Revision of chromosome numbers of *Potamogetonaceae*: a new basis for taxonomic and evolutionary implications

Revize variability v počtu chromozomů a její důsledky pro taxonomii a evoluci v čeledi *Potamogetonaceae*

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In order to establish a sound basis for systematic and evolutionary research, we determined the chromosome numbers of 181 samples of 47 species and 32 hybrids of *Potamogetonaceae* from 27 countries and areas, ranging from Greenland in the north to New Zealand in the south and reevaluated previously published counts. The first counts are reported here for 10 species and 25 hybrids of *Potamogeton* and for 1 species and 3 hybrids of *Stuckenia*. Both homoploid and heteroploid hybrids were identified, as well as hybrids resulting from the fusion of reduced and unreduced gametes. Three previously undetected hybrids of *Potamogeton* are described and validated as *P.* ×*drepanoides*, *P.* ×*luxurians* and *P.* ×*serrulifer*. The extensive within-species variation in chromosome numbers sometimes reported in the literature was not confirmed. Chromosome numbers appeared to be generally species-specific in *Potamogetonaceae*; the only exceptions were two sterile autotriploid plants detected within two otherwise fertile diploid *Potamogeton* species. Furthermore, chromosome numbers were often uniform even within species groups or genera and to some degree also clade-specific in phylogenetic trees based on nuclear ribosomal markers (ITS and 5S-NTS regions). In the largest genus, *Potamogeton*, there are two base numbers for diploids (x = 13 and x = 14) and three ploidy levels in species (diploids, tetraploids and octoploids; all polyploids were based on x = 13), in *Stuckenia* only hexaploids (also based on x = 13) occur and *Groenlandia* is monotypic with x = 15. A critical evaluation of the published counts revealed three major sources of error: (i) methodological problems due to difficult karyotypes, (ii) approximations based on wrong preconceptions and (iii) poor taxonomic treatments, misidentified species or unrecognized hybrids. We estimate that about 24% of the counts in original publications and up to 41% in chromosome atlases and indices are doubtful or demonstrably erroneous. Most of these were from a relatively few dubious sources whereas the majority of counts reported in the literature correspond to our findings. Two alternative scenarios for the evolution of chromosome numbers in this family are discussed in a phylogenetic context, with either x = 13 or x = 14 as the base chromosome number in the family; the base number of x = 7 suggested by some authors is refuted. In both scenarios, several aneuploid transitions between these karyotypes and a single change towards x = 15 have to be assumed. Polyploidizations are rare in this family and mostly associated with major evolutionary events. A single or a very few events led to a large species group of tetraploids in *Potamogeton*, and two subsequent rounds of polyploidization can explain the cytotype of *Stuckenia*, in which speciation took place entirely at the hexaploid level. Three octoploid species of *Potamogeton* had allopolyploid origins. This study gives an example of how the careful re-examination of chromosome numbers can substantially ameliorate interpretations of systematic and phylogenetic patterns.

K e y w o r d s: aneuploidy, base number, chromosome number, cytotaxonomy, evolution, heteroploid hybrid, internal transcribed spacer, karyology, phylogeny, ploidy level, polyploidy, reduced gamete, 5S non-transcribed spacer

Introduction

Chromosome number is an important cytological character that substantially influences various biological and evolutionary characteristics of an organism and is used for evaluating relationships and taxonomic decisions (Stace 2000). Its value is acknowledged in many recent taxonomic and biosystematic studies (e.g. Felix & Guerra 2010, Krahulec et al. 2011, Rooks et al. 2011, Rotreklová et al. 2011, Šafářová et al. 2011, Štěpánek et al. 2011, Dančák et al. 2012, Koutecký et al. 2012a, b, Kúr et al. 2012). However, both systematic and geographical representation of chromosome records is highly uneven. Bennett (1998) estimated that chromosome numbers of more than 70% of angiosperm species are unknown. Most chromosome counts are for relatively well-known plants from temperate and boreal regions of Europe. Even for these species, this information may be incomplete because the counts are often made on only one individual. The proportion of incorrect counts and counts made on misidentified material is unknown, but may be surprisingly high, as can be deduced from the high number of recent studies commenting on previous doubtful or erroneous records (e.g. Krahulcová 2003, Marhold et al. 2005, Mártonfi et al. 2008, Mráz et al. 2008, Rotreklová et al. 2011, Dančák et al. 2012, Letz et al. 2012, Vít et al. 2012). Many taxonomically difficult groups and plants from poorly explored regions are largely neglected. The proportion of species for which the chromosome number is known is less than 1% in some little-collected tropical areas (Stace 2000).

The *Potamogetonaceae* are one of the most diverse and taxonomically difficult families of aquatic plants (Wiegleb & Kaplan 1998, Kaplan 2002a). The main sources of taxonomic complexity include their reduced morphology, which limits the number of taxonomic characters that can be used to separate species (Preston & Croft 1997, Kaplan & Štěpánek 2003, Kaplan et al. 2009), an extensive phenotypic plasticity (Kaplan 2002b, 2008, Kaplan & Zalewska-Gałosz 2004), partitioning of genetic variation between rather than within populations (Hettiarachchi & Triest 1991, Kaplan & Štěpánek 2003) and the occurrence of numerous hybrids (e.g. Preston 1995, Wiegleb & Kaplan 1998, Kaplan & Fehrer 2007, Kaplan et al. 2009, Kaplan 2010a). The *Potamogetonaceae* include about 80 species and 105 hybrids classified into three genera, namely *Potamogeton* with about 72 species and 99 hybrids, monotypic *Groenlandia*, and *Stuckenia* with 7 species and 6 hybrids (Kaplan 2008, 2010a, c, and unpublished data). The highest species and hybrid diversity is found in temperate regions of the Northern Hemisphere.

Previous records indicate considerable variation in chromosome number in *Potamogeton* and *Stuckenia*. There are two reviews of the chromosome numbers in *Potamogeton* s. l. (i.e. incl. *Stuckenia*). (i) Les (1983) intended to bring together reports of chromosome numbers in the literature and discuss them in the context of taxonomic and phylogenetic relationships within *Potamogeton*. He concludes that the genus is characterized by two different polyploid lineages (one based on x = 7 and the other on x = 13) and that the diploid level of the genus is 2n = 14. Unfortunately, this study included many errors that mainly resulted from poor taxonomic background and reliance on secondary chromosome compilations rather than on the original publications (see below). It was criticized by Wiegleb (1988) because the listing of chromosome counts is incomplete, the attribution of synonymy is incorrect in some cases and the assignment of species to morphological groups erroneous. Consequently, this review did not show any reasonably clear pattern and the implications for the evolution of chromosome numbers in pondweeds were

doubtful. (ii) Hollingsworth et al. (1998) thoroughly revised all published counts and compared records abstracted in chromosome indices and compilations with those in the original publication, and highlighted numerous errors in the literature. The most important outcome of this review is the clear demonstration that many chromosome counts in secondary literature are misreported or even never made. The authors emphasize that the use of secondary literature presents many pitfalls.

In their worldwide account of *Potamogeton* species and hybrids, Wiegleb & Kaplan (1998) note that two or more methodologically reliable chromosome counts are available only for about 30 of the 69 recognized species and 4 of 50 confirmed hybrids. All counts are for plants from the Northern Hemisphere. For at least 20% of the published counts, Wiegleb and Kaplan were not sure to which taxon they actually refer. They identified that the obtaining of reliable chromosome counts of well identified specimens, both from the Northern Hemisphere, but in particular from the Southern Hemisphere, is one of the major tasks of future *Potamogeton* research.

Only a small number of new records have been published since the last review by Hollingsworth et al. (1998). Less than half of the species have been studied cytologically. The literature continues to include much contradictory information about chromosome numbers in *Potamogetonaceae*, and there is still no consensus about the base numbers in this family (Hollingsworth et al. 1998, Wang et al. 2007). The aims of this paper are therefore to (i) fill some of the gaps in the knowledge of chromosome numbers in *Potamogetonaceae*, (ii) compare new counts with published records, (iii) evaluate all available chromosome numbers and, as far as possible, distinguish repeatedly confirmed counts from unique doubtful records never confirmed by other researchers, (iv) analyse the pattern in the variation in chromosome numbers against morphological and taxonomic groups and in a phylogenetic framework based on well identified specimens and representative intraspecific genetic variation, and (v) identify the base chromosome number.

Material and methods

Plant material

Samples of plants of *Potamogetonaceae* from 27 countries from all over the world were collected in the field from 1993–2012 and cultivated in the Experimental garden of the Institute of Botany, Průhonice, Czech Republic. Altogether, 242 samples of species and hybrids of *Potamogeton*, *Stuckenia* and *Groenlandia* were studied. All samples were identified based on the latest knowledge and an ongoing taxonomic revision of the family for the world monograph of the family within the project Species Plantarum – Flora of the world (Z. Kaplan, in preparation). DNA analysis was used to confirm the identity of all hybrids listed in this study (J. Fehrer & Z. Kaplan, unpublished data, if not indicated otherwise). Many of the samples investigated were also used in parallel studies on phenotypic plasticity (Kaplan 2002b), artificial hybridization (Kaplan & Fehrer 2006), genetic variation (Kaplan & Štěpánek 2003) and hybrid identification based on molecular markers (Kaplan et al. 2002, 2009, 2011, Kaplan & Fehrer 2004, 2007, 2009, 2011, 2013, Kaplan & Wolff 2004, Kaplan 2007). The system of reference numbers used here is common to all these studies. Voucher specimens are preserved in the herbarium of the Institute of Botany, Průhonice (acronym PRA).

Chromosome counts

Cultivated plants were used for most of the chromosome counts. Exceptions are samples 1533, 1534, 1535, 2072, 2074, 2076, 2077, 2251 and 2252, material of which was collected in the field. Chromosome numbers were determined at mitotic metaphase in somatic cells of actively growing root tips; only the gametic number for sample 2072 was established using pollen mother cells from young flower buds. The root tips were collected from roots growing from horizontal shoots (rhizomes) or, less frequently, from stem bases. Usually, about 10 root tips per sample were taken.

The root tips were pre-treated in a saturated water solution of p-dichlorbenzene for approximately two hours then fixed in a 3:1 mixture of 96% ethanol and acetic acid, macerated in a 1:1 mixture of ethanol and hydrochloric acid for 30 s, washed in water and stained with lacto-propionic orcein. The flower buds were treated in a similar way to the roots, only the pre-treatment was omitted. The number of chromosomes was determined under a Carl Zeiss Jena NU microscope equipped with an Olympus Camedia C-2000 Z camera and Olympus E – 510 Digital SRL Camera.

Root sampling and chromosome counting of each sample was repeated (up to eight times) until at least three clear and well-spread metaphase preparations allowed the identification of a reliable count. Approximate counts (uncertain interpretations of the karyotypes ± 1–2 chromosomes) are indicated in the following text by „ca“. Of the 242 samples studied, chromosome numbers of only 181 are recorded here. The remaining preparations contained only mitotically inactive cells or the observations were inconclusive and were excluded to avoid publishing incorrect counts.

In order to avoid shaping the results according to expectations, samples were generally collected by ZK and studied karyologically under anonymous numbers by VJ. Taxon names were assigned to the samples only after the chromosome numbers were established.

Molecular procedures and data analyses

In order to assess the distribution patterns in chromosome number in a phylogenetic framework, two nuclear ribosomal sequence regions were chosen, the internal transcribed spacer (ITS) and the 5S non-transcribed spacer (5S-NTS). Both markers have been used previously in phylogenetic studies of *Potamogetonaceae* (Wang et al. 2007, Lindqvist et al. 2006) and differ in their level of sequence variation. To visualize the relationships of the three genera the less variable ITS region was used. In this analysis, all available *Stuckenia* species and monotypic *Groenlandia* were represented by two accessions each whereas for *Potamogeton*, each available diploid species was represented by one plant. Based on the ITS analyses, the basal-most *Potamogeton* taxa were chosen as an outgroup for addressing the intrageneric relationships with the 5S-NTS, because this region was too variable to allow a reliable alignment with the other two genera. For 5S-NTS analyses, diploid and tetraploid species were mostly represented by two accessions each; additional representative genotypes were included if the intraspecific variation was high (J. Fehrer et al., unpublished data). From among these, we selected preferentially those individuals for which the chromosome numbers were determined, and for which the geographic origins were as broad as possible. Hybrids were not used in the construction of the tree, with the exception of cloned 5S-NTS sequences (see below) of the diploid sample identified as *P. groenlandicus*, which turned out to be of hybrid origin. Octoploid taxa were also excluded as they represent

allopolyploids originating from various combinations of tetraploid parental species (J. Fehrer et al., unpublished data). A list of the samples used in the molecular analyses is given in Appendix 1.

DNA isolations, PCR-amplification and sequencing of the ITS region were done as described in Kaplan & Fehrer (2004). For amplification of the 5S-NTS, primers 5SPI2 (5'-tgggaagtcctcgtgttgca-3') and 5SPIIm (5'-gtagtgctggtatgatcgca-3') were used; both were modified after Cox et al. (1992). PCRs were performed in 25 µl reactions containing 1 mM $MgCl_2$, 200 µM of each dNTP, 0.2 mM of each primer, 2.5 µl of Mg^{2+}-free reaction buffer and 0.5 units of *Taq* DNA-polymerase (MBI Fermentas / Thermo Fisher Scientific, Ontario, Canada) and a few nanograms of genomic DNA. Pre-denaturation was done at 95°C for 5 min, followed by 32 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 20 s, and a final extension step at 72°C for 10 min. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced with both PCR primers at GATC Biotech (Cologne, Germany). Sequence editing, treatment of polymorphisms and cloning of one hybrid sample (see above) were done as described in Fehrer et al. (2009). Sequences were submitted to the GenBank database; accession numbers are included in Appendix 1.

Sequence alignments of both datasets were done by hand in Bioedit V7.0.9.0 (Hall 1999) and incrementally improved using the Guidance Server (Penn et al. 2010a) and a combination of the algorithms Guidance (Penn et al. 2010b) and MAFFT (Katoh et al. 2005). The final alignments used for analyses are available upon request. Indel coding for both datasets was done with FastGap V.1.2 (Borchsenius 2009) based on the simple method of Simmons & Ochoterena (2000). Phylogenetic analyses were performed employing Neighbor Joining (ITS), Maximum Parsimony (ITS, 5S-NTS) and Bayesian inference (5S-NTS) using PAUP* 4.0b10 (Swofford 2002) and MrBayes (Ronquist & Huelsenbeck 2003). Neighbor Joining analysis was done using P-distances; bootstrapping was performed with 1000 replicates. Maximum parsimony analyses (both datasets) were done as heuristic searches with 100 random addition sequence replicates and TBR branch swapping, saving no more than 100 trees with length ≥ 1 per replicate. Bootstrapping was done using the same settings and 1000 replicates, but without branch swapping. Prior to Bayesian analysis, the model of molecular evolution best fitting the data was determined using Modeltest V3.5 (Posada & Crandall 1998). In hierarchical Likelihood Ratio Tests, a K81uf+G model was determined of which the basic model parameters (six substitution rates and gamma distribution) were used as priors for Bayesian inference along with the default settings. Chains were computed for 2 million generations, sampling every 1000th tree. The first 25% of the trees per run were discarded as burn-in and the remaining trees summarized.

New chromosome counts and discussion on particular species and hybrids

In the genera *Potamogeton* and *Stuckenia*, species are listed first (in alphabetic order), followed by hybrids; hybrids with a binomial are listed first followed by unnamed ones. The samples under each taxon are ordered according to the reference numbers that are given in bold.

Groenlandia densa (L.) Fourr.

2n = 30

970: Switzerland, canton Graubünden: western edge of Champfèrer See (lake) by Silvaplana near St. Moritz, ca 46°28'N, 09°48'E, alt. 1789 m, 17 VI 1998, coll. Z. Kaplan 98/70, cult. & coll. Z. Kaplan 970. – **988**: Austria, Vorarlberg, Bregenz: narrow inlet on sandy alluvium on left bank of mouth of Bregenzer Aach River flowing into Bodensee (Lake Constance) at the N–NNE edge of Hard, 47°30'04"N, 09°41'47"E, alt. 396 m, 23 VI 1998, coll. Z. Kaplan 98/136, cult. & coll. Z. Kaplan 988.

This is the only species of the genus *Groenlandia*. It is distributed mainly in western, central and southern Europe, in south-western Asia and northernmost Africa. Our counts agree with previous records for Sweden (Palmgren 1939), Czech Republic (Krahulcová 1988), Slovakia (Uhríková in Májovský et al. 2000) and Austria (Hasitschka-Jenschke 1959). The only record that differs is 2n = 12 for Italy (Peruzzi & Cesca 2002). The figure presented in that paper shows a karyotype with chromosomes too robust and of a different shape to those of *G. densa*. We therefore suspect that this count was made on misidentified plant material from another family.

Potamogeton acutifolius Link

2n = 28

321: Czech Republic, distr. Pardubice: Baroch fispond 0.7 km SSW of Hrobice, 50°05'51.6"N, 15°46'55.3"E, alt. 224 m, 9 IX 1996, coll. Z. Kaplan 96/628, cult. & coll. Z. Kaplan 321 (Fig. 1g). – **1542**: Czech Republic, distr. Česká Lípa: Držník fishpond in forest 1.1 km ESE of Hradčany, 4 km S of Mimoň, 50°36'37"N, 14°43'23"E, alt. 273 m, 11 IX 2004, coll. Z. Kaplan, cult. & coll. Z. Kaplan 1542. – **2074**: Czech Republic, distr. Hradec Králové: Dolní Flajšar fishpond 0.6 km WSW of Štít, 50°06'50.6"N, 15°28'02.5"E, alt. 216 m, 23 V 2009, coll. Z. Kaplan 09/58.

2n = ca 28

1158: Czech Republic, distr. Jindřichův Hradec: Velký Panenský fishpond 1.3 km SSE of Lomnice nad Lužnicí, 49°04'05"N, 14°43'50"E, alt. 420 m, 8 IX 1999, coll. Z. Kaplan 99/157, cult. & coll. Z. Kaplan 1158.

So far, only a single chromosome count has been published for this species of temperate regions of Europe. This was determined by Palmgren (in Löve & Löve 1942) as 2n = 26 based on material from Scandinavia, presumably southern Sweden. As all our samples of *P. acutifolius* and all other species of the *P. compressus* group (sensu Kaplan & Marhold 2012) investigated in this study invariably have 2n = 28 at the diploid level, we consider Palmgren's count as inexact. It may have been estimated, rather than counted exactly, under the mistaken belief that all *Potamogeton* diploids have 2n = 26. The karyotype of sample 321 includes a pair of chromosomes with satellites (Fig. 3a).

Potamogeton alpinus Balb.

2n = 52

338: Czech Republic, distr. Hradec Králové: backwater pool (detached river arm) of Orlice River at north-eastern edge of Malšova Lhota, 50°12'30"N, 15°53'16"E, alt. 232 m, 8 X 1996, coll. Z. Kaplan 96/681, cult. & coll. Z. Kaplan 338 (Fig. 2a).

According to published data, this species has the same chromosome number throughout its circumboreal distribution. The new chromosome count presented here is in accordance with all previous records, which came from Sweden (Palmgren 1939, as n = 26),

Iceland (Löve & Löve 1956), Japan (Harada 1956, Takusagawa 1961), Siberia (Probatova et al. 2008a), Russian Far East (Probatova & Sokolovskaya 1986, 1988, as *P. tenuifolius*), Canada (Löve 1954a, Löve & Löve 1975b, 1981) and Greenland (Dalgaard 1989). The record for Spain (Löve & Kjellqvist 1973, as 2n = 26, "corrected" in Löve & Kjellqvist 1974 to 2n = 52) is erroneous as material they studied was actually of *P. polygonifolius* (see under this species).

Potamogeton amplifolius Tuckerm.

2n = 52

1689: USA, New Hampshire, Carroll Co.: Upper Danforth Pond 5 km WNW–W of Freedom, 43°49'33"N, 71°06'26"W, alt. 124 m, 29 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/414, cult. & coll. Z. Kaplan 1689.

Our count for this North American species is in agreement with both previous records based on plants from Minnesota, USA (Stern 1961, as n = 26) and Manitoba, Canada (Löve & Löve 1981).

Potamogeton berchtoldii Fieber

2n = 26

174: Russia, Siberia, Buryatia, Baikal area, distr. Barguzinskiy, Barguzinskaya Basin: oxbow of Ina River 4 km NNW of Jubilejnyj, ca 53°45'28"N, 110°11'26"E, alt. 495 m, 2 VIII 1993, coll. Z. Kaplan 93/518a, cult. & coll. Z. Kaplan 174. – **910**: Czech Republic, distr. Ústí nad Orlicí: second of five fishponds in castle park at south-eastern edge of Žamberk, 50°04'59.1"N, 16°28'35.1"E, alt. 413 m, 14 IX 1997, coll. Z. Kaplan 97/907, cult. & coll. Z. Kaplan 910. – **925**: Czech Republic, distr. Hradec Králové: ditch at pond in forest 2.8 km NE of Vysoké Chvojno, 50°07'43.7"N, 16°00'22.9"E, alt. 269 m, 4 IX 1996, coll. Z. Kaplan 97/837, cult. & coll. Z. Kaplan 925. – **927**: Czech Republic, distr. Rychnov nad Kněžnou: drainage ditch in forest sand-pit 1.3 km E of Týniště nad Orlicí, 50°09'03.8"N, 16°06'23.4"E, alt. 260 m, 5 IX 1997, coll. Z. Kaplan 97/852, cult. & coll. Z. Kaplan 927. – **930**: Czech Republic, distr. Ústí nad Orlicí: pool in abandoned part of granite quarry at northern edge of Litice nad Orlicí, 0.6 km NE of castle, 50°05'26.9"N, 16°21'16.4"E, alt. 460 m, 11 IX 1997, coll. Z. Kaplan 97/877, cult. & coll. Z. Kaplan 930. – **1160**: Czech Republic, distr. Přerov: ditch in meadows at west-north-western edge of Chropyně, 49°21'48.5"N, 17°21'17.1"E, alt. 194 m, 14 IX 1999, coll. Z. Kaplan 99/158, cult. & coll. Z. Kaplan 1160. – **1648**: USA, Vermont, Addison Co.: fast flowing section of Otter Creek just at confluence with New Haven River 1 km W of Brooksville, Weybridge, 44°03'45"N, 73°10'39"W, alt. 67 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/381, cult. & coll. Z. Kaplan 1648 (Fig. 1b). – **1736**: USA, Maine, Penobscot Co.: ditch in peat bog 0.5 km N–NNW of crossroads of Essex Street and Forest Road 7 km W of Orono, 44°53'17"N, 68°47'18"W, alt. 36 m, 4 VIII 2005, coll. Z. Kaplan & C. B. Hellquist 05/461, cult. & coll. Z. Kaplan 1736. – **2140**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), distr. Ziminskiy: stream at bridge 0.8 km NW of Ignay, 8 km SSW of Batama, 53°47'46.5"N, 101°36'03.5"E, alt. 485 m, 20 VIII 2009, coll. V. Chepinoga & Z. Kaplan 09/366, cult. & coll. Z. Kaplan 2140.

