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# Comparative chromosome banding of two South-American species of rice rats of the genus *Oligoryzomys* (Rodentia, Sigmodontinae)

V. M. Aniskin[1] & V. T. Volobouev[2,3]*
[1]*Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, Moscow, 117071 Russia;*
[2]*Laboratoire de Zoologie, Mammifères et Oiseaux, Muséum National d'Histoire Naturelle, 55, rue Buffon, 75005 Paris, France; Tel: 331 40 79 30 65; Fax: 331 40 79 30 63; E-mail: vitaly@mnhn.fr;* [3]*Cytogénétique Moléculaire et Oncologie, Institut Curie, Section de Biologie, Paris, France*
* *Correspondence*

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**Abstract**

Comparative analysis of G- and C-banding patterns in two species of pygmy rice rats, namely *Oligoryzomys microtis* from Peru (Ucayali and Loreto departments) and *O. flavescens* from Bolivia (Tarija department) established that the diploid number of the former species is 64 (NFa = 66), whereas, in the latter, it varies between 64 and 66 (NFa = 66–68) due to the presence of 0–2 heterochromatic supernumerary or B chromosomes. The G-banding pattern of the euchromatic part of their karyotypes is similar in spite of differences in morphology of the largest and smallest autosomal pairs caused by a centromeric shift and the presence of heterochromatic arms, respectively. In addition, the total quantity of C-heterochromatin is smaller in the karyotype of *O. microtis* than in that of *O. flavescens*, resulting in differences in the number and size of chromosome pairs (including sex chromosomes) bearing C-blocks. It follows from present and previous data that these karyotypic features are stable in each of these species and thus may be used as species-specific markers.

## Introduction

Pygmy rice rats of the genus *Oligoryzomys* are an important component of the Neotropical fauna. The systematics of this genus is difficult owing to the morphological homogeneity of its species, the number of which may vary from one to 30 depending on taxonomists (Hershkovitz 1966, Tate 1932 cited by Musser & Carleton 1993). The use of karyological data for species discrimination turned out to be very useful in this group, and until now the karyotypes of 10 of the 15 recognized species of *Oligoryzomys* (Musser & Carleton 1993) have been described (Brum-Zorilla *et al.* 1988, Espinosa & Reig 1991, Gallardo & Palma 1990, Kasahara & Yonenaga-Yassuda 1984, Yonenaga-Yassuda *et al.* 1976). Although most of the karyotypes appeared invariant and species specific, some of the species share quite similar karyotypic characteristics and display intraspecific variation in diploid number (2n) and chromosome morphology. This is the case of *O. microtis* characterized by a diploid number of 64 and a number of autosomal arms (NFa) of 66 (Gardner & Patton 1976), and of *O. flavescens* with 2n and NFa varying between 64 and 66 and 66 and 68, respecitvely. As revealed by chromosome banding, the variation in both 2n and NFa in the latter species is caused by the presence of 0–2 supernumerary or B chromosomes

(Bs) (Brum-Zorilla *et al.* 1988, Espinosa & Reig 1991, Sbalqueiro *et al.* 1991). However, the Bs notwithstanding, these karyotypes appeared morphologically different after conventional Giemsa staining in spite of a similar 2n and NFa. As chromosome banding data were available for only one of the species, we carried out a comparative chromosome banding analysis (G- and C-bands) of both *O. microtis* and *O. flavescens* to quantify the level of karyotypic divergence and the nature of chromosomal changes which have occured between these two species of *Oligoryzomys*.

## Materials and methods

Specimens of pygmy rice rats of the species *Oligoryzomys microtis* Allen 1916 and *O. flavescens* Waterhouse 1837 were caught and karyotyped during field trips in Peru and Bolivia between 1988 and 1993. Twenty-five specimens of the former species were captured in Peru, departments of Ucayali (08°22′ S, 74°31′ W: 11 females and 12 males) and Loreto (04°51′ S, 73°39′ W: 2 males). Eight specimens (3 females and 5 males) of *O. flavescens* came from Bolivia, Tarija department, (21°30′ S, 64°25′ W). Chromosome analysis was performed from bone marrow preparations by standard colchicine methods. The chromosomes of all specimens were studied by G- (Seabright 1971), and C-banding (Sumner 1972). At least twenty metaphases were analysed for each specimen.

