

BIOLOGY OF THE TRAPLESS RHEOPHYTIC *UTRICULARIA NEOTTIOIDES*:
IS IT POSSIBLE TO GROW THIS SPECIALIZED SPECIES IN CULTIVATION?

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Abstract: The nearly trapless aquatic bladderwort *Utricularia neottioides* is one of the most remarkable and curious *Utricularia* species, growing attached to bare rock in streams of South America. It has never been cultivated due to its rheophytic habit and unknown ecological demands. As late as in 2012, one of us (KP) was able to grow this species in tissue culture from seed, so that the species has become available for cultivation and for anatomical and ecophysiological investigations. In this paper, we describe the biology of the species based on observations made in natural habitat and cultivation, its growth in *in-vitro* and *ex-vitro*, and raise the question whether it can be grown long-term *ex vitro* at all.

Introduction

The small *Utricularia* section *Avesicaria* (the sectional name, proposed by Kamiński (1891), means “without vesicles”, i.e. without traps) comprises only two South American amphibious species which grow strictly attached to bare rock in shallow streaming or seeping waters (so-called “rheophytes”), *Utricularia neottioides* A.St.-Hil. & Girard and *U. oliveriana* Steyerl. (Taylor 1989). *Utricularia oliveriana* reminds one of a typical, tiny terrestrial *Utricularia* species with distinct, spatulate leaves (actually these foliar organs represent modified shoots), bearing the typical bladder traps on stolons and petioles (Fig. 1), whereas *U. neottioides* has greatly modified, finely filamentous leaf-like shoots which gently swiftly move in swift-running waters, and which are practically trap-less (Figs. 2, 3, 4). Yet the flowers are very similar in both species.

Probably the most detailed morphological studies on the exceptional *U. neottioides* have been performed by Luetzelburg (1910). He and Kuhlman (1938) were among the very few botanists who ever found this species with a few traps present, on material collected in Brazil. A few of their specimens are the source of the scarce trap material available, on which subsequent studies (Lloyd 1932, 1942; Taylor 1989) were based. Peter Taylor mentions: “traps few or often apparently absent”, but also that “[...] traps are usually present, as in all other *Utricularia* species, and I have observed them on many of the [...] specimens that I have seen” (Taylor 1989). However Lloyd did not find any traps on the Kew material studied at his time.

Utricularia neottioides personally examined by one of us (AF) both in the field at several locations in Brazil and on rich herbarium material did not show a single trap on any of their vegetative organs. This species therefore even has been considered a trap-less, hence “non-carnivorous”, bladderwort (Kamiński 1891; Fleischmann 2011). Furthermore, on all cultivated



Figure 1: *Utricularia oliveriana* growing attached to sandstone rock in shallow, running water at a river margin, Gran Sabana, Venezuela. Photo by Andreas Fleischmann.



Figure 2: *Utricularia neottioides* growing in a small stream in the Serra do Cabral, Minas Gerais, Brazil. Photo by Andreas Fleischmann.

