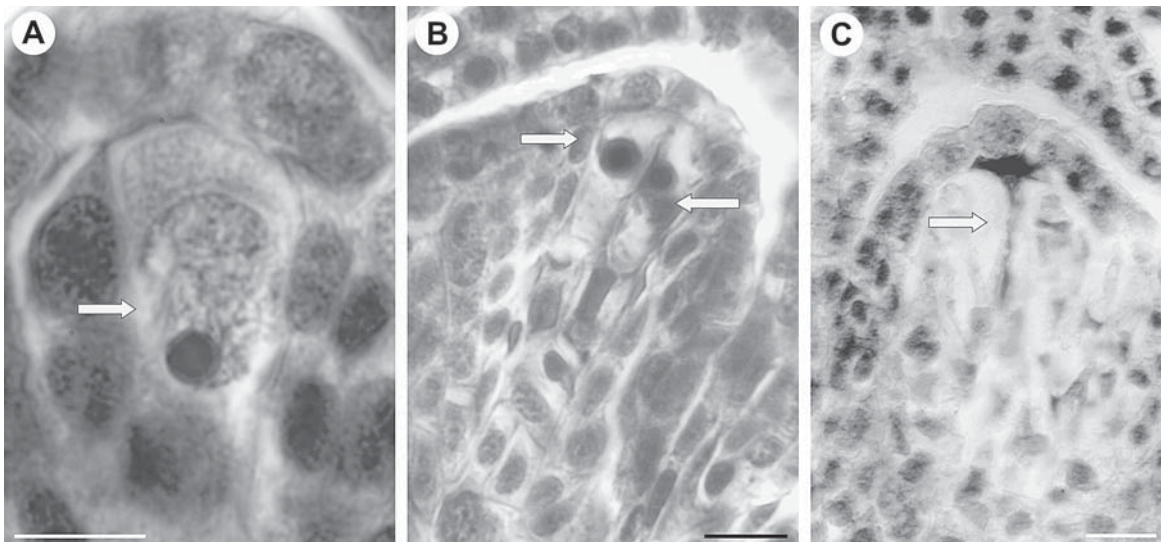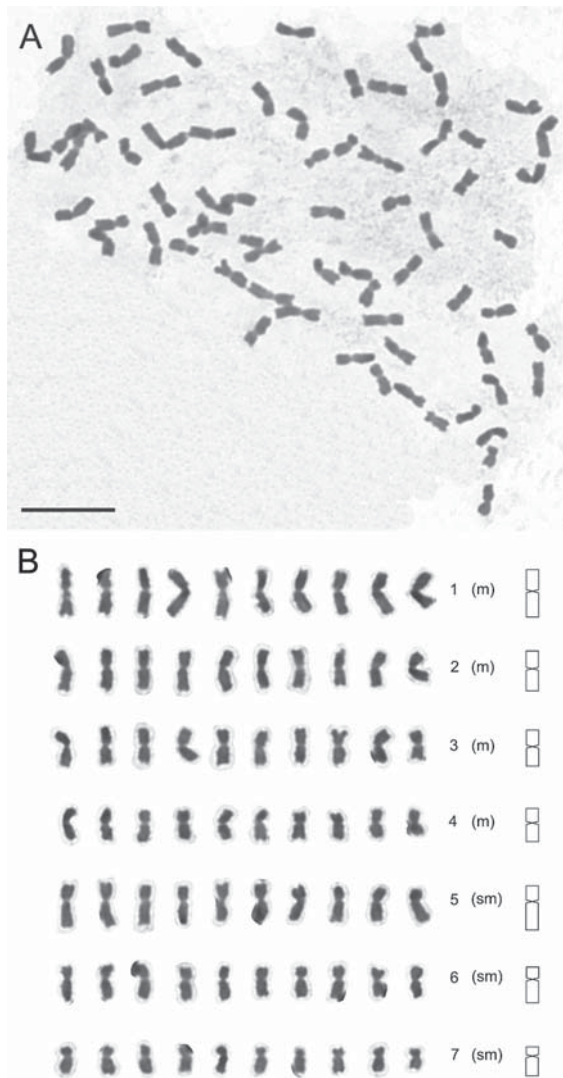

Fig. 4. Embryo sac development in apomictic *Poa gymnantha*. A, single celled archesporium; **B**, bicellular archesporium; **C**, degenerating tetrad of megaspores. Scale bars: 20 µm. (A), 15 µm (B, C).
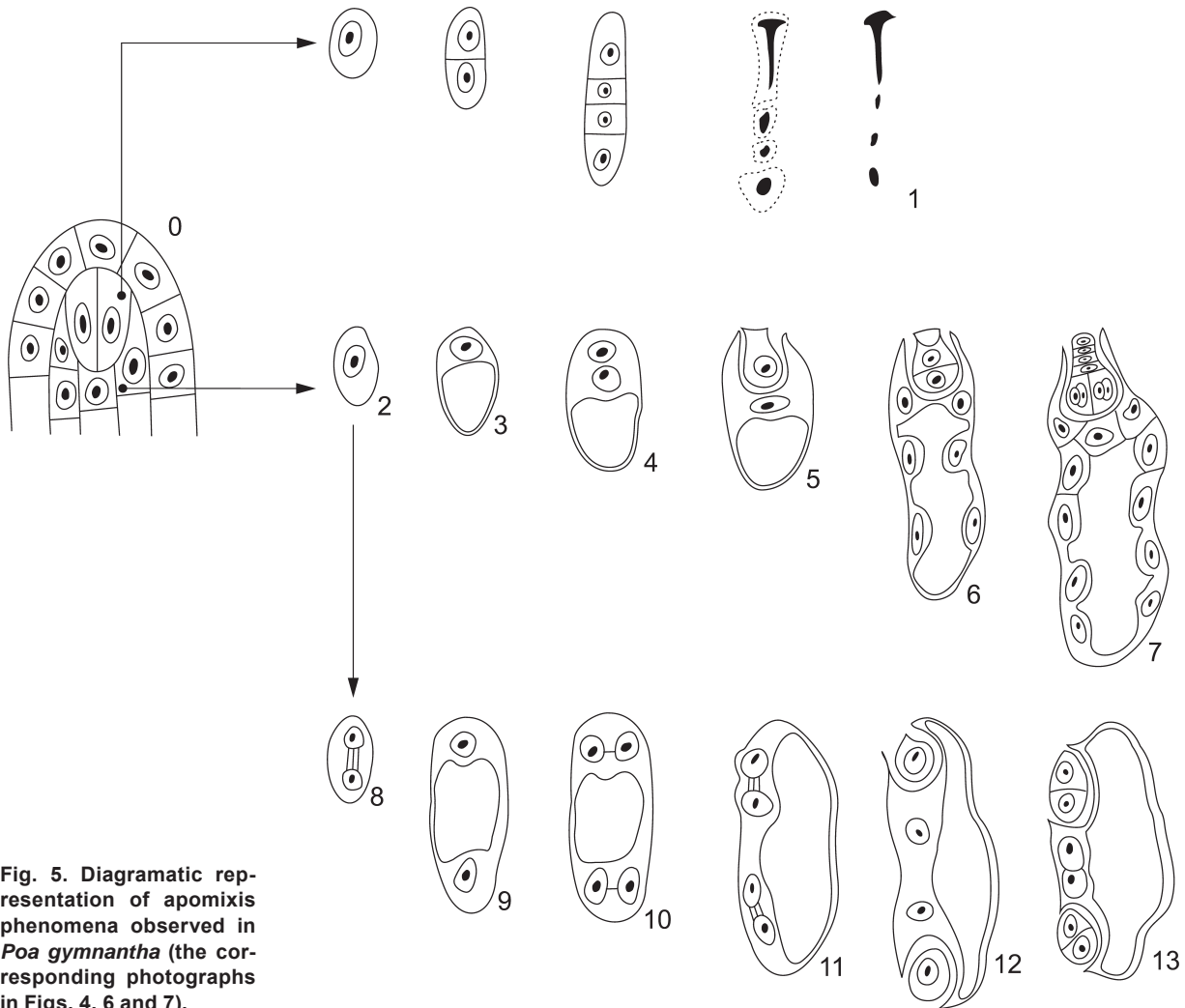
