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WATERY VARIETIES: AQUARIUM PLANT DIVERSITY FROM AESTHETIC, COMMERCIAL, AND SYSTEMATIC PERSPECTIVES

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Chapter 1

WATERY VARIETIES: AQUARIUM PLANT DIVERSITY FROM AESTHETIC, COMMERCIAL, AND SYSTEMATIC PERSPECTIVES

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ABSTRACT

A review of the paradigmatic changes in the role of plants in the 160 years of modern aquarium keeping reveals their position at the intersection of aesthetics, technology, and commerce. Decoration styles in aquaria have changed slowly, and have remained relatively unaffected by the other cultural arenas of visual expression. The aquarium plant trade has developed into a multimillion-euro industry with more than 30 specialized European nurseries serving mainly the European market alone. The genera *Echinodorus* and *Helanthium*, the latter recently reinstated as valid taxon separate from the first, entered this developing scene some 75 and 60 years ago, respectively, and became instantly popular because of their uncomplicated culture and distinct appearances fitting different roles in the aquascapes. The relative ease of *Echinodorus* hybridization has led to an explosion of colorful new *Echinodorus* cultivars since the late 1980s. Nowadays, with respect to the number of traded species and cultivars, *Echinodorus* is the most diverse genus in the international aquatic plant market, and is economically among the most important genera. The problems arising from the international ornamental plant trade, such as property rights or invasions of alien species, call for proper identification of the taxa involved. In order to determine which species are involved in the commercial cultivation, we conducted a series of DNA analyses in the framework of molecular systematics. A number of most common cultivars were included in order to expose their genetic background. The sampled members belonging to the *E. grisebachii* complex, the commercially most important group of *Echinodorus*, reveal no hybrid origins and very few differences on the studied molecular markers. Most of the cultivars belong to *E. uruguayensis* complex, composed of hybrids with a red form of *E. uruguayensis* forming the maternal lineage. The examined specimens of the taxonomically complicated genus *Helanthium* express diversity in growth forms, however, with only one recent cultivar.

We recognize the fundamentally different nature of natural species and artificial cultivars with a discussion on the taxonomical consequences of this distinction.

INTRODUCTION

Correct identification of cultivated aquarium plants is often highly desired. In the countries of aquarium plant production, many decorative plant species are collected from the wild with possibly devastating impact on rare species [Yapabandara and Ranasinghe 2002], or nonindigenous species are introduced into nature either accidentally or purportedly to support commercial production [McLane 1969]. Effective conservation and management of populations require detailed knowledge of the traded plants. Due to its global scale and economic importance, the aquarium plant trade has become a major source of invasive species to aquatic ecosystems [Padilla and Williams 2004]. For this reason the trade of numerous species is currently restricted, but difficulties in species identification seriously hampers both monitoring and controlling the trade [Champion and Clayton 2001; Padilla and Williams 2004; Brunel 2009]. The use of genetic resources in the plant breeding has recently become controlled by the intellectual property rights and the Convention on Biological Diversity [Bhat 2008]. The precise identification of the economically important plants is, therefore, a fundamentally important task in protecting the interests of the plant breeders and the nations alike [Bhat 2008]. Naturally, aquarium enthusiasts are also interested in identifying the plants they are growing, not only so that they can give proper care for their plants, or communicate about them, but also for the pure pleasure of having names for them.

However, the correct identification of an aquarium plant is often a tremendously difficult task. Aquatic plants in general pose great taxonomic difficulties due to their reduced reproductive structures and plasticity in the vegetative characters [Schulthorpe 1967]. Furthermore, the cultivated aquarium plants are rarely in reproductive stage, and their identification can not be helped by geographical origin as any species can be cultivated anywhere. The lack of diagnostic characters makes identification difficult and results in an unreliable taxonomy: the actual species boundaries for many aquatic plants are simply unknown. The ornamental plant trade, on the other hand, deals largely in selected cultivars and hybrids instead of pure natural species [Kasselmann 2003]. The horticultural interest may also result in a proliferation of uninformative names and classifications [Compton et al. 2004]. Explicit, well-formalized and repeatable analyses of high quality data are required to solve these sort of problems [Compton et al. 2004].

The sword plants (genera *Echinodorus* and *Helanthium*, Alismataceae) are among the economically most important ornamental aquatic plants [Brunel 2009], and have been in cultivation since the early 20th century [Wendt 1952]. The sword plants are bottom rooted and have leaves in rosettes, and vary from the large to medium-sized *Echinodorus* to the generally rather small *Helanthium* species (chain swords) (Figure 1). Both genera naturally occur in the New World with the highest species richness in the tropical areas [Haynes and Holm-Nielsen 1994]. In nature most species inhabit transitional environments between the dry land and the water, such as the inundated savannas and floodplains, with only a couple of species adapted to a truly aquatic life [Lehtonen 2008]. However, various naturally semi-aquatic sword plants can be grown permanently submerged in aquaria without any difficulties [Kasselmann 2001]. The sword plants have found a niche in most of the aquarium design styles: for example, the

so-called “low tech” aquaria house little demanding cultivars, a large *Echinodorus* fills a focal position in a “high tech” tank; the aquaria with rough fish are greened with some robust sword plants; the elegant Iwagumi designs grow *Helanthium* species among their rock scapes and other styles use them as “lawns.” The bigger *Echinodorus* can hide equipment and provide cover or spawning sites for fish [Whitern 1956], while the biggest species which grow easily out of the aquarium can be used in an open-topped tank both for the vertical accent of the petioles in the water and for the spread of the green oasis a little bit more into the living room [Hiscock 2005]. The sword plants have not yet had a commercial use as houseplants. There has been a trial in using them as summer flowers in a container culture outdoors with other decorative aquatic plants [see Kaliebe 2004]. So far, the outdoor use has not been popularized among the plant producers or the end consumers.

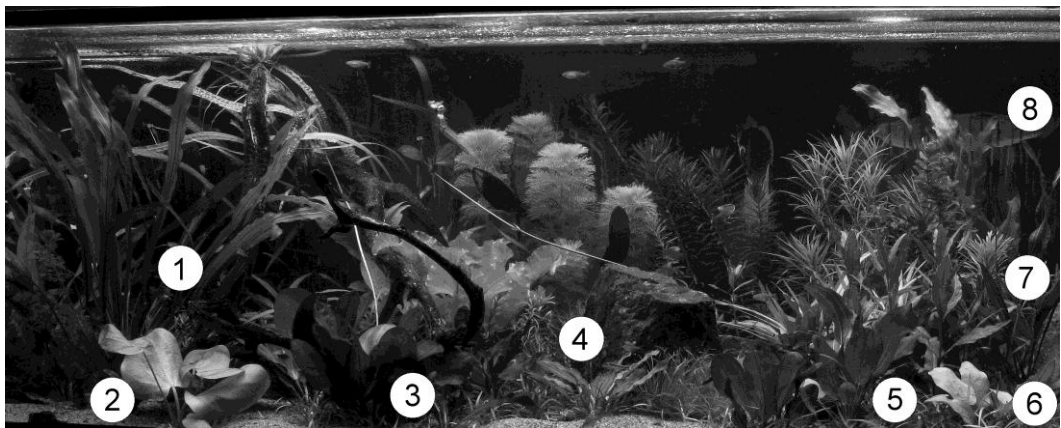


Figure 1. Various *Echinodorus* species and cultivars growing in an aquarium. 1) *E. uruguayensis*, 2) *E.* ‘Yellow Sun,’ 3) *E.* ‘Kleiner Bär,’ 4) *E.* ‘Oriental,’ 5) *E.* ‘Regine Hildebrandt,’ 6) *E.* “cordifolius ssp. ovalis,” 7) *E.* ‘Fantastic Color,’ 8) *E. major*.

Helanthium was considered as a distinct genus by Britton [1905], but since then, most botanists have treated *Helanthium* as a subgenus of *Echinodorus* [e.g., Fassett 1955, Rataj 1975, Haynes and Holm-Nielsen 1994]. This classification has been followed by the aquarium literature as well [e.g., Kasselmann 2001]. The recent phylogenetic studies have revealed that *Helanthium* and *Echinodorus* are not closely related at all, and, consequently, the taxonomic validity of the genus *Helanthium* has been reinstated [Lehtonen and Myllys 2008; Lehtonen 2009a]. It is not only the generic circumscription of the sword plants that has presented difficulties, but especially the species-level taxonomy within both *Echinodorus* and *Helanthium*. The species delimitation has often been confounded by the great morphological plasticity related to the ecological factors [Hauman 1915], the unavailability of collections from many regions [Fassett 1955], the wide use of often sterile material obtained from the aquarium trade [Cook 1978], and by the presence of numerous commercial hybrids [Kasselmann 2001]. In addition, as they are economically important ornamental plants, the *Echinodorus* and *Helanthium* taxonomy has attracted amateur naturalists and plant traders alike. Hence, the number of species rapidly grew from the 18 species listed by Fassett [1955] at the time when aquarium plant trade was developing in South America [Lehtonen and Rodríguez Arévalo 2005] to the 47 species recognised by 1975 [Rataj 1975], many of which were described based on cultivated plants [e.g., Rataj 1970]. This trend has continued,

although, in the recent years, the new forms have been more often named as cultivars following the International Code of Nomenclature for Cultivated Plants [ICNCP; Brickell et al. 2004] instead of as new species under the International Code of Botanical Nomenclature [ICBN; McNeill et al. 2006]. Nevertheless, the new revisions have revealed errors in the previous classifications and suggested that far too many species were described earlier [Haynes and Holm-Nielsen 1994; Lehtonen 2008]. These conclusions, on the other hand, were not accepted by the aquarium hobbyists, who insisted that the botanical classification failed to recognise the great morphological variation hobbyists observed in their aquaria [Kasselmann 1998, Jez 2001, Rataj 2001]. The latest botanical classification lists 28 *Echinodorus* species, but considers five of them to be only doubtfully accepted [Lehtonen 2008]. Systematics of the *Helanthium* has remained even more ambiguous [Lehtonen and Myllys 2008].

The molecular systematic studies have shed new light on the origin and classification of many horticulturally important plants in widely different taxonomical groups, such as, on lotus (*Nelumbo*, Nelumbonaceae; [Han et al. 2007]), geraniums (*Pelargonium*, Geraniaceae; [James et al. 2004]), and *Cyclamen* (Myrsinaceae; [Compton et al. 2004]). Simultaneously, the use of various DNA based techniques has become a standard in the cultivar identification [Bhat 2008]. The molecular techniques have also confirmed presumed hybrid origins and revealed previously unrecognised hybrids in several aquatic plant groups, including *Potamogeton* [Kaplan and Fehrer 2004; Les et al. 2009], *Aponogeton* [Les et al. 2005], *Myriophyllum* [Moody and Les 2002], and *Nuphar* [Padgett 2002]. Since the chloroplast DNA (cpDNA) is generally inherited maternally in the angiosperms, it is assumed, on the one hand, that the cpDNA provides information about the directionality of hybridization events by revealing the maternal lineage [e.g., Kaplan and Fehrer 2004]. On the other hand, the biparentally inherited nuclear DNA (nrDNA) is expected to show genetic patterns of both parental species, especially in the recent hybrids [Moody and Les 2002]. The taxonomic problems within *Echinodorus* and *Helanthium* have been investigated using the molecular systematic methods only recently, but so far the sampling has focused on the natural taxa [Lehtonen and Myllys 2008]. Therefore, the systematic status and the correct identity of the cultivated sword plants have remained obscure.

In this article we aim to provide the first molecular systematic review of the complicated origins of the cultivated *Echinodorus* and *Helanthium*, and to provide an overview of the aesthetic, technological and commercial selection pressures determining the direction of the human-driven evolution of the ornamental aquarium plants.

AESTHETICS

The modern aquarium keeping can be said to have started with the “Aquarium mania” resulting from the opening of the first public aquarium at the Regents Park Zoological Garden in London in 1853 [Hibberd 1860]. There certainly are earlier examples of keeping plants, fish – especially gold fish – and other aquatic life in various containers, but that event marks the time when the wider public became aware of the possibility. The contemporary invention of the cheap sheet glass made the building of the glass aquaria affordable: it was in the 19th century when the keeping of exotic plants in the tropical glasshouses really took off –

exemplified, for example, by the building of the Lily House designed by the gardener Joseph Paxton for the newly discovered *Victoria regia* Lindl. in Chatsworth in 1849-50, and his Crystal Palace for the world fare in London in 1851 [Sinisalo 1997; Murphy and McCloud 2010]. One of the precursors to the aquaria can be said to be the so-called ‘Wardian Case,’ a miniature glasshouse introduced in 1833 by Nathaniel Ward for growing plants in enclosed containers and revolutionising the transportation of living specimens from all the corners of the world [Loxton 2005]. The “mania” soon spread to Germany and German-speaking countries [Rossmässler 1857] and to the USA [West 1865; Edwards 1858].

The very first aquarium books advocated the use of local and native aquatic plants firstly for the practical and utilitarian reasons (oxygenation and purification of water for fishes and other animals). The aquarium was defined as a balanced system where the plant and animal life promoted each other’s health. The aesthetic qualities of the plants held the second place. This article of faith is repeated in all the books in our review until the early 1900s. These first aquarium setups were not for pure aesthetic pleasure of an attractive object, “a domestic toy,” but for using as instructional, contemplative and empirical tools (this “Naturalism” culminating in the publication of Darwin’s *On the Origin of Species* in 1859). The weekly maintenance routines such as water changes were seen objectionably as striving for maximizing the enjoyment of keeping as many fish as possible, and not for studying the “ways of God in Nature” [Hibberd 1860]. Partly this seems to be a reaction against the earlier style of “imperfectly developed taste” in keeping gold fish in the glass globes [Humphreys 1858] with such artificial objects like arches, sunken cities, mermaids, castles, etc. [Lankester 1856; Innes 1936b].

A devolvement of the plants’ status as purifiers of water and placing of their appreciation as decoration to the first place seem to have been first intimated in the late 1900s [Wolf 1908; Eggeling and Ehrenberg 1908]. Their role as the best means of keeping the aquarium water clean had been overthrown by the end of 1960s and relegated to the filters and the regular water changes [Brünner 1969; Weigel 1973]. The plants were there to help design aquarium interior aesthetically [Brünner 1969; Weigel 1973]. A new upswing, or just a continuation of the old, has been seen in the recent years: some authors hold that aquarium plants absorb heavy metals from the water and even have some antibiotic effects [Walstad 2003; Beck 2000].

As late as the 1930s, Innes [1936b] thought that the artificial light was “worthless” for plants in the aquaria. Some 10 years later there already were fluorescent lights – first commercialized in 1938 in the USA [Thayer 1991, 2007] – available for the aquarium use [Aurell, Jacobsson and Lindgren 1949]. After the 2nd World War, other electrically driven machines, too, started to gain in popularity: air pumps, filters and heaters with thermostat soon became standard equipment [e.g., Ladiges 1949].

