

KARYOTYPE ANALYSIS AND NEW CHROMOSOME NUMBER REPORTS OF THE GENUS *ECHINOPS* L. (ASTERACEAE, CARDUEAE) FROM IRAN

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Echinops is a genus of Asteraceae that is represented in Iran by 72 species, karyotype analysis was performed on 18 population of 7 *Echinops* species in southern slope of Central Albourz of Iran. The taxa revealed chromosome counts of $2n=30, 32, 34$ and 36 . Chromosome numbers of 5 species are reported for the first time including *E. cephalotes*, *E. chorassanicus*, *E. macrophyllus*, *E. robustus* and *E. elbursensis*. There are multiple basic chromosome numbers in a few *Echinops* species. Differences in basic chromosome numbers point towards the possible role played by centric fusion/fission in the karyotypic evolution of the genus. The chromosomes were mainly metacentric or sub-metacentric and their size varied from $41.40 \mu\text{m}$ in *E. cephalotes* of Khojir (1) to $79.02 \mu\text{m}$ in *E. elbursensis* of Emamzadeh Hashem population. The species occupied classes 1A, 1B and 2B of Stebbin's karyotype classification, indicating them to have primitive karyotype. Cluster analysis of karyotype features indicated that cytological studies didn't support sectional classification.

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Key words: *Echinops*; karyotype; sectional classification; chromosome number; Iran

بررسی کاربوتیپی و گزارش جدید عدد کروموزومی جنس *Echinops* L. از ایران
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Echinops یکی از جنس‌های تیره گل آفتابگردان است که با ۷۲ گونه در ایران معرفی شده است. بررسی کاربوتیپی بر روی ۱۸ جمعیت از ۷ گونه *Echinops* از شیب جنوبی بخش مرکزی البرز ایران صورت گرفت تاکنون اعداد کروموزومی $2n=30, 32, 34, 36$ را نشان دادند اعداد کروموزومی ۵ گونه شامل *E. cephalotes*, *E. chorassanicus*, *E. macrophyllus*, *E. robustus*, *E. elbursensis* برای اولین بار گزارش شدند. چندین عدد کروموزومی پایه در تعداد کمی از گونه‌های *Echinops* وجود دارد. تفاوت در عدد کروموزومی پایه احتمالاً به وسیله هم‌جوشی یا تقسیم بر روی تکامل کاربوتیپی جنس نقش بازی کرده است. کروموزوم‌ها غالباً متاسانتریک یا ساب متاسانتریک هستند و اندازه آنها از $41/40 \mu\text{m}$ در *E. cephalotes* جمعیت خجیر به $79/02 \mu\text{m}$ در *E. elbursensis* جمعیت امامزاده هاشم متغیر است. گونه‌ها در رده‌های 1A،

۱B و ۲B طبقه‌بندی کاربوتیبی *stebbin* هستند که نشان می‌دهد دارای کاربوتیبی ابتدایی می‌باشند. آنالیزهای خوشه‌ای صفات کاربوتیبی تقسیمات موجود بخش‌ها را در جنس تأیید نمی‌کند.

INTRODUCTION

The genus *Echinops* L. (Cynareae Lam. & DC., Asteraceae Bercht. & J.Presl) comprises of around 120-130 species organized in a different number of sections and distributed all over the world, mostly, in the northern hemisphere (Garnatje & al. 2004a), 54 species of which occur in Iran and some of the species are endemic for Iran (Rechinger 1979). Subsequently, 18 new species of this genus have been introduced in Iran (Mozaffarian & Ghahreman, 2002; Mozaffarian, 2006). *Echinops* is distributed in several parts in Iran specially in Tehran (Mozaffarian 2005).

Cytogenetic studies of *Echinops* taxa are in general very limited (Goldblatt & Johnson 1983; Strid & Franzen 1981; Moore 1982) and there are a few reports from Iran (Sheidai & al. 2000; Ghaffari 1999, Garnatje 2004a, 2004b). The three most common chromosome numbers in *Echinops* are $2n=28$, 30 , 32 and a fourth less common $2n=34$ (Sheidai & al. 2000). There are B chromosomes of the *Echinops grijsii* Hance (Peng 1986).

