

Chromosome studies in the aquatic monocots of Myanmar: A brief review with additional records

Yu Ito[‡], Nobuyuki Tanaka[§]

[‡] University of Canterbury, Christchurch, New Zealand

[§] Kochi Prefectural Makino Botanical Garden, Kochi, Japan

Corresponding author:

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Abstract

Myanmar (Burma) constitutes a significant component of the Indo-Myanmar biodiversity hotspot, with elements of the Indian, the Indochina, and the Sino-Japanese floristic regions, yet thus far only a few reliable sources of the country's flora have been available. As a part of a contribution for the floristic inventory of Myanmar, since it is important in a floristic survey to obtain as much information as possible, in addition to previous two reports, here we present three more chromosome counts in the aquatic monocots of Myanmar: *Limnocharis flava* with $2n = 20$, *Sagittaria trifolia* with $2n = 22$ (Alismataceae), and *Potamogeton distinctus* × *P. nodosus* with $2n = 52$ (Potamogetonaceae); the third one is new to science. A brief review of cytological researches in the floristic regions' 45 non-hybrid aquatic monocots plus well investigated two inter-specific hybrids that are recorded in Myanmar is given, indicating that the further works with a focus on species in Myanmar that has infra-specific chromosome variation in the floristic regions will address the precise evolutionary history of the aquatic flora of Myanmar.

Keywords

Aquatic plants, chromosome counts, *Limnocharis*, Myanmar, *Potamogeton*, *Sagittaria*

Introduction

With its wealth of plant diversity, Myanmar (Burma) constitutes a significant component of the Indo-Myanmar biodiversity hotspot with elements of the India, the Indochina, and the Sino-Japanese floristic regions (ca. 13,500 vascular plants: Van Dijk et al. 2004; Tanaka 2010). Yet, while neighboring countries' floristic diversity has been exposed through international projects, such as Flora of China, Flore du Cambodge, du Laos et du Vietnam, and Flora of Thailand, thus far no reliable sources of Myanmar's flora have been published except a checklist of spermatophytes contributed by Kress et al. (2003). In order to revise the flora of Myanmar, a decade-long continuous inventory has been conducted by

Japanese botanists (Tanaka 2005), which thus far partly contributed a local checklist (Mt. Popa: Tanaka et al. 2006) and a taxon-specific checklist (aquatic plants: Ito and Barfod 2014).

The aim of floristic research is not only to count the total number of species but also to evaluate the native flora's evolutionary origins by comparing with related floristic regions. From this aspect, it is useful to obtain as much information as possible, e.g., chromosome data (Sanders et al. 1983). This is especially important for floristic surveys for aquatic plants, in which infra- or inter-specific chromosome variation is widely known (Les and Philbrick 1993). The proportion of species for which the chromosome number is known is less than 1% in some little-collected tropical areas (Stace 2000), probably including the southeast Asian country of Myanmar.

Aquatic plants, which is polyphyletically evolved in fern and fern allies, basal angiosperms, monocots, and eudicots, is known as having numerous chromosomal variation, thus an excellent model for this aim. Here, in addition to the previous contributions of chromosome counts for new or noteworthy aquatic plants from Myanmar (*Najas tenuis*: Ito et al. 2014b; *Nechamandra alternifolia*: Ito et al. 2009), we present three more chromosome counts for the aquatic monocots of Myanmar: *Limnocharis flava* (Alismataceae), *Sagittaria trifolia* (Alismataceae), and *Potamogeton distinctus* × *P. nodosus* (Potamogetonaceae). A brief review of cytological researches in 45 non-hybrid aquatic monocots plus two well-investigated inter-specific *Potamogeton* hybrids in Myanmar is also given with a broad focus on those distributed in neighboring areas, i.e., the Indian, the Indochina, and the Sino-Japanese floristic regions.

Materials and methods

Chromosome observation

Plant materials of *Limnocharis flava* (Alismataceae), *Sagittaria trifolia* (Alismataceae), *Najas tenuis* (Hydrocharitaceae), *Nechamandra alternifolia* (Hydrocharitaceae), and *Potamogeton distinctus* × *P. nodosus* (Potamogetonaceae) were collected in the expeditions to Myanmar (Bago Division and Shan State) in 2008. The collections were rigorously identified based on morphological characters using the original protologues as well as a previous taxonomic treatment by Cook (1996). *Potamogeton distinctus* × *P. nodosus* (Potamogetonaceae) was identified by DNA barcoding method (Ito et al. 2014a). The first set of the voucher specimens was retained in Forest Department Office, Ministry of Environmental Conservation and Forestry, Union of Myanmar (RAF); the duplicates are deposited in two Japanese herbaria: Makino Botanical Garden (MBK) and the University of Tokyo (TI).