This species of the morphologically defined *P. pusillus* agg. (Kaplan & Štěpánek 2003) has a circumpolar distribution particularly in boreal and temperate regions throughout the Northern Hemisphere. Our counts are in accordance with the previous records of 2n = 26 based on plants from Iceland (Löve & Löve 1956, as *P. pusillus* [sensu Hagström]), Sweden (Palmgren 1939, as *P. pusillus* [sensu Hagström]), Slovakia (Murín 1992), Japan (Harada 1956, Takusagawa 1961, as *P. pusillus* [sensu Hagström]) and Canada (Taylor & Mulligan 1968, Löve & Löve 1981). The karyotype of this species seems to be characterized by the presence of a pair of bigger chromosomes with satellites of the size of small chromosomes, which were observed in samples 174, 930, 1160, 1648 and 1736. One chromosome pair in samples 174, 930 and 1736 had conspicuous centromeres.

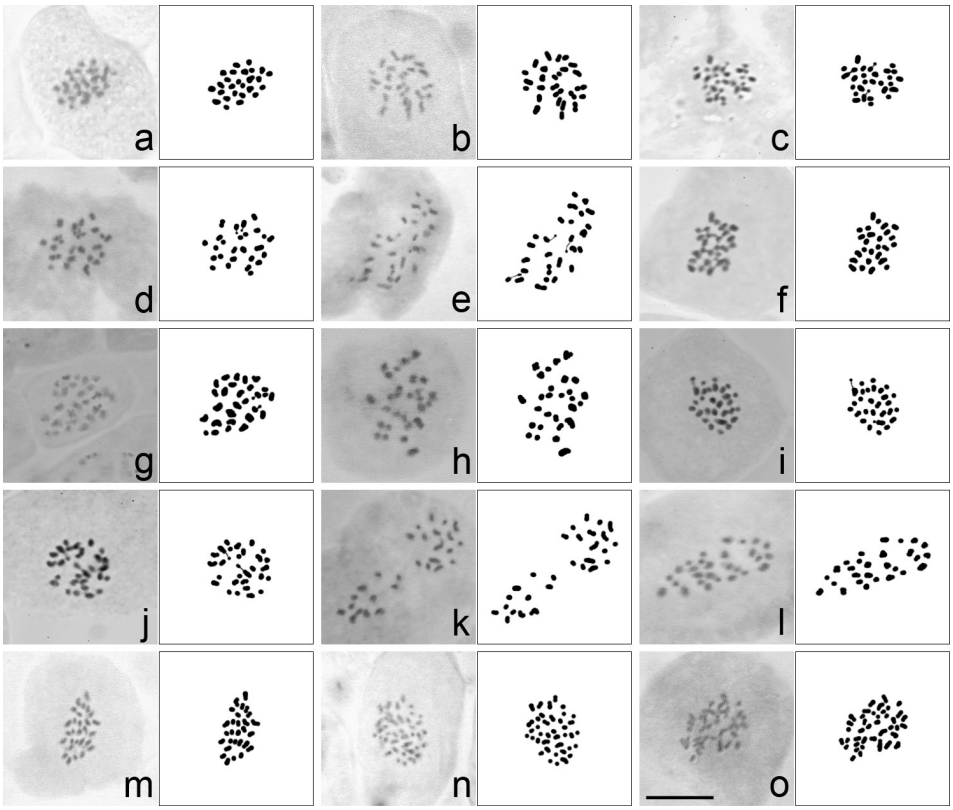


Fig. 1. – Chromosomes (photograph of the cytological preparation on the left with its interpretation on the right in each pair) of selected species and hybrids of *Potamogetonaceae* at the metaphase of the first meiotic division of the pollen mother cells (**a**) or at mitotic metaphase in somatic cells (**b–o**), arranged according to increasing chromosome number: **a** – *Potamogeton gramineus*, sample 2072, n = 26; **b** – *Potamogeton berchtoldii*, sample 1648, 2n = 26; **c** – *P. berchtoldii* s. l., sample 1619, 2n = 26; **d** – *P. foliosus*, sample 1593, 2n = 26; **e** – *P. pusillus*, sample 1715, 2n = 26; **f** – *P. trichoides*, sample 1903, 2n = 26; **g** – *P. acutifolius*, sample 321, 2n = 28; **h** – *P. cheesemanii*, sample 950, 2n = 28; **i & j** – *P. compressus*, sample 1962, 2n = 28; **k** – *P. polygonifolius*, sample 1535, 2n = 28; **l** – *P. spirillus*, sample 1695, 2n = 28; **m** – *P. zosteriformis*, sample 1491, 2n = 28; **n** – *P. pusillus*, sample 1133, 2n = 39; **o** – *P.* ×*gessnacensis*, sample 1286, 2n = 40. Scale bar identical for all figures = 10 μm.

Potamogeton berchtoldii Fieber s. l.

2n = 26

1603 & **1605**: USA, Massachusetts, Berkshire Co.: Berkshire Pond at Lanesborough, 42°30'28"N, 73°11'50"W, alt. 301 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1603 & 1605. – **1619**: USA, Vermont, Washington Co.: Curtis Pond 0.5 km NW of Maple Corner, Calais, 44°22'36"N, 72°30'03"W, alt. 371 m, 22 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1819 (Fig. 1c). – **1708**: USA, Maine, Aroostook Co.: Nickerson Lake at south-eastern edge of New Limerick, Linneus Twp., 5 km WSW of Houlton, 46°05'32"N, 67°54'43"W, alt. 116 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1708.

2n = ca 26

1677: USA, Massachusetts, Franklin Co.: northern part of Lake Rohunta near Route 2, 1.5 km SE of Orange, 42°33'47"N, 72°16'23"W, alt. 162 m, 27 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan

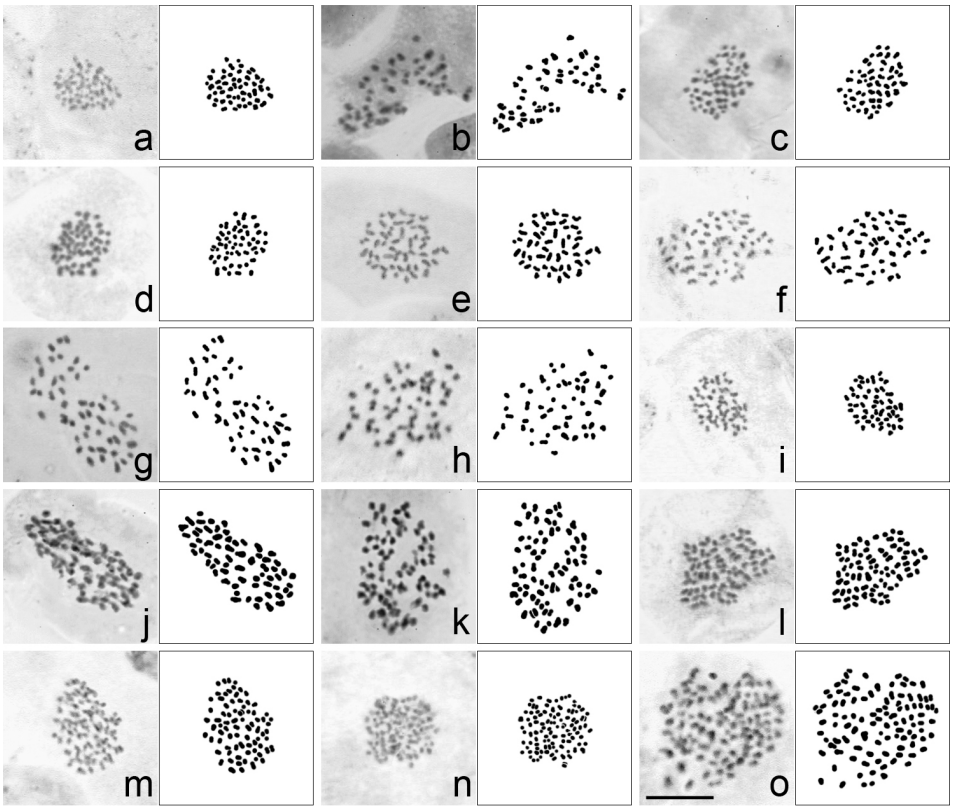


Fig. 2. – Chromosomes (photograph of cytological preparation on the left with its interpretation on the right in each pair) of selected species and hybrids of *Potamogetonaceae* at mitotic metaphase in somatic cells, arranged according to increasing chromosome number: **a** – *Potamogeton alpinus*, sample 338, 2n = 52; **b** – *P. gramineus*, sample 885, 2n = 52; **c** – *P. maackianus*, sample 1768, 2n = 52; **d** – *P. natans*, sample 977, 2n = 52; **e** – *P. perfoliatus*, sample 985, 2n = 52; **f** – *P. perfoliatus*, sample 1002, 2n = 52; **g** – *P. richardsonii*, sample 1056, 2n = 52; **h** – *P.* ×*nitens*, sample 879, 2n = 52; **i** – *P.* ×*undulatus*, sample 1025, 2n = 52; **j** – *P.* ×*lintonii*, sample 2119, 2n = 65; **k** – *P.* ×*salicifolius*, sample 1017, 2n = 78; **l** – *P.* ×*torssanderi*, sample 1006, 2n = 78; **m** – *Stuckenia filiformis*, sample 1187, 2n = 78; **n** – *Potamogeton illinoensis*, sample 856, 2n = 104; **o** – *P. schweinfurthii*, sample 861, 2n = 104. Scale bar identical for all figures = 10 μm.

1677. – **1699**: USA, Maine, Aroostook Co.: Nickerson Lake at south-eastern margin of New Limerick, Linneus Twp., 5 km WSW of Houlton, 46°05'32"N, 67°54'43"W, alt. 116 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1699. – **1719**: USA, Maine, Aroostook Co.: oxbow lake of Pettingrill Brook 1.5 km above its mouth in Aroostook River, just S of Route 164, Washburn, 6 km NNW of Presque Isle, 46°44'20"N, 68°01'59"W, alt. 130 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1719.

These plants from a relatively limited area in New England, USA, would be morphologically identified as *P. berchtoldii* as currently understood, but they deviate slightly from the typical widespread form of this species. Their exact taxonomic identity requires further study. The karyotype of sample 1619 has a pair of bigger chromosomes with satellites and a pair of chromosomes with conspicuous centromeres (Fig. 3b).

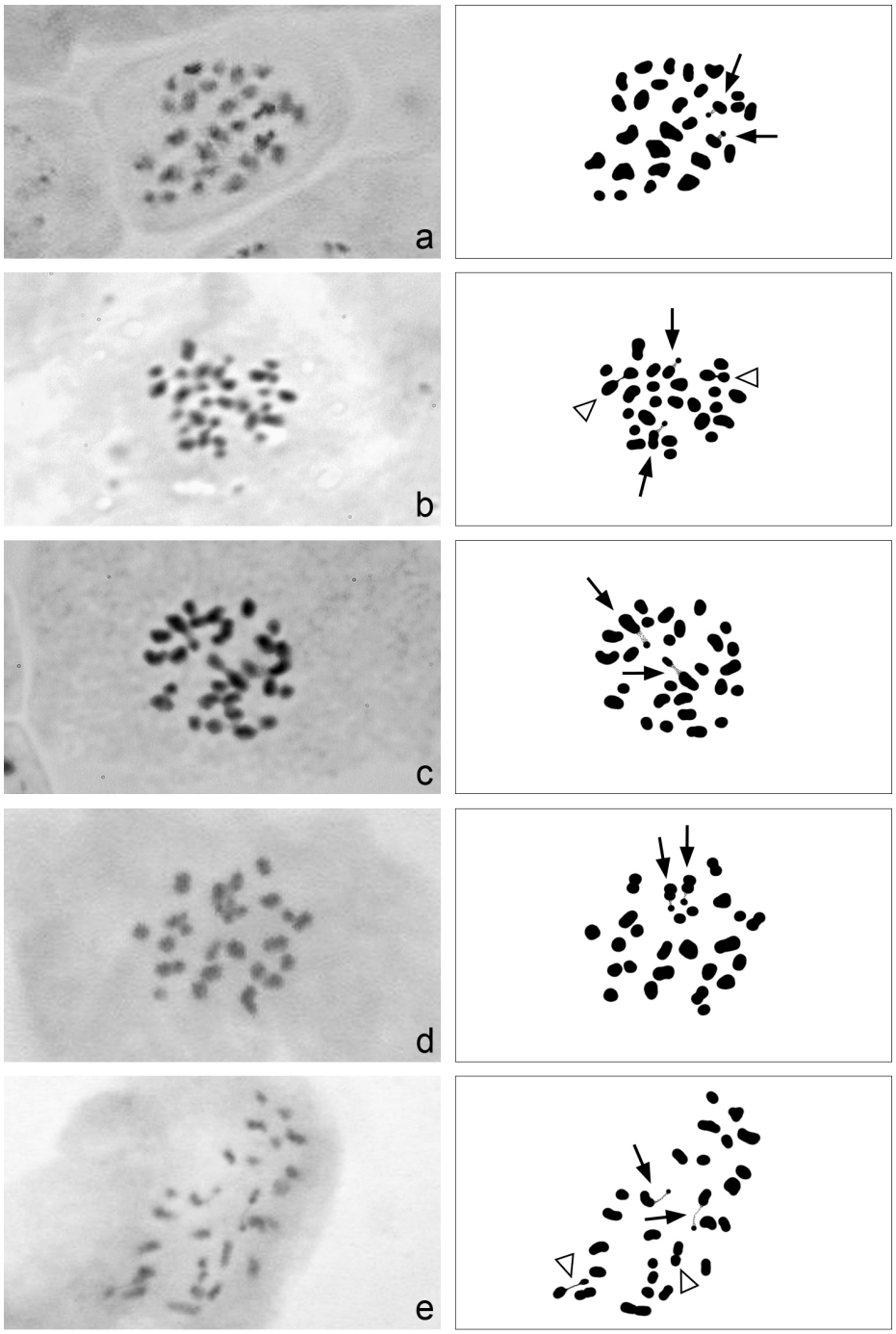


Fig. 3. – Selected karyotypes with satellites (solid black arrows) and chromosomes with conspicuous centromeres (hollow arrowheads): **a** – *Potamogeton acutifolius*, sample 321, 2n = 28; **b** – *P. berchtoldii* s. l., sample 1619, 2n = 26; **c** – *P. compressus*, sample 1962, 2n = 28; **d** – *P. foliosus*, sample 1593, 2n = 26; **e** – *P. pusillus*, sample 1715, 2n = 26.

Potamogeton cheesemanii A. Benn.

2n = 28

950: New Zealand, South Island, region Canterbury: small drain near Christchurch, III 1998, coll. A.-M. Schwarz, cult. & coll. Z. Kaplan 950 (Fig. 1h). – **1070**: New Zealand, North Island, region Auckland: Lake Parkinson 7 km SW of Waiuku, 37°19'S, 174°41'E, 19 III 1999, coll. D. Hofstra, cult. & coll. Z. Kaplan 1070.

This species occurs in New Zealand, Australia and Tasmania. So far, only an approximate count of 2n = ca 28 is published by De Lange et al. (2004) for plants from New Zealand.

Potamogeton clystocarpus Fernald

2n = ca 26

1671 & **1672**: USA, Texas, Jeff Davis Co.: Davis Mountains, Willow Canyon, Willow Creek, isolated pool in dry creek bed, 30°52'13"N, 104°03'54"W, 2005, coll. C. B. Hellquist, cult. & coll. Z. Kaplan 1671 & 1672.

This species is a Texas endemic closely related to *P. berchtoldii* (Les et al. 2009, Kaplan & Reveal 2013, and this study). The chromosome count published here is the first for this species.

Potamogeton coloratus Hornem.

2n = 28

2252: Czech Republic, distr. Nymburk: pool in E part of Hrabanovská černava National Nature Reserve 0.8 km NNW of Lysá nad Labem, 50°13'03.8"N, 14°50'10.1"E, alt. 189 m, 23 VI 2010, coll. Z. Kaplan 10/171, cult. & coll. Z. Kaplan 2252.

This species is distributed mainly in western, central and southern Europe, with a few localities in south-western Asia and northernmost Africa. Two chromosome records were published previously, n = 13 for Sweden by Palmgren (1939) and an approximate count of 2n = ca 26 for the British Isles by Hollingsworth et al. (1995a).

Potamogeton compressus L.

2n = 28

1012: Sweden, prov. Uppland: northern edge of Sparren Lake near Smedsmora, 10 km ENE–E of Närtuna, 59°42'35"N, 18°21'10"E, alt. 17 m, 14 VIII 1998, coll. Z. Kaplan 98/350, cult. & coll. Z. Kaplan 1012. – **1922**: Poland, Województwo Świętokrzyskie: small artificial pond in NW part of Starachowice near Skarżysko-Kamienna, alt. 51°03'13.2"N, 21°02'38.2"E, alt. 203 m, 29 VII 2007, coll. J. Zalewska-Gałosz, cult. & coll. Z. Kaplan 1922. – **1962**: Sweden, prov. Uppland, Stockholm county, municipality (kommun) of Norrtälje, Vätö parish: Storträsket Lake 0.8 km S–SSW of Harg, 59°47'55.5"N, 18°57'06.2"E, alt. 4 m, 28 VIII 2008, coll. Z. Kaplan & A. Svenson 08/633, cult. & coll. Z. Kaplan 1962 (Fig. 1i & j). – **2138**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), distr. Alarskiy: water reservoir at north-north-eastern edge of Kutulik, 53°22'12.7"N, 102°46'48.1"E, alt. 514 m, 20 VIII 2009, coll. Z. Kaplan & V. Chepinoga 09/364, cult. & coll. Z. Kaplan 2138.

2n = 42

1022: Denmark, Jylland (Jutland), region Midtjylland (Central Denmark Region): Gudenå River below motorway bridge at west-south-western edge of Randers, 56°27'19"N, 9°59'30"E, alt. 1 m, 18 VIII 1998, coll. Z. Kaplan 98/368, cult. & coll. Z. Kaplan 1022.

This species occurs in boreal and temperate regions of Europe and Asia. Our diploid counts agree with the records of Takusagawa (1961, as *P. monoginus*, n = 14, 2n = 28) for Japan. Some variation (2n = 26, 28, 38–41) is reported by Harada (1956, as *P. monoginus*), indicating that he also might have detected triploids. Our samples of other species of the *P. compressus* group (sensu Kaplan & Marhold 2012) also have 2n = 28 (see under *P. acutifolius* and *P. zosteriformis*), which indicates that this is the main chromosome number for the entire group. In contrast, the early study by Palmgren (1939) gave n = 13 for Sweden and Probatova et al. (2008a) and Chepinoga et al. (2012) recently recorded 2n = 26 for Russia. Another record of 2n = 26 for Canada by Löve & Löve (1981) is erroneous because *P. compressus* does not occur in North America and the count was presumably made on another taxon (see also discussion under *P. zosteriformis*).

The karyotype of *P. compressus* includes a pair of bigger chromosomes with satellites (Figs 1i & 3c). The triploid count was determined in a single sample. The plant was sterile, but neither morphology nor DNA sequences (nuclear and plastid markers) indicated any vestige of hybridization with another species. We therefore interpret this plant as an autotriploid. For a similar sporadic occurrence of a sterile autotriploid individual within a fertile diploid species, see the discussion under *P. pusillus*.

Potamogeton crispus L.

2n = 52

2077: Czech Republic, distr. Hradec Králové: Horní Flajšar fishpond 0.5 km SW of Štít, 50°06'43.5"N, 15°28'20.6"E, alt. 218 m, 8 VI 2009, coll. Z. Kaplan 09/153.

2n = ca 52

2339: South Korea, prov. Gyeongsangbuk-do (North Gyeongsang): Nakdong River (Nakdongang) 1.5 km E of Buncheon, 36°44'06"N, 128°51'54"E, 26 IX 2010, coll. P. Petřík, cult. & coll. Z. Kaplan 2339.

Our chromosome counts for this widely distributed species correspond with the most frequent literature records. The same numbers are reported for Sweden by Palmgren (1939, as n = 26), for Germany by Scheerer (1939, as 2n = ca 52), for Poland by Jankun (in Pogan et al. 1983), for Slovakia by Uhríková (in Májovský et al. 1978) and Hindáková & Schwarzová (1980), for India by Sharma & Chatterjee (1967), Bhattacharya & Ghosh (1978) and Ghosh & Bhattacharya (1980), for China by Wan et al. (2012) and for Japan by Harada (1942) and Takusagawa (1961). Different counts are rarely reported: n = 13, 2n = 26 by Bhattacharya & Ghosh (1978) and Ghosh & Bhattacharya (1980), 2n = 48 by Wan et al. (2012), 2n = 50 by Misra (1972), 2n = 56 by Nakata & Nagai (1998) and 2n = 78 by Sharma & Chatterjee (1967). In the context of the 13 records of 2n = 52 cited above, the other numbers are discounted unless they are confirmed and clearly documented by other studies.

Potamogeton distinctus A. Benn.

2n = ca 52

1916: Japan, Honshu, Niigata Prefecture (Niigata-ken), Muramatsu: Kalibane, 8 IX 2005, coll. N. Tanaka, cult. & coll. Z. Kaplan 1916.

This species is closely related to the widespread *P. nodosus* (Wiegleb 1990a, Wiegleb & Kaplan 1998, this study). It occurs in eastern and southeastern Asia, on Pacific islands and in northern Australia. Our observation is consistent with previous counts for Japan by Harada (1942, as 2n = 52) and Takusagawa (1961, as n = 26, 2n = 52) and for China by Wan et al. (2012). A slightly different and apparently erroneous count of n = 28 is reported in an early study by Takusagawa (1939). Wan et al. (2012) report "one cell with 2n = 26" in an individual with 2n = 52. We consider this observation to be an artefact, apparently based on an incomplete karyotype produced during preparation for FISH.

Potamogeton epihydrus Raf.

2n = ca 26

1040: USA, Maine, Penobscot Co.: Harrington Cove, Pushaw Lake, Orono, 16 VII 1998, coll. C. B. Hellquist, cult. & coll. Z. Kaplan 1040.

The core of the distribution of this amphiatlantic species is in North America north of Mexico, with an isolated occurrence located in north-western Europe in the British Isles. The same chromosome number as here was recorded also from other states in the USA: for Colorado by Löve et al. (1971) and Wisconsin by Parfitt & Harriman (1981, as n = 13), as well as for Canada: for Manitoba by Löve & Löve (1981), Quebec by Löve & Löve (1982) and for two populations on Queen Charlotte Islands, British Columbia by Taylor & Mulligan (1968).

