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ORIGINAL ARTICLE

# Sporophytic apomixis in polyembryonic *Handroanthus serratifolius* (Vahl) S.O. Grose (Bignoniaceae) characterizes the species as an agamic polyploid complex

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**Abstract** Neopolyploidy has been associated with gametophytic apomixis and breakdown of gametophytic self-incompatibility. Nevertheless, Bignoniaceae presents agamic polyploid complexes with neopolyploidy associated to sporophytic apomixis. Apomictic populations are commonly polyploid, polyembryonic and self-fertile, while diploids are mostly late-acting self-incompatible (LSI) and monoembryonic. Contrastingly, *Handroanthus serratifolius* shows hexaploid monoembryonic and polyembryonic populations, although breeding system has been studied only for monoembryonic individuals, which are LSI. Our aim here was to investigate breeding system and early embryology in polyembryonic individuals of *H. serratifolius* to define if they form an agamic polyploid complex. Experimental pollinations and histological analyzes of ovules and young seeds were carried out. Megasporogenesis and megagametogenesis occurred as in other sexual species of Bignoniaceae. The polyembryonic individuals were self-fertile and double fertilization was observed both after self and cross-pollinated pistils. Adventitious embryos originated from the hypostasis and integument of the ovule, indicating sporophytic apomixis. Adventitious embryo precursor cells occurred in all pistils, including unpollinated ones. But unpollinated pistils aborted possibly due to absence of endosperm, and pollination was required for fruit-set (pseudogamy). It is possible that the self-fertility in polyembryonic individuals ensues as the initial endosperm of self-fertilized ovules supply early adventitious embryos development, and these embryos would later prevent the abortion of selfed pistils. The sporophytic apomixis in polyembryonic populations and the occurrence of sexual monoembryonic populations of *H. serratifolius* allows us to consider the species part of an agamic polyploid complex. But in contrast with other *Handroanthus* agamic complexes, both apomictic and sexual LSI plants were hexaploid.

**Keywords** Megagametogenesis · Megasporogenesis · Neopolyploidy · Polyembryony · Pseudogamy · Self-fertility

## Introduction

Euploidy is a case of polyploidy that consists in whole genome duplication, with the somatic chromosome number being an integral multiple of the monoploid number (Ramsey and Schemske 2002). Neopolyploidy is viewed as recent euploidy events (Ramsey and Schemske 2002), and has been commonly associated with the breakdown of gametophytic self-incompatibility systems (GSI) (Richards 1986; de Nettancourt 1997), as well as with the expression of gametophytic apomixis in angiosperms (Carman 2007; Whitton et al. 2008; Hörandl 2010). But the mechanism of this self-incompatibility breakdown is yet to be clearly understood.

The diploid Bignoniaceae show a predominance of $2n = 40$ chromosomes (Goldblatt and Gentry 1979; Gentry

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1980), and most of them present late-acting self-incompatibility—LSI (Gibbs and Bianchi 1993, 1999; Bittencourt et al. 2003; Bittencourt and Semir 2005; Gandolphi and Bittencourt 2010). Neopolyploidy has been observed in species of *Anemopaegma*, *Dolichandra*, *Pyrostegia* and *Handroanthus*, with records of $2n = 60$, 80 and 120 chromosomes (Goldblatt and Gentry 1979; Piazzano 1998; Firetti-Leggieri et al. 2011; Alves et al. 2013; Sampaio et al. 2013b). Most neopolyploid species of this family are self-fertile, although there are no evidences that neopolyploidy per se is the primary cause of self-fertility in these species. Among the seven neopolyploid species studied for their breeding systems, six are reported as self-fertile (Gobatto-Rodrigues and Stort 1992; Bittencourt and Moraes 2010; Firetti-Leggieri et al. 2013; Sampaio et al. 2013a), with the exception of *H. serratifolius* (Vahl) S.O. Grose that is self-sterile (Alves et al. 2013).

In Bignoniaceae, neopolyploidy also seems to be related to sporophytic apomixis expression (Bittencourt and Moraes 2010; Sampaio et al. 2013b), which contrasts to the classical view of sporophytic apomixis occurring mostly in diploid or paleopolyploid species (Carman 2007; Whitton et al. 2008). Based on histological analysis of the tetraploid *Anemopaegma acutifolium* DC., *H. chrysotrichus* (Mart. ex DC.) Mattos and *H. ochraceus* (Cham.) Mattos, sporophytic apomixis generates polyembryonic seeds in which sexual and adventitious embryos seem to coexist (Costa et al. 2004; Bittencourt and Moraes 2010; Sampaio et al. 2013b). Actually, six out of the seven neopolyploid species mentioned above present high rates of polyembryony (Costa et al. 2004; Bittencourt and Moraes 2010; Mendes-Rodrigues et al. 2012; Firetti-Leggieri et al. 2013; Sampaio et al. 2013a, b), with the exception of *Pyrostegia venusta* (Ker-Gawl) Miers (Sampaio 2010).

