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RESEARCH ARTICLE

Chromosome conspectus of Kashmir Himalayan species of the genus *Potamogeton* L.

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Abstract
The present study represents an original investigation on the chromosome number and meiotic behaviour of eleven *Potamogeton* species inhabiting lotic and lentic freshwater bodies in the Kashmir Himalaya. The chromosome number of *P. berchtoldii* (2n = 4x = 28), *P. crispus* (2n = 12x = 84) recorded during the present study differed from earlier reports, while chromosome count for *P. amblyphyllus* (= *Stuckenia amblyphyla*) is newly reported for the genus. In *P. natans* two cytotypes were identified: tetraploid (2n = 4x = 52) and octoploid (2n = 8x = 104), the latter being the first report. The species characterized by abnormal anaphasic segregation also had low pollen fertility and fruit set. The present study also correlates base number and ploidy level with leaf habit, pollination efficiency and fruit set.

Keywords Chromosome number · PMC meiosis · *Potamogeton* · Ploidy level

Introduction

The genus *Potamogeton* is quite interesting chromosomally because of widespread aneuploidy and polyploidy (Les 1983; Hollingsworth et al. 1998; Kaplan et al. 2013). The genus *Potamogeton* s. l. (incl. *Stuckenia* and *Groenlandia*) is an exceptional case having five base numbers: x = 7, 11, 12, 13 and 15; hence, its variation in chromosome number has been used by some authors to hypothesize various taxonomic relationships as well as the putative ancestral group. Some high level polyploids, also are quite widespread; whereas, others are confined to specific habitats in Kashmir Himalaya. The 11 *Potamogeton* species that inhabit various water bodies of the Kashmir valley have never been investigated before for their chromosome number or ploidy status. Thus, the present study was undertaken to: (a) record the chromosome number/s of Kashmir Himalayan species of *Potamogeton*; (b) evaluate pollen mother cell (PMC) meiotic behaviour with respect to polyploidy; and (c) to compare base number and ploidy level with leaf habit, pollination efficiency, pollen fertility and seed set.

Materials and methods

Species sampled

During the present study only 11 well identified species of the genus *Potamogeton* were selected, which include *P. lucens* L., *P. natans* L., *P. pusillus* L., *P. amblyphyllus* C.A. Meyer, *P. berchtoldii* Fieb., *P. crispus* L., *P. nodosus* Poir., *P. distinctus* A. Bennett., *P. pectinatus* L., *P. perfoliatus* L. *and P. wrightii* Morong. Standard herbarium methods (Bridson and Foreman 1992) were used during collection, processing and preparation of the herbarium specimens. The voucher specimens have been deposited at the University of Kashmir Herbarium (KASH). The specimens were identified with the help of relevant literature and morphologically characterized. Identifications of some species were confirmed by Dr. Zdenek Kaplan, Institute of Botany, Academy of Science of Czech Republic, CZ-252 43 Pruhonice, Czech Republic.

Analysis of pollen mother cell meiosis

Chromosome counts were obtained and PMC meiosis examined in eleven well identified *Potamogeton* species

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from 13 sites of the Kashmir Valley ranging in altitude from 1590–1622 m asl. These sites included urban and rural valley lakes, rivulets and streams both oligotrophic and eutrophic in nature and also with different altitudinal gradient (Table 1). The species were assigned to five habit groups namely: heterophyllous (floating-leaved) (HET); submerged broad-leaved (SBL); intermediate between submerged broad and linear-leaved (ISBL); submerged linear-narrow leaved (SLNL) and submerged-filiform leaved (SFL) species. Not all species could be sampled in all the sites, but in average, each species was sampled in 2 or 5 sites depending on their occurrence (Table 2).

Floral spikes were collected while still inside the leaf sheath and were fixed in Carnoy's fixative [ethanol: acetic acid (3:1)] between 1000–1300 h for 60–90 min, transferred to freshly prepared Carnoy's fluid for about 22 h and preserved in 70% ethanol at 4 °C. 2% propionocarmine solution was used for staining. Randomly collected floral spikes of each species from the selected sites inhabited by the species were analyzed for PMC meiosis and this procedure

Table 1 Salient features of the aquatic habitats of Kashmir valley (Jammu and Kashmir, India), where *Potamogeton* species were collected for the present investigation

Site (District)	Salient features of the water body					
	Nature	Location	Altitude (m.a.s.l.)	Latitude (north)	Longitude (east)	Species recorded /collected
Anchar lake (Srinagar)	Urban Valley lake	12 km NW of Srinagar*	1595	34°10′55″	74°48′10″	*P. crispus*, *P. lucens*, *P. natans*, *P. pectinatus* (=*S. pectinata*), *P. pusillus*
Dal lake (Srinagar)	Urban Valley lake	3 km of Srinagar	1595	34°08′48″	74°52′51″	*P. crispus*, *P. lucens*, *P. natans*, *P. nodosus*, *P. pectinatus* (=*S. pectinata*), *P. wrightii*
Manasbal lake (Bandipora)	Rural Valley lake	25 km NW of Srinagar	1590	34°15′26″	74°41′26″	*P. lucens*, *P. natans*, *P. nodosus*, *P. pectinatus* (=*S. pectinata) P. perfoliatus*, *P. pusillus*, *P. wrightii*
Hokhersar (Srinagar)	Urban Valley	10 km NW of Srinagar	1600	34°05′36″	74°42′37″	*P. natans*, *P. nodosus*, *P. pectinatus* (=*S. pectinata*), *P. pusillus*
Nigeen lake (Srinagar)	Urban Valley lake	10 km NW of Srinagar	1595	34°08′50″	74°52′55″	*P. lucens*, *P. pectinatus*, (=*S. pectinata*), *P. pusillus*
Wular lake (Bandipora and Baramulla)	Rural Valley lake	35 km NW of Srinagar	1595	34°17′25″	74°30′48″	*P. crispus*, *P. lucens*, *P. natans*, *P. nodosus*, *P. pectinatus* (=*S. pectinata*), *P. pusillus*, *P. trichoides*, *P. wrightii*
Achabal stream (Anantnag)	Stream	58 km SE of	1610	33°41′03″	75°13′12″	*P. amblyphyllus* (=*S. amblyphylla*), *P. berchtoldii*, *P. pectinatus*, (=*S. pectinata*), *P. crispus*
Aarpath rivulet of Brakapor (Anantnag)	Rivulet	56 km SE of Srinagar	1600	33°43′09″	75°11′04″	*P. pectinatus* (=*S. pectinata*), *P. crispus*
Kandizal irrigation channel (Pulwama)	Irrigation channel	27 km NW of Srinagar	1622	33°58′01″	74°52′10″	*P. distinctus*
Irrigation canal of Sundoo (Anantnag)	Stream	56 km of SE of Srinagar	1605	33°42′00″	75°11′31″	*P. amblyphyllus*, *P. berchtoldii*, *P. nodosus*
Nambal rivulet of Barakapora (Anantnag)	Stream	55 km SE of Srinagar	1605	33°43′09″	75°11′04″	*P. amblyphyllus*, *P. crispus*, *P. pectinatus*
Nagrad stream of Sundoo (Anantnag)	Stream	56 km SE of Srinagar	1605	33°42′00″	75°11′31″	*P. amblyphyllus*, *P. berchtoldii*
Spring stream of ThajIwara (Anantnag)	Stream	57 km SE of Srinagar	1605	33°42′18″	75°12′15″	*P. crispus*, *P. perfoliatus*,, *P. pectinatus* (=*S. pectinata*)