A Karyological study was performed on species which are distributed in the southern slope of Central Alburz of Iran. The aim of the present work is to determine the relationships between karyological characters and sectional classification among studied populations and the chromosomal changes that have taken place during species diversification. Here we report the chromosome numbers and karyotype for 18 *Echinops* population of 7 species, from which six species including *E. cephalotes* DC., *E. robustus* Bunge, *E. macrophyllus* Boiss. & Hausskn., *E. ritrodes* L., *E. elbursensis* Rech.f., *E. chorassanicus* Bunge are endemics.

MATERIALS AND METHODS

Seed collected from wild plants was done in Tehran, Elburz and Ghom regions, during 2012. Cytological studies were performed in 18 populations of *Echinops* species growing wild in these regions. Voucher specimens are deposited in Herbarium of IRAN and HSBU. Details of which have been given in table 1.

For Karyological study freshly grown root tips were collected from the seeds of at least ten randomly selected plants in each species, pretreated with 0.002 mol/L 8-hydroxyquinolin and fixed in absolute ethanol: glacial acetic acid (3:1) for 24 hrs. The fixed tips were then washed thoroughly in distilled water and hydrolyzed in 40°C 1N HCl for about 18 min. Squash

technique was used the root tips which were stained with 2% aqueous aceto-orcein. The somatic chromosome number and karyotype details were studied in at least 5 well-prepared metaphase plates. The chromosomes were photographed by digital camera and measured. The chromosomes were identified according to Levan & al. (1964), karyotype symmetry was determined according to Stebbins (1971), while other karyotype parameters like total form percentage (TF %), coefficient of variation (CV) of the chromosome size as well as A1 and A2 indices according to Romero-Zarco (1986), total chromosome length (TL), size of the longest chromosome pair (L), size of the shortest chromosome pair (S), mean chromosome length (X), Stebbins symmetry class (ST) and karyotype formulae (KF) were determined. Grouping of the taxa was performed using clustering methods (single linkage and WARD) and ordination of taxa on the first two principal components axes (PCA), Pearson correlation among karyotype characters and PCA were performed with PAST software (Hamer, 2012).

RESULT AND DISCUSSION

The species studied had $2n=30$, 32 , 34 , 36 chromosome numbers. There are multiple basic chromosome numbers in a few *Echinops* species. *Echinops robustus* showed $2n=30$ in Vardavard population and showed $2n=32$ in Eshtehard population. These are new reports for this species. *Echinops elbursensis* showed $2n=32$ in Emamzadeh Hashem population, which is new report for this species. *Echinops cephalotes*, showed $2n=32$ in Khojir (1), Ghom, Gharchak, Saveh populations and showed $2n=34$ in Toochal population and showed $2n=36$ in Khojir (2) population. *Echinops leiopolyceas* showed $2n=32$ in all populations, but the previous reports of *E. leiopolyceas* was $2n=30$ (Sheidai & al. 2000). *Echinops chorassanicus* showed $2n=32$ in all populations, which are new reports. *Echinops ritrodes* showed $2n=34$ in all population but Sheidai & al. (2000) reported $2n=32$ for it. Details of Karyotypes are presented in table 1, figs 1 & 2.

The chromosomes of the species studied were mostly of m (centromere at median region) and sm (sub-metacentric) types.

Among the species with $2n=32$ the highest value for total length of chromosomes, size of the longest chromosome, size of the shortest chromosome and

mean chromatin length was observed in *E. elbursensis* Emamzadeh Hashem (79.02, 7.08, 2.82, 4.95) while the lowest values occurred in *E. cephalotes* Khojir (1) (41.40, 3.58, 1.55, 2.59) (table 1). Among the species with $2n=34$ the highest value for total length of chromosomes, size of the longest chromosome, size of the shortest chromosome and mean chromatin length was observed in *E. ritroides* Abali while the lowest values occurred in *E. ritroides* (Chenar) and *E. cephalotes* (Toochal) (64.73, 5.88, 2.52, 4.05) (table 1).