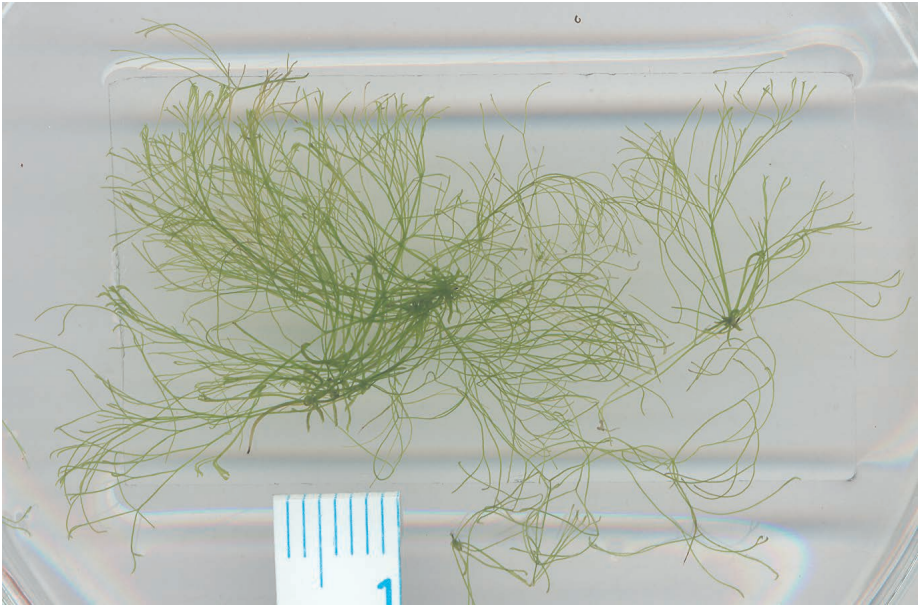


Figure 3: Shoots of *Utricularia neottioides* from *in-vitro* culture. Scale = 1 mm. Photo by Lubomír Adamec.

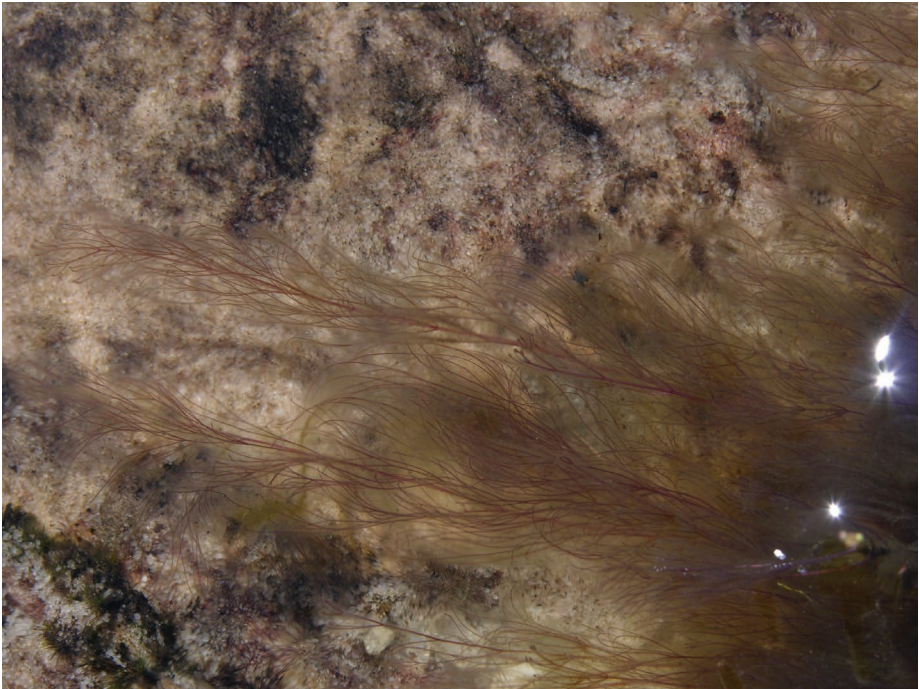


Figure 4: The usually trap-less, filamentous leaves of *U. neottioides* in quickly running water, Minas Gerais, Brazil. Photo by Andreas Fleischmann.

specimens, we have never found any traps. However, Luetzelburg (1910) and Lloyd (1932, 1942) studied and illustrated some scattered traps of the species from field-collected Brazilian material. Apparently the material collected by von Luetzelburg and by Kuhlman is among the very few specimens of *U. neottioides* that possess any traps; Taylor (1989) later based his studies and illustrations on two additional, more recently collected specimens with few traps present on leaves and stolons. Lloyd (1932) and Taylor (1989) describe the traps as ovoid (or “streamlined”, following Lloyd 1942) with a deep overhang, ca. 1 mm long, dark brown in color, and apparently normally functioning. The quadrifid trap glands have been illustrated by Lloyd (1942) and Taylor (1989).

Quite in contrast to our observations (and those made by Lloyd 1932 and Taylor 1989), Kuhlman (1938), who studied the species in Brazil on several occasions, reports that this species apparently always bears traps (with three antennae on one side), although in much lower numbers than any other *Utricularia* species.