One of the most important recent aquarium technologies is the Internet in the 1990s. First the newsgroups and later the aquarium forums have had a huge impact on the distribution of knowledge and know-how among the aquarists. Posting of photographs of aquaria has given inspiration and visual aid to designing a home aquarium. The aquarium design competitions (e.g., the ones of the Aquatic Gardeners Association, US, and of the Aqua Design Amano, Japan) have created venues for the hobbyists to show their skills and creations. The Internet has also created a new way for the plant trade: contacts are made internationally and even anonymously now.

AQUARIUM DESIGN STYLES

The early aquarium books give few instructions on how to design the aquarium exactly [cf. Frank 1996]. The situation has not gotten much better in this regard as the newer books dedicate much more space to the construction, chemistry, technology, care, propagation and maintenance, placing of the aquaria in the room, and plant descriptions than on the design plan [Weigel 1973; Nieuwenhuizen 1982; Brünner 1984; Tepoot 1998; Kasselmann 2003; Hiscock 2005; Barber and Wilson 2005]. One of the early exceptions is the horticulturist and garden designer [Lecerf 2001] Marcel François with his ten unique aquarium layouts set in a manner of theatre stages [see François 1951]. Still, it is possible to find references that illustrate a developing practice in direct lineage to the aquascaping nowadays. Most of the aquarium books reviewed refer to gardening, if only in passing and metaphorically. Yet, at least some of the authors of the early aquarium books have a direct linkage to the garden designing: James Shirley Hibberd was famous for his long-time garden journalism [Wilkinson 1998], and the artist and author Henry Noel Humphreys worked for such famous garden designers as J. C. Loudon and W. Robinson [Leathlean 1995]. Therefore, we find it fruitful to make a comparison with the philosophies of garden design in order to explicate those implied in the aquarium design practices.

The Western culture has had a long-lasting relationship to the late Roman period Neoplatonic encapsulation of Plato's Theory of Forms in the axiom: "Art should imitate nature." The different times have interpreted the three terms differently thereby creating different styles of, for example, architecture, music, and garden design. It has swept from the Medieval "nature of ideas," through Cartesian rationalism of the "laws of Nature" to the "unadorned nature" of empiricism of the 18th century England, with the inserting of the last in the designs becoming the hallmark of the English landscape or Serpentine style gardens. [Turner 2005.] In our opinion, this idea can be applied to studying the various aquarium designs. Even though "art" has not been mentioned often in the aquarium literature until perhaps the 1990s and the 2000s, the "imitation of nature" has been central and explicit since the beginning: an aquarist must imitate [Taylor 1876] – if not reproduce [Innes 1936b] – natural conditions for the health of plants and animals; stones ought to be placed so that they imitate a river bed [Humphreys 1857], and the "unnatural" objects should be avoided [Lankester 1858]; an aquarium is "nature on a small scale" [Edwards 1858] or a microcosm [Lankester 1856]; etc..

Another take on this axiom is that the aquarium design should be composed in the same way as a painting of natural scenery. This is shown in Humphrey's suggestion of placing plants *picturesquely* among a few *picturesque* stones [Humphrey 1857], and in West's [1865] opinion that the "aquatic forest may, in fact, be made as picturesque as the primeval woods or the artistically arranged shrubbery." The Picturesque style of garden design at the end of the 18th century wanted to "stimulate the mind with scenery comprised according to the principles of landscape painting." The former smoothly curving lines were to be replaced by irregular ones as the empiricists wanted to create a more 'natural' look. In the end, the Picturesque gardens were not distinguishable from nature. This dead-end was resolved by four different approaches to garden design, one of which is relevant for our analysis: the landscape style. [Turner 2005.]

In the landscape style, the aim was to create a transition from the realm of art or “the Beautiful” (of smooth, feminine curves) in the foreground to the realm of wild, threatening nature or “the Sublime” in the background *via* the transitional middle ground of “the Picturesque.” The principle of forming gardens with the transition from art to nature was set around 1793 and remained central to the English garden design throughout the 19th century and up until 1947. Turner considers the aforementioned Crystal Palace to represent this style. [Turner 2005.] It is no wonder, then, that it has influenced the Western aquarium design schemes as well, and therefore, no wonder that Aurell, Jacobsson and Lindgren [1949] desired an aquarium to look “like an overgrown English garden.” The transitions from art to nature, and *vice versa*, are very much in the heart of the aquarium aesthetics. This tripartite division is echoed in the much-repeated instruction of placing the short plants in the foreground of the aquarium and the tall ones in the back [e.g., Brorsson 1942; Ramshorst 1953; Stilton 1986; Nieuwenhuizen 1982; Hiscock 2005]. One of the first to use this design scheme was Hibberd [1856] who suggested setting the plants of “massive and decided character” in back with “lighter plants before them, just the same as a painter sets his chestnuts and elms in mid-distance, and his lady birches in the fore-ground.” The large plants were “well seen through the interstices of [--] fragile and delicate structures” (note the implicit sexualization in the description). The threefold structure became so pervasive that even the commerce adopted these categories [see e.g. Oriental Aquarium 2002].

This planting scheme is behind one of the most recognizable aquarium design styles: the Dutch style. It developed to its peak in the 1960s and 1970s with the recently developed aquarium technology enabling the keeping of the more demanding plants. Its reliance on such compositional techniques as the use of the Golden Section to define central or focal points, and the decentralized diagonal plant or sand “streets” to create more depth and perspective, can be said to have their roots in the Baroque style of the garden design (1600-1750). The Golden Section was discovered in the Antique, the Renaissance found the perspective, and both of these were used in the Baroque. Behind their use was the belief in the mathematical principles as conveying the truth of nature. Still, the used aquascaping techniques could be said to be ‘abstractions of orderly design methods from the chaotic past’ which would be in line with the early 20th century Arts and Crafts garden design style. [Turner 2005.] This view is strengthened with the notion that the grouping of the plants in contrasting colors and leaf-shapes in the Dutch style created a look more like the English cottage garden border plantings than a formal Baroque garden. These borders were central to the Arts and Crafts design style that relied strongly on the “honest manual labor” and good plantsmanship. The borders are set before a vertical surface against which the plants displayed are viewed. The taller plants are interspersed with lower creating a varied texture. [Douglas and al. 1984; Turner 2005.] These same elements are found in the Dutch style. [See Nieuwenhuizen 1982; also Barber and Wilson 2005.]

The larger sword plants have been used as focal or central points in the aquaria since their introduction [cf. Innes 1938], and have, thus, filled one of the positions (or functions) reserved for the plants in the aquaria: the idea of a solitary plant as eyecatcher was there already in the 1850s [e.g., Hibberd 1856; Edwards 1858]. They were used in such a manner in the Dutch style aquaria, too [Wit 1964; O’Connell 1973; Nieuwenhuizen 1982]. The list of species includes, for example, *Echinodorus grisebachii* Small, *E. major* (Micheli) Rataj, *E. osiris* Rataj and *E. berteroi* (Spreng.) Fassett. The newer, more colorful hybrids have largely taken this role as eyecatchers in many aquaria. The large sword plants have had their place

also in the background of the aquaria giving contrast to the plants with different habitus placed in front of them.

Another position for plants created by the tripartite division of space is the foreground “lawn” or “carpet.” Even though the space behind the frontal glass has been seen as a place for the fish to show themselves [Brorsson 1942; Wit 1964], the references to the grassy lawns echo the garden designs, and hence, their aesthetic philosophy. One of the first aquarists to cultivate a carpet seems to be West, who in 1864 used *Lemna trisulca* L. and algae for this purpose [West 1864]. Since their introduction, the pseudostoloniferous *Helanthium* have found a steady home in this position of the aquarium design. They have also been used for the “streets” in the Dutch style tanks [Kasselmann 2003; Nieuwenhuizen 1982].

Today, the Dutch planted tanks are classified as “high tech” (i.e. a lot of technology is used in them), and high-maintenance setups. In contrast, the so-called “low tech” aquaria have their origins in the 19th century idea of a balanced system. The central speaker for this kind of a “natural aquarium” has been Diana Walstad [2003], who, basing her ideas of planted aquarium maintenance on the ecological studies on the water plants claimed the “high tech” tanks “unnatural, expensive, and laborious.” These aquaria do not have any special planting schemes as the plants are let grow “wild” following the idea of the survival of the fittest. These aquaria often house native plants, fish and invertebrates. [Walstad 2003; Barber and Wilson 2005.] The “natural style” creates a demand for the plants of easy culture and great adaptability: *Echinodorus* and *Helanthium* are included in them (Walstad [2003] mentions *E. bleherae* Rataj and *E. major* among the plants that can be counted on). Perhaps one could speak of an idea of “organic aquarium” in the sense of “organic food” as there seems to be a preference for consuming less non-renewable resources.

If the “natural aquarium” style is interpreted through the axiom “art should imitate nature,” then its imitation of nature is moved from the realm of the visual to the processes abstracted from the nature by sciences. Designing an aquarium following these abstractions of the processes – whether or not these processes occur in a similar fashion, alone or together, in the imitated biotope – connects this style with the modernism as a style. According to Turner [2005] the aesthetics of the modernist garden design were based on the understanding of nature through scientific analysis and reflecting this in the designs following the principles of abstraction. However, visually abstract aquarium designs (cf. abstract paintings, etc.) are still lacking, with, perhaps, one exception: Christian [2000].

The imitation of nature has had another swing within the “low” and “high tech” setups of the so called “biotope aquaria.” This style of aquarium design began in the 1950s, first as an aid to narrow down the choices of plants by their original continent, or by a large part of it [e.g., Wendt 1952; Brünner 1953]. These have been called “geotope aquaria,” often with such ‘unnatural’ results as a frequent combination of the sword plants with the angelfish [e.g., Whitem 1956] because both come from South America. They can end up in “natural-appearing”, though imagined, results, even when they are not actualizations of a “piece of underwater nature” [Yoshino and Kobayashi 1996]. A more narrow view is an attempt at a replication of a small piece of a natural body of water with the plant and animal species that do occur together [Barber and Wilson 2005; Tullock 2007]. The aesthetic value is not derived so much from an artistic expression in designing the tank – though this certainly can be incorporated – but from the belief (or the ‘suspension of disbelief’) in the idea of a remote location recreated. In this, they function in a similar way as the zoos or public aquaria transporting the aquarist where she or he might not otherwise be able to go to [Tullock 2007].

In terms of the garden design philosophies, they are descendants of the Picturesque style (see above). In relation to the sword plants, the “biotope aquaria” create a demand for the natural species instead of the cultivars.

The aquarium decorations that have been shunned with trepidation as artificial and kitsch, such as divers and mermaids [Hagenbüchli 1956], can nowadays be relegitimated with the postmodern approach to the aquarium design. These designs have been seen intermittently on the internet aquarium forums in the recent years (e.g., The Planted Tank forum, <http://www.plantedtank.net/forums/>) presented with somewhat self-belittling comments but received with obvious enjoyment. To our knowledge, only one aquarium book incorporates postmodern designs [see Christian 2000]. With the postmodernism, the garden designs incorporated beliefs in “complexity, pluralism, conceptualism, layering and recontextualization” [Turner 2005]. The “nature” becomes one concept among others that can be used. A natural setting in the postmodern style could incorporate, for example, some *Echinodorus* cultivars among other man-made hybrids.

Practically at the same time with the rise of the Internet, a new aquarium design style stormed the world with a new conceptualization of “nature.” The Japanese nature photographer Takashi Amano started already in the 1970s but his works were discovered in the Occident only in the 1990s, greatly helped with the translation of his books [Amano 1994, 1996, 1997a, 1997b]. This “high tech,” high maintenance style is called the “Nature Aquarium.” His aquaria can be seen as attempts to recreate the emersed, not submersed scenes of nature [Adams 2009], though the exact philosophical background is more complicated. The arrangement of the elements, especially of the so-called “hardscape” of rocks, wood and sand, is highly regulated in the “Nature Aquarium” style. Amano [1997b] has referred to the “karesansui style” of the Japanese rock garden that harks to a strict system of rules of composition in use already by the 13th century. These gardens were developed from the representations of the old Taoist *sansui* paintings of stream, waterfall and mountains by reducing the scale and by the abstract use of substitutes: rocks, gravel and sand [Davidson 1982] with only mosses used as vegetation [Douglas and al. 1984]. The elements of a Japanese garden have metaphorical associations: they don’t reproduce but “symbolize the harmonies of natural design” by conceptualizing nature and interpreting it “abstractly and artificially” [Douglas and al. 1984]. The “Nature Aquarium’s” submersed transposition of the rock garden designs have become called Iwagumi style incorporating just rocks, sand and very few species of mainly low growing plants [Wazeter 2009].

The “Nature Aquarium” style is actually many styles - Iwagumi being one among them. Amano uses focal points, often calculated with the Golden Section but in a different manner from the Dutch style. Where, in the Dutch style aquaria, the space is filled with plants, in the “Nature Aquarium” style, a lot of space is left empty [Barber and Wilson 2005; Amano 1997b]; where the Dutch tanks are mostly planted in a “Front-slope style” (term from [Tepoot 1998]), Amano’s stylistic means include what Tepoot [1998] has called “Side-slope style” (focal point at one end of an aquarium, shortest plants at the other), “Mount style” (focal point off-centre, plants trimmed to slope down to the ends of the aquarium) and “Valley style.” The last one can be seen as a development of the Dutch style as well: the planted “street” has become a path – often of sand – meandering in an off-centre diagonal from the front to the back of the tank.

What we see in the “Nature Aquarium” style is a confluence of the driving forces that shape the aquarium plant demand: there is a joining of the aesthetics of aquascaping, and of

taking great pictures of them, with the technological means (“high tech” aquaria) and the commercialization of the products. It all comes together in Amano’s enterprise: Aqua Design Amano Co., Ltd. (ADA). From the sword plants’ point of view this development has been detrimental: the larger *Echinodorus* have had little place in the “Nature Aquaria,” and there has been a notable decline in their demand [Kaminski 2009]. The rhizomatous ferns, mosses and liverwort (*Riccia fluitans* L.), and plants propagated from stem cuttings (“stem plants”) have had a heyday. However, *Helanthium*, especially *H. “tenellus,”* have been in much demand among the other small, “runner-producing” plants.

To sum up: there has been a change from the coldwater to the tropical tanks which went together with the shift from the local and native to the imported and exotic plants (see below). A contemporary change seems to be from the plants as water purifiers first to the plants as decoration first. There has been a move from the reproduction of nature (nature ‘actualized’) to the “Nature Style” (nature conceptualized), from the painterly to the photographic (one has to remember that the photography saw the day in the late 1820s). This is echoed by the transition from the representation of a submersed view to an emersed scenery, often, especially in Iwagumi style, involving a reduction in scale (e.g. small plants used to represent trees, rocks to represent mountains, etc.): here the move is from the ‘documentary’ to the ‘panoramic,’ even ‘cinematic.’ Some of these changes have not been so much paradigmatic as branching into parallel, sometimes strongly adhered “schools of thought.” The sword plants have a place in all of the above aquarium design styles, which helps to explain why they are so central commercially.