* = Summer capital of J&K state, India

Source: Survey of India Toposheets (1971) on 1:50,000 scale and present study

Table 2 Chromosome counts observed for eleven species of the genera *Potamogeton* and *Stuckenia* from the Kashmir valley, India. *HET* = heterophyllous (floating-leaved); *SBL* = submerged broad-leaved; *ISBL* = intermediate between submerged broad and linear-leaved; *SLNL* = submerged linear-narrow leaved, and *SFL* = submerged-filiform leaved species

Habit group	Species	Sampling site/s	Present count			Earlier count and ploidy level	References
			Base number (x)	Chromosome count and ploidy level	No. of bivalents per PMC		
HET	*P. natans* Cytotype A Cytotype B	Anchar lake, Manasbal lake, Dal lake, Hokhersar, Wular lake, Anchar lake, Manasbal lake, Hokhersar	13 13	2n = 4x = 52 2n = 8x = 104*	26 52	2n = 4x = 52 (n = 13) 2n = 6x = 42 (x = 7)	Palmgren (1939), Harada (1942a, 1956), Wan et al. (2012), Löve and Löve (1956), Hollingsworth et al. (1995), Stren (1961), Probatava and Sokolovskaya (1984), Kaplan et al. (2013)
	P. distinctus	Kandizal irrigation channel	13	2n = 4x = 52	26	2n = 4x = 52 (x = 13)	Harada (1942b, 1956), Takusagawa (1961), Wan et al. (2012), Kaplan et al. (2013)
	P. nodosus	Manasbal lake, Dal lake, Hokhersar, Wular lake, Irrigation canal of Sundoo	13	2n = 4x = 52	26	2n = 4x = 52 (x = 13)	Taylor and Mulligan (1968), Ottonello et al. (1985), Talavera and García Murillo (1992), Wan et al. (2012), Kaplan et al. (2013)
	P. wrightii	Manasbal lake, Dal lake, Wular lake, Nambal rivulet of Barakapora	13	2n = 4x = 52	26	2n = 4x = 52 (x = 13)	Harada (1956), Takusagawa (1961), Weigleb and Kadano (1990), Wan et al. (2012), Kaplan et al. (2013)
SBL	*P. lucens*	Anchar lake, Manasbal lake, Dal lake, Nigeen lake, Wular lake	13	2n = 4x = 52	26	2n = 4x = 52 (x = 13)	Palmgren (1939), Uhriková and Feráková (1978), Hollingsworth et al. (1998), Wan et al. (2012)
	P. perfoliatus	Manasbal lake, Spring stream of ThajIwara	13	2n = 4x = 52	26	2n = 4x = 52 (x = 13) 2n = 2x = 26 (x = 13) 2n = 4x = 48 (x = 12)	Palmgren (1939), Sehreer (1939), Harada (1942a, b, 1956), Felföldy (1947), Löve and Ritchie (1966), Ficini et al. (1980), Kaplan et al. (2013), Hollingsworth et al. (1998), Löve (1954a, b), Wiśniewska (1931)
ISBL	*P. crispus*	Anchar lake, Manasbal lake, Dal lake, Wular lake, Aarpath rivulet, Nagrad stream sundoo	7	2n = 12x = 84#	42	2n = 4x = 52 (x = 13) 2n = 6x = 78 (x = 13)	Palmgren (1939), Harada (1942a, b, 1956), Takusagawa (1961), Junkun in Pogan et al. and Izamallow (1983), Kaplan et al. (2013), Sharma and Chatterjee (1967)
SLNL	*P. berchtoldii*	Achabal stream, Spring stream sundoo, Nagrad stream of Sundoo	7	2n = 4x = 28#	14	2n = 2x = 26 (x = 13)	Palmgren (1939), Harada (1956), Löve and Löve (1956), Taylor and Mulligan (1968), Löve and Löve (1981), Murin (1992), Kaplan et al. (2013)
	P. pusillus	Anchar lake, Manasbal lake, Hokhersar, Wular lake	7	2n = 4x = 28	14	2n = 2x = 26 (x = 13) 2n = 4x = 28 (x = 7)	Palmgren (1939), Harada (1956), Takusagawa (1961), Wan et al. (2012), Kaplan et al. (2013), Harada (1942b), fide Harada (1956)

Table 2 (continued)

Habit group	Species	Sampling site/s	Present count			Earlier count and ploidy level	References
			Base number (x)	Chromosome count and ploidy level	No. of bivalents per PMC		
SFL	*P. amblyphyllus* (=*S. amblyphylla*)	Nambal rivulet of Barakapora, Nagrad stream of Sundoo, Irrigation canal of Sundoo	7	2n = 12x = 84*	42		
	P. pectinatus (=*S. pectinata*)	Anchar lake, Manasbal lake, Dal lake, Wular lake, Aarpath rivulet, Nagrad stream sundoo	7	2n = 12x = 84	42	2n = 6x = 78 (x = 13) 2n = (78 = 82, 84) and (76, 78, 82) 2n = 12x = 84 (x = 7) 2n = 86	Scheerer (1939), Palmgren in Löve and Löve (1942), Löve and Löve (1981), Arohonka (1982), Hollingsworth et al. (1998), Kaplan et al. (2013), Kolkman and Van Wijk (1984), Uchiyama (1989), Yurtsev et al. (1975)

Figures in parenthesis represent base number

* = First report

Chromosome number differed from earlier records (first counts)

was repeated for four consecutive years. Bivalents and chromosomes were counted at diakinesis, metaphase-I and anaphase-I to authenticate the correct chromosome count for each species. Only very good preparations were used for chromosome counts.

Pollen fertility estimation

Pollen fertility was estimated following Stanley and Linskens' (1974) method; wherein mature and un-dehisced anthers were placed in 1% triphenyltetrazolium chloride for one hour and squashed. The well stained pollen grains were considered as viable.

Calculation of fruit set

Fruit set was estimated by randomly selecting plants in different populations of each species, tagged and scored for the number of spikes per ramet and the number of flowers and fruits per spike following Lubber and Christensen (1966).

Percentage fruit set was calculated as follows:

$$\% \text{ age fruit set per ramet} = \frac{\text{Total number of fruits set}}{\text{Total number of ovules borne}} \times 100$$

Results

PMC meiosis

Cytological observations indicated that meiosis is asynchronous among the Pollen Mother Cells (PMCs) of an anther, anthers of a flower and flowers of a spike (flower development being acropetal). Owing to the small size of chromosomes, a detailed study of their morphology was not possible. Chromosomes were most readily countable and bivalent morphology was reasonably evident at diakinesis, metaphase-I (MI) and anaphase-I (AI) (Fig. 1, 2, 3). The heterophyllous or broad-leaved submersed species i.e. *Potamogeton lucens*, *P. distinctus*, *P. natans*, *P. nodosus*, *P. perfoliatus* and *P. wrightii* shared the base number x = 13 and all are tetraploid (2n = 4x = 52); however, an octoploid cytotype of *P. natans* (2n = 8x = 104) also was recorded from Anchar lake, Manasbal lake, and Hokhersar. In this cytotype, some bivalents remained linked to each other by chromatin bridges in 22% PMCs at diakinesis and MI (Fig. 1d, g). The submerged intermediate broad to linear and narrow to filiform-leaved species shared a base number of x = 7. Three of these species, namely *P. crispus*, *P. amblyphyllus* (=*Stuckenia amblyphylla*), and *P. pectinatus* (=*Stuckenia pectinata*) were 12-ploid (2n = 12x = 84); whereas, *P. berchtoldii* and *P. pusillus* were tetraploid (2n = 4x = 28; Table 2).