As perfectly illustrated by Luetzelburg (1910) and Taylor (1989), *U. neottioides* bears stiff, coral-loid, claw-like rhizoids 0.2-0.4 mm thick, by use of which the plants affix themselves to the bedrock of the streams (Fig. 5). These are located on creeping thick stolons several cm long, which also bear 1-4 cm long, sparsely pinnatisect, filamentous “leaves” (in fact the “leaves” are modified leaf-like shoots, as in all aquatic *Utricularia*; see e.g. Rutishauser & Isler 2001). These leaves are bright green to bronze-colored or red, depending on light levels, and densely covered by long, unicellular, transparent hairs (Fig. 7). Generally, the filamentous leaves (diameter of ca. 60 µm) are truly reminiscent of filamentous green algae, e.g. of the genus *Cladophora*. Interestingly, the “leaves” of *U. neottioides* are not only formed from the stolons in this species (like in all other *Utricularia*), but (uniquely among *Utricularia*) also from the lowermost bracts of the flower scape (Luetzelburg 1910; Fernández-Pérez 1964; Taylor 1989) – this strange morphology is paralleled in the unusual riverweeds (Podostemaceae), likewise rheophytes adapted to very similar habitats.

Not surprisingly, due to the turbulent habitat of *U. neottioides*, the leaves and shoots of this species are inhabited comparatively by much fewer periphytic algae, such as desmids (Desmidiaceae), although aquatic *Utricularia* are generally a good habitat for these algae: Förster (1964) found only seven different taxa of desmids growing on *U. neottioides*, while other Brazilian aquatic *Utricularia* had 13-94 different desmid inhabitants (for comparison: five were found on the terrestrial *U. subulata*).

Flowering in *U. neottioides* is apparently sometimes induced by low water levels (Rivadavia 1991). The scapes can be up to 30 cm long (Taylor 1989; Rivadavia 1993). The cream-white corolla has a deeply trilobed lower lip, and the palate consists only of a shallow ring-like rim, so that the entrance to the tube and spur remains open (as seen in front view, Fig. 6). The flowers of this species (and *U. oliveriana*) have a sweet scent (AF, pers. obs.), which probably attracts a specific pollinator. Seeds are different from all other *Utricularia* (Taylor 1989), probably in adaptation to dispersal and seedling establishment in the demanding riparian habitat. The seeds become mucilaginous when wet (Lloyd 1942; van Steenis 1981) and thus can attach to rocks and stones of the riverbed, especially during the dry season when water levels are low.

Interestingly, a similar rheophytic habit has evolved in parallel in an African lineage of aquatic *Utricularia*, again a species pair (*U. rigida* Benj. and *U. tetraloba* P.Taylor), comprising *U.* section *Avesicarioides* (Taylor 1989). Molecular phylogenetic results show that these two species, which are superficially very similar to *U. neottioides*, are only distantly related to it. Thus, the rheophyte habit indeed evolved independently at least twice in *Utricularia*, and in both cases this happened from terrestrial lineages (Müller *et al.* 2006).

