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## Serial block face SEM visualization of unusual plant nuclear tubular extensions in a carnivorous plant (*Utricularia*, Lentibulariaceae)

Bartosz J. Płachno<sup>1\*</sup>, Piotr Świątek<sup>2</sup>, Richard W. Jobson<sup>3</sup>, Karol Małota<sup>2</sup> and Wojciech Brutkowski<sup>4</sup>

<sup>1</sup>Department of Plant Cytology and Embryology, Jagiellonian University in Kraków, 9 Gronostajowa St., Cracow, Poland, <sup>2</sup>Department of Animal Histology and Embryology, University of Silesia in Katowice, 9 Bankowa St., 40-007 Katowice, Poland, <sup>3</sup>National Herbarium of New South Wales, Mrs Macquaries Road, Sydney, NSW 2000 Australia and <sup>4</sup>Laboratory for Imaging Tissue Structure and Function, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur St., 02-093 Warszawa, Poland

\*For correspondence. E-mail bartosz.plachno@uj.edu.pl

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● **Background and Aims** In *Utricularia nelumbifolia*, the nuclei of placental nutritive tissue possess unusually shaped projections not known to occur in any other flowering plant. The main aim of the study was to document the morphology and ultrastructure of these unusual nuclei. In addition, the literature was searched to find examples of nuclear tubular projections in other plant groups, and the nuclei of closely related species of *Utricularia* (i.e. sects *Iperua*, *Orchidioides*, *Foliosa* and *Utricularia*) were examined.

● **Methods** To visualize the complexity of the nuclear structures, transmission electron microscopy (TEM) was used, and 3-D ultrastructural reconstructions were made using the serial block face scanning electron microscopy (SBEM) technique. The nuclei of 11 *Utricularia* species, i.e. *U. nelumbifolia*, *U. reniformis*, *U. cornigera*, *U. nephrophylla* (sect. *Iperua*), *U. asplundii*, *U. alpina*, *U. quelchii* (sect. *Orchidioides*), *U. longifolia* (sect. *Foliosa*), *U. intermedia*, *U. minor* and *U. gibba* (sect. *Utricularia*) were examined.

● **Key Results** Of the 11 *Utricularia* species examined, the spindle-like tubular projections (approx. 5 µm long) emanating from resident nuclei located in placental nutritive tissues were observed only in *U. nelumbifolia*. These tubular nuclear extensions contained chromatin distributed along hexagonally shaped tubules. The apices of the projections extended into the cell plasma membrane, and in many cases also made contact at the two opposing cellular poles, and with plasmodesmata via a short cisterna of the cortical endoplasmic reticulum. Images from the SBEM provide some evidence that the nuclear projections are making contact with those of neighbouring cells.

● **Conclusions** The term chromatubules (chromatin-filled tubules) for the nuclear projections of *U. nelumbifolia* placental tissue was proposed here. Due to the apparent association with the plasma membrane and plasmodesmata, it was also speculated that chromatubules are involved in nucleus–cell–cell communication. However, further experimental evidence is required before any functional hypothesis can be entertained.

**Key words:** Chromatubules, nucleus, plant nuclear shape, plasmodesmata, placental nutritive tissue, micropylar embryo sac haustorium, micropylar endosperm haustorium, SBEM, ultrastructure.

### INTRODUCTION

*Utricularia* (Lentibulariaceae, Lamiales) are carnivorous plants that possess suction traps for trapping small organisms. The genus contains many habitat-specific forms including aquatic, terrestrial, epiphytic, lithophytic and rheophytic species (Taylor, 1989). Some highly specialized species, such as *U. nelumbifolia*, are aquatic epiphytes that inhabit Bromeliad tanks (Taylor, 1989; Płachno and Świątek, 2010). Although *Utricularia* inflorescences share a typical angiosperm floral bauplan, their rootless vegetative bauplan is quite divergent (Rutishauser, 2016).

Genome size is variable in *Utricularia*, with a tendency for genome miniaturization; the smallest genomes were recorded in *U. sect. Vesiculina* (*U. purpurea*, 79 Mbp), *U. sect. Foliosa* (*U. longifolia*, 97 Mbp) and *U. sect. Utricularia* (*U. floridana*, 100 Mbp; *U. gibba*, 103 Mbp) (Veleba *et al.*, 2014). *Utricularia* and sister genus *Genlisea* exhibit the fastest rates of nucleotide substitution known within angiosperms (Jobson and Albert, 2002; Müller *et al.*, 2004; Wicke *et al.*, 2013), with an

observed high rate of gene turnover linked to the evolution of a compact genome (Carretero-Paulete *et al.*, 2015a, b).

In most angiosperms, the female gametophyte remains in the ovule surrounded by sporophytic tissue. However, in many *Utricularia* species, the female gametophyte grows outside the ovule where it is partially exposed and able to make contact with sporophytic tissue (i.e. placenta). The haustorial component of the female gametophyte penetrates the placenta and makes contact with a specialized spherically shaped cluster of cells called ‘nutritive tissue’ (Fig. 1A). This tissue differentiates in close proximity to the ovule base during the early stages of female gametophytic development (Khan, 1954; Płachno and Świątek, 2008, 2012; Płachno, 2011).