Fig. 5. Diagramatic representation of apomixis phenomena observed in *Poa gymnantha* (the corresponding photographs in Figs. 4, 6 and 7).
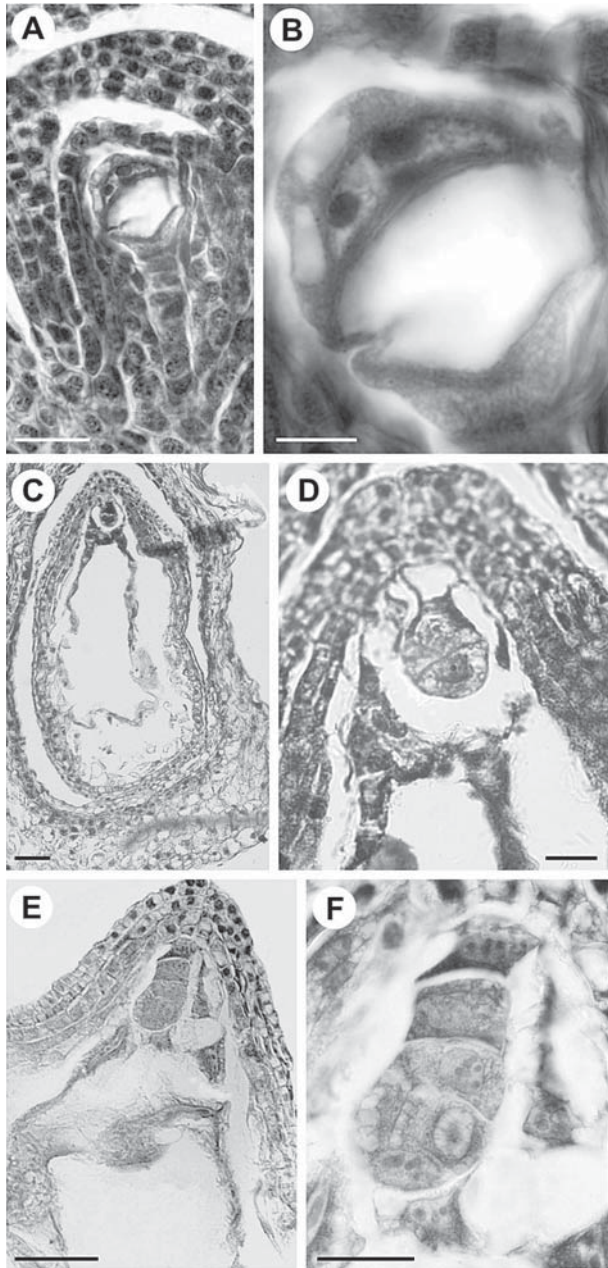
Pattern 2. When vacuolization follows nuclear division (Fig. 5,8–13), a 2-nucleate embryo sac is formed, bearing a central vacuole and two nuclei, one at each pole (Figs. 5,9; 7A). Nuclear division occurs in both nuclei giving rise to a four nucleate bipolar embryo sac (Figs. 5,9–10; 7B). At this point, cytokinesis might occur originating two egg-cell like structures and a 2-nucleate coenocyte in between (Figs. 5,12–13; 7C). The coenocytic nuclei may fuse and both egg-cell structures may reach at least the 2–3-celled stage by mitotic division. Apparently, there is no caryopsis formation because of the absence of endosperm development.