The size of the longest chromosome pair varied from $7.08\mu\text{m}$ in *E. elbursensis* (Emamzadeh Hashem) to $3.95\mu\text{m}$ in *E. leiopolycerus* (Tizab) while the size of shortest chromosome pair ranged from $3.07\mu\text{m}$ in *E. ritroides* (Absard) to $1.55\mu\text{m}$ in *E. cephalotes* (Khojir 1), similarly the mean chromosome length varied from $4.95\mu\text{m}$ in *E. elbursensis* (Emamzadeh Hashem) to $2.59\mu\text{m}$ in *E. cephalotes* (Khojir1).

The total form percentage value (TF%) varied from 44% in *E. leiopolyceras* (Tizab, Damavand) to 40% in *E. cephalotes* (Saveh) and *E. ritroides* (Absard, Abali, Chenar), higher value of TF% indicates the presence of a relatively more symmetrical karyotype. Also Karyotype formula of studied species is different (table 1). According to Stebbin's karyotype classification, these species fell into 1A, 1B and 2B classes among species that placed in class 2B. *Echinops ritroides* (Abali, Chenaran) shows a higher value of A1 index (0.33) of Romero Zarco. PCA analysis of the karyotype characters revealed that the one PCA components comprised about 74 % of the total variation. The TL (total chromosome length), x (mean chromosome length), size of the longest chromosome, size of the shortest chromosome and Stebbins symmetry class characters are the first PCA axis component, therefore these characters are the most variable karyotype characters. Pearson correlation determined among the karyotype characters showed a positive significant correlation between the TL and S (size of the shortest chromosome pair) ($r > 0.68$, $p < 0.05$). Therefore, the significant quantitative change in the chromatin material has occurred in the size of only short chromosome arm during the species diversification. Also, total chromosome length and total form percentage had negative correlation ($r < 0.76$, $P < 0.01$). Different clustering methods and PCO ordination of the *Echinops* species based on karyotype data were similar therefore Ward dendrogram is discussed here (fig. 3). In general, two major clusters are formed. The species belonging to sections are located in various positions of dendrogram (fig. 3).

$2n=2x=34$, 36 and 32 is reported in sec. *Oligolepis* that $2n= 28$, 30 previously was recorded for sec. *Oligolepis* (Sheidai & al. 2000; Garnatje & al. 2004a) $2n=2x=32$ is reported in sec. *Ritropsis* and $2n=2x=32$, 34 is reported in sec. *Echinops*. The basic chromosome number $x = 9$ are recorded for most of the species in the Compositae family. Semple & Watanabe 2009; Garnatje & al. 2004b suggested $x = 8$ as the ancestral basic chromosome number that has happened during polyploidization and genome downsizing. Leitch & Bennett 2004; Leitch & al. 2008; Sapci & al. 2015, suggested an ancient basic chromosome number of $x = 9$. polyploidy, dysploidy, chromosome breakage and elimination have been the main mechanisms of chromosomal evolution in the *Echinops* (Sapsi & al. 2015). Also basic chromosome number is $x=8$, 9 in *Echinops* species that indicated influences of these events during *Echinops* diversification. In present study all three studied section placed in variable situation in dendrogram and there were different chromosome number among members of sections. Thus chromosome number did not confirm sectional classification in *Echinops*. This result is in agreement with the previous studies based on chromosome number and molecular data (Garnatje & al. 2004b, 2005) In Contrary to sectional classification, the karyotype study is approximately helpful in species delimitation.

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Table 1. Voucher specimens and Karyotype features of the *Echinops* species and population studied. Abbreviation: TL= Total chromosome length, L=Size of the longest chromosome, S= Size of the shortest chromosome, L/S=Ratio of the longest chromosome / shortestest chromosome, X= the mean chromosome length, ST= Stebbins symmetry class, A1 and A2=Romero-Zarco indices, TF%= Total form percentage and KF=Karyotype formulae.