Nuclei of the placenta nutritive tissue in *Utricularia nelumbifolia* have spindle-like tubular projections (unknown among other flowering plants), and the aim of our study was to document in detail their morphology and ultrastructure. We examined whether or not the projections occur in plants from different genera and in other *Utricularia* species (sects *Iperua*,

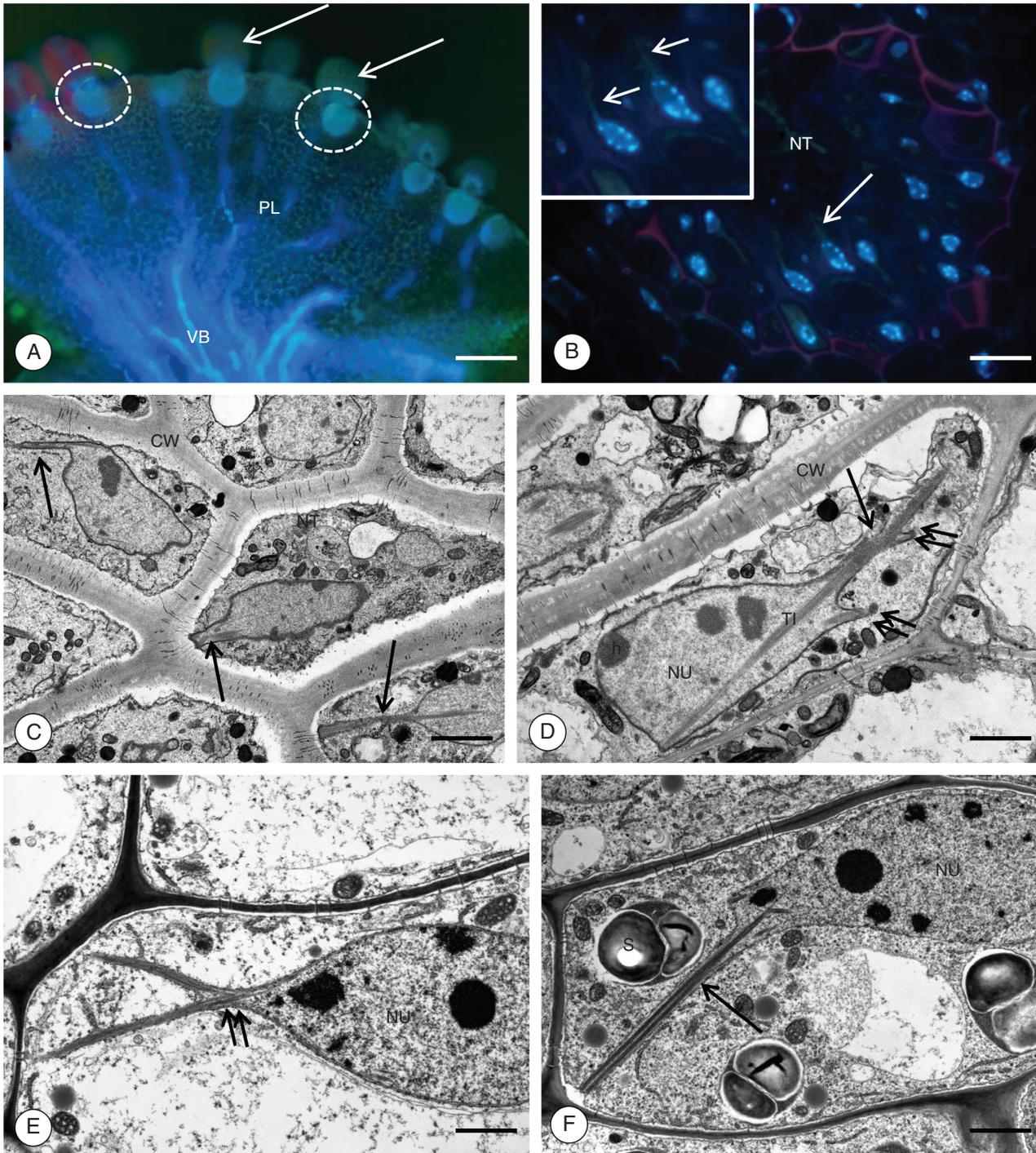


FIG. 1. *Utricularia nelumbifolia*. The localization and general structure of the placental nutritive tissue. (A, B). The general morphology and histology of *U. nelumbifolia* placenta and nutritive tissue. (A) PL, placenta; VB, vascular bundles; arrows, ovules; encircled, nutritive tissue. Whole mounted preparation stained with DAPI. Fluorescence microscopy. Scale bar = 180  $\mu\text{m}$ . (B) NT, nutritive tissue, the arrow marks the nuclear projection. Inset: a fragment of nutritive tissue at higher magnification; arrows point to nuclear projections. Histoacryl semi-thin section stained with DAPI. Fluorescence microscopy. Scale bar = 20  $\mu\text{m}$ . (C, D) Ultrastructure of nutritive tissue cells (NT). CW, cell walls; NU, nucleus; h, chromocentre; arrows, nuclear projections; double arrows, nuclear projection branching; TI, tubule-like inclusions. Material prepared for the SBEM method, transmission electron microscopy (TEM). Scale bars = 2.75  $\mu\text{m}$  in (C) and 1.80  $\mu\text{m}$  in (D). (E) Nutritive cell ultrastructure after fixing for microtubules. Note bifurcating nuclear projection. NU, nucleus. TEM. Scale bar = 1.5  $\mu\text{m}$ . (F) Nutritive cell ultrastructure after fixation in modified Karnovsky fixative. The arrow points to the nuclear projection; NU, nucleus; S, starch grain. TEM. Scale bar = 1.4  $\mu\text{m}$ .

*Orchidioides*, *Foliosa* and *Utricularia*). We also examined nuclei of vegetative tissue for the presence of the projections, and performed the search across several growing seasons.

Finally, we used serial block face scanning electron microscopy (SBEM) to construct a three-dimensional model of the placental nutritive cells that allowed visualization of the number of projections per nucleus, and whether or not the projections extend into, and interacted with, neighbouring cell nuclei.

## MATERIALS AND METHODS

### *Plant material*

Inflorescences and vegetative tissue (phylloclades) of *Utricularia nelumbifolia* Gardner (sect. *Iperua*) were obtained from two sources: the greenhouse collection at the Botanic Garden of the Jagiellonian University in Kraków, Poland and Liberec Botanical Garden, Czech Republic. Material was collected four times over a period of 10 years. Flowers of other species: *U. reniformis*, *U. cornigera*, *U. nephrophylla* (sect. *Iperua*), *U. asplundii*, *U. alpina*, *U. quelchii* (sect. *Orchidioides*), *U. longifolia* (sect. *Foliosa*), *U. intermedia*, *U. minor* and *U. gibba* (sect. *Utricularia*) were collected from the Liberec Botanical Garden, Czech Republic, Prague Botanical Garden, Czech Republic, Botanic Garden of the Jagiellonian University in Kraków and from a locality in southern Poland (the Jeleniak–Mikuliny Nature Reserve, Lubliniec). Material from the Nature Reserve was collected under a permit from the Polish Ministry of the Environment (Płachno and Świątek, 2011).

