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New Chromosome Numbers and Karyotype Analysis of *Tordylium* (Apiaceae) Species with Emphasis on Their Systematics and Evolutionary Significance

Sistematiği ve Evrimsel Önemi Vurgulanarak *Tordylium* (Apiaceae) Türlerinin Yeni Kromozom Sayıları ve Karyotip Analizi

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ABSTRACT

In Turkey, there are eighteen *Tordylium* (Apiaceae) species out of twenty in the worldwide and seven of them are endemic. In this research, karyotypes of *T. apulum, T. pestalozzae, T. syriacum, T. trachycarpum* and karyotypes and chromosome numbers of *T. cappadocicum, T. aegaeum, T. hasselquistiae, T. ketenoglui* (endemic), *T. macropetalum* (endemic), *T. pustulosum* (endemic) are provided for the first time. Tordylium species are separated into five cytotypes according to chromosome numbers as 2n=8, 16, 18, 20 and 22. Besides, idiograms of haploid chromosome set, length of the chromosome arms, arm ratios, centromeric index, relative length and haploid karyotype formulas are given. *T. ketenoglui* has longest chromosome lengths (3.39-1.66 µm) while *T. aegaeum* has shortest (1.10-0.59 µm). Additionally, principal component analysis (PCA) has been performed by quantified karyological characters. PCA results show that the median type and haploid chromosome length have significant variants among karyological characters. Also, correlation among karyological data, current infrageneric classification and the phylogenetic hypothesis based morphological dataset of *Tordylium* are discussed. Karyological characters are incompatible with current infrageneric classification, similarly with conflict between the phylogenetic hypothesis and infrageneric classification. In the light of the phylogeny, it could be stated that the ancestral number is 2n=16.

Key Words

Cytotype, karyotype evolution, principal component analysis, *Tordyliinae.*

ÖZ

Dünya çapında yirmi türü bulunan Tordylium (Apiaceae) türlerinin onsekizi Türkiye'de bulunmaktadır ve bunların yedisi endemiktir. Bu araştırmada *T. apulum, T. pestalozzae, T. syriacum* ve *T. trachycarpum*'un karyotipleri ve *T. cappadocicum, T. aegaeum, T. hasselquistiae, T. ketenoglui* (endemik), *T. macropetalum* (endemik), *T. pustulosum* (endemik)'un ise karyotipleri ve kromozom sayıları ilk kez verilmiştir. *Tordylium* türleri kromozom sayılarına göre 2n=8, 16, 18, 20 ve 22 olmak üzere beş sitotipe ayrılmıştır. Bunun yanında, haploid kromozom setinin idiyogramları, kromozom kollarının uzunluğu, kol oranları, sentromerik indeks, nispi uzunluk ve haploid karyotip formülleri verilmiştir. En uzun kromozom boyuna *T. ketenoglui* (3,39-1,66 µm), en kısasına ise *T. aegaeum* (1,10-0,59 µm) sahiptir. Ayrıca, kantitatif karyolojik karakterler kullanılarak temel öğeler analizi (PCA) yapılmıştır. PCA sonuçları, karyolojik karakterler arasından medyan tipinin ve haploid kromozom uzunluğunun anlamlı değişkenler olduğunu göstermiştir. Ayrıca, *Tordylium*'un karyolojik verileri, mevcut infragenerik sınıflandırması ve filogenetik hipoteze dayalı morfolojik veri seti arasındaki korelasyonu tartışılmıştır. Mevcut infragenerik sınıflandırma ile filogenetik hipotez arasındaki uyumsuzluğa benzer bir durum karyolojik karakterlerle de söz konusudur. Filogeninin ışığında, atasal kromozom sayısının 2n=16 olduğunu söylenebilir.

Anahtar Kelimeler

Sitotip, karyotip evrimi, temel öğeler analizi, *Tordyliinae*.

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INTRODUCTION

Apiaceae (Umbelliferae) is a family of herbs, rarely shrubs and widely distributed. It comprises 300-455 genera and 3000-3750 species in the worldwide [1-2]. In the family, the haploid chromosome counts range from n=4 to 84 [3]. In Turkey, Apiaceae is represented by 101 genera, 479 species of which 159 are endemic [4]. While the Apiaceae family has remarkable diversity in Turkey, limited number of species of this family have been investigated in terms of cytogenetic [5-14].

The genus *Tordylium* Tourn. ex L. is accepted in subtribe *Tordyliinae* Drude based on phylogenetic analysis of nrDNA ITS sequences [15]. The genus is annual which is generally has a distribution in Europe and Mediterranean area with 20 species [16-18]. Its crenate leaf margin, compound umbels, strongly dorsally flattened mericarp, thickened mericarp margin and mericarp indumentum are considered as the crucial diagnostic characters [19]. In the historical review, as a result of many taxonomic treatments, genera *Ainsworthia* Boiss., *Synelcosciadium* Boiss., *Condylocarpus* Hoffm. and *Hasselquistia* L., have been reduced to the synonyms of *Tordylium* [16, 20-24]. According to the last revision [16], the infrageneric classification has been formed such as *Ainsworthia* as subgenus; *Condylocarpus* and *Hasselquistia* as sections. However, the phylogenetic topology based morphological data contradicts Al-Eisawi and Jury's [16] infrageneric classification of the genus [25]. Therefore, it is revealed that the current subgeneric and sectional divisions are unnatural. Additionally, *Tordylium*, which has been considered a 'putative natural' genus, proved non-monophyletic because genus *Ormosciadium* Boiss. has been nested in *Tordylium* clade.

In Turkey, *Tordylium* is represented with 18 species of which 7 are endemic [16, 26-27]. Generally, the species of *Tordylium*, except *T. cappadocicum* Boiss., are distributed southern and western Anatolia which are Mediterranean phytogeographic region in Turkey. *T. apulum* L. is more widespread than the others, occurs both Mediterrranean and Irano-Turanian phytogeographic regions. *T. cappadocium* grows in eastern Anatolia which is Irano-Turanian phytogeographic area in Turkey.

