RHYNCHOSPORA ALBESCENS (MIQUEL) KÜKENTHAL (CYPERACEAE) – AN UNUSUAL AQUATIC SPECIES

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SUMMARY

An aquarium plant that has been sold in the aquarium trade for over 10 years was identified as *Rhynchospora albescens* (Cyperaceae) and molecularly analysed. For the first time [in English] the DNA sequence of this species as well as SEM images of young flowers are shown in this article. While three stamens are normal for the species of the genus *Rhynchospora* section *Dichromena*, two stamens were found in most flowers of *R. albescens* examined here. This is followed by a description and experiences with aquarium culture and a summary of the previous knowledge about the ecology of the species.

PREFACE FROM C. KASSELMANN

For more than 10 years an aquatic plant has been marketed in the aquarium trade under the name Eriocaulaceae sp. "Type 2". From the very beginning I doubted whether it belonged to this family due to morphological characteristics, which is why in the 3rd edition of my book "Aquarienpflanzen" (2010) I called the species Cyperaceae sp. For many years no flowering plants were available.

In the summer of 2018, two inflorescences first developed

in my winter garden on a small, emersed stand in daylight, warm temperatures and humidity. As they referred to the Cyperaceae family, I sent photos of plants and the very small spikelet to botanists of the Royal Botanic Gardens Kew (England) who specialize in this family. The family Eriocaulaceae could be excluded, but the family Cyperaceae could not be confirmed.. The determination of the species did not seem to be easy.

Through the mediation of Josef Bogner (Gersthofen) I then turned to Dmitry Sokoloff (Moscow). The botanist published articles on various genera and families (e.g. the Hydatellaceae) and described some new species. Sokoloff answered immediately. This was followed by extensive correspondence—more than 70 emails—which may make it clear to the reader what a challenge the determination of this species was. I sent living plants, herbarium specimens and the few available inflorescences to Moscow. My material was morphologically and molecularly examined by Dmitry Sokoloff and his colleague Galina Degtjareva. In November 2018, a DNA comparison with the sequence database (GenBank) revealed that it was a species of the genus *Rhynchospora* (family Cyperaceae, order Poales).

THE DIFFICULT PATH TO DETERMINATION

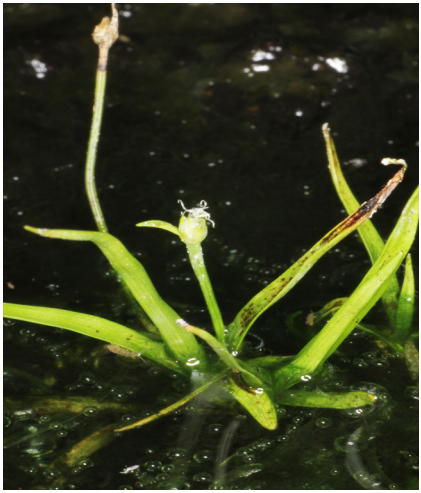
From the beginning of our study we realized that our plants fit the most important characters of the family Cyperaceae, such as the overall leaf morphology, the flowers arranged in a spikelet with glumes and no bracteoles, the reduced perianth (which is just absent in our plant), the gynoecium with a superior unilocular ovary and single basal ovule. For further identification of the plant, a remarkable character was the occurrence of single terminal spikelet. Most Cyperaceae possess inflorescences composed of several to many spikelets. Some Cyperaceae, however, do possess a single spikelet, for example the genus *Eleocharis*. We carefully checked all genera of the family normally or frequently possessing a single spikelet per inflorescence and found that our plant fit none of them. This is why we used the molecular phylogenetic approach.