### *Electron microscopy*

Placentas with ovules (from buds of different sizes from mature flowers), and other tissues (parts of corolla, phylloclades) were fixed in 2.5 % formaldehyde and 2.5 % glutaraldehyde in a 0.05 M cacodylate buffer (pH 7.0) for 2 d. The material was post-fixed in 1 % OsO<sub>4</sub> in a cacodylate buffer for 24 h at approx. 4 °C, rinsed in the same buffer, treated with 1 % uranyl acetate in distilled water for 1 h, dehydrated with acetone and embedded in an Epoxy Embedding Medium Kit (Fluka). Semi-thin sections were stained with methylene blue and examined using an Olympus BX60 microscope. Ultrathin sections were cut on a Leica ultracut UCT ultramicrotome. After contrasting with uranyl acetate and lead citrate, the sections were examined using a Hitachi H500 electron microscope at 75 kV.

A second type of fixation for TEM was used to check whether the morphology of the nuclei was independent of the fixation methodology. Thus a part of the material for ultrastructural studies was fixed in a mixture of 4 % formaldehyde (freshly prepared from paraformaldehyde) and 0.25 % glutaraldehyde in a piperazine buffer (fixing as for microtubules). The material was post-fixed in 1 % OsO<sub>4</sub> in a cacodylate buffer for 24 h at approx. 4 °C, rinsed in the same buffer, treated with 1 % uranyl acetate in distilled water for 1 h, dehydrated with acetone and embedded in an Epoxy Embedding Medium Kit (Fluka).

### *Serial block face scanning electron microscopy (SBEM)*

Tissue fixation and preparation was adopted from Deerinck et al. (2010) with some modifications. *Utricularia nelumbifolia* placentas were fixed in 2.5 % formaldehyde and 2.5 % glutaraldehyde in a 0.05 M cacodylate buffer (pH 7.0) for 2 d. After fixation, tissue was washed three times for 15 min with the same buffer. After washing, samples were post-fixed with 3 % potassium ferrocyanide in 0.3 M cacodylate buffer mixed with an equal volume of 4 % aqueous solution of osmium tetroxide for 1 h. Tissue was then washed three times for 5 min in ddH<sub>2</sub>O and incubated in 1 % solution of thiocarbonylhydrazide (Ted Pella) for 20 min at 60 °C. After that, samples were washed three times for 5 min in ddH<sub>2</sub>O and placed in 2 % aqueous osmium tetroxide for 30 min, then tissue was washed again three times for 5 min in ddH<sub>2</sub>O and incubated overnight in 1 % aqueous uranyl acetate in 4 °C. The samples were then rinsed three times for 5 min in ddH<sub>2</sub>O and put into a freshly prepared Walton's lead aspartate for 30 min at 60 °C, washed five times for 3 min in ddH<sub>2</sub>O and dehydrated for 10 min in each of 30, 50, 70 and 96 % ethanol solution, then placed in anhydrous 100 % ethanol three times for 20 min, a 1:1 solution of acetone and ethanol for 15 min and twice for 15 min in 100 % acetone. After dehydration, samples were placed in a mixture of 25 % Epoxy Embedding Medium (Sigma, St. Louis, MO, USA) in acetone for 24 h, then in 50 % epoxy resin in acetone for 24 h and in 75 % resin in acetone for another 24 h and, after saturation, samples were left overnight for evaporation of acetone, then embedded between two layers of Aclar (EMS) and left for polymerization.

