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Research Article

Karyotype Symmetry/ Asymmetry and Karyotypic Relationships of Cervidae (Artiodactyla) Taxa

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Abstract | Karyotype asymmetry is one of the most widely used approaches in cytotaxonomic studies. The symmetry/asymmetry index (S/A_I) is used to determine karyotype asymmetry in higher animals and humans. The formula designed by the number of chromosome types is $S/A_I = (1 \times M) + (2 \times SM) + (3 \times A) + (4 \times T) / 2n$. The S/A_I value varies from 1.0000 (full symmetric) to 4.0000 (full asymmetric). After a detailed literature review, the chromosomal data of 36 female species and 32 male species of family Cervidae were detected, namely (i) karyotype formulae, (ii) symmetry/asymmetry index values (iii) karyotype types. According to the chromosomal data, two phylogenetic trees were formed. The phylogenetic trees were showed karyotypic relationships among the taxa.

Novelty Statement | This is the first report on karyotype asymmetry of Cervidae. Karyotypic relationships are useful to infer processes of evolution and speciation. Karyotype asymmetry is an important parameter that helps to establish phylogenetic relationships among various species.

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Introduction

Cervidae (Goldfuss, 1820) is placed in the order Artiodactyla (Owen, 1848). The family consists of at least 51 wild species in 19 genera. The Cervidae are the most widespread family of extant Artiodactyla after Bovidae (ITIS, 2019). They are distributed naturally in all continents except Antarctica and Australia (Gilbert *et al.*, 2006). The number of cervids decreases with human effects as a result of uncontrolled hunting, especially in Europe, Asia and America. According to the International Union for the Conservation of Nature (IUCN) Red List, the seven species are categorized as Endangered (EN). In addition, *Axis kuhlii* (bawean deer), *Elaphurus davidianus* (père David's deer) and *Rucervus schomburgki* (Schomburgk's deer) are categorized as Critically Endangered (CR), Extinct in the Wild (EW) and Extinct (EX), respectively (IUCN, 2018).

The family Cervidae is one of the most important members of the world's wildlife. Therefore, many taxonomic and cytotaxonomic studies have been reported related cervids till now and some of them have been summarized. The chromosome numbers of Cervidae are generally $2n$ = 50–70 (Wurster and Benirschke, 1967a, 1967b; Hsu and Benirschke, 1973a, 1973b; Jorge and Benirschke, 1977). The majority of species have 66–70 chromosomes (Naik *et al.*, 1964; Wurster and Benirschke, 1967a, 1967b; Gustavsson and Sundt, 1968; Koulischer *et al.*, 1972; Sokolov *et al.*, 1978; Wang and Du, 1983; Herzog, 1987). As an extreme example, *Muntiacus muntjak* has the lowest diploid chromosome number among mammals (female $2n$ = 6; male $2n$ = 7) (Tanomtong *et al.*, 2005). The chromosome numbers of genus *Muntiacus* are extremely diverse, ranging from 6 in *Muntiacus muntjak* (Tanomtong *et al.*, 2005) to the relatively high number of 46 in *Muntiacus reevesi reevesi* (Wurster and Benirschke, 1967a; Chiang *et*

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al., 2004) and *Muntiacus reevesi micrurus* (Chiang *et al*., 2004). In addition, there are B chromosomes in the family Cervidae (Abril and Duarte, 2008; Resende *et al*., 2011; Fiorillo *et al*., 2013). B chromosomes, are not necessarily necessary chromosomes, are extra chromosomes that do not comply with Mendelian inheritance rules. They are commonly found in mammals (Palestis *et al*., 2004).

Karyotype asymmetry is an important parameter in karyotype studies. Many parameters have been proposed in the calculation of karyotype asymmetry, which are parameters of Stebbins classification, TF (total form percentage), AsK (karyotype asymmetry index percentage), Syi (symmetric index), Rec (ratios of each chromosome), A1 (intrachromosomal asymmetry index), A2 (interchromosomal asymmetry index), DI (dispersion index), A (asymmetry index), CV_{CL} (coefficient of variation of chromosome length), CV_{CI} (coefficient of variation of centromeric index), M_{CA} (mean centromeric asymmetry) and S/A_I. All except S/A_I have been proposed for the calculation of plant karyotype asymmetries (Eroğlu *et al*., 2013; Peruzzi and Eroğlu, 2013; Eroğlu, 2015). The S/A_I is used to determine karyotype asymmetry in higher animals and humans (Eroğlu, 2015). All methods use basic measurement data such as long and short arms of chromosomes. The symmetrical karyotype is characterized by chromosomes with central centromere that do not differ much between long and short arm lengths. On the contrary, the asymmetric karyotype is characterized by chromosomes with very different long and short arm lengths and without a central centromere (Peruzzi and Eroğlu, 2013). The aim of this study is to reveal the karyotypic relationships of Cervidae taxa by determining the karyotype asymmetries.