Runemark [5] has recorded chromosome numbers of 6 Aegean *Tordylium* species. Moore [28] has revealed chromosome information for the *Tordylium* and *Ainsworthia* taxa. To date, 13 *Tordylium* species have been recorded by previous researchers [16, 29-42]. According to these chromosome counts, chromosome numbers in *Tordylium* species are 2n=8, 16, 20 and 22. However, there are still unknown chromosome numbers and karyotypes of *Tordylium* species, especially Turkish endemic ones.

Runemark [5] has two hypotheses that the basic chromosome number in this genus is x=10 or x=5. The basic number x=10 easily explains 2n=20. Alternatively, he has proposed that x=5, so 2n=10 and 2n=20 (tetraploidy) can be occur. However, both of two hypotheses cannot explain 2n=8 (*T. hirtocarpum* Candargy) or 2n=16. According to Al-Eisawi and Jury [16], x=4. It easily explains 2n=8 and 2n=16 (tetraploidy). They have proposed the counts such as 2n=20 and 2n=22 by the mechanism occurs crossing between tetraploid and hexaploid taxa or by losing/gaining 4 chromosomes. Additionally, they have showed irregular meiosis in *T. aegyptiacum* (L.) Poir., so they have deduced that hybridization might occur in *Tordylium*. The last hypothesis of Al-Eisawi and Jury [16] concerning chromosomes range from x=4 to x=11.

In this research, our aim is i) to determine the chromosome numbers of *Tordylium* species still unknown, ii) to investigate the chromosome morphology of *Tordylium*, iii) to reveal the karyotypic variation within the genus, and iv) to understand the cytological relationship between *Tordylium* species.

MATERIALS AND METHODS

Sampling

During the course of a project on the pollen morphology and chromosome account of *Tordylium* genus from Turkey, ca. 60 specimens belonging to 11 species have been collected between 2009 and 2011. The localities and collector numbers of the specimens are given in Table 1. The voucher specimens have been collected from Turkey and deposited in Herbarium of Hacettepe University (HUB) by their collector numbers.

Karyological Studies

Karyotype analyses have been done on mitotic metaphase chromosomes that are prepared by the squash technique. After sodium hypochlorite sterilization procedure, the seeds have been germinated in petri dishes. The root tips have been pretreated with 8 hydroxyquinoline at 18 °C for 4 h. The material has been fixed in acetic alcohol (3:1 ethanol and glacial acetic acid) and stored in 70% ethanol at 4 °C. Material has been hydrolyzed with 1 N HCl for 12 min at 60 °C, washed with distilled water, stained with Feulgen

Table 1. Localities of studied Tordylium specimens. "HA" indicates Haşim Altınözlü.

Species	Locality	Collector number	Species	Locality	Collector number
T. ketenoglui	Antalya	HA 2512	*T. syriacum*	Hatay	HA 2960
T. elegans	Osmaniye	HA 2394A	*T. pestalozzae*	Muğla	HA 2921
T. cappadocicum	Erzincan	HA 2634	*T. hasselquistiae*	Şanlıurfa	HA 2547
T. trachycarpum	Hatay	HA 2967	*T. aegaeum*	Muğla	HA 2692
T. macropetalum	İzmir	HA 2812	*T. pustulosum*	Antalya	HA 2547
T. apulum	Muğla	HA 2620	-	-	-

