



ARE THERE SEED PEDESTALS IN LENTIBULARIACEAE?

BARTOSZ J. PŁACHNO^{1*}, DÉBORA CLIVATI², VÍTOR F. OLIVEIRA DE MIRANDA²,
AND PIOTR ŚWIĄTEK³

¹Department of Plant Cytology and Embryology, Jagiellonian University,
Grodzka 52, 31–044 Cracow, Poland

²Plant Systematics Laboratory, Herbarium Mogiense – HUMC,
Universidade de Mogi das Cruzes – UMC, Av. Dr. Cândido Xavier de Almeida Souza,
n.200, CEP 08780-911, Mogi das Cruzes, SP, Brasil

³Department of Animal Histology and Embryology, University of Silesia,
Bankowa 9, 40–007 Katowice, Poland

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The term "seed pedestal" was introduced recently to describe a structure of placental origin connecting a seed with the placenta. Seed pedestals are widespread in Scrophulariaceae and a few adjacent families, but have not been found in Lentibulariaceae so far. Here their presence is reported for *Utricularia reniformis* from Brazil, and their formation during seed development is described. We observed that the formation of this structure was strictly associated with seed development; seed pedestals were not formed under aborted (unfertilized) ovules.

Key words: Ovule, seed development, placenta, seed pedestal, *Utricularia*, micromorphology, carnivorous plants.

INTRODUCTION

Lentibulariaceae, the largest carnivorous plant family, comprises a highly evolved and specialized family of the Lamiales (e.g., Fischer et al., 2004; Müller et al., 2006). They show several morphological modifications ("relaxed morphology") (Brugger and Rutishauser, 1989; Rutishauser and Isler, 2001; Ellison and Gotelli, 2009), traps for catching small invertebrates (e.g., Juniper et al., 1989; Płachno et al., 2007, 2008), and also unusual embryological characters (e.g., Khan, 1954; Płachno and Świątek, 2008). As in other angiosperms, in Lentibulariaceae the seeds provide important taxonomic information. Taylor (1989) described the great diversity of seed morphology in *Utricularia*. Seed shape and seed testa structure are important characters in *Utricularia* taxonomy on both section and species levels. In *Pinguicula* some characters of seed coat structure may be significant at the section level, and other characters at specific or infraspecific levels (Degtjareva et al., 2004, 2006). Many authors have studied seed for-

mation in Lentibulariaceae (mainly in *Utricularia*), including Merz (1897), Lang (1901), Merl (1915), Lloyd (1942), Khan (1954) and Farooq (1965), but less attention has been paid to placental changes during seed development.

The term "seed pedestal" was recently introduced by Rebernig and Weber (2007) to denote a structure which is developmentally placental in origin and thus not part of the ovule, and which connects a seed with the placenta. They examined in detail members of Scrophulariaceae (s.l.) (45 species of 27 genera) and also samples of 11 other (mainly derived) families of Lamiales, including Lentibulariaceae (*Pinguicula* and *Utricularia*). However, they did not find evidence for seed pedestals or comparable structures in Lentibulariaceae.

This study was intended to determine whether the seed pedestal occurs in Lentibulariaceae. The paper is also part of a larger project on the biology of reproduction in Lentibulariaceae carried out by B.J.P. (grant N N304 002536 from the Polish Ministry of Science and Higher Education).

*e-mail: bartek78pl@poczta.onet.pl

MATERIALS AND METHODS

PLANT MATERIAL

Material was taken from two sources: directly from nature and from greenhouse collections. Buds, flowers and fruits of *Utricularia reniformis* A.St.-Hil. (large form) were collected by V.F.O.M. in the Pedra do Garrafão from a population on a mountain slope facing the sea, near the town of Biritiba Mirim, São Paulo State, Brazil (Fig. 1a). Voucher specimens are deposited in HUMC. Another form, *Utricularia reniformis* (A.St.-Hil.) "Enfant Terrible" (B.Rice & M.Studnicka) (Rice and Studnička, 2004), was provided by the Liberec Botanical Garden (Czech Republic) and then cultivated successfully in the Botanical Garden of the Jagiellonian University in Cracow, Poland. The plants produced flowers in 2008, and after hand-pollination fruits were obtained.