Materials and Methods

The karyotype symmetry/asymmetry index

Eroğlu (2015) reported a formula to calculate karyotype symmetry/asymmetry index with two important matters. Firstly, while other parameters use chromosome arm lengths, the S/AI parameter uses the karyotype formula. Secondly, the chromosomal lengths may contain small differences in different karyotype studies of the same species. The differences do not affect the karyotype formula and S/A_I value. The S/A_I formula is shown below.

$$S/A_I = (1 \times M) + (2 \times SM) + (3 \times A \text{ or } ST) + (4 \times T) / 2n$$

The formula was designed according to the number of chromosome types, namely the chromosome number of metacentric (M), submetacentric (SM), acrocentric (A), subtelocentric (ST) and telocentric (T). In addition, a classification model was given according to the S/A_I value, namely $1.0 = S/A_I$ (full symmetric), $1.0 < S/A_I \leq 2.0$ (symmetric), $2.0 < S/A_I \leq 3.0$ (between symmetric and asymmetric), $3.0 < S/A_I < 4.0$ (asymmetric) and $4.0 = S/A_I$ (full asymmetric).

Sample application of symmetry/asymmetry on taxa

The karyotypes of the sample application belong to Cervidae taxa. The Cervidae includes the ruminant mammals commonly known as deer, elk, moose and caribou. After a detailed literature review, the information about family Cervidae was detected namely; (i) scientific/common names and author(s), (ii) karyotype formulae with B-chromosomes, if any (iii) symmetry/asymmetry index values (iv) karyotype types (Table 1).

Table 1 includes the scientific/common names and author(s) of the taxa. The databases were used for the control of scientific names, namely IUCN Red List (IUCN, 2018) and the Integrated Taxonomic Information System (ITIS, 2019), because sometimes scientific names can be expressed with different names in different sources. The genus *Rucervus* is an important example. The names of genus are *Cervus* (Chandra *et al*., 1967; Chavananikul *et al*., 1995; Thevenon *et al*., 2000; Bonnet-Garnier *et al*., 2003) and *Rucervus* (IUCN, 2018; ITIS, 2019).

In addition, Table 1 includes the symmetry/ asymmetry index values and the karyotype types. Calculation of these values is given below with *Cervus albirostris* karyotype. In Table 1, the karyotype formulae are $2n = 66 = 2M + 2SM + 62A$ in female and $2n = 66 = 2M + 3SM + 61A$ in male.

$$S/A_I\ (female) = (1 \times M) + (2 \times SM) + (3 \times A) + (4 \times T) / 2n$$
$$S/A_I\ (female) = (1 \times 2) + (2 \times 2) + (3 \times 62) / 66$$
$$S/A_I\ (female) = 2.9091$$
$$2.0 < S/A_I\ (female) \leq 3.0\ (between\ symmetric\ and\ asymmetric)$$
$$S/A_I\ (male) = (1 \times M) + (2 \times SM) + (3 \times A) + (4 \times T) / 2n$$
$$S/A_I\ (male) = (1 \times 2) + (2 \times 3) + (3 \times 61) / 66$$
$$S/A_I\ (male) = 2.8939$$
$$2.0 < S/A_I\ (male) \leq 3.0\ (between\ symmetric\ and\ asymmetric)$$

The phylogenetic trees formed by chromosomal data in Table 1 present the karyotype relationships among the taxa of Cervidae (Figures 1 and 2). The Figures 1 and 2 include 36 female taxa and 32 male taxa, respectively. For two reasons, four taxa are not included in the male phylogenetic tree: (i) The male subjects were not studied in *Capreolus pygargus*, *Mazama gouazoubira* and *Ozotoceros bezoarticus*; only female reports (Sokolov *et al*., 1978; Spotorno *et al*., 1987; Resende *et al*., 2011). (ii) In the karyotype study of *Hippocamelus bisulcus*, there are small Y chromosomes that cannot be examined. Therefore, chromosome cannot be classified (Spotorno *et al*., 1987). Finally, drawings of some species were added to the figures to further clarify the karyotype relationships among taxa.

Table 1: The karyotype formulae, index values and karyotype types of the taxa.