The self-incompatible species of Bignoniaceae show a kind of LSI on which double fertilization occurs regardless the kind of pollination, promoting endosperm formation and proembryonal tube development before abscission of selfed pistils (Gibbs and Bianchi 1993, 1999; Bittencourt et al. 2003; Bittencourt and Semir 2005; Gandolphi and Bittencourt 2010). In this sense, some studies suggest that the self-fertility found in these apomictic species would be caused by the development of adventitious embryos in selfed pistils, whose presumed hormonal signaling for the mother plant would prevent selfed pistil abortion (Oliveira et al. 1992; Bittencourt and Semir 2005; Bittencourt and Moraes 2010; Sampaio et al. 2013b). But it is not clear how the sporophytic apomixis is triggered in these Bignoniaceae and how the polyploidy may affect gene dosage effect or developmental asynchronies usually associated with the loss of self-incompatibility (Pandey 1968; Richards 1986; Carman 2007).

The coexistence of sexual and apomictic populations give rise to agamic polyploid complexes that commonly involve intricate evolutionary patterns, many lineages, and high variability rates (Asker and Jerling 1992). Among the Bignoniaceae, agamic polyploid complexes have been reported for three species of *Anemopaegma* (Firetti-Leggieri et al. 2011, 2013; Sampaio et al. 2013b) and two of *Handroanthus*. *Handroanthus ochraceus* presents apomictic/polyploid (Costa et al. 2004; Sampaio 2010) and also sexual/diploid populations (Gibbs and Bianchi 1993; Barros 2001; Sampaio 2010), while for *H. chrysotrichus* only apomictic polyploid populations were reported so far (Piazzano 1998; Bittencourt and Semir 2005; Bittencourt and Moraes 2010).

*Handroanthus serratifolius* is taxonomically closely related to *Handroanthus ochraceus* and *H. chrysotrichus* and widely distributed in the Neotropics (Gentry 1992; Grose and Olmstead 2007). They are hexaploid ($2n = 120$ chromosomes) trees, with self-sterile and monoembryonic populations (Alves et al. 2013), but also with polyembryonic populations with unknown breeding system (Mendes-Rodrigues et al. 2012). Monoembryonic populations of *H. serratifolius* occur in Minas Gerais State (Sampaio 2010; Alves et al. 2013) and polyembryonic populations have been observed both in Goiás and Minas Gerais states, in Southeastern Brazil (Mendes-Rodrigues et al. 2012). The self-sterility found in hexaploid individuals of *H. serratifolius* suggests that polyploidy is not the main cause of self-fertility in neopolyploid Bignoniaceae (Alves et al. 2013).

In this context, our aims here were to compare the breeding system of polyembryonic populations of *H. serratifolius* with the already studied monoembryonic ones and to verify if polyembryony is also the result of sporophytic apomixis expression. The data would make it possible to define if this species are also part of agamic polyploid complexes as other *Handroanthus* and *Anemopaegma* species, adding important information to the evolution of those complexes and also to the relationship among ploidy and breeding systems in the Bignoniaceae.

## Materials and methods

### Species and studied sites

*Handroanthus serratifolius*, one of the most common South American yellow trumpet tree species, is an up to 30 m tall tree with ample morphological variability (Gentry 1992). It possesses palmately 5–7 foliolate leaves with serrate, mostly glabrous or inconspicuously lepidote leaflets. The calyx is campanulate, sparsely pubescent and the yellow corolla is tubular-infundibuliform. The fruit takes about

30 days to reach maturity, and is a linear, almost glabrous, inconspicuously lepidote capsule, which shows longitudinal grooves and many extrafloral nectaries. These nectaries resemble warty lumps (Gentry 1992). The species is deciduous, showing a massive flowering during the dry season, which has been recognized as a "big bang" type flowering phenology (sensu Gentry 1974).

The studies were conducted in 2012 and 2013 using ten native polyembryonic individuals (the same trees used in the study of Mendes-Rodrigues et al. 2012), which have been proven to be hexaploid (Sampaio 2010), located in Luziânia, Goiás, Brazil (16°14′14.4″S, 47°56′31.8″W). We also used three hexaploid and polyembrionic ($2n = 120$ chromosomes and 78 % of polyembryonic seeds; M. F. Alves personal observation) cultivated individuals located in Uberlândia, Minas Gerais, Brazil (18°55′04.43″S, 48°15′38.40″W). Vouchers were deposited in *Herbarium Uberlandense* (HUFU), Uberlândia, Minas Gerais, Brazil, under the following registration numbers: Luziânia—HUFU 48937; Uberlândia—HUFU 60076.

### Experimental pollinations

Cross- and self-pollinations were carried out in the individuals in Luziania (2012), and only self-pollinations were done in the individuals in Uberlândia (2013). Flower buds were bagged with nylon mesh to avoid contact with possible floral visitors. Hand self- and cross-pollinations were done in previously emasculated first-day flowers. In self-pollinations, we used the pollen grains of the same flower, and in cross-pollinations we used pollen from individuals at least 10 m distant. In Luziania, some bagged buds were marked to check for spontaneous self-pollination and others were emasculated before isolation to confirm the absence of autonomous apomixis. Natural fruit set was estimated by monitoring marked, non-bagged flowers. Fruit set was used to evaluate breeding system (Bittencourt and Moraes 2010; Alves et al. 2013).