DISCUSSION

Poa gymnantha is a decaploid with $x = 7$. The fact that its chromosomes can be arranged in ten groups, based on their size and morphology, suggests that it is an autopolyplo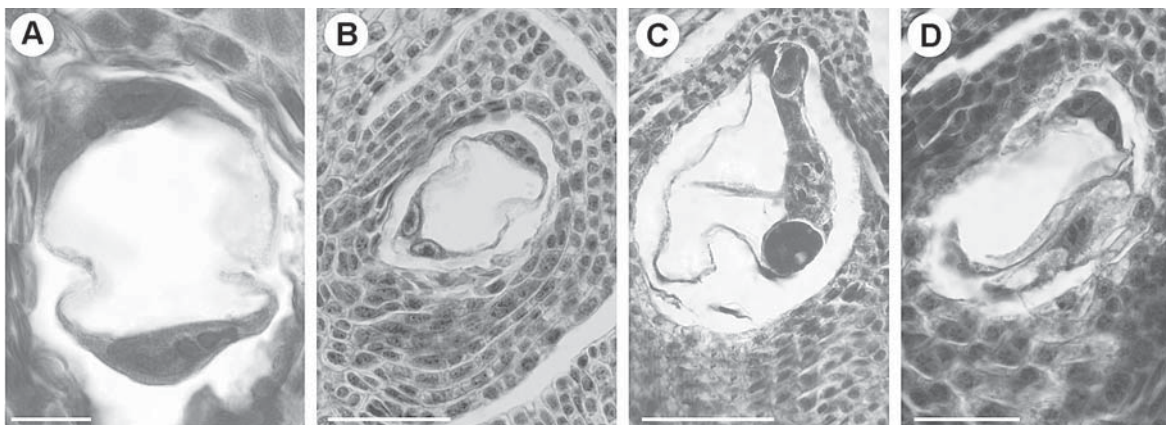id (Fig. 2). So far, $2n = 70$ has been reported only in 22 species of *Poa* (cf. Fedorov, 1969; Moore, 1973, 1977; Goldblatt, 1981, 1984, 1988; Goldblatt & Johnson, 1990); thus, it should be considered as an infrequent chromosome number for the genus, which includes around 500 species (Negritto & Anton, 2006) with ca. 235 of them investigated karyologically.

The general association between apomixis and polyploidy in flowering plants is well known (Savidan, 2000; Richards, 2003). Grass polyploids comprise roughly 44% of the species of the family, with basic chromosome numbers of $x = 2$, 4–13, 15 and 19 (Gaut, 2002). Furthermore, polyploidy is considered by Hilu (2004) one of the prominent features of grass genome evolution. In parallel, it has been demonstrated that gametophytic apomixis is always related to polyploidy; the species involved in such cases, conform agamic complexes in which sexual and apomictic genotypes coexist. A good example is *Paspalum simplex*, in which diploids are sexual and polyploids apomictic (Quarin, 1992; Cáceres & al., 2001).

Megasporogenesis is highly disturbed in *Poa gymnantha* because of irregularities during chromosome segregation in meiosis I, in a way that all four tetrads are aborted. Embryo sac development occurs through apospory (Gustafsson, 1947), being the first record of aposporic apomixis in South American *Poa*. Soreng (1990: 1384) mentioned the occurrence of apomixis in *P. chamaeclinos* Pilg.—another pistillate species from South America closely related to *P. gymnantha*—but the mechanism was not described.

Apospory is by far the most common mechanism of apomixis in grasses, accounting for more than 95% of known apomictic species (Bashaw & Hanna, 1990); furthermore, the majority of the apomictic genera cited by Kellogg (1990) are aposporous. Diplospory is known to occur in *Poa* (Muntzing, 1940) but there is no evidence for it in *P. gymnantha*.

During megagametogenesis, two patterns were detected in *Poa gymnantha*, only one of them leading to embryo formation. Vacuolization prior to nuclear division appears to be necessary to form a viable caryopsis (Pattern 1). In general, more than one embryo sac per ovule starts its development, but only one succeeds. The low number of full developed caryopsis observed is always associated with abortive structures, which are the result of the abnormal behaviours of some embryo sac initials (Fig. 7D).