Polymerized samples were cut out and glued with cyanoacrylate on aluminium specimen pins (Gatan, Pleasanton, CA, USA) and mounted in the Gatan 3View system installed in a Zeiss Sigma scanning electron microscope. The serial block face images were then collected, section thickness was 60 nm and pixel size of collected images was 60 × 60 nm. The size of the imaged area was 32.3 × 32.3 μm which provided an image resolution of 5500 × 5500 pixels. The number of collected images was 700; however, after data analysis, only 204 images were selected for further processing, which provided 12.24 μm of overall analysed sample thickness. Because of the large volume of data, images for analysis were resized from the original resolution of 5500 × 5500 pixels to 1000 × 1000 pixels, with this operation performed using Fiji Software (Schindelin et al., 2012). Resized images were manually segmented, and 3-D models created in Imaris software (Bitplane Inc.).

### *Visualization of nuclei using epifluorescence microscopy*

The placentas of *U. nelumbifolia* were fixed in 4 % formaldehyde (for 30–40 min), washed in phosphate-buffered saline (PBS), dehydrated in a graded ethanol series, and then infiltrated and embedded in Histocryl resin (London Resin Company Ltd, Basingstoke, Hampshire, UK). The Histocryl sections (1 μm) were stained with 4',6-diamidino-2-phenylindole (DAPI; 1 μg mL<sup>-1</sup>). Whole mounted preparations and Histocryl sections were examined using an Olympus BX60 epifluorescence microscope equipped with the appropriate filters.

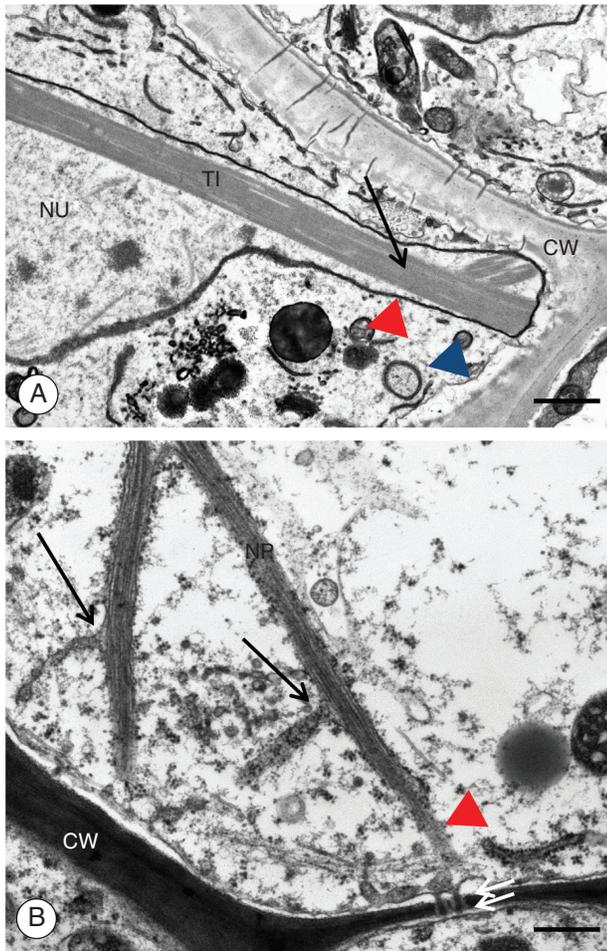


Fig. 2. *Utricularia nelumbifolia*. Ultrastructure of the placental nutritive tissue cells with emphasis on the ultrastructure of the nuclei. (A) A fragment of nuclear projection. CW, cell wall; NU, nucleus; TI, tubule-like inclusions; red arrowhead, nuclear envelope; blue arrowhead, cell membrane. Material prepared for the SBEM method, TEM. Scale bar = 0.85  $\mu$ m. (B) A magnified fragment of the nutritive cell nucleus shown in Fig. 1E. CW, cell wall; NP, nuclear projection; arrows show continuity between the outer nuclear membrane and the endoplasmic reticulum; double arrows, plasmodesmata; the red arrowhead marks the continuity of the outer nuclear membrane with the endoplasmic reticulum and plasmodesmata. Tissue fixed as for microtubules. TEM. Scale bar = 0.3  $\mu$ m.