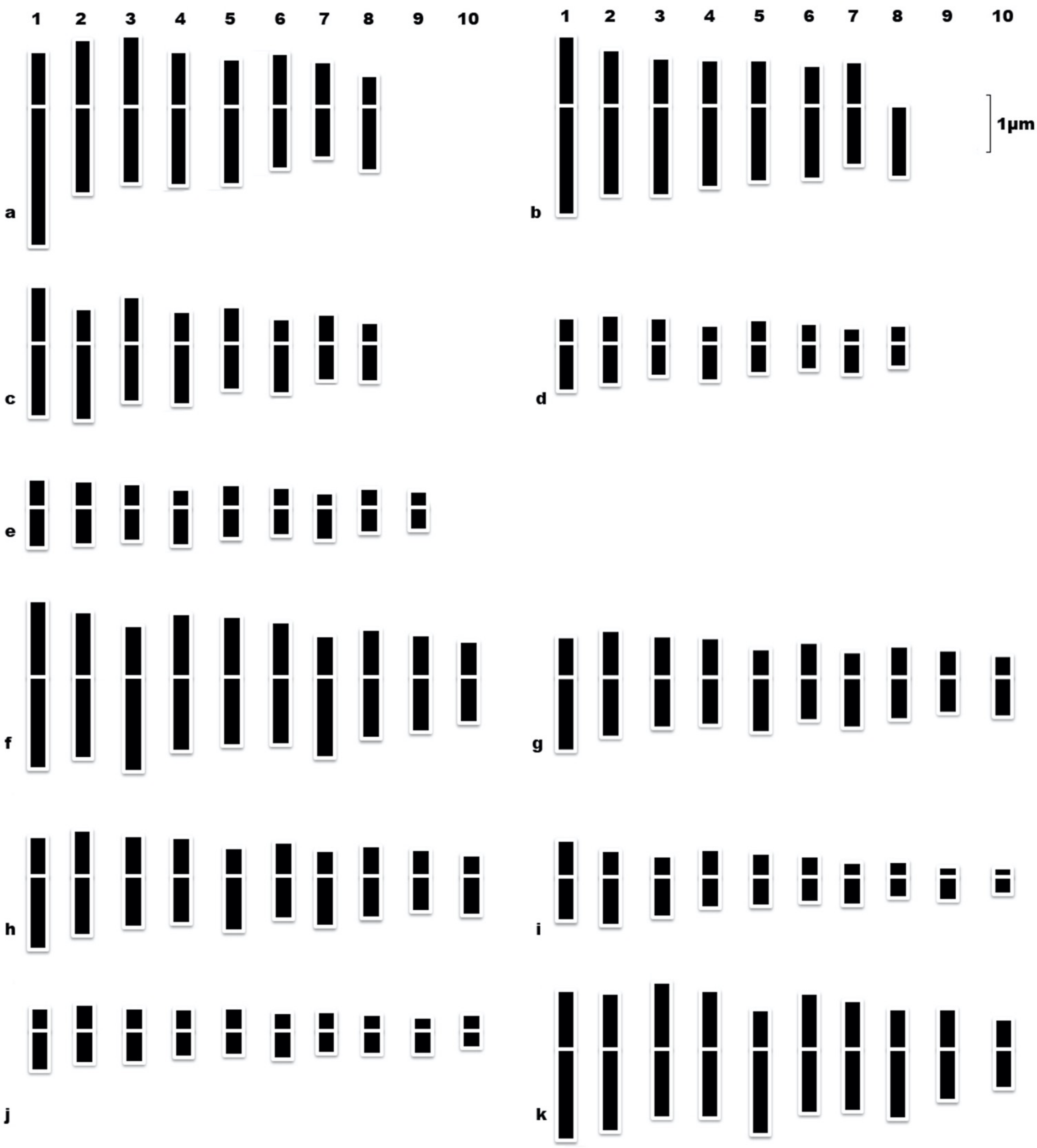

Figure 1. Haploid idiograms of *Tordylium* species: a) *T. ketenoglui*, b) *T. elegans*, c) *T. cappadocicum*, d) *T. trachycarpum,* e) *T. macropetalum*, f) *T. apulum*, g) *T. syriacum*, h) *T. pestalozzae*, i) *T. hasselquistiae*, j) *T. aegaeum*, k) *T. pustulosum* (Bar=1µm).

and squashed in 45% acetic acid. Preparations have been mounted in Canada balsam for permanent preparations. At least well-spread 5 metaphase plates have been measured for each taxon. The chromosomes in the karyotype have been ordered by decreasing length. The detection of the homologous chromosomes and the determination of their position in the karyograms have been carried out following the method proposed by Levan et al. [43]. The measurements obtained from ten metaphase plates for the long and short arm length have allowed the construction of the idiograms of the species. The idiogram of haploid chromosome set is presented in Figure 1. Long and short arms, total lengths, arm ratios, centromeric indexes and relative lengths of chromosomes are given in Tables 2-4. Permanent slides have been stored in the Department of Biology, Hacettepe University, Ankara.

Besides, chromosome numbers of *Tordylium* species are assembled in Table 5.

Multivariate Analysis

To understand the evolution of chromosome numbers we have used phylogenetic hypothesis inferred by Maximum Parsimony analysis based morphological characteristics from Doğru-Koca [25] (Figure 2). The classification of the genus according to the last revision by Al-Eisawi and Jury [16] has been implemented to this cladogram. Scatter plot of Principal Component Analysis (PCA) has been obtained by PAST 3.06 [44] (Figure 3). The dataset comprises herein species and is given in Appendix Table S1. The analysis has been computed by variance-covariance matrices. The eigenvalue variants of first five principal components and loadings of them have been documented in Appendix Table S2 and S3, respectively.

Table 2. Measurements of somatic chromosomes of *Tordylium* species, 2n=16.

Chromosome Pairs	Chromosome length (µm)			Arm ratio r=L/S	Centromeric index i=100 xs/c	Relative length (%)	Centromere position
	Long arm	Short arm	Total				
T. ketenoglui (2n=16)							
I	*2.44*	0.95	3.39	1.59	27.93	18.23	sm
II	*1.53*	1.17	2.70	1.31	43.70	14.56	m
III	*1.35*	1.22	2.57	1.11	47.46	13.86	m
IV	*1.39*	0.95	2.34	1.46	40.60	12.62	m
V	*1.36*	0.83	2.19	1.64	37.99	15.10	m
VI	*1.09*	0.93	2.02	1.17	46.13	10.95	m
VII	*0.91*	0.78	1.69	1.17	46.15	9.06	m
VIII	*1.12*	0.54	1.66	2.07	32.98	8.95	sm
T. elegans (2n=16)							
I	*1.93*	1.22	3.15	1.59	38.73	17.83	m
II	*1.59*	0.99	2.58	1.61	38.37	14.60	m
III	*1.59*	0.84	2.43	1.89	37.17	13.75	sm
IV	*1.45*	0.81	2.26	1.79	35.84	12.79	sm
V	*1.35*	0.80	2.15	1.69	37.21	12.17	sm
VI	*1.30*	0.71	2.01	1.83	35.32	11.38	sm
VII	*1.06*	0.77	1.83	1.38	42.08	10.35	m
VIII	*1.26*	0.00	1.26	0.00	0.00	7.13	T
T. cappadocicum (2n=16)							
I	1.28	1.00	2.28	1.30	43.86	17.85	m
II	1.35	0.61	1.96	2.17	31.12	15.35	sm
III	1.03	0.83	1.87	1.25	44.39	14.64	m

IV	1.07	0.56	1.64	1.94	34.15	12.84	sm
V	0.81	0.65	1.47	1.28	44.22	11.51	m
VI	0.88	0.44	1.32	1.98	33.33	10.33	sm
VII	0.66	0.52	1.18	1.23	44.07	09.24	m
VIII	0.67	0.37	1.05	1.85	35.24	08.22	sm
T. trachycarpum (2n=16)							
I	0.83	0.45	1.28	1.92	35.16	16.33	sm
II	0.72	0.51	1.23	1.36	41.46	15.69	m
III	0.57	0.45	1.02	1.30	44.12	13.01	m
IV	0.65	0.33	0.98	2.03	33.67	12.50	sm
V	0.52	0.42	0.94	1.22	44.68	11.99	m
VI	0.46	0.36	0.82	1.24	43.90	10.46	m
VII	0.54	0.28	0.82	2.19	34.15	10.46	sm
VIII	0.42	0.33	0.75	1.37	44.00	09.57	m

Table 3. . Measurements of somatic chromosomes of *Tordylium macropetalum*, 2n=18.

