CHROMOSOME NUMBERS AND KARYOTYPE ANALYSES OF SPECIES OF SUBFAMILY SALICORNIOIDEAE (CHENOPODIACEAE) FROM IRAN

S. M. Ghaffari, L. Saydrasi, H. Ebrahimzadeh, & H. Akhani

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Chromosome counts are reported from 19 populations representing seven genera and 11 taxa of the subfamily *Salicornioideae* (*Chenopodiaceae*) from Iran. In all studied accessions a basic chromosome number of x = 9 is reported. First chromosome numbers are reported for *Halostachys belangeriana* (2n=8x=72), *Halopeplis pygmaea* (2n=2x=18) and *Kalidium capsicum* (2n=4x=36). The species of the genus *Salicornia* are divided into tetraploid and diploid groups. The newly described endemic species *S. persica* is only tetraploid (2n=4x=36). But the diploid group (2n=2x=18) may represent three different taxa.

Seyed Mahmood Ghaffari, Institute of Biochemistry and Biophysics, University of Tehran, P. O. Box 13145-1384, Tehran, Iran. E-mail: ghaffari@ibb.ut.ac.ir*; L. Saydrasi, H. Ebrahimzadeh, and H. Akhani, School of Biology, College of Science, University of Tehran, P. O. Box 14155-6455, Tehran, Iran. E-mail:* akhani@khayam.ut.ac.ir

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شمارش کروموزومی و بررسی کاریوتیپ گونه های زیرتیره Salicornioideae از تیره اسفناج در ایران

سید محمود غفاری، لیلا سیدراثی، حسن ابراهیم زاده و حسین آخانی

شمارش کروموزومی ۱۹ جمعیت متعلق به ۷ جنس و ۱۱ آرایه ازتیره اسفناج (زیرتیره *Salicornioideae*) از ایران گزارش می شود. کلیه واحدهای مورد مطالعه دارای عدد پایه کروموزومی x=۹ می باشد. اولین گزارش کروموزومی برای گونه های *Halostachys belangeriana* ، *Halopeplis pygmaea* و *Kalidium caspicum* ارائه می شود. گونه های جنس *Salicornia* به دو گروه تتراپلوییید و دیپلوییید تقسیم‌بندی می شوند. گونه *Salicornia persica* که اخیرا به عنوان گونه بومی مرکز ایران شرح داده شده است، تتراپلوییید می باشد. گروه دیپلوییید را می توان در سه آرایه متفاوت تقسیم بندی کرد.

INTRODUCTION

The family *Chenopodiaceae* comprises about 100 genera and 1500 species. Chenopods are common in deserts and especially in saline or alkaline soils. The Irano-Turnian region is one of the major center of origin and diversification of *Chenopodiaceae.* According to Flora Iranica 38 genera and 178 species have been known from Iran (Hedge & al. 1997). However, some more new species and new records have been added in subsequent publications which resulted addition of two more genera and seven more species (Akhani 2003, Assadi 2001; Ghobadnejhad *& al.* 2003, Akhani *& al.* 2005, Rahiminejad *& al.* 2004; Rahiminejad & Ghaemmaghami 2005).

The subfamily *Salicornioideae* with ca. 15 genera and ca. 90 species is a monophyletic lineage with a cosmopolitan distribution (Kadereit & al. 2006; Kapralov & al. 2006). These are succulent species which are characterized by their distinctive reduced leaves which may be modified to form an articulated, photosynthetic stem. These specialized plants generally have spike-like compound inflorescence, comprised of paired cymules of tiny flowers that are sessile within succulent free or fused bracts (Shepherd & al. 2005; Kühn 1993, Scott 1977). The species of *Salicornioideae* are among the most salt-tolerant land plants which inhabited in hygrohalopyhtic and coastal communities. In spite of only seven genera and nine

reported species in Iranian *Saliocornioideae* (Hedge & al. 1997, Akhani 2003), they play a major role in large parts of coastal and inland halophytic communities in Iran (Akhani 2004).