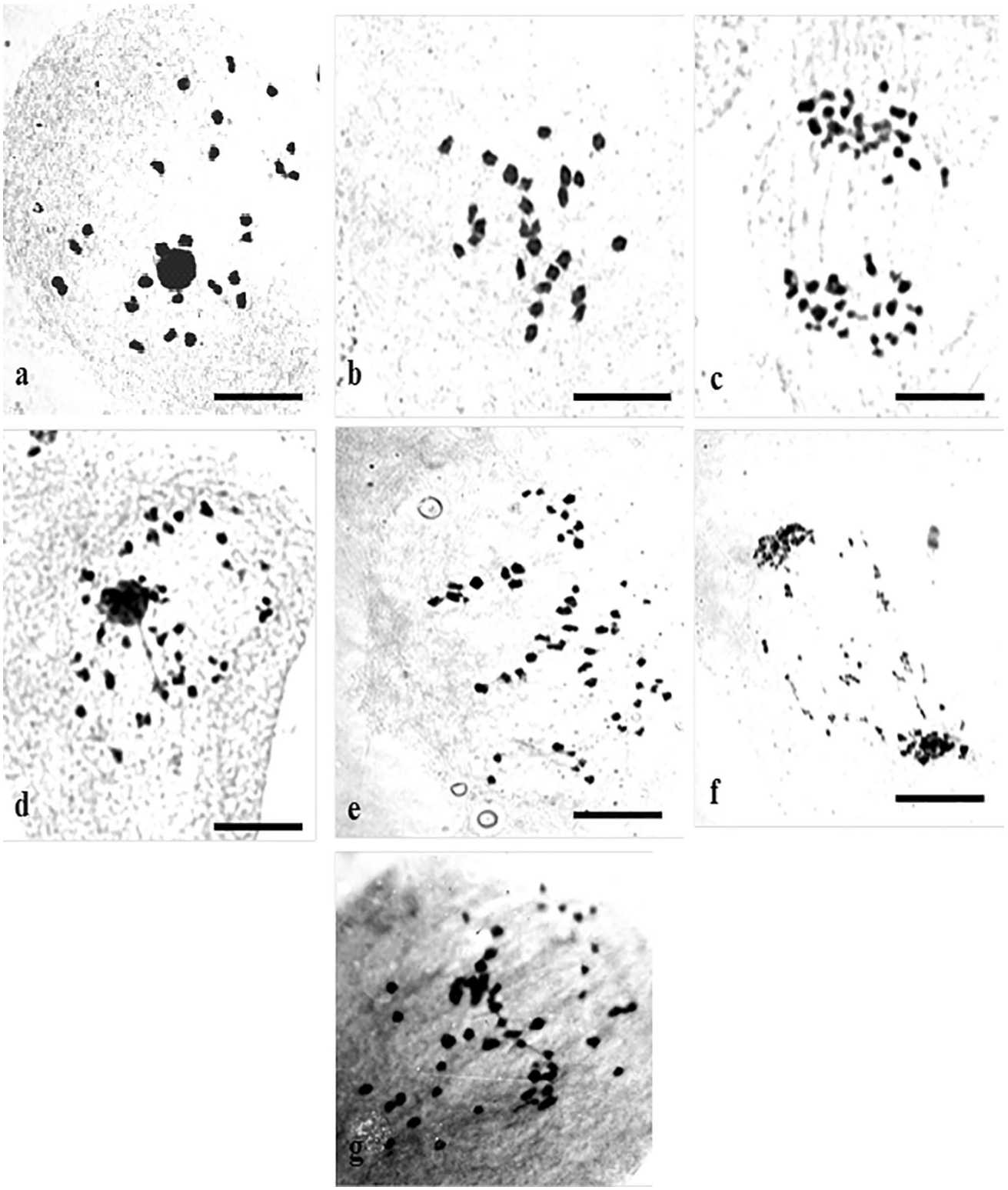

Fig. 1 Meiotic behavior and chromosome count of broad-leaved species of *Potamogeton* (**a**–**v**) growing in Kashmir valley. PMC meiosis in *P. natans* cytotype A (2n = 4x = 52) (**a**, **b**, **c**) and *P. natans* cytotype B (2n = 8x = 104) (**d**, **e**, **f**, **g**). **a**, **b** PMCs at diakinesis and MI; note 26 bivalents per PMC; **c** AI; note normal segregation of 26 chromosomes at each pole; **d**, **e** PMCs at diakinesis and MI; note 52 bivalents per PMC; **f** AI; note abnormal segregation (lagging chromosome between the anaphasic poles; **g** PMC at MI (*P. natans*–cytotype B). Note chromatin bridges between the bivalents. (Scale: 10 µm) PMC meiosis in *P. nodosus* (2n = 4x = 52) (**h**, **i**, **j**); *P. distinctus* (2n = 4x = 52) (**k**, **l**, **m**) and *P. lucens* (**n**, **o**, **p**). PMCs at Diakinesis, MI and AI. Note 26 bivalents per PMC and normal segregation of 26 chromosomes at each pole. (Scale: 10 µm) PMC meiosis in *P. perfoliatus* (2n = 4x = 52) (**q**, **r**, **s**) and *P. wrightii* (2n = 4x = 52) (**t**, **u**, **v**) PMCs at MI and AI; note 26 bivalents per PMC and normal segregation of 26 chromosomes at each pole. (Scale: 10 µm)