Root tips collected in the field were pretreated with 0.002 M 8-hydroxyquinoline at 4°C in 12h, and fixed with freshly mixed Carnoy's fixative (3 : 1 ethyl alcohol : acetic acid) for at least 30 min, and then preserved at 4°C in 12h. For microscopic observation, root tips were soaked in 1 N HCl for 1 h followed by 10 min at 60°C. After being immersed in tap water,

the materials were stained in a drop of 1.5% orcein acetate solution on a slide glass in 5 min., and then squashed. Then somatic chromosome numbers of the three taxa were obtained by light microscopic examination. For each species, at least two cells were used to confirm the numbers.

Distribution for each species follows Ito and Barfod (2014).

Literature review

Chromosome researches for aquatic monocots of Myanmar were reviewed with a broad focus on Myanmar and related floristic regions, i.e., the Indian, the Indochina, and the Sino-Japanes floristic regions. The focal species include 45 non-hybrid aquatic monocots listed in Ito and Barfod (2014), Ito et al. (2014a) as well as well-investigated two inter-specific *Potamogeton* hybrids (Ito et al. 2014a). Initial literature search was carried out with Fedorov (1969) as well as Index to Plant Chromosome Numbers (Missouri Botanical Garden, <http://mobot.mobot.org/W3T/Search/ipcn.html>), followed by extensive literature review with original references. For some species, mostly cosmopolitan ones, only a few representative literature references are given for each chromosome number. Since a comprehensive cytological review was given for aquatic plants (Les and Philbrick 1993), including almost all the taxa listed in the present study, our literature review focused on literature published in 1993 or later. Due to incapability of original references, some rare chromosome counts are not included; those references are mostly published in 1970 or earlier, and written not in English. No detailed references are given for Potamogetonaceae and *Ruppia* because an exhaustive cytological review was published by Kaplan et al. (2013), Talavera et al. (1993).

Chromosome counts for the aquatic monocots of Myanmar

Order Alismatales

Family Alismataceae

Genus *Limnocharis* Bonpl., 1808

Limnocharis flava (L.) Buchenau, 1868

Material

- a. country: Myanmar; stateProvince: Bago; municipality: Pyat Township; locality: along the roadside, paddy field, ca. 30 km east of Pyat; verbatimLatitude: 18°49'44"N; verbatimLongitude: 95°18'06"E; eventDate: 7 Dec 2008; recordedBy: Y. Ito; collectionID: N. Tanaka & al. 080776; institutionCode: MBK, RAF, TI; occurrenceID: 7FEB0822-ACA8-56D5-BAD4-FF799490C2B4

Distribution: Native to Americas; naturalized to tropical Asia.

Notes: Chromosome counts: $2n = 20$ (Fig. 1; obtained in this study).

Genus *Sagittaria* L., 1753

Sagittaria trifolia L., 1753

Material

- a. country: Myanmar; stateProvince: Shan; verbatimLocality: Pindaya; verbatimLatitude: 20°59'57"N; verbatimLongitude: 96°39'59"E; eventDate: 1 Dec 2008; recordedBy: Y. Ito; collectionID: N. Tanaka & al. 080623; occurrenceID: 8BBD49F3-D906-5165-B975-79EE832DD2C0

Distribution: Bangladesh, Bhutan, China (nationwide), India (nationwide), Indonesia (Borneo, Java, Sulawesi), Japan, Malaysia (Peninsular), Myanmar, Nepal, Pakistan, Philippines, Thailand; Oceania.

Notes: Chromosome counts: $2n = 22$ (Fig. 2; obtained in this study).