HISTOLOGICAL ANALYSIS

Buds, flowers and fruits were fixed in 2.5% formaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.0), rinsed in the same buffer, dehydrated in a graded ethanol series (10%, 30%, 50%, 70%, 96%) for 15 min at each concentration and then kept 1 h in absolute ethanol. Later, samples were infiltrated in mixtures of absolute ethanol and Technovit-7100 (2-hydroxyethyl-methacrylate) (Heraeus Kulzer) (3:1, 1:1, 1:3 v/v; 1 h in each mixture) and stored for 12 h in pure Technovit. The resin was polymerized with the addition of hardener at 37°C. The material was sectioned 5 µm and 7 µm thick with a rotary microtome (Microm, Adamas Instrumenten), stained with toluidine blue or methylene blue and mounted in Entellan (Merck). Part of the material was embedded in Epon 812 (Fullam, Latham, NY). Semithin and ultrathin sections were cut on a Leica Ultracut UCT ultramicrotome and stained with methylene blue. Microscope sections were photographed with a Zeiss Axio Cam MRe digital camera with Zeiss Axio Vision 3.0 software and an Olympus BX60 microscope.

SCANNING ELECTRON AND EPIFLUORESCENCE MICROSCOPY

The procedures for preparing samples for SEM were as described earlier (Plachno et al., 2005a,b). Briefly, flowers and fruits were fixed as for histological analysis. The dried tissues were sputter-coated with gold and viewed in a HITACHI S-4700 microscope (Scanning Microscopy Laboratory of Biological and Geological Sciences, Jagiellonian University). Additionally, whole ovules were examined by epifluorescence microscopy.

RESULTS

The stalked spherical placenta is covered with numerous sessile, unitegmic ovules (Fig. 1b). The ovule is ~165 µm long. During ovule development a small spherical group of cells differentiates near its base, which forms the placental nutritive tissue (Fig. 1c; see also Merl, 1915, Fig. 30f). The placental epidermal cells are papilla-shaped and form an epithelium (Fig. 1c). During seed development the placental tissues form elaborations (Fig. 1d). Each seed has one placental elaboration. The seed is attached at the apex of this elaboration (pedestal) (Figs. 1d,e). The pedestal is formed by placental parenchyma, epidermis and nutritive tissue. The epidermal and parenchyma cells that form the pedestal change their shape and enlarge. The seed pedestal is formed only if the ovule is fertilized and the seed is developing (Fig. 1e). After maturation, the seeds very easily drop away from the pedestals (the seed detaches from the site of the endospermal micropylar haustorium; Fig. 1f).

DISCUSSION

Our results are in contrast to Rebernick and Weber's (2007) finding that the Lentibulariaceae lack seed pedestals. However, the focus of that work was on Scrophulariaceae (s.l.) and the sample size for the remaining families of the Lamiales was small. Regarding Lentibulariaceae, Rebernick and Weber examined *Pinguicula* (*P. agnata*, *P. esseriana*, *P. moranensis*) and *Utricularia* (*U. livida*, *U. sandersonii*). All were found to lack seed pedestals. The genus *Pinguicula* is the most basal genus of the Lentibulariaceae family and a sister group of *Utricularia-Genlisea* clade (Jobson et al., 2003; Müller et al., 2004, 2005). *U. livida* and *U. sandersonii* are classified in sect. *Calpidisca*, which is not very evolutionarily advanced according to Taylor (1989). *Utricularia reniformis*, on the other hand belongs to the derived section *Iperula* according to the phylogenetic hypotheses of *Utricularia* (Jobson et al. 2003; Müller and Borsch, 2005). At this point it is difficult to suggest any taxonomic implications, because our knowledge of Lentibulariaceae placenta and seed development is poor. The seed pedestal in *Utricularia reniformis* is similar to the *Digitalis* type of Rebernick and Weber's (2007) classification.

Our data show that in *Utricularia reniformis* the formation of the seed pedestal is strictly connected with seed development; it did not form under aborted (unfertilized) ovules. This finding is not in agreement with Rebernick and Weber (2007), who stated that the pedestal develops fully even in the case of ovule abor-