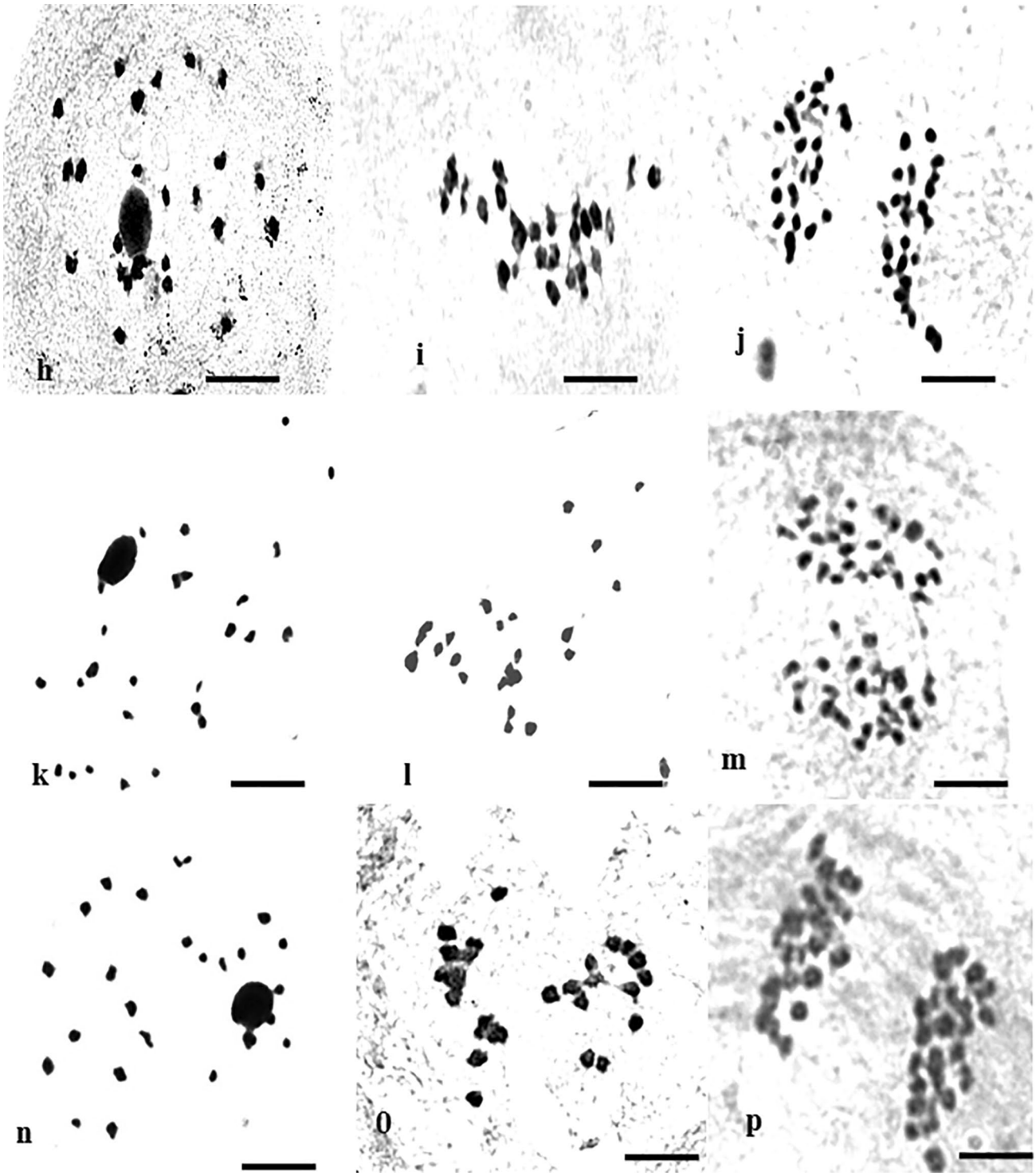

Fig. 1 (continued)

The chromosomes paired regularly into 26 and 52 bivalents in HET and SBL species and into 14 and 42 bivalents in ISBL, SNL and SFL species. Due to their very small size, there is one chiasma per bivalent; the bivalents are mostly rod-shaped with a few ring-shaped. Despite differences in chromosome number, anaphasic segregation proceeded normally in all the species studied with an equal number of chromosomes moving to each pole without error (Figs. 1, 2, 3). The octaploid cytotype of *P. natans* was an exception as almost all the PMCs in this

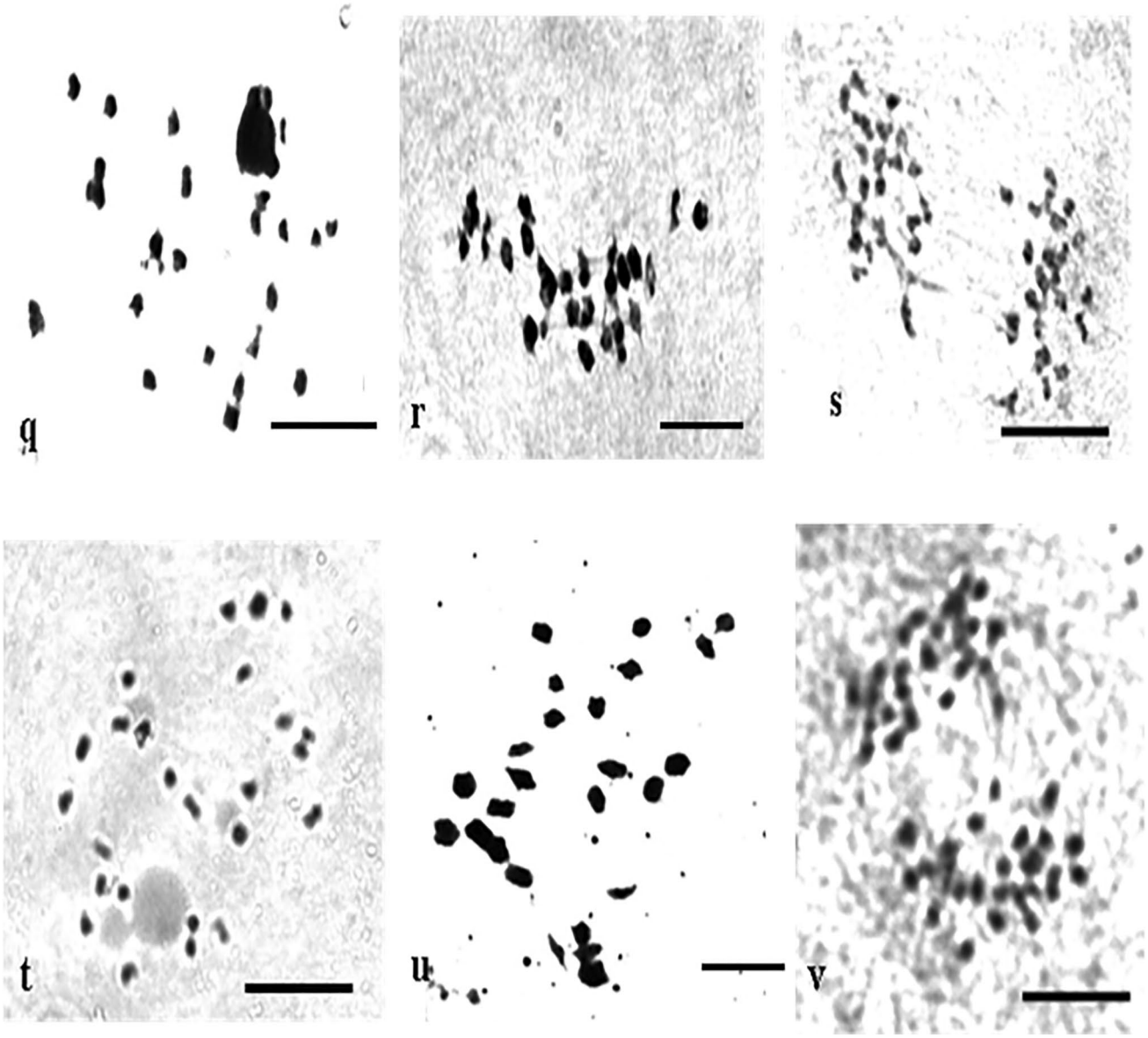

Fig. 1 (continued)

species exhibited abnormal anaphasic segregation with lagging chromosomes (Fig. 1f). In SLNL species normal anaphasic disjunction was observed in roughly 90–95% of cells; however, in some cells a few chromosomes failed to reach the poles and were visibly lagging and in some PMCs abnormal anaphase was also observed (Fig. 2e). The number of nucleolar bivalents at diakinesis and number of nucleoli observed in PMCs are summarized in Table 3 and Fig. 4. Among the broad-leaved species maximum number of nucleolar bivalents was observed in octoploid *P. natans*. The number of nucleolar bivalents and nucleoli was highest in octoploid *P. natans* and their number was almost similar in all the four groups.

Pollen fertility

The species presently investigated produced high percentage of healthy and stainable pollen. The percentage of viable pollen grains ranged from 75.60 ± 0.91 to 90.22 ± 1.30. The floating and submerged broad-leaved species had high pollen fertility as compared with linear to filiform submerged-leaved species (Table 4).