Chromosome Pairs	Chromosome length (µm)			Arm ratio r=L/S	Centromeric index i=100 xs/c	Relative length (%)	Centromere position
	Long arm	Short arm	Total				
T. macropetalum (2n=18)							
I	*0.70*	0.50	1.20	1.43	41.67	14.25	m
II	*0.65*	0.47	1.12	1.36	41.96	13.30	m
III	*0.59*	0.43	1.02	1.37	42.16	12.11	m
IV	*0.67*	0.33	1.00	2.02	33.33	11.87	sm
V	*0.55*	0.40	0.95	1.38	42.11	11.28	m
VI	*0.50*	0.36	0.86	1.40	41.86	10.21	m
VII	*0.57*	0.26	0.83	2.18	31.33	9.86	sm
VIII	*0.45*	0.35	0.80	1.29	43.75	9.50	m
IX	*0.40*	0.30	0.70	1.80	42.86	8.31	m

Table 4. Measurements of somatic chromosomes of *Tordylium* species, 2n=20.

Chromosome Pairs	Chromosome length (µm)			Arm ratio r=L/S	Centromeric index i=100 xs/c	Relative length (%)	Centromere position
	Long arm	Short arm	Total				
T. apulum (2n=20)							
I	1.60	1.34	2.94	1.21	45.58	13.31	m
II	1.42	1.14	2.56	1.26	44.53	11.59	m
III	1.65	0.91	2.56	1.82	35.55	11.59	sm
IV	1.30	1.12	2.42	1.18	46.28	10.96	m
V	1.20	1.06	2.26	1.13	46.90	10.23	m
VI	1.18	0.97	2.15	1.22	45.12	09.73	m
VII	1.41	0.73	2.14	2.15	34.11	09.69	sm

Chromosome Pairs	Chromosome length (µm)			Arm ratio r=L/S	Centromeric index i=100 xs/c	Relative length (%)	Centromere position
	Long arm	Short arm	Total				
VIII	1.08	0.84	1.92	1.32	43.75	08.69	m
IX	0.96	0.75	1.71	1.27	43.86	07.74	m
X	0.80	0.63	1.43	1.28	44.06	06.47	m
T. syriacum (2n=20)							
I	1.28	0.70	1.98	1.85	35.35	13.64	sm
II	1.04	0.81	1.85	1.31	43.78	12.74	m
III	0.89	0.71	1.60	1.28	44.38	11.02	m
IV	0.83	0.68	1.51	1.23	45.03	10.40	m
V	0.96	0.50	1.46	1.95	34.25	10.05	sm
VI	0.75	0.60	1.35	1.26	44.44	09.30	m
VII	0.88	0.45	1.33	1.99	33.83	09.16	sm
VIII	0.74	0.54	1.28	1.41	42.19	08.82	m
IX	0.62	0.47	1.09	1.32	43.12	07.51	m
X	0.69	0.38	1.07	1.87	35.51	07.37	sm
T. pestalozzae (2n=20)							
I	1.34	0.75	2.09	1.52	35.89	13.43	m
II	1.33	0.66	1.99	2.36	33.17	12.79	sm
III	1.08	0.73	1.81	1.54	40.33	11.63	m
IV	1.04	0.65	1.69	1.41	38.46	10.86	m
V	1.01	0.62	1.63	1.97	38.04	10.47	sm
VI	0.9	0.61	1.51	1.60	40.40	10.28	m
VII	0.94	0.50	1.44	2.16	34.72	09.25	sm
VIII	0.76	0.55	1.31	1.38	41.98	08.42	m
IX	0.65	0.46	1.11	1.25	41.44	07.13	m
X	0.56	0.42	0.98	1.45	42.86	06.29	m
T. hasselquistiae (2n=20)							
I	0.79	0.65	1.44	1.21	45.14	15.75	m
II	0.87	0.47	1.34	1.86	35.07	14.66	sm
III	0.72	0.36	1.08	2.02	33.33	11.81	sm
IV	0.55	0.48	1.03	1.15	46.60	11.27	m
V	0.53	0.42	0.95	1.28	44.21	10.39	m
VI	0.46	0.36	0.82	1.31	43.90	08.97	m
VII	0.51	0.26	0.77	2.03	33.77	08.42	sm
VIII	0.37	0.27	0.64	1.41	42.19	07.00	m
IX	0.42	0.18	0.60	2.33	30.00	06.56	sm
X	0.31	0.16	0.47	2.03	34.04	05.14	sm
T. aegaeum (2n=20)							
I	0.70	0.40	1.10	1.74	36.36	13.33	sm
II	0.57	0.46	1.03	1.24	44.66	12.48	m
III	0.56	0.40	0.96	1.39	41.66	11.63	m
IV	0.47	0.38	0.85	1.22	44.71	10.30	m
V	0.43	0.40	0.83	1.07	48.19	10.06	m
VI	0.50	0.32	0.82	1.54	39.02	09.94	m
VII	0.40	0.33	0.73	1.20	45.21	08.85	m

VIII	0.41	0.28	0.69	1.48	40.58	08.36	m
IX	0.42	0.23	0.65	1.80	35.38	07.87	sm
X	0.30	0.29	0.59	1.04	49.15	07.15	m
T. pustulosum (2n=20)							
I	1.39	0.89	2.28	1.36	42.50	13.03	m
II	2.26	0.85	2.10	1.76	34.66	12.21	sm
III	1.04	1.02	2.06	1.50	42.25	11.56	m
IV	1.04	0.88	1.92	2.17	31.82	10.75	sm
V	1.31	0.59	1.90	1.27	43.75	10.42	m
VI	0.98	0.84	1.82	2.07	33.33	09.28	sm
VII	0.95	0.74	1.69	1.18	45.61	09.28	m
VIII	1.07	0.60	1.67	1.32	43.14	08.31	m
IX	0.79	0.61	1.40	1.72	38.00	08.14	sm
X	0.61	0.45	1.06	1.24	44.19	07.00	m

Table 5. Chromosome numbers of *Tordylium* species.