So far, there are ca. 19 reports of chromosome counts of Iranian *Chenopodiaceae* (Uotila 1973, Hekmat–Shoar 1978, Hekmat–Shoar & Manafi 1982, Ghaffari 1986, 1988, Ebrahimzadeh & al. 1994; Akhani & al. 2005). In this paper the chromosome counts and karotypes of all members of Iranian *Salicornioideae* is reported.

MATERIALS AND METHODS

The seeds and voucher specimens have been collected from most parts of Iran and one location in United Arab Emirates. The seeds were then germinated on moist filter paper in the laboratory (ca. 21˚-25˚C). The growing root tip of ca. 1.0 – 1.5 cm long were cut and pretreated in 0.002 M 8 – hydroxyquinoline (3 hours) at 20 ˚C. After treatment, the root tips were fixed in Piennaŕs solution (6 parts ethanol, 3 parts chloroform and 2 parts propionic acid) for 24 hours. The chromosomes were stained by the Feulgen method after hydrolysis in 1 N Hcl for 10 minutes at 60˚C. Root tips were then squashed in 2% acetocarmine. The photomicrographs were taken with an Olympus universal research microscope fitted with a photographic attachment, using an oil immersion objective (100X). The voucher specimens are deposited in the herbaria of Research Institute of Forests and Rangelands (TARI), Natural History Museum of Iran (MMTT) and the private herbarium of H. Akhani.

RESULTS

Arthrocnemum macrostachyum (Moric.) C. Koch
Bushehr: Between Gavbandi and Kangan, Bidkhoon, 2.12.1987, Assadi & Akhani 64014 (TARI). 2n=18.

A. macrostachyum is a coastal species with a range from W Indian through SW Asia to the Mediterranean coast (Freitag 1991). The studied population from the Persian Gulf coasts show a diploid level, 2n=18 (Fig.1A). According to previous reports four different ploidy levels have been reported i.e. diploid 2n=2x=18, tetraploid 2n=4x=36, hexaploid 2n=6x=54 and octaploid 2n=8x=72 (Fedorov 1974; Goldblatt 1981-1988; Goldblatt & Johnson 1990-2003; Castroviejo & Coello 1980; Runemark 1996). Karyotype in this species consists of 4 submetacentric and 5 metacentric chromosomes (Fig. 2A).

Halocnemum strobilaceum (Pall.) M. Bieb.
Yazd: Corner of Kavire Marvast, on the road from Mehriz to Marvast, 1600m, 25.11.1987, Assadi & Akhani 61761 (TARI). 2n=18; Bushehr: 11km NE of Borazjan towards Kazeroun, 20.11.1991, Akhani 7955 (Hb. Akhani). 2n=18; Tehran: Karaj, W of Mardabad, 1200m, 4.12.1987, Assadi & Akhani 61717 (TARI). 2n=18 (Fig. 1B).

Halocnemum strobilaceum has a wide distribution range in the Irano-Turanian, Mediterranean and Saharo-Sindian regions. Three collections of this taxon were studied, that all were diploid with 2n=2x=18 (Fig. 1B). The karyotype of this species was considerably symmetrical, and consisted of 3 pairs of metacentric and 6 pairs of submetacentric chromosomes (Fig. 2B). Five previous counts for this species were 2n=18 (Tarnavschi 1938, Hekmat-Shoar & Manafi 1982, Khatoon & Ali 1993, Al-Turki *& al.* 2000) and 2n=4x=36 from Iraq (Najaf lake) by Murin & Sheikh (1971).

Halopeplis perfoliata (Forssk.) Schweinf. & Aschers.
United Arab Emirates: Near Dubai, near sea coast, 16.12.1990. H. Akhani 9173 (Hb. Akhani). 2n=18.

H. perfoliata is a coastal halophyte distributed around the Red Sea and the Persian Gulf coasts with a Nubo-Sindian range (Freitag 1991). During the project we had only viable seeds from a population collected from UAE. Our diploid count (2n=18) (Fig. 1C) is in accordance with previous report of this species by Al-Turki *& al.* (2000). The karyotype of this species consisted of five pairs acrocenteric, two pairs metacenteric and two pairs telocenterc chromosomes (Fig .2C).