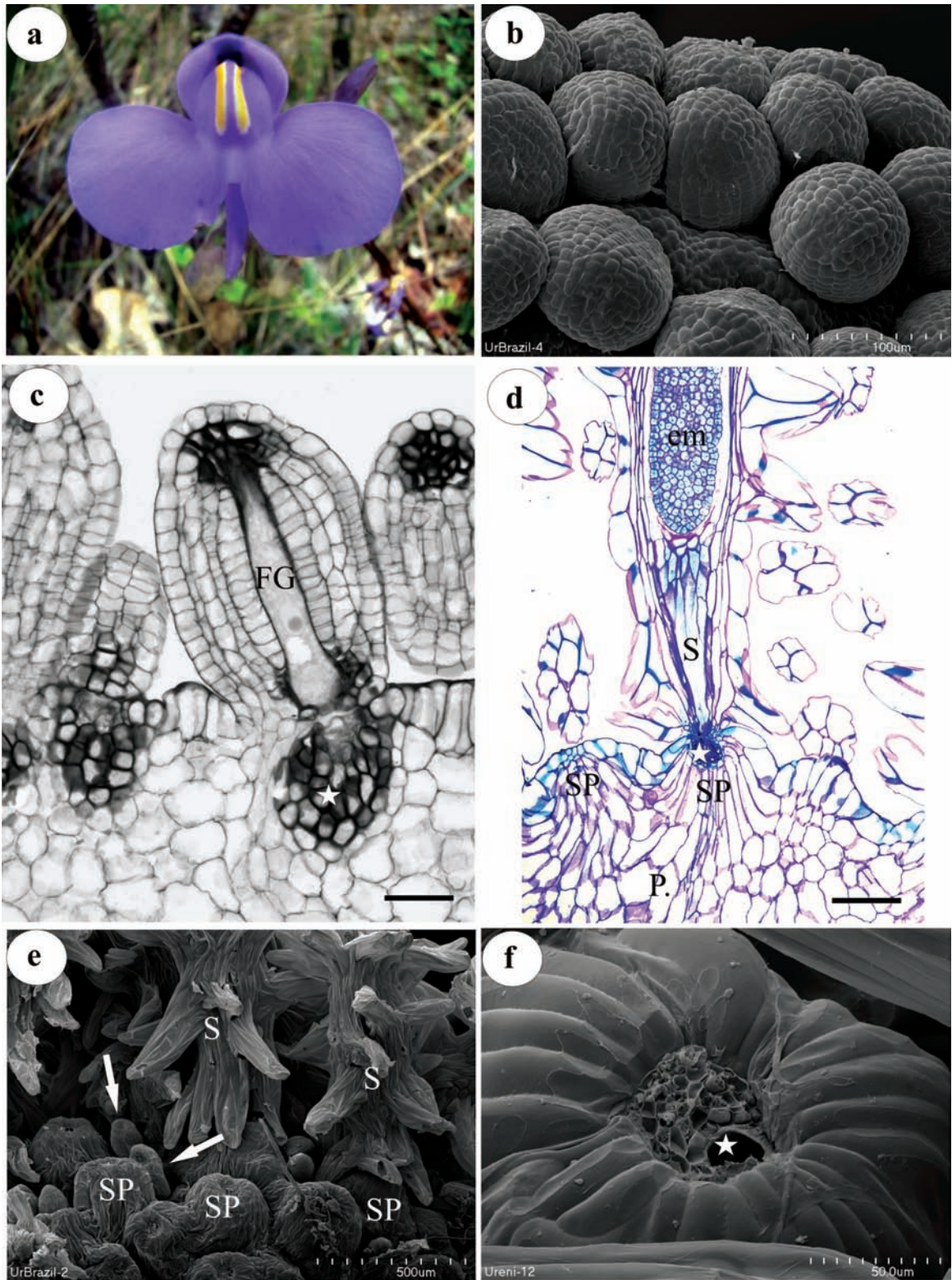


Fig. 1. *Utricularia reniformis* A.St.-Hil. **(a)** Flower of large form from the Pedra do Garrafão, Brazil, **(b)** Ovules on the placenta, **(c)** Section through ovule and female gametophyte (FG) and nutritive tissue (star), **(d)** Section through placenta (P), seed pedestal (SP) and seed (S), embryo (em), **(e)** Placenta surface with seed pedestal (SP), seeds (S) and aborted (unfertilized) ovules (arrow), **(f)** Apical surface of seed pedestal after seed detachment, haustorium of endosperm (star). Bar in (a–c) = 36 μm in (d) = 137 μm .

tion. It may be speculated that seed pedestals facilitate detachment of seeds from the placenta.

The presence or absence of seed pedestals needs to be examined in other *Utricularia* species. In species exhibiting a very similar pattern of seed development, the presence of seed pedestals is especially probable.