Infrageneric classification according to Al-Eisawi and Jury [16]	Species	Endemism	Chromosome number	References
Subgen. *Tordylium* Sect. *Tordylium*	*T. macropetalum*	endemic	2n=18	Present study
	T. maximum		2n=22 2n=20	Runemark [5], Tamaschjan [29], Silvestre [34], Strid and Franzén [35], Geldykhanov [36], Baltisberger and Baltisberger [39], Dobes et al. [40]
	T. carmelii	-	n=10	Kamari et al. [42], Al-Eisawi and Jury [16]
Subgen. *Ainsworthia* Sect. *Condylocarpus*	*T. apulum*	-	2n=20	Runemark [5], Capineri et al. [33], Present study
	T. cappadocicum	endemic	2n=16	Present study
	T. hirtocarpum	-	2n=8	Runemark [5]
	T. ketenoglui	endemic	2n=16	Present study
	T. officinale	-	2n=18 n=9	Runemark [5] Silvestre [38]
	T. pestalozzae	endemic	2n=20	Runemark [5], Present study
	T. aegaeum	endemic	2n=20	Present study
	T. pustulosum	endemic	2n=20	Present study
	T. syriacum	-	2n=20 n=10	Garde and Garde [30] Vogt and Aparicio [41], Silvestre [38], Present study
Subgen. *Ainsworthia* Sect. *Hasselquistia*	*T. trachycarpum*	-	2n=16 n=8 n=9 n=10	Present study Constance et al. [31], Al-Eisawi and Jury [16] Constance et al. [32] Constance et al. [32]
	T, aegyptiacum	-	2n=20	Al-Eisawi and Jury [16]
	T. cordatum	-	2n=20 n=10	Garde and Garde [30] Constance et al. [32]
	T. elegans	endemic	2n=16	Gömürgen et al. [45]
	T. hasselquistiae	-	2n=20	Present study

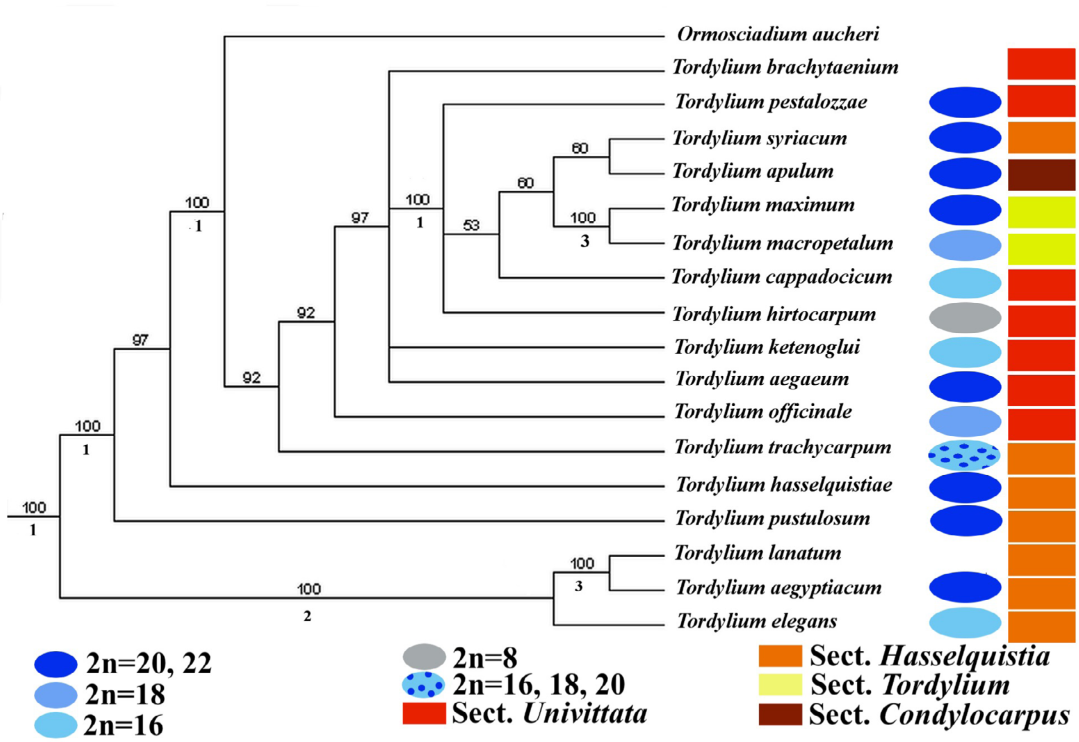

Figure 2. The *Tordylium* clade from the phylogeny based morphological data has been modified from Doğru-Koca [25]. It is a maximum parsimony hypothetical tree. The somatic chromosome numbers and current infrageneric classification have been indicated by ellipses and rectangles, respectively. The data of *T. brachytaenium* and *T. lanatum* are unknown. The upper branch numbers are bootstrap branch support values, and the bottoms are Bremer support values.

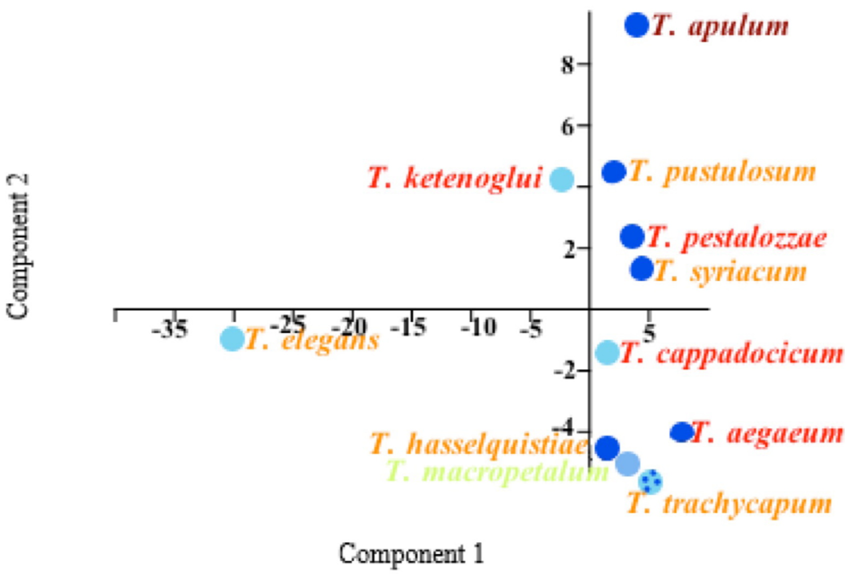

Figure 3. Scatter plot of principal component analysis (PCA) of karyogram characters of currently studied 11 *Tordylium* species, according to principal component 1 and principal component 2 axes. The colors indicate the chromosome numbers and species like in the legend of Figure 2.