## Results

The diploid number of all the *O. microtis* specimens studied is 64 and the NFa is 66 (Figure 1a). One pair of large and one pair of small metacentric autosomes are present as well as 29 pairs of acrocentric autosomes gradually decreasing in size from intermediate to small. The X chromosome is a middle-sized subtelocentric characterized by size variation of its short arm and the Y chromosome is a small metacentric.

The diploid number in *O. flavescens* varies between 64 and 66 owing to the presence of 0–2 Bs (Figure 2a). Among the eight specimens studied, three possessed 2 Bs, two 1 B and three no Bs. This variation in B chromosome number resulted in a NFa varying between 66 and 68. The karyotype of this species basically comprises two pairs of small metacentric chromosomes (1 and 2), one pair of large (pair 3) and 28 pairs of intermediate to small (pairs 4 to 32) acrocentric autosomes. The X chromosome is submetacentric and characterized by size variation of its short arm. The Y chromosome is a small metacentric similar in size to that of *O. microtis*.

It thus appears that, the Bs of *O. flavescens* aside, both species share the same diploid and fundamental numbers while showing clear differences in the morphology of several chromosomes. In particular, the biarmed chromosomes of *O. flavescens* include two small metacentric pairs, whereas, in *O. mictrotis*, they comprise one large and one small metacentric pair. Acrocentric chromosomes in *O. microtis* form a gradual series of decreasing size, whereas, in *O. flavescens*, one acrocentric pair is much larger than the others, being the largest of the whole chromosomal set.

The G-banding technique allowed the unambiguous identification of all chromosome pairs in both species and the establishment of the nature of the differences revealed by the standard chromosomal analysis (Figures 1b & 2b). The comparative analysis showed that the largest pairs of automsomes in both karyotypes (metacentric in *O. microtis* and acrocentric in *O. flavescens*) share an identical banding pattern (Figure 3). This suggests that both are the result of a tandem fusion involving the same ancestral chromosomes, followed by the inactivation of a different centromere in each species. The banding pattern of acrocentric chromosome 31 of *O. microtis* corresponds to the long arm of chromosome 2 in *O. flavescens*, the short arm of which is heterochromatic. All the other chromosome pairs show identical banding patterns as well as morphology in the two species.

After C-banding, all autosomes in *O. flavescens* display pericentric blocks of C-heterochromatin, and several pairs show entirely heterochromatic short arms. The short arm of the X chromosome is also entirely heterochromatic, with interindividual size variation. The Y chromosome has a C-band positive block at the distal part of its long arm. The Bs are entirely C-band positive. In *O. microtis*, only moderate blocks of centromeric C-heterochromatin are detected in most pairs of autosomes and on the short arm of the X chromosome. The Y chromosome is entirely C-band positive. Six pairs of autosomes