We used two molecular markers, namely (1) nuclear internal transcribed spacers (ITS) of ribosomal DNA and (2) portion of the coding plastid gene rbcL, because they are the most characterized regions in GenBank, with wide representation from all major groups of plants. Total DNA was extracted from dried leaves. Newly obtained sequences have been deposited in GenBank under the accession numbers MN515402 (for ITS) and MN515403 (for rbcL). The BLAST search in the GenBank database (www. ncbi.nlm.nih.gov) has shown that species of *Rhynchospora* were most similar to our accession. Similarity values from the BLAST

search were: rbcL–96% (*R. nervosa, R. colorata, R. nitens, R. robusta*); ITS–91-93% (*R. colorata, R. nervosa*). As rbcL data are more abundantly presented in the GenBank in relation to *Rhynchospora* species than ITS (57 vs. 7), this marker was chosen for further analysis. The Bayesian analysis was performed using MrBayes version 3.2.6 (Ronquist et al., 2012).

Our phylogenetic tree (see Fig. 1, page 26) clearly shows that our plant belongs to the genus *Rhynchospora* and, more precisely, to one of its clades that comprises species of the section *Dichromena*. The section *Dichromena* is a group that is interesting with respect to its reproductive biology. While most Cyperaceae are wind-pollinated, inflorescences of species of this section are visited by insects such as flour beetles, syrphid flies, and bees and correspondingly possess white glumes (Thomas 1984). The whitish glumes are also characteristic of our species.

Rhynchospora is one of the largest genera of the family Cyperaceae. A complete monograph of the large genus *Rhynchospora* was written by the German botanist GEORG Kükenthal (1938–1952) who recognized 211 species. Today, about 250 to 300 species of *Rhynchospora* are recognized (Thomas et al. 2009). Almost all species of this genus possess inflorescences comprising many spikelets (see e.g. Lucero and Vegetti 2012). This is why



^ *Emersed plant of* R. albescens *with two very small spikelets.*

v *Inflorescence (spikelet) of* R. albescens*: A small green leaf at the base of the spikelet, several white glumes and the filiform stigmas are visible, behind them an anther.*

we overlooked *Rhynchospora* during our initial search based on morphology. Having the molecular phylogenetic evidence, we looked morecarefullythroughthetaxonomic literature on the section *Dichromena* species, only a few of which were sequenced earlier and are available as GenBank sequences. While Kükenthal listed 14 species for the section *Dichromena*, the American botanistWaytThomas(TheNewYork Botanical Garden), who worked on this plant group in 1984, describes already 23 species. We found that there is a species of the section *Dichromena* that constantly possesses only one spikelet, namely *R. albescens*. Further comparisons with morphological descriptions of this species and its available images, including scanned specimens from various Herbaria convinced us of this identification. Finally, our determination was approved by the main expert in taxonomy of this group, Wayt Thomas.

As the published data on molecular phylogenetics of the section *Dichromena* were incomplete, we here provide the first molecular evidence on the phylogenetic placement of *R. albescens (see Fig. 1, p. 24)*.

v *Magnificent stock of* Rhynchospora albescens *in the Discus aquarium of Eddy Leysen.*

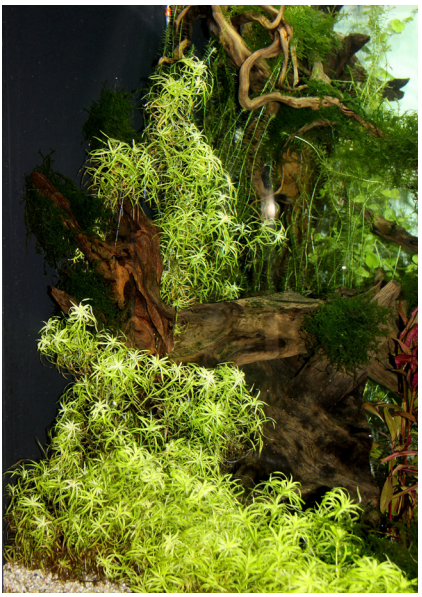


Photo by E. Leysen

v *Over 40 cm long shoot of* R. albescens *from the aquarium showing the foliage with the leaf rosettes.*