Halopeplis pygmaea (Pall.) Bunge ex Ungern-Sternb.
Fars: 23 km W Abadeh Tashk, saline shores of Tashk lake, 27.11.1987, Assadi & Akhani 61790 (TARI). 2n= 18.

Halopeplis pygmaea is almost an omni-Irano-Turanian species and occur in moist high saline soils from the Caucasus to China. Its distribution extends to Hormozgan province in S. Iran. The habitat of this species is hygrohalophytic communities and occurs with, or close to *Salicornia* communities. Mitotic metaphase showed chromosome compliment of 2n=18 in *H. pygmaea* (Fig. 1D). Apparently, it is a first chromosome number report for this species. Previously the Mediterranean vicariant species *H. amplexicaulis* (Vahl) Cesati, Passerini & Gibelli was reported with the same chromosome number (Blanché & Molero 1987). The chromosome sets consisted of five pairs metacentric, two pairs submetacenteric, one pair telocenteric and one pair acrocenteric (Fig 2D).

Halostachys belangeriana (Moq.) Botsch.

Yazd: Corner of Kavire Marvast, on the road from Mehriz to Marvast, 25.11.1987, Assadi & Akhani 61757 (TARI); 2n=72; Semnan: Touran Protected Area, between Delbar and Ahmad-abad, along Kal-Shur river, in high salty soils, 31.10.1987, Akhani 4210 (MMTT, Hb. Akhani). 2n=72 (Fig. 1E).

The monotypic genus *Halostachys* is distributed from Caucasus, E. Turkey and through Iran and Central Asia to China (Hedge & al. 1997) on most salty and moist soils. Two studied samples were octaploid with 2n=8x=72 chromosomes (Fig. 1E). The karyotype was consisted of 5 pairs of metacentric, 26 pairs of submetacentric and 5 pairs of acrocentric chromosomes (Fig. 2E). We did not find any chromosome report for this species in available references and indexes.

Kalidium capsicum (L.) Ung.-Sternb.
Semnan: Touran Protected Area, ca. 8 km NE Razeh, river bed in *Tamarix* tickets, 30.9.1987, H. Akhani 4136 (Hb. Akhani, MMTT). 2n=36.

From six known species of the genus *Kalidium*, only *K. capsicum* is known in saline habitats from Semnan, Golestan and East Azerbaijan provinces (Hedge & al. 1997, Assadi 2001). This species has a wide distribution from the Caspian area to most parts of Central Asia (Nikolskaya 1972). The presence of *K. foliatum* (Pall.) Moq., as has been reported based on a sterile specimen (Assadi 2001) is doubtful because the young plants of *K. capsicum* have well developed leaves which reduce in mature shoots. Apparently the only reported chromosome counts from the genus *Kalidium* is a diploid report (2n=18) for *K. foliatum* by Lomonosova & Krasnikov (1993). Our count for *K. capsicum* was tetraploid with chromosome complement of 2n=36 (Fig. 1F). The karyotype consisted of one pair of metacentric, 14 pairs of submetacentric and three acrocentric chromosomes (Fig.2F).

Microcnemum coralloides (Loscos & Pardo) Buen
Arak (Ostane Markazi): N. of Kavir-e- Meyghan, near Deh-Namak, 27.8.1986. H. Akhani 1112 (Hb. Akhani; TARI). 2n=18.

Microcnemum is a monotypic genus containing *M. coralloides* with two known subspecies: subsp. *coralloides* is endemic in Spain and subsp. *anatolicum* Wagenitz has a range in Central Turkey, Iran, Armenia and Syria (Hedge & al. 1997). There is only one previous count (2n=18) for subsp. *coralloides* by Castroviejo & Coello (1980). Therefore it is a first report for Iranian population and the subsp. *anatolicum*. Our count yielded a diploid mitotic chromosome number of 2n=18 (Fig.1G). The karyotype formula was determined as follows: 2n=2x=18: 2m + 3sm + 4st (Fig. 2G).

Salicornia spp.

