

Karyological study of the white tailed mole, *Parascaptor leucura*, from Myanmar

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Abstract: Conventionally and differentially stained karyotypes of the white-tailed mole, *Parascaptor leucura*, collected in northern and eastern Myanmar, were examined. The diploid number was $2n = 34$, which is rare for Asian species of the subfamily Talpinae. The karyotype comprised nine metacentric, four subtelocentric, and three acrocentric pairs and a sex pair. Therefore, the fundamental number of autosomes is $FNa = 58$. This differs from FNa of the Chinese *P. leucura*, indicating that chromosome variation may occur in this species. The Y chromosome was clearly identified as a small acrocentric chromosome. The G-band pattern of some middle-size meta-submetacentric chromosomes was identical to those of most species in the subfamilies Talpinae and Scalopinae, indicating the conservative nature of these chromosomes. One of the large metacentric pairs was clearly larger than the remainder of the karyotype complement. This marker chromosome is thought to be identical to those of Scalopinae species, although they belong to a different subfamily. *P. leucura* appears to have a unique karyotype among Asian species of talpine mole.

Key words: Assam mole, Talpinae, karyotype, chromosome, G-band, C-band

Introduction

The monotypic genus *Parascaptor* consists of the white-tailed mole, *Parascaptor leucura*, and is a talpid genus with a unique dental formula. This species is distributed from northeastern India to southwestern China through Myanmar (Hutterer, 2005). Other species have been proposed to belong to this genus, e.g., *P. leptura* and *P. grandidens* (see Stroganov, 1948), based on

their dental formulas. However, these species are now considered junior synonyms of *Scaptochirus moschatus* (see Hutterer, 2005) because dentitions of holotypes of *P. leptura* and *P. grandidens* are thought to be dental anomaly of *Scaptochirus*. The dental formula of the genus *Parascaptor* is distinct among talpid moles, i.e., $I3/3, C1/1, P3/4, M3/3 = 42$ based on a reduction in upper premolars from the ancestral formula of the genera *Talpa* and *Euroscaptor*, $I3/3, C1/1,$

P4/4, M3/3 = 44. The genus *Scaptochirus* has a more advanced formula, I3/3, C1/1, P3/3, M3/3 = 40, and Stroganov (1948) hypothesized that it was derived from the genus *Parascaptor* through a reduction in the lower premolars. Consequently, the genus *Parascaptor* is key group in the phylogeny of talpine moles.

Recent phylogenetic research has made progress based on molecular findings, but important information from karyological studies is needed (Kawada, 2006). Karyological information helps to determine species relationships based on reproductive isolation, which is sometimes achieved through chromosomal changes. The karyology of three genera of Asian talpine moles has been studied: *Euroscaptor* (Kawada *et al.*, 2001, 2005, 2006a, 2012a), *Mogera* (Kawada, 2001, 2007, 2010), and *Scaptochirus* (Kawada *et al.*, 2002a). He *et al.* (2012) listed the diploid number ($2n$) and fundamental number of autosomes (FNa) of *Parascaptor* as $2n=34$ and FNa = 64, respectively, although a detailed chromosome study and karyotype figure have not been published. Therefore, we studied conventional and differentially stained karyotypes of *P. leucura* from northern Myanmar and compared them with those of allied genera.

Materials and Methods

Two male specimens of *P. leucura* collected from two localities in Myanmar were examined. One of them (NSMT-M38354) was collected in Yikyawdi Village, Putao, Kachin State and the other (NSMT-M38401) in Pyin Oo Lwin, in the Mandalay Region. These specimens were collected in hung-type mole traps set in a rice field and a botanical garden, respectively. The details of the field work have been reported elsewhere (Kawada *et al.*, 2012b). The voucher specimens are deposited in the National Museum of Nature and Science, in Tsukuba, Japan.