No	Family/ Species Scientific name/common name	Sample number	2n	Autosomes and sex chromosomes	References	S/A_I	Karyotype type
1	*Hydropotes inermis* (Swinhoe, 1870) (Water deer)	1 F 1 M	70	68A X = A, Y = A	Hsu and Benirschke, 1973b	3.0000 (F) 3.0000 (M)	BSA
2	*Muntiacus reevesi reevesi* (Ogilby, 1839) (Chinese muntjac)	1 F 1 M	46	44A X = A, Y = SM	Wurster and Benirschke, 1967a; Chiang *et al.*, 2004	3.0000 (F) 2.9783 (M)	BSA
3	*Muntiacus reevesi micrurus* (Sclater, 1875) (Formosan muntjac)	1 F 1 M	46	44A X = A, Y = SM	Chiang *et al.*, 2004	3.0000 (F) 2.9783 (M)	BSA
4	*Muntiacus feae* (Thomas & Doria, 1889) (Fea's muntjac)	4 F 2 M	14	13A X^{*}, Y = SM	Tanomtong *et al.*, 2005	3.0000 (F) 2.8571 (M)	BSA
5	*Muntiacus muntjak* (Zimmermann, 1780), (Indian muntjac)	3 F 2 M	6 (F) 7 (M)	2M + 4A X^{**}, Y = M	Tanomtong *et al.*, 2005	2.3333 (F) 2.2857 (M)	BSA
6	*Capreolus capreolus* (Linnaeus, 1758) (European roe deer)	1 F 1 M	70	68A X = SM, Y = ST	Wurster and Benirschke, 1967b	2.9714 (F) 2.9857 (M)	BSA
7	*Capreolus pygargus* (Pallas, 1771) (Siberian roe deer)	—	70	68A X = SM, Y $?^{***}$	Sokolov *et al.*, 1978	2.9714 (F)	BSA
8	*Mazama gouazoubira* (G. Fischer [von Waldheim], 1814) (Gray brocket)	1 F	70 + B (rarely)	2SM + 66A X = A, Y $?^{***}$	Resende *et al.*, 2011	2.9714 (F)	BSA
9	*Mazama nemorivaga* (F. Cuvier, 1817) (Amazonian brown brocket)	4 F 3 M	68 + B (2-7)	2SM + 64A X = SM, Y = A	Fiorillo *et al.*, 2013	2.9412 (F) 2.9559 (M)	BSA
10	*Mazama temama* (Kerr, 1792) (Central American red brocket)	1 F 2 M	50	8M + 12SM + 28A X = SM, Y = M	Jorge and Benirschke, 1977	2.4000 (F) 2.3800 (M)	BSA
11	*Mazama nana* (Hensel, 1872) (Brazilian dwarf brocket)	11 F 13 M	36 + B (1-6)	12M + 8SM + 14A X = M, Y = M	Abril and Duarte, 2008	2.0000 (F) 2.0000 (M)	S
12	*Rangifer tarandus* (Linnaeus, 1758) (Reindeer)	1 F 1 M	70	2SM + 66A X = SM, Y = A	Gripenberg *et al.*, 1986	2.9429 (F) 2.9571 (M)	BSA
13	*Alces americanus* (Clinton, 1822) (Siberian elk)	—	70	2SM + 66A X = SM, Y = A	Hsu and Benirschke, 1969	2.9429 (F) 2.9571 (M)	BSA
14	*Alces alces* (Linnaeus, 1758) (Eurasian elk)	4 F 2 M	68	2M + 2SM + 62A X = SM, Y = SM	Gustavsson and Sundt, 1968	2.8824 (F) 2.8824 (M)	BSA
15	*Odocoileus virginianus virginianus* (Zimmermann, 1780) (White-tailed deer)	1 F 1 M	70	2SM + 66A X = SM, Y = M	Wurster and Benirschke, 1967a	2.9429 (F) 2.9286 (M)	BSA
16	*Odocoileus virginianus borealis* (Miller, 1900) (White-tailed deer)	—	70	2SM + 66A X = SM, Y = M	Benirschke *et al.*, 1963	2.9429 (F) 2.9286 (M)	BSA
17	*Odocoileus hemionus* (Rafinesque, 1817) (Mule deer)	1 F 1 M	70	2SM + 66A X = SM, Y = M	Wurster and Benirschke, 1967a	2.9429 (F) 2.9286 (M)	BSA
18	*Hippocamelus bisulcus* (Molina, 1782) (Patagonian huemul)	1	70	2SM + 66A X= SM, Y minute^{****}	Spotorno *et al.*, 1987	2.9429 (F)	BSA
19	*Pudu puda* (Molina, 1782) (Southern pudu)	—	70	2SM + 66A X = SM, Y = M	Koulischer *et al.*, 1972	2.9429 (F) 2.9286 (M)	BSA
20	*Dama dama* (Linnaeus, 1758) (Fallow deer)	1 F 1 M	68	2M + 64A X = A, Y = SM	Wurster and Benirschke, 1967a; Gustavsson and Sundt, 1968	2.9412 (F) 2.9265 (M)	BSA
21	*Elaphurus davidianus* (Milne–Edwards, 1866) (Père David's deer)	1 F 1 M	68	2M + 64A X = A, Y = M	Hsu and Benirschke, 1971	2.9412 (F) 2.9118 (M)	BSA
22	*Axis porcinus* (Zimmermann, 1780) (Hog deer)	1 F 1 M	68	2M + 64A X = A, Y = A	Wurster and Benirschke, 1967b	2.9412 (F) 2.9412 (M)	BSA
23	*Axis axis* (Erxleben, 1777) (Chital)	1 F 3 M	66	2M + 2SM + 60A X = A, Y = A	Naik *et al.*, 1964	2.9091 (F) 2.9091 (M)	BSA

Table continue on next page..................