This is a widespread genus in most salty and moist soils at the margin of salt lakes and salty rivers and the coastal area of Caspian Sea and Persian Gulf. Taxonomically, *Salicornia* is an extremely difficult genus. The reports of *S. europaea* from Iranian plants by Hedge & al. (1997) and Assadi (2001) is very doubtful as parts of our present cytological studies and unpublished morphological and molecular data have shown (Akhani, in preparation). *S. persica* as a distinct endemic species in Central Iranian salines with unique morphological features has been described by Akhani (2003). In this study eight populations of *Salicornia* including three populations of *S. persica* and five populations of suspected three other species – here designated as *Salicornia* A, B and C – are presented.

TETRAPLOID GROUP

Salicornia persica Akhani
Fars: North of Maharlu Lake, 9 km ESE of Kaftarak, 1460m,18.11.1991. H. Akhani 7916 (MMTT, Hb. Akhani). 2n=36, (Fig. 1H, 2H); Fars: Between Arsanjan and Abadeyh, margin of Task Lake, near Gomban, 1560, 17.11.1991, H. Akhani 7907 (MMTT, Hb. Akhani). 2n=36; Yazd: Kavir-e Marvast, near Rahmatabad, 25.11.1987, Assadi & Akhani 61771 (TARI). 2n=36.

Three studied collections of this species were tetraploid with 2n=36 (Fig. 1H) which is in agreement with the previous report (Akhani 2003). Karyotype was consisted of 5 pairs of metacentric, 12 pairs of submetacentric and one pair of acrocentric chromosomes (Fig. 2H).

DIPLOID GROUP

Salicornia spec. A.
Tehran: Karaj: near Eshtehard, 1200m, 17.11.1987, Assadi & Akhani 61728 (TARI). 2n= 18.

In this population which represents North-Central and Northwestern Iranian populations characterized by usually short spike branches. Eighteen chromosomes have been observed at metaphase (Fig. 1I). They include three pairs metacentric, five pairs submetacentric and one pair acrocentric chromosomes (Fig. 2I).

Salicornia spec. B.
Fars: Between Arsanjan and Abadehe-Tashk, margin of Tashk Lake, near Gomban, 1560m, 17.11.1991, H. Akhani 7906 (MMTT, Hb. Akhani), 2n=18; Fars: 21

km to Perspolis on the road towards Shiraz, Assadi & Akhani 62050. 2n=18. (Fig. 1J); Fars: West of Tashk Lake, between Gomban and Kotak, 1700m, 27.11.1987, Assadi & Akhani 61806 (TARI). 2n=18 (Fig. 1K, 2J); Fars: ca. 20 km N. Kharameh, near Soltanabad, 1200m, 27.11.1987, Assadi & Akhani 61828. 2n=18.

All three populations originated from salty soils around Tashk Lake and salines in Fars province. These plants occur in the same habitat as *S. persica*, but they are well distinguished by their erect habit versus ascending to prostrate habit of *S. persica*. Their upper branches are opposite and the central flowers are obtuse. In *S. persica* the upper branches are verticillate and the central flowers are truncate (Akhani 2003). All three populations were diploid with chromosome compliment of 2n=18 (Fig. 1J, K).

The karyotypes showed that most chromosomes are metacentric and submetacentric and in two cases one pair of acrocentric chrosmosome have been detected (Fig. 2J).

Salicornia spec. C.
Bushehr: 17 km from Borazjan towards Bandare-Genaveh, Dalaki river margin, c. 40 m, 21.11.1991, H. Akhani 7995 (MMTT).

This species which differs morphologically and geographically from the Central and South-Central Iranian *Salicornia* species is a diploid (2n=18) (Fig. 1L). The chromosome compliment consists of six pairs of submetacentric and three pairs of metacentric chromosomes. (Fig. 2K).

DISCUSSION

All reported species in this study show a basic number of x=9, congruent with other members of *Salicornioideae* and family *Chenopodiaceae* (Shepherd & Yan 2003, Contandriopoulos 1968, Turner 1994). The species of the genera *Arthrocnemum*, *Halopeplis*, *Microcnemum*, *Halocnemum* and many of *Salicornia* species show diploidy.