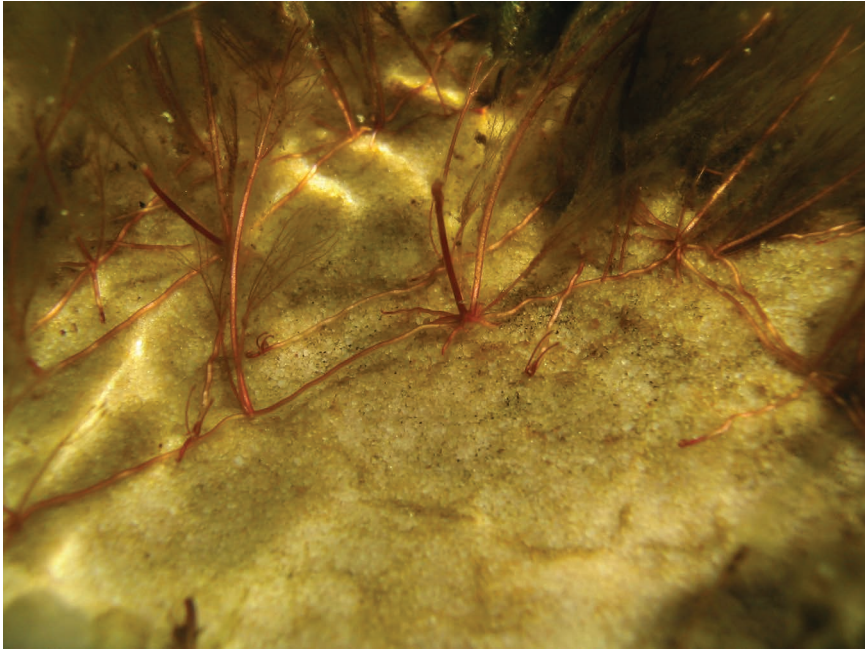


Figure 5: The claw-like rhizoids of *U. neottiioides* which anchor the plant firmly to the sandstone bedrock. Leaves and flower scapes are produced from the thick stolons. Photo by Andreas Fleischmann.



Figure 6: Flowers of *U. neottiioides* from Minas Gerais. Photo by Andreas Fleischmann.

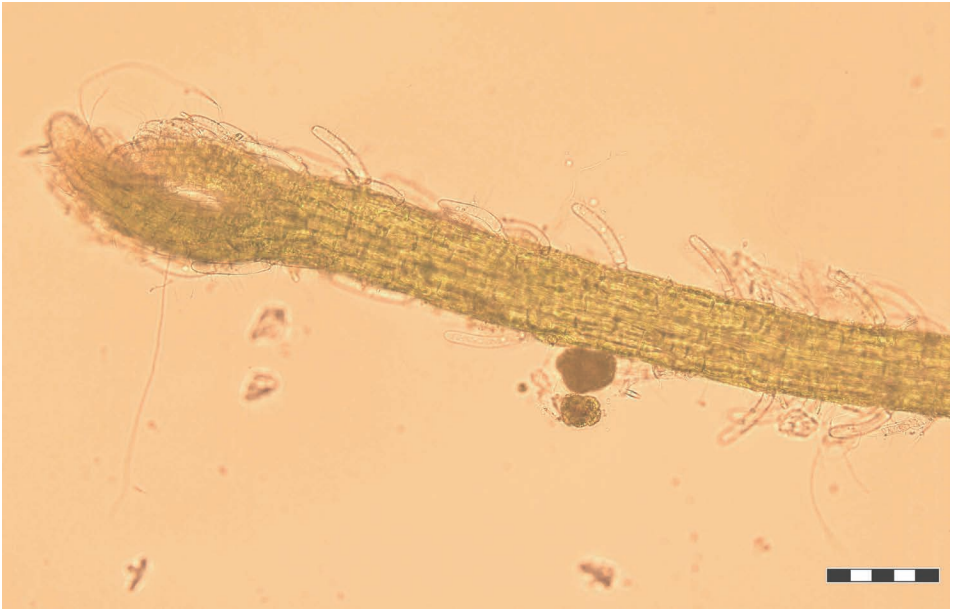


Figure 7: Microscopic photo of an *U. neottioides* leaf from *in-vitro* culture. Scale = 100 μm . Photo by Lubomír Adamec.

Natural Habitats

Utricularia neottioides is a so-called “torrenticolous rheophyte” (van Steenis 1981), as all vegetative parts of the plant are permanently submerged in quickly running water, with only the flower stalks produced above the water-line. The plants can form large carpets in suitable habitats, where they are often the only flowering plant inhabiting the stream (Fig. 2). In shallow pools they can remain in full growth during the dry season as a perennial. However in habitats where the streams become fully dry, this species can also grow as an annual. According to Taylor (1989), *U. neottioides* is distributed across a relatively vast territory of tropical South America: it is recorded from Colombia, Venezuela, Bolivia, and Brazil (majority of states except southern-most). Typical habitats are shallow, swiftly flowing, cool, acidic, dystrophic (“Coca-Cola-colored”, Rivadavia 1996) torrents and streams of mountainous areas, and the plants are affixed only on rocks and stones (predominately sandstone rocks), but never on a soft sediment or sand (AF, pers. obs.). A detailed description of the habitat of this species is given by Rivadavia (1996). The species occurs at altitudes from ca. 300 to 1800 m a.s.l., but is generally restricted to cooler highland waters. The majority of these streams are dark brownish (i.e., dystrophic or humic): this fact can be important both for nutrition of this species and for the requirement of the plants for irradiance. However, at most sites (see Fig. 2), the plants grow under bright sunshine as a result of which the leaves/shoots are red and relatively thick (Figs. 4, 5) – as heliophytes; they have only very rarely been found growing in more shaded habitats (F. Rivadavia, pers. comms.).

