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KARYOLOGY OF SOME COASTAL AND WATER PLANTS FROM FAR EAST (RUSSIA)

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Cytogenetic studies on four species of vascular coastal and water plants from Russian Far East are presented. During the present investigation the next chromosome numbers have been revealed: *Gypsophila pacifica* (2*n* = 34), *Allium sacculiferum* (2*n* = 32), *Mertensia maritima* (2*n* = 24), and *Nelumbo komarovii* (2*n* = 16). Unusual chromosome numbers for these species have not been noted but it was the first case of karyological studies of *Nelumbo komarovii* from the Jewish Autonomous Region and most northern habitat. The number of nucleoli in interphase nuclei of these species was counted. Interphase nuclei of studied species contain 1–4 nucleoli except in *A. sacculiferum* so far which have 1–2 nucleoli per cell. Different points of view on polyploidy of studied species are discussed.

Key words*:* chromosome number, karyotype, nucleoli, seacoast halophytes

INTRODUCTION

Seacoast halophytes are exceptional subjects for study of adaptation mechanisms to constantly changing environments. Seacoasts are the area with high rate of speciation. Species, populations and plant communities are continuously and intensively modifying due to extremely dynamic and tense ecological factors. It was demonstrated that di-, tetra-, hexa- and octoploid species were found among vascular plants of seashores and the ecological amplitudes of polyploids are wider than those of diploids (Probatova and Seledets 1999). Different cytotypes are found within the same genus and even species. Generally, they vary in ploidy, often with the same basic number (x) or rarely with various basic numbers. Such a phenomenon is characteristic of species differentiation (Grant 1981, Pershina 2009, Probatova 2014).

This requires extensive cytogenetical analysis of seacoast species from different areas. The article is based on the data obtained by the authors while studying the karyotypes of four seacoast species from previously unexplored habitats.

MATERIALS AND METHODS

In September 2015, one of the authors (E. V. Burkovskaya) collected seeds and plants on the coast of the Uglovoy bay near Prokhladnoie village on the middle section of the flat sea coast (Fig. 1). Dr V. Yu. Barkalov checked the definition of the species. *Nelumbo komarovii* seeds, collected on the lake Utinoie of Oktyabrsky district of the Jewish Autonomous Oblast, were kindly provided by Prof. Yu. N. Zhuravlev (Fig. 1). The seeds were stored in refrigerator at 2–4 °C. Herbarium specimens that confirm the cytological screening are kept at the Cell Biology Lab of the Federal scientific centre of the East Asia terrestrial biodiversity, Russian Academy of Sciences, Far East Branch. Plants' names follow Kharkevych (1985–1996).

The plant material (root tips) for the cytogenetic studies was collected from seedlings. Seedlings grown from seeds were germinated on moist filter paper under laboratory conditions at room temperature. *Nelumbo komarovii*'s seeds were scarified before germination and placed into glass container with water as described in the previous report (Khrolenko *et al*. 2019). The materials were prepared and analysed according to generally accepted techniques for seed plants (Smirnov 1968) with some our modifications. Actively grow-

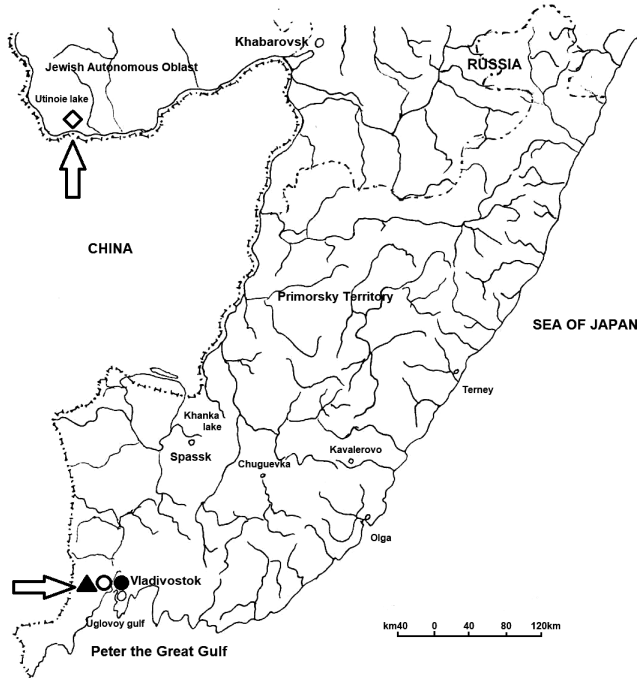

Fig. 1. Location of studied species (shown by arrows): ▲ = *Gypsophila pacifica*, ● = *Allium sacculiferum*, ○ = *Mertensia maritima*, and ◇ = *Nelumbo komarovii*