RESULTS

Tordylium ketenoglui Duman & A. Duran (Figure 1a, Figure 4a, Table 2)
Chromosome number and karyotype analysis of endemic *T. ketenoglui* have been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=16. The haploid karyotype formula is n=6m+2sm, 6 pairs are of the median type and 2 pairs submedian. The longest chromosome length is 3.39 µm, the shortest is 1.66 µm and the haploid chromosome length is 18.55 µm. Chromosome arm ratios have been calculated between 2.57-1.11. The centromeric index values are varying between 47.46-27.93 and relative lengths are 18.23-8.95 µm.

Tordylium elegans (Boiss. and Bal.) Alava and Hub.-Mor. (Figure 1b, Figure 4b, Table 2)
The data about chromosome number and karyotype analysis of endemic *T. elegans* have been taken from Gömürgen et al. [45]. According to Gömürgen et al. [45], the somatic mitotic chromosome number has been stated as 2n=16. Karyotype formula of *T. elegans* is n=2x=1T+3m+4sm, 1 pair is of the terminal type, 3 pairs

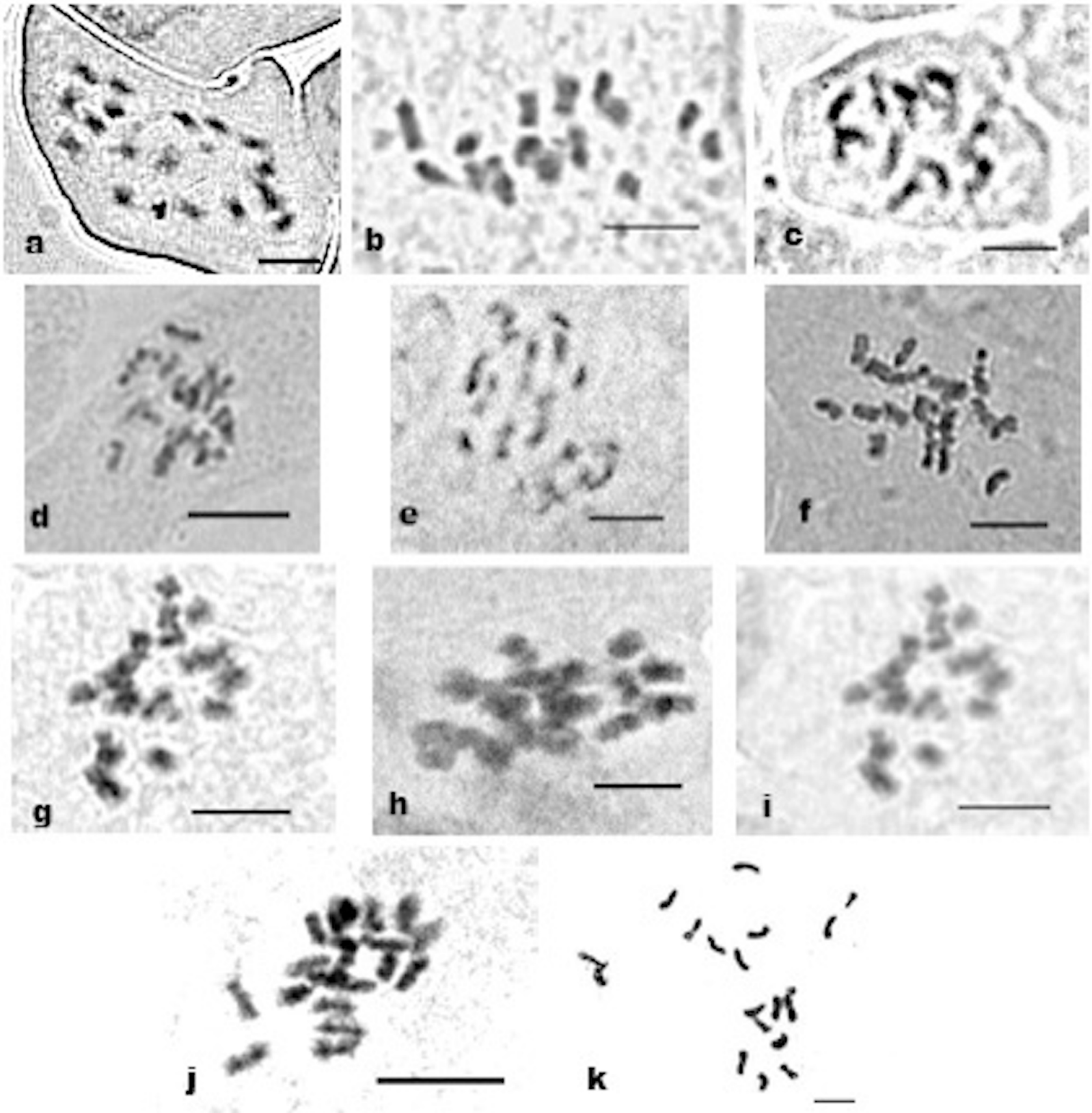

Figure 4. Somatic chromosomes of *Tordylium* species: a) *T. ketenoglui*, b) *T. elegans*, c) *T. cappadocicum*, d) *T. trachycarpum*, e) *T. macropetalum*, f) *T. apulum*, g) *T. syriacum*, h) *T. pestalozzae*, i) *T. hasselquistiae*, j) *T. aegaeum*, k) *T. pustulosum* (Bar=5 µm).

median and 4 pairs submedian. The shortest chromosome length is 1.26 µm and the longest is 3.15 µm, and the haploid chromosome length is 17.67 µm. Chromosome arm ratios have been calculated between 0,00-1.38. The centromeric index values are varying between 42.08-0.00 and relative lengths are 17.83-07.13 µm.

Tordylium cappadocicum Boiss. (Figure 1c, Figure 4c, Table 2)
Chromosome number and karyotype analysis of *T. cappadocicum* has been reported for the first time in this study. The somatic mitotic chromosome number has been stated as 2n=16. The haploid karyotype formula is n=4m+4sm, 4 pairs are of the median type and 4 pairs submedian. The range of metaphase chromosome lengths is 1.05 to 2.28 µm. The total length of the haploid set is 12.77 µm. Chromosome arm ratios have been calculated between 1.23-2.17. The centromeric index values are varying between 44.39-31.12 and relative lengths are 17.85-08.22 µm.