Family Hydrocharitaceae

Genus *Najas* L., 1753

Najas tenuis Magnus, 1870

Material

- a. country: Myanmar; stateProvince: Shan; verbatimLocality: Inlay Lake, Nyaung Shwe Township; verbatimLatitude: 20°32'02"N; verbatimLongitude: 96°53'53"E; eventDate: 3 Dec 2008; recordedBy: Y. Ito; collectionID: N. Tanaka & al. 080642; institutionCode: MBK, RAF, TI; occurrenceID: D716C22F-0CA0-5278-86E9-ADAB7EEC44BE

Distribution: India (Central, Southern), Myanmar, Sri Lanka.

Notes: Chromosome counts: $2n = 24$ (Fig. 3; After Ito et al. 2014b; reproduced with publisher's permission).

Genus *Nechamandra* Planch., 1849

Nechamandra alternifolia (Roxb.) Thwaites, 1864

Material

- a. country: Myanmar; stateProvince: Shan; verbatimLocality: Near Yae Aye Kan Dam, Yae Aye Kan, Kalaw Township; verbatimLatitude: 20°35'37"N; verbatimLongitude: 96°31'46"E;

eventDate: 26 Nov 2008; recordedBy: Y. Ito; collectionID: N. Tanaka & al. 080058;
institutionCode: MBK, RAF, TI; occurrenceID: 79D0F260-14BE-5B2C-
A212-278CE0A44FAF

Distribution: Bangladesh, China (Southern), India (Eastern, Northern, Southern), Myanmar, Nepal, Sri Lanka, Thailand, Vietnam, Yemen, and Sudan.

Notes: Chromosome counts: $2n = 16$ (Fig. 4; After Ito et al. 2009; reproduced with publisher's permission).

Family Potamogetonaceae

Genus *Potamogeton* L., 1753

Potamogeton distinctus A. Benn. x *P. nodosus* Poir.

Material

- a. country: Myanmar; stateProvince: Shan; verbatimLocality: Inle Lake; verbatimLatitude: 20°27'28"N; verbatimLongitude: 96°50'37"E; eventDate: 4 Dec 2008; recordedBy: Y. Ito; collectionID: N. Tanaka & al. 080662; institutionCode: MBK, RAF, TI; occurrenceID: 4F231568-A5D5-5572-B0D2-1CFB3401D83F

Notes: Chromosome counts: $2n = 52$ (Fig. 5; obtained in this study). The chromosome count for this taxon is new to science.

Analysis

The chromosome counts given for 45 non-hybrid species of aquatic monocots of Myanmar as well as well-investigated two *Potamogeton* hybrids among them were reviewed with a focus on infra-specific chromosome variation (Table 1). The cited literature references also include chromosome counts obtained from related floristic regions, i.e., the Indian, the Indochina, and the Sino-Japanese floristic regions. For widespread species, cytological information from other regions is cited.

Discussion

Of 45 non-hybrid aquatic monocots and two interspecific hybrids among them, more than two thirds have no chromosome variation. Meanwhile, the following nine species have infra-specific chromosome variation, i.e., *Acorus calamus*, *Cryptocoryne crispata*, *Blyxa echinosperma*, *Hydrilla verticillata*, *Najas graminea*, *Ottelia alismoides*, *Vallisneria spiralis*, *Monochoria hastata*, and *M. vaginalis* (Table 1). Among the cytologically variable aquatic monocots are *A. calamus*, *O. alismoides*, *V. spiralis*, and *M. vaginalis*, for which unique chromosome counts are obtained from each floristic region. Myanmar is known as including borders among the Indian, the Indochina, and the Sino-Japanese floristic regions

(Tanaka 2010), yet in the aquatic flora, it is unknown which flora is more influenced. Future research with a focus on such species will address this issue.

Potamogeton is known as having numerous inter-specific hybrids, and each parental combination is varied from intra-ploidy crosses to inter-ploidy ones (Kaplan et al. 2013). The present study revealed *P. distinctus* × *P. nodosus* as another intra-ploidy hybrid of *Potamogeton* at tetraploid level.

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Author contributions

Conceived and designed the study: YI NT. Collected the samples in the field: YI NT. Performed the chromosome observation: YI. Analyzed the data: YI. Wrote the paper: YI. Corrected and revised manuscript: NT.