When vacuolization follows nuclear division (Pattern 2), two parthenogenetic egg-cells-like structures

◀ **Fig. 6. A, 2-nucleate unipolar embryo sac; B, magnification of A; C, 2-celled embryo and endosperm, D, magnification of C; E, 8-cellular proembryo with cellular endosperm near the embryo and coenocytic in the rest; F, magnification of E. Scale bars: 60 μm (A, C, E), 10 μm (B), 20 μm (D), 50 μm (F).**

Fig. 7. A, 2-nucleate embryo sac; B, 4-nucleate embryo sac; C, 2 egg-cell-like structures and a 2-nucleate coenocyte in between; D, two embryo sacs. Scale bars: 25 μm (A), 60 μm (B), 100 μm (C), 50 μm (D).

▼

are formed, but they do not go any further because of the absence of endosperm development. As a rule, in the normal process of embryogenesis, the endospermogenic nucleus divides prior to the zygote, giving rise to a great number of endosperm nuclei while the zygote is still at a resting stage. Later on, cellularization starts to surround the zygote from the micropylar end toward the chalazal one.

Fertilization of the polar nuclei is believed to be essential for seed development in all known apomictic grasses (Bashaw & Hanna, 1990). Greene (1984) however, revealed autonomous development of embryo and endosperm in diplosporic variants of *Calamagrostis stricta* subsp. *inexpansa* (A. Gray) Greene. Furthermore, Anton & Connor (1995) cited *Cortaderia* and *Lamprothyrsus* as autonomous apomictics (embryo and endosperm), in which precocious seed development occurs before inflorescence emergence in species represented only by pistillate populations. The behaviour of *Poa gymnantha* is identical to these, since all the processes we refer to, have been found in female inflorescences still included in the sheath of the flag leaf, so syngamy or pseudogamy are prevented.

Since *Poa gymnantha* produces only functionally pistillate flowers with highly reduced staminodia, microsporogenesis and microgametophyte formation are suppressed as well as pollination.

Sexually reproducing flowering plants require a ratio of two maternally to one paternally derived genomes for normal endosperm and seed development (Vinkenoog & Scott, 2001). For autogamous endosperm development without fertilization, an alteration of these requirements is necessary (Talent & Dickinson, 2007). Vinkenoog & Scott (2001) propose that, in either genome, a set of genes is silenced in a sex-specific way by genomic imprinting. Removal of the imprints from the maternal derivates lead to the expression of normally maternally silenced genes, and effectively supplies the missing paternal genome. This might be applicable to the case of *Poa gymnantha*.

It is interesting to note that apomixis is not distributed randomly, but prevails in certain families (Poaceae, Rosaceae, Asteraceae) at high latitudes, suggesting that certain taxa in these habitats are preadapted to apomixis (Richards, 2003). It has been demonstrated that some Arctic species of *Poa* have three asexual mechanisms within the same population, i.e., vegetative spread, vegetative proliferation and apomixis (Richards, 1990). Soreng & van Devender (1989) indicate that, in general, the frequency of apomixis in species of *Poa* in western North America is associated with cold climates and alpine zones. The occurrence of apomixis in a high Andean species of *Poa* might suggest a similar situation in South America.

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Appendix. Material used in this study.