COMMERCIAL HISTORY

The commercial trade of the ready-made aquarium tanks, on the one hand, and collecting plants and animals for the dealers, on the other, was established already by 1857 in England and in the USA [Sowerby 1857; Small 1893]. The first tropical aquatic plants are mentioned already in the books from the 1850s but these, *Aponogeton distachyos* L.f. and *Euryale ferox* Salisb. among them [Lankester 1856] would be considered pond plants nowadays. The water lilies gained popularity in the late 1880s in the USA as well as in the Europe with the introduction of new hardy cultivars by the French specialist Marliac [Bisset 1907]. Since then the crossing of water lilies grew fast with 17 species or subspecies and 71 cultivars available on the US market alone by 1923 [Olmstead, Coville and Kelsey 1923]. The first tropical plants which could be sustainably cultivated in the aquaria appeared in the late 1870s in the USA and in the late 1880s in Germany (cf. Mulertt’s description of his introduction of “*Cabomba viridifolia*” from Brazil in 1878, and his discovery of two “*Sagittaria natans*” seedlings among the rootstock of tropical water lilies from South America in 1879, in Mulertt 1902; *Heteranthera zosteraefolia* [sic] was the first in Germany according to Mühlberg [1982]).

Mulertt [1883] wrote that the number of plants for aquaria was large but listed only 12 – assumedly best – in 1883. The earlier books listed even more species, but many of these were not recommended for long time culture [e.g., Hibberd 1860; Taylor 1876]. Kasselmann’s [2001] assessment of ca. 20 water and bog plants being in cultivation in 1900, ca. 30 in 1910 and ca. 40 in 1930, can be refined further. The situation in the North America in 1900 can be

seen in the Cyclopedia of American Horticulture: it listed 28 plants in the aquarium usage [Bailey and Miller 1900a, 1900b, 1901, 1902]. Some fifteen years later in the mid-1910s, Bailey's Standard Cyclopedia of Horticulture mentions 45 plants in the aquarium context [Bailey 1914, 1915, 1916a, 1916b, 1917a, 1917b].

By the turn of the 20th century a number of commercial aquarium plant nurseries existed. For example, Adolf Kiel had a large nursery in Frankfurt am Main, Germany, servicing the international market [Tinnappel 2005; Bleher 2009]. Paul Matte had an aquatic plant nursery beside his fish one, established in 1878 near Berlin in Germany [Posseckert 1984]. In the USA, the examples include import-export-nursery of Mulertt established in 1874, first in Cincinnati and later in Brooklyn, N.Y. Edmund Sturtevant founded his water lily and aquatic plant nursery in Bordentown, N.J. in 1876 (See the advertisements in *The Aquarium*, Nr 25 (Vol. III), 1892 and Nr. 28 (Vol. III), 1893).

The family Alismataceae has been present in the aquarium guides since the beginning. There are mentions of the genera *Alisma*, *Damasonium*, and *Sagittaria* with species native to the United Kingdom and to the USA [e.g. Hibberd 1856; Hibberd 1860; Sowerby 1857; Edwards 1858]. The first intended Alismataceae hybrid was made by Mulertt in 1890 with two *Sagittaria* plants – the first being his cultivar 'New Era' from a cross-pollination of the two aforementioned South American "*S. natans*," the other a "*S. lanceolata*" [Mulertt 1897]. In this light, it is very surprising that we had to wait until the 1980s for the first intentional *Echinodorus* hybrid.

THE SWORD PLANTS ENTER THE SCENE

According to Wendt [1952], the first cultivated *Echinodorus* was *E. grandiflorus* (Cham. & Schldl.) Micheli, imported to Germany in 1905 by the nursery Henkel in Darmstadt and sold under the name *Sagittaria guyanensis*. From Wendt's description it seems that this plant actually was *E. floribundus* (Seub.) Seub.. Aquarists had to wait till the 1930s for the next *Echinodorus* species, which seems surprisingly late considering that there are two native *Echinodorus* and one *Helanthium* species in the USA, and the outspoken urging of the early aquarium books to go out and collect your own plants [Edwards 1858; Hibberd 1860]. *Echinodorus cordifolius* (L.) Griseb. entered the US market in 1934, with the same incorrect name *Sagittaria guyanensis* [Innes 1936]. The latter part of the 1930s saw the introduction of three more species: *E. grandiflorus*, *E. grisebachii* and *E. berteroi*. The first of these was brought from Buenos Aires to Germany in 1936, but misidentified as *E. paniculatus* Micheli [Schreitmüller 1936]. Later Wendt [1952] identified it as *E. longistylis* Buchenau, a name that was later synonymized with *E. subalatus* (Mart.) Griseb.. This identification has been accepted for example by Kasselman [2001]. However, Schreitmüller's [1937] description of the emerged plant makes us lean towards *E. grandiflorus* (*sensu* Lehtonen [2008]). See also Rataj and Horeman [1977], who consider the cultivated *E. longistylis* as incorrect name for *E. argentinensis* Rataj, a synonym of *E. grandiflorus*).

The species that ultimately became the most important *Echinodorus* in aquarium trade [Brunel 2009], *E. grisebachii* (the Amazon sword plant), appeared in cultivation among a number of American aquarists in 1937 [Innes 1938]. It was still during the 1930s that this plant reached Germany [Baum 1941] and the Scandinavia [Jacobsson and Lindgren 1949].

The Amazon sword plant has had many names attached to it: it has been variously called *Echinodorus tenellus* (Mart. ex R. & S.) Buchenau [Baum 1941], *E. brevipedicellatus* (Kunze) Buchenau and *E. paniculatus* [Wendt 1952], *E. intermedius* (Mart.) Griseb. [cf. Wendt 1952], *E. amazonicus* Rataj and *E. bleherae* [Rataj 1970], and so on. Where Rataj and aquarists split the taxon into two or more species, Haynes and Holm-Nielsen [1994] lump them into one, *E. grisebachii*. The results from our current study support this latter view of one polymorphic species (see below).

The last taxon to enter the German aquarium scene in the 1930s was *E. berteroi* in 1939. Wendt [1952] identified it as *E. nymphaeifolius* (Griseb.) Buchenau, but his pictures clearly show *E. berteroi*. *Echinodorus berteroi* was available in Sweden already by 1949 [Aurell, Jacobsson and Lindgren 1949], and was being sold in the USA at that time as the “Cellophane plant” (see the Everglade Aquatic Nurseries, Inc. advertisement in Frank [2008]).

The economically second most important *Echinodorus* species nowadays [Brunel 2009] – *E. major* – was introduced in Germany as *E. martii* in 1949 [Wendt 1952]. The first *Helanthium* species, representing a wide leaved species, was introduced soon after the second world war [Aurell, Jacobsson and Lindgren 1949]. The number of available taxa remained practically the same during the 1950s. At this time the growing demand for the sword plants was satisfied by exporting locally cultivated plants from the Amazonian countries [Lehtonen and Rodríguez Arévalo 2005]. For example, *E. grisebachii* and *E. horizontalis* Rataj were cultivated for commercial purposes in large volumes in the Peruvian Amazonia during 1950–1970 [Lehtonen and Rodríguez Arévalo 2005]. Plants were cultivated by the rural communities in the fields located at the river floodplains, with growing and harvesting following the seasonal water level fluctuations [Lehtonen and Rodríguez Arévalo 2005]. Although the Amazonian countries still produce large quantities of ornamental fish today [Chao and Prang 1997], the local aquarium plant production faded away with the introduction of the new colorful cultivars and the more developed methods of mass production [Lehtonen and Rodríguez Arévalo 2005].

The explosion of the new *Echinodorus* forms started in the 1960s with the introduction of the first reddish *Echinodorus*: *E. osiris* became available in 1961 as spec. *rubra* [Kasselmann 2001]. Its status as a species has been in question for the last 40 or so years, for example, Schöpfel [1986] suggested that it should be classified as a hybrid. Our research concludes that it is a natural hybrid. By the end of the 1960s there were at least ten *Echinodorus* and three *Helanthium* types in cultivation in England [Roe 1967]. The number of the different forms seems to be even higher in the Eastern Europe at the time: Schöpfel [1969] reports ca. 15 *Echinodorus* and four *Helanthium* species (*amazonicus*, *bleherae* and *parviflorus* here counted as one taxon) available for the aquarists in Europe. Later in 1969, the editorial board of the magazine *Aquarium Terrarium* lists 47 *Echinodorus* plants (including the *Helanthium*) following the yet unpublished but obviously available work-in-progress of the revision of the genus by Karel Rataj [ZAG Wasserpflanzen 1969]. However, according to the list only ca. 20 *Echinodorus* and five *Helanthium* species of the total number were available [ZAG Wasserpflanzen 1969]. Most of the newly imported plants were originally from Amanda Bleher’s Brazilian nursery “Lotus Osiris” [Schöpfel 1969]. However, the mass cultivation of *Echinodorus* in Southeast Asia had already begun [Rataj 1970], and the growing *Echinodorus* trade no longer profited South American economies as the plants were mostly exported for mass cultivation elsewhere [Lehtonen and Rodríguez Arévalo 2005].

The technological innovations in plant breeding and aquarium keeping at home have dramatically shaped the aquarium plant industry since the 1980s. The novel breeding technologies used in East Europe to create new *Echinodorus* forms involved crossbreeding and mutations induced by chemical or radiation treatments [Andersen, Christensen and Pedersen 2006]. In 1984, just before the first named *Echinodorus* cultivars stormed the market, there were about 15 *Echinodorus* species, three natural hybrids and five *Helanthisum* species in cultivation, at least in Germany [see Brünner 1984]. The cultivar boom started with *E. parviflorus* ‘Tropica’ which entered cultivation in 1982 and was described as a cultivar in 1985 [cf. Schöpfel 1988]. It continued with the first apparently intentionally created hybrid, *E. ×barthii* H.Mühlberg, the first appearance of which was in 1984 as “Double Red Osiris,” later described as a new species [Mühlberg 1986], and recognized as a hybrid only afterwards [cf. Kasselmann 2003]. In the late 1980s Barth [1988] introduced *E. schlueteri* ‘Leopard’, the first lastingly spotted cultivar, and *E.* ‘Rosé’, a hybrid that was said to originate from the cross between *E. horizontalis* and *E. horemani* “red”.

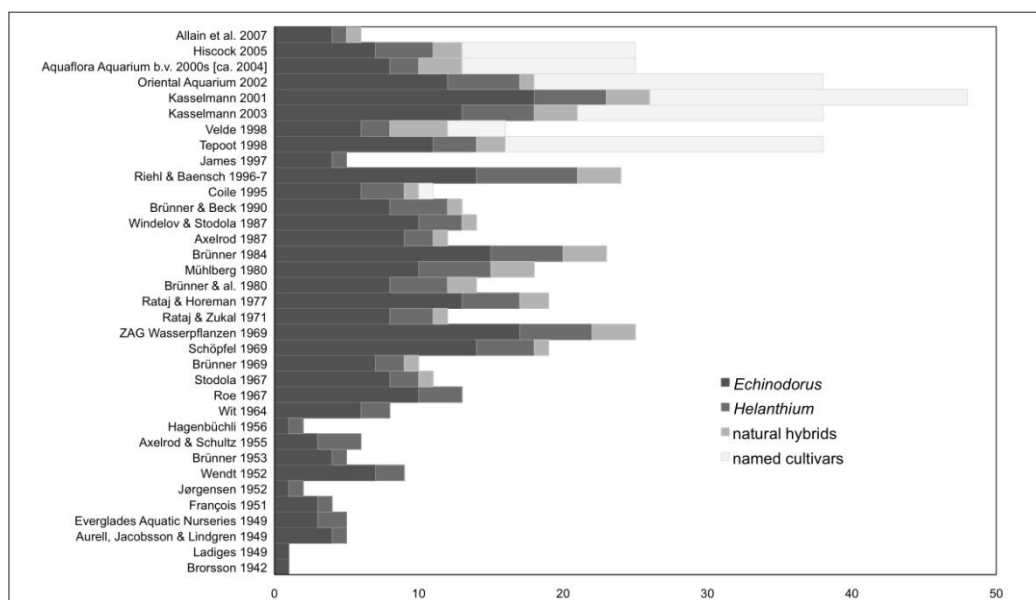


Figure 2. The number of *Echinodorus* and *Helanthisum* species, natural hybrids, and cultivars listed in various aquarium plant books since the beginning of their cultivation.

By the mid to late 1990s, the number of the cultivated species was almost the same but the number of the cultivars had doubled the number of the available *Echinodorus* forms (see Figure 2). By that time the aquarium plant nurseries had established the hydroponical cultivation (i.e. soilless cultivation in mineral nutrient solution) of plants in the emersed conditions, and the mass propagation of aquatic plants by tissue culture [Christensen 1996]. During this time, the protection of intellectual property rights began with the first patented *Echinodorus* cultivars [e.g., Barth 1998]. By the year 2000, there were so many *Echinodorus* hybrids available that it prompted Kasselmann [2001] to say it was impossible even for an expert to have a general view of the field. Our survey on the Internet of the named and not-too-obscure *Echinodorus* cultivars marketed for sale in March 2010 revealed that there are over 110 cultivars more or less available in Europe alone. The first *Helanthisum* cultivar,

marketed as *Echinodorus* ‘Vesuvius,’ entered the trade in 2006 [Kaminski 2009]. Large quantities of aquarium plants are currently produced by sophisticated plant nurseries in Europe, USA, Singapore, Sri Lanka, South-East Asia, and elsewhere [Christensen 1996; Yapabandara and Ranasinghe 2002; Brunel 2009; Winkelman et al. 2006], servicing their customers worldwide over the Internet [Kay and Hoyle 2001].

Despite of their great importance in the aquatic plant trade, *Echinodorus* species have not yet caused serious problems as invasive plants. They have become introduced at least in Florida [Lehtonen 2009] and Thailand [Office of Natural Resources and Environmental Policy and Planning 2009], and the trade of some species has been restricted, for example *E. cordifolius* is considered weedy and therefore its entry into western Australia has been prohibited [Champion and Clayton 2001]. Nevertheless, the aquarium plants in the international trade pose a threat as vectors of serious pests: *Echinodorus* have been intercepted many times with, for example, whiteflies (*Bemisia tabaci* (Gennadius), see e.g., Eppo 2000; Eppo 2001; Eppo 2008), mango shield scale (*Milviscutulus mangiferae* (Green), Eppo 2008), pathogenic nematodes (*Hirschmanniella*, Eppo 2009; *Meloidogyne*, Eppo 2004), and African cotton leafworm (*Spodoptera littoralis* (Boisduval), Eppo 1999).