Potamogeton foliosus Raf.

2n = 26

1043: USA, Maine, Aroostook Co.: pond on west side of Presque Isle airport by dump, Presque Isle, 14 VII 1998, coll. C. B. Hellquist 16293, cult. & coll. Z. Kaplan 1043. – **1044**: USA, Vermont, Orleans Co.: Lake Parker, West Glover, 21 VII 1998, coll. C. B. Hellquist & R. G. Popp 16326, cult. & coll. Z. Kaplan 1044. – **1593**: USA, Massachusetts, Berkshire Co.: pond on Kinderhook Creek at crossroads of Route 43 and Whitman Road in Hancock, 42°34'40"N, 73°17'51"W, alt. 385 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1593 (Fig. 1d). – **1621**: USA, Vermont, Orleans Co.: Lake Parker 1 km WSW of West Glover, 44°43'34"N, 72°13'53"W, alt. 396 m, 22 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1621. – **1668**: USA, Vermont, Rutland Co.: NE corner of Lake Hortonia 2.5 km NE of Hortonia, Sudbury, 43°45'09"N, 73°12'38"W, alt. 148 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1668.

The chromosome number of this North and Central American species has long been controversial. Two counts were published corresponding to the diploid level, 2n = 26 for Manitoba, Canada (Löve & Löve 1981) and 2n = 28 for Minnesota, USA (Stern 1961). The latter may have resulted from the misinterpretation of two satellites that are present in our samples (Fig. 3d). An unusually low number of n = 7 (with some cells n = 8) appeared in an early study by Wiegand (1899). This count requires special attention because it is discussed in the literature as a potential base chromosome number of the family. However, Wiegand's own figures show chromosomes that are too large to belong to *Potamogeton* and it is therefore likely that he used misidentified material. This controversial count has never been confirmed. We therefore consider Wiegand's anomalous count as erroneous (see also general discussion below).

Potamogeton friesii Rupr.

2n = 26

1658: USA, Vermont, Rutland Co.: Burr Pond 2.5 km SSE of Hyde Manor, Sudbury, 43°46'06"N, 73°10'57"W, alt. 157 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1658. – **1949**: Austria, Oberösterreich: western edge of Traunsee Lake at Altmünster, 1.5 km SW of Gmunden, 47°54'06.1"N, 13°46'11.2"E, alt. 422 m, 9 VIII 2008, coll. Z. Kaplan 08/606, cult. & coll. Z. Kaplan 1949.

Our counts established on this circumpolar species are the same as two previous records, one for Sweden (Palmgren 1939) and the other for Canada (Löve & Löve 1981).

Potamogeton '*gayi*' auct. hort., non A. Benn.

2n = 26

1194: Original locality unknown, plant samples from the Botanical Garden of the University of Wrocław, Poland, provided by R. Kamiński in 2000, cult. & coll. Z. Kaplan 1194.

Proper *P. gayi* A. Benn. occurs in South America. Plants designated with this name are sometimes grown in aquaria and in water tanks in greenhouses. The garden plant that we used for chromosome counting was also so named. Our record is the first chromosome count for this cultivated taxon. The sample studied differs slightly morphologically from typical natural plants, is consistently sterile, and DNA sequencing indicates its hybrid origin (J. Fehrer & Z. Kaplan, unpublished data). The exact identity of these aquarium plants and their comparison with samples from natural populations of *P. gayi* and of similar taxa in South America require further study. The chromosome count of proper *P. gayi* from the field has yet to be determined.

Potamogeton gemmiparus (J. W. Robbins) Morong

2n = 26

1721: USA, Maine, Hancock Co.: SE shore of Graham Lake 3 km NNE of Ellsworth, 44°35'36"N, 68°24'40"W, alt. 31 m, 3 VIII 2005, coll. Z. Kaplan & C. B. Hellquist 05/445, cult. & coll. Z. Kaplan 1721.

This is the first chromosome count for this species, which is confined to a relatively small area in northeastern USA and adjacent southeastern Canada.

Potamogeton gramineus L.

n = 26

2072: Czech Republic, distr. Hradec Králové: Dolní Flajšar fishpond 0.6 km WSW of Štít, 50°06'50.6"N, 15°28'02.5"E, alt. 216 m, 23 V 2009, coll. Z. Kaplan 09/54 (Fig. 1a).

2n = 52

885: Czech Republic, distr. Náchod: eastern edge of Rozkoš Reservoir by Šeřeč, 50°23'02"N, 16°05'14"E, alt. 280 m, 22 VIII 1997, coll. Z. Kaplan 97/829, cult. & coll. Z. Kaplan 885 (Fig. 2b). – **897**: Czech Republic, distr. Česká Lípa: Držník fishpond in forest 1.1 km ESE of Hradčany, 4 km S of Mimoň, 50°36'37"N, 14°43'23"E, alt. 273 m, 18 IX 1996, coll. Z. Kaplan 96/638, cult. & coll. Z. Kaplan 897. – **1156**: France, Bretagne, Morbihan, Lorient: Scorff River ca 30 km from its mouth, VI 1998, coll. J. Květ, cult. & coll. Z. Kaplan 1156. – **1285**: France, Lorraine, Moselle: Saarkohlenkanal at Rémelfing, ca 49°05'25"N, 07°05'55"E, alt. 200 m, 21 July 2001, coll. P. Wolff, cult. & coll. Z. Kaplan 1285. – **2240**: Greece, region (periphery) of Epirus (Ípiros), Thesprotía

Pref.: northern edge of Limni Prontani (Lake Prontani) 2 km NNW of Ampelia, 39°26'55"N, 20°25'20"E, alt. 240 m, 10 VI 2010, coll. Z. Kaplan 10/146, cult. & coll. Z. Kaplan 2240.

This species has an extensive circumpolar range in boreal and temperate regions throughout the Northern Hemisphere and appears to be invariable in chromosome number. Our counts are consistent with the cytotype 2n = 52 previously recorded for Sweden (Palmgren 1939, as n = 26), Iceland (Löve & Löve 1956), Russia (Probatova et al. 2008a), China (Wan et al. 2012), Japan (Harada 1956), Canada (Taylor & Mulligan 1968, as n = 26, Löve & Löve 1981) and USA (Stern 1961, as n = 26, Pringle 1969).

Potamogeton groenlandicus Hagstr.

2n = 26

1153: Greenland, Disko Fjord: lakes between Eqalunguit and Kangerdluarssuk, 69°31'13.5"N, 53°43'36.5"W, 29–30 VII 1999, coll. F. Rune & S. Bernstein, cult. & coll. Z. Kaplan 1153.

This is a relatively little-known taxon whose present concept includes all linear-leaved plants similar to the *P. pusillus* agg. (sensu Kaplan & Štěpánek 2003) that occur in Greenland. Our karyological observations confirm two previously published records 2n = 26 (Jörgensen et al. 1958, Dalgaard 1989). DNA sequencing indicates that the sample analysed is apparently a hybrid between *P. berchtoldii* and *P. sibiricus* (see below). Consequently, *P. sibiricus*, for which no counts are available as yet, should have the same chromosome number. The present concept of *P. groenlandicus* may change in future taxonomic revisions.

Potamogeton hillii Morong

2n = 26

1607: USA, Vermont, Washington Co.: Bliss Pond 2.5 km N of Adamant, Calais, 44°21'04"N, 72°30'05"W, alt. 369 m, 22 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/349, cult. & coll. Z. Kaplan 1607.

This is the first chromosome record for this species whose distribution is scattered in north-eastern USA and adjacent Canada.

Potamogeton illinoensis Morong

2n = 104

856: Argentina, prov. Río Negro: main irrigation system at Viedma, ca 40°48'S, 63°07'W, provided by P. Denny in 1997, cult. & coll. Z. Kaplan 856 (Fig. 2n).

This species occurs in North and South America from southern Canada in the north through Mexico and the West Indies southwards to northern Argentina. The two chromosome records published so far, for Manitoba, Canada (Löve & Löve 1981) and Minnesota, USA (Stern 1961, as n = 52), both correspond to our count of 2n = 104. So far, the octoploid level has been recorded in *Potamogeton* only for this species. Our record is the first count determined on a South American plant of *P. illinoensis*.

Potamogeton lucens L.

2n = 52

858: The Netherlands, prov. Limburg: Arcen, ca 51°28'N, 06°12'E, coll. P. Denny, cult. & coll. Z. Kaplan 858. – **912**: Czech Republic, distr. Mladá Boleslav, region of Český ráj: fishpond at west-south-western edge of Arnoštice near Žehrov, 50°31'42.8"N, 15°05'11.2"E, 247 m, 18 IX 1997, coll. Z. Kaplan 97/914, cult. & coll. Z. Kaplan 912. – **1762**: Japan, Honshu, Chiba Prefecture (Chiba-ken): Tegagawa (Tega River) NE of Tokyo, alt. 1 m, 2006, coll. N. Tanaka, cult. & coll. Z. Kaplan 1762. – **2135**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), border of Alarskiy & Cheremkhovskiy distr.: Golumet' River at bridge 2.2 km SW of Nygda, 11 km SE of Alar', 52°59'36.4"N, 102°40'01.3"E, alt. 445 m, 19 VIII 2009, coll. Z. Kaplan & V. Chepinoga 09/361, cult. & coll. Z. Kaplan 2135.

This species occurs in Europe, Asia and rarely also in northern and eastern Africa. Our counts correspond to records published for Sweden (Palmgren 1939, as n = 26), Slovakia (Uhríková in Májovský et al. 1978), Russia (Probatova et al. 2008a), China (Wan et al. 2012) and Japan (Harada 1942, Takusagawa 1961, both as *P. dentatus*).

Potamogeton maackianus A. Benn.

2n = 52

1570: China, prov. Hubei: Xiliang Lake NW of Xianning, 29°55'N, 114°05'E, 2000, coll. W. Li, cult. & coll. Z. Kaplan 1570. – **1768**: Japan, Honshu, Kanagawa Prefecture (Kanagawa-ken): Ashino-ko Lake, 2006, coll. N. Tanaka, cult. & coll. Z. Kaplan 1768 (Fig. 2c). – **2020**: China, prov. Heilongjiang, Mishan county: pond, IX 2008, coll. X.-L. Zhang, cult. & coll. Z. Kaplan 2020.

Our chromosome counts for this species of eastern and south-eastern Asia agree with the literature records for Japan by Harada (1942) and Takusagawa (1961). The count 2n = 26 for the Russian Far East reported by Probatova & Sokolovskaya (1984) is considered erroneous, and 2n = 56 for Japan by Uchiyama (1989) is also doubtful (see also general discussion below).

Potamogeton natans L.

2n = 52

977: Switzerland, canton Sankt Gallen: ditch with running water 200 m SSW of Altenrhein near Rorschach, 47°29'08.3"N, 09°32'56.4"E, alt. 396 m, 23 VI 1998, coll. Z. Kaplan 98/122, cult. & coll. Z. Kaplan 977 (Fig. 2d). – **1890**: Czech Republic, distr. České Budějovice: side pool of Stropnice stream 640 m NE of Třebeč, 48°52'41.4"N, 14°41'16.8"E, alt. 450 m, 29 VI 2007, coll. Z. Kaplan 07/215, cult. & coll. Z. Kaplan 1890.

This species is widespread in circumpolar, boreal and temperate regions of the Northern Hemisphere. The chromosome numbers presented here are in accordance with the most frequently recorded cytotype previously reported for Sweden (Palmgren 1939, as n = 26), Iceland (Löve & Löve 1956), British Isles (Hollingsworth et al. 1995b, as 2n = ca 52), Poland (Pogan in Skalińska et al. 1966), Slovakia (Murín in Májovský et al. 1976), China (Wan et al. 2012), Japan (Harada 1942, Takusagawa 1961) and Canada (Löve & Löve 1981). Another cytotype corresponding to 2n = 42 is recorded for the USA (Stern 1961, as n = 21) and the Russian Far East (Probatova & Sokolovskaya 1984, as 2n = ca 42). In light of the 12 observations of 2n = 52 we consider the two lower counts as erroneous. This view is also supported by the fact that all our samples of the respective group of broad-leaved

species invariably have 2n = 52 (see also the discussion below on the pattern in the variation in the chromosome numbers).

Potamogeton nodosus Poir. [syn.: *P. fluitans* auct.]

2n = 52

2251: Greece, region (periphery) of Epirus (Ípiros), Préveza Pref.: shallow southern edge of Limni Zirós (Lake Ziros) 1.5 km N–NNW of Romia, 39°14'07"N, 20°50'45"E, alt. 53 m, 13 VI 2010, coll. Z. Kaplan 10/165.

Our count for this subcosmopolitan species is in accordance with the records (apparently for Poland) by Kuleszanka (1934, as *P. fluitans*, n = 26), Italy by Ottonello et al. (1985), Morocco by Talavera & García Murillo (1992, as *P. fluitans*, as n = 26), India by Sivakamasundari & Selvaraj (1999), China by Wan et al. (2012) and Canada by Taylor & Mulligan (1968, as n = 26). Some of the records given for India under the name *P. indicus* may also refer to *P. nodosus*, e.g. the record 2n = 52 by Ghosh & Bhattacharya (1980). Only recently Wan et al. (2012) report for China also 2n = 48 and "one cell with 2n = 24" in an individual with 2n = 52. We consider these observations to be artefacts based on preparations for FISH.

Potamogeton obtusifolius Mert. et W. D. J. Koch

2n = 26

1138: Czech Republic, distr. Nový Jičín: Kotvice fishpond 1–1.5 km SE of railway station in Studénka, 49°41'58"N, 18°04'52"E, alt. 232 m, 9 VII 1999, coll. Z. Kaplan 99/126, cult. & coll. Z. Kaplan 1138.

2n = ca 26

1051: Canada, Manitoba: Snow Creek at west end of Snow Lake, town of Snow Lake, 60 mi. ENE of Cranberry Portage, 20 VIII 1998, coll. C. B. Hellquist & C. E. Hellquist 16369, cult. & coll. Z. Kaplan 1051. – **2075**: Czech Republic, distr. Hradec Králové: Dolní Flajšar fishpond 0.6 km WSW of Štít, 50°06'50.6"N, 15°28'02.5"E, alt. 216 m, 23 V 2009, coll. Z. Kaplan 09/59, cult. & coll. Z. Kaplan 2075.

This species is circumpolar, distributed in Europe, western and northern Asia and in northern North America. So far, there is only one chromosome count of 2n = 26 for this species, published for Sweden by Palmgren (1939).

Potamogeton ochreatus Raoul

2n = 26

1071: New Zealand, North Island, region Bay of Plenty: Lake MacLaren SW of Tauranga, ca 37°49'S, 176°02'E, 19 III 1999, coll. D. Hofstra, cult. & coll. Z. Kaplan 1071.

2n = ca 26

1072: New Zealand, North Island, region Waikato: Lake Rotoaira 9 km SW of Turangi, 39°03'S, 175°43'E, 23 III 1999, coll. D. Hofstra, cult. & coll. Z. Kaplan 1072.

This species is endemic to south-western and south-eastern Australia, New Zealand and a few surrounding Pacific islands. The chromosome counts presented here are the first records for this species.

Potamogeton octandrus Poir.

2n = 28

1915: Japan, Honshu, Aomori Prefecture (Aomori-ken): Nakazato, Pond Osawauchi, 30 VIII 2006, coll. N. Tanaka, cult. & coll. Z. Kaplan 1915.

This species exhibits a disjunct distribution in central and southern Africa, southern and eastern Asia and Australia. Our count is the same as all previously published records: for Japan published by Harada (1956, as *P. numasakianus*), Takusagawa (1961, as *P. numasakianus*), Uchiyama (1989) and Nakata & Nagai (1998) and for China by Wan et al. (2012).

Harada (1942) and Takusagawa (1961) record 2n = 28 for Japan under the name *P. vaseyi*. Later revisions showed that *P. vaseyi* does not occur in Japan (Kadono 1982, Ka 1984, Wiegleb & Kaplan 1998) and that this name was misapplied to plants of *P. octandrus*. This is probably also the actual identity of plants on which the records published for *P. vaseyi* were determined. The species *P. vaseyi* is confined to North America (Wiegleb & Kaplan 1998) and its chromosome number has not been determined yet.

Potamogeton oxyphyllus Miq.

2n = 26

1765: Japan, Honshu, Fukushima Prefecture (Fukushima-ken), 2006, coll. N. Tanaka, cult. & coll. Z. Kaplan 1762.

This species occurs in eastern and south-eastern Asia. The same chromosome number as we identified is recorded for Japan by Harada (1942) and Takusagawa (1961). Uchiyama (1989) records 2n = 28 for this species.

Potamogeton perfoliatus L.

2n = 52

840: Czech Republic, distr. Uherské Hradiště: lake in sand pit 2.1 km NW of railway station in Ostrožská Nová Ves, 49°00'58"N, 17°25'36"E, alt. 173 m, 25 VI 1997, coll. Z. Kaplan 97/524, cult. & coll. Z. Kaplan 840. – **979**: Switzerland, canton Sankt Gallen: southern edge of Bodensee (Lake Constance) 0.8 km N–NNE of Altenrhein near Rorschach, 47°30'03"N, 09°33'18"E, alt. 396 m, 23 VI 1998, coll. Z. Kaplan 98/125, cult. & coll. Z. Kaplan 979. – **985**: Austria, Vorarlberg, Bregenz: southern edge of Fussacher Bucht (bay) of Bodensee (Lake Constance) 1.5 km N–NNE of Fußach, 47°29'43"N, 09°40'06"E, alt. 396 m, 23 VI 1998, coll. Z. Kaplan 98/131, cult. & coll. Z. Kaplan 985 (Fig. 2e). – **1002**: Sweden, prov. Skåne: Björkaån River 0.5 km ENE of church in Björka, 4 km WNW of Sjöbo, 55°39'28"N, 13°38'35"E, alt. 24 m, 12 VIII 1998, coll. Z. Kaplan 98/338, cult. & coll. Z. Kaplan 1002 (Fig. 2f). – **1470 & 1471**: Germany, Bavaria: small backwater at Main River 1.2 km ENE of Ebing, ca 50°02'N, 10°55'E, 11 VI 2003, coll. L. Meierott, cult. & coll. Z. Kaplan 1470 & 1471. – **1531**: Italy, Trentino-Alto Adige, prov. Bolzano: Lake Muta (Lago di Muta, Haider See) S of San Valentino alla Muta, 46°44'53"N, 10°32'01"E, 8 VI 2004, coll. Z. Kaplan & J. Štěpánková 04/63, cult. & coll. Z. Kaplan 1531.

This species occurs in Europe, northern and central Africa, Asia, Australia, eastern North America and Central America. In addition to our seven counts for six countries there are 11 identical records for Iceland (Löve & Löve 1956), British Isles (Hollingsworth et al. 1998, as 2n = ca 52), Sweden (Palmgren 1939, as n = 26), Germany (Scheerer 1939, as 2n = ca 52), Slovakia (Váchová & Feráková 1986, Váchová in Májovský et al. 1987), Hungary (Felföldy 1947, as n = 26), an unspecified record for

Europe (Löve 1954b), China (Guo in Du et al. 2009, Wan et al. 2012) and Japan (Harada 1942, Takusagawa 1961).

Besides these 11 tetraploid records, different chromosome numbers are reported in the literature: n = 7 is recorded by Takusagawa (1939) for Japan, 2n = 26 by Löve (1954a, b, as *P. bupleuroides*) for North America and by Probatova & Sokolovskaya (1984) and Probatova et al. (2008b) for the Russian Far East, 2n = ca 40 by Probatova & Sokolovskaya (1984) for Russia, n = ca 24 by Wiśniewska (1931) for Poland, and 2n = 78 by Arohonka (1982) for Finland and by Probatova & Sokolovskaya (1986) for the Russian Far East. It is the goal of further studies to show whether these deviant chromosome counts reflect real variation in chromosome number of *P. perfoliatus* or whether they are erroneous. In any case it should be noted that they all came from only five authors (or author teams) who either conducted only a single count in this family or published highly doubtful or even demonstrably erroneous counts for other species as well. These few controversial records for *P. perfoliatus* contrast to 18 tetraploid counts of 2n = 52 for 13 different countries. We are sceptical that these deviant counts are correct. Taken together with the placement of this species in phylogenetic analysis (see the general discussion below), we discount all counts below the tetraploid level. The extremely low count of n = 7 given by Takusagawa (1939) is particularly incredible. On the dubious records in the 1954 papers by Löve, see the discussion under *P. zosteriformis*.

Potamogeton polygonifolius Pourr. [syn.: *P. oblongus* Viv.]

2n = 28

1533: Czech Republic, distr. Cheb: Nový fishpond 0.3 km WNW of Studánka railway station by Novosedly near Hranice, 50°16'35"N, 12°10'24"E, alt. 610 m, 13 VII 2004, coll. Z. Kaplan 04/168. – **1534**: Czech Republic, distr. Cheb: small pool in peat bog 0.7 km SSW of Pastviny, 4.9 km W–WSW of Studánka railway station by Novosedly near Hranice, 50°16'09"N, 12°08'45"E, alt. 605 m, 13 VII 2004, coll. Z. Kaplan 04/172. – **1535**: Czech Republic, distr. Cheb: small pool at NE bank of Vodárenská nádrž reservoir 1.4 km NNW–NW of Studánka railway station by Novosedly near Hranice, 50°17'14"N, 12°09'57"E, alt. 585 m, 13 VII 2004, coll. Z. Kaplan 04/173 (Fig. 1k).

Potamogeton polygonifolius is a species with an amphiatlantic distribution. It occurs in western, northern, central and southern Europe, on the Azores, Madeira, and rarely also in limited areas of northernmost Africa and easternmost North America. The literature reports two similar chromosome numbers for this species: 2n = 26 (Palmgren 1939, as n = 13, for Sweden; Fernandes 1950 for Portugal; and Ficini et al. 1980, as *P. oblongus*, for Italy) and 2n = 28 (Hollingsworth et al. 1998 for three localities in the British Isles). Hollingsworth et al. (1998) published their new chromosome counts based on careful examination of plants from three different populations from England. The authors commented that although they experienced difficulties in chromosome counting of other *Potamogeton* taxa, for their samples of *P. polygonifolius* clear metaphase preparations were achieved and all unambiguously gave a count of 2n = 28 (see also Preston et al. 1998). To establish the chromosome number for the Czech plants, we therefore intentionally sampled three populations thoroughly and studied carefully altogether more than 40 root tips from different plants to correctly distinguish between the 26 and 28 chromosomes recorded for this species in the literature. We came to unequivocal counts of 2n = 28 in at least 20 well-spread mitotic preparations from 12 different root tips from the three popula-

tions. Thus the Czech plants agree in their chromosome number with British counts for this species. This number is also indirectly confirmed by *P.* ×*gessnacensis*, a hybrid with *P. natans*, and by *P.* ×*rivularis*, a hybrid with *P. berchtoldii* (see below). The other three published counts of 2n = 26 are therefore interpreted here as only approximate counts, potentially influenced by the mistaken belief in the earlier literature, that all *Potamogeton* diploids have 2n = 26.