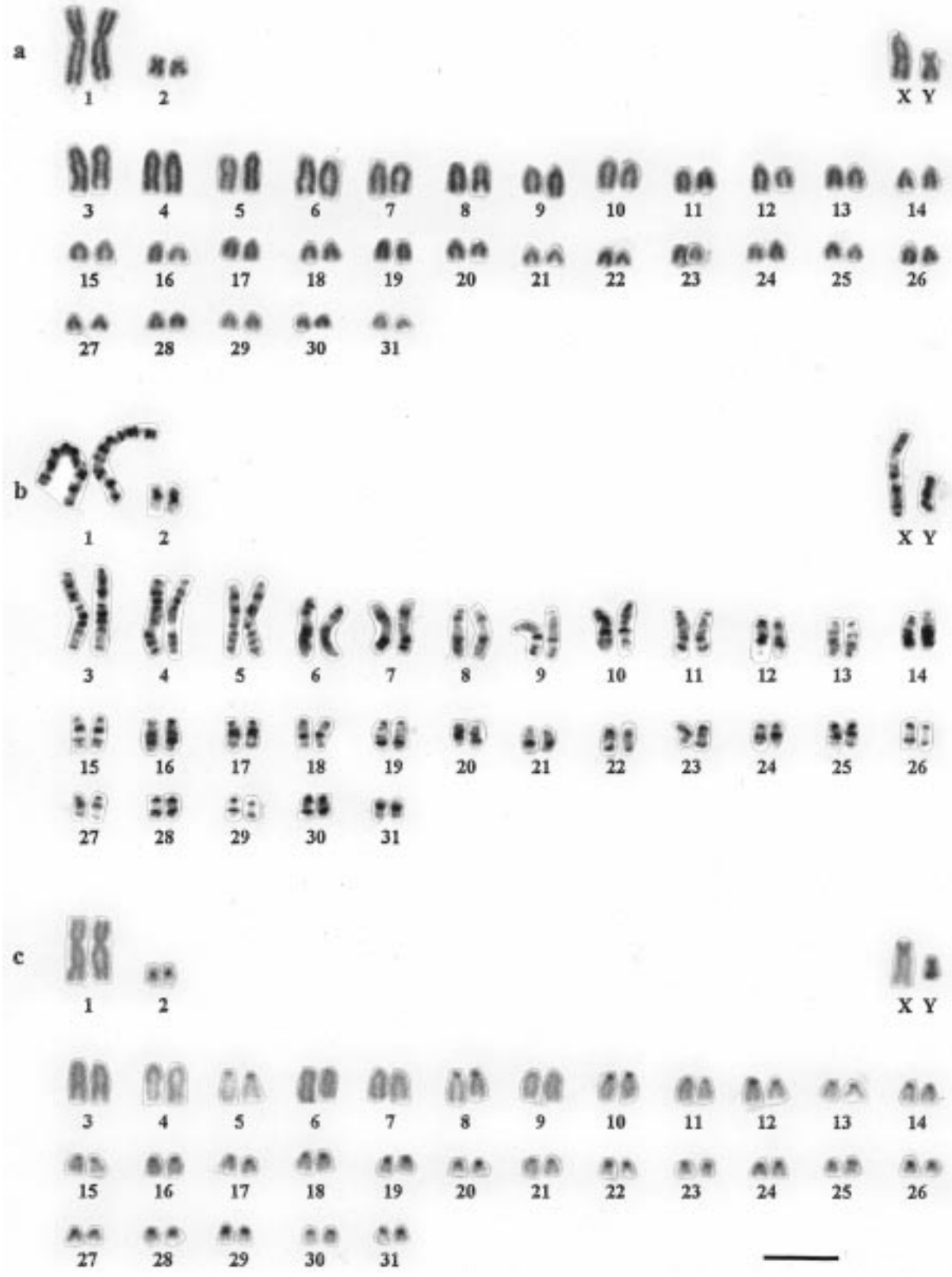


*Figure 1.* Karyotype of *Oligoryzomys microtis*. (**a**) Standard Giemsa staining. (**b**) G-banding. (**c**) C-banding. Scale bar represents 10 $\mu$m.