SYSTEMATICS

Detecting Hybrids and their Parental Species

We studied the hybrid origins of the cultivated sword plants by employing methods of molecular and morphological systematics. Total genomic DNA was extracted from the selected specimens using E.Z.N.A. SP Plant DNA Kit (Omega Bio-tek, Doraville, GA). Consequently we amplified and sequenced *psbA-trnH* intergenic spacer from the *Echinodorus* samples using primers *trnH* [Tate and Simpson 2003] and *psbA3'f* [Sang et al. 1997], and *trnL-trnF* intergenic spacer from the *Helanthisum* samples using primers *e* and *f* [Taberlet et al. 1991]. In addition to these noncoding chloroplast sequences, we amplified and sequenced the second intron of the nuclear *LEAFY* gene from both *Echinodorus* and *Helanthisum* using primers *FLint2-F1* and *FLint2-R1* [Grob et al. 2004]. The PCR products were purified and sequenced in both directions under BigDye™ terminator cycling conditions by Macrogen Inc., Seoul, South Korea (www.macrogen.com). It is assumed that in the angiosperms the chloroplast DNA is predominantly maternally inherited, although exceptions to this rule are known [Hansen et al. 2007]. We follow this assumption here, but our assumption must be considered speculative until the maternal inheritance of the cpDNA in *Echinodorus* and *Helanthisum* is verified with controlled crosses. In either case, none of the *psbA-trnH* sequences indicated biparental inheritance. The nrDNA, on the other hand, is biparentally inherited. The presence of biparental hybrid genotype is evidenced by mixed DNA chromatograms showing two fluorescent signals in positions that differ between the parental species [Moody and Les 2002].

The usual approach to detect the parental copies of hybrids from mixed PCR products is via subcloning and consequent sequencing of the different clones [e.g., Moody and Les 2002]. However, among Alismataceae the second intron of the *LEAFY* gene generally produces very clear DNA chromatograms in the sequencing, with very little background noise

and a strong signal. Hence, we avoided the laborious and expensive subcloning by applying, instead, an algorithm implemented in RipSeq program (iSentio; www.isentio.com), which is specifically designed to sort out different signals directly from the mixed chromatograms [Kommedal et al. 2008]. The RipSeq algorithm applies a cutoff value to avoid a low-intensity noise signal, and an average peak distance to deal with the relative displacement of the fluorescence peaks in the corresponding sequence positions [Kommedal et al. 2008]. After the determination of the corresponding peaks and filtering out the noise, the mixed signals are matched against a reference database in order to reveal overlapping sequences [Kommedal et al. 2008]. The RipSeq algorithm has proven to be highly effective in identifying gene sequences from the mixed chromatograms of polybacterial samples [Kommedal et al. 2009]. We visually examined all the sequence chromatograms produced previously [Lehtonen and Myllys 2008] and in this study, and created a *LEAFY* reference database from the chromatograms where no overlapping signal existed. The chromatograms that included overlapping fluorescent peaks were analyzed with the RipSeq and matched against the reference database.

Our sampling of the natural *Echinodorus* and *Helanthisum* was mostly based on our previous analyses [Lehtonen and Myllys 2008], with some new specimens sequenced for this study. The expanded sampling covers some natural taxa of questionable taxonomic status but widely recognized among the aquarium community, such as *E. portoalegrensis* Rataj and *E. osiris*. We also sampled some of the earliest cultivars that originated in the aquarium plant nurseries, for example *E. 'Rosé'* and *E. ×barthii*. The number of the traded cultivars has exploded within the past 15 years, and from these we selected a representative sample of common or morphologically most distinct ones for the study. The sampling was mostly focused on the plants available in the European markets and the studied plants were largely obtained from the Tropica Aquarium Plants nursery, Denmark. Nevertheless, some of the studied cultivars have originated in Asian nurseries, or were obtained from the USA. The voucher specimens of the DNA data are deposited in the herbarium TUR, and the sequences in the GenBank database (Table 1). We failed to produce readable *LEAFY* sequences from one of the studied *H. "latifolius"* specimens, and from *H. "tenellus red"*. Otherwise, we obtained all the target sequences from all the studied specimens. Throughout the text we follow the latest [Lehtonen 2008] classification for the natural taxa. For the cultivated plants we mostly apply the names under which they were sold, but in some cases we use the informal names common among the aquarium hobbyists. The taxonomy of the genus *Helanthisum* is highly controversial, with some authors accepting only one species [Matias 2007], where Rataj [2004] lists nine species. Only the names *H. parvulum* Small, *H. tenellum* (Mart. ex R. & S.) Britton, *H. bolivianum* (Rusby) Lehtonen & Myllys and *H. zombiense* (Jérémie) Lehtonen & Myllys have been formally placed in the *Helanthisum*, the remaining names have been described or combined to the *Echinodorus*. We are not proposing any new taxonomic changes here, instead we apply the available names, either the botanical names or those used by the hobbyists, but write them in double quotation marks to indicate that they are not botanically valid combinations.

A short inversion (16 bp) associated with a stem-loop secondary structure was observed in the *psbA-trnH* noncoding region of several *Echinodorus*. Short inversions are common in the noncoding sequences, including the *psbA-trnH* region, and are generally considered to be highly homoplastic [e.g., Kim and Lee 2005; Kelchner and Wendel 1996; Storchova and Olson 2007]. Due to the often homoplasious origin and the problems with the character non-

independence in the inverted regions, inversions may present serious problems for the phylogenetic analyses [e.g., Borsch and Quandt 2009; Lehtonen et al. 2009; Catalano et al. 2009]. We treated the inverted sequence regions by replacing them with their complement sequences, and by coding an additional binary character to record the original orientation of the fragment. This way we corrected the erroneous character correspondences without losing the possible phylogenetic information of the inversion event itself.

In order to combine the plastid and the nuclear sequences of the putative hybrids we had to recognize the maternal and paternal copies of the mixed *LEAFY* sequences. We did this by first combining one version of the *LEAFY* sequences with the *psbA-trnH* sequence obtained from the same specimen, and adding this combination to the analysis of the non-hybrid natural taxa. Then we repeated the analysis by combining the *psbA-trnH* sequence with the other version of the *LEAFY* sequence. We assumed that in the case of a hybrid plant the combination of the paternally inherited nuclear sequence with the maternally inherited chloroplast sequence would result in a conflicting phylogenetic signal and thus, in a less parsimonious tree than the combination of the maternally inherited nuclear and chloroplast sequences. In some cases the tree lengths were identical for both possible combinations. In these cases we compared the results of the separate analyses of the *LEAFY* and *psbA-trnH* sequences. We assumed that those *LEAFY* sequences, which in the separate analyses were grouped with the same natural species as the *psbA-trnH* sequences, represented maternally inherited copies. Yet, two cultivars (*E.* ‘Oriental’ and *E.* ‘Fantastic Color’) had pure *LEAFY* chromatograms. However, these sequences were associated with different species in the separate analyses than the chloroplast sequences from the same specimens. We excluded these cultivars from the phylogenetic analysis of the molecular data only, as their inclusion dramatically reduced the tree resolution. As well, we excluded the paternal *LEAFY* copy of *E. osiris*, *E. cf. osiris*, *E. horemani* “red” and *E.* ‘Roter Oktober’ from the molecular analysis, because an identical sequence is present in several species belonging to the different clades in the combined analysis of the chloroplast and nuclear markers. In this case also the inclusion of the paternal *LEAFY* copy without the associated *psbA-trnH* sequence collapsed the tree. Otherwise we combined the maternally originating *LEAFY* copy with the *psbA-trnH* sequence for the combined molecular analysis and the paternally originating *LEAFY* copies were added into the analysis as such. Hence, the final combined molecular data set included natural species that were represented by the *LEAFY* and *psbA-trnH* sequences, maternal hybrid lineages that were also represented by these two sequences, and the paternal hybrid lineages that were represented by the *LEAFY* sequence only.

Table 1. Voucher specimens and GenBank accession codes

Terminal; Origin; Voucher; Genbank accession numbers
<i>LEAFY</i> ; <i>psbA-trnH</i> : <i>Echinodorus amazonicus</i> Rataj; cultivated; <i>Quester s.n.</i> (TUR); HM367203; HM367280. <i>E. amphibius</i> Rataj; cultivated; <i>Quester s.n.</i> (TUR); HM367199; HM367276. <i>E.</i> ‘Apart’; cultivated; Falck 92 (TUR); ♂ HM367229, ♀ HM367228; HM367331. <i>E. ×barthii</i> ; cultivated; Lehtonen 702 (TUR); ♂ HM367221, ♀ HM367220; HM367327. <i>E. berteroi</i> (Sprengel) Fassett; Mexico; Lehtonen & Ramírez 412 (TUR); EF088181; HM367270. <i>E. bleherae</i> Rataj 1; cultivated; Lehtonen 701 (TUR); HM367198; HM367275. <i>E. bleherae</i> Rataj 2; cultivated; <i>Quester s.n.</i> (TUR); HM367204; HM367281. <i>E. bracteatus</i> Mich.; Ecuador; Lehtonen & Navarrete 491 (TUR); EF088170; HM367301. <i>E. cordifolius</i> (L.) Griseb 1; Mexico; Lehtonen

& Rámirez 417 (TUR); EF088173; HM367303. *E. cordifolius* (L.) Griseb. 2; Venezuela; Lehtonen 457 (TUR); EF088172; HM367302. *E. cordifolius* (L.) Griseb. 3; USA; Keener 275 (UNA); EF088190; HM367311. *E. cordifolius* ‘Marble Queen’; cultivated; Lehtonen 704 (TUR); HM367207; HM367315. *E. “cordifolius ssp. fluitans”*; Lehtonen 703 (TUR); ♂ HM367216, ♀ HM367217; HM367325. *E. “cordifolius ssp. ovalis”*; cultivated; Falck 89 (TUR); HM367209; HM367317. *E. decumbens* Kasselm.; cultivated; Lehtonen 392 (TUR); EF088163; HM367296. *E. emersus*; Peru; Lehtonen 140 (TUR); EF088147; HM367286. *E. ‘Fantastic Color’*; cultivated; Falck 98 (TUR); HM367212; HM367320. *E. floribundus* (Seub.) Seub. 1; Bolivia; Lehtonen 161 (TUR); EF088153; HM367288. *E. floribundus* (Seub.) Seub. 2; Bolivia; Lehtonen 188 (TUR); EF088150; HM367287. *E. floribundus* (Seub.) Seub. 3; Venezuela; Lehtonen & Pacheco 485 (TUR); EF088175; HM367305. *E. gabrielii* Rataj; cultivated; Bogner s.n. (TUR); ♂ HM367225, ♀ HM367224; HM367329. *E. glaucus* Rataj; cultivated; Mühlberg s.n. (TUR); EF088178; HM367307. *E. grandiflorus* (Cham. Schltdl.) Mich. 1; Uruguay; Lehtonen & Delfino 358 (TUR); EF088180; HM367309. *E. grandiflorus* (Cham. Schltdl.) Mich. 2; Argentina; Lehtonen 391 (TUR); EF088160; HM367294. *E. grandiflorus* (Cham. Schltdl.) Mich. 3; cultivated; Lehtonen 393 (TUR); EF088164; HM367297. *E. “grandiflorus ssp. aureus”*; cultivated; Lehtonen 718 (TUR); ♂ HM367240, ♀ HM367239; HM367337. *E. gracilis* Rataj; cultivated; Mühlberg s.n. (TUR); EF088177; HM367273. *E. grisebachii* Small 1; Peru; Lehtonen & Rodríguez 74 (TUR); EF088142; HM367271. *E. grisebachii* Small 2; Bolivia; Lehtonen 151 (TUR); EF088149; HM367272. *E. grisebachii* Small 3; cultivated; Quester s.n. (TUR); HM367201; HM367278. *E. ‘Harbich’*; cultivated; Falck 100 (TUR); ♂ HM367244, ♀ HM367243; HM367339. *E. heikobleheri* Rataj; cultivated; Quester s.n. (TUR); EF088187; HM367274. *E. horemani* Rataj “red”; cultivated; Falck 96 (TUR); ♂ HM367231, ♀ HM367230; HM367332. *E. horizontalis* Rataj; Peru; Lehtonen & Rodríguez 99 (TUR); EF088143; HM367282. *E. inpai* Rataj; cultivated; Mühlberg s.n. (TUR); EF088186; HM367310. *E. ‘Kleiner Bär’*; cultivated; Falck 93 (TUR); HM367211; HM367319. *E. longipetalus* Mich.; Paraguay; Lehtonen & Burguez 271 (TUR); EF088162; HM367284. *E. longiscapus* Arechav. 1; Argentina; Lehtonen & Dematteis 204 (TUR); HM367245; HM367289. *E. longiscapus* Arechav. 2; Uruguay; Lehtonen 341 (TUR); EF088158; HM367292. *E. longiscapus*-hybrid; Uruguay; Lehtonen & Delfino 334 (TUR); ♂ HM367246, ♀ HM367247; HM367322. *E. “macrophyllus”*; cultivated; Lehtonen 700 (TUR); HM367206; HM367314. *E. maculatus* Somogyi; cultivated; Lehtonen 719 (TUR); ♂ HM367237, ♀ HM367238; HM367336. *E. major* (Micheli) Rataj; cultivated; Lehtonen 394 (TUR); EF088165; HM367298. *E. cf. opacus* Rataj; cultivated; Quester s.n. (TUR); ♂ HM367215, ♀ HM367214; HM367323. *E. ‘Oriental’*; cultivated; Falck 91 (TUR); HM367213; HM367321. *E. cf. osiris* Rataj; cultivated; Quester s.n. (TUR); ♂ HM480485, ♀ EF088188; HM367324. *E. osiris* Rataj; cultivated; Lehtonen 698 (TUR); ♂ HM367223, ♀ HM367222; HM367328. *E. palaefolius* J.F.Macbr. var. *latifolius* (Mich.) Rataj; cultivated; Lehtonen 705 (TUR); HM367234; HM367334. *E. paniculatus* Mich. 1; Bolivia; Lehtonen 168 (TUR); EF088144; HM367285. *E. paniculatus* Mich. 2; Venezuela; Lehtonen & Pacheco 469 (TUR); EF088176; HM367306. *E. parviflorus* Rataj; cultivated; Quester s.n. (TUR); HM367202; HM367279. *E. parviflorus* ‘Tropica’; cultivated; Quester s.n. (TUR); HM367200; HM367277. *E. portoalegrensis* Rataj; Bogner s.n. (TUR); ♂ HM367227, ♀ HM367226; HM367330. *E. pubescence* (Mart.) Seub. ex Warm.; Brazil; Harley et al. 20019 (AAU); EF088193; HM367312. *E. ‘Red Diamond’*; cultivated; Falck 90 (TUR); ♂ HM367242, ♀ HM367241; HM367338. *E. reptilis*; Paraguay; Lehtonen & Burguez 261 (TUR); EF088156; HM367290. *E. ‘Rosé’*; cultivated; Lehtonen 699 (TUR); ♂ HM367218, ♀ HM367219; HM367326. *E. ‘Roter Oktober’*; cultivated; Falck 101 (TUR); ♂ HM367232, ♀ HM367233; HM367333. *E. ‘Rubin’*; cultivated; Lehtonen 706 (TUR); HM367205; HM367313. *E. scaber* Rataj; Venezuela; Lehtonen & Pacheco