## RESULTS

Of the 11 examined species of *Utricularia*, nuclei with spindle-like tubular projections (chromatubules) were only observed in the placental cells of *U. nelumbifolia*. Nuclei from all other examined cell types contained typical proteinaceous paracrystalline inclusions.

The nuclei of the placental nutritive tissue of *U. nelumbifolia* possess long chromatubules often extending to the cell membrane (Figs 1–5). TEM analysis revealed that chromatubules are a continuation of the inner and outer membranes of the nuclear envelope and are either simple or bifurcating structures (Figs 1C–E, 2B and 3A, B). They are usually approx. 5  $\mu$ m in length, and vary in width from 0.31 to 1.48  $\mu$ m near the base, and from 0.25 to 1.16  $\mu$ m near the apical tip (Table 1). The apices extend towards the cell periphery (Figs 1C, F and 2A), often

forming direct contact with the plasma membranes (Figs 1C, F, 2A, B, 4B and 5A, B). TEM analysis revealed that chromatubules contain electron-dense material adjoining the inner nuclear envelope (Fig. 3B), with this material here interpreted as chromatin. The striking feature of each chromatubule is the presence of proteinaceous tubule-like inclusions forming orderly structures quite unlike the randomly arranged analogous structures located in the central regions of nuclei (Figs 1C–F, 2A, B and 3A, B). We confirmed the organized structure of these inclusions using transversal sectioning, which revealed a bundled array of hexagonal shaped lamellar tubules (Fig. 3B). These tubule-like inclusions seem to modify the shape of the chromatubule (Figs 1A–F and 2A). Ultrastructural analysis showed that the nuclear envelope of the chromatubules may be in direct contact with the plasma membrane, with contact sites observed numerous times (Figs 1C, F, 2A and 5A, B), in positions where plasmodesmata are situated (Fig. 5A, B). Sometimes we observed the endoplasmic reticulum extending from the outer nuclear envelope of the chromatubules towards plasmodesmata (Fig. 2B). Regardless of the fixation methods used (such as modified Karnovsky fixative, fixing for microtubules, and highly membrane-contrasting chemicals used for the SBEM methodology), the morphological and ultrastructural properties of nuclear tissue nuclei were broadly the same (Fig. 1C–F).

To better characterize the number and shape of the chromatubules and determine the spatial relationship between chromatubules from neighbouring cells, we made ultrastructural 3-D reconstructions of placenta tissue fragments using SBEM methodology. Our reconstructions (Fig. 4A, B) revealed that each nucleus may possess up to five chromatubules. The orientation of these nuclear extensions is not specific, i.e. they seem to be arranged randomly in different directions (Fig. 4A, B). In some instances, the apices of chromatubules from neighbouring cells were observed seemingly to conjugate, separated only by the plasma membrane and cell wall (Figs 4B and 5C). It is interesting that both TEM and SBEM analysis showed that the region in which the chromatubules make contact with the cell periphery is usually rich in plasmodesmata (Fig. 5A–C).

## DISCUSSION

The generalized image of the plant nucleus as a uniform, more or less rounded organelle, is correct for the majority of plant tissue types. However, nuclei of specialized cells are known to possess characteristic grooves and invaginations (Collings *et al.*, 2000), especially in those involved in secretory or nutritional functions (e.g. endosperm or suspensor haustoria), or due to physiological hypertrophy (Nagl, 1992). Nuclear grooves and invaginations increase the surface area of the nuclear envelope – the site of exchange between the nucleus and cytoplasm.

In *Utricularia aurea*, irregularly shaped giant nuclei, from the endosperm haustoria and endosperm–placental syncytia, have been described and illustrated by Khan (1954). Following on from Khan (1954), Płachno and Świątek (2012) examined two giant nuclei from tissue of the endosperm–placental syncytium equalling the ‘micropylar endosperm haustorium’ in Khan’s paper 1954 in *U. intermedia*, describing it as being lobed and surrounded by a 3-D microtubule ‘cage’. Within