Fruit set

Only the species inhabiting standing water habitats produced fruits, while those occurring in running water habitats

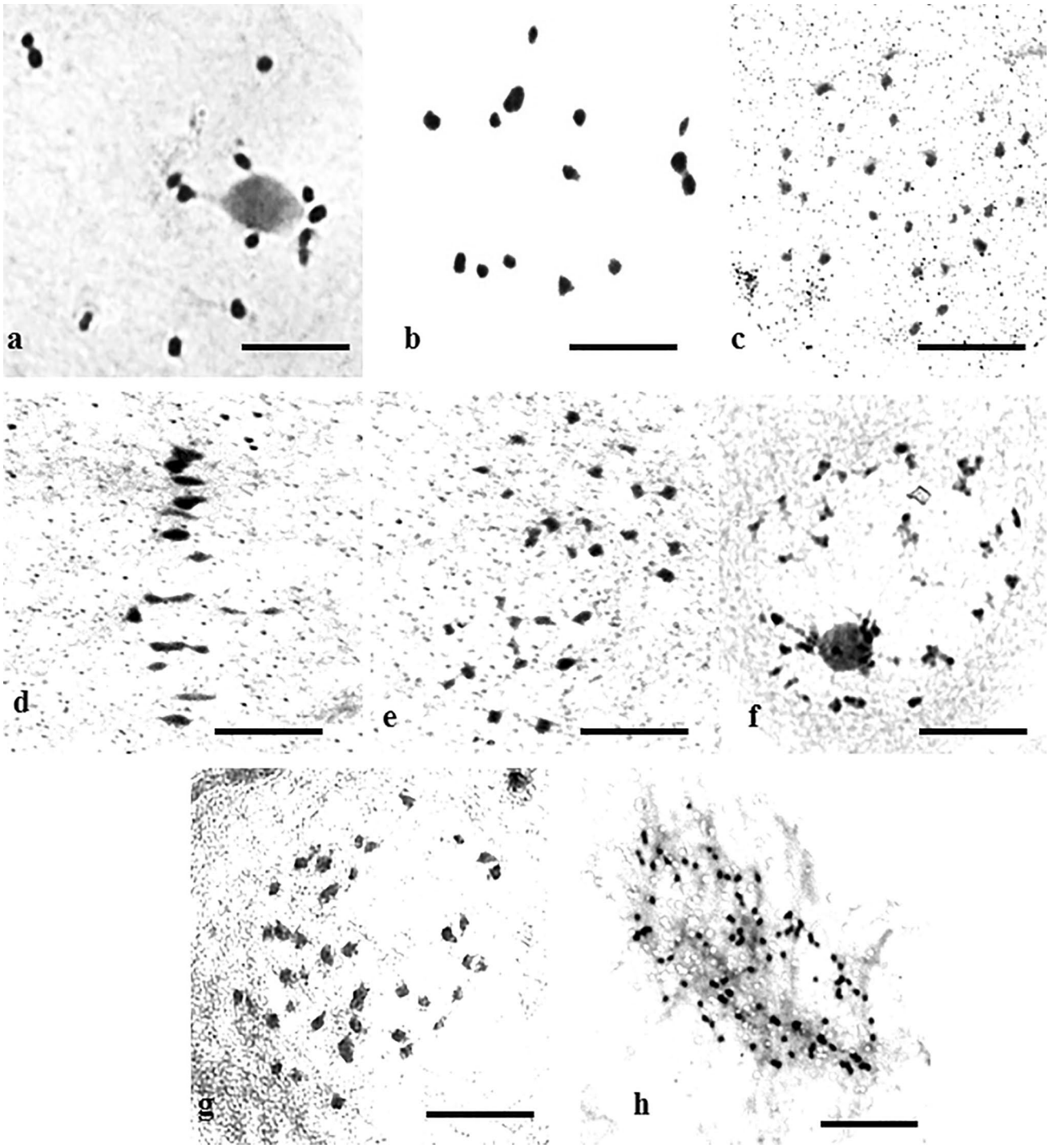

Fig. 2 Meiotic behavior and chromosome count of linear-leaved species of *Potamogeton* (**a**–**h**). PMC meiosis of *P. berchtoldii* (2n = 4x = 28) (**a**, **b**, **c**), *P. pusillus* (2n = 4x = 28) (**d**, **e**) and *P. crispus* (2n = 12x = 84) (**f**, **g**, **h**). **a**, **b** PMCs at Diakinesis, MI and AI. Note 14 bivalents per PMC; **c** PMC at AI; note normal distribution of chromosomes at two poles; **d**, **e** PMCs at MI and AI. Note 14 bivalents per PMC and abnormal segregation of 12 and 16 chromosomes at each pole; **f**, **g**, **h** PMCs at diakenesis, MI and AI. Note 42 bivalents per PMC and 42 chromosomes at each pole (Scale: 10 μm)

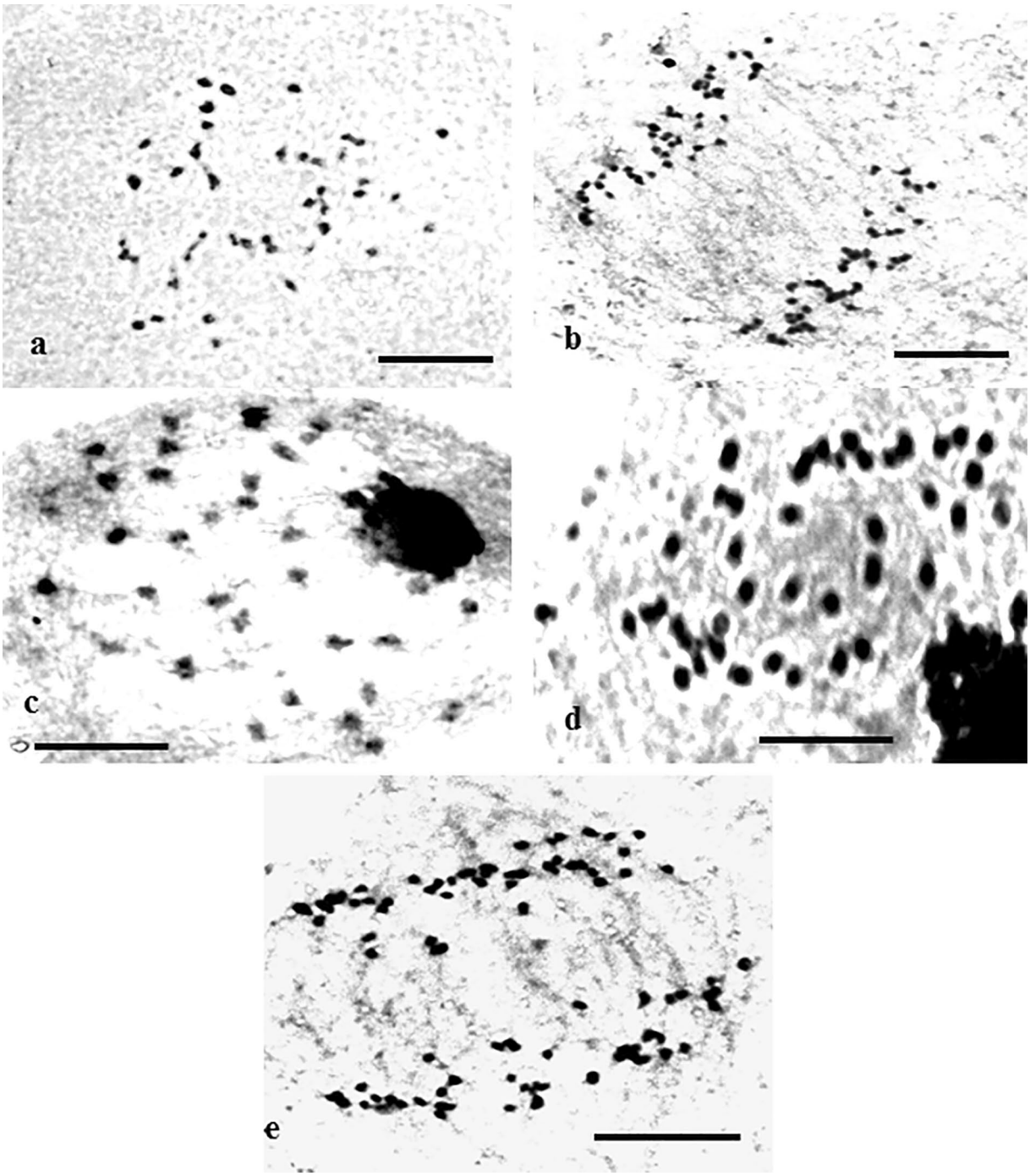

Fig. 3 Meiotic behavior and chromosome count of filiform-leaved species of *Potamogeton* (**a**–**e**) PMC meiosis in *P. amblyphyllus* (=*S. amblyphylla*) (2n = 12x = 84) (**a**, **b**) and *P. pectinatus* (=*S. pectinata*) (2n = 12x = 84) (**c**–**e**). **a**, **b** PMCs at MI and AI. Note 42 bivalents per PMC and normal segregation of 42 chromosomes at each pole; **c**–e PMC at diakinesis, MI and AI. Note 42 bivalents per PMC and 42 chromosomes at each anaphasic pole (Scale: 10 µm)