ing root tips were pretreated with 0.2% colchicine solution for 2 to 3 hours at room temperature (about 22 °C), rinsed in water and fixed in Farmer's fluid (ethanol – 3 parts: glacial acetic acid – 1 part) overnight. The material was then mordanted with 4% iron alum for 15 min and stained with 1% aceto-haematoxylin solution for 6–12 hours. *N. komarovii*'s preparations were stained with 5% Giemsa solution (Merck, Germany).

The 1.5 to 2 mm long root tips were cut out separately and places in a drop of chloral hydrate solution on a microscope slide. The slides were prepared from root tip meristem cells using the improved squash technique. Non-overlapping metaphase spreads with whole chromosome set ($2n$) were chosen and examined under Leica DMLS ("Leica Microsystems", Germany). The cells were photographed using an Axioscop-40 microscope equipped with a digital camera AxioCam HRc (Zeiss, Germany). The present study was made by technical support from the Microscopy Objects' Collective Use Centre of the Federal scientific centre of the East Asia terrestrial biodiversity, Russian Academy of Sciences, Far East Branch, Vladivostok.

RESULTS AND DISCUSSION

Gypsophila pacifica Kom. (Caryophyllaceae) is a perennial herbaceous plant and a petrophyte (Pavlova and Bezdeleva 1996). The study determined diploid number of chromosomes $2n = 2\times = 34$ in this species (Fig. 2a). *G. pacifica* had really small chromosomes' size (1.5–2 μm), that is why the mitotic chromosomes at their late prophase were studied in addition to metaphase during the karyological analysis (Fig. 2b). In late prophase the chromosomes are longer in comparison with metaphase, where they are fully condensed. As previously reported, the same number of chromosomes was determined a few times for this species collected from Primorsky Territory (Probatova 2014). One more chromosome number ($2n = 68$) was registered for this species from France and commercial cultivars from Italy (Favarger 1946, Vettori *et al*. 2015).

Additionally, the number of nucleoli in interphase nuclei of *G. pacifica* was determined. In this case, 765 nuclei were studied and their number varies from 1 to 4 (Fig. 3a). The average number of nucleoli per cell is 1.16±0.017. Among the studied nuclei, 88.89% were interphase nuclei with one nucleolus, 6.93% with two nucleoli, 3.92% with three nucleoli and 0.26% with four nucleoli. According to these results, it is possible to assume that the karyotype of this species contains 1–2 pairs of nucleolar chromosomes.

For closely related species *Gypsophila violacea* (Ledeb.) Fenzl., Rudyka (1990) determines $2n = 36$ chromosomes. Some experts suggest it to be incorrect (Probatova 2014), because previously Zhukova (1982) determined $2n = 34$ chromosomes in specimens from Magadan region. Analysis of the latest data from comparative genomics shows that species with high basic chromosome