440 (TUR); EF088174; HM367304. *E. schlueteri* Rataj; cultivated; Lehtonen 720 (TUR); ♂ HM367236, ♀ HM367235; HM367335. *E. schlueteri* ‘Leopard’; cultivated; Falck 99 (TUR); HM367210; HM367318. *E. sp3*; Paraguay; Lehtonen & Burguez 275 (TUR); EF088157; HM367291. *E. sp4*; Paraguay; Lehtonen & Burguez 309 (TUR); EF088179; HM367308. *E. subalatus* (Mart.) Griseb.; Venezuela; Lehtonen & Pacheco 472 (TUR); EF088169; HM367300. *E. tunicatus* Small; Peru; Lehtonen 133 (TUR); EF088154; HM367283. *E. trialatus* Fassett; Venezuela; Lehtonen & Pacheco 441 (TUR); EF088168; HM367299. *E. uruguayensis* Arechav. 1; Uruguay; Lehtonen & Delfino 364 (TUR); EF088159; HM367293. *E. uruguayensis* Arechav. 2; Argentina; Lehtonen et al. 237 (TUR); EF088161; HM367295. *E. uruguayensis* Arechav. 3; cultivated; Falck 94 (TUR); HM367208; HM367316. *Sagittaria sprucei* Mich.; Peru; Lehtonen & Rodríguez 31 (TUR); EF088151; HM367269. **LEAFY**; *trnL-trnS*: *Helanthis* “angustifolius”; cultivated; Mühlberg s.n. (TUR); HM367249; HM367348. *H. “australis”*; cultivated; Mühlberg s.n. (TUR); HM367267; HM367350. *H. “austro-americanus”*; cultivated; Mühlberg s.n. (TUR); HM367251, HM367254; HM367355. *H. “bolivianus”*; cultivated; Mühlberg s.n. (TUR); HM367257, HM367264; HM367345. *H. bolivianum* (Rusby) Lehtonen & Myllys; Ecuador; Øllgaard et al. 57161 (AAU); HM480481, EF088192; HM367357. *H. bolivianum* (Rusby) Lehtonen & Myllys; Argentina; Lehtonen et al. 213 (TUR); HM480482, EF088155; HM367354. *H. “latifolius”*; cultivated; Mühlberg s.n. (TUR); HM367256, HM367268; HM367358. *H. “latifolius”*; cultivated; Falck 104 (TUR); XXXX; HM367359. *H. parvulum* (Engelm.) Small; USA; MacDonald 11345 (UNA); EF088191; HM367341. *H. “quadricostatus”*; cultivated; Mühlberg s.n. (TUR); HM367252, HM367260, HM367265; HM367356. *H. sp*; Venezuela; Lehtonen & Pacheco 482 (TUR); EF088184; HM367352. *H. “tenellus”*; cultivated; Falck 103 (TUR); HM367259, HM367263; HM367343. *H. “tenellus micro”*; cultivated; Lehtonen 721 (TUR); HM367258, HM367262; HM367344. *H. “tenellus red”*; cultivated; Lehtonen 723 (TUR); ; HM367346. *H. “tenellus regular”*; cultivated; Lehtonen 722 (TUR); HM367266; HM367353. *H. tenellum* (Mart) Britton; Bolivia; Lehtonen 156 (TUR); HM480483, EF088152; HM367342. *H. “Vesuvius”*; cultivated; Falck 102 (TUR); HM367250, HM367255; HM367347. *H. “xinguensis”*; cultivated; Mühlberg s.n. (TUR); HM367253, HM367261; HM367349. *H. zombiense* (Jérémie) Lehtonen & Myllys; Guadeloupe; Christenhusz 4040 (TUR); HM480484, EF088166; HM367351. *Ranalisma rostrata* Stapf; cultivated; Lehtonen 695 (TUR); HM367248; HM367340.

Morphological intermediacy has often predicted hybridism [e.g., Moody and Les 2002; Kaplan and Fehrer 2004], and morphological characters may provide valuable additional information on the identity of parental species when the genetic variation between morphologically distinct parental species is low, as was case with many *Echinodorus*. The hybrid plants typically show intermediate morphologies in comparison to their parental species, with an equal expression of the maternal and paternal characteristics [McDade 1990; Kaplan and Fehrer 2004]. A cladistic analysis of artificial F1 hybrid plants indicated that majority of the hybrids were placed as the basal members of the lineage that included the most apomorphic parental species [McDade 1992]. We coded 31 morphological characters for the *Echinodorus* (Tables 2 and 3). The characters were selected and in some cases slightly modified from Lehtonen and Myllys [2008] or Lehtonen [2009a]. The morphological characters were mostly selected so that they would help to distinguish those taxa that were unidentifiable using the studied DNA sequences. We also included several new characters to

accommodate the variation observed in the cultivars into our data matrix. We had most of the studied cultivars in cultivation at the Botanical Garden of the University of Turku, and the character coding for the cultivars was based on a critical literature review and on the observations directly made from the cultivated plants. Many characters, especially those related to the reproductive structures, were excluded from this study since no adequate data were available for the cultivars.

The morphological characters were analyzed separately and together with the molecular evidence. In the combined analysis each hybrid was represented by the maternal and paternal lineages, so the morphological characters had to be coded for these lineages as well. No algorithms exist for optimizing the parental origin of the hybrid morphologies. Instead, we based the coding for the molecular evidence by comparing the morphological characters of hybrids with the characters of those species they were associated with based on the molecular analysis. Whenever a hybrid had a character state that was present only in the other parental lineage (as determined by the molecular analysis), we coded the observed state for this lineage and a question mark for the other lineage. If a character state observed in a hybrid was not present in any species of the inferred parental lineages, or if it was present in both lineages, we coded the character with a question mark for both the paternal and the maternal lineages. For the combined analysis of the morphological and the molecular data we included also those paternal *LEAFY* copies that were excluded in the molecular data analysis (*E. osiris*, *E. cf. osiris*, *E. horemani* “red” and *E. ‘Roter Oktober’*), and those *trnH-psbA* sequences that were not associated with any *LEAFY* sequence (*E. ‘Oriental’* and *E. ‘Fantastic Color’*).

Table 2. Morphological character descriptions coded for *Echinodorus*. Character 31 was analyzed as a part of molecular, not morphological data analysis

0. Rhizome orientation: (0) erect; (1) horizontal
1. Rhizome length: (0) short, <1 cm; (1) long, >5 cm
2. stamen number: (0) 9; (1) 12; (2) ca. 13; (3) 15-22; (4) 24-35; (5) 40-60
3. leaves: (0) unicolor; (1) spotted
4. leaf color: (0) green; (1) very dark green to red
5. leaf base: (0) attenuate-truncate; (1) cordate
6. leaf apex: (0) acuminate; (1) acute-obtuse; (2) retuse
7. parallel veins: (0) less than 7; (1) 7-15; (2) up to 25
8. pellucid markings: (0) absent; (1) network; (2) dots; (3) lines
9. veins pseudopinnate: (0) no; (1) yes
10. indument: (0) glabrous; (1) pubescent; (2) trichomes
11. petiole cross-section: (0) terete; (1) triangular; (2) half-circle; (3) irregularly angular; (4) polygonal
12. inflorescence: (0) erect; (1) creeping
13. order of branching: (0) one; (1) two; (2) three
14. whorls: (0) three or less; (1) five-12; (2) 13 or more
15. rachis: (0) alate; (1) non-alate
16. bracts: (0) persistent; (1) deciduous
17. bract tip: (0) acute; (1) acuminate
18. pedicel length: (0) <1 cm; (1) 1-4 cm; (2) >4 cm
19. pedicel orientation: (0) recurved in fruit; (1) spreading in fruit

20. sepal orientation: (0) erect and appressed to the receptacle; (1) spreading to reflexed
21. sepal veins: (0) <10; (1) 10-20; (2) >20
22. carpels: (0) few; (1) many; (2) numerous
23. petals basally: (0) clawed; (1) not clawed
24. flowers: (0) small \varnothing <2 cm; (1) large \varnothing 2-5 cm; (2) very large, \varnothing >5 cm
25. flowers: (0) open for one day; (1) open for a few morning hours
26. petals: (0) entire; (1) crisped
27. anther: (0) short, 0.5-1 mm; (1) long, 1-2.5 mm; (2) very long, c. 3 mm
28. style length: (0) short, < 1mm; (1) long > 1mm
29. flowers: (0) non-spotted; (1) spotted
30. flowers odorous: (0) no; (1) yes
31. *psbA-trnH* inversion orientation: (0) CAA-type; (1) TTG-type.

Table 3. Morphological data matrix. For the combined analysis of molecular and morphological data characters of hybrids were coded to originate either from maternal or paternal parent, or were ignored when the origin was uncertain (see text for more details). Underlined character states were assigned to maternal lineage of a hybrid, double underlined to paternal lineage, italicized states were ignored. Polymorphism are marked as: a=[0,1]; b=[1,2]; c=[2,3]; d=[0,2,3]. Character 31 (orientation of *psbA-trnH* inversion) is presented here together with morphological data, but was analyzed together with molecular data

	11111111111222222222233 01234567890123456789012345678901
<i>Echinodorus</i> 'Apart'	11301 <u>1</u> 1001020111011111111100100?1
<i>E.</i> <i>×barthii</i>	113 <u>a</u> 10103102001101111111110??00 <u>1</u> 1
<i>E.</i> 'Fantastic Color'	11?0 <u>1</u> 0100 <u>1</u> 010??101111111110??0?0
<i>E.</i> 'Harbich'	11 <u>4</u> 00121000 <u>1</u> 0011011111111100100?0
<i>E.</i> 'Kleiner Bär'	11301010002b0001012111111101100?0
<i>E.</i> 'Oriental'	11301 <u>1</u> 100001a00101211111110 <u>1</u> 100?1
<i>E.</i> 'Red Diamond'	11 <u>3</u> 0101010 <u>1</u> 01a001011111111100100?1
<i>E.</i> 'Rosé'	113 <u>a</u> 101031220 <u>1</u> 1101211111110010011
<i>E.</i> 'Roter Oktober'	11301 <u>1</u> 1000011011011111111110??00 <u>1</u> 1
<i>E.</i> 'Rubin'	113010103102a0?10111111111100100?1
<i>E.</i> "macrophyllus"	113001100021101101211111110110000
<i>E. amazonicus</i>	11000000310111110100100100000000
<i>E. amphibius</i>	11000000310111110100100100000001
<i>E. berteroi</i>	002001113003021100111111000001000
<i>E. bleherae</i> 1	11000000310111110100100100000001
<i>E. bleherae</i> 2	11000000310111110100100100000001
<i>E. bracteatus</i>	11300111c020012001001111110010001
<i>E. cordifolius</i> 'Marble Queen'	113001110001101101211111110010000
<i>E. cordifolius</i> 1	113000103020100101211111110010000
<i>E. cordifolius</i> 2	114001113020111101211111110010000

<i>E. cordifolius</i> 3	11300111302010110121111110010000
<i>E. cordifolius</i> ssp. <i>fluitans</i>	<u>113</u> 000 <u>100</u> 00 <u>1</u> 001101 <u>2</u> 1111110010000
<i>E. cordifolius</i> ssp. <i>ovalis</i>	11300a11000110010121111110??00?0
<i>E. decumbens</i>	11100010300410110101111100011101
<i>E. emersus</i>	11300111002001210101111110010000
<i>E. floribundus</i> 1	11400122002001210111111110010000
<i>E. floribundus</i> 2	11400122202001210111111110010000
<i>E. floribundus</i> 3	11400122202001210111111110010000
<i>E. gabrielii</i>	11400111c002000101111111101100?0
<i>E. glaucus</i>	11400111000001210111122110010001
<i>E. gracilis</i>	11000000310100110100100100000000
<i>E. grandiflorus</i> 1	114a0011c02001110111111110110010
<i>E. grandiflorus</i> 2	114a0011c02001110111111110110010
<i>E. grandiflorus</i> 3	114a0011c02001110111111110110010
<i>E. grandiflorus</i> ssp. <i>aureus</i>	<u>1140012</u> <u>1202</u> <u>001</u> 110111111110??0000
<i>E. grisebachii</i> 1	11000000310111110100100100000001
<i>E. grisebachii</i> 2	11000000310101110100100100000001
<i>E. grisebachii</i> 3	110000003101?1110100100100000000
<i>E. heikobleheri</i>	11000000310110110100100100000001
<i>E. horemani</i> "red"	<u>11301</u> <u>0100</u> <u>12</u> <u>b00</u> 1101111111100100 <u>1</u> 1
<i>E. horizontalis</i>	01400101100010111110022101000000
<i>E. inpai</i>	111000103004010001011111000011?1
<i>E. longipetalus</i>	11500010110100110110022120020001
<i>E. longiscapus</i> 1	114a0111c00000110111111110010000
<i>E. longiscapus</i> 2	114a0111c00000110111111110010000
<i>E. longiscapus</i> -hybrid	<u>114a</u> 0111c0 <u>2</u> 00 <u>1</u> 110111111110010000
<i>E. maculatus</i>	<u>11?</u> <u>a</u> 0111c0 <u>22</u> 11110121111110??00?0
<i>E. major</i>	11100010310400010101111100000001
<i>E. cf. opacus</i>	<u>11?01</u> 1 <u>10000</u> 200 <u>1</u> 10111111110??00?0
<i>E. osiris</i>	11300010c <u>122</u> a011011111111001000 <u>1</u>
<i>E. cf. osiris</i>	113000103 <u>102</u> a0110111111110 <u>1</u> 100?1
<i>E. palaefolius</i> var. <i>latifolius</i>	11100010310400200101111100011101
<i>E. paniculatus</i> 1	11400010000101110111111110010000
<i>E. paniculatus</i> 2	11400010000101110111111110010000
<i>E. parvifloru</i> s	11000000310111110100100100000001
<i>E. parviflorus</i> "Tropica"	11001000310100110100100100000001
<i>E. portoalegrensis</i>	<u>11301010010200</u> 1101111111100100?0
<i>E. pubescens</i>	111000110010002101011111000111?1
<i>E. reptilis</i>	11300010300210010121111110010000
<i>E. scaber</i>	11300111002001210111111100000000
<i>E. schlueteri</i>	<u>11300111300</u> 210 <u>01012</u> 111111100100?0
<i>E. schlueteri</i> 'Leopard'	11310111c02111?10121111110??00?0
<i>E. sp3</i>	11300010302011110111111110010000
<i>E. sp4</i>	11400111202011210111111110010000
<i>E. subalatus</i>	11100010300400200111111100011101

<i>E. trialatus</i>	11100010010100200100111100000000
<i>E. tunicatus</i>	01400101100000111110022101000000
<i>E. uruguayensis</i> 1	11300010310ba01101111111110010011
<i>E. uruguayensis</i> 2	11300010310ba01101111111110010011
<i>E. uruguayensis</i> 3	11300010310ba01101111111110010011
<i>Sagittaria sprucei</i>	00000112100100111000022000001000

In the case of the *Helanthisum*, the noncoding chloroplast sequence (*trnL-trnF*) produced a well resolved tree (see results). Most specimens had mixed *LEAFY* sequences, suggesting either a presence of wide polymorphism within the species, or a common hybridism. We tried similar approach as with the *Echinodorus*, but failed to produce any resolution in the combined analysis of the *Helanthisum trnL-trnF* and *LEAFY* sequences.