### Histological analysis

In order to analyze if megasporogenesis and megagametogenesis occured as in other sexual species of Bignoniaceae, or if there was any evidence of gametophytic apomixis in hexaploid polyembryonic individuals, flower buds of 1–6 cm long and first-day flowers were collected in four individuals in Luziânia and one individual in Uberlândia. In order to verify the possible emergence of adventitious embryos in different pollination treatments, pistils from hand self- and cross-pollinations were collected 24, 48, 72, 96, 120 and 144 h after pollinations in individuals in Luziânia. Moreover, unpollinated pistils were collected 24, 48, 72, 96 and 120 h after the onset of anthesis in Luziânia to verify if adventitious embryos or parthenogenetic embryos from unreduced megagametophytes were formed. To analyze further stages of embryos development, fruits at different stages (1–12.5 cm long) were collected from two individuals in Luziânia and one in Uberlândia.

Pistils and fruits were fixed in 1 % glutaraldehyde and 4 % formaldehyde solution in sodium phosphate buffer 0.1 M, pH 7.2 (Mc Dowel and Trump 1976). Ovary and fruit walls were removed with the aid of scalpel and tweezers under stereomicroscope Olympus SZX12 so as to expose the ovules or developing seeds stuck to the septum. The material were dehydrated in an ethanol series and passed through a series ethanol: chloroform (3:1, 1:1, 3:1) to remove epicuticular waxes. Embedding was carried out with hydroxyethylmethacrylate (Gerrits and Smid 1983) and serial sections from 3 to 5 µm thick were obtained using a rotary microtome Leica RM2135 with an 8-mm wide Leica glass knife. The sections were stained with Toluidine Blue O 0.05 % in sodium benzoate buffer, pH 4.4 (Feder and O'Brien 1968) and slides were sealed with Permount®. Analyses and photomicrographs were made using a light microscope Olympus BX51 equipped with a digital camera Olympus DP70.

## Results

### Fruit set from experimental pollinations in polyembryonic individuals

Fruits from cross-, self- and natural pollinations started to develop (Table 1) indicating self-fertility. But fruit-set was very low for all treatments in 2012, being higher only for self-pollinations in Uberlândia, in 2013. A few mature fruits were obtained. A single mature fruit was obtained from cross-pollinations, measuring 22.5 cm long and bearing 70 seeds. The five mature fruits obtained from self-pollinations were $24.5 \pm 4.5$ cm long with $123 \pm 18.9$ seeds per fruit (mean $\pm$ standard deviation); and the only mature fruit obtained from natural pollination was 21.5 cm long with 91 seeds. All seeds analyzed in those fruits showed well developed embryos.

### Megasporogenesis and megagametogenesis

*Handroanthus serratifolius* presents anatropous, unitegmic and tenuinucellate ovules (Fig. 1a). The megaspore mother cell (MMC) was elongated in shape, with a conspicuous nucleus (Fig. 1b). The characteristic cytological steps of meiosis were verified starting from the MMC. In meiosis I, a metaphasic plate perpendicular to the longer axis of the MMC was observed (Fig. 1c), followed by the formation of

**Table 1** Results of experimental pollinations in hexaploid polyembryonic individuals of *Handroanthus serratifolius* (Vahl) S.O. Grose in Luziânia, GO, and Uberlândia, MG, Brazil

| Experimental pollinations | *H. serratifolius* This work | *H. serratifolius* Alves et al. (2013) |
|---|---|---|
| Natural pollination | 2.52 % (3/119)[a] | 0 % (0/126)[c] |
| Hand cross-pollination | 0.9 % (1/101)[a] | 34.9 (36/103)[c] |
| Hand self-pollination | 1.92 % (2/104)[a] | 1.8 % (2/110)[c] |
| Hand self-pollination | 6.4 % (5/78)[b] | |
| Spontaneous self-pollination | 0 % (0/100)[a] | 0 % (0/110)[c] |
| Emasculation | 0 % (0/104)[a] | 0 % (0/110)[c] |

Similar data for monoembryonic sexual individuals studied by Alves et al. (2013) is presented for comparative purpose

[a] Treatments in Luziania population in 2012. Fruit-set % (fruits/flowers) 15 days after treatments

[b] Treatments in Uberlândia individuals in 2013. Fruit-set % (fruits/flowers) 30 days after treatments

[c] Data from Alves et al. (2013). Treatments in Uberlândia and Uberaba individuals in 2011. Fruit-set % (fruits/flowers) 24–37 days after treatments

a dyad of megaspores (Fig. 1d). Five ovules were observed just after the second cycle of meiosis division and in four of them, synchronous meiosis II events occurred with the formation of linear tetrads. However, in the remaining ovule, we observed a triad of megaspores (Fig. 1e, f), indicating an asynchronous meiosis II in the dyad. In this ovule, the second division of meiosis II seemed to occur in the chalazal cell, due to a thinner cell wall between the chalazal megaspores (Fig. 1e). The three micropylar megaspores degenerate in most ovules observed, while the chalazal one remained intact (Fig. 1g). Of the 63 ovules analyzed in the stage of megaspores degeneration, one of them presented the two chalazal megaspores intact and only the two micropylar ones degenerating (Fig. 1h).