Tordylium trachycarpum (Boiss.) Holmboe (Figure 1d, Figure 4d, Table 2)
Karyotype analysis of *T. trachycarpum* has been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=16. This chromosome number is in correlation with the previous chromosome counts [16, 31-32]. The haploid karyotype formula is n=5m+3sm, 5 pairs are of the median type and 3 pairs submedian. The range of metaphase chromosome lengths is from 1.28 to 0,75 µm. The total length of the haploid set is 7.84 µm. Chromosome arm ratios have been calculated between 2.19-1.22. The centromeric index values are varying between 44.68-33.67 and relative lengths are 16.33-09.57 µm.

Tordylium macropetalum Boiss. (Figure 1e, Figure 4e, Table 3)
Chromosome number and karyotype analysis of endemic *T. macropetalum* have been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=18. The haploid karyotype formula is n=7m+2sm, 7 pairs are of the median type and 2 pairs submedian. The range of metaphase chromosome lengths is from 1.20 to 0.70 µm. The total length of the haploid set is 8.42 µm. Chromosome arm ratios have been calculated between 2.18-1.29. The centromeric index values are varying between 43.75-31.33 and relative lengths are 14.25-8.31 µm.

Tordylium apulum L. (Figure 1f, Figure 4f, Table 4)
Karyotype analysis of *T. apulum* has been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=20. This chromosome number is in correlation with the previous chromosome counts [5, 33]. The haploid karyotype formula is n=8m+2sm, 8 pairs are of the median type and 2 pairs submedian. The range of metaphase chromosome lengths is 2.94 to 1.43 µm. The total length of the haploid set is 22.09 µm. Chromosome arm ratios were measured at 2.15-1.13. The centromeric index values are varying between 46.90-34.11 and relative lengths are 06.47-13.31 µm.

Tordylium syriacum L. (Figure 1g, Figure 4g, Table 4)
Karyotype analysis of *T. syriacum* has been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=20. This chromosome number is in correlation with the previous chromosome counts [30, 38, 41]. The haploid karyotype formula is n=6m+4sm, 6 pairs are of the median type and 4 pairs submedian. The range of metaphase chromosome lengths is 1,98 to 1.07 µm. The total length of the haploid set is 14.52 µm. Chromosome arm ratios were measured at 1.99-1.23 µm. The centromeric index values are varying between 45.03-33.83 µm and relative lengths are 13.64-07.37 µm.

Tordylium pestalozzae Boiss. (Figure 1h, Figure 4h, Table 4)
Karyotype analysis of *T. pestalozzae* has been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=20. This number was in correlation with the previous count [5]. The haploid karyotype formula is n=7m+3s, 7 pairs are of the median type and 3 pairs submedian. The longest chromosome length is 2.09 µm, the shortest is 0.98 µm and the haploid chromosome length is 15.56 µm. Chromosome arm ratios have been calculated between 2.36-1.25. The centromeric index values are varying between 42.86-33.17 and relative lengths are 13.43-06.29 µm.

Tordylium hasselquistiae DC. (Figure 1i, Figure 4i, Table 4)
Chromosome number and karyotype analysis of *T. hasselquistiae* have been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=20. The haploid karyotype formula is n=5m+5sm, 5 pairs are of the median

type and 5 pairs submedian. The range of metaphase chromosome lengths is 1.44 to 0.47 µm. The total length of the haploid set is 9.14 µm. Chromosome arm ratios have been calculated between 2.33-1.15. The centromeric index values are varying between 46.60-30.00 and relative lengths are 15.75-05.14 µm.

Tordylium aegaeum Runemark (Figure 1j, Figure 4j, Table 4)
Chromosome number and karyotype analysis of *T. aegaeum* has been reported for the first time in this study. The somatic mitotic chromosome number has been stated as 2n=20. The haploid karyotype formula is n=8m+2sm, 8 pairs are of the median type and 2 pairs submedian. The range of metaphase chromosome lengths is from 1.10 to 0.59 µm. The total length of the haploid set is 8,25 µm. Chromosome arm ratios have been calculated between 1.80-1.04. The centromeric index values are varying between 49.15-35.38 and relative lengths are 13.33-07.15 µm.

Tordylium pustulosum Boiss. (Figure 1k, Figure 4k, Table 4)
Chromosome number and karyotype analysis of endemic *T. pustulosum* have been determined for the first time in this study. The somatic mitotic chromosome number of this taxon has been stated as 2n=20. The haploid karyotype formula is n=6m+4sm, 6 pairs are of the median type and 4 pairs submedian. The range of metaphase chromosome lengths is from 2.28 to 1.06 µm. The total length of the haploid set is 17.9 µm. Chromosome arm ratios have been calculated between 2.17-1,18. The centromeric index values are varying between 45.61-31.82 and relative lengths are 13.03-7.00 µm.

DISCUSSION

Chromosome counts of *Tordylium* species based on previous studies and present study are given in Table 5. The somatic chromosome numbers of *T. ketenoglui, T. cappadocicum, T. trachycarpum* and *T. elegans* have been determined as 2n=16; for *T. macropetalum*, 2n=18; and for *T. apulum*, *T. aegaeum, T. hasselquistiae, T. pestalozzae, T. pustulosum* and *T. syriacum* 2n=20. Chromosome numbers of four *Tordylium* species (*T. pestalozzae, T. apulum, T. trachycarpum and T. syriacum*) are in correlation with the previous studies [5, 16, 30-33, 38, 41]. Our chromosome counts of *T. aegaeum, T. pustulosum, T. hasselquistiae, T. pestalozzae, T. syriacum* and *T. apulum* (2n=20) support Runemark [5]. Additionally, our chromosome counts of *T. cappadocicum, T. elegans* and *T. ketenoglui* (2n=16) also support Al-Eisawi and Jury [16].