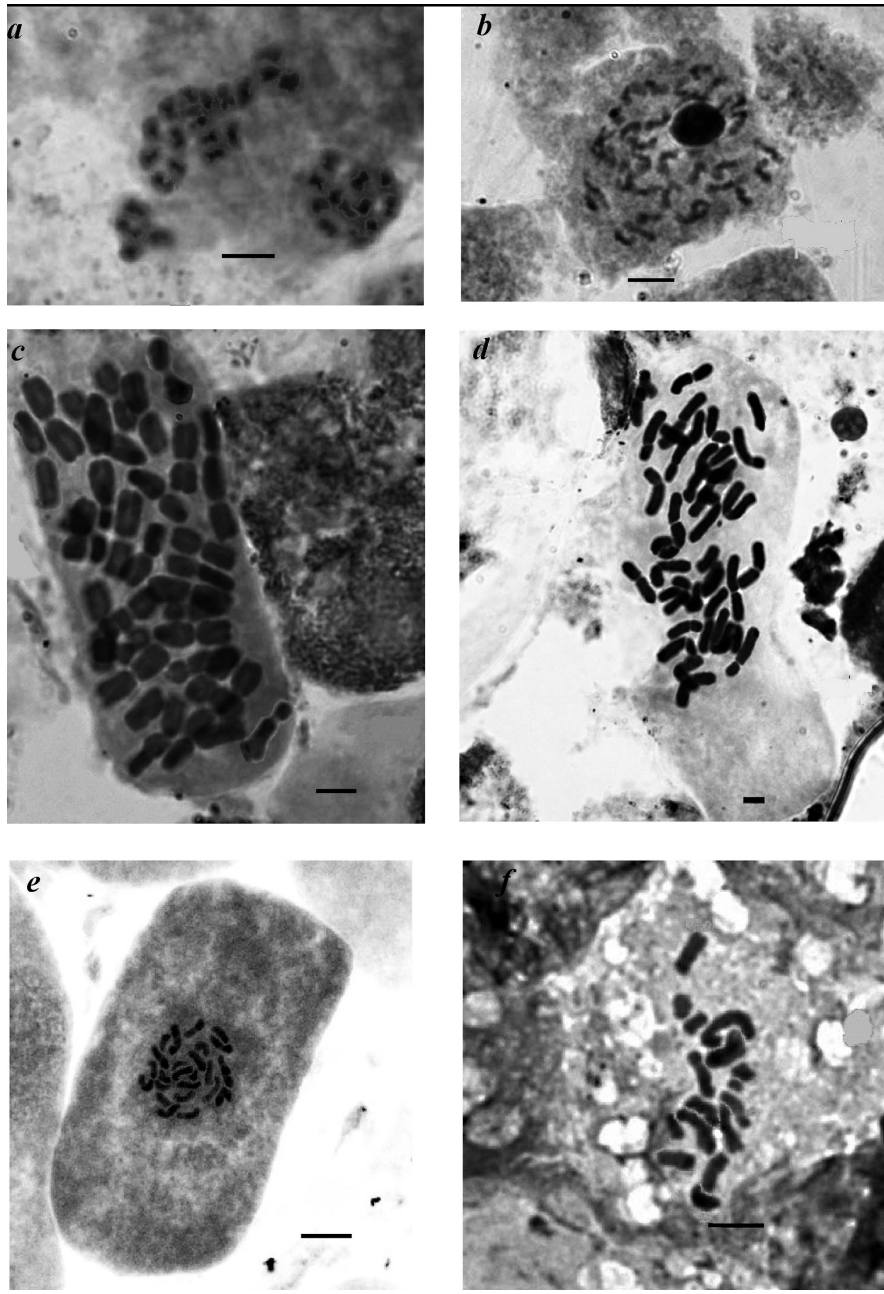

Fig. 2. Chromosome complements of studied species: a) *Gypsophila pacifica*, b) *Gypsophila pacifica* (late prophase), c) *Allium sacculiferum*, d) *Allium sacculiferum* (late prophase), e) *Mertensia maritima*, and f) *Nelumbo komarovii*. Bar = 5 μm. Ocular lens W-PI 10×/23; objective lens oil immersion 100×

numbers can be ancient polyploids, often allopolyploids. After one or several rounds of polyploidisation, they return to the diploid state (Alix *et al*. 2017, Pershina 2009, Rodionov 2013, Soltis and Soltis 2009, Soltis *et al*. 2014). Such events could have happened in the evolution of the genus *Gypsophila* L.

Allium sacculiferum Maxim., (Alliaceae) (syn. *Allium pseudojaponicum* Makino; *Allium yuchuanii* Y. Z. Zhao et J. Y. Chao) is a perennial bulbous herbaceous plant (IPNI 2020). The study of the plant's karyotype showed $2n$ = 4x = 32 number of chromosomes (Fig. 2c–d). Our definition corresponds to the previously determined number of chromosomes in this species in Primorsky Territory is $2n$ = 32 (Gritsenko and Gurzenkov 1983, Probatova 2014). In Primorsky Territory, the mainly studied habitats of *A. sacculiferum* are in Khasansky district (Ryazanovka settlement, Talmi Lake, Gamova Cape). This report presents the results of karyological study of *A. sacculiferum* from a previously unexplored habitat of Nadezhdinsky district. The chromosome number $2n$ = 32 is determined in several habitats of this species in China and Republic of Korea (Choi and Oh 2003, Ko *et al*. 2009, Seo and Kim 1989, Seo *et al*. 2007). In this species from Korea Peninsula, one B-chromosome ($2n$ = 32+1B) was observed (Ko *et al*. 2009). For this species from South Korea, the somatic chromosome number $2n$ = 42 was reported (Lee 1967). Furthermore, for this species (under the species name *A. thunbergii* G. Don) two chromosome numbers $2n$ = 16 and $2n$ = 32 are given (Shukherdorj *et al*. 2018).