After the first mitosis of the megagametogenesis, the binucleate megagametophyte began to expand and showed several small vacuoles in the cytoplasm (Fig. 1i). Subsequently, a large central vacuole was formed between the nuclei and each nucleus moved into one pole of the megagametophyte (Fig. 1j). The two nuclei undergone simultaneously a second mitotic division, giving rise to a tetranucleate stage of the megagametogenesis (Figs. 1k; 2a, b). The third and final mitotic cycle of divisions of the four nuclei gave rise to an octonucleate megagametophyte (not shown). Since the beginning of the expansion of the megagametophyte, cells of the nucellar epidermis around the micropylar region of the embryo sac started to degenerate and ended up collapsing (Fig. 1i–k). From this stage up to the embryo sac maturity, the collapse of the nucellar epidermis was followed by the degeneration and collapse also of the inner cells of the integument, at the same time as the micropylar region of the megagametophyte expanded laterally (Fig. 2a–e).

One nucleus of the micropylar pole and one of the chalazal pole migrated to the center of the megagametophyte, constituting the polar nuclei of the central cell (Fig. 2c, e). During the cellularization of the megagametophyte, the two synergids and the egg cell showed small vacuoles and are organized in a triangular arrangement to form the egg apparatus (Fig. 2c, d). Subsequently, these cells undergone a pronounced expansion, after which each synergid showed a large vacuole toward the chalazal pole, and a small vacuole adjacent to the micropylar region (Fig. 2e), while the egg cell showed the cytoplasm and nucleus in the chalazal pole and a large vacuole in the micropylar pole of the cell (Fig. 2e). The three nuclei in the chalazal region, in turn, cellularize to form the antipodals (Fig. 2c–e). Of the 89 ovules analyzed during megagametogenesis or in mature megagametophyte stage, only one presented two megagametophytes (not shown).

## Double fertilization and endosperm development

After ovule penetration, the pollen tube discharged its cytoplasmic contents into one of the synergids, which a bit later showed dense cytoplasmic staining with a cytoplasmic loop between the central cell and the chalazal pole of the egg cell (Fig. 3a). Among the analyzed ovules (ca. 400 per treatment from 72 h onwards), 43 from self-pollinations and 54 from cross-pollinations presented the cytoplasmic loop evidencing fertilization, similar to the observed in all Bignoniaceae studied to date (e.g. Gibbs and Bianchi 1993; Bittencourt and Semir 2005). Sixty ovules from self-pollinations and 48 from cross-pollinations showed endosperm. After the triple fusion, the primary endosperm cell undergone karyokinesis (Fig. 3b) followed by cytokinesis. This first division was transverse and originated two cells, one in the micropylar chamber and one in the chalazal chamber (Fig. 3c). The second division of the endosperm was longitudinal in both chambers, giving rise to a four-celled endosperm (not shown). This cycle of divisions also occurred asynchronously among chambers, being faster in the chalazal one and giving an intermediate three-celled endosperm stage (Fig. 3d). The third cycle of divisions of the endosperm was transverse and occurred in the cells of the micropylar chamber, originating a six-celled endosperm (Fig. 3e). The next divisions observed in the endosperm 120 h after pollination were always transverse, originating a bisseriate endosperm (Fig. 3f). The two chalazal cells of the endosperm showed a dense cytoplasmic staining and were identified as the endosperm chalazal haustorium (Fig. 3d).

In unpollinated pistils, ovules showed a mature and unchanged megagametophyte from the onset of anthesis to