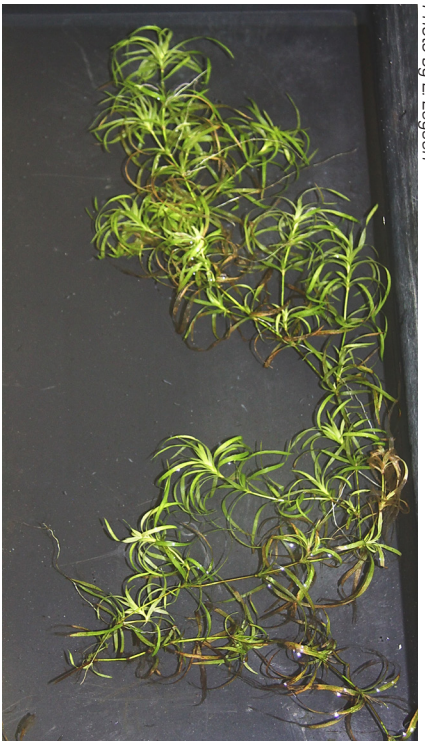


Photo by E. Leysen

TO THE SCIENTIFIC NAME

Rhynchospora albescens is a botanically old species, which was already described in 1847 by the Dutch botanist F. A. W. Miquel as *Isolepis albescens*, which Kükenthal then placed into the genus *Rhynchospora* in 1951. The type was collected in Surinam.

FLOWER MORPHOLOGY AND DEVELOPMENT

With living plants in our hands, we had an opportunity to study for the first time details of flower morphology and early flower development in *R. albescens* using scanning electron microscopy (SEM).

Our data show that the flowers are all bisexual and lack perianth or any traces of reduced perianth members. The absence of the perianth is characteristic of the section *Dichromena*. Almost all flowers possess two lateral stamens. Among all the examined material, only one flower with three stamens was found. There are two carpels in transverse position. The two stigmas appear late in development (see Fig. 2, 3, p. 28-29).

Our data add some information to the extensive study of Lucero et al. (2014) who studied nine species of *Rhynchospora*. Published taxonomic data indicate the occurrence of three stamens in flowers of the members of the section *Dichromena*. Our data show that this character varies at the level of species in this group.

ETYMOLOGY

The generic name *Rhynchospora* is composed of the Greek words *rhynchos* = beak and *spora* = seed, which refers to the beak-like base of the style; *albescens* means whitening and refers to the white glumes of the inflorescence.

DISTRIBUTION

The genus *Rhynchospora* is widespread worldwide. Most species of the section *Dichromena* are found in tropical South America. Although relatively few collections of *Rhynchospora albescens* have been found, they are found almost in the entire tropical area of South America, extending into Central America. While Kükenthal (1938–1952) only mentions Guyana and northern Brazil as distribution areas, more recent collections show that the occurrence is considerably larger. Thomas (1984) documents the distribution mainly north of the equator in Colombia, Venezuela, Brazil, Guyana and Surinam; the occurrence in French Guiana is suspected. A locality from Mato-Grosso (Brazil) far south of the equator is reported. And the species was also collected much further north in Costa Rica (e.g. Thomas 1992).

DESCRIPTION

Rhynchospora albescens is a persistent, delicate swamp plant with a predominantly aquatic way of life. Submerged plants become much larger than terrestrial plants. In the aquarium, 20 to approximately 60 cm long, light green, loosely leafed stoloniferous shoots are formed,

Figure 1: Phylogenetic tree of Rhynchospora *inferred from Bayesian analysis of sequences of the plastid marker rbcL. GenBank accession numbers are indicated in parentheses after species names. Our new sequence (*R. albescens*) is underlined. Members of the section* Dichromena *are indicated.*