	Species	Locality	Voucher no.	Collector	2n	Ploidy Level	TL (μm)	L (μm)	S (μm)	L/S (μm)	X	ST	A1	TF%	KF
1	<i>E. robutus</i>	Vardavard	HSBU-2019900	Alijanpoor	30	2x	47.29	4.43	2.00	2.22	3.15	1B	0.28	43	M
2	<i>E. robutus</i>	Eshtehard	HSBU-2019901	Alijanpoor	32	2x	61.00	5.95	2.12	2.80	3.81	1B	0.29	41	M
3	<i>E. elbursensis</i>	Emamzadeh Hashem	IRAN -67131	Alijanpoor	32	2x	79.02	7.08	2.82	2.51	4.95	1B	0.29	41	M
4	<i>E. cephalotes</i>	Khojir(1)	HSBU-2019902	Alijanpoor	32	2x	41.40	3.58	1.55	2.32	2.59	1B	0.25	42	M
5	<i>E. cephalotes</i>	Ghom	HSBU-2019903	Alijanpoor	32	2x	54.06	4.56	2.33	1.96	3.38	1A	0.23	43	M
6	<i>E. cephalotes</i>	Gharchak	HSBU-2019904	Alijanpoor	32	2x	78.61	6.68	2.70	2.48	4.91	1B	0.26	42	14 m+2sm
7	<i>E. cephalotes</i>	Saveh	IRAN -67126	Alijanpoor	32	2x	70.91	6.26	2.96	2.11	4.43	2B	0.31	40	13m+3sm
8	<i>E. cephalotes</i>	Toochal	HSBU-2019905	Alijanpoor	34	2x	64.73	5.88	2.52	2.34	4.05	1B	0.29	41	M
9	<i>E. cephalotes</i>	Khojir(2)	IRAN -6132	Alijanpoor	36	2x	52.75	5.13	1.46	3.51	2.93	1B	0.28	42	M
10	<i>E. leiopolyceras</i>	Tizab	HSBU-2019906	Alijanpoor	32	2x	45.99	3.95	1.89	2.09	2.87	1B	0.19	44	M
11	<i>E. leiopolycerus</i>	Damavand	IRAN -67126	Alijanpoor	32	2x	46.51	4.08	1.92	2.12	2.92	1B	0.20	44	M
12	<i>E. leiopolycerus</i>	Abanjirak	IRAN -67128	Alijanpoor	32	2x	50.11	4.44	2.09	2.12	3.13	1B	0.26	42	M
13	<i>E. chorassanicus</i>	Ploor	HSBU-2019907	Alijanpoor	32	2x	55.89	4.52	2.22	2.04	3.49	1B	0.24	43	M
14	<i>E. chorassanicus</i>	Gadok	HSBU-2019908	Alijanpoor	32	2x	61.48	5.45	2.20	2.48	3.84	1B	0.27	42	15m+1sm
15	<i>E. macrophyllus</i>	Jajrod	IRAN -67129	Alijanpoor	32	2x	57.39	5.63	2.30	2.45	3.59	2B	0.25	43	M
16	<i>E. ritrodes</i>	Abali	HSBU-2019909	Alijanpoor	34	2x	78.12	6.22	2.95	2.11	4.60	2B	0.33	40	12m+5sm
17	<i>E. ritrides</i>	Absard	HSBU-2019910	Alijanpoor	34	2x	73.48	5.77	3.07	1.88	4.32	2B	0.25	40	13m+4sm
18	<i>E. ritrodes</i>	Chenar	HSBU-2019911	Alijanpoor	34	2x	66.97	5.65	2.26	2.50	3.94	2B	0.33	40	12m+5sm

