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Comparison of the chromosome banding patterns in three species of social voles (*Microtus irani karamani, M. schidlovskii, M. anatolicus*) from Turkey

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Abstract: The karyotypes of three species of social voles recently discovered in Turkey (*Microtus irani karamani*, *M. schidlovskii*, and *M. anatolicus*) were investigated. All specimens examined revealed similar karyotypes comprising 60 chromosomes in the diploid complement. All autosomes and the X chromosome were acrocentric. The subtelocentric Y chromosome was recorded in *M. anatolicus* but it was acrocentric in the other species. Dark C-bands were observed in centromeric/pericentromeric areas of all the acrocentric autosomes. The X chromosome had a centromeric C-positive area and the Y chromosome was completely heterochromatic in all specimens examined. AgNORs were recorded in the pericentromeric region of seven autosome pairs in *M. irani karamani*, ten autosome pairs in *M. schidlovskii*, and eight autosome pairs of *M. anatolicus*. Differences in the NOR distribution between the species were quantified in a neighbor-joining tree. The individuals of *M. anatolicus* appeared as the basal branch in relation to the derived sister group of *M. schidlovskii* and *M. irani karamani*.

Key words: Karyotype, C-banding, AgNOR staining, divergence pattern

1. Introduction

Social voles include arvicoline species of small or medium size distributed in southeastern Europe, Asia Minor, the Caucasus, and the Middle East (Musser and Carleton, 2005). This group represents a monophyletic lineage in the tree of arvicoline voles that is included in the nominate subgenus *Microtus* as the *socialis* group, together with the sister *arvalis* group (Jaarola et al., 2004; Martínková and Moravec, 2012). The taxonomic relationships within the *socialis* group have not yet been satisfactorily resolved. Musser and Carleton (2005) recognized eight species in this group but this treatment has been under intensive discussion. Kryštufek et al. (2009, 2012) and Zorenko et al. (2014) distinguished two basic phylogenetic branches within this group, i.e. the *socialis* and the *guentheri* lineages. The taxonomic position of some species, such as *M. irani*, still remains uncertain (Kryštufek and Kefelioğlu, 2001; Kryštufek et al. 2010).

Contrary to the *arvalis* group, chromosomal investigations have not contributed substantially to solution of the taxonomic problems in social voles (Zima et al., 2013). An important exception is *M. dogramacii*, which possesses a karyotype distinctly different from other species (Kefelioğlu and Kryštufek, 1999; Şekeroğlu et al., 2011). The two basic lineages distinguished in molecular studies differ in their standard karyotype characteristics (2n = 54 and 60–62, respectively), but there are also unexpected findings in the enigmatic *M. irani* that contradict this general divergence (Çolak et al., 1997; Mahmoudi et al., 2014).

The aim of the present study is to provide a detailed cytogenetic comparison of the karyotypes of three different taxa of social voles from Turkey sharing the same diploid number of 60 chromosomes. These species were only recently added to the fauna of Turkey (*Microtus irani karamani*: Kryštufek and Kefelioğlu, 2001; Kryštufek et al., 2010; *M. schidlovskii*: Yiğit et al., 2006; *M. anatolicus*: Kefelioğlu and Kryštufek, 1999). The karyotypes of these three taxa seem to be quite similar according to studies that applied conventional staining of chromosomes (Kefelioğlu, 1995; Golenishchev et al., 1999, 2002; Kefelioğlu and Kryštufek, 1999; Kryštufek and Kefelioğlu, 2001; Yiğit et

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al., 2006; Kryštufek et al., 2010). Investigations involving chromosomal banding techniques were, however, exceptional (Yavuz et al., 2009). Therefore, an analysis was made with the use of chromosome C-banding and AgNOR staining to reveal possible differentiation between karyotypes of the studied species.

2. Materials and methods

Cytogenetic analyses were performed for 10 specimens of *M. irani karamani*, *M. schidlovskii*, and *M. anatolicus* from several Turkish populations. The specimens were caught with live traps. The number of specimens analyzed and location of the collection sites of *Microtus* species are shown in Figure 1 and Table 1. Standard voucher specimens (skins and skulls) are deposited in the Department of Biology, Faculty of Science, Selçuk University, Konya, Turkey.