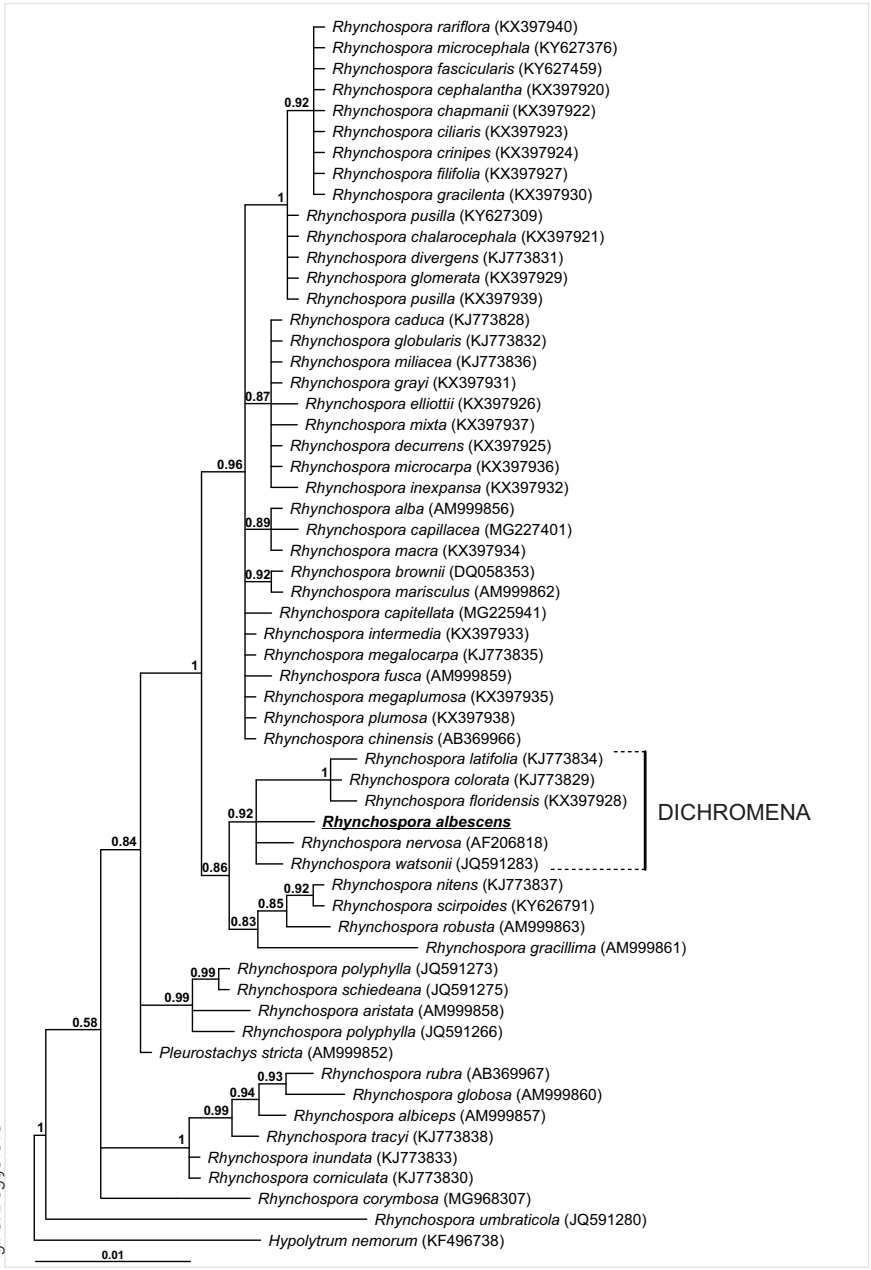


Fig.: G. Degtjareva

that strive upright (not horizontally) to the water surface. These stolons are thin and round in cross-section, branching and rooting at the nodes. The spirally arranged, cauline, soft leaves develop densely packed leaf rosettes. The internodes between the rosettes are from 1.5–3 cm long. A short, closed leaf sheath is located at the base of the leaves. A ligule is missing. The submerged leaf blade is linear, 3.0–5.0 cm long and 1.5–2.0 mm wide, thin, delicate, glabrous and with even parallel veins. The water leaves are light green above and whitish below.

Emersed plants form densely packed, low stands that reach about 4 to 6 cm in height. The plants are clearly smaller (leaves up to 3.5 cm long) and coarser in structure.

Inflorescences form on emersed plants and consist of a single, upright, ovoid to broadly ovoid spikelet with few flowers. The peduncle usually protrudes above the plant after the anthesis, even when no pollination has taken place. At the base, the spikelet has 1 green, 1.4–6 mm long small leaf and up to 20 white glumes. According to Thomas (1984), the spikelet is 2.5–4 mm long and 1.5–2.2 mm wide. In our plants, the possibly not fully developed inflorescences with a length of about 2 mm remained below this size. In contrast to other species of the genus *Rhynchospora*, where the length of the glumes usually increases toward the tip of the spike, the lowest glumes of *R. albescens* are the longest.