BOLIVIA. **La Paz:** Omasuyos, Cantón Huarina, Comunidad de Moco-Moco, 3,810 m, *Loza de la Cruz 46* (LPB); Próximo al camino que conduce a la cumbre del Huayna Potosí, 4,800 m, *R. López s.n.* (CORD 1130); B. Saavedra, Cotapampa, isla con rocas calcáreas, en la laguna Sosa Khocha, en estepa graminosa, *Menhofer 2087* (LPB); Caxata, Cotacocha, Sierra de Tres Cruces, *Ceballos & al. 500* (SI); Aroma, Huaraco, Abra Cuñamani, jenseits, *Ruthsatz B1725* (Herb. Ruthsatz); Murillo, Valle del Zongo, entre Ullumani y el Zongo, Estación Zongo, *Cocucci, Negritto & Romanutti 906* (CORD); Murillo, Valle del Zongo, entre Ullumani y el Zongo, Estación Zongo, *Cocucci, Negritto & Romanutti 911* (CORD, LPB); Murillo, Strasse nach Santiago de Collana, südl. Cerro Cuñamani, *Ruthsatz 1637* (LPB); Murillo, Camino al Valle del Zongo, Valle del Zongo, Pasando la represa del Zongo, 16°11′59″ S, 68°07′00″ W, 4,700 m, *Negritto & Meneses 128* (CORD, LPB); Bajando hacia la represa hidroeléctrica Zongo, *Negritto & Meneses 137* (CORD); Murillo, a 10 km de Unduavi, *Negritto & Ortuño 152* (CORD); Murillo, Valle de La Paz, Putupampa, *R. López 329* (LPB); Murillo, Calacoto, a 64 km hacia el Nevado Illimani, sobre el pueblo de Pinaya, al pie del Illimani, *Beck 9080* (LPB); Murillo, 6.6 km N of the road to Valle del Zongo, on the road to Chacaltaya, *Solomon 11775, 11781* (LPB); Murillo, Zongo Pass: Grassland with rock outcrops and intermittent swamps and pools, *Renvoize 5202* (K, AAU); Murillo, 4.5 km N of Milluni on road to Zongo pass (ca. 3 km S of pass), *Solomon 13206*, "moist puna, grassy slopes with rock outcrops" (LPB); Murillo, pass at the head of the Valle del Zongo and lower slopes of Nevado Huayna Potosí, *Solomon 13222* (LPB, SI); Murillo, Vicinity of the dam at Lago Zongo, *Solomon 13368* (LPB, SI); Murillo, a 1 km al N (abajo) de la represa del Lago Zongo, Puna húmeda, *Solomon 15840* (LPB; SI); Omasuyos, a 49 km del camino principal a la Peñas, vía Mina Fabulosa, *Beck 2908* (LPB; SI). **Oruro:** Abaroa, Laguna Tacagua, Abel Kom, *Ruthsatz 10390* (Herb. Ruthsatz); Sajama, Sururua-Tal, *Ruthsatz 2829, B2837* (Herb. Ruthsatz). **Potosí:** Nor Lípez, 16 km N de San Pablo, *Peterson & al. 13005* (LPB); Sud Lípez, 44 km SW de San Antonio, *Peterson & Soreng 13050* (LPB); Sud Chichas, 8 km N de San Vicente, *Peterson & Annable 12865* (LPB).

CHILE. **I Región**: Parinacota, a 4 km al N de Termas Chirigualla, SW Chungara, *Peterson & Soreng 15758* (CONC 151895), *Peterson & Soreng 15768* (CONC 151900); Arica, Portezuelo de Chapiquiya, 18°19′ S, 69°28′ W, 4,250 m, *Ricardi & al. 204* (CONC 27737); Parinacota, 66 km NW de Colchane hacia Chilcayo, 18°55′ S, 68°56′ W, 4,464 m, *Peterson & Soreng 15675* (CONC 152369); Iquique, 106 km NE de Huara, camino hacia Colchane, 19°34′ S, 68°58′ W, 4,340 m, *Peterson & Soreng 15656* (CONC 152442).

PERÚ. **Ancash:** 41 km east of Yunga, *Renvoize & Laegaard 5095B* (K); Bolognesi, Paso de Chonta, distrito de Ticllos, *Cerrate 2650* (USM); Huaraz, encima de Huaraz, cerca de Cajamarquilla, *Ferreyra 14313* (USM). **Arequipa:** Arequipa, Sumbay, *Weberbauer 6905* (BAA, F, MOL, S). **Ayacucho:** Betroren Pisco und Ayacucho, *Weberbauer 5440* (S); Lucanas, Pampa Galeras, a 1 km E del Campamento, *O. Tovar 6642* (USM). **Huancavelica:** Castrovirreina, Pisco por Ayacucho, *Weberbauer 5440* (F). **Junín:** La Oroya, Pachacayo, Hacienda Cochas, minas von Calzado, *Gutte 3039* (CORD); Mancocutana, Loive, *Infantes 2226* (BAA). **Tacna:** Tarata, Laguna Casire, *La Torre 2406* (USM).