In *Arthrocnemum macrostachyum* we found a diploid level. However, most of the reports from the Mediterranean area are tetraploid, hexaploid and heptaploid (Fedorov 1974; Goldblatt 1981-1988; Goldblatt & Johnson 1990-2003; Castroviejo & Coello 1980, Runemark 1996). But there is one report from India by Subramanian (1988) which shows a diploid level. This may interpret that the eastern populations from the Persian Gulf and Indian Ocean are original and the species has migrated later westwards. Based on ITS phylogeny (Kadereit & al. 2006) this species form a clade with *Microcnemum* with weak bootstrap support. Both genera show a rather common geographical pattern, *Arthrocnemum* as a coastal species adapted in warmer sea shores but *Microcnemum* as an inland species grow in temperate climate with disjunct distribution.

The two investigated *Halopeplis* species are diploid but with distinct differences in their karyotype supporting their specific separation.

In *Halostachys* a first chromosome number report is given. Both populations surrounding the great Kavir in Yazd and Semnan provinces represent a high ploidy (2n=72). Although rather rare in *Chenopodiaceae*, but octaploid chromosome compliments have been reported in *Arthrocnmeum* (Zeybek 1969) and *Sarcocornia fruticosa* (Castroviejo & Coello 1980) from *Salicornioideae* and in *Suaeda baluchestanica* Akhani & Podlech and *S. fruticosa* Forssk. ex Gmelin (Ebrahimzadeh & al. 1994) from *Suaedoideae*. In spite of high number of chromosomes, they represent comparatively large chrosmosome (Fig. 1E). As *H. belangeriana* is a widespread species in Central Asian deserts, this is of interest to check the chromosome numbers from other populations. Based on ITS phylogeny *Halostachys* and *Halocnemum* form a clade which morphology, geography and habitat support their affinity (Kadereit & al. 2006). However, their chromosomes differ both in number and length. The large shrubs of *Halostachys belangeriana* which sometimes form thick trunk versus much smaller subshrubs of *Halocnemum strobilaceum* are congruent with both their different ploidy level and chromosome length.

The chromosome data of the genus *Salicornia* show that the Central Iranian species are well separated into diploid and tetraploid groups. The tetraploid populations belong to *S. persica* s.l. and the diploid one to other species. In various studies of European *Salicornia* two groups of species were distinguished based on ploidy level (Davy *& al.* 2001, Contandriopoulos 1968, Piirainen 1991, Ball & Brown 1970). *S. pusilla*, *S. ramosissima* and *S. europaea* are considered diploid and *S. dolichostachya*, *S. fragilis* and *S. nitens* are defined as tetraploid. The tetraploid species of N. and Central European species are characterized by the lateral flowers which are only slightly smaller than the Central flowers. Furthermore they are obtuse and not reach to the upper segments. The tetraploid species in Iran is characterized by remarkably large central flowers which reach to the upper segments and are truncate at apex (Akhani 2003). The diploid *Salicornia* species in Iran are heterogenous and as the variation of karyotypes and ITS marker suggest they represent inter-population and

geographical variation. A detailed morphological and molecular analysis of the genus will be published in an ongoing publication by H. A.