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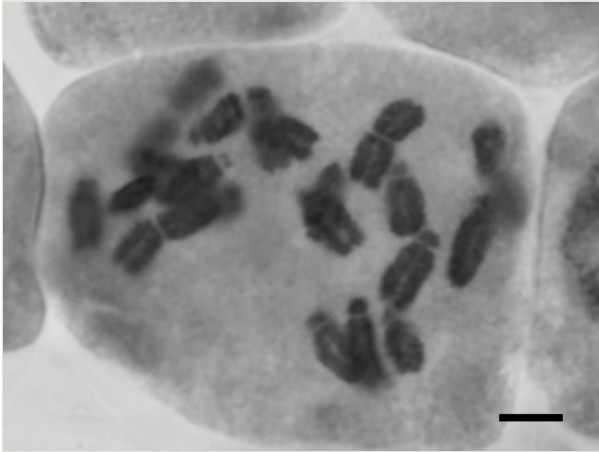


Figure 1.
Somatic chromosome of *Limnocharis flava*. Bar indicates 5 μm .

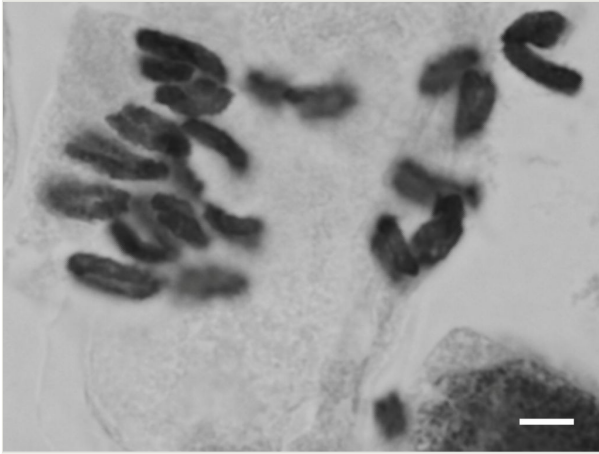


Figure 2.
Somatic chromosome of *Sagittaria trifolia*. Bar indicates 5 μm .

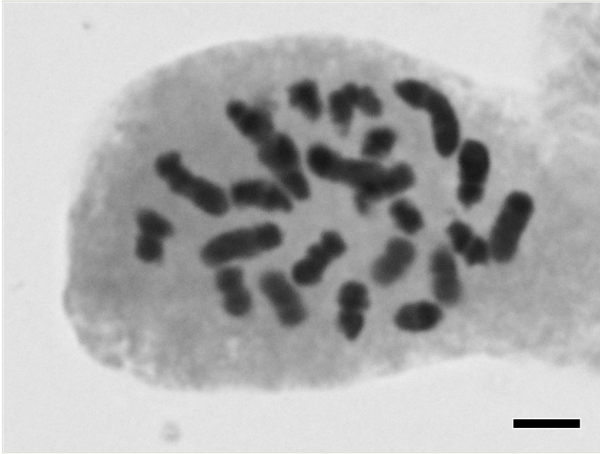


Figure 3.
Somatic chromosome of *Najas tenuis*. Bar indicates 5 μm .

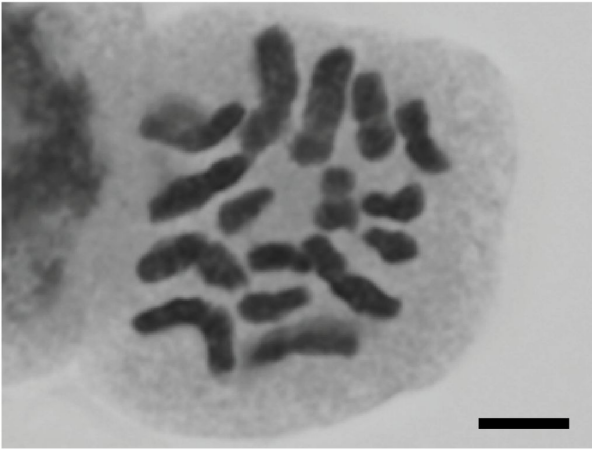


Figure 4.
Somatic chromosome of *Nechamandra alternifolia*. Bar indicates 5 μm .