No	Family/Species Scientific name/common name	Sample number	2n	Autosomes and sex chromosomes	References	S/A_I	Karyotype type
24	*Cervus elaphus* (Linnaeus, 1758) (Red deer)	22	68	2M + 64A X = A, Y = M	Herzog, 1987	2.9412 (F) 2.9118 (M)	BSA
25	*Cervus canadensis* (Erxleben, 1777) (Wapiti)	—	68	2M + 64A X = A, Y = A	Koulischer *et al*., 1972	2.9412 (F) 2.9412 (M)	BSA
26	*Cervus nippon* (Temminck, 1838) (Sika deer)	1 F 1 M	67	3M + 62A X = A, Y = SM	Gustavsson and Sundt, 1968	2.9104 (F) 2.8955 (M)	BSA
27	*Cervus albirostris* (Przewalski, 1883) (White–lipped deer)	1 F 1 M	66	2M + 2SM + 60A X = A, Y = SM	Wang and Du, 1983	2.9091 (F) 2.8939 (M)	BSA
28	*Blastocerus dichotomus* (Illiger, 1815) (Marsh deer)	18 F 18 M	66	4M + 2SM + 58A X = M, Y = SM	Duarte and Giannoni, 1992	2.7879 (F) 2.8030 (M)	BSA
29	*Ozotoceros bezoarticus* (Linnaeus, 1758) (Pampas deer)	1 F	68	4M + 62A X = M, Y ?***	Spotorno *et al*., 1987	2.7429 (F)	BSA
30	*Rusa marianna* (Desmarest, 1822) (Philippine deer)	1 F 1 M	65 (F) 64 (M)	4M + 1SM*****+ 58A X = A, Y = A	Hsu and Benirschke, 1973a	2.8615 (F) 2.8750 (M)	BSA
31	*Rusa timorensis* (de Blainville, 1822) (Rusa deer)	—	60	6M + 4SM + 48A X = A, Y =A	Bonnet–Garnier *et al*., 2003	2.7333 (F) 2.7333 (M)	BSA
32	*Rusa unicolor* (Kerr, 1792) (Sambar deer)	1	58	6M + 6SM + 44A X = A, Y =A	Chandra *et al*., 1967	2.6897 (F) 2.6897 (M)	BSA
33	*Rucervus eldii siamensis* (Lydekker, 1915) (Thai brow–antlered deer)	2 F 6 M	58	6M + 6SM + 44A X = A, Y =A	Thevenon *et al*., 2000; Bonnet–Garnier *et al*., 2003	2.6897 (F) 2.6897 (M)	BSA
34	*Rucervus eldii thamin* (Thomas, 1918) (Thamin brow–antlered deer)	1 F 1 M	58	6M + 6SM + 44A X = A, Y =A	Thevenon *et al*., 2000	2.6897 (F) 2.6897 (M)	BSA
35	*Rucervus eldii eldii* (M'Clelland, 1842) (Brow–antlered deer)	—	58	6M + 6SM + 44A X = A, Y =SM	Chavananikul *et al*., 1995	2.6897 (F) 2.6724 (M)	BSA
36	*Rucervus duvaucelii* (G. Cuvier, 1823) (Barasingha)	1 F 1 M	56	6M + 8SM + 40A X = A, Y = M	Chandra *et al*., 1967; Wurster and Benirschke, 1967b	2.6429 (F) 2.6071 (M)	BSA

Abbreviations: M: metacentric; SM: submetacentric; A: acrocentric; ST: subtelocentric; T: telocentric; F: female; M: male; B: B-chromosomes; BSA: between symmetric and asymmetric; S: symmetric.

*The X chromosomes were fused to the chromosome paired 1; **The X chromosomes were fused to the chromosome paired 3; ***There is no male in the karyotype study; ****Could not determine the type of chromosome; *****In only female karyotype.

Results and Discussion

The ancestral cervid karyotype is 2*n* = 70 (Bonnet-Garnier *et al*., 2003). The diploid chromosome number in the family Cervidae is a wide range. According to the Table 1, it is observed from 6–7 in the *Muntiacus muntjak* to 70 in many taxa. Although the diploid chromosome number in many taxa is greater than 50, there are 50 or fewer chromosomes in only *Muntiacus reevesi reevesi*, *Muntiacus reevesi micrurus*, *Muntiacus feae*, *Muntiacus muntjak*, *Mazama temama* and *Mazama nana*.