Karyotype preparations were obtained from the bone marrow of animals treated with colchicine (Ford and Hamerton, 1956). After preparation of chromosome slides, conventional Giemsa staining was carried out. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected in individual autosomal and sex chromosome pairs via C-banding (Sumner, 1972) and AgNOR staining (Howell and Black, 1980),

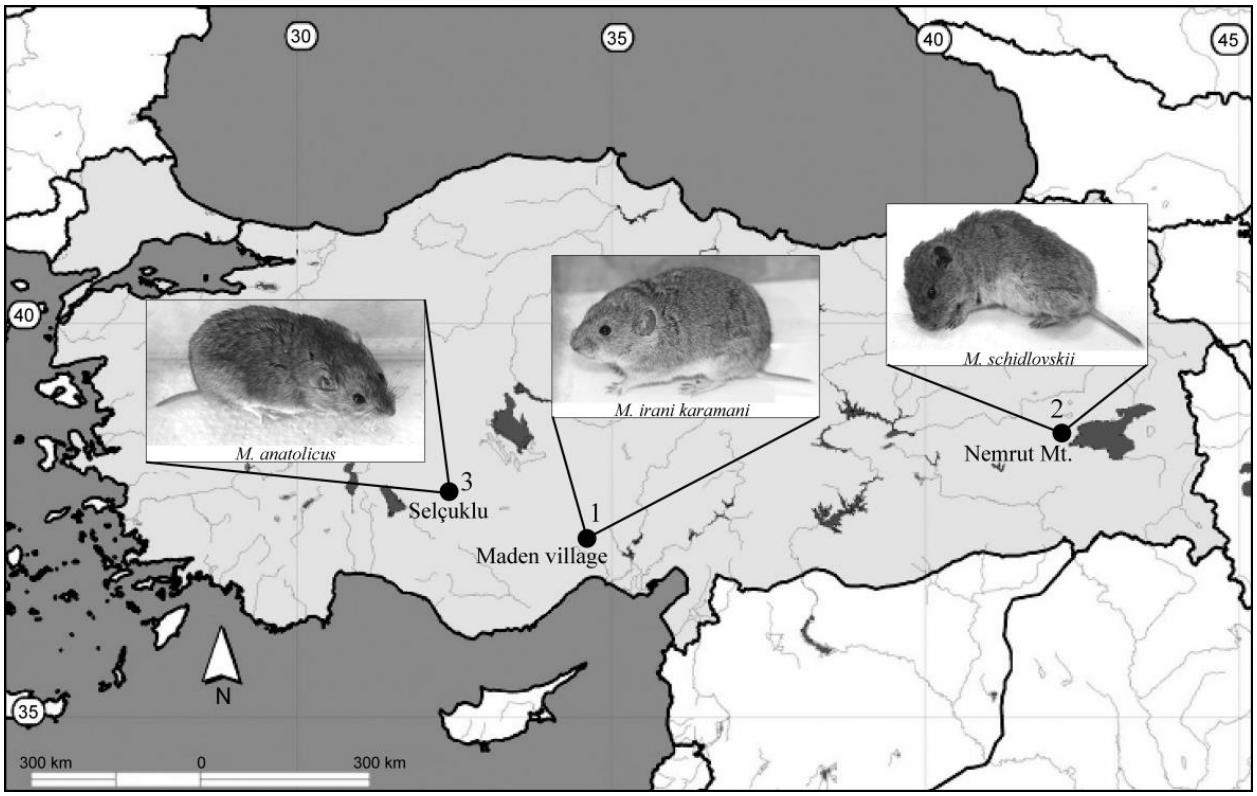

Figure 1. Collecting sites of *M. irani karamani* (1), *M. schidlovskii* (2), and *M. anatolicus* (3) in Turkey. The numbering of sampling localities corresponds to data in Table 1.

Table 1. Studied localities of three *Microtus* species in Turkey. The numbering of the sampling sites corresponds to data in Figure 1.