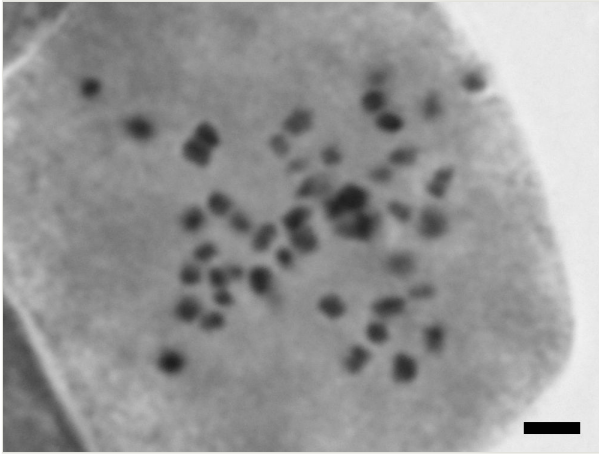


Figure 5.
Somatic chromosome of *Potamogeton distinctus* × *P. nodosus*. Bar indicates 2.5 μm.

Table 1.

The chromosome counts given for 45 non-hybrid species of aquatic monocots of Myanmar as well as well-investigated two *Potamogeton* hybrids among them. Those recorded from neighboring regions are also provided. The species that have no chromosome counts anywhere in the world are shown with n/a. For some species, mostly cosmopolitan ones, only a few representative literature references are given for each chromosome number. Note that due to incapability of original references, some rare chromosome counts are not included in this table: $2n = 18, 42, 48$ for *Acorus calamus*; $2n = 18, 22$ for *Acorus gramineus* (Acoraceae), $2n = 28$ for *Pistia stratiotes* var. *cuneata* Engl.; $2n = 28$ for *Pistia stratiotes* var. *spathulata* (Michx.) Engl.; $2n = 20, 50, 60, 80$ for *Lemna aequinoctialis*; $2n = 44$ for *Lemna trisulca*; $2n = 30, 50$ for *Spirodela polyrrhiza* (Araceae); $n = 14$ ($2n = 28$), $2n = 10, 12$ for *Alisma plantago-aquatica*; $2n = 22$ for *Caldesia parnassifolia*; $2n = 26, 39$ for *Limnocharis flava*; $2n = 22$ for *Sagittaria trifolia* var. *longiloba* (Turr.) Mak.; $2n = 22$ for *Sagittaria trifolia* var. *sinensis* Sims; $2n = 22$ for *Sagittaria trifolia* var. *edulis* (Sieb.) Ohwi (Alismataceae); $2n = 24$ for *Blyxa aubertii*; $2n = 60$ for *Najas marina*; $2n = 12+1B$ for *Najas marina* var. *intermedia* (Gorski) A. Braun; $2n = 22, 52, 72, 88, 132$ for *Ottelia alismoides*; $2n = 16, 22, 28, 33$ for *Vallisneria spiralis* (Hydrocharitaceae); $2n = 64$ for *Eichhornia crassipes*; $2n = 26, n = 40$ ($2n = 80$) for *Monochoria vaginalis* (Pontederiaceae); $2n = 60$ for *Typha angustifolia* (Typhaceae). Also refer to previous cytological reviews (aquatic plants: Les and Philbrick 1993; Potamogetonaceae: Kaplan et al. 2013; *Ruppia*: Talavera et al. 1993).

Order	Family	Species	Chromosome number	Floristic region	
				Indian	M
Acorales	Acoraceae	<i>Acorus calamus</i> L.	$2n = 24$	Subramanian and Munian (1988)	
Acorales	Acoraceae	<i>Acorus calamus</i> L.	$2n = 35$		
Acorales	Acoraceae	<i>Acorus calamus</i> L.	$2n = 36$		
Acorales	Acoraceae	<i>Acorus calamus</i> L.	$2n = 44$		
Acorales	Acoraceae	<i>Acorus calamus</i> L.	$2n = 45$	Ramachandran (1978)	
Acorales	Acoraceae	<i>Acorus calamus</i> L.	$2n = 66$		
Acorales	Acoraceae	<i>Acorus gramineus</i> Sol. ex Aiton	$2n = 24$		
Acorales	Araceae	<i>Cryptocoryne crispatula</i> Engl.	$2n = 36$	Arends et al. (1982)	
Acorales	Araceae	<i>Cryptocoryne crispatula</i> Engl.	$2n = 54$	Jacobsen (1977)	
Acorales	Araceae	<i>Cryptocoryne cruddasiana</i> Prain	n/a		
Acorales	Araceae	<i>Pistia stratiotes</i> L.	$2n = 28$	Ramachandran (1978), Subramanian and Munian (1988)	