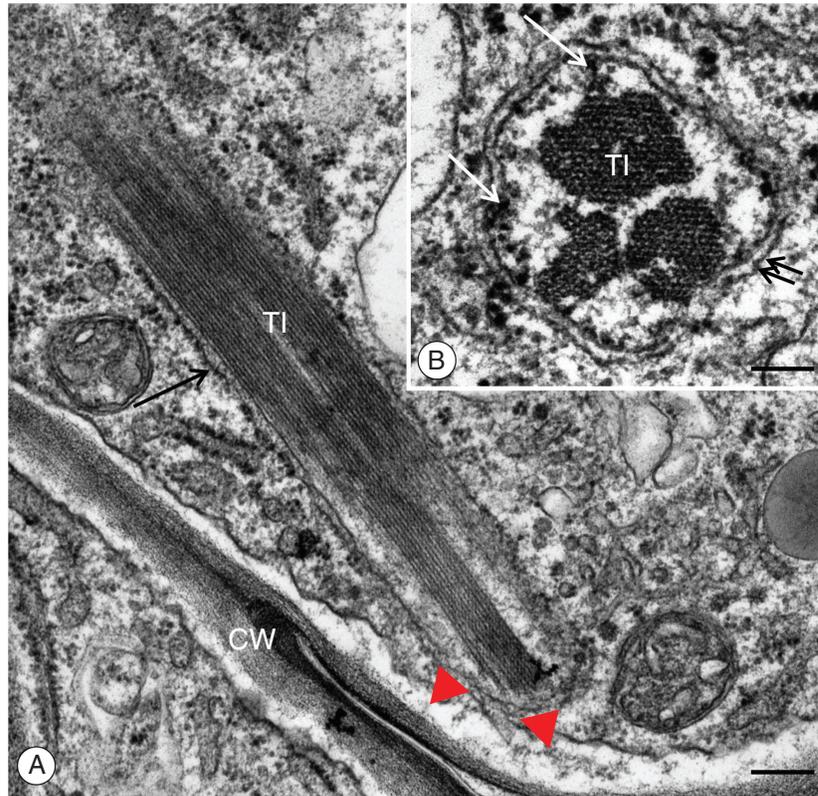


FIG. 3. *Utricularia nelumbifolia*. Ultrastructure of the placental nutritive tissue cells with emphasis on the ultrastructure of nuclear projections. (A) The ultrastructure of the projection apex. CW, cell wall; TI, tubule-like inclusions; arrows marks the outer nuclear membrane decorated with ribosomes; red arrows point to the contact area between the nuclear envelope and the cell membrane. Modified Karnovsky fixative. TEM. Scale bar = 0.18  $\mu\text{m}$ . (B) A cross-section through the nuclear projection. TI, tubule-like inclusions; long white arrows point to chromatin associated with the nuclear envelope; double arrows mark the outer nuclear membrane. Modified Karnovsky fixative. TEM. Scale bar = 0.1  $\mu\text{m}$ .

each endosperm–placental syncytium, Płachno and Świątek (2012) observed two giant nuclei from the endosperm haustorium and many small sized nuclei that originated from the placental nutritive cells.

The first report of syncytium formation in *Utricularia* was by Merz (1897), who observed two differently shaped large haustorium nuclei that were often lobed or indented.

Merz (1897) had already determined that *Torenia* (Linderniaceae) had a haustorial embryo sac for food supply that was derived from the surrounding sporophytic tissue (i.e. the funiculus rather than the placenta). As mentioned above, in *Utricularia* the female gametophyte grows outside of the ovule where it is partially exposed and able to make contact with other sporophytic tissue for supply of nutrients. In contrast to *Torenia*, *Utricularia* possesses an extra-embryonic sac in which the central cell develops externally, while the egg apparatus remains in the ovule (Płachno, 2011). In *Utricularia*, the embryo sac haustorium formed by the central cell is the first stage (after fertilization) in the development of the micropylar endosperm haustorium and the placenta–endosperm syncytium (thus far only known in insects and *Utricularia*).

In other families of the order Lamiales, there have been observed aggressive endosperm haustoria (Johri *et al.*, 1992). Examples include branching micropylar haustorium with amoeboid-like nuclei in the parasitic *Orobancha cernua* (Orobanchaceae) (Tiagi, 1951; Wardlaw, 1955), and the giant

hypertrophied nuclei, described from the endosperm haustorium in *Pedicularis sylvatica* (Orobanchaceae; Berg, 1954) and *Rhinanthus serotinus* (Schrophulariaceae; Świerczyńska *et al.*, 2013).

Based on previous evidence, it seems clear that there is a tendency for irregularly shaped giant nuclei inside their micropylar endosperm haustoria within the order Lamiales. However, nuclei of the placental nutritive cells (e.g. *U. nelumbifolia*) differ from the above-mentioned nuclei of the endosperm haustoria in that they maintain a regular cell shape (as compared with amoeboid-shaped endosperm nuclei) and size (i.e. not hypertrophied).

Large haustorium nuclei do not have projections with internal hexagonally shaped tubules (Płachno and Świątek, 2012; Płachno *et al.*, 2013). The cytological differentiation of endosperm haustoria has been linked to polyploidization (D'Amato, 1989; Nagl, 1992), and nuclei from haustoria possess characters indicative of polyploidization (e.g. giant size, polytenic chromosomes and abundant nucleoli). In contrast to the above, placental nutritive cells in *U. nelumbifolia* have none of these characters, and the literature is devoid of any observations of similar shaped nuclei.

Although endosperm–placental syncytia have been described for *Utricularia* species from sect. *Utricularia* (Płachno and Świątek, 2012), the development of endosperm in *U. nelumbifolia* (sect. *Iperua*) has not yet been described.