No.	Species	Locality / Province	Latitude, longitude	2n	No. of specimens		NF	NFa	X	Y
					Male	Female				
1	*M. irani karamani*	Madenköy / Niğde	37°27′N, 34°37′E	60	2	1	60	58	A	A
2	*M. schidlovskii*	Nemrut Mt. / Bitlis	38°33′N, 42°12′E	60	2	-	60	58	A	A
3	*M. anatolicus*	Selçuklu / Konya	38°02′N, 32°27′E	60	3	2	62	58	A	St

respectively. From each specimen, 10 to 20 slides were prepared, and at least 20 well-spread metaphase plates were analyzed. Chromosome morphologies were determined after calculating centromeric indices. The system of classification of chromosomes according to the centromere position was adopted after Hsu and Benirschke (1967–1977), and almost all chromosomes were distinguished as uniarmed (acrocentric – A). The fundamental number of autosomal arms (NFa) and the number of all chromosomal arms in the female complement (NF) were calculated. The distribution of the AgNOR sites on individual chromosomes was summarized in the presence/absence matrix and a neighbor-joining clustering analysis was performed based on the character dataset using the PAST program (Hammer et al., 2001).

3. Results

3.1. Conventionally stained karyotypes

The karyotype of a male and a female of *M. irani karamani* consisted of 60 chromosomes including 29 acrocentric autosomal pairs of gradually diminishing size (NFa = 58). The X chromosome was large acrocentric and the Y chromosome was small acrocentric (NF = 60) (Figure 2, set 1).

The karyotype of a male and a female of *M. schidlovskii* consisted of 60 chromosomes including 29 acrocentric autosomal pairs of gradually diminishing size (NFa = 58). The X chromosome was large acrocentric and the Y chromosome was small acrocentric (NF = 60) (Figure 2, set 2).