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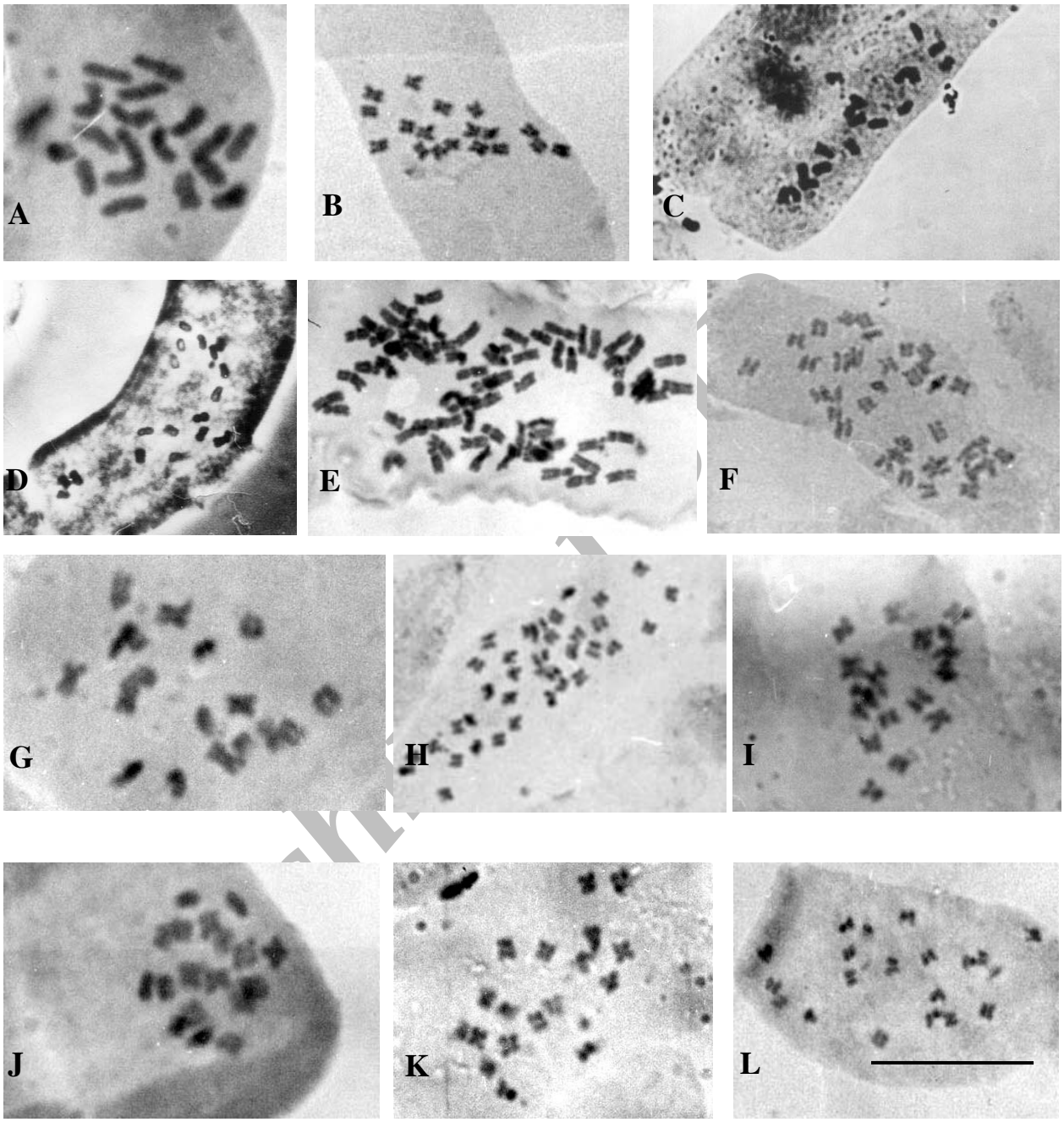

Fig. 1 Somatic metaphase chromosome complement. A: *Arthrocnemum macrostachyum* (2n=18). B: *Halocnemum strobilaceum* (2n=18). C: *Halopeplis perfoliata* (2n=18). D: *Halopeplis pygmaea* (2n=18). E: *Halostachys belangeriana* (2n=72). F: *Kalidium capsicum* (2n=36). G: *Microcnemum coralloides* (2n=18). H: *Salicornia persica* (2n=36). I: *Salicornia* spec. A. (2n=18). J: *Salicornia* spec. B. (2n=18). K: *Salicornia* spec. B. (2n=18). L: *Salicornia* spec. C. (2n=18). Bar=10µm.