did not. The floating and submerged broad-leaved species namely; *P. distinctus*, *P. natans*, *P. lucens*, *P. nodosus*, and *P. wrightii* had high percent fruit set (63.03, 68.4, 65.1, 63.88 and 46.5%, respectively). The linear to filiform-leaved species have low fruit set as compared with broad-leaved ones (Table 5).

Table 3 Number of nucleoli and nucleolar bivalents in PMCs of eleven species of *Potamogeton* and *Stuckenia* from Kashmir valley, J&K, India. *HET* = heterophyllous (floating-leaved); *SBL* = submerged broad-leaved; *ISBL* = intermediate between submerged broad and linear-leaved; *SLNL* = submerged linear-narrow leaved, and *SFL* = submerged-filiform leaved species

Habit	Species	No. of bivalents attached to nucleoli	Modal no. of nucleolar bivalents per PMC	Percentage of PMCs showing highest number of nucleolar bivalents	Percentage PMCs showing modal no of nucleolar bivalents	No. of nucleoli per PMC	Modal no. of nucleoli per PMC	Percentage of PMCs showing modal number of nucleoli	Percentage of PMCs showing highest number of nucleoli per bivalent
HET	*P. natans*								
	Cytotype A	1–5	4	10	70	1–6	1	80	5
	Cytotype B	1–10	8	8	85	1–11	1	80	7
	P. nodosus	1–5	3	11	70	1–6	1	72	5
	P. distinctus	1–5	3	11	72	1–6	1	75	5
	P. wrightii	1–5	4	11	72	1–8	1	75	5
SBL	*P. lucens*	1–5	3	9	75	1–6	1	77	4
	P. perfoliatus	1–5	3	9	70	NR	NR	NR	NR
ISBL	*P. crispus*	1–4	3	12	75	1–8	1	75	6
SNL	*P. berchtoldii*	NR	NR	NR	NR	NR	NR	NR	NR
	P. pusillus	NR	NR	NR	NR	NR	NR	NR	NR
	P. amblyphyllus (=*S. amblyphylla*)	1–5	5	13	73	1–10	1	76	6
	P. pectinatus (=*S. pectinata*)	1–5	4	13	70	NR	NR	NR	NR

NR Not Recorded

Discussion

The genera *Potamogeton* and *Stuckenia* are extremely interesting with respect to their broad range of 2n chromosome counts and high levels of polyploidy observed in many species. At least four base numbers have been reported (x = 7, 11, 12, 13), which characterize various diploid, polyploid and aneuploid species. The range of polyploidy extends from triploids to even some 12-ploids (Fig. 5).

In the present study, chromosome counts of HET species (*P. distinctus*, *P. natans* and *P. nodosus*) conformed with earlier records (Table2). Based on x = 13, these species are tetraploid (2n = 4x = 52). In the same group, an octoploid cytotype (cytotype B) of *P. natans* (2n = 8x = 104) is reported for the first time. When viewed in light of previous studies reporting a chromosome number of 2n = 6x = 42 (based on x = 7) for *P. natans* (Stern 1961; Probatova and Sokolovskaya 1984) provided those counts are accurate, then this species would be characterized by two base numbers, i.e., x = 7 and x = 13.

In SBL species (*P. lucens and P. perfoliatus*) the chromosome counts from Kashmir populations agreed with those presented in earlier accounts (Table 2). Based on x = 13, these species are tetraploid having 2n = 4x = 52. For *P. perfoliatus*, a chromosome number of 2n = 2x = 26 based on x = 13 was recorded by Löve (1954a, b) and 2n = 4x = 48 based on x = 12 by Wiśniewska (1931). If these counts are correct, then the difference indicated also reflects a dibasic nature of the species, with two base numbers (x = 12, 13), and two corresponding diploid and tetraploid cytotypes (2n = 2x = 24 and 2n = 4x = 52).

Chromosome counts for Kashmir populations of several SLNL and ISBL species (*P. berchtoldii*, *P. crispus*) do not agree with previous records (Table 2) and additionally reveal the dibasic nature of these species (x = 7 and 13). The chromosome count in *P. pusillus* (2n = 4x = 28) is in conformity with that of Harada [(1942b) fide Harada (1956)] but differed with the earlier count (2n = 2x = 26) reported by some other workers (Table 2), which also points towards dibasic nature of the species.

Based on x = 7 the SFL species [*P. amblyphyllus* (= *S. amblyphylla*), *P. pectinatus* (= *S. pectinata*)] are 12-ploid (2n = 12x = 84). The count for *P. amblyphyllus* (= *S. amblyphylla*) is the first report for the species. However, in some PMCs in both these species 39 bivalents were also observed. The 42 bivalents in both these species were most prevalent in the PMCs with good preparations and 84 chromosomes were also observed in well spread preparations at anaphase I.