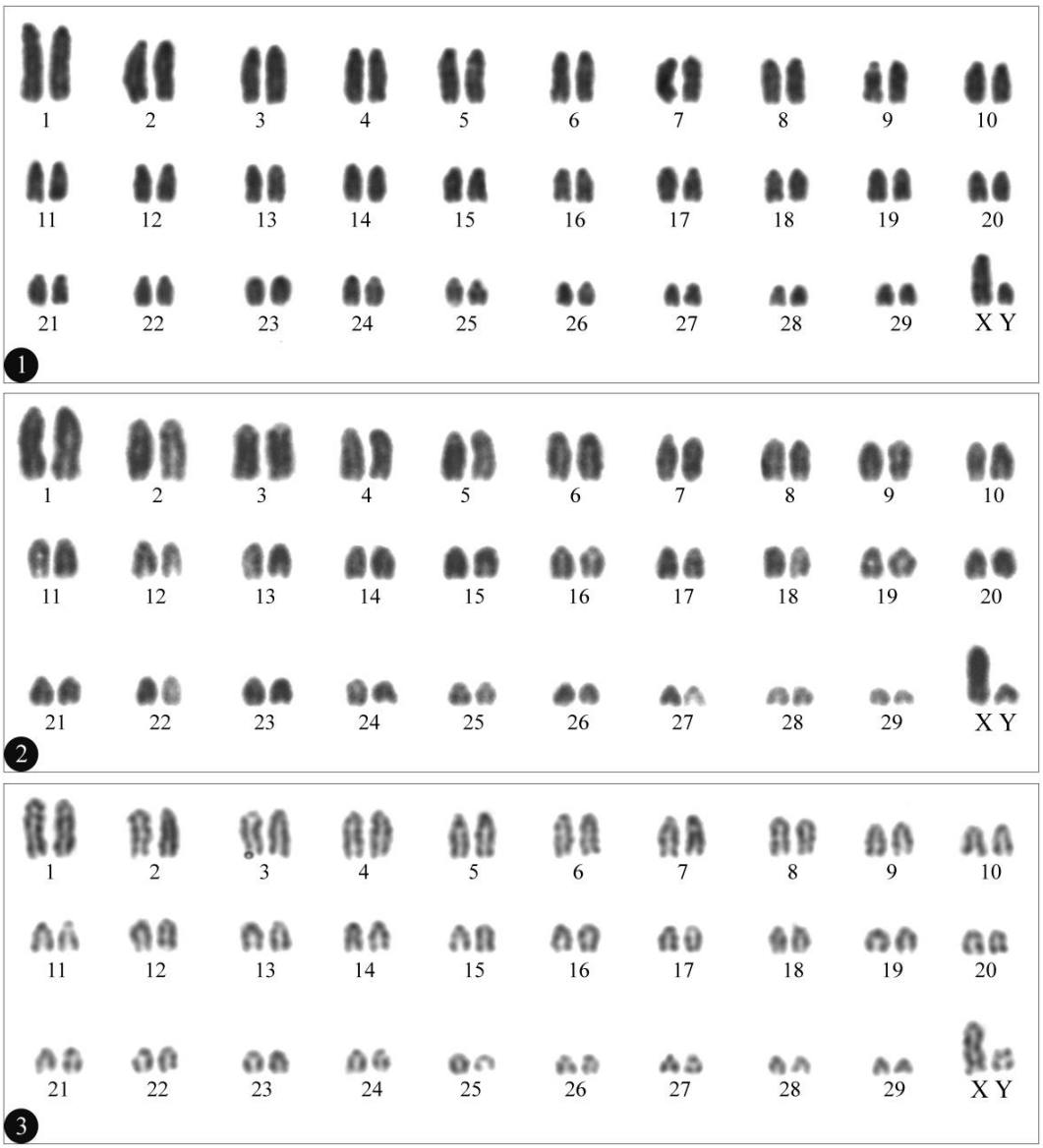

Figure 2. Standard karyotypes of *M. irani karamani* (1), *M. schidlovskii* (2), and *M. anatolicus* (3).

The karyotype of a male and a female of *M. anatolicus* consisted of 60 chromosomes including 29 acrocentric autosomal pairs of gradually diminishing size (NFa = 58). The X chromosome was large acrocentric and the Y chromosome was small subtelocentric (NF = 62) (Figure 2, set 3).

3.2. C-banding patterns

In the complement of *M. irani karamani*, dark C-bands were observed in centromeric/pericentromeric areas of all the acrocentric autosomes. The X chromosome had a centromeric C-positive area and the Y chromosome was completely heterochromatic (Figure 3, set 1).

In the complement of *M. schidlovskii*, dark C-bands were recorded in centromeric/pericentromeric areas of all the acrocentric autosomes. The X chromosome had a centromeric C-positive area and the Y chromosome was completely positively stained (Figure 3, set 2).

In *M. anatolicus*, dark C-bands were observed in centromeric/pericentromeric areas of all the acrocentric autosomes. The X chromosome had a centromeric C-positive area and the Y chromosome was entirely C-negative (Figure 3, set 3).

3.3. Silver staining and NORs distribution

In the complement of *M. irani karamani*, AgNORs were recorded in the pericentromeric region of seven autosome pairs (2, 9, 14, 21, 22, 25, 27). In some cells, only one homologue of pair 21 bore the positive silver signal (Figure 4, set 1).

Figure 3. C-banded karyotypes of *M. irani karamani* (1), *M. schidlovskii* (2), and *M. anatolicus* (3).