In Figure 1 while the karyotype is between symmetric and asymmetric in the 17 genera and 35 taxa, it is symmetric in the only one genus and taxon. The female karyotype symmetry/asymmetry values of 36 taxa are between 2.0000 and 3.0000 with an average of 2.8343 ± 0.21.

In Figure 2 while the karyotype is between symmetric and asymmetric in the 15 genera and 31 taxa, it is symmetric in the only one genus and taxon. The male karyotype symmetry/asymmetry values of 32 taxa are between 2.0000 and 3.0000 with an average of 2.8132 ± 0.23.

In Figures 1 and 2 while the karyotype of *Mazama nana* is symmetric, others are between symmetric and asymmetric. The chromosome numbers are polymorphic in genus *Mazama*. The genus has different chromosome number as 2*n*= 36, 50, 68, 70 and different types of chromosomes (Jorge and Benirschke, 1977; Abril and Duarte, 2008; Resende *et al*., 2011; Fiorillo *et al*., 2013). Therefore, *Mazama gouazoubira*, *Mazama nemorivaga* and *Mazama temama* are characterized by greater number of chromosomes than *Mazama nana*. Their karyotype is between symmetric and asymmetric. *Mazama temama* was earlier classified as a subspecies of *Mazama americana*.

Jorge and Benirschke (1977) reported that the karyotype of *Mazama temama* was different from *Mazama americana*. The chromosome numbers of *Mazama nemorivaga* and *Mazama nana* have been determined as 2*n*= 67–69 (Fiorillo *et al*., 2013) and 2*n*= 36–39 (Abril and Duarte, 2008), respectively. B chromosomes or microchromosomes can cause variant karyotype. These chromosomes were not considered in the calculation of the diploid numbers because there was interindividual and intraindividual variations (Abril and Duarte, 2008). Fiorillo *et al*. (2013) showed that there are variable (2–7) numbers of B chromosomes in the *Mazama nemorivaga*. They are very small and similar to the acrocentric Y chromosome. Abril and Duarte (2008) stated that there are variable (1–6) numbers of B chromosomes in the *Mazama nana*. Besides *Mazama gouazoubira* has B chromosomes (Resende *et al*., 2011). It was believed that B chromosomes in genus *Mazama* could originate from Robertsonian translocations involving biarmed chromosomes, which lose centromeric fragments (Palestis *et al*., 2004).