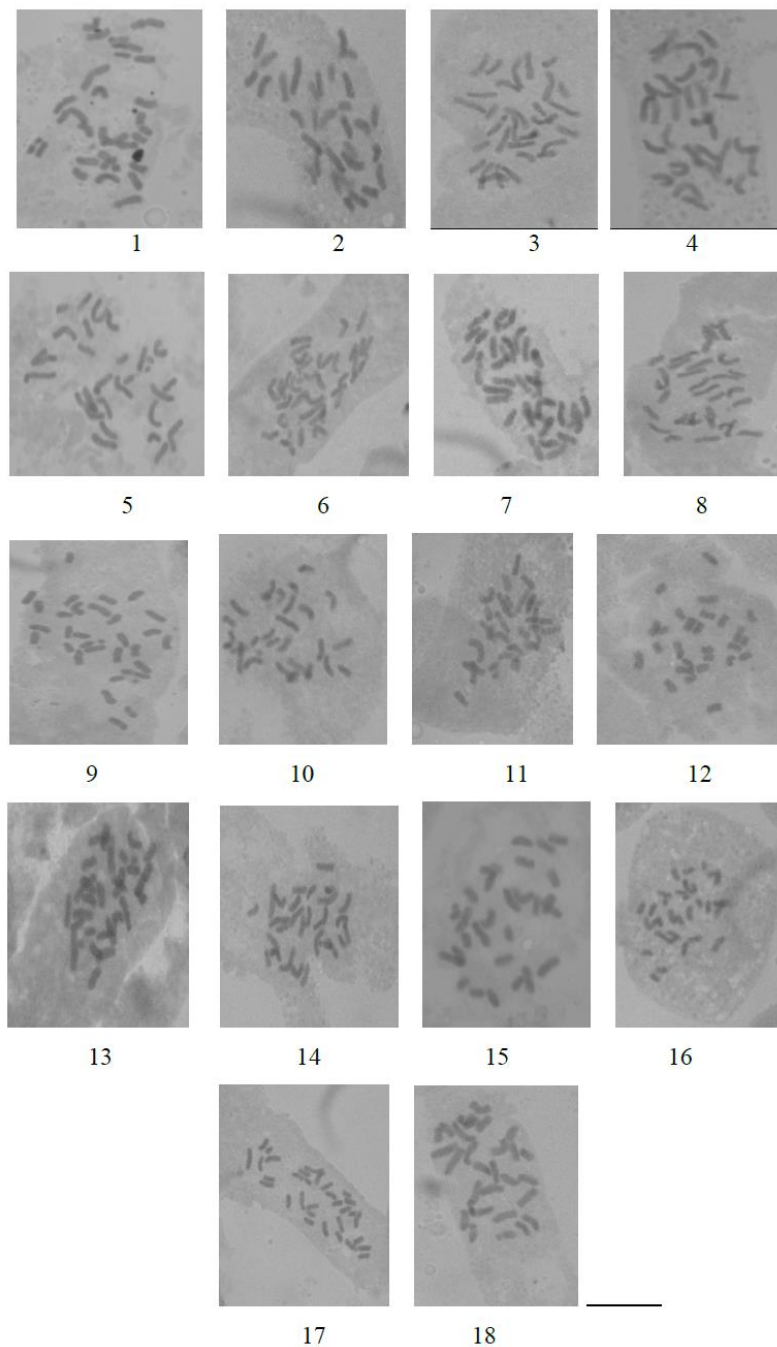


Fig. 1. 1-18. Representative somatic cells of the studied *Echinops* species. Scale bar = 10 μ m. 1: *E. macrophyllus*, (Jajrod) $2n=2x=32$; 2: *E. leiopolyceras*, (Abanjirak) $2n=2x=32$; 3: *E. elbursensis* (Emamzadeh Hashem) $2n=2x=32$; 4: *E. chorassanicus* (Gadok) $2n=2x=32$; 5: *E. cephalotes* (Khojir) $2n=2x=32$; 6: *E. ritroides* (Absard) $2n=2x=34$; 7: *E. ritroides*, (Abali) $2n=2x=34$; 8: *E. ritroides* (Chenar) $2n=2x=34$; 9: *E. chorassanicus* (Ploor) $2n=2x=32$; 10: *E. cephalotes* (Khojir 2) $2n=2x=36$; 11: *E. cephalotes* (Saveh) $2n=2x=32$; 12: *E. cephalotes* (Ghom) $2n=2x=32$; 13: *E. cephalotes* (Gharchak) $2n=2x=32$; 14: *E. robustus* (Eshtehard) $2n=2x=32$; 15: *E. robustus* (Vardavard) $2n=2x=30$; 16: *E. leiopolyceras* (Tizab) $2n=2x=32$; 17: *E. leiopolyceras* (Damavand) $2n=2x=32$; 18: *E. cephalotes* (Toochal) $2n=2x=34$.