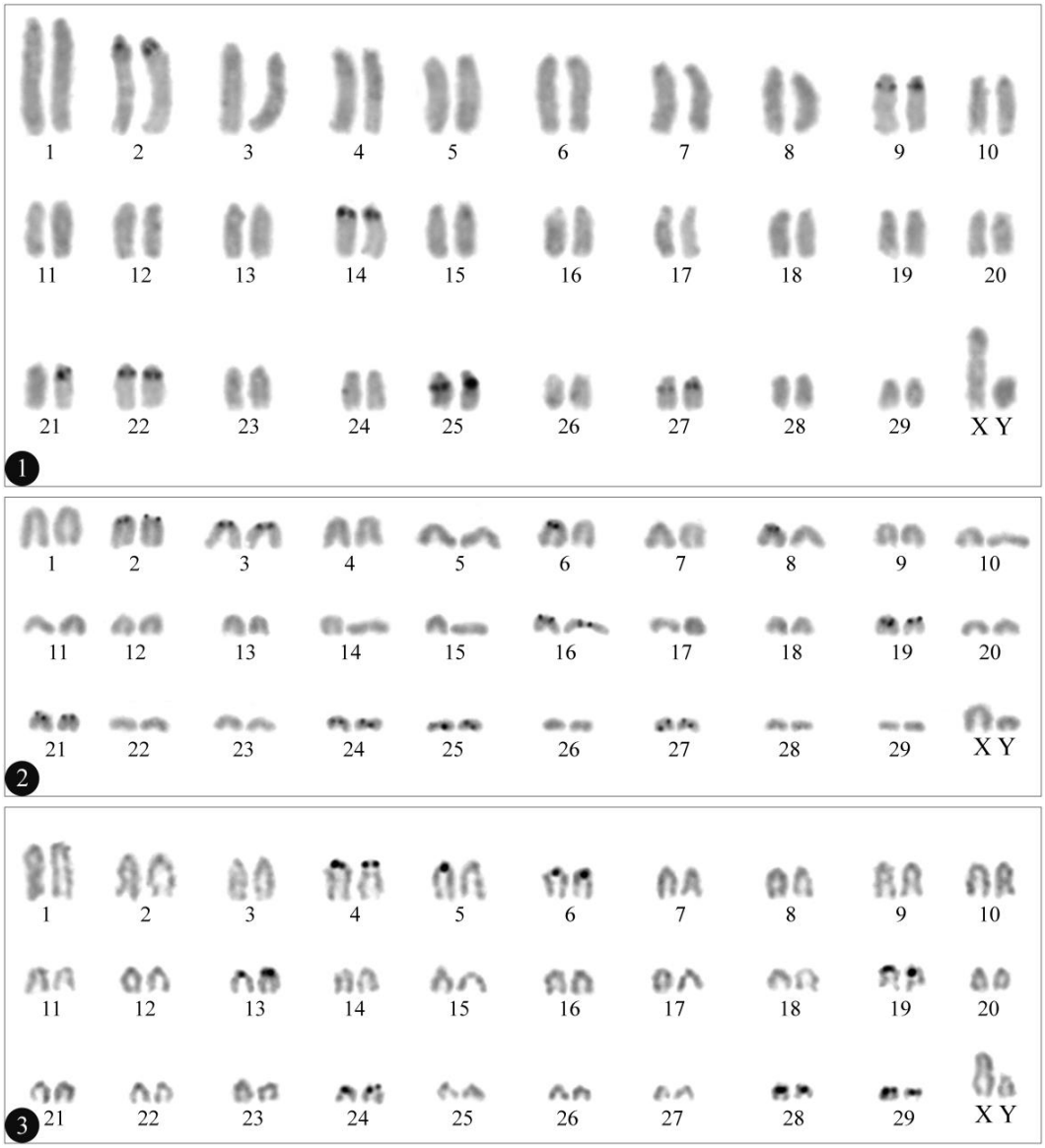

Figure 4. Silver-stained karyotypes of *M. irani karamani* (1), *M. schidlovskii* (2), and *M. anatolicus* (3).

In *M. schidlovskii*, AgNORs were localized in the pericentromeric region of ten autosome pairs (2, 3, 6, 8, 16, 19, 21, 24, 25, 27). In some cells, only one homologue of pairs 6 and 8 bore the positive silver signal (Figure 4, set 2).

In *M. anatolicus*, AgNORs were localized in the pericentromeric region of eight autosome pairs (4, 5, 6, 13, 19, 24, 28, 29). In some cells, only one homologue of pair 5 bore the positive silver signal (Figure 4, set 3).

3.4. Karyotypic relationships between the populations

The distribution pattern of the AgNOR sites in individual chromosomes is summarized in Table 2, and the resulting neighbor-joining tree derived from the presence or absence of the characters is shown in Figure 5. The individuals of *M. irani karamani* appeared as the basal branch in relation to the derived sister group of *M. schidlovskii* and *M. irani karamani*.