An additional chromosome number recorded for this species comes from Spain, although it was not published under the name *P. polygonifolius*. First, Löve & Kjellqvist (1973) published a diploid count of 2n = 26 under the name *P. alpinus*. This was rather surprising as all previous records for this species are tetraploid, with 2n = 52 (see above). This record was "corrected" in a subsequent paper that claimed the count was actually 2n = 52 (Löve & Kjellqvist 1974). This "correction" was apparently motivated by an unfortunate effort to fit the new record from Spain to other records published for this species. We have examined the herbarium specimen A. Löve & E. Kjellqvist 512 preserved at LD, which is the voucher for the above chromosome count(s) for *P. alpinus*. However, this specimen is undoubtedly *P. polygonifolius*. The original herbarium label attached to the voucher clearly documents that "2n = 26" was indeed the chromosome number actually determined on this specimen. Not 2n = 52, but 2n = 26 was therefore the original count, the only one established on this material. Although inexact, it is at least in accordance with the diploid level known for *P. polygonifolius*.

In his summary of chromosome counts, Les (1983) treated *P. oblongus* with 2n = 26 as a separate species from *P. polygonifolius*, for which he claimed 2n = 52. As demonstrated by Preston et al. (1998) and Hollingsworth et al. (1998), the record 2n = 52 claimed for *P. polygonifolius* is erroneous. The only primary record that could be traced was that attributed to Takusagawa (1961) by Moore (1973). However, this count was never made, but was merely the chromosome number expected by Takusagawa for this species on the basis of the chromosome number of similar species (Preston et al. 1998, Hollingsworth et al. 1998). It should also be noted that *P. polygonifolius* does not occur in eastern Asia and that the names "*P. oblongus*" and "*P. polygonifolius*" were in Japan widely misapplied to *P. distinctus*, which is indeed tetraploid.

On the incorrect assumption of the karyological differentiation, Crow & Hellquist (2000) separated American populations under the name *P. oblongus* from "the closely related *P. polygonifolius* of Europe, which is tetraploid". As described above, the European populations of *P. polygonifolius* are diploid. Furthermore, the choice of the name adopted for the American plants was incorrect, because *P. oblongus* was the name given to plants described from north-western Italy. If the classification suggested by Crow & Hellquist was correct, *P. oblongus* would become a synonym of *P. polygonifolius* and not the correct name for the American populations. The separate taxonomic position of North American plants was based solely on erroneous reports of chromosome numbers, as there are no morphological differences. In addition, ITS sequences of American samples from Newfoundland, the western margin of the distribution area of that species, are identical to European material from Portugal and Denmark (J. Fehrer et al., unpublished data). We therefore consider *P. oblongus* as conspecific with *P. polygonifolius* from Europe, northern Africa and the Atlantic islands.

Potamogeton praelongus Wulfen

2n = 52

881: Germany, Mecklenburg-Vorpommern, Güstrow: Lake Gültz (Gültzsee) 2.2 km SSW of Dobbin, 53°35'23"N, 12°19'04"E, 15 VIII 1997, coll. Z. Kaplan 97/824, cult. & coll. Z. Kaplan 881.

Our count corresponds to the only chromosome number recorded for this circumboreal species distributed throughout the Northern Hemisphere. The same karyotype is reported for Sweden by Palmgren (1939, as n = 26), for Iceland by Löve & Löve (1956), for Japan by Harada (1942) and Takusagawa (1961), and for Canada by Löve & Löve (1981).

Potamogeton pusillus L. s. str. [syn.: *P. panormitanus* Biv.]

2n = 26

307: Czech Republic, distr. Jindřichův Hradec: Šimanov pond near Třeboň town, 1.4 km NE of Třeboň railway station, 49°01'27"N, 14°46'41"E, alt. 428 m, 21 VIII 1996, coll. Z. Kaplan, cult. & coll. Z. Kaplan 307. – **959**: Czech Republic, distr. Litoměřice: small pond in valley at northern edge of Pokratice suburb of Litoměřice, 50°33'18"N, 14°07'43"E, alt. 260 m, 27 V 1998, coll. Z. Kaplan 98/52, cult. & coll. Z. Kaplan 959. – **987/2**: Austria, Voralberg, Bregenz: southern edge of Fussacher Bucht (bay) of Bodensee (Lake Constance) 1.5 km N–NNE of Fußach, 47°29'43"N, 09°40'06"E, alt. 396 m, 23 VI 1998, coll. Z. Kaplan, cult. & coll. Z. Kaplan 987/2. – **1159**: Czech Republic, distr. Jindřichův Hradec: Velký Panenský fishpond 1.3 km SSE of Lomnice nad Lužnicí, 49°04'09"N, 14°43'50"E, alt. 420 m, 8 IX 1999, coll. Z. Kaplan, cult. & coll. Z. Kaplan 1159. – **1212**: Czech Republic, distr. Jindřichův Hradec: Velký Dubovec pond 1.3 km SSE of Lomnice nad Lužnicí, 49°04'06"N, 14°43'19"E, alt. 425 m, 15 VI 2000, coll. Z. Kaplan 00/57, cult. & coll. Z. Kaplan 1212. – **1601**: USA, Massachusetts, Berkshire Co.: Berkshire Pond at Lanesborough, 42°30'28"N, 73°11'50"W, alt. 301 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1601. – **1712 & 1714 & 1715**: USA, Maine, Aroostook Co.: pond on Prestile Stream in Mars Hill, at crossroads of Routes 1 and 1A, 46°31'06"N, 67°52'01"W, alt. 125 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist 05/437 & 05/439 & 05/440, cult. & coll. Z. Kaplan 1712 & 1714 & 1715 (Fig. 1e). – **2076**: Czech Republic, distr. Hradec Králové: Dolní Flajšar fishpond 0.6 km WSW of Štít, 50°06'50.6"N, 15°28'02.5"E, alt. 216 m, 23 V 2009, coll. Z. Kaplan 09/60.

2n = 39

1133: Czech Republic, distr. Jičín: Rokytňanský rybník fishpond 0.4 km W of Dolní Rokytňany, 50°22'34,4"N, 15°07'13,7"E, alt. 234 m, 11 VI 1999, coll. Z. Kaplan 99/69, cult. & coll. Z. Kaplan 1133 (Fig. 1n).

The taxonomic delimitation of this species followed here is the narrow concept established by Hagström (1916, as *P. panormitanus*), developed by Dandy & Taylor (1938), and adopted e.g. by Dandy (1980), Preston (1995), Kaplan & Štěpánek (2003) and Kaplan (2010b). This species is distributed in Europe, Africa, Asia and North America. Particularly Scandinavian and Japanese botanists long followed the nomenclature established by Hagström (1916). Their chromosome records for *P. pusillus* are therefore referable to *P. berchtoldii* (see under this species).

The chromosome number 2n = 26 established for plants from the nine above-cited fertile populations is consistent with the most frequently reported count. This was previously recorded for Sweden (Palmgren 1939, as *P. panormitanus*), Slovakia (Mičieta in Májovský et al. 2000), Morocco (Talavera & García Murillo 1992, as n = 13), China (Wan et al. 2012) and Japan (Harada 1956, Takusagawa 1961, both as *P. panormitanus*). A slightly different count of 2n = 28 was claimed for Japan by Harada (1942, as *P. panormitanus*). This may have been caused by misinterpretation of two conspicuous satellites that we observed in samples 1159, 1601 and 1715 (Fig. 3e) or of chromosomes with conspicuous centromeres that we detected in sample 1715 (Fig. 3e). In light of alto-

gether 15 records of 2n = 26 cited above, we consider this count to be the only correct diploid chromosome number for *P. pusillus*.

A different chromosome number is recorded for sample 1133. We repeated the sampling three times (each count was made on several reliable mitotic plates) to be absolutely sure of the chromosome number. We arrived at an unambiguous count of 2n = 39 (Fig. 1n). No triploid plants of *P. pusillus* have been recorded so far. Sample 1133 was totally sterile. Although it flowered freely and produced abundant spikes each season during eight years of cultivaton, it failed to produce a single fruit and persisted only vegetatively. The whole spikes rotted soon after flowering instead of setting fruit. In contrast, 16 other *P. pusillus* samples cultivated under the same conditions, 9 of which were identified as diploid (see the list above), regularly set well-formed and viable fruit.

The development of the flowers of sample 1133 was similar to that of sterile hybrids (see Preston 1995:46, Preston et al. 1998, Kaplan & Wolff 2004, Kaplan & Fehrer 2004). However, no sign of hybridization was observed in this sample. The plant was morphologically typical *P. pusillus* s. str. and all diagnostic features indicated this species. Isozyme phenotypes were found to be species-specific in the *P. pusillus* agg. (Kaplan & Štěpánek 2003) and can be used to identify hybrids (e.g. Hollingsworth et al. 1995b, Preston et al. 1998, Kaplan et al. 2002, Kaplan & Wolff 2004, Kaplan 2007). However, the isozyme pattern of sample 1133 corresponded to pure *P. pusillus* and no bands indicating hybridization with other species were found (Kaplan & Štěpánek 2003). ITS sequencing also did not detect any indication of hybridization (J. Fehrer, unpublished data). We therefore interpret this plant as an autotriploid resulting from the combination of an unreduced (n = 26) and a normal reduced (n = 13) gamete. Production of unreduced gametes in plants has been thoroughly studied and is considered to be the main process involved in the origin of polyploid plants (Bretagnolle & Thompson 1995). Alternatively, the triploid plant might have arisen from the mating of a diploid with a tetraploid. As tetraploid plants have not been detected so far in *P. pusillus*, we consider the first alternative as more probable. The observed sterility of the triploid sample probably results from difficulties involved in chromosome pairing.

Potamogeton richardii Solms [syn.: *P. thunbergii* auct.]

2n = ca 104

2442: Tanzania, Rukwa Region: small pond fed by permanent spring in Wipanga, 7 km N–NNE of Sumbawanga, 7°52'09.4"S, 31°38'05.8"E, alt. 2144 m, 21 X 2011, coll. R. v. Blittersdorff, cult. & coll. Z. Kaplan 2442.

This species is confined to Africa and Madagascar (Kaplan & Symoens 2005). The only chromosome record published for this species is 2n = 42 for Burundi (Symoens et al. 1979). Our karyological observation was confirmed using flow cytometry and the genome size (2C-value) of the sample was found to be almost identical to that of the octoploid (2n = 104) sample 856 of *P. illinoensis* and significantly different from that of tetraploids and hexaploids (P. Trávníček & Z. Kaplan, unpublished data).

Potamogeton richardsonii (A. Benn.) Rydb.

2n = 52

1056: CANADA, Manitoba: Snow Creek at west end of Snow Lake, town of Snow Lake, 60 mi. ENE of Cranberry Portage, 20 VIII 1998, coll. C. B. Hellquist & C. E. Hellquist, cult. & coll. Z. Kaplan 1056 (Fig. 2g). – **1595**: USA, Massachusetts, Berkshire Co.: Berkshire Pond at Lanesborough, 42°30'28"N, 73°11'50"W, alt. 301 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/343, cult. & coll. Z. Kaplan 1595.

Our counts for this North American species are in accordance with the previous records for Minnesota, USA (Stern 1961, as n = 26) and Manitoba, Canada (Löve & Ritchie 1966, Löve & Löve 1975a). There is a different count of 2n = 26 in two publications, which also contain other dubious or demonstrably incorrect numbers: one is unspecified, for North America (Löve 1954a, b; see also the discussion under *P. zosteriformis*), the other is for the Russian Far East by Probatova & Sokolovskaya (1986).

Potamogeton robbinsii Oakes

2n = ca 52

1596: USA, Massachusetts, Berkshire Co.: Berkshire Pond at Lanesborough, 42°30'28"N, 73°11'50"W, alt. 301 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/344, cult. & coll. Z. Kaplan 1596. – **1667**: USA, Vermont, Rutland Co.: NE corner of Lake Hortonia 2.5 km NE of Hortonia, Sudbury, 43°45'09"N, 73°12'38"W, alt. 148 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/398, cult. & coll. Z. Kaplan 1667.

Our counts for this North American species correspond to both published records, one for Minnesota, USA (Stern 1961, as n = 26) and the other for Manitoba, Canada (Löve & Löve 1981).

Potamogeton rutilus Wolfg.

2n = 26

1431: POLAND, Województwo Lubelskie, Łęczyńsko-Włodawskie Lakeland: Rotcze Lake near Urszulin, 51°22'39"N, 23°06'46"E, 22 VIII 2002, coll. J. Zalewska-Gałosz, cult. & coll. Z. Kaplan 1431. – **2115**: FINLAND, Uusimaa, Espoo: western edge of Matalajärvi Lake at Högnäs, 60°15'14.2"N, 24°41'03.9"E, alt. 23 m, 18 VII 2009 coll. Z. Kaplan & P. Uotila 09/323, cult. & coll. Z. Kaplan 2115.

There is a single chromosome record published for this species, which occurs in the northern half of Europe and western Asia: Palmgren (in Tischler 1950) records 2n = 26 for material that perhaps originated from Scandinavia. Another record claiming 2n = 52 for this species is published for Canada by Löve & Löve (1981). However, because *P. rutilus* does not occur in North America, the record must be discarded as it was presumably established on another taxon (see also the discussion under *P. zosteriformis*).

Potamogeton sarmaticus Mäemets

2n = 52

1918: RUSSIA, region Voronezh (Voronezhskaya oblast'): small swampy water reservoir in nature reserve Khopërskiy zapovednik 6 km SW of Varvarino, 24 VIII 2007, coll. E. Pechenyuk, cult. & coll. Z. Kaplan 1918.

This species was described only recently (Maemets 1979) and is closely related to *P. gramineus* (Kaplan & Fehrer 2011; see also below). It is endemic to Kazakhstan, southern

and eastern Ukraine and small adjacent regions of southern European Russia. The chromosome number presented here is the first one for this species.

Potamogeton schweinfurthii A. Benn.

2n = 104

861: Kenya, coll. P. Denny, cult. & coll. Z. Kaplan 861 (Fig. 2o). – **1883**: Portugal: prov. Algarve, distr. Faro: local irrigation channel system 2 km SW of Rogil, 5 km N of Aljezur, 37°21'54"N, 8°48'32"W, alt. 90 m, 16 I 2007, coll. U. Schwarzer, cult. & coll. Z. Kaplan 1883. – **1951**: Italy, Tuscany, prov. Arezzo: artificial lakes along Tevere (Tiber) River 3.5 km N of Anghiari: the lake 0.9 km NNW of Viaio, 43°34'33.4"N, 12°03'21.0"E, alt. 330 m, 12 VIII 2008, coll. Z. Kaplan et al. 08/608, cult. & coll. Z. Kaplan 1951.

This species has its main distribution in Africa, Madagascar, Mascarene Islands and the Azores (Kaplan & Symoens 2005). Recently it was also discovered in southern Europe (Kaplan 2005b) and subsequently identified to be more widespread in Mediterranean Europe (Lastrucci et al. 2010, Aymerich et al. 2012). The chromosome counts presented here are the first for this species. The occurrence of octoploids is exceptional in *Potamogeton*. Besides *P. schweinfurthii*, they are recorded only in the morphologically similar and closely related *P. illinoensis*, which is allopatric in North and South America, and another African species, *P. richardii*.

Potamogeton spirillus Tuckerm.

2n = 28

1695: USA, New Hampshire, Carroll Co.: mouth of West Branch Brook on Ossipee Lake (NW shore) 3 km ESE of West Ossipee, 43°48'33"N, 71°09'49"W, alt. 124 m, 29 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/418, cult. & coll. Z. Kaplan 1695 (Fig. 1l).

This species is distributed in north-eastern USA and adjacent south-eastern Canada. Our chromosome count is the first for this species.

Potamogeton strictifolius A. Benn.

2n = 26

1707: USA, Maine, Aroostook Co.: Nickerson Lake at south-eastern margin of New Limerick, Linneus Twp., 5 km WSW of Houlton, 46°05'32"N, 67°54'43"W, alt. 116 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist 05/434, cult. & coll. Z. Kaplan 1707.

This species occurs in north-western, central and eastern North America. Only a single chromosome count is recorded for this species: 2n = 52 by Löve (1954a) without any details of its origin. This count is rather surprising, because no linear-leaved species proved to be tetraploid in this study (see the general discussion below). This count appeared in a study that also included other doubtful records (see discussion under *P. zosteriformis*). We therefore dismiss this record as untrustworthy and consider our count as the first one that is actually for this species.

Potamogeton trichoides Cham. et Schlecht.

2n = 26

1903: Czech Republic, distr. Karlovy Vary: Bražecké Hliňáky fishponds 0,5–0,9 km WSW of Bražec near Bochov, 50°10'19,7"N, 13°02'17,4"E, alt. 692 m, 23 VII 2007, coll. Z. Kaplan 07/357, cult. & coll. Z. Kaplan 1903 (Fig. 1f).

This species occurs in Europe, northern, eastern and southern Africa and Asia. Our observation is in accordance with the previous records of 2n = 26 for Sweden by Palmgren (1939), for Slovakia by Uhríková (in Májovský et al. 1978) and for Morocco by Talavera & García Murillo (1992, as n = 13).

Potamogeton wrightii Morong [syn.: *P. malaianus* auct.]

2n = 52

1239: Japan, Honshu, Chiba Prefecture (Chiba-ken): Tegagawa (Tega River) NE of Tokyo, 2000, coll. N. Tanaka, cult. & coll. Z. Kaplan 1239.

This species occurs in south-eastern and eastern Asia and on Pacific islands (Wiegleb 1990b, 2002). Almost all published chromosome numbers for this species correspond to our observation. They are recorded for the Russian Far East by Probatova et al. (2006, as *P. malaianus*), for Japan by Harada (1942, as *P. malaianus*) and Takusagawa (1961, as *P. malaianus*) and for China by Guo (in Du et al. 2009). Only recently Wan et al. (2012) reported aneuploid variation in chromosome numbers (2n = 48, 50, 51 and 52), which has never been reported for any broad-leaved *Potamogeton*. The figures presented in that study are not of the quality to allow such precise chromosome counting.

Potamogeton zosteriformis Fernald

2n = 28

1490 & 1491: Canada, British Columbia: Shuswap River under bridge at Cliff Avenue in Enderby, 18 km SSE of Salmon Arm, 50°33'08"N, 119°08'00"W, alt. 366 m, 21 IX 2003, coll. F. Lomer, cult. & coll. Z. Kaplan 1490 & 1491 (Fig. 1m). – **1591**: USA, Massachusetts, Berkshire Co.: pond on Kinderhook Creek at crossroads of Route 43 and Whitman Road in Hancock, 42°34'40"N, 73°17'51"W, alt. 385 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/339, cult. & coll. Z. Kaplan 1591. – **1612**: USA, Vermont, Washington Co.: Bliss Pond 2.5 km N of Adamant, Calais, 44°21'04"N, 72°30'05"W, alt. 369 m, 22 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/353, cult. & coll. Z. Kaplan 1612.

This species is a North American vicariant of the Eurasian *P. compressus*. These allopatric species are only weakly differentiated morphologically (Kaplan & Marhold 2012) and are genetically closely related (see below).

There is only a single chromosome count reported for this species, which appeared in a problematic phytogeographical paper by Löve (1954a). He aligned pairs of substitutional taxa in a table, with one species in each pair occurring in Europe and the other confined to North America. The first couple included European *P. compressus*, with 2n = 26, and American *P. zosteriformis*, for which 2n = 52 is indicated. The second pair consisted of European *P. rutilus*, with 2n = 26, and American *P. strictifolius*, for which 2n = 52 is claimed. No details on chromosome counting and the origin of the samples are provided in this phytogeographical paper. Two tetraploid counts 2n = 52 recorded for

linear-leaved species, a group that was found here to consist exclusively of diploids (see the discussion on the pattern of chromosome number variation below), is rather surprising. In spite of the clear-cut differentiation claimed in the first paper, in a later publication Löve & Löve (1981) report both *P. compressus* and *P. rutilus* (i. e. the European members of the species pairs) as diploids for Manitoba, Canada (i. e. in the area where these European species should be, according to the Löve's own observations, substituted by the North American tetraploids). The table in Löve (1954a) includes also two other doubtful records: diploid counts for *P. perfoliatus* (as *P. bupleuroides*) and *P. richardsonii*, which both proved to be tetraploids in this study. This was changed again in the later study that cites *P. richardsonii* as tetraploid. The herbarium vouchers listed by Löve & Löve (1981) are all dated between 1951 and 1953 (specimens seen in DAO and S, see also below), that is, prior to the publication of the 1954 paper. This indicates that the chromosome records given in both papers were apparently based on the same plants but may have been confused during the preparation of the manuscripts.

When attempting to disentangle this confusing story, the possibility of incorrect identification of samples was also taken into account. We intended to examine voucher specimens but, unfortunately, no details on the origin of the plants or the herbarium where the respective vouchers were preserved are given by Löve (1954a). In the later paper, Löve & Löve (1981) indicate the vouchers are "in COLO or WIN". However, none of the *Potamogeton* vouchers (and of many other plants) were actually presented to COLO (Tim Hogan, personal communication) or WIN (Bruce A. Ford, personal communication). We attempted to trace the collections also in other institutions where the Löves are known to have worked. No *Potamogeton* vouchers of Löves are located at MT (Stuart G. Hay, personal communication). We also found no North American material collected by Löves at LD in spite of searching through all of the collections. Eventually, five vouchers for chromosome counts published by Löve & Löve (1981) were discovered by Gisèle Mitrow at DAO. We examined these specimens and found that only three of these vouchers (for *P. berchtoldii*, *P. gramineus* and *P. richardsonii*) were correctly identified. However, the voucher Löve & Löve 5260, on which the chromosome count for *S. pectinata* is based (Löve & Löve 1981, as *P. pectinatus*) actually belongs to *S. vaginata*. Another collection, Löve & Löve 5590, which is a voucher for the chromosome record for *S. vaginata* (Löve & Löve 1981, as *P. vaginatus*), consists of two herbarium sheets, one of which is *S. vaginata* but the other is *S. pectinata*. It is thus unclear which of these two species was actually used for the chromosome counts and this particular record must therefore be considered doubtful. A voucher for a count made on *P. berchtoldii* was unexpectedly found at S. The vouchers for the dubious records described above are considered as missing, as they have not been located in any of the herbaria consulted.

This peculiarly unfortunate combination of problematic circumstances prompted us to consider all six dubious records discussed above (i. e., the diploid counts 2n = 26 for *P. perfoliatus* and *P. richardsonii* and the tetraploid counts 2n = 52 for *P. zosteriformis* and *P. strictifolius* from Löve 1954a, and the records from Löve & Löve 1981 on the diploid counts of 2n = 26 for *P. compressus* and *P. rutilus* from North America where these species do not occur) as unreliable and to exclude them from further consideration.