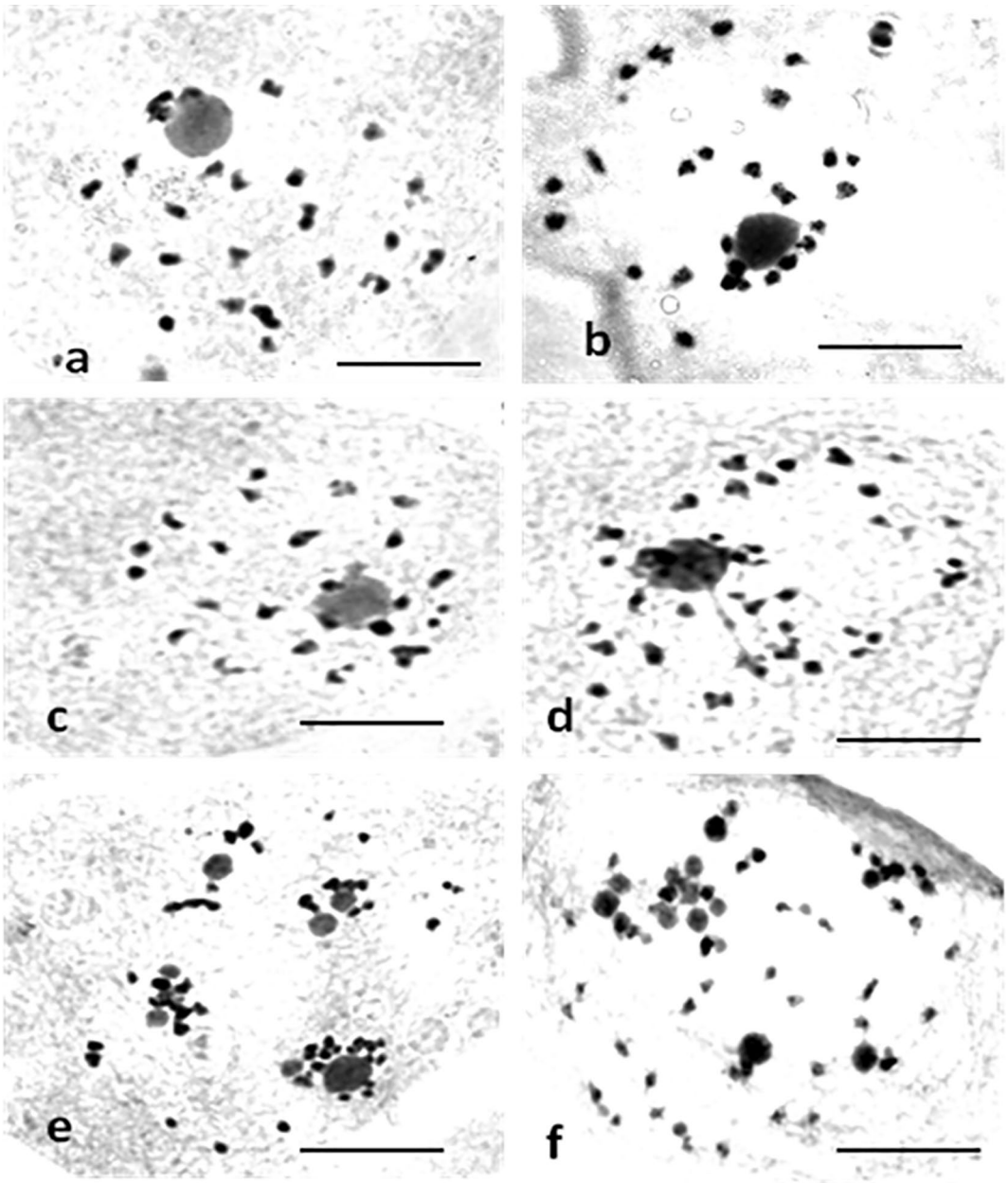

Fig. 4 Variation in the number of nucleolar bivalents and number of nucleoli in PMCs of presently studied species of *Potamogeton and Stuckenia* (**a**) a pair of bivalents attached to nucleolus (*P. natans*–cytotype A) (**b**) and (**c**) four bivalents attached to nucleolus (*P. nodosus and P. lucens*) (**d**) eight bivalents attached to nucleolus (*P. natans*–cytotype B) (**e**) seven nucleoli in a PMC (*P. amblyphyllus;* = *S. amblyphylla*); (**f**) ten nucleoli in a PMC (*P. natans* – cytotype B) (Scale: 10 µm)

The present count obtained for *P. pectinatus* (= *S. pectinata*) is in agreement with that of Uchiyama (1989) and Kalman and Van wijk (1984). The latter authors, however, recorded a range of 2n numbers for the species (2n = 76, 78, 82, 84), with the prevalent count being 2n = 78. Kalman and Van Wijk (1984) also observed that some "good" counts revealed a number distinctly higher than 78, which they emphasized should not be neglected. In *P. crispus,* Sharma and Chatterjee (1967) reported that counts for the species typically were 2n = 52 but some cells were 2n = 36 and some plants had cells possessing 2n = 72, and 2n = 78. Wiegand (1899) reported that some cells of *P. foliosus* had n = 8 rather than the more usual n = 7; (evaluated as 2n = 16 and 2n = 14, respectively). Hollingsworth et al. (1998) later emphasized that some of the variation in chromosome number could be attributed to technical difficulties or even misidentification, resulting in errors of counting or interpretation. In order to minimize this discrepancy, only well identified species were

Table 4 Pollen fertility in the species of *Potamogeton* presently studied. *HET* = heterophyllous (floating-leaved); *SBL* = submerged broad-leaved; *ISBL* = intermediate between submerged broad and linear-leaved; *SLNL* = submerged linear-narrow leaved, and *SFL* = submerged-filiform leaved species

Habit group	Species	Number of pollen grains scanned (mean ± SE)	Number of viable pollen grains (mean ± SE)	Percentage of viable pollen (mean ± SE)
HET	*P. distinctus*	2538 ± 1734	2178 ± 143.33	86.21 ± 1.43
	P. natans			
	Cytotype A	2432 ± 186.61	2188 ± 171.34	90.22 ± 1.30
	Cytotype B	2123 ± 178.23	0	0
	P. nodosus	2560 ± 186.01	2252 ± 151.80	88.14 ± 1.27
	P. wrightii	2720 ± 213.07	2076 ± 214.70	81.86 ± 2.56
SBL	*P. lucens*	2300 ± 187.08	2083 ± 155.31	89.72 ± 0.43
	P. perfoliatus	2240 ± 208.80	1986 ± 197.79	88.44 ± 0.92
SLNL	*P. crispus*	1926 ± 478.66	1893 ± 199.38	81.68 ± 1.42
	P. berchtoldii	2480 ± 177.20	1876 ± 141.01	75.60 ± 0.91
SFL	*P. pusillus*	2466 ± 183.18	2098 ± 168.82	85.32 ± 1.02
	P. pectinatus	2300 ± 158.11	1926 ± 104.57	84.06 ± 1.80
	P. amblyphyllus	2580 ± 86.02	207 ± 91.75	77.74 ± 2.01

Table 5 Fruit set in different species of the genus *Potamogeton* in lentic water bodies of Kashmir valley. *HET* = heterophyllous (floating-leaved); *SBL* = submerged broad-leaved; *ISBL* = intermediate between submerged broad and linear-leaved; *SLNL* = submerged linear-narrow leaved, and *SFL* = submerged-filiform leaved species

Habit	Species	No. of spikes per ramet	No. of flowers per ramet	No. of fruits per ramet	% fruit set
HET	*P. distinctus*	1.56 ± 0.26	84.53 ± 15.21	191.20 ± 40.89	63.03
	P. natans				
	Cytotype A	1.53 ± 0.16	50.06 ± 5.90	120.13 ± 13.76	68.4
	Cytotype B	1.20 ± 0.11	66.23 ± 3.90	0	0
	P. nodosus	1.66 ± 0.27	85.53 ± 16.26	197.20 ± 39.90	63.88
	P. wrightii	2.13 ± 0.27	78.33 ± 10.74	135.26 ± 16.39	46.5
SBL	*P. lucens*	1.66 ± 0.18	60.13 ± 6.87	153.73 ± 17.42	65.1
	P. perfoliatus	2.66 ± 0.47	21.66 ± 1.40	5.53 ± 2.02	10.26
SLNL	*P. berchtoldii*[a]	1.58 ± 0.17	6.23 ± 0.84	0	0
	P. crispus	3.73 ± 0.62	19.46 ± 3. 07	19.30 ± 4.09	19.3
SFL	*P. amblyphyllus*[a]	1.33 ± 0.12	10.46 ± 0.98	0	0
	P. pectinatus	3.66 ± 0.63	24.40 ± 3.36	28.33 ± 6.12	25.90
	P. pusillus	2.44 ± 0.47	9.66 ± 1.95	1.70 ± 0.47	6.76

[a]Species inhabiting running water only

selected during the present study, the chromosome count was recorded at diakinesis, metaphase-I and anaphase-I, and PMC meiosis was worked out for all the species across selected sites for four consecutive years.

Regardless of their ploidy-level, the chromosomes in all species presently investigated showed perfect homologous pairing of their small, rod shaped bivalents, which had only one chiasma per bivalent. Anaphasic segregation proceeded normally. This observation is consistent with the presumed allopolyploid nature of these species which, however, needs to be established with more definitive evidence. Molecular studies by Wang et al. (2007) revealed that *P. natans* (2n = 52) probably is an allotetraploidand also proposed that *P. lucens* and *P. maakianus* also are allotetraploids of uncertain parentage.