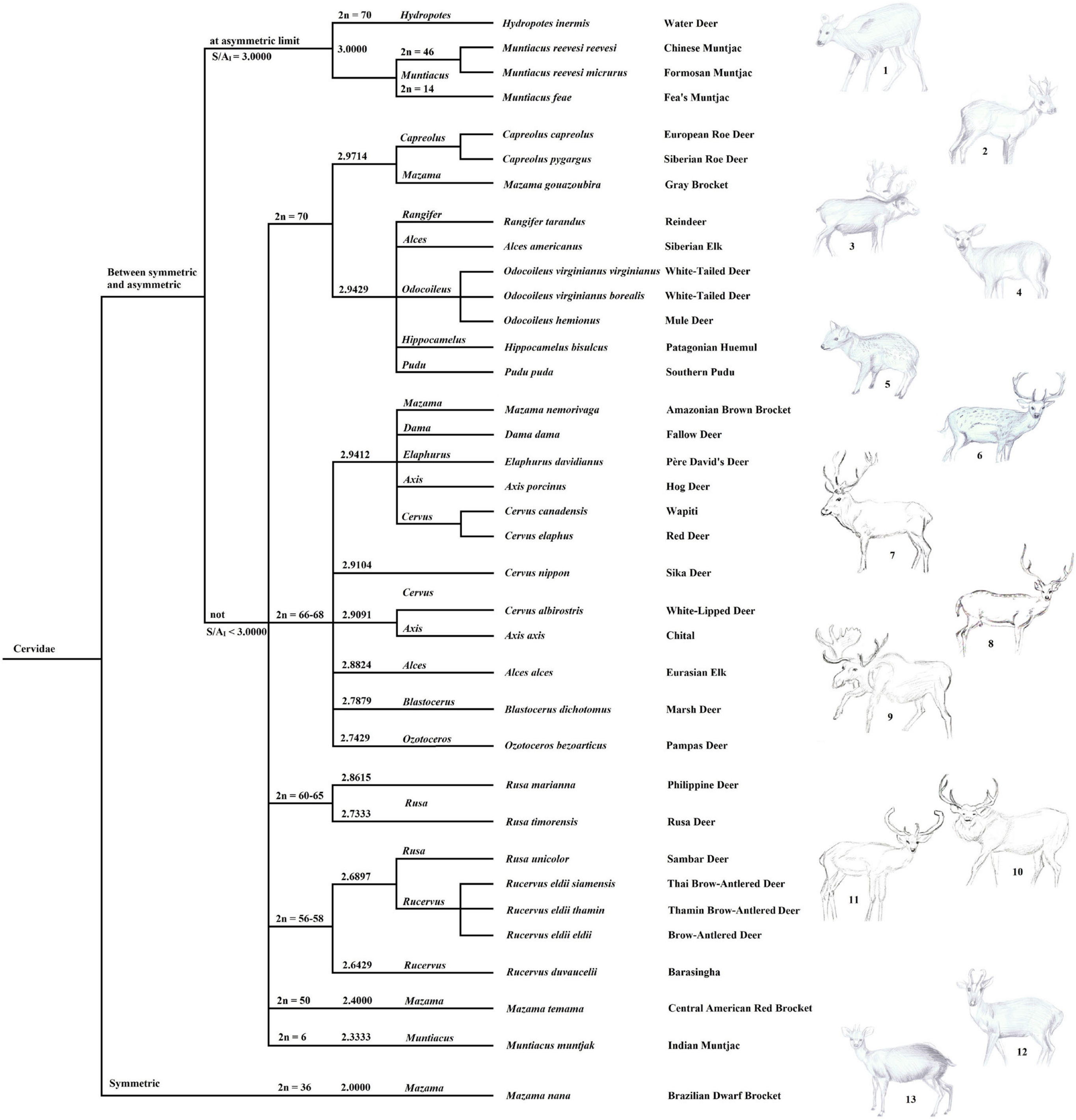

Figure 1: Phylogenetic tree showing relationships of the female index values among the taxa of cervids.
(1) Hydropotes inermis; (2) Capreolus capreolus; (3) Rangifer tarandus; (4) Odocoileus virginianus virginianus; (5) Pudu puda; (6) Dama dama; (7) Cervus elaphus; (8) Axis axis; (9) Alces alces; (10) Rusa unicolor; (11) Rucervus eldii siamensis; (12) Muntiacus muntjak; (13) Mazama nana.