Acorales	Araceae	<i>Landoltia punctata</i> (G. Mey.)Les & D.J. Crawford	n/a	
Acorales	Araceae	<i>Lemna aequinoctialis</i> Welw.	2n = 40	Urbanska-Worytkiewicz (1975) (<i>L. perpusilla</i> Torr.)
Acorales	Araceae	<i>Lemna trisulca</i> L.	2n = 20	
Acorales	Araceae	<i>Lemna trisulca</i> L.	2n = 40	
Acorales	Araceae	<i>Lemna trisulca</i> L.	2n = 60	
Acorales	Araceae	<i>Lemna trisulca</i> L.	2n = 80	
Acorales	Araceae	<i>Spirodela polyrrhiza</i> (L.) Schleid.	2n = 40	
Acorales	Araceae	<i>Spirodela polyrrhiza</i> (L.) Schleid.	2n = 42	
Acorales	Araceae	<i>Spirodela polyrrhiza</i> (L.) Schleid.	2n = 80	
Acorales	Araceae	<i>Wolffia globosa</i> (Roxb.)Hartog &Plas	n/a	
Alismatales	Alismataceae	<i>Alisma plantago-aquatica</i> L.	2n = 14	Mehra and Pandita (1984)
Alismatales	Alismataceae	<i>Caldesia parnassifolia</i> (Bassi ex L.)Parl.	n/a	

Alismatales	Alismataceae	<i>Limnocharis flava</i> (L.) Buchenau	2n = 20		T S
Alismatales	Alismataceae	<i>Sagittaria trifolia</i> L.	2n = 22		T S
Alismatales	Hydrocharitaceae	<i>Blyxa aubertii</i> Rich.	2n = 40		
Alismatales	Hydrocharitaceae	<i>Blyxa echinosperma</i> (C.B. Clarke) Hook. f.	2n = 42		
Alismatales	Hydrocharitaceae	<i>Blyxa echinosperma</i> (C.B. Clarke) Hook. f.	2n = 74		
Alismatales	Hydrocharitaceae	<i>Blyxa japonica</i> (Miq.) Maxim. ex Asch. & Gürke	2n = 42		
Alismatales	Hydrocharitaceae	<i>Blyxa japonica</i> (Miq.) Maxim. ex Asch. & Gürke	2n = 72		
Alismatales	Hydrocharitaceae	<i>Egeria densa</i> (Planch.) Casp.	2n = 46		
Alismatales	Hydrocharitaceae	<i>Egeria densa</i> (Planch.) Casp.	2n = 48		
Alismatales	Hydrocharitaceae	<i>Elodea nuttallii</i> (Planch.) H. St. John	2n = 48		
Alismatales	Hydrocharitaceae	<i>Hydrilla verticillata</i> (L. f.) Royle	2n = 16	Chaudhuri and Sharma (1978), Pandita and Mehra (1984)	
Alismatales	Hydrocharitaceae	<i>Hydrilla verticillata</i> (L. f.) Royle	2n = 24	Chaudhuri and Sharma (1978)	
Alismatales	Hydrocharitaceae	<i>Hydrilla verticillata</i> (L. f.) Royle	2n = 32		
Alismatales	Hydrocharitaceae	<i>Hydrocharis dubia</i> (Blume) Backer	2n = 16	Pandita and Mehra (1984)	
Alismatales	Hydrocharitaceae	<i>Najas graminea</i> Delile	2n = 12		
Alismatales	Hydrocharitaceae	<i>Najas graminea</i> Delile	2n = 24		
Alismatales	Hydrocharitaceae	<i>Najas graminea</i> Delile	2n = 36		