NSMT-M38354 was dead upon collection. Therefore, a skin sample was stored in AmnioMAX II medium (Gibco), brought to Japan, and then cultured in the laboratory. NSMT-M38401

was collected live. Therefore, a colchicine solution was injected at $1.25\mu\text{g}$ per body weight. After 40 minutes, bone marrow cells were collected in a centrifuge tube and rinsed with hypotonic solution (0.075M KCl) for 18 minutes. The tube was then centrifuged using a handle centrifugal manipulator and modified Carnoy's solution was used for fixation. Sampled tissues for cell culture were minced and small amounts of tissue were placed in tissue flasks (Iwaki) to which 6 ml of AmnioMAX II medium (Gibco) were added. Every 7 days, half of the medium was replaced with fresh medium. After 20 days, the cells were cultivated in trypsin solution supplemented with EDTA (Gibco) and hypotonic treatment and fixation were performed in the same manner as that used for bone marrow cells. Chromosome preparations were stained with 4% Giemsa solution for conventional staining. To visualize the character of each chromosome, the ASG method of Sumner *et al.* (1971) and BSG method of Sumner (1972) were used for G- and C-banding, respectively.

The chromosomes were categorized as metacentric (M), submetacentric (ST), and acrocentric (A) based on the work of Levan *et al.* (1964).

Results

The diploid number of *P. leucura* was determined to be $2n=34$. Figure 1a shows the conventionally stained karyotype of *P. leucura*. The karyotype comprised 13 banded and 3 acrocentric pairs; therefore, the autosomal fundamental number was FNa = 58. Chromosome M1 was clearly the largest metacentric chromosome. Chromosome M2 had a secondary constriction on the proximal short arm. Chromosomes M3 – M8 were of decreasing size and M9 was the smallest in the metacentric series. Chromosomes ST1 – ST4 were large submetacentric chromosomes of similar size, so each chromosome pair was unidentifiable. The karyotype included three acrocentric pairs, which were large, medium-sized, and small chromosomes, respectively.