As a consequence, we consider our four records of 2n = 28 for *P. zosterifomis* as the first counts actually established on this species. The same number was also found in its vicariant species *P. compressus*. The validity of the newly established chromosome

number gains further indirect support from counts of 2n = 27 determined for hybrids involving *P. zosterifomis* and several linear-leaved species with 2n = 26 (see below).

Potamogeton ×*absconditus* Z. Kaplan, Fehrer et Hellq. [= *P. perfoliatus* × *P. richardsonii*]

2n = 78

1720: USA, Maine, Aroostook Co.: oxbow of Pettingrill Brook 1.5 km above its mouth to Aroostook River, just S of Route 164, Washburn, 6 km NNW of Presque Isle, 46°44'20"N, 68°01'59"W, alt. 130 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist 05/444, cult. & coll. Z. Kaplan 1720.

The existence of this hybrid was only recently documented by molecular analysis (Kaplan et al. 2009). It is known to occur in Maine and Vermont and is likely to be more widespread in the north-eastern USA where both species co-occur (Kaplan & Reveal 2013). The sample used here for chromosome counting showed a stronger signal of the ITS variant from *P. richardsonii* than that from *P. perfoliatus*. This is interpreted as a possible later generation backcross to *P. richardsonii* (Kaplan et al. 2009).

The chromosome number identified here provides additional information for determining the origin of this hybrid plant. As both parental species are tetraploid and this hybrid is hexaploid, it apparently resulted from the combination of an unreduced gamete (n = 52) and a normal reduced gamete with 26 chromosomes. Hybrids are more likely to produce unreduced gametes than pure species because of potentially disturbed meiosis. We therefore assume that an unreduced egg cell of *P. perfoliatus* × *P. richardsonii* was fertilized by pollen from *P. richardsonii*. Other *P. perfoliatus* × *P. richardsonii* hybrids may not necessarily be hexaploid but tetraploid like the parental species.

Potamogeton ×*angustifolius* J. Presl [= *P. gramineus* × *P. lucens*]

2n = 52

333: Czech Republic, distr. Česká Lípa: Držník fishpond in forest 1.1 km ESE of Hradčany, 4 km S of Mimoň, 50°36'37"N, 14°43'23"E, alt. 273 m, 18 IX 1996, coll. Z. Kaplan 96/640, cult. & coll. Z. Kaplan 333.

This is a relatively well-known hybrid that is recorded for many countries in Europe and a few regions of Asia. The chromosome number determined in this study is consistent with the theoretical assumption based on the chromosome numbers of the parental species (2n = 52 repeatedly recorded for each of them). This is the first chromosome count for this hybrid.

Potamogeton ×*assidens* Z. Kaplan, Zalewska-Gałosz et M. Ronikier [= *P. nodosus* × *P. perfoliatus*]

2n = 52

1944: Montenegro, distr. Bar: shallow edge of Skadarsko jezero (Lake Scutari, Lake Skadar) at abandoned port of Pristan, 1.1 km N–NNE of Seoca, 5 km ESE of Virpazar, 42°13'47.7"N, 19°08'50.6"E, alt. 6 m, 13 VII 2008, coll. Z. Kaplan 08/573, cult. & coll. Z. Kaplan 1944. – **1948**: Lithuania, Verknë River under bridge 1 km S of Lielionys, 54°33'26.0"N, 24°20'36.8"E, 24 VII 2008, coll. W. Gałosz & J. Zalewska-Gałosz, cult. & coll. Z. Kaplan 1948.

This hybrid was discovered and described only recently, based on fresh material from Poland and herbarium collections from Africa (Zalewska-Gałosz et al. 2010). It is here for

the first time recorded for Montenegro and Lithuania. The chromosome number of this hybrid is identified here for the first time and is identical to that of its parental species.

Potamogeton ×*billupsii* Fryer [= *P. coloratus* × *P. gramineus*]

2n = ca 40

2055: United Kingdom, Wales, Isle of Anglesey: Talwrn, shallow pools in Cors Bodeilio National Nature Reserve, 53°16'16.4"N, 04°14'52.1"W, alt. 34 m, VI 2002, coll. R. V. Lansdown, cult. & coll. Z. Kaplan 2055.

This is an extremely rare hybrid known from only a few sites in the British Isles. The chromosome count was identified here for the first time and it is consistent with the number expected for a hybrid between the parental species (2n = 28 in *P. coloratus*, 2n = 52 in *P. gramineus*).

Potamogeton ×*cognatus* Asch. et Graebn. [= *P. perfoliatus* × *P. praelongus*]

2n = 52

1226: Denmark, Jylland (Jutland), region Midtjylland (Central Denmark Region): Gudenå River near Randers, 17 VIII 2000, coll. C. D. Preston, cult. & coll. Z. Kaplan 1226.

This hybrid is known from only a few countries in the northern half of Europe. The identity of the sample studied was recently confirmed using DNA analysis (Kaplan & Fehrer 2013). The chromosome count presented here is the first for this hybrid and is the same as for its parental species.

Potamogeton ×*cooperi* (Fryer) Fryer [= *P. crispus* × *P. perfoliatus*]

2n = 52

1248: United Kingdom, Wales, Pembrokeshire Co.: River Solva, 8 VI 2001, coll. T. D. Dines & C. D. Preston, cult. & coll. Z. Kaplan 1248. – **1420**: Czech Republic, distr. Břeclav: small water reservoir "Malá laguna" 1.4 km ESE of Pasohlávky village, 48°53'45"N, 16°34'01"E, alt. 170 m, 21 VI 2002, coll. J. Rydlo, cult. & coll. Z. Kaplan 1420.

This hybrid is known from several countries mainly in the northern half of Europe. Both samples used for determining the chromosome number were previously analysed genetically to confirm their origin (Kaplan & Fehrer 2004). Our chromosome counts are the first established for this hybrid and are identical to those of the parental species.

Potamogeton ×*drepanoides* Z. Kaplan [= *P. berchtoldii* × *P. oxyphyllus*]

2n = 26

1236: Japan, Honshu, Hiroshima Prefecture (Hiroshima-ken): fishpond 1 km ENE of University campus SW of city centre of Higashi-Hiroshima, 34°24'23"N, 132°43'47"E, alt. 227 m, 4 X 2000, coll. L. Adamec, cult. & coll. Z. Kaplan 1236. – **1238**: Japan, Honshu, Hiroshima Prefecture (Hiroshima-ken): water reservoir in Yamanaka park at University campus SW of city centre of Higashi-Hiroshima, 34°24'12"N, 132°43'09"E, alt. 234 m, 4 X 2000, coll. L. Adamec, cult. & coll. Z. Kaplan 1238.

This hybrid is known only from two close localities. It has the same chromosome number as its parental species. It is described in terms of its morphology on p. 467.

Potamogeton ×*faxonii* Morong [= *P. illinoensis* × *P. nodosus*]

2n = 78

1654: USA, Vermont, Addison Co.: fast flowing section of Otter Creek just at confluence with New Haven River 1 km W of Brooksville, Weybridge, 44°03'45"N, 73°10'39"W, alt. 67 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/387, cult. & coll. Z. Kaplan 1654.

The exact distribution of this hybrid is poorly known because the variation in its morphology overlaps that of its parental species. It may be widespread in North and Central America. The chromosome count presented here is the first recorded for this hybrid. It is intermediate between the chromosome numbers of its parental species (2n = 52 in *P. nodosus*, 2n = 104 in *P. illinoensis*).

Potamogeton ×*gessnacensis* G. Fisch. [= *P. natans* × *P. polygonifolius*]

2n = 40

1286: United Kingdom, Wales, Powys Co.: small pool at Afon Marteg (river) at Gilfach, ca 3.5 km N of Rhayader, alt. 240 m, 16 VII 2001, coll. A. O. Chater & C. D. Preston, cult. & coll. Z. Kaplan 1286 (Fig. 1o).

This is an extremely rare hybrid reliably documented for only two countries in Europe. So far, only a single chromosome count has been published for this hybrid: 2n = ca 39 by Preston et al. (1998). Although inexact, it was sufficient to show that their plant was a hybrid between a diploid and a tetraploid. Our chromosome count exactly corresponds to the number that may be expected for a hybrid between species with 2n = 28 (*P. polygonifolius*) and 2n = 52 (*P. natans*).

Potamogeton ×*hagstroemii* A. Benn. [= *P. gramineus* × *P. richardsonii*]

2n = ca 52

1647: USA, Vermont, Addison Co.: fast flowing section of Otter Creek just at confluence with New Haven River 1 km W of Brooksville, Weybridge, 44°03'45"N, 73°10'39"W, alt. 67 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/380, cult. & coll. Z. Kaplan 1647.

This hybrid occurs in North America but its exact distribution is poorly known. It has the same chromosome number as its parental species. This is the first chromosome record for this hybrid.

Potamogeton ×*haynesii* Hellq. et G. E. Crow [= *P. strictifolius* × *P. zosteriformis*]

2n = 27

1673: Canada, Saskatchewan: Egg Lake S of Cumberland House, coll. C. B. Hellquist, cult. & coll. Z. Kaplan 1673.

This hybrid is known from about 30 localities in the north-eastern United States and adjacent south-eastern Canada (Hellquist & Crow 1986). The chromosome count presented here is the first for this hybrid. It is intermediate between the chromosome numbers of its parental species (2n = 26 in *P. strictifolius*, 2n = 28 in *P. zosteriformis*).

Potamogeton ×*lanceolatifolius* (Tiselius) C. D. Preston [= *P. gramineus* × *P. nodosus*]

2n = 52

1005: Sweden, prov. Småland, Kalmar: Alsterån River 0.8 km NNW(–N) of bridge in Strömsrum, 3 km SE of Ålem, 56°56'36"N, 16°24'52"E, alt. 6 m, coll. Z. Kaplan 98/342, cult. & coll. Z. Kaplan 1005.

This taxon was first described as one of several forms of *P. gramineus* by Tiselius (1894–1897, see also Kaplan 2010c). Based on a careful morphological investigation of the type collection, Hagström (1916) correctly noted that these plants have sterile pollen, suggested their hybrid origin and interpreted them as a hybrid *P. gramineus* × *P. polygonifolius*. This taxonomic view was adopted by later researchers and held for almost a century. However, a recent molecular investigation of plants from the original clones still growing at the type locality demonstrated that one of the parental species was not *P. polygonifolius* as previousy believed, but the morphologically and anatomically similar species *P. nodosus* (Kaplan & Fehrer 2011). The chromosome count presented here, the first one established for this hybrid combination, is identical to that of its parental species, whereas *P. polygonifolius*, one of the previously believed parental species, differs in being diploid with 2n = 28.

Potamogeton ×*lintonii* Fryer [= *P. crispus* × *P. friesii*]

2n = 65

2119: Germany, Nordrhein-Westfalen (North Rhine-Westphalia), Lower Rhine Basin (Niederhein): ditch between fields (550 m upstream of its mouth to Kleine Niers River) at bridge on road Kempen – Straelen, 2.7 km W of Kerken, 51°27'11.6"N, 06°19'32.8"E, alt. 25 m, coll. Z. Kaplan, K. van de Weyer & J. Bruinsma 09/326, cult. & coll. Z. Kaplan 2119 (Fig. 2j).

This is an extremely rare hybrid currently known only from the British Isles (Dandy 1975, Preston 1995) and the Netherlands (Ploeg 1990, Meijer & Ploeg 1994) and tentatively recorded for Germany (Wiegleb et al. 2008). The records for Belgium (Vannerom & Andriessen 1987) are erroneous, based on underdeveloped plants of *P. crispus* with plane leaves (Z. Kaplan, unpublished data). *Potamogeton* ×*lintonii* is a hybrid between highly unrelated species, diploid *P. friesii* (2n = 26) and tetraploid *P. crispus* (2n = 52). This may be the reason why the parental species rarely hybridize. The chromosome count presented here is the first for this hybrid. The recorded pentaploid chromosome number is best explained as a result of fusion of an unreduced gamete of *P. crispus* (n = 52) and a normal reduced gamete of *P. friesii* (n = 13).

Potamogeton ×*luxurians* Z. Kaplan [= *P. amplifolius* × *P. illinoensis*]

2n = 78

1625: USA, Vermont, Orleans Co.: Lake Parker 1 km WSW of West Glover, 44°43'34"N, 72°13'53"W, alt. 396 m, 22 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/362, cult. & coll. Z. Kaplan 1625.

This is a little-known North American hybrid sometimes incorrectly annotated as *P.* ×*scoliophyllus* (Hagström 1916, Scoggan 1978, Brayshaw 2000). Its nomenclature is analysed on p. 469–471. The chromosome count presented here is the first recorded for this hybrid. It is intermediate between the chromosome numbers of its parental species (2n = 52 in *P. amplifolius*, 2n = 104 in *P. illinoensis*).

Potamogeton ×*malainoides* Miki [= *P. distinctus* × *P. wrightii*]

2n = ca 52

1763: JAPAN, Kyushu, Fukuoka Prefecture (Fukuoka-ken), Kitakyusyu: Kokura-Minami, Jingu-ji, 16 V 1999, coll. N. Tanaka, cult. & coll. Z. Kaplan 1763.

This hybrid is known from several regions of south-eastern and eastern Asia where the parental species co-occur. The chromosome number of our sample is in agreement with previous records for this hybrid (Harada 1942, Takusagawa 1961) and corresponds to the count of 2n = 52 for both parental species.

Potamogeton ×*nitens* Weber [= *P. gramineus* × *P. perfoliatus*]

2n = 52

879: GERMANY, Mecklenburg-Vorpommern, Güstrow: Lake Gültz (Gültzsee) 2.2 km SSW of Dobbin, 53°35'23"N, 12°19'04"E, 15 VIII 1997, coll. Z. Kaplan 97/828, cult. & coll. Z. Kaplan 879 (Fig. 2h). – **999**: SWEDEN, prov. Skåne: Björkaån River 0.5 km ENE of church in Björka, 4 km WNW of Sjöbo, 55°39'28"N, 13°38'35"E, alt. 24 m 12 VIII 1998, coll. Z. Kaplan 98/335, cult. & coll. Z. Kaplan 999. – **1824**: SWEDEN, prov. Skåne, municipality (kommun) of Kristianstad: shallow bay at E bank of SE part of Hammarsjön Lake 1.7 km SW of Rinkaby, 55°57'59"N, 14°14'45"E, sea level, 11 VIII 2006, coll. Z. Kaplan, S. Skällberg, J. Svensson & A. Jacobson 06/359, cult. & coll. Z. Kaplan 1824. – **2148**: RUSSIA, Siberia, prov. Irkutsk (Irkutskaya oblast'), distr. Ziminskiy: stream (side arm of Oka River) below road in village Osipovskiy, 8.5 km WSW of Maslyanogorsk, 53°31'13.7"N, 101°33'10.6"E, alt. 517 m, 21 VIII 2009, coll. Z. Kaplan & V. Chepinoga 09/373, cult. & coll. Z. Kaplan 2148.

This hybrid is widespread but only locally common in boreal and temperate regions of the Northern Hemisphere. Molecular confirmation of the identities of samples 879 and 999 was published previously (Kaplan & Fehrer 2006). The chromosome counts presented here are all identical with those of its parental species. Until now, only a single approximate chromosome number 2n = ca 52 was published for the British Isles by Hollingsworth et al. (1998).

Potamogeton ×*ogdenii* Hellq. et R. L. Hilton [= *P. hillii* × *P. zosteriformis*]

2n = 27

1590: USA, Massachusetts, Berkshire Co.: pond on Kinderhook Creek at crossroads of Route 43 and Whitman Road in Hancock, 42°34'40"N, 73°17'51"W, alt. 385 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/338, cult. & coll. Z. Kaplan 1590.

This hybrid is known to occur at about 20 localities in the north-eastern United States and one site in adjacent south-eastern Canada (Hellquist & Hilton 1983, Haynes & Hellquist 2000, Hellquist & Mertinooke-Jongkind 2002). This taxon is not a species of hybrid origin as suggested by the original authors and recognized by the recent American literature but a recent hybrid involving *P. hillii* and *P. zosteriformis* as the ITS copies of both parental species are clearly recognizable and not yet homogenized by concerted evolution as in pure species of *Potamogeton* (J. Fehrer & Z. Kaplan, unpublished data). The chromosome count presented here is the first for this hybrid. It is intermediate between the chromosome numbers of its parental species (2n = 26 in *P. hillii*, 2n = 28 in *P. zosteriformis*).

Potamogeton ×*rivularis* Gillot [= *P. berchtoldii* × *P. polygonifolius*]

2n = 27

2056: France, Burgundy, Dept. Nièvre: granitic mountainous massif Le Morvan, La Cure River (rivière la Cure) at site called "le Furtiau" 1.3 km NE of Montsauche-les-Settons, 0.8 SSW of Nataloup, 47°13'34"N, 04°02'20"E, alt. 517 m, 2 IX 2003, coll. R. V. Lansdown & O. Bardet, cult. & coll. Z. Kaplan 2056.

This is an extremely rare hybrid so far recorded only for France. The sample investigated originates from the type locality. Its chromosome count corresponds to the number expected for a hybrid between species with 2n = 26 (*P. berchtoldii*) and 2n = 28 (*P. polygonifolius*). This is the first chromosome record for this hybrid.

Potamogeton ×*salicifolius* Wolfg. [= *P. lucens* × *P. perfoliatus*]

2n = 52

972: Italy, Lombardy, prov. Como: eastern shore of northern edge of Lago di Como (lake) 0.8 km SSE of Gera Lario, 3.3 km ENE of Domaso, 46°09'46"N, 09°22'48"E, alt. 200 m, 18 VI 1998, coll. Z. Kaplan, cult. & coll. Z. Kaplan 972. – **1958**: Sweden, prov. Uppland, Stockholm county, municipality (kommun) of Stockholm, Stockholm parish: channel Riddarholmskanalen in Gamla Stan (Old Town) in center of Stockholm City, 59°19'28.2"N, 18°03'59.3"E, sea level, 28 VIII 2008, coll. Z. Kaplan & A. Svenson 08/629, cult. & coll. Z. Kaplan 1958.

2n = ca 52

2137: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), border of Alarskiy & Cheremkhovskiy distr.: Golumet' River at bridge 2.2 km SW of village Nygda, 11 km SE of Alar', 52°59'36.4"N, 102°40'01.3"E, alt. 445 m, 19 VIII 2009, coll. Z. Kaplan & V. Chepinoga 09/363, cult. & coll. Z. Kaplan 2137.

2n = 78

1017: Sweden, prov. Uppland: river connecting lakes Skarren and Oxundasjön 300 m SE of Rosendal settlement, 2 km ENE of Runsa, 5 km NW of Upplands Väsby, 59°33'48"N, 17°51'06"E, alt. 5 m, 15 VIII 1998, coll. Z. Kaplan 98/361, cult. & coll. Z. Kaplan 1017 (Fig. 2k).

This hybrid is recorded for several European countries and Siberia. The majority of our records correspond to the only published record that comes from the British Isles (Hollingsworth et al. 1998, as 2n = ca 52) as well as the chromosome counts of both parental species. The exception is sample 1017, which is hexaploid. It apparently resulted from a fusion of reduced and unreduced gametes.

Potamogeton ×*schreberi* G. Fisch. [= *P. natans* × *P. nodosus*]

2n = 52

1276: France, Lorraine, Moselle: Blies River upstream of Bliesbruck, ca 49°08'10"N, 07°11'03"E, alt. 205 m, 14 VII 2001, coll. P. Wolff, cult. & coll. Z. Kaplan 1276. – **1278**: Germany, Saarland: Blies River above bridge at south-eastern edge of Reinheim, ca 49°08'15"N, 07°11'20"E, alt. 207 m, 14 VII 2001, coll. P. Wolff, cult. & coll. Z. Kaplan 1278. – **1279**: Germany, Saarland: Blies River below bridge at south-eastern edge of Reinheim, ca 49°08'08"N, 07°10'57"E, alt. 207 m, 14 VII 2001, coll. P. Wolff, cult. & coll. Z. Kaplan 1279. – **1889**: Czech Republic, distr. České Budějovice: Stropnice stream 0.9 km ENE of Třebeč, 48°52'39.3"N, 14°41'31.5"E, alt. 450 m, 29 VI 2007, coll. Z. Kaplan 07/214, cult. & coll. Z. Kaplan 1889.

This hybrid is known from only a small number of European countries. Identities of all the samples studied were confirmed using molecular methods (Kaplan & Wolff 2004, Kaplan & Fehrer 2009). Our chromosome counts are the first for this hybrid. They are identical to those of the parental species.

Potamogeton ×*serrulifer* Z. Kaplan [= *P. crispus* × *P. schweinfurthii*]

2n = 78

1953: Italy, Tuscany, prov. Siena: water reservoir 1.1 km ENE of San Fabiano farm, 1.6 km NE of Monteroni d'Arbia, 43°14'33.7"N, 11°26'11.3"E, alt. 180 m, 12 VIII 2008, coll. Z. Kaplan, L. Lastrucci, F. Frignani & B. Foggi 08/612, cult. & coll. Z. Kaplan 1953.

This hybrid is currently only known from a single site in a relatively small area of the overlap of the distributional ranges of its parental species. Its chromosome number is intermediate between those of its parental species (2n = 52 in *P. crispus*, 2n = 104 in *P. schweinfurthii*). It is described morphologically on p. 471.

Potamogeton ×*spathuliformis* (J. W. Robbins) Morong [= *P. gramineus* × *P. illinoensis*]

2n = 78

1663: USA, Vermont, Rutland Co.: Burr Pond 2.5 km SSE of Hyde Manor, Sudbury, 43°46'06"N, 73°10'57"W, alt. 157 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1663.

This hybrid is known from scattered locations mainly in eastern USA. The chromosome count presented here is the first for this hybrid. It is intermediate between the chromosome numbers of its parental species (2n = 52 in *P. gramineus*, 2n = 104 in *P. illinoensis*).

Potamogeton ×*torssanderi* (Tiselius) Dörfler [= *P. gramineus* × *P. lucens* × *P. perfoliatus*]

2n = 78

1006: Sweden, prov. Södermanland: north-eastern edge of Sillen Lake near Vårdinge, 59°01'19"N, 17°21'29"E, alt. 11 m, 13 VIII 1998, coll. Z. Kaplan 98/343, cult. & coll. Z. Kaplan 1006 (Fig. 2l).

This is the only unequivocally documented triple hybrid in this family. It is known only from its type locality. After more than a century of taxonomic uncertainty about this taxon, Kaplan & Fehrer (2007) used DNA sequencing of plant material from the original population and identified it as a triple hybrid between *P. gramineus*, *P. lucens* and *P. perfoliatus*. Plants from the same clone were used also for chromosome counting. This triple hybrid has apparently arisen from combination of an unreduced gamete (n = 52) of a primary binary hybrid and a normal reduced gamete (n = 26) of a third species.