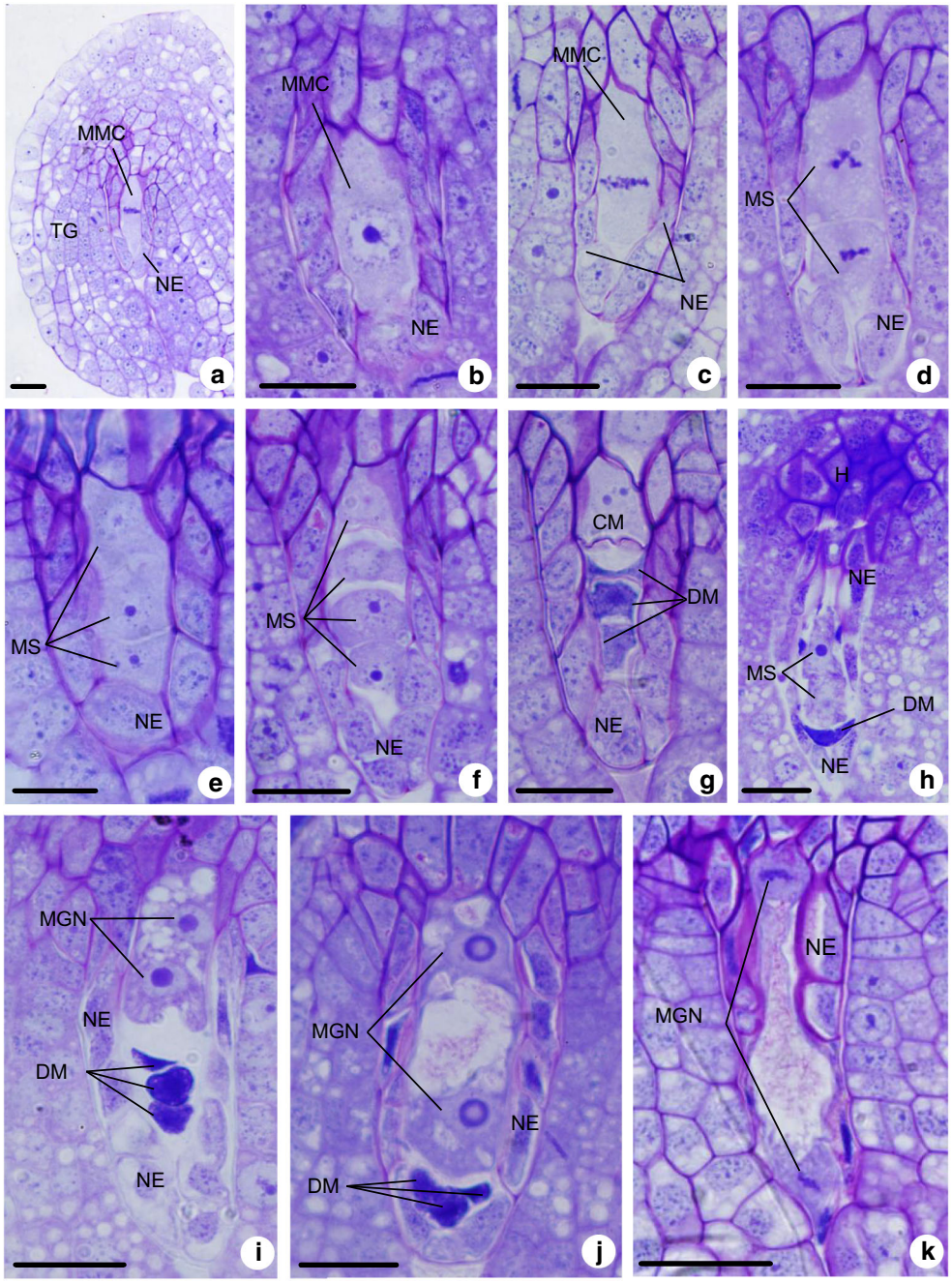


**Fig. 1** Longitudinal sections of ovules of *Handroanthus serratifolius*. **a** General view of the anatropous, unitegmic and tenuinucellate ovule, **b** megaspore mother cell, **c** megaspore mother cell during the metaphase of meiosis I, **d** dyad of megaspores during the metaphase of meiosis II, **e** triad of megaspores, **f** linear tetrad of megaspores, **g** degeneration of three megaspores located in the micropylar pole, **h** tetrad of megaspores presenting two chalazal megaspores intact and one micropylar megaspore degenerating. The second micopylar megaspore is also degenerating, however, it appears in a sequential section that has not been shown, **i** megagametophyte in the binucleate stage. Three degenerating megaspores can be observed in the micropylar region, **j** binucleate megagametophyte with a large central vacuole. Three degenerating megaspores can be observed in the micropylar region, **k** megagametophyte during the second mitotic division. *CM* chalazal megaspore, *DM* degenerating megaspores, *MMC* megaspore mother cell, *MGN* megagametophyte nuclei, *MS* megaspores, *NE* nucellar epidermis, *TG* integument. *Scale bars* 20 μm

120 h after flower opening. The polar nuclei in those pistils remained intact in all intervals after flower opening, pointing out that fusion only occurs after pollination ($n = 395$ ovules). From the fourth day after flower opening onwards, antipodals showed signs of degeneration, with the collapse of the nucleus and the absence of cytoplasm, and