4. Discussion

All three populations studied revealed the same karyotype as described in previous studies (Kefelioğlu, 1995; Kefelioğlu and Kryštufek, 1999; Kryštufek and Kefelioğlu, 2001; Yiğit et al., 2006; Yavuz et al., 2009, Kryštufek et al., 2010; Arslan and Zima, 2014). All chromosomes in the complement revealed distinct dark C-bands in the pericentromeric position. The only variation in the C-banding pattern was found in the staining of the Y chromosome. This sex chromosome was stained C-negatively in *M. anatolicus*, whereas it was C-heterochromatic in other species. This

Table 2. The distribution of the AgNOR sites on individual chromosome pairs according to presence (1) and absence (0). For population numbers see Table 1. The out groups are *Nannospalax xanthodon* (2n = 60) from Konya (Arslan et al., 2011) and Aksaray (Arslan and Bolukbas, 2010).

Species	Chromosome no.																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
M. irani karamani	0	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	1	0	1	0	0
M. schidlovskii	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	1	0	0	1	1	0	1	0	0
M. anatolicus	0	0	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1	1
First out group	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Second out group	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

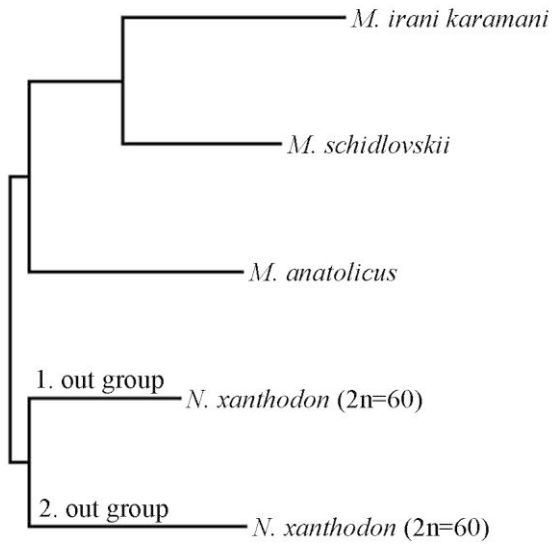

Figure 5. The neighbor-joining tree of the relationships among the studied *Microtus* species based on the distribution of AgNOR sites on individual chromosome pairs. The out groups are *Nannospalax xanthodon* (2n = 60) from Konya (Arslan et al., 2011) and Aksaray (Arslan and Bolukbas, 2010).

C-banding pattern found in all specimens examined is similar to that reported previously in *M. anatolicus* (Yavuz et al., 2009) and *M. socialis* (Zima et al., 2013). Contrary to our results, Yavuz et al. (2009) found a distinct dark C-band in the centromeric region of the Y chromosome in *M. anatolicus*.

Zima et al. (2013) reported up to 16 NOR sites (i.e. they occurred on at least eight chromosome pairs) in the karyotype of *M. socialis* from Armenia. This finding is within the extent of variation ascertained in our study, which ranged from seven to ten autosomal pairs bearing AgNOR sites. The same is also the case for the localization of the AgNOR sites, which were mainly observed in the long arms near the centromeric region of acrocentric autosomes. Albayrak et al. (2012) recorded NORs in centromeric regions of four acrocentric pairs and also studied the C-banding pattern in *M. dogramacii* (2n = 48).

Our findings demonstrate fairly distinct differentiation between the three studied species, particularly in the pattern of NOR distribution. We are aware that distinguishing of individual pairs in C-banded and AgNOR stained complements is uncertain and the identity of pairs determined according to their size is only tentative. The distribution of the AgNOR sites nevertheless clearly differed between the three species, and some of these differences were quite distinct. Some doubts were also raised concerning the reliability of the NOR distribution as a marker of phylogenetic relationships (Sánchez et al., 1990). However, other studies considered this pattern of NOR distribution as an important character to reveal divergence between various lineages (Ivanitskaya et al., 1997, 2008).

The resulting tree depicting the relationships between species cannot be directly compared to available molecular trees because different species were employed in individual analyses (Jaarola et al., 2004; Kryštufek et al., 2012; Martínková and Moravec, 2012). However, the close relationships between *M. irani* and *M. schidlovskii* were also supported in the tree proposed by Zorenko et al. (2014).

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