Potamogeton ×*undulatus* Wolfg. [= *P. crispus* × *P. praelongus*]

2n = 52

1024 & 1025: Denmark, Jylland (Jutland), Region Syddanmark (Region of Southern Denmark): Ribeå River at western edge of Varming, 4 km ESE of Ribe, 55°19'07"N, 08°50'52", alt. 1 m, 19 VIII 1998, coll. Z. Kaplan 98/374, cult. & coll. Z. Kaplan 1024 & 1025 (Fig. 2i).

This hybrid is known from several countries mainly in the northern half of Europe and from a single location in the USA. The identity of the samples studied was recently confirmed using DNA analysis (Kaplan & Fehrer 2013). The identified chromosome number is the first for this hybrid and it is the same as that of its parental species.

Potamogeton ×*variifolius* Thore [= *P. berchtoldii* × *P. natans*]

2n = 39

1589: France, Vosges du Nord, Bas-Rhin: downstream from Philippsbourg, fast flowing cool stream of Falkensteinerbach near Breitenwasen, 2000, coll. Š. Husák & G. Thiébaut, cult. & coll. Z. Kaplan 1589.

This remarkable hybrid between a narrow-leaved and broad-leaved species is known from Ireland, France and Germany. Its chromosome number, identified for the first time for this hybrid, is intermediate between that of its parental species (2n = 26 in *P. berchtoldii*, 2n = 52 in *P. natans*).

Potamogeton ×*vepsicus* A. A. Bobrov et Chemeris [= *P. natans* × *P. praelongus*]

2n = 52

1739: Russia, prov. Vologda, distr. Babaevo: Nozhema River at Pyazhelka, ca 60°09'N, 35°44'E, alt. 190 m, 8 VIII 2005, coll. A. Bobrov & E. Chemeris, cult. & coll. Z. Kaplan 1739.

This hybrid was only recently described from Russia and believed to be *P. alpinus* × *P. natans* (Bobrov & Chemeris 2006). However, subsequent molecular investigation of shoots from the type clone showed that the taxon had been misunderstood and its correct identity is *P. natans* × *P. praelongus* (Kaplan & Fehrer 2011). The same plant was also used for the first karyological analysis of this hybrid. The chromosome number is the same as that of its parental species.

Potamogeton berchtoldii × *P. zosteriformis*

2n = 27

1598 & 1600 & 1604: USA, Massachusetts, Berkshire Co.: Berkshire Pond at Lanesborough, 42°30'28"N, 73°11'50"W, alt. 301 m, 21 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/346, cult. & coll. Z. Kaplan 1598 & 1600 & 1604. – **1618**: USA, Vermont, Washington Co.: Curtis Pond 0.5 km NW of Maple Corner, Calais, 44°22'36"N, 72°30'03"W, alt. 371 m, 22 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1618. – **1674**: USA, New England, exact locality unknown, cultivated by C. B. Hellquist in Adams, Massachusetts, USA, cult. & coll. Z. Kaplan 1674.

This is one of several similar North American hybrids among linear-leaved species, which was identified only recently. Its chromosome number is intermediate between those of its parental species (2n = 26 in *P. berchtoldii*, 2n = 28 in *P. zosteriformis*).

Potamogeton distinctus × *P. natans*

2n = 78

2179: India, Kashmir, Bandipora district: outlet of Manasbal Lake at Naninara, 34°14'47"N, 74°39'18"E, alt. 1582 m, IX 2009, coll. A. H. Ganie, cult. & coll. Z. Kaplan 2179.

This is a very recently detected hybrid. The chromosome number indicates that the investigated plant apparently resulted from the fusion of a reduced and unreduced gamete of its parental species, which are both tetraploid with 2n = 52.

Potamogeton friesii × *P. pusillus*

2n = 26

1669: USA, Vermont, Rutland Co.: NE corner of Lake Hortonia 2.5 km NE of Hortonia, Sudbury, 43°45'09"N, 73°12'38"W, alt. 148 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist, cult. & coll. Z. Kaplan 1669.

This is a little-known hybrid that is difficult to distinguish from its parental species. It has the same chromosome number as its parental species.

Stuckenia filiformis (Pers.) Börner

2n = 78

975: Switzerland, canton Sankt Gallen: canal with fast-running water (Rheintaler Binnenkanal) 0.8 km NW of Kriessern near Altstätten, 47°22'27"N, 09°36'12"E, alt. 407 m, 22 VI 1998, coll. Z. Kaplan 98/105, cult. & coll. Z. Kaplan 975. – **1187**: Switzerland, canton Sankt Gallen: southern edge of Bodensee (Lake Constance) 0.8 km N–NNE of Altenrhein near Rorschach, 47°30'03"N, 09°33'18"E, alt. 396 m, 23 VI 1998, coll. Z. Kaplan, cult. & coll. Z. Kaplan 1187 (Fig. 2m). – **1703**: USA, Maine, Aroostook Co.: Nickerson Lake at south-eastern edge of New Limerick, Linneus Twp., 5 km WSW of Houlton, 46°05'32"N, 67°54'43"W, alt. 116 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist 05/426, cult. & coll. Z. Kaplan 1703. – **2134**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), distr. Cheremkhovskiy: Verkhnaya Iret' River at south-western edge of Russkaya Alar', 1 km upstream of its mouth into Bol'shaya Belaya River, 7.5 km SW of Parfenovo, 52°56'51.4"N, 102°45'29.8"E, alt. 438 m, 19 VIII 2009, coll. Z. Kaplan & V. Chepinoga 09/360, cult. & coll. Z. Kaplan 2134. – **2543**: Switzerland, canton Sankt Gallen: canal with fast-running water (Rheintaler Binnenkanal) 0.8 km NW of Kriessern near Altstätten, 47°22'27"N, 09°36'12"E, alt. 407 m, 12 VII 2012, coll. Z. Kaplan 12/195, cult. & coll. Z. Kaplan 2543.

This species is widely distributed in Europe, western, central and northern Asia, North America and South America. Our records of its chromosome number are the same as the prevailing count for this species (all as *Potamogeton filiformis*), as reported for Scandinavia (Palmgren in Löve & Löve 1942, as 2n = ca 78), Iceland (Löve & Löve 1956), Russian Far East (Yurtsev & Zhukova 1978), Canada (Löve & Ritchie 1966, Löve & Löve 1981) and Greenland (Jörgensen et al. 1958). The only deviant count, 2n = ca 66, came from an early report by Palmgren (1939). However, this appears to be erroneous. The author himself indicated it as inexact and did not include it in the chromosome catalogue of Scandinavian plants (Palmgren in Löve & Löve 1942), but replaced it with the count "2n = ca 78".

Stuckenia pectinata (L.) Börner

2n = 78

841: Czech Republic, distr. Hodonín: Velička navigation canal at northern edge of Strážnice, 48°54'38.5"N, 17°18'42.5"E, alt. 170 m, 25 VI 1997, coll. Z. Kaplan 97/509, cult. & coll. Z. Kaplan 841. – **1841**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), Ust-Ordyn-Buryat autonomous okrug, distr. Alarskiy: Kuda River near Kapsal, 52°41'N, 104°40'E, 2 IX 2006, coll. V. Chepinoga, cult. & coll. Z. Kaplan 1841. – **2026**: India, Kashmir, Bandipora district: outlet of Manasbal Lake at Naninara, 34°14'47"N, 74°39'18"E, alt. 1582 m, 27 VI 2008, coll. A. H. Ganie, cult. & coll. Z. Kaplan 2026. – **2238**: Switzerland, canton Thurgau: Untersee (Lower Lake Constance), at outflow of Seerhein into Ermatinger Becken, NE of Triboltingen, 47°40'00"N, 9°07'20"E, alt. 395 m, 7 IV 2010, coll. D. Richter, cult. & coll. Z. Kaplan 2238.

2n = ca 78

981: Switzerland, canton Sankt Gallen: ditch with running water 200 m SSW of Altenrhein near Rorschach, 47°29'08.3"N, 09°32'56.4"E, alt. 396 m, 23 VI 1998, coll. Z. Kaplan 98/127, cult. & coll. Z. Kaplan 981. – **1650**: USA, Vermont, Addison Co.: fast flowing section of Otter Creek just at confluence with New Haven River 1 km

W of Brooksville, Weybridge, 44°03'45"N, 73°10'39"W, alt. 67 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/383, cult. & coll. Z. Kaplan 1650. – **1837**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), distr. Alarskiy: Golumet' River near Nygda, ca 52°59'N, 102°40'E, 29 VIII 2006, coll. V. Chepinoga, cult. & coll. Z. Kaplan 1837.

There are several published records of the chromosome number of this cosmopolitan species (all as *Potamogeton pectinatus*). The most frequent count is 2n = 78. This is recorded for Germany (Scheerer 1939, as 2n = ca 78), Scandinavia (Palmgren in Löve & Löve 1942), Finland (Arohonka 1982), British Isles (Hollingsworth et al. 1998, as 2n = ca 78), Slovakia (Uhríková & Feráková 1978), Russia (Chepinoga et al. 2012, as 2n = ca 78), India (Bhat et al. 1975, as n = 39), Japan (Harada 1942, as 2n = ca 78, Takusagawa 1961) and Canada (Löve & Löve 1981).

Other records are more or less controversial. Kalkman & Van Wijk (1984) report extensive aneuploidy in this species around 2n = 78 and the occurrence of 17 cytotypes (2n = 70, 71, and all ranging between 73 and 87). They record high variation in chromosome number both within and between populations. For some populations as many as 10 different cytotypes are reported. It is not clear from their paper how many plants were studied from each population (i. e., what was the probability of recording such high variation) and thus if the variation was between individuals or between different cells within a single or a few individuals. An extensive variation in chromosome number, ranging from 70 to 87, is reported also by Ceccarelli et al. (2008). Variable counts are recorded even for metaphases in one and the same root. The most common chromosome number is 78, which they consider to be the euploid chromosome number of this species. Two other records indicate counts within this variation: Uchiyama (1989) reports 2n = 84 for Japan and Yurtsev et al. (1975, as "*P.* cf. *pectinatus*") record 2n = 86 for the Russian Far East. Given the obvious technical difficulties of exactly counting the small chromosomes at the hexaploid level in *Potamogetonaceae*, this variation probably does not reflect genuine variation in chromosome number but is rather due to counting errors.

Lower numbers of chromosomes are reported only exceptionally. Probatova & Sokolovskaya (1986) publish for the Russian Far East an approximate count of 2n = ca 66 and Misra (1972) report an even less credible count of 2n = 42 for India. Considering the fact that no cytotype below the hexaploid level is confirmed for the entire genus, which represents a monophyletic group well separated from other genera (see below), we consider these records as erroneous.

We examined the voucher specimen (Löve & Löve 5260, DAO) for the chromosome count of 2n = 78 for "*Potamogeton pectinatus*" given by Löve & Löve (1981) and found that it is actually *Stuckenia vaginata*. This chromosome record must therefore be transferred to the latter species.

Stuckenia striata (Ruiz et Pav.) Holub

2n = 78

855: Argentina, prov. Buenos Aires: irrigation canals in Bahía Blanca, provided by P. Denny in 1997, cult. & coll. Z. Kaplan 855.

This species is closely related to *S. pectinata* and occurs in western and south-western North America and South America. The chromosome count presented here is the first for this species. Although the sample investigated morphologically fits the present concept of

S. striata, ITS sequencing indicated that this particular plant may be a hybrid rather than a pure species (J. Fehrer, unpublished data). The relation between morphology, genetic structure and taxonomy in *Stuckenia* requires further study.

Stuckenia vaginata (Turcz.) Holub

2n = 78

2052: Russia, Siberia, Chita, distr. Uletovskiy: lake at Tanga, 50°58'07"N, 111°32'37"E, 8 VIII 2008, coll. S. Rosbakh & K. Fleckenstein, cult. & coll. Z. Kaplan 2052.

2n = ca 78

1063: Canada, Manitoba: inlet at northernmost large portion of Tramping Lake at start of Grass River, SE of town of Snow Lake, 19 VIII 1998, coll. C. B. Hellquist & C. E. Hellquist, cult. & coll. Z. Kaplan 1063.

This species is circumboreal, distributed in northern Europe, northern Asia and northern North America. The same count as we determined was published for Canada by Löve & Löve (1961, 1981). Only an approximate number of 2n = ca 88 is reported for Sweden by Palmgren (1939). Another count was published under an incorrect name. We examined the voucher specimen (Löve & Löve 5260, DAO) for the chromosome count of 2n = 78 for "*Potamogeton pectinatus*" given by Löve & Löve (1981) and found that it is actually *Stuckenia vaginata*.

Stuckenia ×*bottnica* (Hagstr.) Holub [= *S. pectinata* × *S. vaginata*]

2n = 78

1027: Denmark, Jylland (Jutland), region Syddanmark (Region of Southern Denmark): Sonderå stream at northern edge of Rens, 54°54'00"N, 09°05'34"E, alt. 8 m, 19 VIII 1998, coll. Z. Kaplan 98/379, cult. & coll. Z. Kaplan 1027. – **1839 & 1840**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), distr. Alarskiy: Golumet' River near Nygda, ca 52°59'N, 102°40'E, 29 VIII 2006, coll. V. Chepinoga, cult. & coll. Z. Kaplan 1839 & 1840.

This hybrid is known from only a few boreal regions of the Northern Hemisphere. It is here for the first time recorded for Denmark and Siberia. The chromosome count for this hybrid is reported here for the first time.

Stuckenia ×*fennica* (Hagstr.) Holub [= *S. filiformis* × *S. vaginata*]

2n = 78

1710: USA, Maine, Aroostook Co.: Prestile Stream just N of bridge on Pierce Road, Blaine, 1.5 km SSE of Mars Hill, 46°29'45"N, 67°50'46"W, alt. 115 m, 2 VIII 2005, coll. Z. Kaplan & C. B. Hellquist 05/4358, cult. & coll. Z. Kaplan 1710. – **2141**: Russia, Siberia, prov. Irkutsk (Irkutskaya oblast'), distr. Ziminskiy: Zima River at eastern edge of Ignay, 8.5 km S of Batama, 53°47'21.6"N, 101°37'31.6"E, alt. 479 m, 20 VIII 2009, coll. Z. Kaplan & V. Chepinoga 09/367, cult. & coll. Z. Kaplan 2141.

2n = ca 78

1651: USA, Vermont, Addison Co.: fast flowing section of Otter Creek just at confluence with New Haven River 1 km W of Brooksville, Weybridge, 44°03'45"N, 73°10'39"W, alt. 67 m, 25 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/384, cult. & coll. Z. Kaplan 1651.

This hybrid is known from only a few boreal regions of the Northern Hemisphere. The samples investigated had the same chromosome number as their parental species. These are the first chromosome counts for this hybrid.

Stuckenia ×*suecica* (K. Richt.) Holub [= *S. filiformis* × *S. pectinata*]

2n = 78

1009: Sweden, prov. Södermanland: brackish water along sea shore at south-south-eastern edge of Dalarö, ca 59°08'N, 18°25'E, 13 VIII 1998, coll. Z. Kaplan, cult. & coll. Z. Kaplan 1009.

The occurrence of this hybrid is only documented in several countries in the northern half of Europe. Our chromosome count is the first made on this hybrid. It is the same as the chromosome number of its parental species.

General discussion

Pattern of chromosome number variation

Chromosome numbers of 181 samples of 47 species and 32 hybrids were counted. In *Potamogeton*, six cytotypes (2n = 26, 28, 39, 42, 52, 104) were identified in species and seven cytotypes (2n = 26, 27, 39, 40, 52, 65, 78) in hybrids. The somatic numbers 26 and 52 predominated among species while counts of 39 and 42 were recorded for only two species (a single plant in each, interpreted as autotriploids produced by diploid species, see under *P. compressus* and *P. pusillus* for details). Les (1983) in his review indicates the frequent occurrence of within-species variation in chromosome number in *Potamogeton* and later Les & Scheridan (1990) suggest that extensive chromosome number modifications have occurred within many modern species. Our observations do not support this view. In contrast, our data indicate that within-species variation is very rare in *Potamogetonaceae*, restricted to an exceptional rise of autotriploids. Similarly we did not detect any aneuploid plants, although occurrence of aneuploids is recorded by some authors (Harada 1956, Kalkman & Van Wijk 1984, Ceccarelli et al. 2008, Wan et al. 2012). The other two genera were uniform, all taxa of *Stuckenia* shared the number 2n = 78 while the single species of *Groenlandia* had 2n = 30. Our study provides the first chromosome counts for 10 species and 25 hybrids of *Potamogeton* and for 1 species and 3 hybrids of *Stuckenia*, which fill a considerable gap in the karyological data for this family. Nevertheless, for 33 of 80 species of *Potamogetonaceae* (41%) there is no information on their chromosome numbers.

There are correlations between the variation in chromosome numbers identified in species in the three genera of *Potamogetonaceae* and with eight basic morphological groups in *Potamogeton* (see Table 1). The unique chromosome number of 2n = 30 exclusive to *Groenlandia densa* supports its long-recognized separate generic position. Species with 2n = 78 are present only in *Stuckenia*, which is in accordance with the present treatment of this group as a separate genus (Les & Haynes 1996, Holub 1997, Kaplan 2008). The

Table 1. – Chromosome numbers of species that were counted in this study and reported in the literature and their relation to supraspecific groups (genera and basic morphological groups) in the *Potamogetonaceae*. Chromosome numbers recorded in this study that were based on multiple samples are given in bold. Sterile autotriploids detected in two diploid fertile species were omitted (see text). The most frequently published chromosome numbers are given in bold, doubtful and unique records not confirmed by other researchers are given in brackets. Erroneous records, mostly made on misidentified material, were excluded (for details see the text under particular taxa). Literature records for *P. cristatus* and *P. fryeri* were taken from Takusagawa (1939, 1961), Harada (1942) and Uchiyama (1989), for the remaining species see the text. ▶

Genus	Informal species group (in *Potamogeton*)	Species	Chromosome number determined in this study (2n)	Literature records (2n)
Groenlandia		*G. densa*	**30**	**30**
Potamogeton	I. linear-leaved species (excl. *P. compressus* group)	*P. berchtoldii*	**26**	**26**
		P. clystocarpus	ca 26	–
		P. foliosus	**26**	26 (28)
		P. friesii	**26**	26
		P. gemmiparus	26	–
		P. groenlandicus	26	**26**
		P. hillii	26	–
		P. obtusifolius	**26**	26
		P. ochreatus	**26**	–
		P. oxyphyllus	26	**26** (28)
		P. pusillus	**26**	26
		P. rutilus	**26**	26
		P. strictifolius	26	–
		P. trichoides	26	**26**
	II. *P. epihydrus* group	*P. epihydrus*	ca 26	**26**
	III. *P. compressus* group	*P. acutifolius*	**28**	(26)
		P. compressus	**28**	26, **28** (38–41)
		P. zosteriformis	**28**	–
	IV. *P. diversifolius* group	*P. spirillus*	28	–
	V. *P. octandrus* group	*P. octandrus*	28	**28**
		P. cristatus	–	**28**
	VI. broad-leaved species of the *P. polygonifolius* group	*P. cheesemanii*	**28**	28
		P. coloratus	28	26
		P. polygonifolius	**28**	26, **28**
	VII. majority of broad-leaved species	*P. alpinus*	52	**52**
		P. amplifolius	52	**52**
		P. crispus	**52**	**52** (26, 48, 50, 56, 78)
		P. distinctus	ca 52	**52** (56)
		P. fryeri	–	**52** (42, 48)
		P. gramineus	**52**	52
		P. lucens	**52**	52
		P. maackianus	**52**	52 (56)
		P. natans	**52**	**52** (42)
		P. nodosus	52	**52**
		P. perfoliatus	**52**	**52** (78)
		P. praelongus	52	**52**
		P. richardsonii	**52**	52
		P. robbinsii	**ca 52**	**52**
		P. sarmaticus	52	–
		P. wrightii	52	**52** (48, 50, 51)
	VIII. broad-leaved species of the *P. illinoensis* group	*P. illinoensis*	104	104
		P. richardii	ca 104	(42)
		P. schweinfurthii	**104**	–
Stuckenia		*S. filiformis*	**78**	**78**
		S. pectinata	**78**	78 (70–87)
		S. striata	78	–
		S. vaginata	**78**	78 (ca 88)

remaining chromosome numbers occur in *Potamogeton*, the most species-rich genus of the family. All counts of 2n = 52 and 2n = 104 are for broad-leaved species (groups VII and VIII, respectively), the latter of which was identified only in three species for which our preliminary molecular data revealed allopolyploid origins from different combinations of broad-leaved species with 52 chromosomes (J. Fehrer & Z. Kaplan, unpublished data). The count of 2n = 26 is confined to the linear-leaved species (group I) and to *P. epihydrus* (group II). The number of 2n = 28 is characteristic of one group of linear-leaved species (III), two groups of heterophyllous species (with broad floating leaves and linear submerged leaves, groups IV and V) and one group of broad-leaved species (VI). Our counts indicate that not only are species invariable in terms of chromosome numbers but also whole basic morphological groups of *Potamogetonaceae* appear to be homogeneous in this respect.

Additional support for the high regularity in the chromosome counts reported here comes from the investigation of hybrids. All *Stuckenia* hybrids (8 counts for 4 hybrids) had 78 chromosomes like their parents. For *Potamogeton*, 46 counts of different plants were made that represented 29 hybrid combinations. The majority were homoploid hybrids with 52 chromosomes like their parents (23 counts for 12 hybrids). Only in 4 cases, the hybrids between parents with 2n = 52 had 78 chromosomes, which was interpreted as a fusion of unreduced and reduced gametes. Two homoploid hybrids had 26 chromosomes like their parents. Heteroploid crosses usually had intermediate numbers of chromosomes. Hybrids between 2n = 26 and 2n = 28 parents had 27 chromosomes (8 counts for 4 hybrids); hybrids with 2n = 78 were derived from crosses between parental species with 52 and 104 chromosomes, respectively (4 counts for 4 hybrids); and for combinations in which one parent had 2n = 52 and the other had 2n = 26 or 2n = 28, the corresponding 39 or 40 chromosomes were recorded (3 counts for 3 hybrids). The only heteroploid cross that did not result in intermediate chromosome numbers was one of a hybrid with 2n = 65, the parents of which had 52 and 26 chromosomes, respectively. In this case, the former parent had apparently contributed an unreduced gamete.