Meiosis in the presently studied species appears to be normal with perfect bivalent pairing. Heterophyllous species produced more than 80% viable pollen. However, most PMCs of the octoploid cytotype of *P. natans* exhibited abnormal anaphasic segregation and a pollen viability close to zero; hence no fruit set occurred in the species. The SNL species yielded 75–85% viable pollen. In these species anaphasic disjunction was abnormal (i.e. lagging chromosomes and unequal segregation) in 5–10% of the PMCs (probably on account of structural hybridity), which may also be one of the causes of low fruit set in these species other than lack

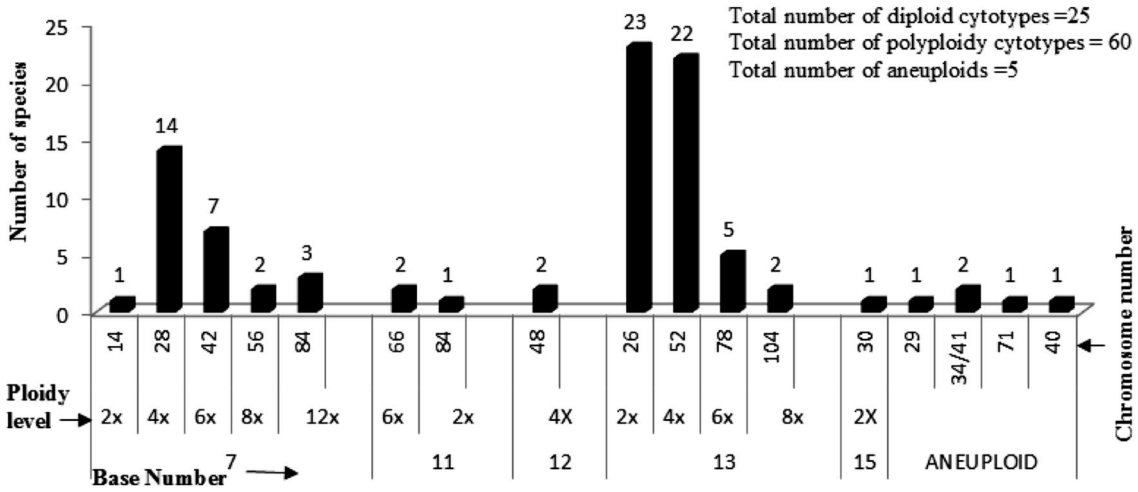

Fig. 5 Chromosome conspectus of the genus *Potamogeton* s.l (compiled from Fedôrov 1969; Hollingsworth et al. 1998; present work). In addition Kaplan et al. (2013) reported 11 new chromosomes counts for the genus *Potamogeton*

of pollination, small size of spikes and fast flowing waters in lentic water habitats (Ganie et al. 2008, 2016).

It has been proposed that the base number x = 7 represents the ancestral base number in *Potamogeton* and that species with x = 13 arose from multiple origins through aneuploidy (Les and Sheridan 1990). The question of ancestral base number in the genus has been considered by various workers (Les 1983; Kaplan et al. 2013), and till date the question has not been resolved fully. Previously, Stern (1961) and Haynes (1974) had interpreted counts of 2n = 26 or 28 as the diploid level in the genus. Goldblatt (1979) suggested x = 7 as the base number of Potamogetonaceae, with all counted species having a 2*n* number in excess of 14 indicating polyploidy. This proposition is in agreement with Grant (1963) and Stebbins (1971), who attributed haploid numbers exceeding n = 10–13 in all plants, as an indication of polyploidy. Ehrendorfer et al. (1968) characterized angiosperm progenitors as having a base number of x = 7 and *Potamogeton* occurs within the early diverging sub-class Alismatidae (Takhtajan 1969; Cronquist 1981). Consequently, Les (1983) concluded that a base number of x = 7 was feasible for *Potamogeton*.

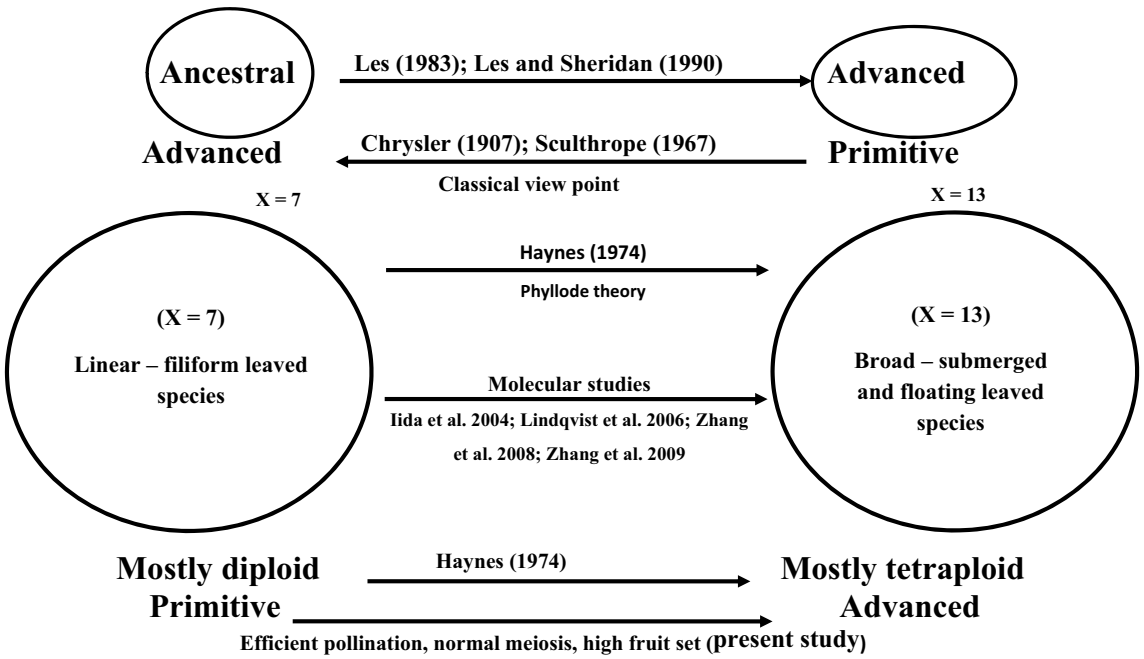

Fig. 6 Different views proposed by various authors regarding evolution of leaf and base number in the genus *Potamogeton*

Counts of 2n = 14 reported for populations of *P. foliosus* (Wiegand 1899) and for *P. perfoliatus* (Moor 1973) cannot be ignored.

In the present study, two groups could be distinguished by their chromosomal base numbers (x = 7 or x = 13) with respect to the overall leaf morphology of species, though the basic origin number of *Potamogeton* has not been entirely explained and confirmed (Wan et al. 2012). The heterophyllous species and broad-leaved submersed species have the base number of x = 13; whereas, the exclusively submerged linear to filiform-leaved species have x = 7 (Table 2). Based on the presumption that x = 13 is derived and x = 7 is ancestral (Les 1983; Les and Sheridan 1990), these broad-leaved species could be regarded as advanced. The results of the present investigations indicate that the base number x = 7 in SLNL species is replaced by x = 13 in HET and SBL species and this is in agreement with Les (1983) and Les and Sheridan (1990) who advocated that x = 7 represented the ancestral base number and x = 13 is derived. The efficient pollination mechanisms, normal meiotic behavior, high pollen fertility and high fruit set observed during the present study in HET and SBL substantiate this view point (Fig. 6).

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