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A karyological study of four European species of *Roncus* (Pseudoscorpiones: Neobisiidae)

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Abstract. We present the results of the first study on the karyotypes of four European species of *Roncus*: *Roncus alpinus* L. Koch, 1873, *Roncus lubricus* L. Koch, 1873, *Roncus transsilvanicus* Beier, 1928 and *Roncus* sp. The diploid number was 2n = 23 in *Roncus* sp., 2n = 43 in *R. alpinus* and *R. transsilvanicus* and 2n = 45 in *R. lubricus*. Telocentric autosomes predominate in species with a high chromosome number and metacentric autosomes in *Roncus* sp. We assume that the ancestral situation for this genus is a high number of chromosomes. A low number of chromosomes is very likely a consequence of centric fusions, which have possibly played a very important role in karyotype evolution in the genus *Roncus*. All the species analyzed have the X0 sex chromosome system. The X chromosome is metacentric and is the smallest element in the karyotypes of all the species analyzed.

INTRODUCTION

Pseudoscorpions are an ancient phylogenetic lineage of arachnids that had developed most of their typical morphological features by the Middle Devonian (Schawaller et al., 1991). With the currently recognized 25 families and more than 3500 described species they represent the fourth largest order of arachnids (Harvey, 2011). Despite this diversity, we have still only fragmentary information about the cytogenetics and karyotype evolution in this group. Currently there are karyotype data for 49 species from 8 families (Šťáhlavský, 2013) and it is evident that there is considerable variation in number of diploid chromosomes in pseudoscorpions ranging from 7 in males of *Olpium turcicum* and *Indolpium* sp. (Olpiidae) (Šťáhlavský et al., 2006) to 143 in males of *Cyclatemnus* sp. (Šťáhlavský et al., 2012). Centric fusions are proposed as important in the karyotype evolution of many groups (e.g. Olpiidae, Neobisiidae) (Troiano, 1990; Šťáhlavský et al., 2006) but tandem fusions and pericentric inversions are also thought to have been important in the differentiation of karyotypes within the family Chthoniidae (Šťáhlavský et al., 2004). The karyology of the genus *Roncus* (Neobisiidae) is better known than that of other pseudoscorpion genera, with published data available for eight species (Table 1) that seem to reveal significant interspecific variability. The diploid numbers of chromosomes range from 16 in *R. cadinensis* (Zaragoza & Šťáhlavský, 2008) to 52 in *R. andreinii* Caporiacco, 1925 (Troiano, 1990). Additionally, the karyotypes of the genus *Roncus* differ not only in chromosome number and morphology, but also in the sex chromosome systems with three species having an XY system and the other five an X0 system (e.g. Troiano, 1990, 1997; Zaragoza & Šťáhlavský, 2008) (Table 1). This genus of pseudoscorpions includes 138 named species and is endemic to Europe, North Africa and the Caucasus region (Harvey, 2011). They primarily occur in leaf litter and soil (Gabbutt & Vachon, 1967), as well as in caves. Until now, the only species of *Roncus* for which the karyology is known originated from north-eastern Spain (Zaragoza & Šťáhlavský, 2008) and north-western Italy (Troiano, 1990, 1997). To complement and extend these studies, we add data for four additional species collected from north-western and central Europe, bringing the total number of *Roncus* species for which the karyology is known to 12. The aim of this study is to add more information about *Roncus* chromosomes from other European regions, which will help us to better understand the overall diversity and karyotype evolution in these arachnids.