The phylogenetic analyses were based on the direct optimization [Wheeler 1996] of unaligned sequence data with the computer program POY [Varón et al. 2010], by building 250 random addition starting trees and swapping these with subtree pruning and reconnection (SPR) and tree bisection and reconnection (TBR) branch swapping, and evaluating all the suboptimal trees within 5% of the best cost, followed by 5 rounds of ratcheting [Nixon 1999], 50 rounds of tree fusing [Goloboff 1999], and a final swapping with SPR and TBR. The morphological data was analyzed with TNT [Goloboff et al. 2008] by building 250 random addition starting trees and swapping with ratcheting. Jackknife support [Farris et al. 1996] was also calculated with the TNT, by using implied alignments and 1000 pseudoreplicate analyses with a deletion probability of e^{-1} , for each pseudoreplicate 100 random addition starting trees were built and swapped with ratcheting. As outgroup taxa we used *Ranalisma rostrata* Stapf in the *Helanthisum* analyses and *Sagittaria sprucei* M.Mich. in the *Echinodorus* analyses.

RESULTS OF THE PHYLOGENETIC ANALYSES

Our combined analysis of the *psbA-trnH* and *LEAFY* sequences, excluding *E.* ‘Oriental’, *E.* ‘Fantastic Color’, and the paternal *LEAFY* copies of *E. osiris*, *E. cf. osiris*, *E. horemani* “red” and *E.* ‘Roter Oktober’, resulted in two equally parsimonious trees with 768 steps. The strict consensus tree with jackknife support values is shown in Figure 3. The morphological data analysis found 810 equally parsimonious trees with a length of 144 steps (the strict consensus shown in Figure 4). The analysis of all molecular data and morphological characters divided between parental lineages for the hybrids as explained above, resulted in a single most parsimonious tree with 926 steps (Figure 5).

The analysis of the *Helanthisum trnL-trnF* data resulted in a single most parsimonious tree with a length of 191 steps (Figure 6). In this tree the genus is divided in two major clades with 100% jackknife support. Both of the major clades are further split in smaller groups with jackknife support varying between 63-100%. Most of the studied *Helanthisum* specimens had two *LEAFY* sequences mixed. The exceptions were two natural collections, *H. parvulum* and *H. sp* (called as *H. cf. bolivianum* 1 in Lehtonen and Myllys [2008]), and three plants of cultivated origin, *H.* “angustifolius”, *H.* “australis” and *H.* “tenellus regular”. They had only

one signal. In addition, a cultivated *H. "quadricostatus"* had three divergent forms of the *LEAFY* sequence. The presence of three versions is in agreement with Kasselmann's [1999] report that *H. "quadricostatus"* is a triploid. The phylogenetic analysis of the *Helanthium LEAFY* sequences yielded 14 equally parsimonious trees with 127 steps. The strict consensus tree showed several clades, but was largely unresolved (Figure 7). The combined analysis of the *trnL-trnF* and *LEAFY* sequences produced an essentially unresolved tree (not shown).

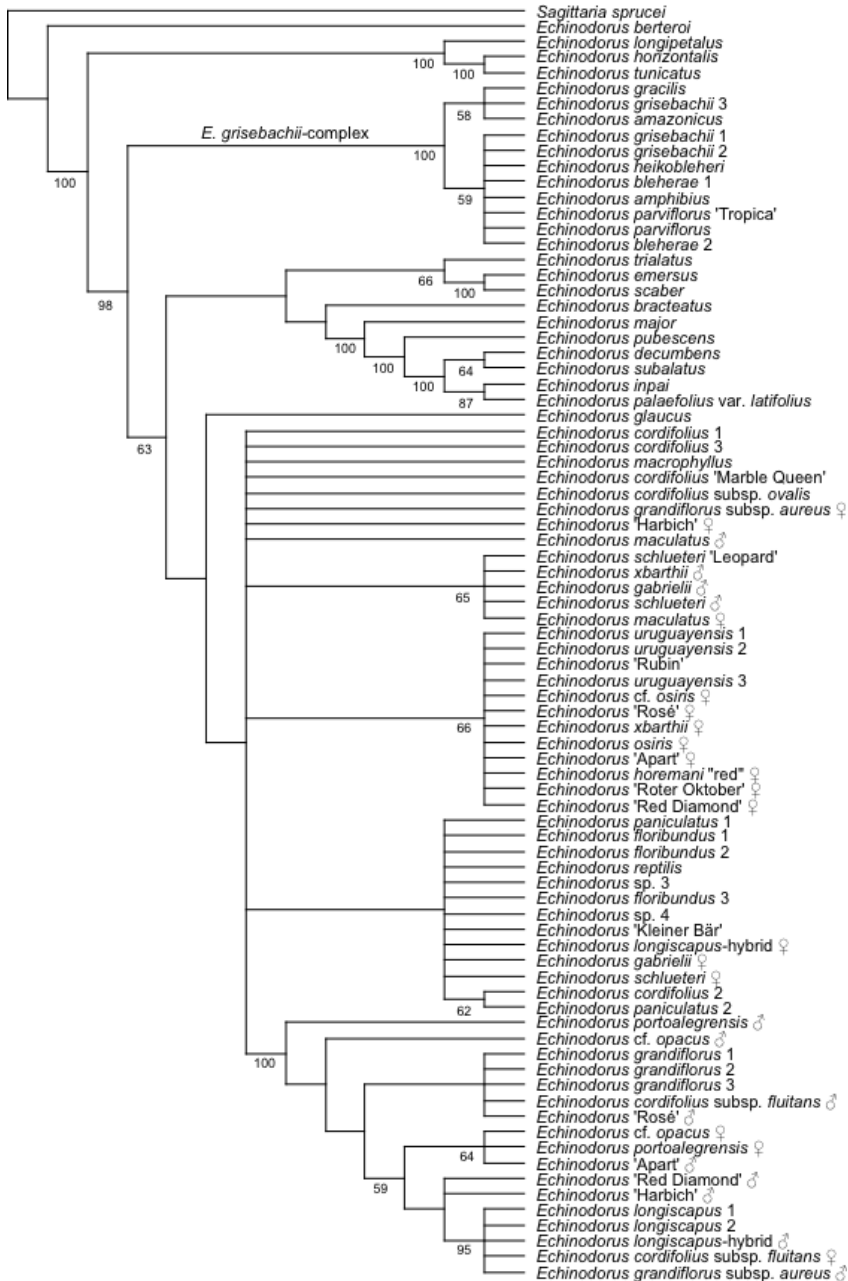


Figure 3. A strict consensus tree of two equally parsimonious trees obtained from the molecular analysis of the *psbA-trnH* and the *LEAFY* sequences.



Figure 4. A strict consensus tree of 810 equally parsimonious trees obtained from the analysis of the morphological characters.

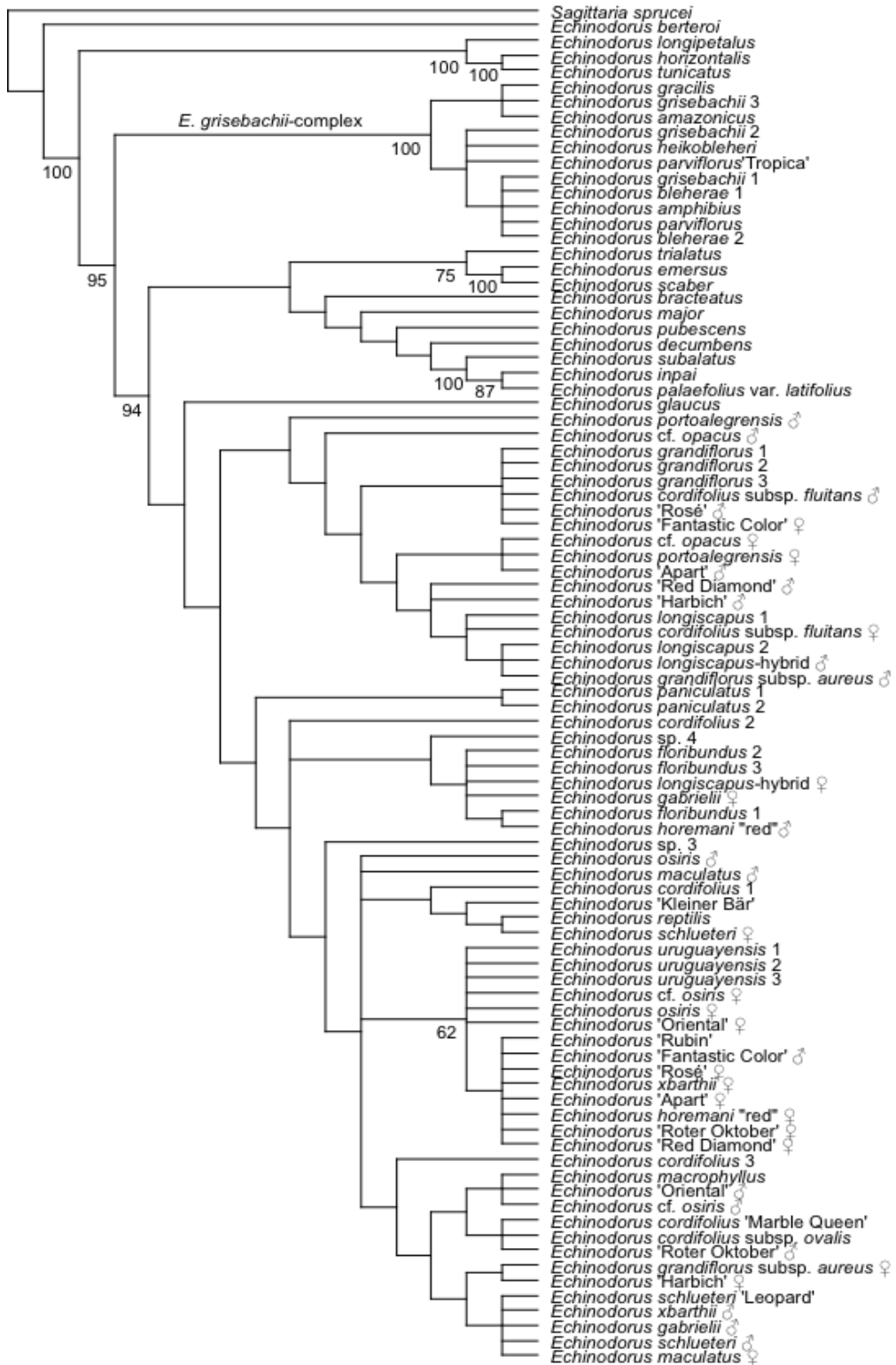


Figure 5. The single most parsimonious tree obtained in the combined analysis of the molecular and the recoded morphological data.

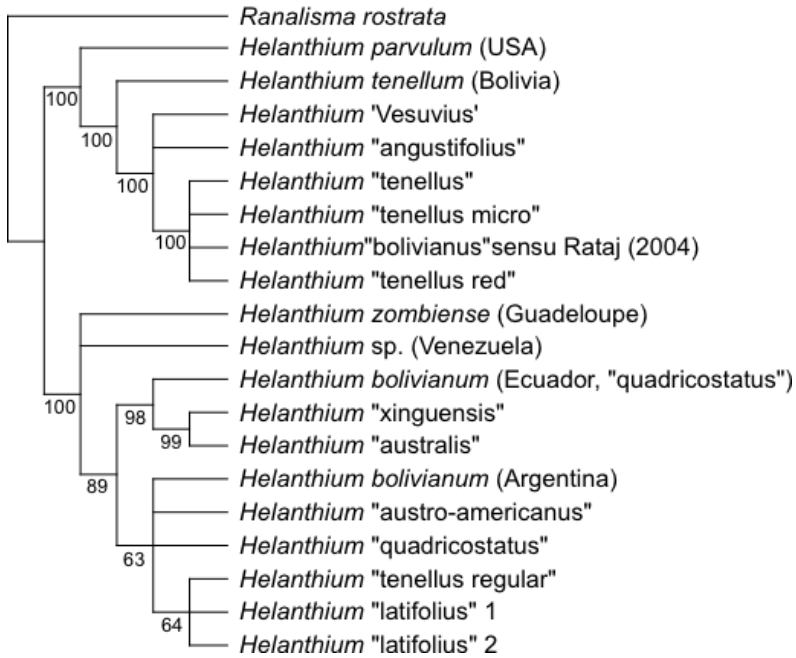


Figure 6. The single most parsimonious tree obtained in the analysis of the *trnL-trnF* spacer region of *Helanthium*.

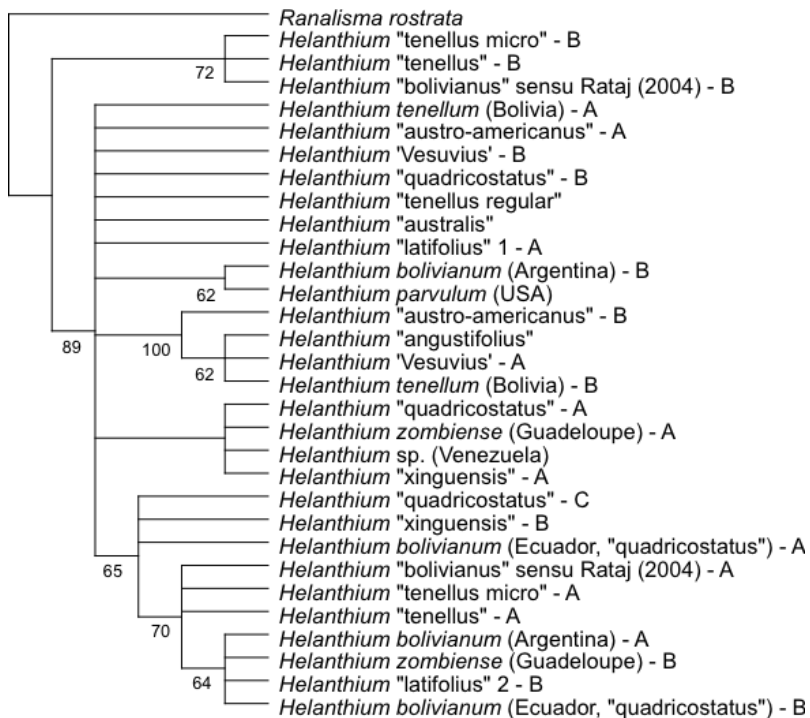


Figure 7. A strict consensus tree of 14 equally parsimonious trees obtained from the *LEAFY* second intron analysis of *Helanthium*.