Relatively regular patterns also emerged when the confirmed chromosome numbers from Table 1 (doubtful ones excluded) were mapped onto phylogenetic trees based on nuclear ribosomal markers (Figs 4 and 5). In most cases there was a close correspondence between morphological groups, main clades and characteristic (or at least dominant) chromosome number. This indicates a close relationship between chromosome number and the systematic division of the family. Both *Groenlandia* and *Stuckenia* are separate monophyletic lineages uniform in chromosome number (Fig. 4). Within *Potamogeton* (Fig. 5), counts of 2n = 52 are confined to broad-leaved species, which all occur in a single clade, while 2n = 26 occurs only among linear-leaved species and in the *P. epihydrus* group. Species with 2n = 28 occur in several species-poor clades. Although not all main clades are uniform in their chromosome number, nor are the morphological groups necessarily monophyletic with this marker, the general pattern is far from random and shows that there is actually very little variation in cytotypes within clades of *Potamogetonaceae*. This is in strong contrast to previous studies (Les 1983, Les & Sheridan 1990, Hollingsworth et al. 1998, Lindqvist et al. 2006) that attempted to identify trends in chromosome number evolution in *Potamogeton* by assigning published chromosome counts to phylogenetic trees or morphological groups. The elementary reason for their failure to find any reasonably clear pattern is the high number of incorrect chromosome records

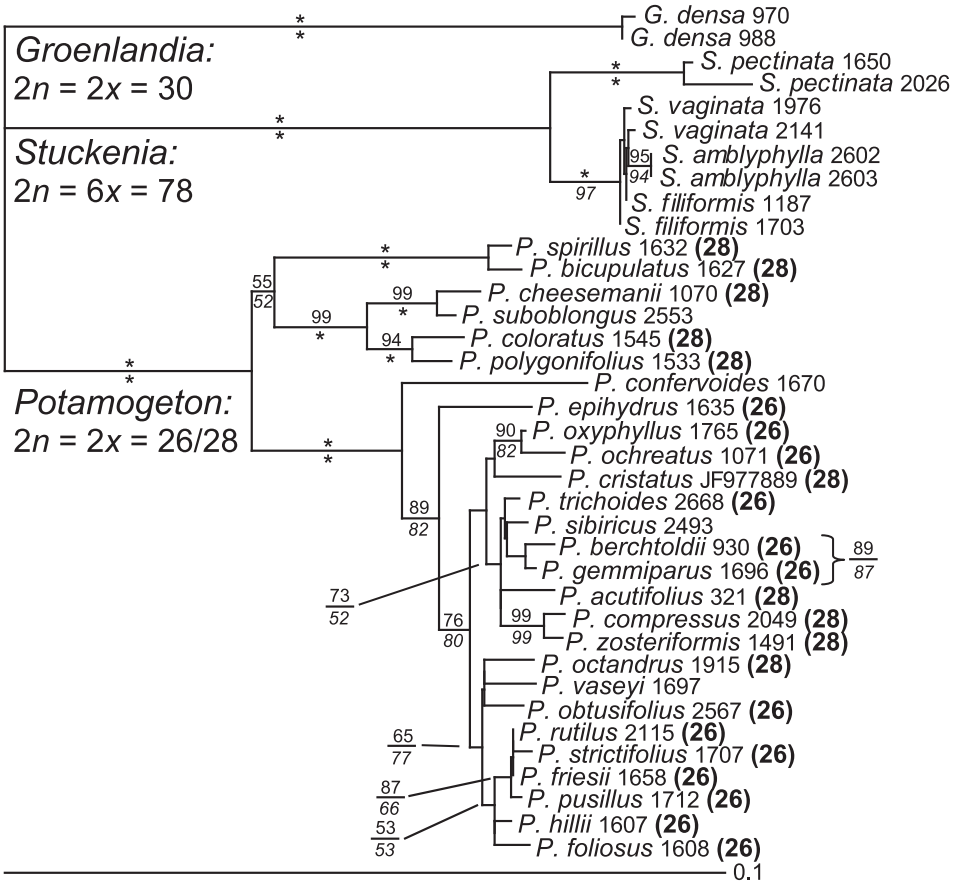
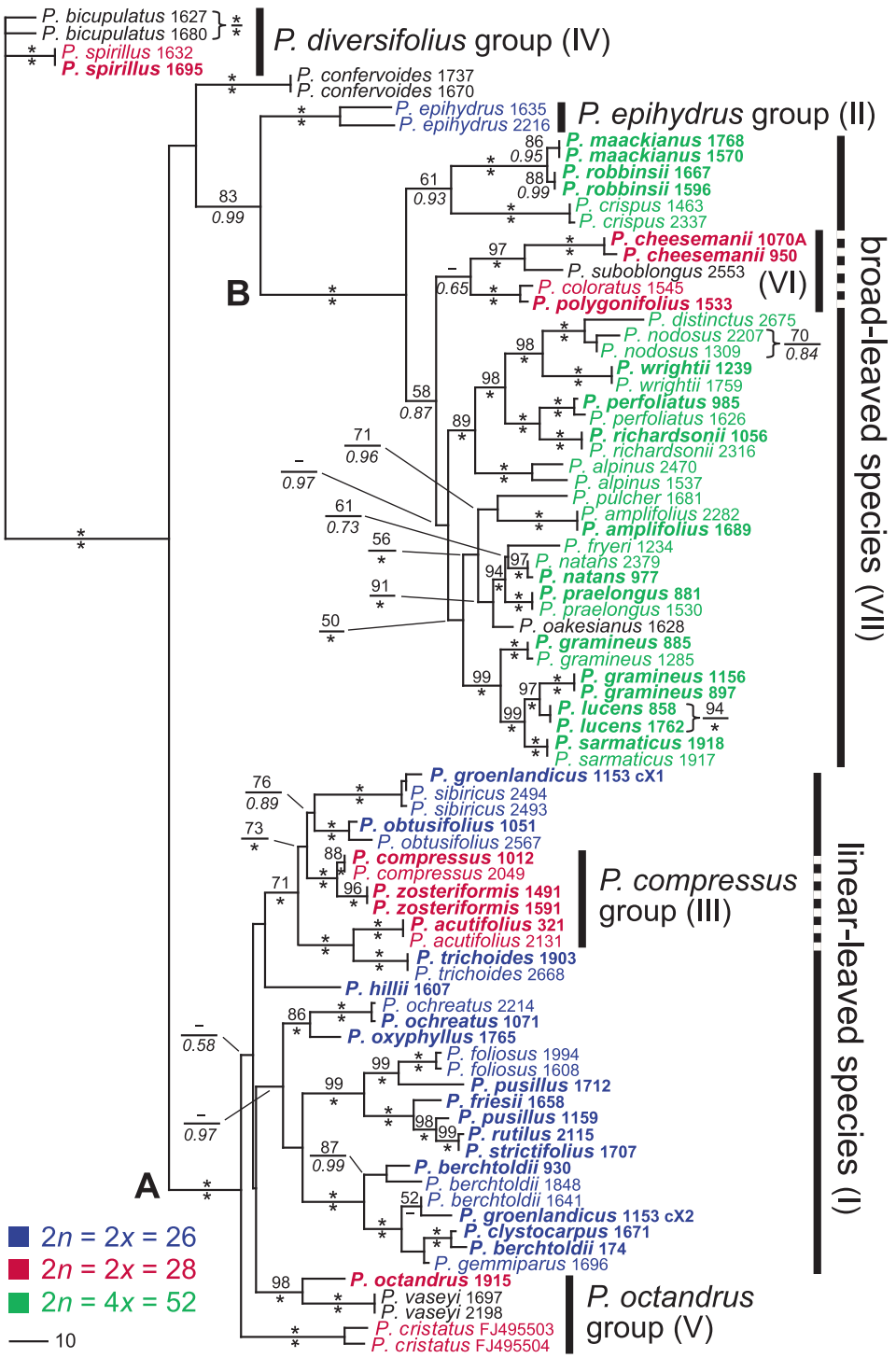


Fig. 4. – Phylogenetic analysis of the three genera of *Potamogetonaceae* based on ITS sequences. The Neighbor joining tree is shown with bootstrap values above branches; bootstrap support for the Maximum parsimony analysis (based on 239 parsimony informative characters incl. coded indels; resulting in 32 equally parsimonious trees of length 413; ci=0.828; ri=0.948) is shown below branches; 100% support is indicated by an asterisk (*). For *Potamogeton*, the main chromosome counts for the respective species are indicated in brackets. All tetraploid species fall into the large clade at the bottom consisting of species with 26 or 28 chromosomes (data not shown). For details about the accessions, see Appendix 1.

included in their data analyses. A review and critical evaluation of the published counts is therefore an inevitable prior step to drawing evolutionary implications.

Reliability of published chromosome records and sources of errors

The main problems in cytotaxonomic studies are erroneous chromosome counts recorded in the literature and incorrect identification of the plants studied (Stace 2000). As karyological investigations are mostly conducted as independent studies of various authors, a critical analysis of the previously obtained data is necessary (Guerra 2000, Mansanares et al. 2002).


P. bicupulatus 1627
P. bicupulatus 1680
P. spirillus 1632
P. spirillus 1695
P. diversifolius group (IV)
P. confervoides 1737
P. confervoides 1670
P. epihydrus 1635
P. epihydrus 2216
P. epihydrus group (II)
P. maackianus 1768
P. maackianus 1570
P. robbinsii 1667
P. robbinsii 1596
P. crispus 1463
P. crispus 2337
P. cheesemanii 1070A
P. cheesemanii 950
P. suboblongus 2553
P. coloratus 1545
P. polygonifolius 1533
(VI)
B
P. distinctus 2675
P. nodosus 2207
P. nodosus 1309
P. wrightii 1239
P. wrightii 1759
P. perfoliatus 985
P. perfoliatus 1626
P. richardsonii 1056
P. richardsonii 2316
P. alpinus 2470
P. alpinus 1537
P. pulcher 1681
P. amplifolius 2282
P. amplifolius 1689
P. fryeri 1234
P. natans 2379
P. natans 977
P. praelongus 881
P. praelongus 1530
P. oakesianus 1628
P. gramineus 885
P. gramineus 1285
P. gramineus 1156
P. gramineus 897
P. lucens 858
P. lucens 1762
P. sarmaticus 1918
P. sarmaticus 1917
broad-leaved species (VII)
P. groenlandicus 1153 cX1
P. sibiricus 2494
P. sibiricus 2493
P. obtusifolius 1051
P. obtusifolius 2567
P. compressus 1012
P. compressus 2049
P. zosteriformis 1491
P. zosteriformis 1591
P. acutifolius 321
P. acutifolius 2131
P. trichoides 1903
P. trichoides 2668
P. compressus group (III)
P. hillii 1607
P. ochreatus 2214
P. ochreatus 1071
P. oxyphyllus 1765
P. foliosus 1994
P. foliosus 1608
P. pusillus 1712
P. friesii 1658
P. pusillus 1159
P. rutilus 2115
P. strictifolius 1707
P. berchtoldii 930
P. berchtoldii 1848
P. berchtoldii 1641
P. groenlandicus 1153 cX2
P. clystocarpus 1671
P. berchtoldii 174
P. gemmiparus 1696
linear-leaved species (I)
A
P. octandrus 1915
P. vaseyi 1697
P. vaseyi 2198
P. cristatus FJ495503
P. cristatus FJ495504
P. octandrus group (V)
2n = 2x = 26
2n = 2x = 28
2n = 4x = 52
10

Literature review indicates extensive variation in chromosome numbers of *Potamogetonaceae*. However, Hollingsworth et al. (1998) has already demonstrated that many chromosome counts given in chromosome indices are erroneous and that some of them were even never made. The comparison of our data, which show both a systematic and phylogenetic pattern (see above), with that in the literature indicate that many of the published records are highly doubtful. For example, Wiegand (1899) in his early study records 2n = 14 for *Potamogeton foliosus*. However, this count has never been confirmed, whereas there are six later counts of 2n = 26 for this species, which is consistent with the number of chromosomes in all species in the respective morphological group (I in Table 1) and with the numerous counts for closely related species with which *P. foliosus* forms a strongly supported monophyletic clade (Fig. 5). Another example is *P. perfoliatus*, which belongs to a morphological group of broad-leaved species (VII in Table 1) and also to a highly supported clade (Fig. 5) where all closely related species have 2n = 52, which can be considered as tetraploids. Thus, at least for significantly supported clusters comprising of several taxa with 52 chromosomes, speciation has taken place at the tetraploid level. This is also in keeping with the general observation that direct sequence reads of ITS and 5S-NTS of tetraploid species of *Potamogeton* (and hexaploid *Stuckenia*, see below) do not show any indications of hybrid origin (in contrast to hybrids whose identifications are based on the same markers). The count 2n = 52 for *P. perfoliatus* was established on 18 samples of this species. In addition, *P. perfoliatus* was involved in 8 different hybrids studied here all of which have karyotypes that are consistent with this chromosome number. In the light of these observations, the existence of counts such as 2n = 26 recorded for *P. perfoliatus* in the literature is highly improbable. A third example concerns the genus *Stuckenia*. Our data indicate that all species (and hybrids) of this genus have 2n = 78 (Table 1), which can be considered as the hexaploid level within the *Potamogetonaceae*. This is also the most frequently recorded number in the literature. The ITS tree (Fig. 4) shows that *Stuckenia* forms a separate lineage and that speciation in this genus took place entirely at the hexaploid level. Singular anomalous counts such as 2n = 42 recorded for *S. pectinata* are therefore considered here as erroneous. Many other dubious and obviously incorrect records are discussed above under the particular species. It should be noted that the majority of the doubtful counts come from only a few authors. Generally speaking, all solitary unusual counts not confirmed by other researchers should be treated with caution, particularly if other authors repeatedly record a different number.

◀ Fig. 5. – Phylogenetic analysis of diploid and tetraploid species of *Potamogeton* based on 5S-NTS sequences. One of 30 equally parsimonious trees is shown (based on 265 parsimony informative characters incl. coded indels; tree length 1097; ci=0.500; ri=0.869) with bootstrap support above branches; posterior probabilities from Bayesian analysis are given below branches. Confidence values of 100% / 1.00 are indicated by asterisks (*). Chromosome numbers of particular species are given in different colours; the number for *P. sibiricus* was inferred from the hybrid origin of the sample of *P. groenlandicus* analysed (see text); the individuals that had their chromosomes counted in this study are in bold. Species names in black indicate that currently there are no known chromosome numbers for these species. Assignments of morphological species groups correspond to Table 1. A and B designate species-rich clades that are characterized by basal polytomies, which may indicate rapid speciation. For details about the accessions, see Appendix 1.

The chromosomes of *Potamogetonaceae* are very small, often dot-like, ranging from ca. 0.8–2 μm in length. Their size frequently differs even within a single cell and the smallest may be less than a half of the size of the biggest. In diploid linear-leaved species (see comments under *P. acutifolius*, *P. berchtoldii*, *P. compressus*, *P. foliosus* and *P. pusillus*) we observed an occasional occurrence (i) of satellites on big chromosomes, which are usually approximately of the same size as the smallest chromosomes (Figs 1i & 3a–e) and (ii) of massive chromosomes with conspicuous centromeres (Figs 3b & e). Particularly in the case of rupture of these unusually shaped big chromosomes or of separation of the satellites during preparation, these detached bodies can be easily misinterpreted by an unexperienced eye as additional chromosomes in a karyotype with 2n = 26. In contrast, two of the small chromosomes in a sample with 2n = 28 may be overlooked in low quality preparations. Several species for which we identified 2n = 28 were previously treated as species with 26 chromosomes and vice versa, which may be to a large degree explained by technical difficulties caused by the karyotype. To establish exact chromosome numbers at higher ploidy levels is even more difficult. The very small and rather numerous chromosomes, which greatly increases the potential for making errors during counting, stimulated many authors to comment that *Potamogeton* is a genus difficult to investigate cytologically (e.g. Kalkman & Van Wijk 1984, Hollingsworth et al. 1998, Preston et al. 1998, Wang et al. 2007, Wan et al. 2012). Thus, distinguishing genuine variation in chromosome numbers from artefacts is particularly difficult in this family (Hollingsworth et al. 1998). Only repeated observations of several clear well-spread metaphase preparations can provide unambiguous counts. In the light of this experience we are sceptical about the recorded occurrence of aneuploid variation and the potential to distinguish actual aneuploids from inexact interpretations of inadequate cytological preparations. Several samples that we suspected at first to be aneuploids proved to be euploids in repeated observations of the same material. In spite of the fact that we studied 242 samples in great detail (see Material and methods), we did not detect any unequivocally aneuploid plants.

The *Potamogetonaceae* are taxonomically difficult and our studies of herbarium material indicate frequent misidentifications, sometimes even with phenotypically similar plants of other families (such as *Callitrichaceae*, *Zannichelliaceae*, *Polygonaceae* and *Hydrocharitaceae*). Unfortunately, we were not able to examine voucher specimens for the majority of the doubtful records (for many records no vouchers were indicated and may not even have been prepared; for others the vouchers are not available on loan from the respective herbaria, especially from institutions in Asia), but those few herbarium vouchers that we could inspect revealed several incorrectly identified specimens (see under *Potamogeton alpinus*, *P. polygonifolius*, *P. zosteriformis*, *Stuckenia pectinata* and *S. vaginata*). These cases are probably not exceptional, but may represent the tip of an iceberg. Chromosome counts recorded from continents where the respective species are unknown to occur (see under *Potamogeton compressus* and *P. rutilus* for examples) indicate additional taxonomic confusions. A further source of mistakes is the misidentification of interspecific hybrids as species. Hybrids are in some regions more common than previously assumed (Kaplan 2005a, 2007, 2010a, Kaplan et al. 2009, 2011, Zalewska-Gałosz et al. 2009, Du et al. 2010, Kaplan & Uotila 2011, Zalewska-Gałosz & Ronikier 2012, Kaplan & Fehrer 2013) and their identification is often very difficult and may require the aid of molecular techniques (Kaplan et al. 2009, 2011, Zalewska-Gałosz & Ronikier 2010, 2011, Kaplan & Fehrer 2011, 2013). It is therefore possible that some doubtful counts

recorded for species were actually established on unrecognized hybrid plants. This is supported by the fact that some chromosome numbers reported in the literature for species (2n = 27, 40, 65) were confined to hybrids in this study.

Some counts, although published as exact, may actually have been only estimated to fit previously published records rather than counted. This is illustrated by *P. polygonifolius*. Although three older records independently indicated 2n = 26, recent careful examination of samples from four populations conducted by two different teams in different countries gave unambiguous counts of 2n = 28. These were indirectly supported by two heteroploid hybrids whose intermediate chromosome numbers showed that the second parent must have had a karyotype with 28 chromosomes (see under *P. polygonifolius* for details). We suspect that the former counts were only approximate, established on the mistaken belief in the older literature that all *Potamogeton* diploids have 2n = 26. The case of one sample of "*P. alpinus*" from Spain (see the discussion under *P. polygonifolius* above) clearly shows that the temptation to fit actual observations to expected chromosome counts may sometimes be high. In order to exclude this type of error a priori, our counts for this study were mostly conducted without prior knowledge of the identity of the karyotyped plants (see Methods).

In summary, the chromosome counts established by this study in combination with a critical and thorough analysis of the original literature indicate that the within-species variation in chromosome number is much lower than one would expect from the published records. Based on the available data, we estimate that about 24% of the chromosome records published for *Potamogetonaceae* in original publications and up to about 41% of the records listed in secondary chromosome compilations (chromosome atlases and indices) are erroneous. Only when these erroneous records are filtered out to minimize the risk of misleading conclusions, will the resulting reliable and confirmed data be suitable for drawing evolutionary implications.

Chromosome base number and evolution of chromosome number

The identification of the base chromosome number is fundamental for understanding the ploidy level of its members and the relationships between them. There is currently no consensus on the base number in *Potamogetonaceae*. As Lindqvist et al. (2006) point out, varying classification, incorrect identification of plants, technical difficulties in counting small chromosomes and use of misleading sources of chromosome counts are the potential problems in studies on chromosome evolution in *Potamogetonaceae*.

Stern (1961) and Haynes (1974) have interpreted the chromosome numbers 2n = 26 or 28 to represent the diploid level in *Potamogeton*, from which one has been derived by aneuploidy. In contrast, Goldblatt (1979) suggested that the base number in *Potamogetonaceae* is likely to be x = 7. This is accepted by Les (1983) who also recognizes 2n = 14 as the diploid level in *Potamogeton*. The main reason for this conclusion is the record of 2n = 14 for *P. foliosus* by Wiegand (1899). In this concept, however, *P. foliosus* would be the only extant diploid species in the family, all other species would be polyploids and the count 2n = 26, which is the number recorded in approximately 36% of *Potamogeton* species, would correspond to the hypotetraploid level. However, this old record has never been confirmed and is here considered as erroneous (see under *P. foliosus* for details). There is only one more record of n = 7 in *Potamogetonaceae*, which comes

from an early study by Takusagawa (1939) of *P. perfoliatus*. This count is even more improbable considering the fact that *P. perfoliatus* belongs to a group of tetraploid (2n = 52) species (see also the discussion above). In the light of these data, x = 7 as the base number in *Potamogeton* is unsubstantiated.

The great majority of our counts are multiples of x = 13, which occurs in about 81% of all species and in two of the three genera, *Potamogeton* and *Stuckenia*. Consequently, plants with 26, 39, 52, 78 and 104 somatic chromosomes would correspond to diploids, triploids, tetraploids, hexaploids and octoploids, respectively. In *Potamogeton*, diploids with 2n = 26 and tetraploids with 2n = 52 are the most frequent as they occur in altogether 78% of the species analysed. The secondary number x = 14 occurs only within *Potamogeton* where it is confined to four species-poor groups (III–VI in Table 1). If x = 13 is considered to be the base chromosome number, according to Fig. 5, about 3–7 independent origins of x = 14 through aneuploid events from species with x = 13 have to be assumed (exact estimates of such transitions cannot be made based on the tree, because the relationships among the basal lineages within clades A and B are largely unresolved). Our observations of two additional satellites of the size of normal chromosomes in several diploid species with 26 chromosomes may give a hint how such transitions could have happened in the past. *Groenlandia* is unique with x = 15 and may have evolved from an ancestral x = 13 diploid through two such events. In contrast, *Stuckenia* may have originated from a diploid ancestor by two subsequent rounds of polyploidization.

An alternative scenario of chromosome number evolution suggests 2n = 28 as the base number. This view is supported by the fact that this count occurs in three of the most divergent *Potamogeton* clades in both trees (Figs 4 and 5) and could therefore be viewed as the relict ancestral constitution. Under this assumption, the transition from x = 14 to either x = 15 (*Groenlandia*) or to x = 13 (others) would require only one step in either direction. These events could have resulted in reproductive isolation and may have triggered speciation and polyploidization in various ways: Chromosome fusion in the lineage leading to *Stuckenia* may have caused problems in meiosis that were overcome by immediate polyploidization (in that case by two rounds, resulting in the hexaploid level) to restore fertility. Within *Potamogeton*, two strongly supported species-rich clades (A and B in Fig. 5) are characterized by unresolved bases that suggest rapid speciation of the respective lineages. A single transition to 26 chromosomes in clade A, a number which is now found in the majority of the linear-leaved species, may have prompted extensive speciation, and incomplete lineage sorting (e.g. Degnan & Rosenberg 2009, Krak et al. 2013) may explain the non-monophyletic distribution of chromosome numbers within clade A. Similarly, an independent transition to 2n = 26 in clade B may have occurred in the ancestor of the *P. polygonifolius* group (which is identified as monophyletic using the ITS marker, see Fig. 4) and was followed by polyploidization to the tetraploid level and by extensive speciation of the broad-leaved species. A third transition to x = 13 then led to the *P. epihydrus* group.