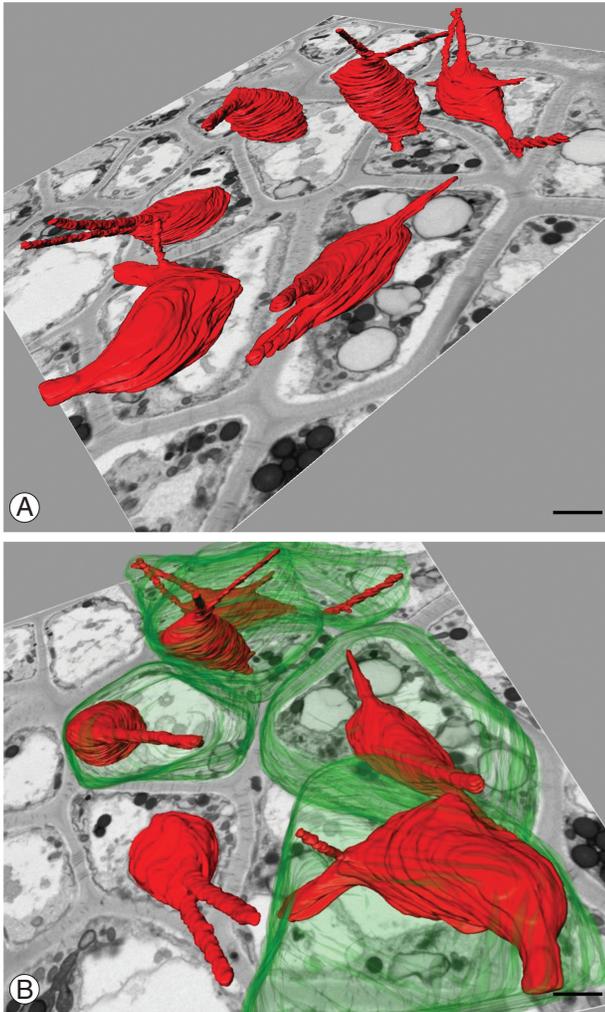


FIG. 4. *Utricularia nelumbifolia*. Three-dimensional reconstruction of a selected fragment of the nutritive tissue. (A) Nuclei are in red – note that there are no specific patterns in spatial orientation of the nuclear projections. Background – the single microphotography showing the ultrastructure of the nutritive tissue. Scale bar = 2  $\mu\text{m}$ . (B) A fragment of the reconstruction shown in (A) rotated and magnified. Cell membranes are in green. Scale bar = 3  $\mu\text{m}$ .

The shape of the nuclei can also be altered by pathological conditions, especially in unstable cytological tissues. One such example involves heterochromatin-filled surface ‘spikes’ that were recorded in long-term callus cells of *Allium fistulosum* L., interpreted by Joachimiak and Ilnicki (2003) as a means for heterochromatin elimination from the nucleus.

It may be the case that chromatubules are of viral origin, and there are examples of virally induced membrane tubule formation (Laporte et al., 2003). However, our examination included *U. nelumbifolia* accessions that were derived from two different sources, making viral infection of both specimens unlikely. In addition, we did not observe any pathological changes in the source specimens, and therefore it is likely that the chromatubules are a stable characteristic of the species.

In all examined members of the family Lentibulariaceae, proteinaceous paracrystalline inclusions in nuclei are present (e.g. Thomas and Gouranton, 1979; Fineran and Lee, 1980; Płachno