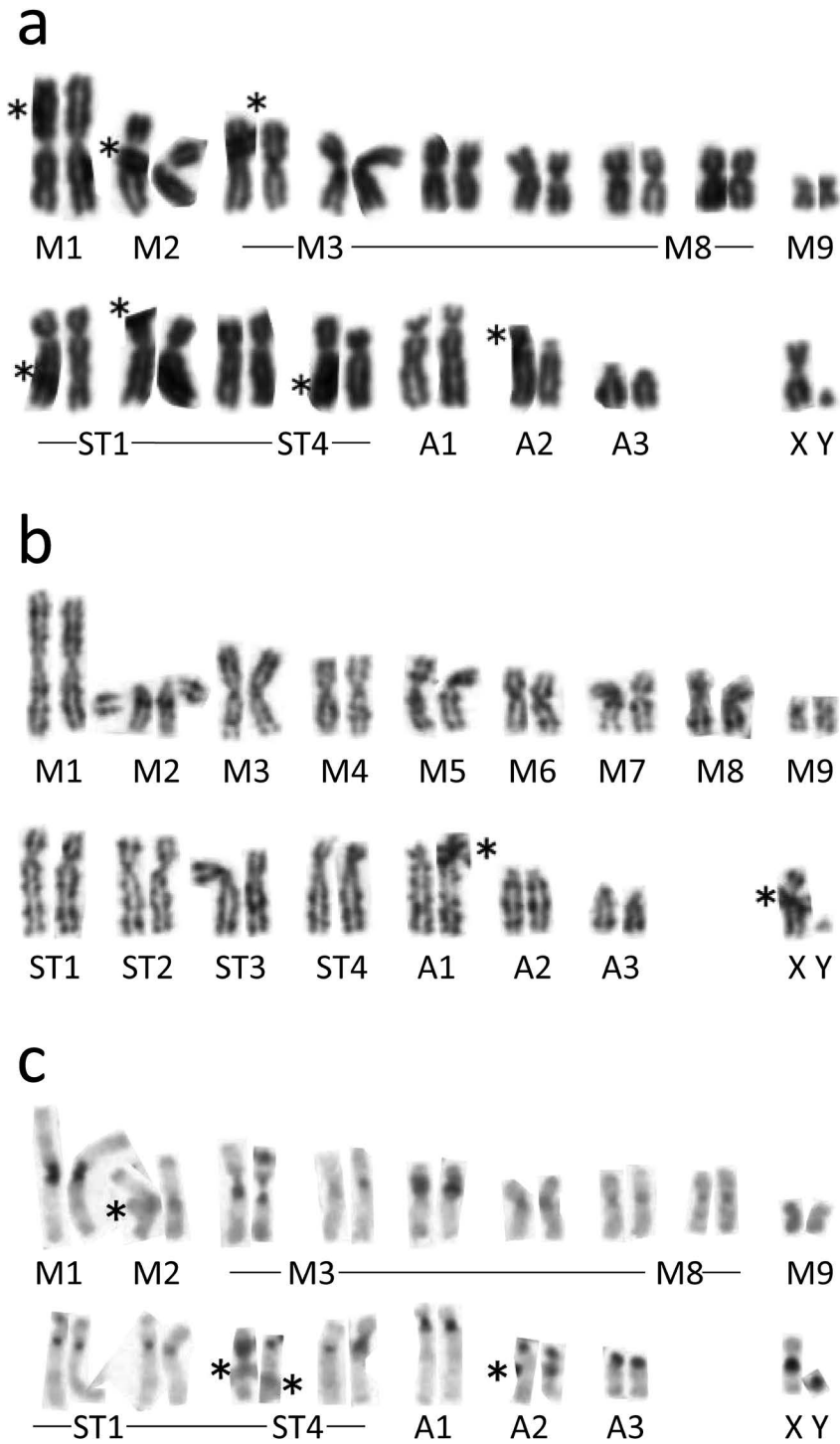


Fig. 1. Conventional (a), G-band (b) and C-band (c) karyotypes of a male *Parascaptor leucura*. Asterisks indicate crossings of chromosomes.

Chromosome A1 was a large, biarmed chromosome with a very short short-arm. The sex chromosomes consisted of a medium-sized metacentric X, which was similar in size to M5 to M8, and a minute Y chromosome. The Y chromosome had an acrocentric shape.

Figure 1b shows the G-banded karyotype of a male *P. leucura*. Each pair had a distinct G-banding pattern and homologs were identified. Figure 1c shows the C-banded karyotype. Generally, centromeric C-bands were seen in all chromosomes. Additional positive bands in chromosome M5 were seen as a rather faint region. The Y chromosome stained positively with the C-band procedure.

Discussion

The diploid and fundamental numbers of autosomes of *P. leucura* from Myanmar were $2n = 34$ and $FNa = 58$. He *et al.* (2012) reported the same diploid number, but $FNa = 64$. Although He *et al.* (2012) did not include a detailed karyological description of this species, $FNa = 64$ means that all of the autosomes were biarmed chromosomes. In our karyotypes, the largest acrocentric pair, A1, had a rather large short arm, which might be considered a biarmed submetacentric, while the medium-sized and small pairs, A2 and A3, were clearly acrocentric. These findings suggest that karyological variation exists in this species. Although He *et al.* (2012) did not mention the locality of their *P. leucura* with a different FNa number, perhaps Myanmar specimens have a distinct karyotype from those collected in China. Based on a morphological comparison of mandibles by He *et al.* (2013), this species can be divided into three groups: northeastern Yunnan, southern Hengduan Mountain, and Indian groups. Our samples were collected in the region between the last two groups. Furthermore, molecular phylogenetic findings indicated that the monotypic genus *Parascaptor* is highly variable and at least two species were recognized in Yunnan Province, China, including one cryptic species (He *et al.*, 2014). It is believed that this species has split into several species or subspecies and the karyological

variation between previous studies and our results may support this idea.

The karyotypes shown in Fig. 1 confirmed several uniformities of Talpidae chromosomes. Chromosome M2 had an obvious secondary constriction at the proximal short arm, which is shared in all talpid species except subfamily Uropsilinae (Kawada *et al.*, 2006b) and genus *Neurotrichus* (Kawada *et al.*, 2008a). Similarly, the chromosome pairs M3, M5, M6, M7, M8, and M9 of *P. leucura* share homologies with chromosomes 1, 2, 3, 6, 4, and 7 of Japanese moles in the genus *Mogera*, respectively, based on the G-band comparison between this study and Kawada *et al.*, (2001). These chromosome pairs also correspond to 2, 8, 9, 10, 12, and 16 in three shrew mole genera, *Dymecodon*, *Scaptonyx*, and *Urotrichus* (Kawada and Obara, 1999; Kawada *et al.*, 2008a), and with 6, 8, 9, 10, 11, and 12 in the European talpine species *Talpa altaica* (Kawada *et al.*, 2002b). These biarmed elements, together with the X chromosome, are thought to be conserved in talpid karyotype evolution.