As the chromosome number of *P. confervoides*, which constitutes another divergent lineage within *Potamogeton* based on both markers (Figs 4 and 5), is unknown and too little information about mechanistic pathways of chromosome fusion or fission in this group is currently available, it is not possible at this point to draw definitive conclusions about the base chromosome number of this family. However, we can clearly refute x = 7 as a base number and establish x = 13 and x = 14 as the main diploid karyotypes in the family between which multiple transitions must be assumed for each scenario. In addition, our

data clearly show that whole genome duplication is a rare event in *Potamogetonaceae*, in contrast to some published chromosome records. It may have occurred only 2 (–3) times during the evolution of *Potamogetonaceae*, once in the lineage leading to *Stuckenia* (Fig. 4) and maybe only once early in the evolution of broad-leaved species of *Potamogeton* (clade B in Fig. 5). A very low level of intra-individual polymorphism even in polyploid species shows that ITS repeats are well homogenized, which also suggests ancient origins of polyploidy (Kaplan et al. 2009). These can be readily distinguished from recent, but also relatively rare, autopolyploidization events (2 cases), from occasional sterile addition hybrids (5 cases) and from octoploid allopolyploid species (3 cases).

To conclude, chromosome numbers are generally uniform not only within species but mostly also within morphological groups and clades. From this it is obvious that changes in chromosome number, like aneuploidy and polyploidy, are rare in *Potamogetonaceae* and mostly associated with major evolutionary events.

Descriptions of new hybrids

The samples counted included also several previously unknown interspecific hybrids, which are recorded here for the first time. Besides morphology and molecular data, intermediate chromosome numbers identified in heteroploid hybrids provided additional evidence of their identities. Three of the hybrids that are sufficiently recognized and documented by adequate material are here described and their binomials validated. Detailed results of molecular hybrid identifications will be presented in a separate paper (J. Fehrer & Z. Kaplan, in prep.).

Potamogeton ×drepanoides Z. Kaplan, **nothosp. nova**
(= *P. berchtoldii* × *P. oxyphyllus*)

Type: Japan, Honshu, Hiroshima Prefecture (Hiroshima-ken): water reservoir in Yamanaka park at University campus SW of city centre of Higashi-Hiroshima, 34°24'12"N, 132°43'09"E, alt. 234 m (coll. L. Adamec 4 X 2000), cult. as Z. Kaplan 1238, pressed 1 VIII 2001 (holotype: PRA; isotypes: BM, GH, K, KRA, KYO, PR, PRA, S, TI, W).

Stem richly branched, with long basal stolons producing new vertical shoots at nodes; nodal glands present on most nodes; turions axillary, shoot-like but densely foliated, 35–67 mm long, with 8–13 leaves. Submerged leaves sessile, linear, often asymetrically turned to one side, 34–92 mm long, 1.1–1.8 mm wide, 26–54 times as long as wide, 3-veined, sometimes with up to 6 inconspicuous and interrupted sclerenchymatous strands, with 1–2 rows of lacunae on each side of the midrib, entire at margins, acute to acuminate at the apex. Floating leaves absent. Stipules axillary, convolute, 7–11 mm long. Inflorescences terminal; peduncle 13–28 mm long; spike 4–6 mm long. Flowers 3–5, with 4 abortive carpels. Fruits not produced (plants sterile). Fig. 6.

This hybrid resembles a slender narrow-leaved form of *P. oxyphyllus*, with which it was originally confused until DNA analyses showed it to be a hybrid (J. Fehrer & Z. Kaplan, unpublished data). The leaves are often asymetrically turned to one side, as in *P. oxyphyllus*, whereas leaves of *P. berchtoldii* are ± strait or only dorsally recurved. The venation of the leaves of the hybrid is more similar to that of *P. berchtoldii* as it shows only 3 veins, but sometimes they exhibit some inconspicuous and interrupted sclerenchymatous strands, which are a vestige of often more numerous veins and sclerenchymatous



Fig. 6. – Holotype of *Potamogeton* ×*drepanoides* (= *P. berchtoldii* × *P. oxyphyllus*).

strands that occur in leaves of typical broad-leaved plants of *P. oxyphyllus*. Turions of *P.* ×*drepanoides* are less morphologically differentiated from normal shoots than those in *P. berchtoldii*; they are longer, with numerous leaves, hence resembling short axillary shoots, which is a feature that the hybrid shares with *P. oxyphyllus*. In contrast, turions of *P. berchtoldii* are born terminally on the tops of shoots and branches, short, with only 2–4 short leaves, hence dissimilar from normal shoots. The hybrid also differs from both parental species in being consistently sterile, like the great majority of *Potamogetonaceae* hybrids (Hagström 1916, Dandy 1975, Preston 1995, Wiegleb & Kaplan 1998, Kaplan 2001, 2010a, Kaplan & Fehrer 2007, Kaplan et al. 2009).

Etymology: *drepanoides* = sickle-like, curved like a sickle; for sickle-shaped leaves asymetrically turned to one side.

Additional specimens examined (paratypes): JAPAN, Honshu, Hiroshima Prefecture (Hiroshima-ken): water reservoir in Yamanaka park at University campus SW of city centre of Higashi-Hiroshima, 34°24'12"N, 132°43'09"E, alt. 234 m (coll. L. Adamec 4 X 2000), cult. as Z. Kaplan 1238, pressed 27 VIII 2001 (PRA), 29 VIII 2002 (PRA), 23 VII 2003 (PRA), 21 VIII 2005 (PRA), 6 VIII 2007 (PRA), 30 X 2007 (PRA). – JAPAN, Honshu, Hiroshima Prefecture (Hiroshima-ken): fishpond 1 km ENE of University campus SW of city centre of Higashi-Hiroshima, 34°24'23"N, 132°43'47"E, alt. 227 m (coll. L. Adamec 4 X 2000), cult. as Z. Kaplan 1236, pressed 1 VIII 2001 (PRA), 27 VIII 2001 (BM, GH, K, KRA, KYO, PR, PRA, S, TI, W), 29 VIII 2002 (PRA), 23 VII 2003 (PRA), 21 VIII 2005 (PRA), 6 VIII 2007 (PRA), 30 X 2007 (PRA).

Potamogeton ×luxurians Z. Kaplan, **nothosp. nova**
(= *P. amplifolius* × *P. illinoensis*)

Type: USA, Vermont, Orleans Co.: Lake Parker 1 km WSW of West Glover, 44°43'34"N, 72°13'53"W, alt. 396 m, 22 VII 2005, coll. Z. Kaplan & C. B. Hellquist 05/362 (holotype: PRA; isotypes: GH, PRA).

Stem unbranched or sparingly branched. Submerged leaves subsessile to petiolate; lamina oblong to broadly elliptical, sometimes slightly dorsally recurved, 55–188 mm long, 13–57 mm wide, 1.9–5.2 times longer than wide, 13–31-veined, apparently entire at margins or very minutely denticulate on young leaves, with denticles fugacious and absent on older leaves, acute to indistinctly mucronate or subobtuse at the apex; petiole 2–71 mm long, 0.02–0.64 times as long as the lamina. Floating leaves present on adult plants that reach the water surface, petiolate; lamina oblong to elliptical, 72–145 mm long, 24–47 mm wide, 1.8–4.7 times as long as wide, membranaceous to subcoriaceous or coriaceous, 17–33-veined, acute at apex; petiole 12–119 mm long, 0.1–0.8 times as long as the lamina. Stipules axillary, convolute, 17–93 mm long. Inflorescences terminal; peduncle 36–193 mm long; spike 12–35 mm long. Flowers numerous, with 4 abortive carpels. Fruits not produced (plants sterile). Fig. 7.

This hybrid is clearly intermediate between the parents, particularly in the shape and size of submerged leaves. The lamina of the submerged leaves is on average narrower than in *P. amplifolius* (25–75 mm wide, mostly 2–4 times longer than wide) but broader than in *P. illinoensis* (mostly 10–40 mm wide, 4–8 times longer than wide). The lamina of submerged leaves of *P. illinoensis* as well as of *P.* ×*luxurians* is ± strait or slightly dorsally recurved whereas that of *P. amplifolius* is typically strongly arcuate. Another quantitative character that shows intermediate values in *P.* ×*luxurians* is the number of veins in leaves. The hybrid has fewer veins than *P. amplifolius* (mostly 25–37 in submerged leaves and 29–41 in floating leaves) but more numerous veins than *P. illinoensis* (mostly 7–17 in submerged leaves and 13–29 in floating leaves). In contrast to the parental species, the hybrid is sterile and its entire spikes rot after flowering instead of setting fruit.



Fig. 7. – Holotype of *Potamogeton* ×*luxurians* (= *P. amplifolius* × *P. illinoensis*).

Etymology: *luxurians* = luxuriant, characterized by profuse growth; based on the growth of plants in the type population.

Additional specimens examined (paratypes): USA, Vermont, Orleans Co.: Lake Parker 1 km WSW of West Glover, 44°43'34"N, 72°13'53"W, alt. 396 m, cult. as Z. Kaplan 1625, pressed 7 IX 2006 (PRA), 27 VII 2007 (PRA), 26 VIII 2008 (PRA). – USA, Massachusetts, Berkshire Co.: small pond on north side of Thomas Island Road at Lake Onota, Pittsfield, 42°28'52"N, 73°16'23.4"W, 18 VIII 2009, coll. C. B. Hellquist 17158 & J. Garrett (PRA). – USA, Massachusetts, Franklin Co.: Leverett Pond, Leverett, 42°27.057'N, 72°30.137'W, alt. 417 ft., 18 VI 2009, coll. C. B. Hellquist 17117, A. Bobrov & E. Chemeris (PRA).

Hagström (1916) describes *P. scoliophyllus* as a hybrid *P. amplifolius* × *P. illinoensis* based on three collections. The first collection, from Michigan, USA, is preserved in S; its duplicates (isosyntypes) are preserved in GH and NY. These plants are morphologically consistent with *P. illinoensis*, the shape and size of leaves correspond to this species, as well as the mucronate leaf apices and the numbers of veins in floating (15–17) and submerged leaves (11). Also, the stem anatomy agrees with that of *P. illinoensis*. The two remaining collections are from Canada and preserved in C. The specimen from Quebec is rather typical *P. illinoensis* with 11–13-veined submerged leaves and that from Ontario has submerged leaves with 11–17 veins and is also consistent with the morphology of *P. illinoensis*. In fact, Hagström (1916) himself comments that his taxon is more similar morphologically and anatomically to *P. illinoensis* than intermediate. The protologue indicates that the plant from Michigan was the main basis for the description of the taxon; the specimen was annotated by Hagström on 17th February 1900 and 9th October 1907, i.e. prior to the publication of its name, its morphology is in agreement with the description given in the protologue, and the specimen is therefore selected here for lectotypification of the name (the herbarium sheet was labelled accordingly by ZK in 2007):

Potamogeton scoliophyllus Hagstr., Kungl. Svenska Vetenskapsakad. Handl. 55/5: 164. 1916. Type: "Herb. Thomas Morong, *Potamogeton lucens* L., …, In lacu "Bar" ad Manistee, Michigan, U.S.A., Leg. T. M[orong], Aug. 18, 1882" (**lectotype**: S, **designated here**).

Potamogeton ×serrulifer Z. Kaplan, **nothosp. nova**
(= *P. crispus* × *P. schweinfurthii*)

Type: Italy, Tuscany, prov. Siena: water reservoir 1.1 km ENE of San Fabiano farm, 1.6 km NE of Monteroni d'Arbia, 43°14'33.7"N, 11°26'11.3"E, alt. 180 m, cult. as Z. Kaplan 1953, pressed 16 IX 2009 (holotype: PRA; isotypes: BM, PRA).

Stem unbranched or rarely only sparingly branched above. Submerged leaves sessile, linear-oblong to oblanceolate, (32–) 52–122 (–137) mm long, (4–) 6–10 (–14) mm wide, (6–) 9–15 (–17) times longer than wide, 5–7-veined, very finely but distinctly undulate along margins, denticulate to serrulate at margins, acute to shortly mucronate at apex. Floating leaves absent. Stipules axillary, convolute, 9–20 (–41) long, acute, delicate, gradually disappearing. Inflorescences terminal; peduncle 20–58 mm long; spike 9–15 mm long. Flowers numerous, with 4 abortive carpels. Fruits not produced (plants sterile). Fig. 8.

At first sight, this hybrid resembles slender plants of *P. schweinfurthii* and it may be difficult to distinguish them. The typical robust plants of *P. schweinfurthii* mostly have longer (up to 250 mm) and wider (up to 28 mm) leaves with more veins (mostly 7–9). While the leaves of *P. schweinfurthii* are only minutely denticulate to almost entire at margins and



Fig. 8. – Holotype of *Potamogeton* ×*serrulifer* (= *P. crispus* × *P. schweinfurthii*)

± flat, those of *P.* ×*serrulifer* are denticulate to serrulate at margins and finely but distinctly undulate along margins, which are clearly characters inherited from *P. crispus*. The leaves of *P.* ×*serrulifer* are more abruptly contracted to the apex than those of *P. schweinfurthii*, which has leaves distinctly mucronate or acuminate at the apex. Stipules of the hybrid are smaller, delicate and gradually disappear whereas those of *P. schweinfurthii* are more robust (20–95 mm long) and persistent. The inflorescence of *P.* ×*serrulifer* is also smaller than that of *P. schweinfurthii*, which has a 35–250 mm long peduncle and a 40–92 mm long spike at fruiting. The other parent, *P. crispus*, can be easily distinguished by its linear to linear-oblong leaves, which are serrate and usually strongly undulate at the margins, and by its characteristic pattern of venation that is unlikely to be confused with that of any other species. The leaves of *P. crispus* usually have two veins on each side of the midrib, with the outer pair of veins being faint and running close along the margins and the inner pair being more prominent and running longitudinally at approximately $^1/_2$–$^2/_3$ of the distance between the midrib and the margins. In contrast, the leaves of *P.* ×*serrulifer* have veins more regularly and evenly spaced, like *P. schweinfurthii*. Further characters that distinguish *P. crispus* from the hybrid include small (4–12 mm long) and early decaying stipules and a small number of flowers (3–8). In contrast to the parental species, the hybrid is sterile and the entire spikes rot after flowering instead of setting fruit.

Etymology: *serrulifer* = bearing small serrations, finely serrulate; refers to the margins of the leaves.

Additional specimens examined (paratypes): ITALY, Tuscany, prov. Siena: water reservoir 1.1 km ENE of San Fabiano farm, 1.6 km NE of Monteroni d'Arbia, 43°14'33.7"N, 11°26'11.3"E, alt. 180 m, 12 VIII 2008, coll. Z. Kaplan, L. Lastrucci, F. Frignani & B. Foggi 08/612 (PRA); material from the type locality, cult. as Z. Kaplan 1953, pressed 27 VII 2009 (PRA), 10 VIII 2009 (PRA).

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Souhrn

Karyologickou variabilitu v čeledi rdestovité (*Potamogetonaceae*) jsme podrobili revizi a zhodnotili z pohledu taxonomie a evoluce. Pro 47 druhů a 32 kříženců pocházejících z různých koutů světa jsme stanovili chromozomové počty, přičemž pro 11 druhů a 28 kříženců se jedná o první karyologické údaje. Tři poprvé rozpoznaní kříženci jsou v článku platně popsáni. Dále jsme analyzovali literární údaje, mezi nimiž jsme zjistili poměrně velké množství chyb. Naše pozorování nepotvrdila udávanou vysokou míru vnitrodruhové variability v počtu chromozomů. Právě naopak, chromozomový počet u taxonů čeledi *Potamogetonaceae* se zdá být relativně stabilní znak, který je specifický nejen pro jednotlivé druhy (výjimkou je pouze ojedinělý vznik sterilních autotriploidních rostlin v populacích fertilních diploidů), ale i pro celé skupiny druhů, a do jisté míry i pro celé klady ve fylogenetické rekonstrukci. Druhově nejbohatší rod *Potamogeton* obsahuje dvě základní čísla (x = 13 a x = 14) a druhy na třech

ploidních úrovních (diploidy, tetraploidy a oktoploidy; všechny polyploidní cytotypy jsou odvozeny od počtu x = 13). Rod *Stuckenia* zahrnuje výhradně hexaploidy (rovněž odvozené od x = 13) zatímco monotypický rod *Groenlandia* se vyznačuje unikátním základním chromozomovým číslem x = 15. Na základě revidovaných počtů chromozomů a fylogenetické analýzy jsme se pokusili identifikovat základní chromozomové číslo a evoluci karyotypu. Předložili jsme dva alternativní scénáře evoluce čeledi, se základním chromozomovým číslem x = 13 nebo x = 14, přičemž každý předpokládá několik aneuploidních změn mezi těmito karyotypy a jednu změnu k číslu x = 15. Hypotéza některých autorů, kteří pokládají za základní číslo x = 7, byla založena na mylných předpokladech a je zde odmítnuta. Zásadní změny genomu jako jsou aneuploidie a polyploidie jsou většinou svázány s významnými evolučními událostmi. Skupina tetraploidů v rodu *Potamogeton* vznikla jako důsledek jedné nebo několika málo polyploidizací, zatímco dvě následné polyploidizace vysvětlují vznik rodu *Stuckenia*, kde se veškerá speciace odehrála na hexaploidní úrovni. Tři oktoploidní druhy rodu *Potamogeton* jsou alopolyploidního původu.

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Appendix 1. – Origin of the accessions used in the molecular analyses and their GenBank accession numbers. Reference numbers in bold indicate that the chromosomes of that particular sample were counted. Some ITS data are from our previous publications; accession numbers KF270900–KF271010 are new for this study. For *P. groenlandicus* 5S-NTR, two cloned sequences corresponding to the parental species are given. Sequences of *P. cristatus* were adopted from GenBank.

Species	Country	Reference number / Isolate	GenBank accession numbers	
			ITS	5S-NTS
G. densa	Switzerland	**970**	KF270900	
	Austria	**988**	KF270901	
P. acutifolius	Czech Republic	**321**	KF270902	KF270930
	Sweden	2131		KF270931
P. alpinus	Czech Republic	1537		KF270932
	Russia	2470		KF270933
P. amplifolius	USA	**1689**		KF270934
	USA	2282		KF270935
P. berchtoldii	Russia	**174**		KF270936
	Czech Republic	**930**	KF270903	KF270937
	USA	1641		KF270938
	Japan	1848		KF270939
P. bicupulatus	USA	1627	FJ151203	KF270940
	USA	1680		KF270941
P. cheesemanii	New Zealand	**950**		KF270942
	New Zealand	**1070A**	KF270904	KF270943
P. clystocarpus	USA	**1671**		KF270944
P. coloratus	Austria	1545	HQ263487	KF270945
P. compressus	Sweden	**1012**		KF270946
	Russia	2049	KF270905	KF270947
P. confervoides	USA	1670	KF270906	KF270948
	USA	1737		KF270949
P. crispus	Czech Republic	1463		KF270950
	South Korea	2337		KF270951
P. cristatus				FJ495503
				FJ495504
	China		JF977889	
P. distinctus	India	2675		KF270952
P. epihydrus	USA	1635	FJ151206	KF270953
	USA	2216		KF270954
P. foliosus	USA	1608	KF270907	KF270955
	USA	1994		KF270956
P. friesii	USA	**1658**	KF270908	KF270957
P. fryeri	Japan	1234		KF270958
P. gemmiparus	USA	1696	KF270909	KF270959
P. gramineus	Czech Republic	**885**		KF270960
	Czech Republic	**897**		KF270961
	France	**1156**		KF270962
	France	1285		KF270963
'*P. groenlandicus*'	Greenland	**1153**		KF270964 (sib)
= *P. sibiricus* × *P. berchtoldii*				KF270965 (ber)
P. hillii	USA	**1607**	KF270910	KF270966
P. lucens	Netherlands	**858**		KF270967
	Japan	**1762**		KF270968
P. maackianus	China	**1570**		KF270969
	Japan	**1768**		KF270970
P. natans	Switzerland	**977**		KF270971
	Hungary	2379		KF270972

P. nodosus	France	1309		KF270973
	USA	2207		KF270974
P. oakesianus	USA	1628		KF270975
P. obtusifolius	Canada	**1051**		KF270976
	Denmark	2567	KF270911	KF270977
P. ochreatus	New Zealand	**1071**	GU814250	KF270978
	Australia	2214		KF270979
P. octandrus	Japan	**1915**	KF270912	KF270980
P. oxyphyllus	Japan	**1765**	KF270913	KF270981
P. perfoliatus	Austria	**985**		KF270982
	USA	1626		KF270983
P. polygonifolius	Czech Republic	**1533**	HQ263525	KF270984
P. praelongus	Germany	**881**		KF270985
	Italy	1530		KF270986
P. pulcher	USA	1681		KF270987
P. pusillus	Czech Republic	**1159**		KF270988
	USA	**1712**	KF270914	KF270989
P. richardsonii	Canada	**1056**		KF270990
	USA	2316		KF270991
P. robbinsii	USA	**1596**		KF270992
	USA	**1667**		KF270993
P. rutilus	Finland	**2115**	KF270915	KF270994
P. sarmaticus	Russia	1917		KF270995
	Russia	**1918**		KF270996
P. sibiricus	Russia	2493	KF270916	KF270997
	Russia	2494		KF270998
P. spirillus	USA	1632	FJ151214	KF270999
	USA	**1695**		KF271000
P. strictifolius	USA	**1707**	KF270917	KF271001
P. suboblongus	New Zealand	2553	KF270918	KF271002
P. trichoides	Czech Republic	**1903**		KF271003
	India	2668	KF270919	KF271004
P. vaseyi	USA	1697	KF270920	KF271005
	USA	2198		KF271006
P. wrightii	Japan	**1239**		KF271007
	Japan	1759		KF271008
P. zosteriformis	Canada	**1491**	KF270921	KF271009
	USA	**1591**		KF271010
S. amblyphylla	Tajikistan	2602	KF270922	
	Tajikistan	2603	KF270923	
S. filiformis	Switzerland	**1187**	KF270924	
	USA	**1703**	KF270925	
S. pectinata	USA	**1650**	KF270926	
	India	**2026**	KF270927	
S. vaginata	USA	1976	KF270928	
	Russia	**2141**	KF270929	