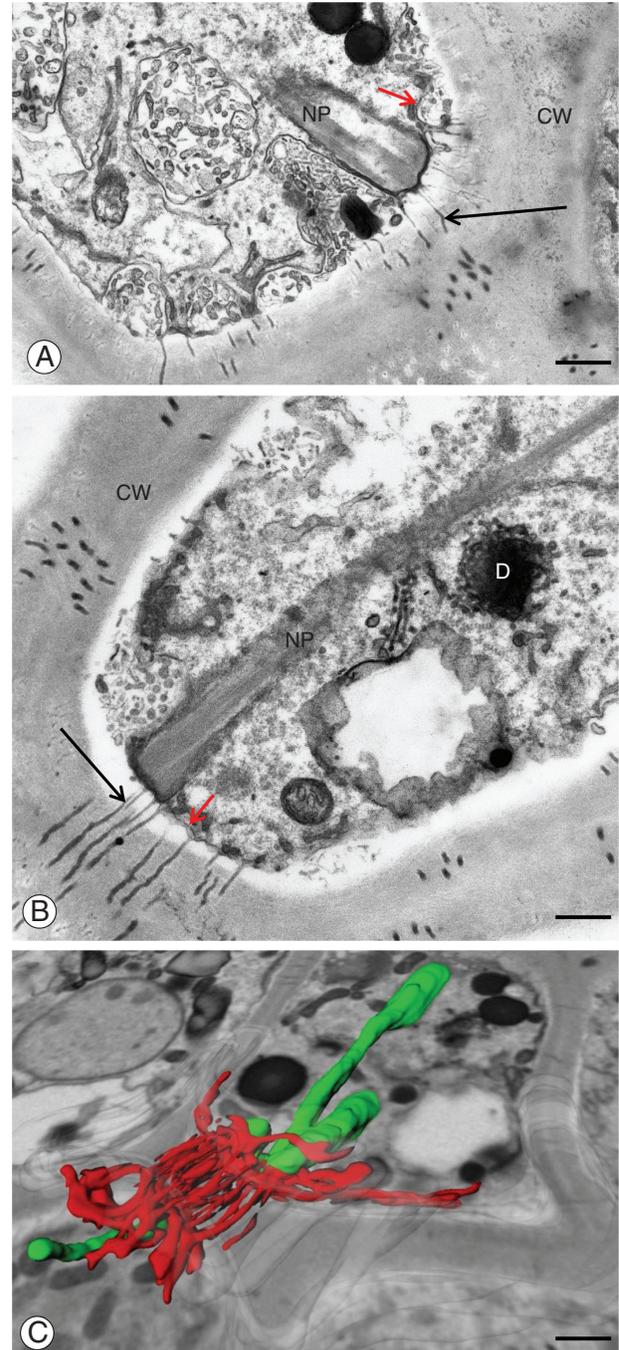


FIG. 5. *Utricularia nelumbifolia*. Cell-cell contact via plasmodesmata in placental nutritive tissue: the contact points between nuclear projections and cell membrane. (A, B) Ultrastructural details. CW, cell wall; D, dictyosome; NP, nuclear projection; black arrows mark plasmodesmata; red arrows point to the cell membrane. Tissue prepared for the SBEM method. Scale bars in (A) and (B) = 0.55  $\mu\text{m}$ . (C) Three-dimensional reconstruction showing the association of nuclear projections (in green) with the endoplasmic reticulum and plasmodesmata (both shown in red). Background: a part of a single microphotograph showing the cell ultrastructure. Scale bar = 1  $\mu\text{m}$ .

et al., 2016). However, the inclusions are observed in the central part of nuclei in all cases except *U. nelumbifolia* in which they are also present in the chromatubules; and it seems that the inclusions have modified the shape of the nucleus.

TABLE 1. Chromatubule length and width

No. of projections	Length (µm)	Width at the base (µm)	Width of the tip (µm)
1	2.0	0.31	0.27
2		1.04	0.45
3	4.8	1.5	1.16
4	4.5	0.75	0.25
5	5.61	0.77	0.84
6	1.92	1.48	0.71
7	3.83	0.58	0.25

Stromules (stroma-filled tubules) are small structures extending from the surface of plastids that superficially resemble chromatubules, with the most obvious differences involving the presence of stroma rather than chromatin and the lack of proteinaceous tubule-like inclusions in stromules (Gunning, 2005; Hanson and Sattarzadeh, 2011).

Chromatubules potentially connect cell poles, and are possibly interacting with the plasma membrane and plasmodesmata, and neighbouring cells. The observed consistent structure and positioning of the chromatubules make it unlikely that they are a random phenomenon, invoking the possibility that they are involved in nucleus–cell–cell communication, thereby constituting a novel functional process for the plant nucleus.

It may be of significance that chromatubules are positioned in the region of the female gametophyte that makes contact with the non-ovular sporophytic tissue. Although this suggests communication between two generations (i.e. sporophyte–gametophyte), the lack of chromatubules in the sporophytic tissue implies that this is unlikely.

Our ultrastructural observations show some evidence that the desmotubules of adjacent plasmodesma are forming a continuous connection with the envelope of the chromatubules. Thus, it is conceivable that macromolecules such as mRNA, proteins and transcription factors are being transported not via the cytoplasm but rather via a direct route from the nucleus to the desmotubule.

Another possible function of the chromatubules involves anchoring and stabilization of the nucleus within the cell. Such a function for ramified, amoeba-like nuclei was suggested in some animal cells, e.g. specialized nurse cells in some insect ovaries (Żelazowska and Biliński, 2001). However, in this case, during late oogenesis there is a phase of rapid transport of nurse cell cytoplasm via wide cytoplasmic channels (termed intercellular bridges or ring canals) towards growing oocytes; long nuclear extensions seem to prevent nuclei from physically blocking the intercellular bridges (Żelazowska and Biliński, 2001).

The germ cells of animals are often interconnected via wide (<10–15 µm) intercellular bridges. Intercellular bridges are stabilized contractile rings that are able to open and close at specific times, and through which flow cytoplasm, organelles, Golgi complexes and centrioles. Macromolecules such as mRNA (maternal information) can also pass through the bridges, and they are therefore analogous to cytoplasmic canals formed during cytomixis in higher plants (Heslop-Harrison, 1966; Guo and Zheng, 2004). Even so, such direct cytoplasm transfer between plant cells has not been observed thus far.

## CONCLUSIONS

We here propose the name chromatubules, i.e. chromatin-filled tubules for nuclear projections of *U. nelumbifolia* placental nuclei (analogously to stromules of plastids). Our discovery provides far more questions than answers: are chromatubules functional in any sense, and if so are they involved in the mediation and trafficking of macromolecules between cells and tissues? Are they the result of overexpression of a gene/genes involved in proteinaceous tubule-like inclusions formation? An integrated approach, using an *in vivo* experimental system and green fluorescent protein (GFP)-marked molecules (e.g. ribonucleoprotein complexes) for the detection of intracellular and cell–cell transport, should help to answer these questions.

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