Alismatales	Hydrocharitaceae	<i>Najas indica</i> (Willd.) Cham.	n/a		
Alismatales	Hydrocharitaceae	<i>Najas marina</i> L.	2n = 12		
Alismatales	Hydrocharitaceae	<i>Najas marina</i> L.	2n = 24		
Alismatales	Hydrocharitaceae	<i>Najas tenuis</i> Magnus	2n = 24		It (2)
Alismatales	Hydrocharitaceae	<i>Nechamandra alternifolia</i> (Roxb.) Thwaites	2n = 16	Sharma and Chatterjee (1967)	It (2)
Alismatales	Hydrocharitaceae	<i>Ottelia alismoides</i> (L.) Pers.	2n = 44		
Alismatales	Hydrocharitaceae	<i>Ottelia alismoides</i> (L.) Pers.	2n = 66	Chaudhuri and Sharma (1978)	
Alismatales	Hydrocharitaceae	<i>Ottelia alismoides</i> (L.) Pers.	2n = 68	Chaudhuri and Sharma (1978)	
Alismatales	Hydrocharitaceae	<i>Ottelia cordata</i> (Wall.) Dandy	n/a		
Alismatales	Hydrocharitaceae	<i>Vallisneria spiralis</i> L.	2n = 20		
Alismatales	Hydrocharitaceae	<i>Vallisneria spiralis</i> L.	2n = 24	Chaudhuri and Sharma (1978)	
Alismatales	Hydrocharitaceae	<i>Vallisneria spiralis</i> L.	2n = 30	Chaudhuri and Sharma (1978)	
Alismatales	Hydrocharitaceae	<i>Vallisneria spiralis</i> L.	2n = 40	Chaudhuri and Sharma (1978), Sarkar et al. (1980)	
Alismatales	Aponogetonaceae	<i>Aponogeton lakhonensis</i> A. Camus	n/a		
Alismatales	Potamogetonaceae	<i>Potamogeton crispus</i> L.	2n = 52		
Alismatales	Potamogetonaceae	<i>Potamogeton crispus</i> L.	2n = 56		
Alismatales	Potamogetonaceae	<i>Potamogeton distinctus</i> A. Benn.	2n = 52		
Alismatales	Potamogetonaceae	<i>Potamogeton distinctus</i> A. Benn. × <i>P. nodosus</i> Poir.	2n = 52		T s

Alismatales	Potamogetonaceae	<i>Potamogeton maackianus</i> A Benn.	2n = 52	
Alismatales	Potamogetonaceae	<i>Potamogeton maackianus</i> A Benn.	2n = 56	
Alismatales	Potamogetonaceae	<i>Potamogeton ×malainoides</i> Miki	2n = 52	
Alismatales	Potamogetonaceae	<i>Potamogeton lucens</i> L.	2n = 52	
Alismatales	Potamogetonaceae	<i>Potamogeton nodosus</i> Poir.	2n = 52	
Alismatales	Potamogetonaceae	<i>Potamogeton octandrus</i> Poir.	2n = 28	
Alismatales	Potamogetonaceae	<i>Potamogeton wrightii</i> Morong	2n = 52	
Alismatales	Potamogetonaceae	<i>Stuckenia pectinata</i> (L.) Börner	2n = 78	Kaplan et al. (2013)
Alismatales	Potamogetonaceae	<i>Stuckenia pectinata</i> (L.) Börner	2n = 84	
Alismatales	Ruppiales	<i>Ruppia maritima</i> L.	2n = 20	Ito et al. (2010)
		<i>Ruppia maritima</i> L.	2n = 40	
Asparagales	Amaryllidaceae	<i>Crinum thaianum</i> J. Schul.	n/a	
Commelinales	Pontederiaceae	<i>Eichhornia crassipes</i> (Mart.) Solms	2n = 32	
Commelinales	Pontederiaceae	<i>Monochoria hastata</i> (L.) Solms	2n = 28	Patwary et al. (1989)
Commelinales	Pontederiaceae	<i>Monochoria hastata</i> (L.) Solms	2n = 80	Patwary et al. (1989)
Commelinales	Pontederiaceae	<i>Monochoria vaginalis</i> (Burm.f.) C. Presl ex Kunth	2n = 24	Christopher (1983) (var. <i>plantaginea</i> (Roxb.) Solms); Patwary et al. (1989)

Commelinales	Pontederiaceae	<i>Monochoria vaginalis</i> (Burm.f.)C. Presl ex Kunth	2n = 48	
Commelinales	Pontederiaceae	<i>Monochoria vaginalis</i> (Burm.f.)C. Presl ex Kunth	2n = 52	Christopher (1983), Patwary et al. (1989)
Commelinales	Typhaceae	<i>Typha angustifolia</i> L.	2n = 30	
Poales	Eriocaulaceae	<i>Eriocaulon setaceum</i> L.	n/a	