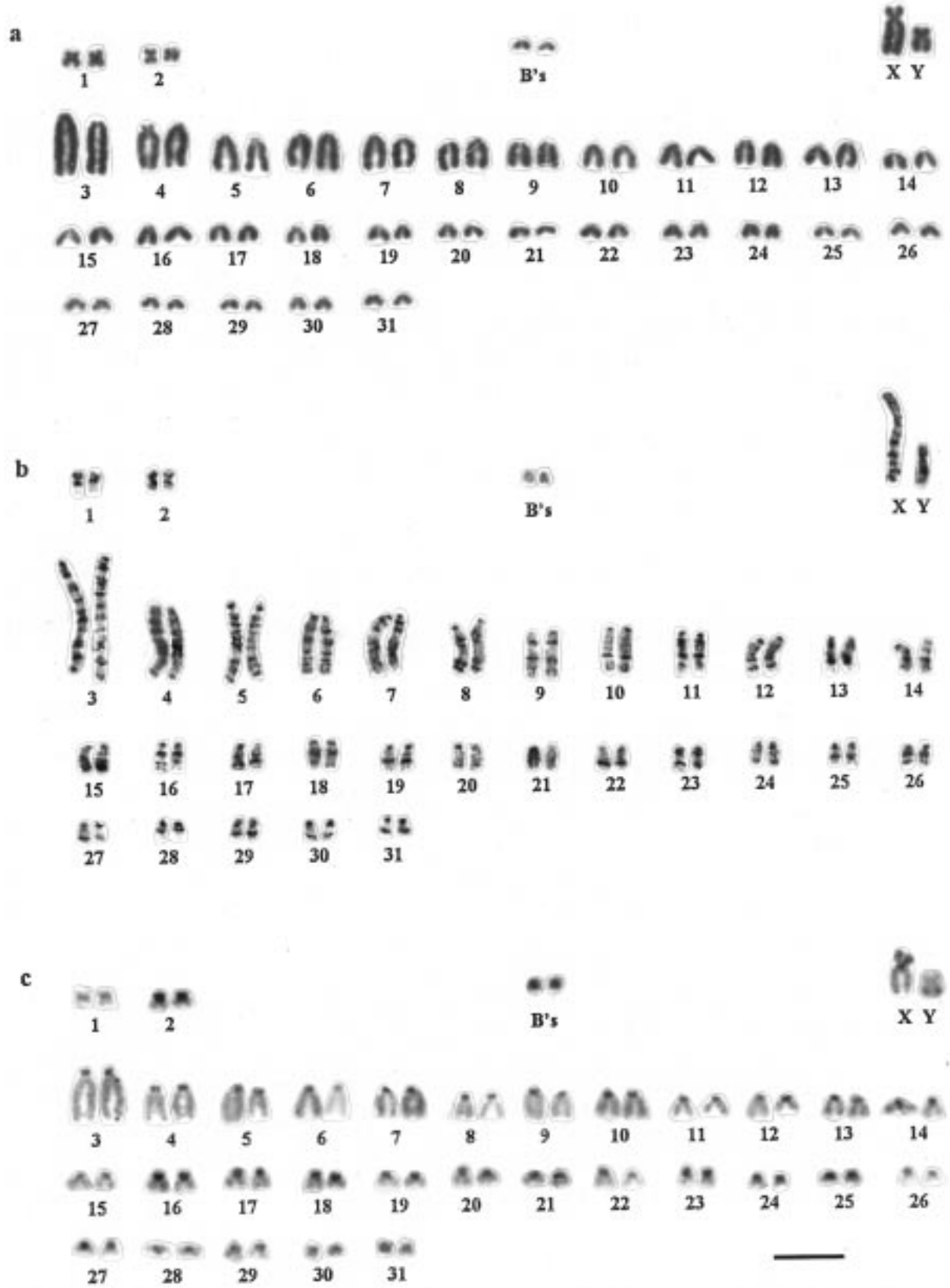


*Figure 2.* Karyotype of *Oligoryzomys flavescens*. (**a**) Standard Giemsa staining. (**b**) G-banding. (**c**) C-banding. Scale bar represents 10 $\mu$m.