Variability is found in *A. sacculiferum* karyotype symmetry, with metacentric, submetacentric and subtelocentric chromosomes. The study of nucleoli in 387 interphase nuclei showed that their number varies from 1 to 2 ones (Fig. 3b). The average number of nucleoli per cell is 1.13±0.071. The percentage of nuclei with one nucleolus is 86.96% and 13.04% with two nucleoli. It suggests the presence of one pair of nucleolar chromosomes.

It is assumed the genome of *A. sacculiferum* ($2n$ = 32) is allotetraploid and consists of two diploid ones with $2n$ = 16. Data on chromosome mapping of the 5S rRNA by applying fluorescence in situ hybridisation give the possibility to suppose that one set of *A. sacculiferum* genome has originated from close relative to *A. deltoide-fistulosum* with diploid cytotype $2n$ = 16 (Seo *et al*. 2007).

The authors of the karyological study of genus *Allium* species noted common polymorphism by the number of chromosomes. For example, di-, tri- and tetraploid cytotypes ($2n$ = 16, 24, 32) are known in *A. monanthum*. Moreover, both *A. senescens* and *A. anisopodium* has two cytotypes with $2n$ = 16 and 32 (Probatova 2014, Volkova and Pshennikova 2013). Friesen and co-authors point out that the process of polyploidy, geographical isolation and hybridogenesis are the grounds for speciation of Asian alliums (Friesen 1988, Friesen *et al*. 2006). Large chromosomal size (10–15 μm) also is a characteristic feature of *Allium* species. Therefore, the genus *Allium* L. is of interest for further karyological studies.

Mertensia maritima (L.) Gray (Boraginaceae) (Syn. *Pulmonaria maritima* L., *Pneumaria maritima* Hill, *Mertensia simplicissima* G. Don, *M. maritima* subsp. *asiatica* H. Takeda, *M. asiatica* J. F. Macbr.) is a circumpolar littoral species. It is a herbaceous perennial with ascending and procumbent thickly foliaceous stems and filamentary rhizome (IPNI 2020, Starchenko 1991). It is the only representative of *Mertensia* Roth genus, common for the coasts of all the oceans in the northern hemisphere (Popov 1953). This species grows on sandy shores and pebbles of the seacoast. The study of *M. maritima* karyotype has showed $2n = 24$ chromosomes (Fig. 2e). This species (including *M. simplicissima*) is known with $2n = 24$ chromosomes (Dalgaard 1988, Dawe and Murray 1981, Gervais and Cayouette 1985, Probatova 2014). To describe karyotype, 253 interphase nuclei have been studied to determine the number of nucleoli. Their number varies from 1 to 3 (Fig. 3c). The average number of nucleoli per cell is 1.25 ± 0.04. Percentage of interphase nuclei with one nucleolus is 77.78%, 19.61% with two nucleoli and 2.61% with three nucleoli. According to these results, it is possible to suppose that the karyotype of the studied species contains 1–2 pairs of nucleolar chromosomes.

There is an opinion that *M. simplicissima* grows in the south of Primorsky Territory, and *M. maritima* in the northern and European part of the Russian Federation (oral communication of M. N. Lomonosova). Different points of view on genus' taxonomy, especially on *M. simplicissima* and *M. maritima* positions are discussed among scientists (Alton and FitzGerald 2009). In our opinion, additional studies are required in order to define *M. simplicissima* as a separate species.

Nelumbo komarovii Grossh. (Nelumbonaceae) (Syn. *Nelumbo nucifera* Gaertn.) is a herbaceous plant and a hydrophyte (IPNI 2020, Tsvelev 1987). *N. komarovii* is known as an amazing water plant, which grown in the lakes, rivers of Jewish Autonomous Region, Khabarovsky and Primorsky Territories. It is a rare relict species and it was listed in the Red Data Book of Russian Federation (1988). The determined number of chromosomes is $2n = 16$ (Fig. 2f). Our definition corresponds to the previously determined number of chromosomes in *N. komarovii* of Primorsky Territory. But it was the first case of chromosome number definition in *N. komarovii* from the Jewish Autonomous Region and most northern habitat. The same number of chromosomes in *N. komarovii* (including species' synonymous name *Nelumbium komarovii*) and in specimens from Primorsky Territory (Lotos lake, Khasansky district and Ilistaya river, Chernigovsky district) was determined three times (Probatova 2006, Probatova and Sokolovskaya 1981).