All the results show that cytotypes of *Tordylium* are 2n=8, 16, 18, 20 and 22. This study is in agreement with Al-Eisawi and Jury's [16] two assumptions that basic chromosome numbers might be x=4 or ranged from x=4 to x=11. If the basic chromosome number is x=4, the somatic chromosome numbers could be 2n=8, 16, and 20 with polyploidy. The species having 2n=18 and 2n=22 chromosomes might be derived by additional 2 chromosomes from 2n=16 and 2n=20 respectively. Alternatively, the cytotypes of 2n=18 and 2n=22 might be derived losing 2 chromosomes from 2n=20 and 2n=24 respectively. However, most *Tordylium* species have been studied and no hexaploid counts have been found. Thus, the present study strongly support that basic numbers of species are ranged from x=4 to x=11.

In the current classification [16], there are two subgenera included four sections in *Tordylium* (Figure 2, Table 5): 1) Subgen. *Tordylium* includes sect. *Tordylium* and is represented by *T. maximum, T. macropetalum* and *T. carmeli*; 2) Subgen. *Ainsworthia* has three sections: a) *Condylocarpus* that is represented only by *T. apulum* and b) *Hasselquistia* (*T. syriacum, T. hasselquistiae, T. trachycarpum, T. pustulosum, T. lanatum, T. aegyptiacum, T. cordatum,* and *T. elegans*) and c) *Univittata* Drude that includes the remaining species. In this study, it is revealed that the chromosome numbers of *Tordylium* species support synonym treatments of genera *Ainsworthia*, *Synelcosciadium*, *Condylocarpus*, and *Hasselquistia* because it is difficult to separate these genera based chromosome counts. Similarly, as appearing in the Table 5, the subgenera and sections do not differ on account of chromosome numbers. In Sect. *Tordylium* the chromosome numbers are 2n=18, 20 and 22, in sect. *Hasselquistia* ranges from 2n=16, 18 and 20. The chromosome number of *T. apulum* is 2n=20. This species has the longest chromosome length (Figure 1f) and morphologically differs from the other species with its equally 2-lobed radiant petals and mericarps with more than 10 vittae. According to Gömürgen et al. [45], *T. elegans* has a terminal chromosome (Figure 1b). This record is unique among *Tordylium* species. *T. elegans* has dimorphic mericarps similarly with *T. aegyptiacum* and *T. lanatum*. Even so the presence of any correlation between the karyology and the mericarp morphology has a remarkable significance to clarify relationships of these three species. So, more cytogenetic investigations are still needed for these three species. Total chromosome length of *T. elegans* (2n=16) and *T. ketenoglui* (2n=16) are recorded 17.67 µm and 18.55 µm respectively in this study. Total chromosome length of the other species which have 16 somatic chromosomes are determined between 7.84 µm and 12.77 µm. It is interesting that *T. ketenoglui* has the longest total chromosome length while it has the smallest mericarps among *Tordylium* species. *T. ketenoglui* was recognized in 1999 and differed from *T. brachytaenium* Boiss. and Heldr. [17]. Thus, *T. ketenoglui* should be placed in the sect. *Hasselquistia*. Accordingly, the chromosome data of *T. ketenoglui* obtained in this study shows compatible with sect. *Hasselquistia*.

To understand the evolution of chromosome numbers the phylogenetic hypothesis of Doğru-Koca [25] has been used (Figure 2). *Tordylium* has too heterogeneous cytotypes. There are no obvious ascending or descending chromosome number series. Therefore, we can say, the genome evolution in this genus has involved by many changes in chromosome numbers. The basal clade contains *T. elegans, T. aegyptiacum* and *T. lanatum* as we have discussed before their mericarp and chromosome morphology. *T. elegans* has 2n=16, *T. aegyptiacum* has 2n=20 chromosomes and *T. lanatum* is still unknown. Thus, we can say that the ancestral number is 2n=16 in the light of phylogenetic hypothesis based on morphological characters.

Also, the principal component analysis (PCA) are performed based on karyotype characters to examine the relationships among the species and infrageneric classification within the genus (Figure 3). The eigenvalues of the first 5 principal components (PCs) are summarized in Appendix Table S2. We have used scatter plot of PC1 and PC2 since first two principal components cumulatively explain 99.654% of the variance according to eigenvalue variables. The most important PCs generated from the karyology data of *Tordylium* and their statistical loadings are listed in Appendix Table S3. The higher than 0.1 contributions are marked in boldface text. PC1 is positively affected by median type and centromeric index values while negatively affected by haploid chromosome and relative length. In respect of PC2, the axis is positively affected by again median type and haploid chromosome length as well as longest chromosome length and minimum centromeric index value; also negatively affected by maximum relative length value. The loadings of median type of chromosomes are positively or negatively higher than 0.1 for all five principal components. Additionally, haploid chromosome length has the highest coefficient loading for PC2 (0.96825) among all loadings. Therefore, based on current data we can deduce that the median type and haploid chromosome length are important karyological characters for *Tordylium.* In respect of infrageneric classification there is a remarkable result based on karyological variables about *T. apulum* which is unique represent of sect. *Condylocarpus*. According to PC2, *T. apulum* is located far from the remaining species (Figure 3). It is compatible with current infrageneric classification while not compatible with phylogenetic relationships. Similarly, *T. elegans* is placed far from other species according to PC1 (Figure 3). This result is in agreement with phylogenetic relationships but not with current classification.

CONCLUSION

The genus *Tordylium* is one of the important genera due to its significant endemism rate and aspect of plant diversity. In conclusion, 11 *Tordylium* species have been studied which five are endemic from Turkey for the first time. *Tordylium* species have been separated into five cytotypes according to chromosome numbers such as 2n=8, 16, 18, 20 and 22. The chromosome numbers are not compatible with current infrageneric classification of the genus. There is not any substantial knowledge of evolutionary interest has been inferred using karyological methods and the chromosome numbers in *Tordylium* hitherto. In this research, we have completed some cytogenetic unknown points and evaluated these results on account of phylogenetic relationships. However, the phylogenetic and the evolutionary relationships within *Tordylium* genus would be solved by further cytogenetic studies on the different populations as well as phylogenetic methods.

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