Interestingly, the leaves of *U. neottioides* are often covered by fine filamentous algae (see Fig. 4) bestowing the leaves with a mucilaginous character. Moreover, as opposed to other aquatic *Utricularia* species growing at the margins of South American streams (e.g. *U. oliveriana*, *U. trichophylla*), the sites inhabited by *U. neottioides* are probably ecologically so extreme that no other aquatic

Utricularia species co-occurs. One would guess a very low pH in these dystrophic waters but the only information on pH from some Brazilian sites available is rather surprising (pH 7.8-8.0; Vitor de Miranda, pers. comm.). Moreover, one of us (AF) several times saw *U. neottioides* surviving also in temporarily, shallow standing pools or ditches remaining from the streams after the water level has sunk in the dry season. Rivadavia (1996; pers. comms.) found the species growing as an affixed aquatic in gravel, red soil, and grey clay. Thus, these findings justify the view that *U. neottioides* may not be a strict rheophyte and support efforts in cultivation. Its rare occurrence in warm waters in lowlands justifies the view that this species may not be strictly adapted to low temperatures. Fernández-Pérez (1964) classified *U. neottioides* as having a “habit intermediate between aquatic and terrestrial” – and indeed the two rheophytes of *U.* section *Avesicaria* are closely related to the terrestrial species of the affinity of *U. subulata* (*U.* section *Setiscapella*), but not to any other aquatic species, a fact already concluded by Taylor (1989) based on common morphological traits. This is also supported by molecular phylogenetic results (Müller *et al.* 2006). Due to its unique habitat and apparently very specific ecological needs, as well as previous failures to maintain wild-collected specimens in cultivation, *U. neottioides* species has been claimed almost un-cultivable (Rivadavia 1996).

Growth in *in-vitro* Culture

Out of several dozens of seeds of *U. neottioides* collected at Itacambira, Minas Gerais, Brazil, and sterilized by a sodium hypochlorite solution, only one germinated in our *in-vitro* culture. Nevertheless, that plant grew very vigorously in a sterile tissue culture, in liquid or solid half-strength Gamborg B5 medium (for all cultivation details, see Adamec & Pásek 2009). The plants grow so rapidly under these conditions that an initial plant stock can occupy a whole E-flask in only 1.5-2 months. Therefore, it is comparatively the most rapid *in-vitro* growing aquatic *Utricularia* species that we know of. As shown in Figs. 3 and 7, *in-vitro* plants form a net of poorly branched, finely filamentous, light green leaves (leaf diam. ca. 60-75 µm) 2-5 cm long, while the thicker and darker rhizoids and stolons are ca. 0.2 mm wide. Most leaf filaments are terminated by a U-shaped bifurcation (Fig. 7). All shoots are densely covered by long translucent hairs. Neither traps nor inflorescences have ever been formed *in-vitro*. Basal rhizoids and stolons become black and decay in old *in-vitro* cultures. The reliable and very rapid growth *in-vitro* has provided sufficient plant material for some experiments in *ex-vitro* cultivation.