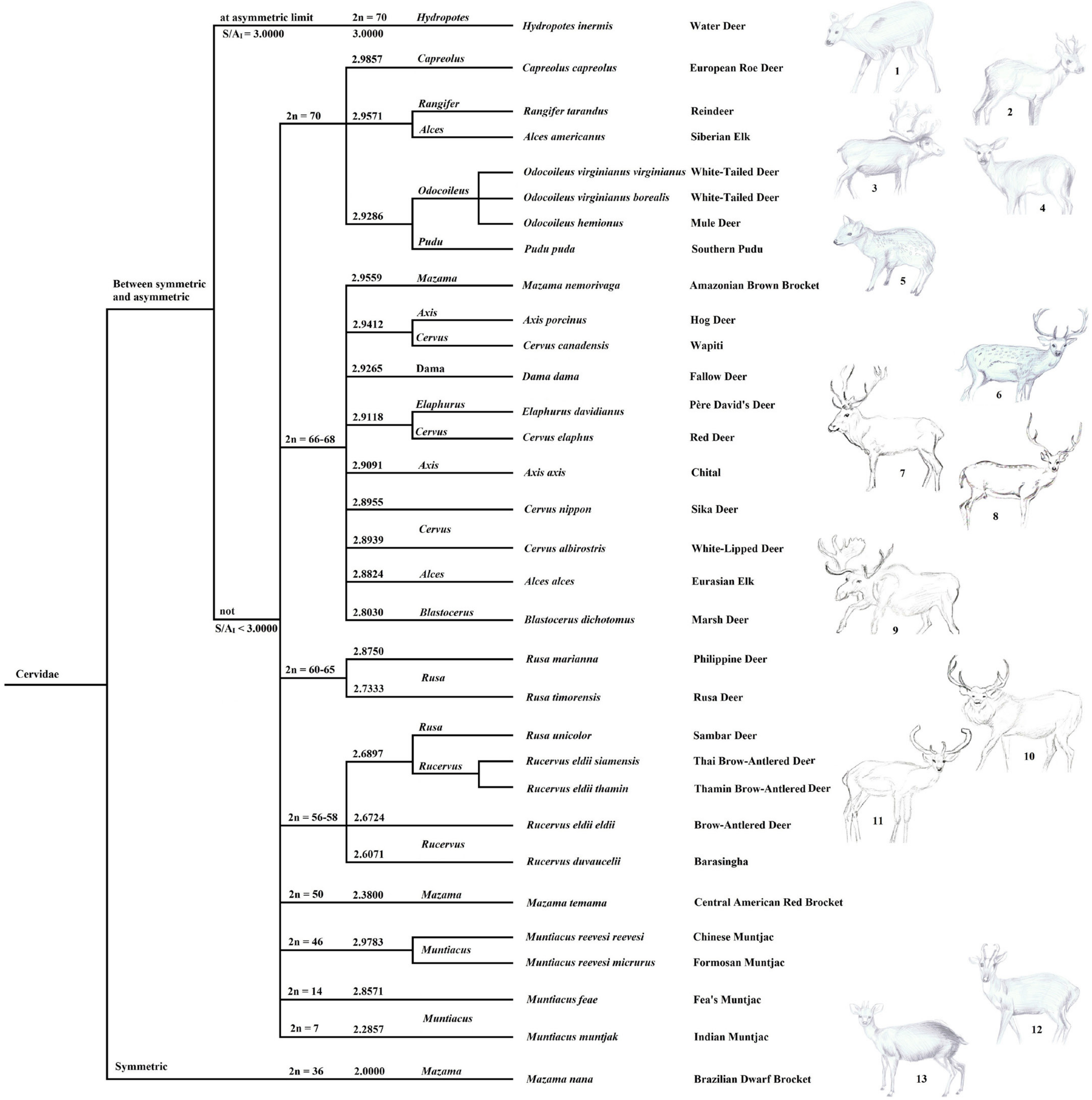

Figure 2: Phylogenetic tree showing relationships of the male index values among the taxa of cervids.
(1) Hydropotes inermis; (2) Capreolus capreolus; (3) Rangifer tarandus; (4) Odocoileus virginianus virginianus; (5) Pudu puda; (6) Dama dama; (7) Cervus elaphus; (8) Axis axis; (9) Alces alces; (10) Rusa unicolor; (11) Rucervus eldii siamensis; (12) Muntiacus muntjak; (13) Mazama nana.

An asymmetric karyotype is characterized by mainly acrocentric/subtelocentric and telocentric chromosomes and the index value is $3.0 < S/A_I < 4.0$ (Eroğlu, 2015). Despite the karyotypes of *Hydropotes inermis* (Figures 1 and 2), *Muntiacus reevesi reevesi*, *Muntiacus reevesi micrurus* and *Muntiacus feae* (Figure 1) are very close to the asymmetric, there is no asymmetric karyotype in all studied species. The similar results are available in the literature. The karyotypes of family Felidae are symmetric in 10 species, and between symmetric and asymmetric in 13 species (Eroğlu, 2017). The karyotypes of infraorder Cetacea are also symmetric in seven species, and between symmetric and asymmetric in 19 species. In addition, human karyotype is symmetric (Eroğlu, 2015). The karyotypes of genus *Alburnus* (Rafinesque, 1820) are symmetric in two species, and between symmetric and asymmetric in five species (Eroğlu, 2016). *Pelophylax ridibundus* (Pallas, 1771) and *Bos taurus indicus* (Linnaeus, 1758) have symmetric, and between symmetric and asymmetric karyotypes, respectively (Canpolat *et al.*, 2018; Amancio *et al.*, 2019).

The chromosome numbers are polymorphic in genus *Muntiacus*. *Muntiacus muntjak* has the lowest diploid chromosome number among mammals (female $2n$= 6; male $2n$= 7) (Tanomtong *et al*., 2005). The chromosome numbers of *Muntiacus feae* ($2n$= 14) and *Muntiacus reevesi* ($2n$= 46) are quite different (Wurster and Benirschke, 1967a; Chiang *et al*., 2004; Tanomtong *et al*., 2005). These species have different index values and positions in Figures 1 and 2. The chromosome numbers of *Muntiacus muntjak* have been determined as $2n$= 6, 7 and 9. The X chromosomes were fused to the chromosome paired 1 in female and 3 in male. The chromosome numbers of *Muntiacus feae* have been determined as $2n$ = 12–14. The X chromosomes were fused to the chromosome paired 1. The centric fusions are thought to have caused the chromosome number and karyotype differences (Tanomtong *et al*., 2005).

The chromosome numbers and index values of *Capreolus* and *Odocoileus* are greater than *Cervus* and *Axis* (Figures 1 and 2). Because of the karyotype homology and other similarities, the genera *Cervus* and *Axis* can interbreed and produce fertile generations (Asher *et al*., 1999). Wilson and Reeder (1993) related that *Cervus canadensis* was classified in *Cervus elaphus*. There is no strong consensus regarding the relationship between *Cervus canadensis* and *Cervus elaphus* (IUCN, 2018). Randi *et al*. (2001) showed that *Cervus canadensis* and *Cervus elaphus* are distinct species by patterns of mtDNA variation. In Figures 1 and 2 *Cervus canadensis* and *Cervus elaphus* are close, but they are distinct species.

Although there is still some debate about the status of species or subspecies of *Alces americanus*, Wilson and Reeder (1993) reported that *Alces americanus* and *Alces alces* are distinct species. In Figures 1 and 2 *Alces americanus* and *Alces alces* have different chromosome numbers, index values and positions, but these values of *Alces americanus* and *Rangifer tarandus* are same. There are several karyotypic similarities between *Rangifer tarandus* and *Alces alces*. The chromosome number of *Alces alces* is $2n$ = 68 as a consequence of centric fusion between two acrocentric chromosomes forming a new metacentric chromosome (Gripenberg *et al*., 1986).