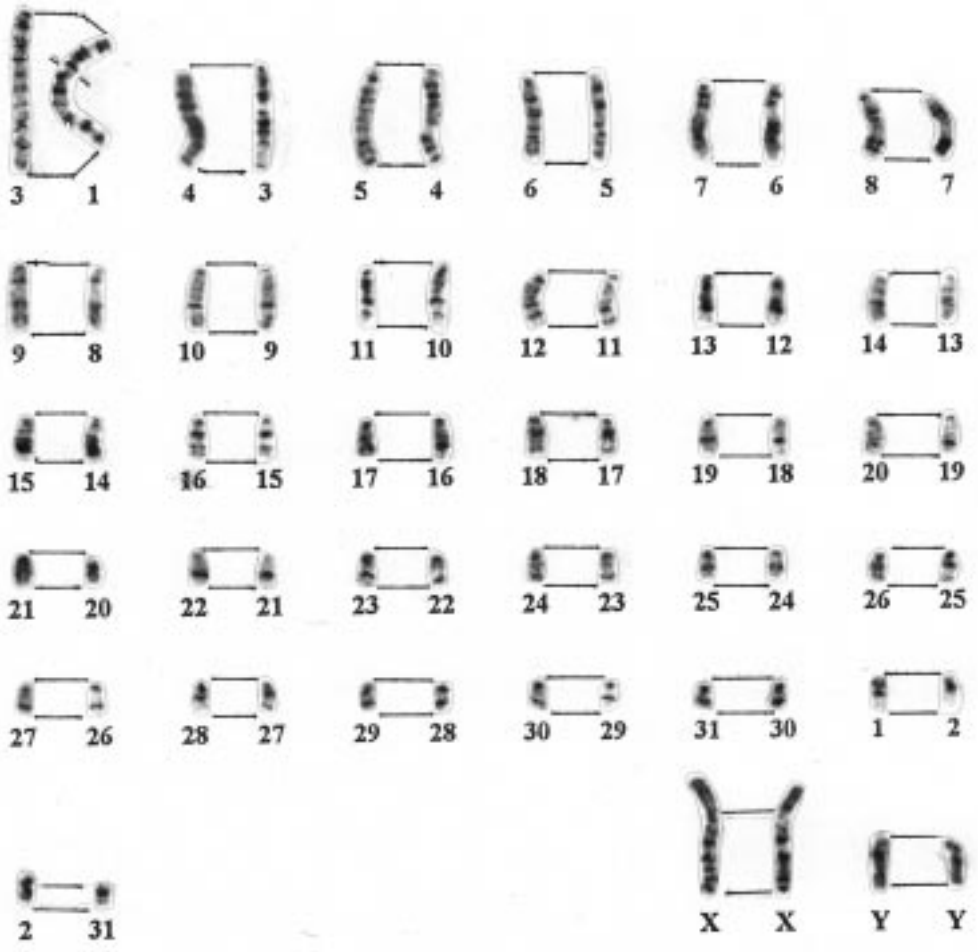


*Figure 3.* Comparison of G-banded chromosomes of *Oligoryzomys flavescens* (left) and *O. microtis* (right).

(pairs 1, 3, 4, 6, 7 and 9) show no centromeric heterochromatin (Figures 1c & 2c).

## Discussion

Previous data on the karyotype of *O. flavescens* came from populations from Uruguay, Argentina and Brazil (Brum-Zorilla *et al.* 1988, Espinosa & Reig 1991, Sbalqueiro *et al.* 1991). These studies all showed a karyotype structure and a variation of the X and B chromosomes similar to those in the present study. Besides, a karyotype identical to that of *O. flavescens* was found in specimens initially referred to as *Oryzomys* sp. from Brazil (Yonenaga-Yassuda *et al.* 1976, Kasahara & Yonenaga-Yassuda 1984) and *Oryzomys fornesi* from Paraguay (Myers & Carleton 1981). The karyotypic identity and the similar nature of their chromosome variation allowed Sbalqueiro *et al.* (1991) to consider *Oryzomys* sp., *O. fornesi* and *O. flavescens* as belonging to the same species with a distribution area including Paraguay, south and south-west Brazil, Uruguay and Argentina. The present study supports this conclusion and adds Bolivia to the distribution area of *O. flavescens*. It must be noted that the above-mentioned conclusion of Sbalquiero *et al.* (1991) is cited by Musser & Carleton (1993) when characterizing *O. flavescens*. However, these authors also used the karyotypic data on *O. fornesi* (Myers & Carelton 1981) to characterize *O. microtis*, considering *fornesi* as its synonym. Such an evident discrepancy needs to be eliminated in a future edition of *Mammal Species of the World*.

The standard karyotype of *O. microtis* was pre-

viously described in Peruvian populations (Loreto and Ayacucho departments) under the name *O. longicaudatus* 'variant 2' but later referred to as *O. microtis* (Musser & Carleton 1993). With the exception of the morphology of the Y chromosome (small metacentric here vs. small acrocentric in Gardner & Patton 1976), the present karyotype description is similar to that of the latter authors and thus allows us to include a new locality (Ucayali department) in the geographical distribution of *O. microtis*.

The present study shows that the karyotypes of *O. microtis* and *O. flavescens* differ from each other by the presence of Bs, the morphology of two autosomal pairs resulting from a centromeric shift and heterochromatic arm addition respectively, and finally by the quantity and distribution pattern of C-heterochromatin. These data as well as previous ones suggest that these chromosomal features seem to be stable within each species and thus may be used as species-specific markers in further studies on the distribution limits on these species.

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