The flowers are bisexual. Each flower develops in the axil of a small glume. The bracteoles and perianth are missing. There are usually 2 stamens whose filaments are long and filamentous. The pistil is unilocular. Ovule 1. The style is divided into 2 filiform stigmas, basally united. The nutlet (according to Thomas 1984) is lenticular, symmetrical on both sides and 0.7-0.9 mm in size.

CULTURE

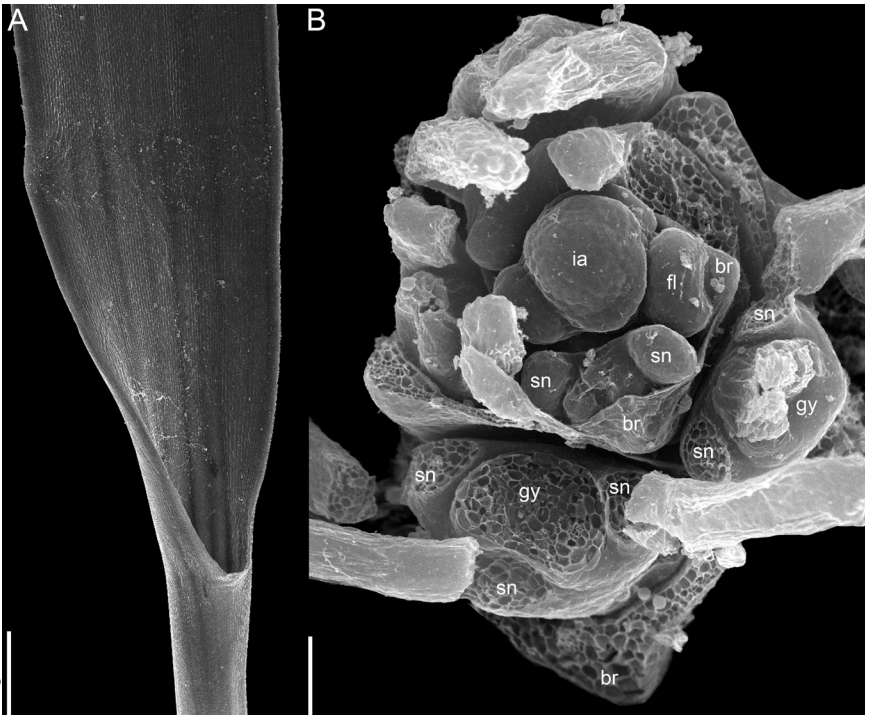
Although *Rhynchospora albescens* occurs in natural habitats in black waters, it can be cultivated very successfully in aquariums under special conditions. A prerequisite for satisfactory growth is very soft, nutrient-poor water with an acid pH value below 6. The species cannot be kept without CO_2 supplementation. At the same time, high temperatures of at least 27°C [80.6°F]—even higher—are required for rapid growth. The light requirements are medium to high. Under optimal conditions, numerous upright stoloniferous shoots develop in the aquarium, which in turn form rooting rosettes. A vegetative propagation succeeds easily and is very productive. *Rhynchospora albescens* has little adaptability to other water values. Attempts to cultivate the species in "normal aquariums" with medium-hard water and a neutral pH value have always failed.

Rhynchospora albescens is particularly recommended for keeping in Discus aquariums with very soft, acid, high temperature

water and good lighting. Eddy Leysen (Belgium), who has propagated the species very successfully over years in an 80 cm high Discus aquarium and has to cut back the populations every 2–3 weeks, cultivates R. albescens under the following conditions: pH-value around 5, CO_2 content 30 mg/l, GH 1.5–2°dH, KH 0.5°dH, conductivity 100–120 µS/cm, NO_2 < 10, Fe < 0.5 mg/l, temperature 30°C, but also 35°C over one month was "survived". The plants grow under very intense lighting (light color 5700 K) and for 13 hours daily. Even flooding shoots continue to grow on the water surface. The bottom of his aquarium consists of fine gravel without any additives. A water change of 15–20% takes place weekly.