Growth *ex-vitro*

We attempted growing *U. neottioides* in many aquatic cultures, both indoors and outdoors. Fresh shoots of *U. neottioides* were inserted into several aquaria or bigger plastic containers with humic water, a method used successfully with other aquatic carnivorous plants for years (see Adamec 1999), where sedge (*Carex* spp.) litter was used as the only substrate. The *U. neottioides* plants were either kept isolated in aquaria, or mixed with other aquatic carnivorous plant species. However, regardless of the indoor or outdoor position of the aquaria and other containers, their volume, irradiance, temperature, water chemistry, presence or absence of other carnivorous species, or bubbling the water gently by air, the *U. neottioides* shoots quickly became greyish, then died and decomposed completely within only 1-3 weeks. Moreover, aquarium snails *Gyraulus chinensis* (Twisted ram's-horn) very eagerly grazed the plants. Yet, even when snails were excluded through nylon netting, *U. neottioides* nonetheless failed to grow. The brighter the natural light was in the setup, the faster the



Figure 8: Terrestrial greenhouse culture of *U. neottioides* on brown peat. The maximum height of the shoots is ca. 2 cm. Photo by Kamil Pásek.

decay occurred. Surprisingly, plants survived relatively better and longer when placed at the bottom of aquaria in deeper shade. Similar to e.g. *U. floridana* shoots, the dense cover of partly hydrophobic hairs on *U. neottioides* shoots renders these hydrophobic. Due to water surface tension, the stolons remain adhered to the water surface. When a small plant fragment was enclosed in a 0.5 liter bottle containing humic water with a high CO₂ concentration, survival was somewhat longer but no new growth was observed.

It has thus become apparent to us that *U. neottioides* cannot be artificially grown as a submerged, freely floating aquatic, for unknown reasons. The same conclusion was also made in the laboratory of V. de Miranda in Brazil (pers. comm.), where again no traps were formed during cultivation trials.

Surprisingly, far better results were achieved when *in-vitro* raised *U. neottioides* shoots were softly placed on the surface of very wet, fibrous brown peat (with or without the addition of quartzitic sand), conditions similar to those used for growing terrestrial carnivorous plants. In a humid greenhouse at 15-25°C and in reduced light (ca. 10-20% light), the initial shoots regenerated within about two weeks, and new growth was observed, yet not as large and vigorous as seen in naturally growing plants – more like a stunted, terrestrial growth of this remarkable aquatic (Fig. 8). We confirmed repeatedly that the regeneration of submerged shoots into newly grown terrestrial ones started as soon as after 5-6 days and proceeded completely very quickly in 2-3 weeks. Evidently, high relative air humidity above the peat substrate is necessary for this regeneration, e.g. in a closed container. In a dry and hot greenhouse, it is possible to put *U. neottioides* shoots on brown peat in a smaller terrarium (2-3 l) which is covered by a translucent lid and which floats on the surface in a larger container filled with water for cooling. Such a thermostatted cultivation works better and

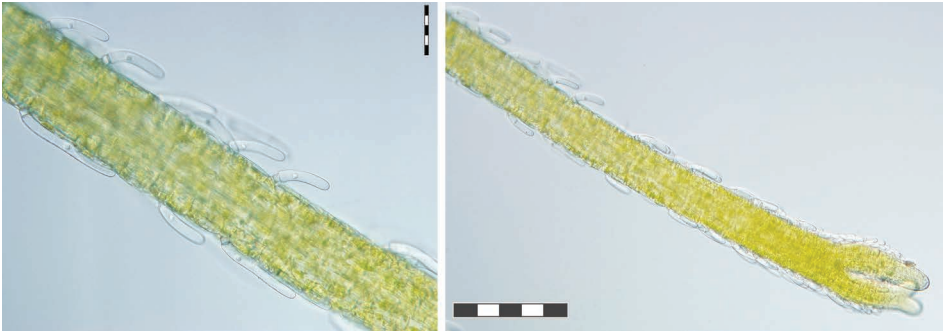


Figure 9: Detailed microscopic view of *U. neottiioides* leaf from *ex-vitro* terrestrial culture (left). The leaf apex is branched into three lobes (right). Scale = 100 µm for the left photo, but 200 µm for the right photo. Photos by Lubomír Adamec.

more reliably. The terrestrial *U. neottiioides* shoots are much shorter (ca. only 8-18 mm) than the submerged shoots *in-vitro* but are significantly wider (ca. 100-120 µm; Fig. 9). The shape and size of the translucent hairs are very similar.