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REFERENCES

- BRUGGER J, and RUTISHAUSER R. 1989. Bau und Entwicklung landbewohnender *Utricularia*-Arten. *Botanica Helvetica* 99: 91–146.
- DEGTJAREVA G, CASPER J, HELLWIG F, SOKOLOFF D. 2004. Seed morphology in the genus *Pinguicula* (Lentibulariaceae) and its relation to taxonomy and phylogeny. *Botanische Jahrbücher* 125: 431–452.
- DEGTJAREVA GV, CASPER SJ, HELLWIG FH, SCHMIDT AR, STEIGER J, SOKOLOFFS DD. 2006. Morphology and nrITS phylogeny of the genus *Pinguicula* L. (Lentibulariaceae), with special attention to embryo evolution. *Plant Biology* 8: 778–790.
- ELLISON AM, and GOTELLI NJ. 2009. Darwin review energetics and the evolution of carnivorous plants – Darwin's' most wonderful plants in the world'. *Journal of Experimental Botany* 60/1: 19–42.
- FAROOQ M. 1965. Studies in the Lentibulariaceae III. The embryology of *Utricularia uliginosa* Vahl. *Phytomorphology* 15: 123–131.
- FISCHER E, BARTHLOTT W, SEINE R, and THEISEN I. 2004. Lentibulariaceae. In: Kubitzki K and Kadereit J [eds.], *The Families and Genera of Vascular Plants*, vol. VII, 276–282. Springer Verlag, Berlin, Heidelberg, New York.
- JOBSON RW, PLAYFORD J, CAMERON KM, and ALBERT VA. 2003. Molecular phylogenetics of Lentibulariaceae inferred from plastid *rps16* intron and *trnL-F* DNA sequences: implications for character evolution and biogeography. *Systematic Botany* 28:157–171.
- JUNIPER BE, ROBINS RJ, and JOEL JM. 1989. *The Carnivorous Plants*. Academic Press, London.
- KHAN R. 1954. A contribution to the embryology of *Utricularia flexuosa* Vahl. *Phytomorphology* 4: 80–117.
- LANG FX. 1901. Untersuchungen über Morphologie, Anatomie und Samenentwicklung von *Polypompholyx* und *Byblis gigantea*. *Flora* 88: 149–206.
- LLOYD FE. 1942. *The Carnivorous Plants*. Chronica Botanica Company, Waltham, Mass., U.S.A.
- MERL EM. 1915. Beiträge zur Kenntnis der Utricularien und Genlisen. *Flora* 108:127–200.
- MERZ M. 1897. Untersuchungen über die Samenentwicklung der Utricularien. *Flora* 84:69–87.
- MÜLLER K, BORSCH T, LEGENDRE L, POREMBSKI S, and BARTHLOTT W. 2006. Recent progress in understanding the evolution of carnivorous Lentibulariaceae (Lamiales). *Plant Biology* 8: 748–757.
- MÜLLER K, BORSCH T, LEGENDRE L, POREMBSKI S, THEISEN I, and BARTHLOTT W. 2004. Evolution of carnivory in Lentibulariaceae and the Lamiales. *Plant Biology* 6: 477–490.
- MÜLLER K, and BORSCH T. 2005. Phylogenetics of *Utricularia* (Lentibulariaceae) and molecular evolution of the *trnK* intron in a lineage with high substitutional rates. *Plant Systematics and Evolution* 250: 39–67.
- PLACHNO BJ, ADAMUS K, FABER J, and KOZŁOWSKI J. 2005a. Feeding behaviour of carnivorous *Genlisea* plants in the laboratory. *Acta Botanica Gallica* 152:159–164.
- PLACHNO BJ, FABER J, and JANKUN A. 2005b. Cuticular discontinuities in glandular hairs of *Genlisea* St.-Hil. in relation to their functions. *Acta Botanica Gallica* 152:125–130.
- PLACHNO BJ, KOZIERADZKA-KISZKURNO M, and ŚWIĄTEK P. 2007. Functional ultrastructure of *Genlisea* (Lentibulariaceae) digestive hairs. *Annals of Botany* 100: 195–203.
- PLACHNO BJ, and ŚWIĄTEK P. 2008. Cytoarchitecture of *Utricularia* nutritive tissue. *Protoplasma* 234: 25–32.
- PLACHNO BJ, KOZIERADZKA-KISZKURNO M, ŚWIĄTEK P, and DARNOWSKI DW. 2008. Prey attraction in carnivorous *Genlisea* (Lentibulariaceae). *Acta Biologica Cracoviensia Series Botanica* 50/2: 87–94.
- RICE B, and STUDNIČKA M. 2004. *Utricularia reniformis* 'Enfant Terrible'. *Carnivorous Plant Newsletter* 33: 53–55.
- REBERNIG CA, and WEBER A. 2007. Diversity, development and systematic significance of seed pedestals in Scrophulariaceae (s.l.). *Botanische Jahrbücher* 127: 133–150.
- RUTISHAUSER R, ISLER B. 2001. Developmental genetics and morphological evolution of flowering plants, especially Bladderworts (*Utricularia*): Fuzzy Arberian morphology complements classical morphology. *Annals of Botany* 88: 1173–1202.
- TAYLOR P. 1989. The genus *Utricularia* – A taxonomic monograph. *Kew Bulletin* 14: 1–735.