Most *N. komarovii* chromosomes have two arms, possibly metacentric and submetacentric. There are dotted chromosomes with an uncertain centromere position. The study of number of nucleoli in interphase nuclei (among 211 cells) showed that their number varies from 1 to 3 nucleoli (Fig. 3d). The aver-

age number of nucleoli per cell is 1.2±0.03. The interphase nuclei with 1 or 2 nucleoli (81.5% and 17.06%, correspondingly) are the most common; nuclei with 3 nucleoli are rarely observed (1.42%). According to these results, it is possible to assume that the karyotype of *N. komarovii* contains 1–2 pairs of nucleolar chromosomes.

Many researchers suggest that *N. komarovii* is a Far Eastern race of *N. nucifera* Gaertn., widely used in medicine, and as a delicacy in Chinese and Japanese cuisine. Karyotype study revealed the same chromosome number ($2n = 16$) in *N. nucifera* (Diao *et al*. 2005, 2006, 2011, Meng *et al*. 2017, Ming *et al*. 2013, Yang *et al*. 1998). Our data on the morphology of *N. komarovii* chromosomes are confirmed by studies of Chinese scientists (Meng *et al*. 2017). They recorded metacentric, submetacentric and acrocentric chromosomes in *Nelumbo* Adans. complex. Karyotypes of some provenances of *N. nucifera* s. l. (including *N. lutea* Willd.) were analysed using fluorescence *in situ* hybridisation (FISH) with 45S ribosomal RNA gene probes. The 45S rDNA loci were mapped in 2–4 of chromosomes per haploid genome for different cultivars and populations of this species. Therefore, chromosome sets of *N. nucifera* have 2–4 nucleolus organising regions (Diao *et al*. 2005, 2011, Meng *et al*. 2017).

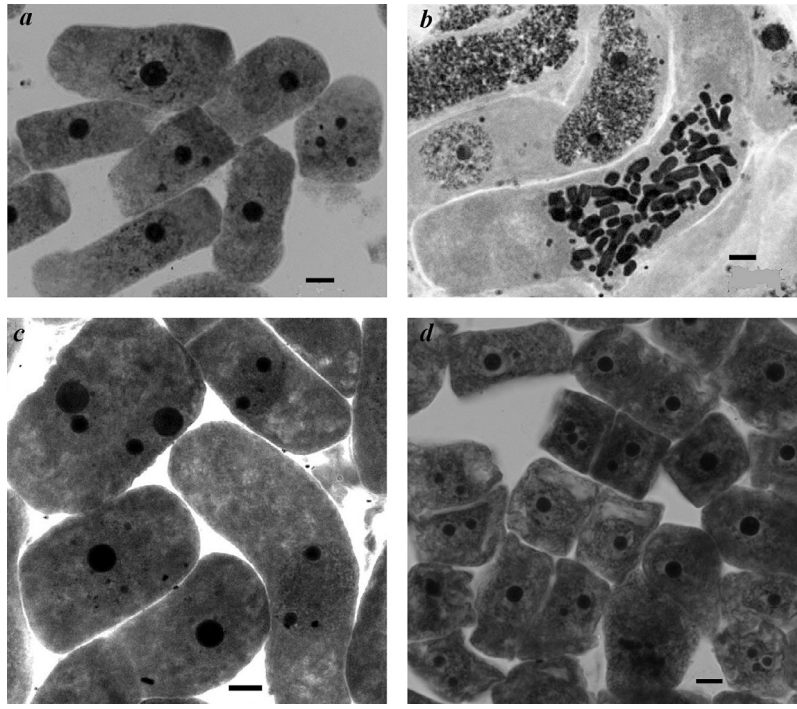

Fig. 3. Nucleoli in interphase nuclei of studied species: a) *Gypsophila pacifica*, b) *Allium sacculiferum*, c) *Mertensia maritima*, and d) *Nelumbo komarovii*. Bar = 5 μm. Ocular lens W-PI 10×/23; objective lens oil immersion 100×

In conclusion, the karyological analysis of three plant species of previously unexplored habitats near Uglovoy bay of Nadezhdinsky district has determined $2n = 34$ number of chromosomes in *G. pacifica*, $2n = 32$ in *A. sacculiferum* and $2n = 24$ in *M. maritima*. The number of chromosomes in *N. komarovii* of the Jewish Autonomous Oblast is $2n = 16$. Present data clearly show that unlike plants of *A. sacculiferum* where karyotype has one pair of chromosomes with secondary constrictions, other species may have more chromosomes with secondary constrictions.

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