DIVERSITY

Helanthium

The inferred *trnL-trnF* phylogeny of the genus *Helanthium* is in full agreement with the phylogenetic hypothesis proposed by Lehtonen and Myllys [2008] on the basis of the different molecular markers. Two major clades were resolved corresponding with the concepts of *Helanthium tenellum* and *H. bolivianum* sensu Haynes and Holm-Nielsen [1994]. The only difference is that *H. “angustifolium”* was considered as a synonym of *H. bolivianum* by Haynes and Holm-Nielsen [1994], but our analysis indicates that it belongs to *H. tenellum* clade instead.

Unlike the clear results obtained from the *trnL-trnF* marker, the results of the *LEAFY* analyses were puzzling. It was interesting that *E. “angustifolius”*, being a triploid [Kasselmann 1999], had only one version of the *LEAFY*, whereas the triploid *H. “quadricostatus”* [Kasselmann 1999] had three divergent *LEAFY* versions. We found several main *LEAFY* types present in *Helanthium*, but failed to propose any phylogenetic explanation for their distribution within the genus. The *LEAFY* tree did not correspond with the biogeography either: for example, the two *LEAFY* versions observed in a *H. bolivianum* collected from northern Argentina were grouped together with a *H. zombiense* from the Caribbean and a *H. parvulum* from the USA! Because the main *LEAFY* versions were quite distinct from each other and only five plants out of the 19 sampled had a non-mixed *LEAFY* sequence, it appears that hybridization may not be the only explanation for the observed pattern. The presence of highly divergent *LEAFY* versions in all the clades observed in the *trnL-trnF* phylogeny would indicate a total absence of interbreeding barriers, but in random interbreeding a higher than observed proportion of the plants might be expected to be homozygous. Also, it would be difficult to explain the well-supported and resolved *trnL-trnF* tree under a free gene flow over the whole range of the genus. Hence, we hypothesize that the *LEAFY* sequence has been duplicated in *Helanthium*, and some of the observed sequences are not truly homologous with each other, but represent paralogous copies. If this is true, we must assume random deletions of some copies in various lineages. Furthermore, it is highly likely that at least some amount of hybridization is also involved. Due to these difficulties we mostly consider the *trnL-trnF* tree in the following paragraphs.

Based on the studied DNA sequences *H. parvulum* appears to be perhaps the most distinct taxon in the genus. It has quite a unique morphology as well, and is probably the only *Helanthium* with North American distribution. Therefore, it is interesting that most authors have considered it as a synonym of South American *H. tenellum* [e.g., Fassett 1955; Rataj 1975, 2004; Haynes and Holm-Nielsen 1994; Lehtonen and Myllys 2008]. These taxa are clearly related, but in our opinion should be treated as separate species.

Kasselmann [2003] assumed that *H. “angustifolius”* could be a hybrid, but the studied specimen had only one version of the *LEAFY* sequence showing that it is at least not an F1 hybrid. *Helanthium* ‘Vesuvius’ is said to be a spontaneous mutation of *E. “angustifolius”* [Mees 2010], and they indeed share identical *trnL-trnF* sequence, and the sole *LEAFY* sequence present in *E. “angustifolius”* was observed also in *H. ‘Vesuvius’*. However, *H. ‘Vesuvius’* had another version of *LEAFY* as well, not present in *E. “angustifolius”*, suggesting a cross between *H. “angustifolium”* and some other *Helanthium*. On the other

hand, we only studied one *E. "angustifolius"* specimen, and may have failed to observe the actual genetic variation within *E. "angustifolius"*. We found that *H. "bolivianus"* sensu Rataj [2004] share identical *trnL-trnF* and *LEAFY* sequences with the plants cultivated as *H. "tenellus"*, *H. "tenellus micro"* and *H. "tenellus red"*. All these plants have very narrow leaves with reddish color in good light conditions, and they clearly belong to the same taxon. The plant known as normal variety of tenellus [Frank 2008], or *H. "tenellus regular"*, among the US hobbyists, was not a member of *H. tenellum* clade at all, but was resolved together with Rataj's [2004] *H. "latifolius"*. These plants share identical *trnL-trnF* and *LEAFY* sequences, but *H. "latifolius"* had an additional copy of the *LEAFY* not present in *H. "tenellus regular"*.

The plant known as *H. "quadricostatum"* in the aquarium trade did not share any identical sequences with the plant bearing the same name in the botany. Haynes and Holm-Nielsen [1994] considered *H. "quadricostatum"* as a synonym of *H. bolivianum*, but in contrast, Fassett [1955] and Rataj [1975; 2004] accepted it as a separate species. However, Rataj's [2004] description of the plant is apparently based on the same cultivated material as we sequenced, and therefore does not represent the natural taxon described by Fassett [1955].

The chain swords pose unusual problems in the morphological taxonomy, molecular systematics, and in the specimen identification. Indeed, some of the plants we received as cultivated *Helanthisum* were, after DNA sequencing, found to actually be *Sagittaria*. In the aquaria *Helanthisum* have also been confused with *Vallisneria* (Hydrocharitaceae) and *Lilaeopsis* (Apiaceae) [Kasselmann 2003]. Generally, it appears that the great taxonomic problems in the *Helanthisum* are not readily solved with molecular systematics. Other low-copy nuclear markers should be studied as it appears that the *LEAFY* may not be suitable for the phylogeny reconstruction in this genus. Also, a much wider sampling of the natural populations is required to clarify the species boundaries. Although we are not willing to suggest any formal taxonomy based on these rather sporadic data, they indicate to us that species diversity within the genus may have been seriously underestimated in some of the recent treatments [e.g., Jérémie et al. 2001; Haynes and Holm-Nielsen 1994; Matias 2007]. The amount of the natural genetic diversity in *Helanthisum* remains mostly unknown, but the cultivated plants represented almost the whole variation observed. It is worth noting that *H. parvulum*, the only species of the genus naturally occurring in the USA, is apparently not in cultivation. It is also worth noting that aquarist's are applying several different names for the plants that obviously belong to one species, emphasizing hobbyists' tendency to over-split the natural variation.

Echinodorus

The previous morphology based phylogenetic analyses of *Echinodorus* relationships have resulted in rather different hypotheses than the molecular analyses [Lehtonen 2006, Lehtonen and Myllys 2008]. In our present analysis several morphological characters from the preceding studies were recoded, while many were rejected because they appeared too homoplastic in the earlier analyses, or we were unable to code them for the hybrids. We also added some new characters. The resulting tree is largely congruent with the molecular trees. Numerous *Echinodorus* cultivars belong to the *E. grisebachii* complex, and they did not show any resolution within the clade. Many other cultivars were also resolved in large polytomies without an indication of the relationships in any more details.

The present analysis of the *psbA-trnH* and *LEAFY* markers generally agreed with the previous phylogenetic results, which were based on a wider molecular sampling [Lehtonen and Myllys 2008]. The first lineages within the genus, *E. berteroi* and the *E. horizontalis* clade, have been similarly resolved in the previous molecular analysis as well [Lehtonen and Myllys 2008]. In contrast, the middle part of the phylogenetic tree is somewhat differently resolved. Unlike in the previous study [Lehtonen and Myllys 2008], the *E. grisebachii* complex was resolved in a deeper position, and the *E. trialatus* Fassett clade, *E. bracteatus* Micheli, and the *E. subalatus* clade together formed a clade. The large clade with *E. glaucus* Rataj as the basal taxon was rather poorly resolved but had some differences in comparison with the previous studies [Lehtonen and Myllys 2008]. *Echinodorus grandiflorus* (including *E. floridanus* R.R. Haynes & Burkhalter) and *E. longiscapus* Arechav. were resolved monophyletic, against the results of previous molecular analysis [Lehtonen and Myllys 2008]. It should be noted that the present study was based on fewer data, but on the other hand, we excluded the multi-copy markers (ITS and 5S-NTS), which are often considered problematic in the phylogenetic inference [e.g., Álvarez and Wendel 2003].

The plants belonging to the *E. grisebachii* complex have remained among the most popular aquarium plants ever since they entered the markets. These plants have also caused considerable difficulties for aquarists trying to name them. Numerous species have been described in this group (up to 7 by Rataj [2004]), but because of the lack of any constant molecular or morphological differences the complex has also been considered as a single variable species [Lehtonen 2008]. Perhaps the most distinct taxa within the complex are *E. bleherae*, a triploid plant of relatively large growth [Kasselmann 1999], and the recently described *E. heikobleheri* Rataj, a plant reported to have unusual morphological characters [Rataj 2004]. Nevertheless, these plants did not show any differences in their *psbA-trnH* or *LEAFY* sequences in comparison with such cultivated plants as *E. amphibius* Rataj, *E. parviflorus* Rataj and *E. parviflorus* 'Tropica', or with a natural *E. grisebachii* collected from the Peruvian Amazonia (most likely the same location from where cultivated *E. parviflorus* originated, see Lehtonen and Rodríguez Arévalo [2005]). The most obvious molecular character observed within the complex was the short inversion in the *psbA-trnH* sequence, separating *E. gracilis* Rataj, *E. amazonicus* Rataj and cultivated *E. grisebachii* (with narrow submersed leaves) from the other accessions. These kind of short inversions are often considered phylogenetically unreliable, and have been observed to occur within species [Kim and Lee 2005]. There appears to be less genetic variation within the *E. grisebachii* complex than there is within some morphologically uniform species, like in *E. paniculatus*. The large growth of *E. bleherae* may be related to its polyploidy, and because of the lack of proper documentation the description of *E. heikobleheri* remains questionable. Because we are unable to delimit any reasonable groupings within the *E. grisebachii* complex we continue to consider the complex as a single species, including *E. heikobleheri*. Only one cultivar has been named in the *E. grisebachii* complex, the small sized *E. parviflorus* 'Tropica.'

Several species of the *E. subalatus* clade are in cultivation, including the economically second most important sword plant – *E. major* [Brunel 2009]. Other examples are the recently introduced *E. decumbens* Kasselmann [Kasselmann 2000], and more commonly sold *E. palaefolius* var. *latifolius*. The taxonomic status of these species remains uncertain [Lehtonen 2008], but it seems possible that *E. palaefolius* (Nees & Mart.) J.F. Macbr. var. *latifolius* (Micheli) Rataj and *E. inpai* Rataj are conspecific. Unfortunately we have been unable to obtain any natural material of *E. palaefolius* for the DNA sequencing, and are not able to

provide a molecular verification of the taxonomic status of the cultivated *E. palaefolius* var. *latifolius*.

Our coding of the morphological characters for the hybrids effectively eliminated all the possible contradiction between the molecular and morphological evidence in the hybrids. However, for the natural non-hybrid taxa, and for the cultivars with non-mixed chromatogram signals, we coded all the morphological data as observed in the studied specimens. Hence, the morphological data certainly could have an effect on the tree topology, but not so much on the placement of the hybrids, which were mostly represented by the question marks in the combined analysis. The idea of this approach was to get a better resolution in the genetically uniform, but morphologically diverse groups. In many cases the resolution was improved indeed, and some of the hybrids that were placed in polytomies in the molecular analysis were better resolved with the aid of the morphological characters, thus pinpointing the potential parental lineages more accurately. The strategy we used is somewhat circular and, consequently, the results of the combined analysis are quite speculative, and they should be verified with additional, rapidly evolving low-copy nuclear markers. For these reasons we are focusing on the results of our molecular analyses in the following discussion, and use the morphological results only as supplementary information.

The molecular analyses did have their own problems as well. Besides the imperfect resolution due to the relatively slow rate of evolution, we only sampled one nuclear marker. This approach is certainly enough to detect F1 hybrids (though only when the parental species have divergent sequences), but easily fails to detect Fn hybrids. It is quite obvious that most of the recent cultivated hybrids in *Echinodorus* are actually hybrids of hybrids. It is impossible to reveal the true origins of these cultivars by sampling only one nuclear marker in addition to the chloroplast. Many of the apparently “pure” cultivars may actually be Fn hybrids that have lost the other version of the *LEAFY* sequence in recombination, and many of the obvious hybrid cultivars may also represent Fn generation. However, if a plant has only one version of the *LEAFY* sequence originating from a different species than its chloroplast, it is certain that the plant is an F2 or further Fn hybrid. We observed two such *Echinodorus* cultivars: *E.* ‘Oriental’ and *E.* ‘Fantastic Color’. *Echinodorus* ‘Oriental’ has been in cultivation since 1994 and is believed to be a spontaneous mutation of *E.* ‘Rosé’ [Kasselmann 2003]. This is certainly not the case, since *E.* ‘Oriental’ lacks both of the two divergent *LEAFY* versions present in *E.* ‘Rosé’ and therefore cannot directly descend from it. *Echinodorus* ‘Fantastic Color’, on the other hand, had only one version of the *LEAFY* sequence otherwise present in the *E. uruguayensis* clade only. Hence, this cultivar must be F2 or Fn hybrid, and within our sampling appears to be the only *E. uruguayensis* Arechav. related cultivar having *E. uruguayensis* as pollen and not seed parent.

All the observed hybrids were confined to the sister clade of *E. glaucus*. This clade is genetically surprisingly uniform given the great morphological and ecological variation within it [Lehtonen 2008]. It seems possible that evolutionary distance does not allow hybridization beyond this clade, but the lack of hybrids within the other clades suggests that in these clades even the closely related species are reproductively isolated from each others, unlike the species in the sister clade of *E. glaucus*. This hybridization potential may in fact be partly responsible for the apparently rapid diversification of the clade. The effect is the same for the aquarists, who have successfully exploited the potential of the genetic recombination between these species.

The taxonomic status of *E. osiris* has remained controversial for a long time. This plant originates from southern Brazil [Wanke and Wanke 1994], but has been variously considered as a distinct species [Rataj 1970b], hybrid [Schöpfel 1986], or conspecific with *E. uruguayensis* [Haynes and Holm-Nielsen 1994; Lehtonen 2008]. Both triploid and tetraploid populations are known to occur, suggesting polyploidization and further hybridization [Dierk Wanke, pers. comm.]. We included in our analyses a triploid *E. osiris* and a tetraploid *E. cf. osiris*. Both individuals had a mixed *LEAFY* signal indicating a hybrid origin. In both cases the chloroplast and the other *LEAFY* sequence were identical with *E. uruguayensis*, assumed here to represent the maternal plant. The paternal *LEAFY* version is shared by several species, thus leaving the pollen parent uncertain. The simultaneous analysis of the molecular and morphological evidence suggested a close affinity with *E. cordifolius* and some related cultivars for the tetraploid *E. cf. osiris*, while the position of the triploid *E. osiris* remained more ambiguous.