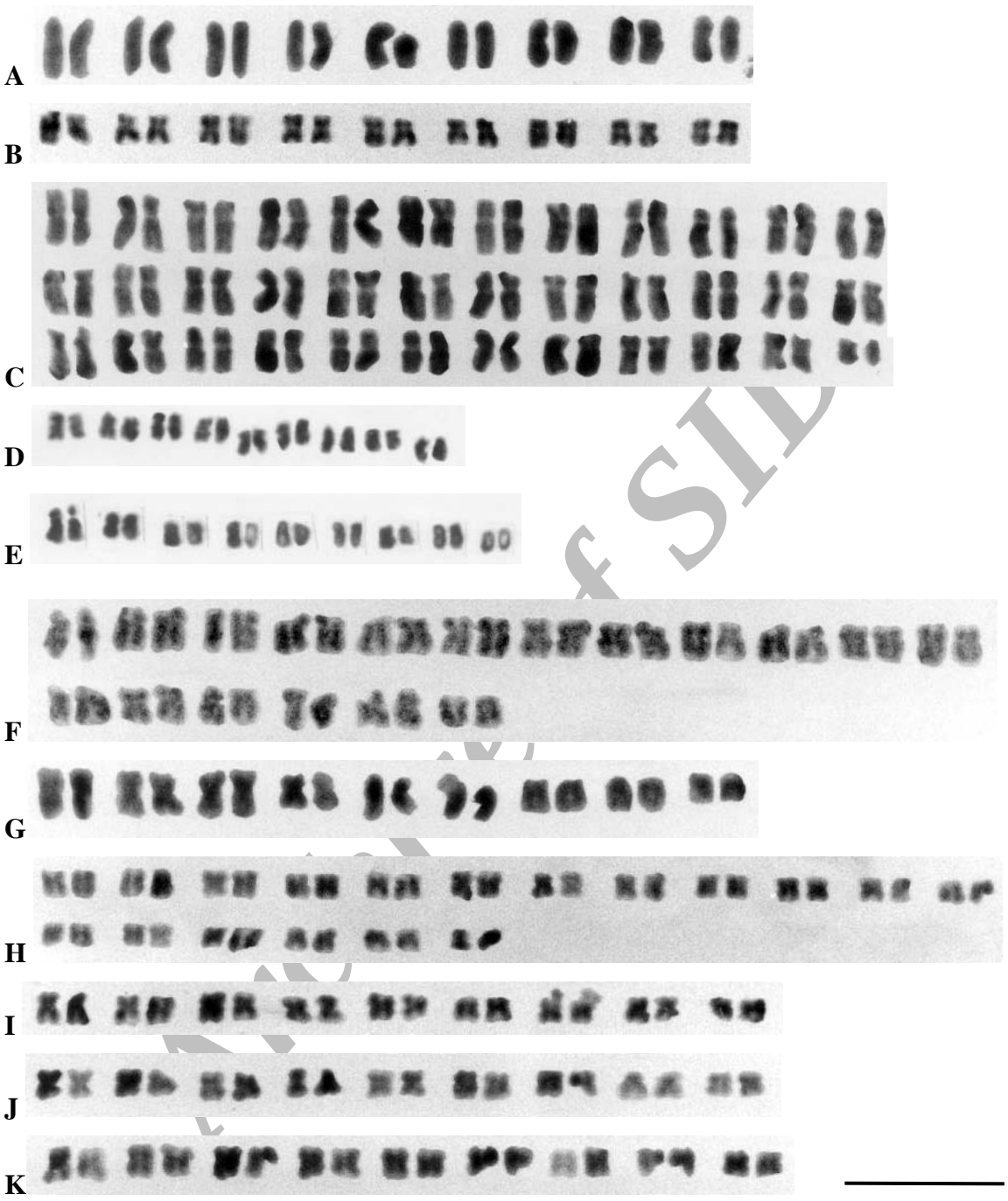

Fig. 2. Karyotype. A: *Arthrocnemum macrostachyum* (2n=18). B: *Halocnemum strobilaceum* (2n=18). C: *Halostachys belangeriana* (2n=72). D: *Halopeplis perfoliata* (2n=18). E: *Halopeplis pygmaea* (2n=18). F: *Kalidium capsicum* (2n=36). G: *Microcnemum coralloides* (2n=18). H: *Salicornia persica*, tetraploid with 2n=36. I: *Salicornia* spec. A., diploid with 2n=2x=18. J: *Salicornia* spec. B diploid with 2n=2x=18. K: *Salicornia* spec. C. diploid with 2n=2x=18. Bar=10µm.