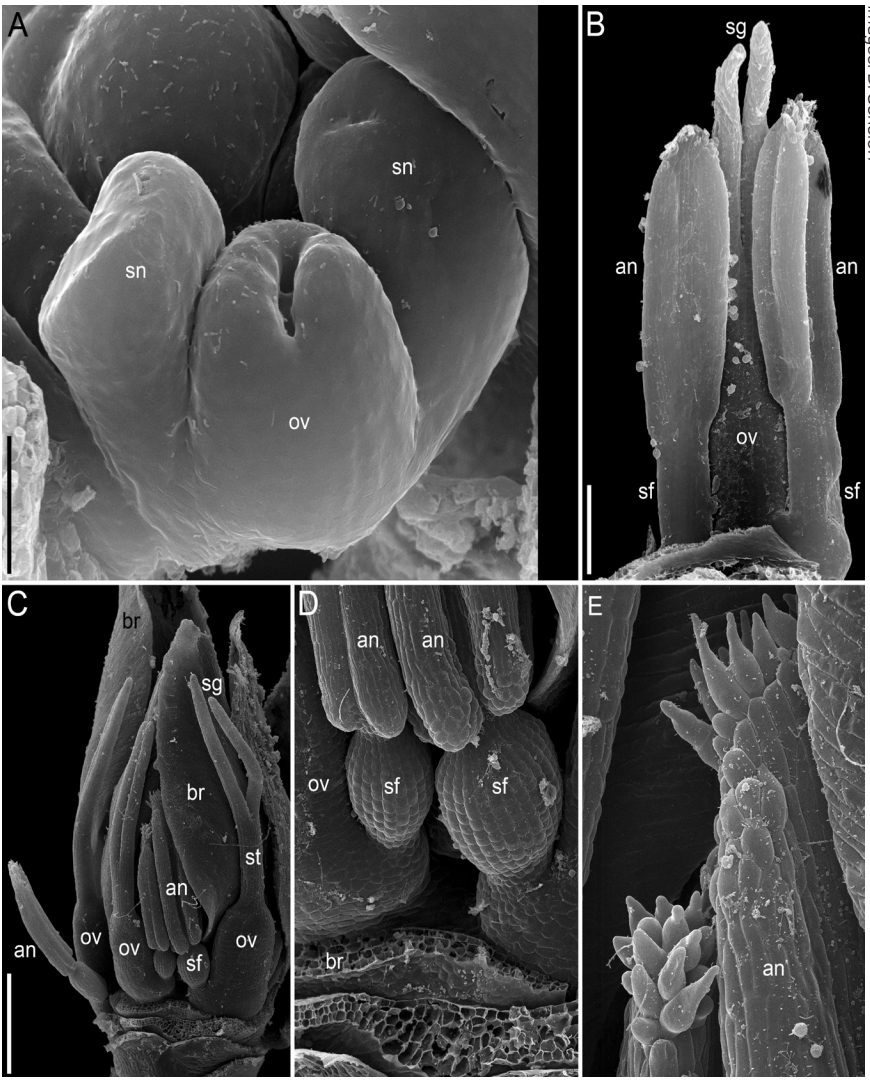
An emersed culture, in which the plants flowered, took place in the soil

Figure 2: Morphology of Rhynchospora albescens *(SEM). A. Detail of leaf morphology showing transition from the sheathing base to the lamina. Scale bar: 0.5 mm. B. Top view of young inflorescence with flowers in different stages of development. Note that one of the oldest flowers has three stamens while the other has two stamens (the stamens as such are removed during preparation, but the places of their attachment are clearly visible). Scale bar: 0.05 mm. Abbreviations: br = glumes (flower-subtending bracts); fl = very young flower before clear initiation of stamens; gy = gynoecium; ia = inflorescence apex; sn = stamen.*