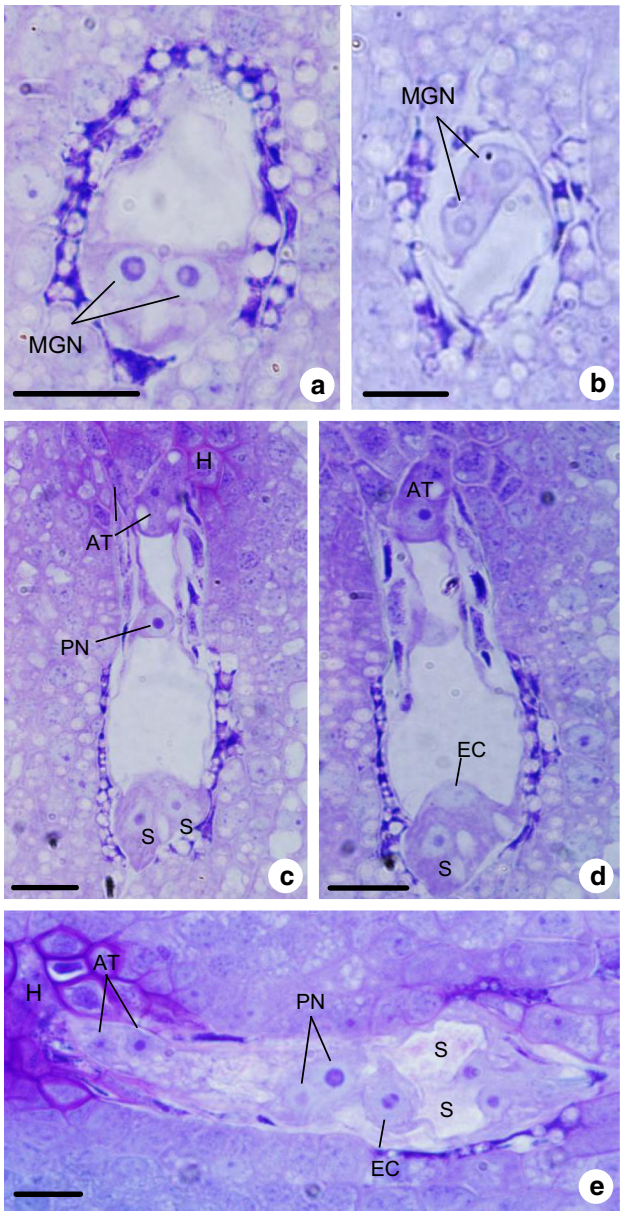


**Fig. 2** Longitudinal sections of ovules of *Handroanthus serratifolius*. **a**, **b** Sequential sections of the same ovule, **a** micropylar region of megagametophyte in tetranucleate stage, **b** chalazal region of megagametophyte in tetranucleate stage, **c**, **d** sequential sections of the same ovule showing the organization and cellularization of megagametophyte after the third mitotic division, **e** mature megagametophyte 24 h after the onset of anthesis of an emasculated flower. *AT* antipodes, *EC* egg cell, *H* hypostasis, *MGN* megagametophyte nuclei, *PN* polar nuclei, *S* synergid. *Scale bars* 20 μm

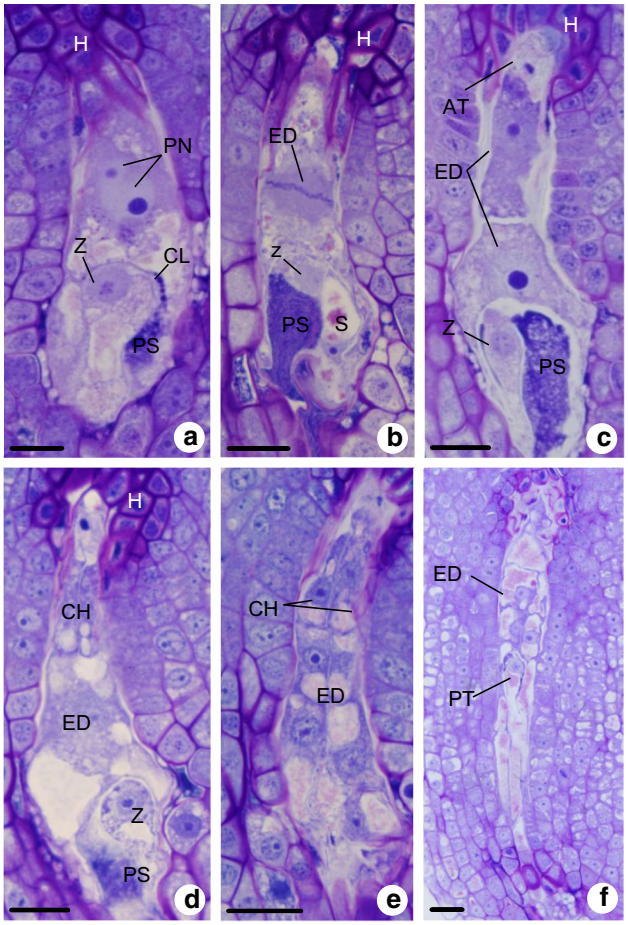


**Fig. 3** Longitudinal sections of ovules and young seeds of *Handroanthus serratifolius*. **a** Penetrated synergid 72 h after cross-pollination. Polar nuclei not yet fused, **b** penetrated and non-penetrated synergids, zygote and primary endosperm cell with a metaphase plate during mitosis 96 h after cross-pollination, **c** penetrated synergid, zygote and two celled endosperm 96 h after self-pollination, **d** penetrated synergid, zygote and three celled endosperm 120 h after cross-pollination, **e** six celled endosperm 144 h after self-pollination, **f** young seeds from a fruit of 1 cm long with bisseriate endosperm and the proembryonal tube. *AT* antipodes, *CH* chalazal haustorium, *CL* cytoplasmic loop, *ED* endosperm, *H* hypostasis, *PN* polar nuclei, *PS* penetrated synergid, *PT* proembryonal tube, *S* synergid, *Z* zygote. *Scale bars* 20 μm

after the fifth day after flower opening, other cells of the megagametophyte also started to degenerate (not shown).

## The origin of supernumerary embryos

Large cells were observed in the micropylar region and also in the hypostasis of the young seeds, as well as of the unfertilized ovules from pollinated and from unpollinated pistils, in all stages analyzed ($n = 26$ ovules of 1.029 observed), from 24 to 144 h after the onset of anthesis (Fig. 4a, b). These cells became elongated, encroaching the interior of the megagametophyte or the young endosperm, and were identified as the adventitious embryos precursor cells (AEP).