In a similar fashion the status of *E. opacus* Rataj and *E. portoalegrensis* has remained unclear. Both species were described based on sterile material [Rataj 1970a-b], and for this reason have been problematic for taxonomists [Haynes and Holm-Nielsen 1994; Lehtonen 2008]. We included in our study *E. portoalegrensis* originating from an old import from Brazil by the company Lotus Osiris and cultivated in the Botanical Garden of Munich. In addition, we included a tetraploid plant originating from Southern Brazil and showing phenotypic similarity with *E. opacus* (Dierk Wanke, pers. comm.), called here as *E. cf. opacus*. Both of these plants produced mixed *LEAFY* sequences and consequently are considered here as natural hybrids. Both plants share identical *psbA-trnH* sequence, and the other version of the *LEAFY* sequence. The other version is almost identical between these plants as well, but does differ slightly. Most interestingly, we have so far been unable to find any pure species having these versions of the *LEAFY* sequence. In the phylogenetic analyses the maternal lineage of these plants was resolved as a sister to *E. longiscapus*, and the paternal versions were resolved in a slightly deeper position, below the common ancestor of *E. grandiflorus* and *E. longiscapus*. Thus, the exact origin of *E. cf. opacus* and *E. portoalegrensis* remain somewhat mysterious, but they seem to be polyploid hybrids very closely related to *E. grandiflorus* and *E. longiscapus*.

We assume that hybridization between different *Echinodorus* species is not rare at all in the nature. One plant identified as *E. longiscapus* in the previous analysis [Lehtonen and Myllys 2008] was in closer inspection revealed to be a hybrid, possibly between *E. floribundus* and *E. longiscapus*. Apparent intermediates between these two species are common in northern Argentina and Paraguay where the species co-occur. One of the sampled specimens identified as *E. cordifolius* and originating from Venezuela was resolved together with one *E. paniculatus* specimen, also collected from Venezuela. In this case both plants had non-mixed *LEAFY* signals and therefore are not F1 hybrids, but the phylogeny points towards gene flow from one species to another.

Echinodorus uruguayensis is clearly the most widely used species in the artificial hybrids. This is not surprising, because *E. uruguayensis* is the only truly aquatic species within the clade of hybridizing species. Even more tempting is the variation in color; in some populations plants are dark red instead of the more regular green color [Wanke and Wanke 1994]. The red color has been introduced in numerous hybrids, apparently via *E. horemani* “red”. This large sized and dark colored cultivar became introduced during the 1970s [Rataj and Horeman 1977]. Kasselmann [2003] noticed great variation in seedlings originating from

a self-pollinated *E. horemani* “red” and concluded that the cultivar must be a hybrid. Her conclusions are supported by the presence of the mixed *LEAFY* sequences, indicating together with the *psbA-trnH* sequence that the seed parent is *E. uruguayensis*, most apparently the red colored form. The pollen parent remains unclear based on the *LEAFY* sequence alone, but simultaneous analysis of the molecular and morphological data suggests that it is *E. floribundus*. *Echinodorus floribundus* and *E. uruguayensis* co-occur in Southern Brazil and we cannot rule out the possibility of a natural hybridization. Numerous red colored cultivars that obviously result from intentional crossings with *E. horemani* “red” have been introduced within the past few decades. The first of them was *E. ×barthii*, a hybrid whose exact origin has remained unknown. Kasselmann [2003] had information that one of the parental species would have been *E. uruguayensis*, and Rataj [2004] wrote that the other parent was *E. osiris*. We suggest here that the seed parent was *E. horemani* “red”, and the pollen parent was most likely *E. maculatus* Somogyi (also known as *E. ‘Rubromaculatus’*, Somogyi [2006]). The paternally originating *LEAFY* sequence of *E. ×barthii* was identical with the one observed in *E. maculatus* and some other cultivars, but within this clade only *E. ×barthii*, *E. maculatus* and *E. schlueteri* ‘Leopard’ have spotted leaves (spots are not always present in the two first mentioned cultivars). *Echinodorus schlueteri* ‘Leopard’, on the other hand, had only one version of the *LEAFY* sequence telling that it may have originated from a self pollinated *E. maculatus*, as Somogyi [2006] suggested. Another early hybrid, *E. ‘Rosé’*, was introduced as a cross between *E. horizontalis* and *E. horemani* “red” [Barth 1988]. The DNA evidence clearly shows, however, that *E. grandiflorus* was used in the crossing instead of *E. horizontalis*. Since then, many new crossings have been made between the original red colored cultivars and the other cultivated *Echinodorus*, for example with *E. portoalegrensis* to create the cultivar named *E. ‘Apart’*.

Echinodorus ‘Kleiner Bär’ is an interesting cultivar, not only because of its beautiful and peculiar growth form, but also because of its claimed origin. This cultivar is said to combine *E. parviflorus* (a synonym of *E. grisebachii*), *E. ×barthii*, and *E. horemani* “red” [Kasselmann 2003]. However, we found no evidence for such a mixture. Actually, *E. ‘Kleiner Bär’* did not share any sequences with these assumed parents, showing that it cannot be a direct offspring of any of them. The analyses imply close affinity between the cultivars *E. ‘Kleiner Bär’* and *E. schlueteri* with the natural species *E. reptilis* Lehtonen. The small size and long, prostrate stem with only a few flower whorls and long pedicels are typical characters for both *E. schlueteri* and *E. reptilis*. However, *E. schlueteri* has broader leaf blades and it does grow somewhat larger than pure *E. reptilis*. Another cultivated plant showing striking similarity with *E. reptilis* and purportedly originating from the same region was described in the older aquarium literature under the name *E. aschersonianus* [Mühlberg 1972; Stallknecht 1977]. This name is not correctly applied in the aquarium trade, since it is a synonym name of *E. uruguayensis* [Lehtonen 2008], but is widely used in the recent aquarium literature still [e.g., Kasselmann 2003; Rataj 2004]. However, the modern description of the cultivated *E. aschersonianus* is somewhat different from the original given in the older aquarium literature. We assume that the originally imported *E. aschersonianus* was in fact *E. reptilis*, but during the decades of cultivation the original plants became replaced with hybrid offspring. According to this view, *E. schlueteri* could have originated from *E. maculatus* pollinated with *E. reptilis* pollen.

Another example of a name misapplication was the plant sold as *E. cordifolius* subsp. *fluitans* by Tropica Aquarium Plants. Based on the studied DNA sequences this plant combines genetic material of *E. longiscapus* and *E. grandiflorus*.

Evolution in Aquaria

According to Rataj and Horeman [1977] there were no *Echinodorus* hybrids on the market in the 1970s. However, these authors gave detailed instructions on how to reproduce *Echinodorus* sexually, and described a method to pollinate self-sterile plants with a pollen mixture containing their own pollen and pollen from other species [Rataj and Horeman 1977]. This was said to induce the mother plant to accept its own pollen, while hybridization was not observed [Rataj and Horeman 1977]. The later hybrid boom together with our own growing experiments show that within the sister clade of *E. glaucus* hybridization readily occurs. It seems evident that the mixed-pollen experiments in the 1970s already created a hybrid swarm in the aquarium market, evidenced by the continuous flow of new species descriptions based on the aquarium plants of unknown origin. Some of these “species”, such as *E. schlueteri* Rataj and *E. gabriellii* Rataj, were here shown to be hybrids, and undoubtedly a large number of other described species with unknown origin are actually hybrids, for example in the *E. uruguayensis* group. Rataj’s [1975] original revision was mostly based on the herbarium material collected from the nature, but later he based most of his studies on the cultivated plants [e.g., Rataj 1981, 1988, 1989]. Indeed, in his later revision many species descriptions were “corrected” based on the experiments with the cultivated plants [Rataj 2004]. As the cultivated species most likely were seriously contaminated with a genetic material from other species already in the 1970s, the growing demarcation between the aquarium based and the botanical classification of *Echinodorus* is fully understandable. The clash between the hobbyists’ and botanists’ *Echinodorus* classification emerges from the different nature of evolution in the nature and in the aquaria – resulting in a fundamentally different kinds of plants growing in each.

The horizontal gene transfer and the intentional artificial selection, not to mention the induced mutations, will result in a rapid evolution, as manifested by the emergence of numerous *Echinodorus* cultivars. This human-driven evolution is carried by the consumers aesthetic desire, plant nurseries economical decisions for maximizing profit, and technological advances – both in the commercial production and in the aquarium keeping at home – allowing, for example, the micropropagation of the plants not reproducing well enough, or the maintenance of the light-demanding plants in the culture (Figure 8). The human-driven, or the artificial, evolution is reticulate and, hence, produces patterns that avoid hierarchy and requires essentially rankless classification [Hettterscheid and Brandenburg 1995]. No matter how species are defined, under the free hybridization and the selection for the desired traits to be used in yet another round of a genetic mix-up, there is nothing that would correspond with a natural species. It has been proposed that, in order to avoid further confusion, the cultivated plants should not be classified using the same concepts as the natural taxa. A cultonomic classification (in contrast to taxonomic one) based on a rankless group category called culton (plural culta) should be applied [Hettterscheid and Brandenburg 1995]. In the cultonomic classification, the species level does not exist, as there are no hierarchic evolutionary groups among cultivars [Hettterscheid and Brandenburg 1995]. This fundamental

difference between the natural taxa and the artificial culta has not been generally realized and, as a result, a continuous confusion exists over the *Echinodorus* classification [e.g., Kasselmann 2003; Rataj 2004]. We highly recommend that in the future all cultivated *Echinodorus* are treated as cultivars and named according to the ICNCP [Brickell et al. 2004], reserving ICBN [McNeill et al. 2006] for the natural taxa exclusively [see Hetterscheid and Brandenburg 1995; Spencer and Cross 2007].

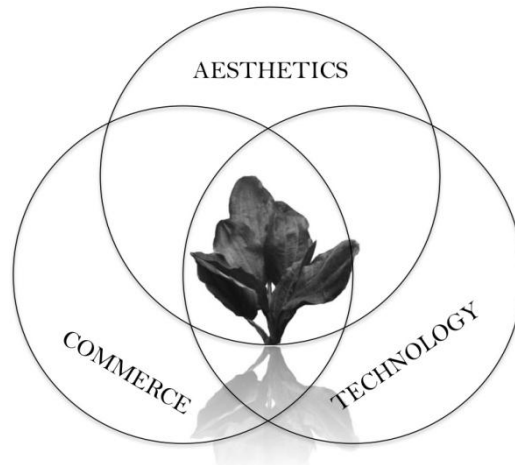


Figure 8. The direction of the human-driven aquarium plant evolution is determined by the customers aesthetic desires, the economics of the commercial production, and the technological advances both in the commercial production and aquarium keeping at home.

In the 1990s the sword plant hybrid-boom overheated so much that in the rush to get new cultivars to the market, many plants were selected that only looked good emerged but grew poorly in the aquaria [Kasselmann 2001]. The characters that are currently searched for in *Echinodorus* breeding are fancy colors associated with a small size on the one hand, but also larger plants with long narrow leaves are desired [Troels Andersen pers. comm.]. Despite of the numerous new fancy cultivars, two natural species imported already in the early years – *E. grisebachii* and *E. major* – continue to be among the most popular aquarium plants. In some aquarium settings, for example in a biotope or a low-tech aquarium, the eye-catching cultivars may not be preferred. Hence, there certainly remains space for the old-fashion natural species in the market. Together with the growing awareness of the environmentally sustainable, certified production practices in the aquarium industry [Harju 2008], this could even allow a local, environmentally sustainable fair trade aquarium plant cultivation to thrive in Amazonia [Lehtonen and Rodríguez Arévalo 2005].

Other important trends in current ornamental plant industry are related to the biosecurity issues and the intellectual property and biodiversity protection [Bhat 2008]. The regulation of the invasive species trade and the risk assessment of the traded plants are extremely important biosecurity questions in the global horticulture [Heywood and Brunel 2009]. The intellectual property rights can be violated through the unauthorized cultivation of the registered varieties, by the intentional selling of plants under a false name of a registered variety, or by the unauthorized commercial exploitation of the plant material collected from the wild [Bhat 2008]. Biopiracy, the unauthorized commercialization of wild organisms or their genes, is

probably a less acknowledged problem in the aquarium trade than, for example, the violation of the patent rights. Prospecting for new species, or populations with new characters or more suitable climatic adaptations, will, most likely, continue to be an integral part of the aquarium plant hobby and industry. This has to be done following the national legislations and respecting the nations' ownership over their own natural resources, including the biodiversity.

The aquarium design styles have witnessed a recent diversification, but still a lot of potential from the historical and recent visual arts and garden design styles remains to be tapped into. The continuously evolving aquarium design styles require new cultivars as well. Members of all major *Echinodorus* clades have been commercialized at some point, but some species have more or less disappeared from the markets. For example, the once popular *E. berteroi* and *E. horizontalis* are no longer commonly available, apparently because of the problems in the commercial production and cultivation [Kasselmann 2003]. Hence, the greatest potential may be among the easily hybridizing species. Surprisingly, we found no evidence that *E. paniculatus*, the most common and widespread sword plant in the nature, would have been used in the commercial crossings. This species has not been considered as suitable aquarium plant [Kasselmann 2003], but as a large, narrow-leaved species, it might provide novel characteristics for the cultivar breeding.

All these issues require correct specimen identification which has remained a difficult task due to the lack of a proper *Echinodorus* cultonomy and molecular identification tools; the genetic markers and the international databases.

Our results illuminated the complex origins of the *Echinodorus* hybrids, but we were unable to reveal the true nature of the proposed *Helanthisum* taxa. The well-structured chloroplast phylogeny suggests the presence of several species, but the independent support for this was not obtained from the studied nuclear marker, the evolution of which seems to be confounded by processes that remained unclear.

CONCLUSION

In this study, we have expanded our previous sampling of the natural taxa to cover the cultivated *Echinodorus* and *Helanthisum*. We found that most of the cultivated *Echinodorus* belong to a hybrid swarm. In order to get new appealing plants to the market, the *Echinodorus* plants with the desired characteristics have been intentionally crossed since the 1980s. The phase of the intentional breeding was obviously preceded with numerous unintentional hybridization events, leading to a confusion in the *Echinodorus* classification and identification. The correct identification of the aquarium plants provide satisfaction for a hobbyist, but it is also tremendously important for monitoring and regulating the trade of the endangered or potentially invasive species, in evaluating a species's invasive risk, and in protecting the intellectual property rights of the plant breeders and the owners of the genetic resources – that is, the nations in the case of wild plants. The identification requires, first of all, a solid natural classification so that the groups identified are meaningful. In the case of the sword plants, this has been difficult to achieve, at least partly because of the unsuccessful attempts to classify cultivars of mixed origins as natural taxa. It is suggested here that the commercially cultivated *Echinodorus* should be considered as such, as artificial commercial products specifically created to fulfil customers' desires. The cultivated *Echinodorus* are not

results of a natural evolution and cannot be reasonably classified under a regular plant taxonomy. Hence, their classification should follow the concepts of the cultivated plant systematics.

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