This species also showed karyological peculiarities. The submetacentric series is confined to this species, for which partial G-band homologies can be estimated. A karyotype with only three acrocentric pairs is unusual in Asian moles. The Y chromosome was clearly acrocentric, while most talpid species have a dot-like Y, in which both arms are unidentifiable. Two more peculiar characters of this species are its diploid number of $2n = 34$ and the one large metacentric chromosome pair M1, which is by far the largest in the complement.

As mentioned, the diploid number $2n = 34$ is rare in Asian talpine moles. The fossorial group of the family Talpidae is classified in the subfamily Talpinae, true moles inhabiting Eurasia, and subfamily Scalopinae, shrew-moles and North American true moles. Shrew-moles are semifossorial species with rather long tails and small paws, showing less adaptation to a tunneling lifestyle than true moles. Previous karyological studies have indicated that shrew-moles in the genera

Dymecodon, *Scaptonyx*, and *Urotrichus* are related to the North American moles in the genera *Scalopus* and *Scapanus*, based on the consistent diploid number $2n = 34$ and the shared character of the largest metacentric chromosome (Kawada *et al.*, 2008a). In addition, most species of European talpine mole, *e.g.*, *Talpa europaea*, *T. romana*, *T. stankovici*, and *T. occidentalis*, have $2n = 34$ (Dzuev, 1982). The previous finding of $2n = 34$ in Asian talpine moles was confined to *Euroscaptor longirostris* (Kawada *et al.*, 2008b), though only a Giemsa-stained karyotype was shown.

Considering the second peculiarity, it is interesting that the karyotype of *P. leucura* included a large M1, which had been considered a good marker of the talpid karyological profile. This chromosome is a common character in the subfamily Scalopinae, *i.e.*, shrew-moles and North American moles (Yates and Schmidly, 1975; Yates *et al.*, 1976; Kawada and Obara, 1999). One large metacentric pair has also been found in the karyotypes of *Mogera insularis* and *Euroscaptor malayana*, but the diploid number of the former species is $2n = 32$, which is different from that of *P. leucura* (Lin *et al.*, 2002), and the shape of this chromosome in the latter species is submetacentric with a unique G-band pattern (Kawada *et al.*, 2005). Therefore, it is obvious that *P. leucura* has several distinct karyological features among Asian mole species.

To further assess the karyological relationships, it is valuable to mention the previous controversy regarding this largest chromosome. In the European genus *Talpa*, only one species, *T. altaica*, possesses this characteristic metacentric pair in its karyotype, which is a similar karyological profile to that of *P. leucura*. Based on this character, Kratochvil and Král (1972) suggested that the *T. altaica* karyotype is closely related to that of the scalopine mole *Parascalops breweri*. As mentioned above, all North American scalopine moles have a diploid number of $2n = 34$, including one much larger metacentric pair (Yates and Moore, 1990). Kawada *et al.* (2002b) debated whether the karyotype of *T. altaica* was

analogically similar to that of North American species and acquired a large metacentric pair through heterochromatic duplication in the short arm of one acrocentric pair from another species of European *Talpa*. It is evident in the C-band karyotype of *T. altaica* that the short arm of the largest metacentric was deeply stained (Kawada *et al.*, 2002b).

The C-band appearance of *P. leucura* comprises ordinal centromeric C-bands without a whole arm C-block (Fig. 1c). These characters differ significantly from those of the C-band karyotype of *T. altaica*. These two talpine species share the same $2n$ value and have similar karyological profiles, but it is believed that they have different karyological histories. The largest metacentric of *P. leucura* had a euchromatic short arm and the G-band pattern of this chromosome is identical to that of scalopine moles from North America and shrew-moles in the genera *Dymecodon*, *Scaptonyx*, and *Urotrichus* (Kawada and Obara, 1999; Kawada *et al.* 2008a). If this chromosome in these different taxa is really derived from a common ancestor, then the genus *Parascaptor* should possess ancestral characters of the subfamily Talpinae.

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