The young fruits showed adventitious embryos under development in both chalazal and micropylar regions. In seeds of a 1 cm long fruit, a bisseriate endosperm provided of a chalazal haustorium, and one zygotic proembryonal tube were observed (Fig. 3f). In seeds of fruits larger than 2 cm, the endosperm was multisseriate, due to the occurrence of

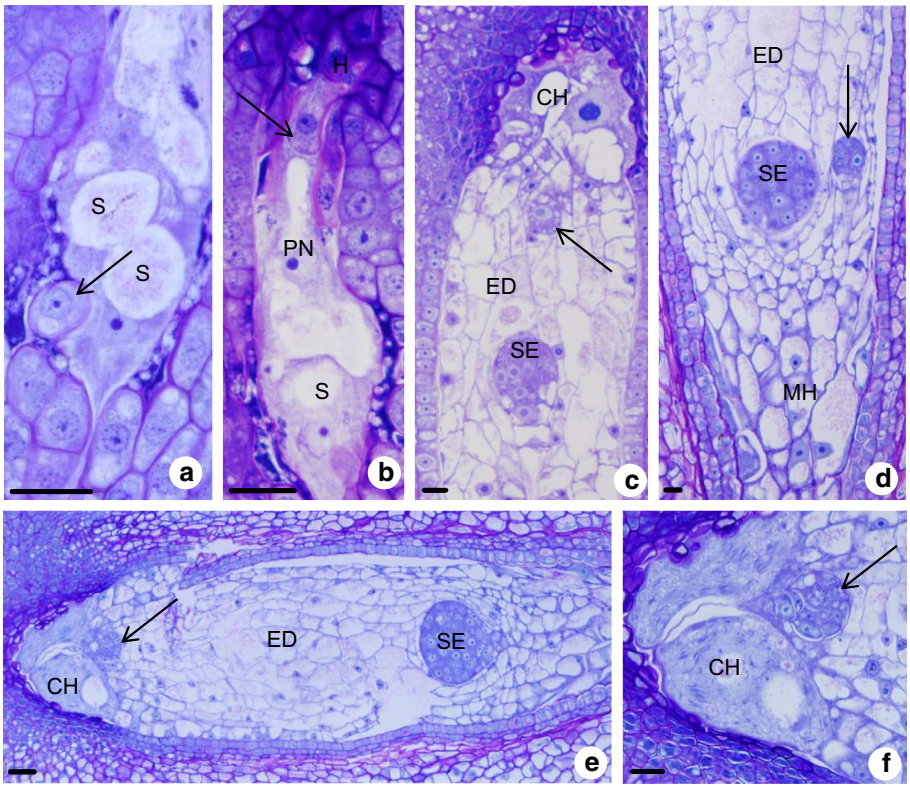


**Fig. 4** Longitudinal sections of ovules and young seeds in *Handroanthus serratifolius*. **a** Adventitious embryo precursor cell (AEP) in the integument of the micropylar region 48 h after self-pollination. This ovule was not fertilized, **b** AEP elongating to the cavity of the megagametophyte of a non-pollinated pistil 48 h after the onset of anthesis, **c** sexual embryo coming from the micropylar region and adventitious embryo coming from the hypostasis. Section of a seed from a fruit with 6.5 cm long, **d–f** sections of a seed from a fruit with 12.5 cm long, **d** sexual embryo and adventitious embryo coming from the micropylar region, **e** sexual embryo coming from the micropylar region and adventitious embryo coming from the hypostasis, **f** detail of figure **e** showing the adventitious embryo crossing the chalazal haustorium. *CH* chalazal haustorium, *ED* endosperm, *H* hypostasis, *MH* micropilar haustorium, *PN* polar nuclei, *S* synergid, *SE* sexual embryo. The *arrows* indicate adventitious embryo precursor cells or adventitious embryos. *Scale bars* 20 μm

periclinal and oblique divisions in this tissue (Fig. 4c–e). The chalazal haustorium, formed by two bulky cells with dense cytoplasmic content, increased in size (Fig. 4c, e, f). The micropylar haustorium differentiated when the endosperm was already multisseriate, and it was composed of many cells that were less deeply stained than those of the chalazal one (Fig. 4d). In some seeds a larger embryo was observed coming from the micropylar region and a smaller one coming from the chalazal region (Fig. 4c, e), which indicates that at least the latter is an adventitious embryo that originated from an AEP from the hypostasis. The larger embryo can be a sexual or another adventitious embryo. Adventitious embryos originating from the chalazal region presented a direct contact with the remaining cells of the hypostasis or the cells of the integument of this region (Fig. 4f). In other cases, two embryos were observed coming from the micropylar region, but one of them was always smaller than the other (Fig. 4d). The central position of some larger micropilar embryos suggests that it may be the sexual ones, but it was impossible to verify.

## Discussion

The experiment of hand self-pollinations indicated that polyembryonic individuals of *H. serratifolius* are self-fertile and developed selfed fruits without any anomaly as compared to those obtained by hand cross-pollinations. This result is very different from those for the monoembryonic individuals (Alves et al. 2013), where 110 self-pollinated flowers resulted in only two fruits (Table 1) with either degenerated seeds or seeds without embryos, and always much smaller (14.0 ± 4.2 cm) than those obtained in the present work. So, despite the low fruit set, the polyembryonic individuals seem to be self-fertile.