Images: D. Sokoloff

v *Figure 3: Flower morphology and development of* Rhynchospora albescens *(SEM). A. Flower with very early stage of gynoecium development, stigmas are yet almost missing. Scale bar = 0.03 mm. B. Young flower with stigmas and anthers well recognizable. Scale bar = 0.1 mm. C. Entire young spikelet with some glumes removed to show the outermost flowers at stages later than in B. Scale bar = 0.3 mm. D. Detail of C showing swollen stamen filaments (these will dramatically elongate just before anthesis). E. Detail of C showing characteristic papillae at the anther tip. Abbreviations: an = anther; br = glumes (flower-subtending bract); ov = ovary; sg = stigma; sf = stamen filaments; sn = stamen.*



Images: D. Sokoloff

"Fluval Stratum"; this is a nutrient-poor, burnt soil. A multiplication by side shoots was not observed. Overall the emersed culture seems to be very difficult, which indicates that *R. albescens* is predominantly a water plant.

ECOLOGY

Most species of the genus *Rhynchospora* live in savannas or open forests. Only three species, to which *Rhynchospora albescens* belongs, live in periodically flooded areas. The other two species, *R. reptans* and *R. papillosa*, also live in the flooded area of black waters with high tannin content and white quartz sand. Black water is characterized by a very low pH value and a low content of nutrients. Many aquarium fish come from such waters, for example neon or scalares.

Rhynchospora albescens grows along streams and rivers with strongly fluctuating water levels and adjacent floodplains. The plants are rooted in small, dense stands on the banks of sandbanks, where they flower and fruit from July to February. The upright runners form under water with the roots growing downward from the rosettes. So on the one hand, the plants anchor themselves in the ground with rising water levels and increased flow speed. On the other hand, however, young plants loosening from the stoloniferous shoots are driven away with the current and provide effective spreading. If the water level rises, the plants grow completely submersed even in deeper water. The locations should be open, shaded or partially shaded. Thomas (1984) found pH values of 4 and 4.5 in soils on which he found *R. albescens*. The author suspects that the plants only survive as seeds during the flood season, but we do not believe this due to our cultural experience and the rapid growth of the plants persisting in the aquarium. We assume that in the natural habitat mainly submersed plants are responsible for the distribution and formation of new emersed and flowering stands. According to our experience with the emersed culture of *R. albescens*, the periodic life cycle in the natural habitat will be as follows: The submersed plants are washed up on sandbanks as the water level falls, where the runners are pressed to the ground and the rosettes can now root in the sand. The water leaves rot and new emersed leaf rosettes with firmer leaves are formed, adapted to the humidity of the air. In the weeks of low water, the plants pollinated by insects flower and fruit. The seeds, which are driven away by the current of the river as the water level rises, also spread the plants.

Herbarium specimens of *R. albescens* were (generally) made by emersed, flowering and fruiting plants; however, they give only an incomplete picture of the life cycle of this plant species. Obviously, the much larger aquatic plants with their multitude of stolons and young plants are hardly known scientifically. This is the only explanation for the

illustration in Thomas (1984), which shows a plant with a horizontal stolon. In reality, the upright stolons with young plants formed under water are likely to be pushed into the horizontal position as the water level falls.

OTHERS

The import of the *R. reptans* and *R. papillosa*, which also spread in the black water and multiply by stolons, would be interesting for the aquaristics. Both come from the same range and form considerably larger leaf blades than *R. albescens*. The rare *Rhynchospora papillosa* from Venezuela has only been collected twice so far.

THANKS

We would like to thank Wayt Thomas (New York) for verifying our determination and for providing additional information on ecology. Josef Bogner (Gersthofen) kindly supported us with discussions about the determination and procurement of herbarium material. We thank Tahir Samigullin (Moscow) for help with phylogenetic analysis, Margarita Remizowa and Anna Platonova (Moscow) for discussion on morphology, and the staff of the Laboratory of electron microscopy at the Biological Faculty of Moscow University for their assistance during the SEM work. Eddy Leysen (Belgium) we owe living plant material and information on aquarium culture. Analysis of flower morphology is performed with support from the Russian Science Foundation (project 19-14-00055, for DDS).

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