Repeated transplantations of *U. neottiioides* shoots from the *in-vitro* culture onto peat substrate revealed several interesting findings. First, as a result of these transplantations and regenerations, new terrestrial shoots were formed quickly during 1-3 weeks, but further growth stopped completely afterwards. The terrestrial shoots only survived for 5-6 months. The more biomass of *U. neottiioides* from *in-vitro* cultures that was placed on the peat, the denser was the resulting terrestrial outgrowth. However, *no* new biomass was produced: the decomposed *in-vitro* biomass was only transformed into the new terrestrial one.

The same regeneration also occurred when terrestrial shoots were carefully laid onto the peat. Under suitable growth conditions (reduced light to ca. 10-15% of that in the open, 15 to 25°C, high relative humidity), terrestrial culture could survive at a constant state for at least 5-6 months. Nevertheless, after this period, the peat was usually overgrown by filamentous algae or mosses which impaired further plant survival.

Terrestrial cultures of *U. neottiioides* confirmed that this species is adapted to lower temperatures. The plants were able to survive a wide temperature range (as daily extremes) between 8 to 35°C and died at temperatures >35°C; suggesting the optimum might be 15-25°C. Similar to the *in-vitro* cultures, flowering and traps have never been observed in plants growing in any *ex-vitro* culture.

Ecophysiological Investigations

Simple investigations were conducted to explain some ecophysiological traits of *U. neottiioides* and, thus, to facilitate its cultivation. Using the 'final pH method' in 1 mM NaHCO₃ solution (Adamec 1995), the CO₂ compensation point of photosynthesis of shoots taken freshly from the *in-vitro* culture at ca. 22°C was 17.1±1.4 µM (n=6). In a similar study of 13 aquatic carnivorous plant species (Adamec & Pásek 2009), the values of CO₂ compensation points usually ranged within 3 to 8 µM for plants grown both *in-vitro* and *ex-vitro* in containers or aquaria. This means that the photosynthetic CO₂ affinity of *U. neottiioides* shoots is rather low and that plants in the wild probably rely on relatively high ambient CO₂ concentration found in fast flowing waters. Furthermore, the aerobic dark respiration rate of *U. neottiioides* shoots in an aquarium, measured using an oxygen sensor, was extremely high: ca. 50 mmol kg⁻¹ (fresh weight) h⁻¹. Compared to other relevant data from shoots/

leaves of several aquatic *Utricularia* species (Adamec 2006, 2013), the respiration rate of *U. neottoides* was around 8-12 times higher! Such a high respiration rate might predetermine plant sensitivity to O₂ shortage in the ambient water. Besides, it also means that a very high net photosynthetic rate is strictly required to counterbalance high respiration rate. Taking into mind the rather low CO₂ photosynthetic affinity of *U. neottoides*, a very high CO₂ concentration (and possibly high irradiance) may be required in the ambient water for positive growth.

Conclusions

To our knowledge, *U. neottoides* cannot be grown successfully in any *ex-vitro* culture for some reasons, but it grows vigorously in an *in-vitro* culture with 2.5% sucrose solution. Meeting the evident requirement of high CO₂ concentration was not sufficient to keep plants alive. It is probably impossible to fulfill the growth needs of *U. neottoides*, except maybe if attached to sandstone in a specially designed stream pool aquarium. Considering that this almost trapless species occurs in dystrophic waters and is densely covered by long hairs, it is logical to assume that its nutrition is to a greater extent dependent on absorption of organic substances from the ambient water (e.g., humic acids) or attached filamentous algae. In this way, the necessary nitrogen might be mostly obtained from these special sources in nutrient-poor waters.

U. neottoides and *U. rigida* are the only *Utricularia* species with enormously suppressed trap formation, though the capability for trap formation is kept, as exceptional traps found on the leaves and shoots of these two species show. For this trait, *U. neottoides* was used as a model trapless species for comparative transcriptomic research (i.e., transcription of functional genes) at the University of South Bohemia at České Budějovice, Czech Republic.

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