The megasporogenesis and megagametogenesis in polyembryonic individuals were similar to that found for the sexual species of the family, with regular meiosis, a linear tetrad and a reduced monosporic megagametophyte of the *Polygonum*-type (Govindu 1950; Bittencourt and Mariah 2002a, b). This regularity confirms the absence of gametophytic apomixis in *H. serratifolius*, which involves

the suppression or anomaly of meiosis and unreduced megagametophytes (Koltunow 1993; Koltunow and Grossniklaus 2003; Whitton et al. 2008). Some observed anomalies, as the presence of an ovule with a triad of megaspores, or two intact megaspores and two degenerating ones, or two megagametophytes were very rare and could not explain the high rates of polyembryony in this species (Mendes-Rodrigues et al. 2012). Our results showed that the polyembryony in these individuals is due to adventive embryony and characterize sporophytic apomixis, which has been also observed in polyembryonic populations of *H. chrysotrichus, H. ochraceus* and *Anemopaegma acutifolium* (Costa et al. 2004; Bittencourt and Moraes 2010; Sampaio et al. 2013b).

The development of the proembryonal tube (of sexual origin) started before the development of adventitious embryos in both self and cross pollinations, which lead us to consider the most robust and central embryo within the seed as the sexual one, as postulated to *A. acutifolium* (Sampaio et al. 2013b). In this sense, the sexual embryo seems to present greater chances of successful germination and establishment than the adventitious embryos. In *H. ochraceus*, although the sexual embryo is not considered the most robust, its development also seems to be possible (Costa et al. 2004). On the other hand, in *H. chrysotrichus*, the zygotic embryo is the less robust and fertilized ovules are able to develop into seeds even in its absence (Bittencourt and Moraes 2010). As we have only histological evidences of sexual embryo development for *H. serratifolius*, the possibility of the polyembryonic seeds formed after self-pollinations possess only adventitious embryos, cannot be ruled out. In any case, we considered these plants self-fertile independent of the sexual embryo complete development, since the endosperm development confirmed double fertilization success.

Our results indicate that the formation of the AEP does not depend on fertilization and early endosperm development, as they were formed even in ovules of unpollinated pistils. However, the absence of endosperm development in these pistils eventually led to abortion. The need of double fertilization to endosperm development indicates the occurrence of pseudogamy, which seems to be common in sporophytic apomictic species (Whitton et al. 2008; Bittencourt and Moraes 2010; Sampaio et al. 2013b). Fertilization and endosperm development in polyembryonic individuals of *H. serratifolius* were similar to those observed in other species of Bignoniaceae (Govindu 1950; Bittencourt et al. 2003; Bittencourt and Semir 2005; Sampaio et al. 2007; Bittencourt and Moraes 2010; Gandolphi and Bittencourt 2010; Sampaio et al. 2013b). The endosperm is cellular, with a two-celled chalazal haustorium differentiating in the early stages of development, as also observed in other *Handroanthus* species (Sampaio et al. 2007; Bittencourt and Moraes 2010), and characterizes the *Catalpa*-type (Mauritzon 1935). The bisseriate endosperm in initial stages of development that later becomes multisseriate with a multicellular micropylar haustorium is also observed in *H. chrysotrichus* and *H. ochraceus* (Sampaio et al. 2007; Bittencourt and Moraes 2010).

Although neopolyploidy is considered capable of breaking down GSI (Pandey 1968; Richards 1986; de Nettancourt 1997), the hexaploid monoembryonic individuals of *H. serratifolius* are self-sterile (Alves et al. 2013). Thus, we believe that neopolyploidy alone was not responsible for LSI break down and self-fertility in *H. serratifolius*. In Bignoniaceae that present LSI, there is an initial endosperm development in self-pollinated pistils and self-sterility seems to be associated with endosperm malfunction (Gibbs and Bianchi 1993; Bittencourt et al. 2003; Bittencourt and Semir 2005; Gandolphi and Bittencourt 2010). So, in the apomictic species of this family, the apparently normal endosperm growth of self-fertilized ovules supply early adventitious embryos development, and the presence of these embryos would later prevent the abortion of selfed pistils (Oliveira et al. 1992; Bittencourt and Semir 2005; Bittencourt and Moraes 2010; Sampaio et al. 2013b).

In any case, our data further corroborate the association between sporophytic apomixis and neopolyploidy (Oliveira et al. 1992; Mendes-Rodrigues et al. 2005; Bittencourt and Moraes 2010; Sampaio 2010; Sampaio et al. 2013b), and indicate a cause and effect relationship similar to the reported for gametophytic apomictic species (Carman 2007; Whitton et al. 2008; Hörandl 2010). The present report of *H. serratifolius* as sporophytic apomictic indicates that this species, together with *H. chrysotrichus* and *H. ochraceus*, presents some features that favor the process of hybridization, polyploidization and apomixis expression. But it is not clear yet and should be better investigated why some hexaploid populations of *H. serratifolius* are monoembryonic and self-sterile. Since allopolyploidy (i.e. hybridization followed by polyploidization) seems to be a crucial factor for apomixis expression (Carman 2007), it is possible that the *H. serratifolius* populations represent distinct lineages, with genome differences that would explain breeding differences in this agamic polyploidy complex. Details of cytogenetic and genomic differences, and hormonal control of pistil abscission will be necessary to understand the evolutionary and ecological relationships in those agamic polyploidy complexes in the Bignoniaceae and in Neotropical apomictic plants as a whole.

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**Compliance with ethical standards**

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