Blastocerus dichotomus and *Ozotoceros bezoarticus* are the only species in genera *Blastocerus* and *Ozotoceros*, and they are close species (IUCN, 2018; ITIS, 2019). Spotorno *et al*. (1987) showed that *Ozotoceros bezoarticus* was classified as *Blastoceros bezoarticus*. In Figure 1 the karyotypes, index values and positions of these species are similar. Neitzel (1987) accepted that the genus *Blastocerus* was included in genus *Odocoileus* by some cytogenetic data. In our study as same as Duarte and Giannonis (1992) study, cytogenetic and morphological differences between two genera are reported.

In Figure 1 the index values of *Dama dama*, *Elaphurus davidianus*, *Axis porcinus*, *Cervus canadensis* and *Cervus elaphus* are identical, in Figure 2 they are not. The main reason is a heteromorphism between X and Y chromosomes in *Dama dama*, *Elaphurus davidianus* and *Cervus elaphus*. The X chromosome is acrocentric and the Y chromosome is metacentric (Hsu and Benirschke, 1971; Herzog, 1987) and submetacentric (Wurster and Benirschke, 1967a; Gustavsson and Sundt, 1968). The main characteristic in many species of Cervidae is identical X chromosome, which is the largest acrocentric chromosome. There are chromosomal polymorphisms in *Dama dama*. The chromosome number of *Dama dama* is $2n$ = 66–68. The centric fusions are the most frequent change in karyotype evolution (Gustavsson and Sundt, 1968).

There are chromosomal polymorphisms in genus *Rusa*. The chromosome numbers of *Rusa unicolor* ($2n$ = 56 to 64–65), *Rusa timorensis* ($2n$ = 60) and *Rusa marianna* ($2n$ = 64–65) are quite different (Chandra *et al*., 1967; Hsu and Benirschke, 1973a; Neitzel, 1987; Bonnet–Garnier *et al*., 2003). The centric fusions are thought to have caused the chromosome number and karyotype differences (Bonnet–Garnier *et al*., 2003). Although there are chromosomal polymorphisms, these species are located close in Figures 1 and 2.

The species of genus *Rucervus* are located close in Figures 1 and 2. The karyotypes of three subspecies of *Rucervus eldii* are identical excluding the Y chromosome of *Rucervus eldii eldii*. In Figures 1 and 2 although the index values is close, the chromosome number of *Rucervus duvaucelii* ($2n$= 56) is different from *Rucervus eldii* ($2n$= 58).

The ancestral karyotype with 70 acrocentric chromosomes, which is varied by intraspecific karyotypic variations, appears to be common in family Cervidae. (Abril and Duarte, 2008). *Hydropotes inermis* has the ancestral karyotype (Hsu and Benirschke, 1973b). In addition, in Table 1 *Capreolus capreolus*, *Capreolus pygargus*, *Mazama gouazoubira*, *Rangifer tarandus*, *Alces americanus*, *Odocoileus virginianus*, *Odocoileus hemionus*, *Hippocamelus bisulcus* and *Pudu puda* have the ancestral karyotype with some metacentric and submetacentric chromosomes. The karyotypic variations were primarily occurred by the centric fusions and hybridization events between different taxa (Abril and Duarte, 2008). Fontana and Rubini (1990) expressed that there are three mechanisms using chromosome arrangement in the evolutionary history of Cervidae. The first mechanisms are centric fusions causing the chromosomes decreasing to 68. The second mechanisms are centric fusions and pericentric inversions that causative to the submetacentric X and one submetacentric autosomal chromosome. The chromosome number is $2n$ = 70. The third mechanisms are centric fusions and tandem fusion chromosome rearrangements resulting with $2n$ = 46.

The karyotypic relationships of Cervidae were determined with the karyotype symmetry/asymmetry index. In this regard, the karyotypic phylogenetic tree is similar to the phylogenetic tree of the relationships among 19 species of family Cervidae based on whole mitochondria genomes made by Zhang and Zhang (2012). Particularly similarity is more pronounced between *Rangifer tarandus* and *Odocoileus virginianus*, and between genus *Muntiacus* and *Hydropotes inermis*. In addition, the karyotypic phylogenetic tree shows parallelism and differences with the cladograms constructed for mitochondrial rRNA genes and nuclear beta-spectrin gene fragment proposed by Kuznetsova *et al.* (2005). The parallelism and differences are more pronounced between *Rangifer tarandus* and *Odocoileus virginianus*, and genus *Cervus* and genus *Muntiacus*, respectively. As a result, the SA_I parameter together with the other parameters will contribute to phylogenetic trees of mammals.

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Conflict of interest

The authors have declared no conflict of interest.

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