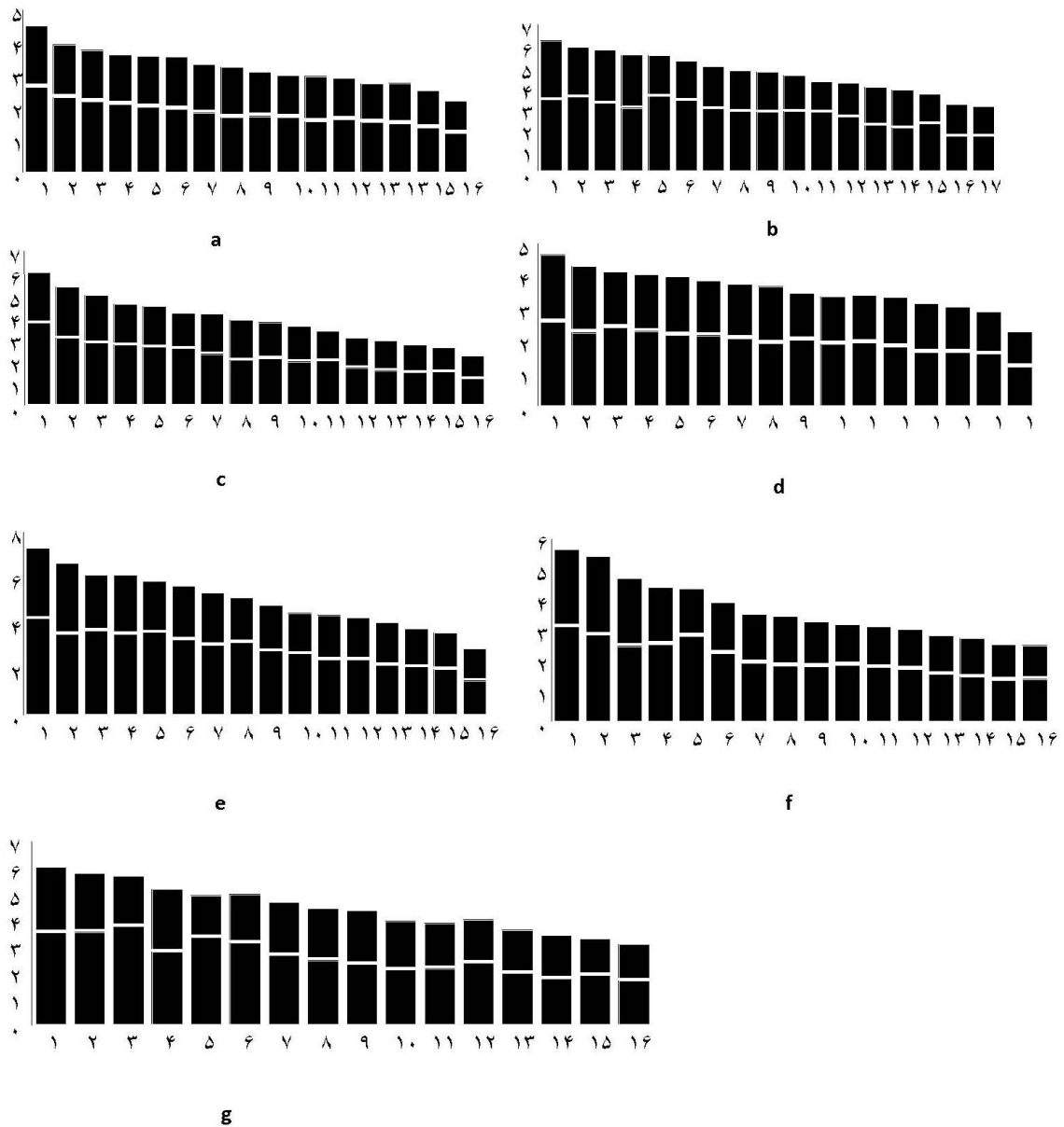


Fig. 2. Idiograms of the studied *Echinops* species, a: *E. leiopolyceras* (Abanjirak) $2n=2x=32$; b: *E. ritrodes* (Abali) $2n=2x=34$; c: *E. robustus* (Eshtehard) $2n=2x=32$; d: *E. chorassanicus* (Ploor) $2n=2x=32$; e: *E. elborensis* (Emamzadeh Hashem) $2n=2x=32$; f: *E. macrophyllus* (Jajrod) $2n=2x=32$; g: *E. cephalotes* (Saveh) $2n=2x=32$.

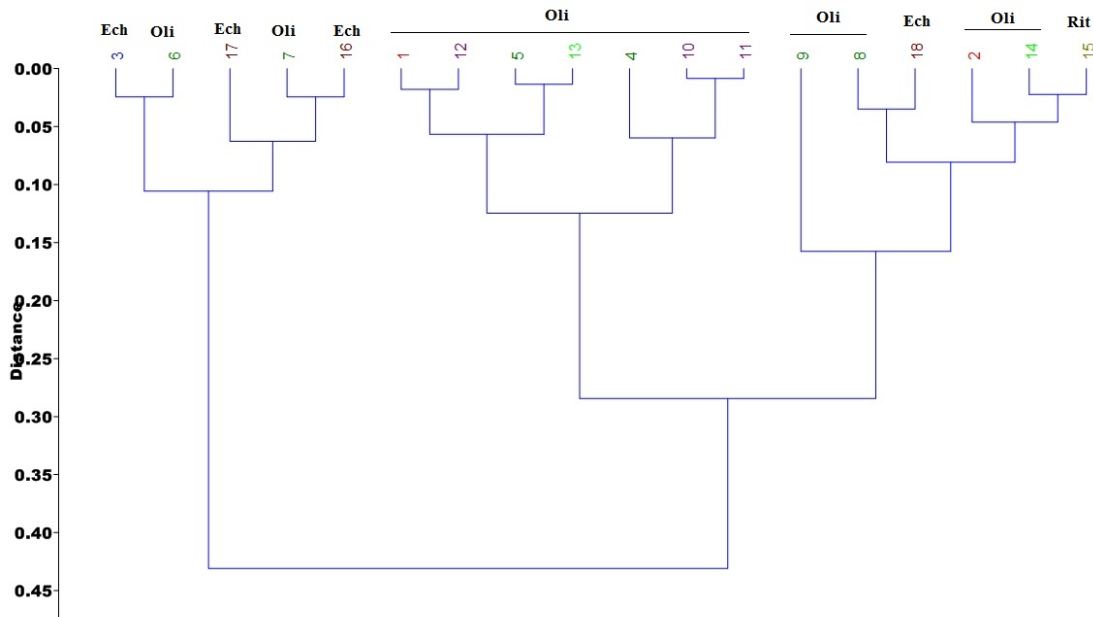


Fig. 3. Ward dendrogram of *Echinops* species based on karyotype data. Abbreviations: Oli: Section *Oligolepis*; Rit: Section *Ritropsis*; Ech: Section *Echinops*. 1-18: according to table 1.

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