MATERIAL AND METHODS

Material for karyological study

The specimens of *Roncus* used in this study were collected from under stones or in caves (Belgium) or sifted from leaf litter (elsewhere). Voucher specimens are deposited in the Department of Zoology, Charles University in Prague. The nomenclature for taxa follows Harvey (2011). The collection details are presented below (all specimens were collected by the first author, unless otherwise stated):

Roncus alpinus L. Koch, 1873: Italy: Castelo Toblino, 46.06°N, 10.97°E (5♂: 9.vi.2007; 1♂: 30.x.2011); Italy: Egna, 46.31°N, 11.29°E (2♂: 22.viii.2008); Italy: Nuova Olonio 46.16°N, 9.43°E (4♂: 23.viii.2008), Italy: Aprica, 46.16°N, 10.19°E (4♂: 31.viii.2008).

TABLE 1. Summary of the cytogenetic data for the genus *Roncus*.

Species	Location	2n ♂/♀	Sex chrom.	Morphology of chromosomes autosomes / sex chromosomes	References
R. andreinii (Caporiacco, 1925)	Italy	51/52	X0	2 sm, 48 a / X m	Troiano, 1990
R. alpinus L. Koch, 1873	Italy	43/44	X0	4 m, 4 sm, 12 st, 22 a / X m	present study
R. belluatii Gardini, 1992	Italy	22/–	XY	m, sm / unknown	Troiano, 1997
R. binaghii Gardini, 1991	Italy	25/26	X0	24 m / X sm	Troiano, 1990
R. cadinensis Zaragoza, 2007	Spain	38/–	XY	2 m, 2 sm, 32 a / X m, Y m	Zaragoza & Šťáhlavský, 2008
R. gestroi Beier, 1930	Italy	43/–	X0	m, sm, st, a / X m	Troiano, 1997
R. ingaunus Gardini, 1991	Italy	24/24	XY	20 m, 2 sm / X m, Y m	Troiano, 1990
R. lubricus L. Koch, 1873	CZ, B, UK	45/46	X0	4 m, 2 sm, 14 st, 24 a / X m	present study
R. montsenyensis Zaragoza & Šťáhlavský, 2008	Spain	16/16	XY	12 m, 2 sm / X m, Y m	Zaragoza & Šťáhlavský, 2008
Roncus sp.	Slovakia	23/24	X0	18 m, 4 sm / X m	present study
R. transsilvanicus Beier, 1928	Romania	43/44	X0	2 m, 2 sm, 10 st, 28 a / X m	present study
R. tuberculatus Gardini, 1991	Italy	40/40	XY	6 m, 2 sm, 4 st, 26 a / X sm, Y a	Troiano, 1990

Abbreviations: a – acrocentric; B – Belgium; CZ – Czech Republic; UK – United Kingdom; m – metacentric; sm – submetacentric; st – subtelocentric.

Roncus lubricus L. Koch, 1873: Czech Republic: Prague – Uhříněves, 50.04°N, 14.60°E (3♂: 28.iv.2004; 1♂: 26.vi.2004; 2♂: 15.v.2005); Belgium: Tilf, 50.56°N, 5.58°E (2♂: 30.iii.2006, lgt. H. Henderickx); Belgium: Engis, 50.58°N, 5.41°E (2♂, 1♀: 13.ii.2006, lgt. H. Henderickx); United Kingdom, South Wales – Mid. Glamorgan, 51.52°N, 3.51°W (1♂: 29.iv.2002, lgt. S.C. Warmingham; 2♂: 18.v.2004, lgt. S.C. Warmingham).

Roncus transsilvanicus Beier, 1928: Romania: Retezat Mts, Gura Zlata, 45.37°N 22.76°E (3♂: 11.vi.2006); Romania: Retezat Mts, Cheile Butii, 45.30°N, 22.98°E, (4♂: 26.viii.2004; 2♂, 1♀: 7.vii.2006; 1♂: 1.x.2007; 2♂: 3.x.2008); Romania: Fagaras Mts, 45.42°N, 24.63°E (1♂: 28.ix.2007).

Roncus sp.: Slovak Republic: Kováčov, 47.83°N, 18.76°E (6♂, 1♀: 5.vii.2001; 4♂, 2♀: 14.iv.2002; 2♂: 24.vi.2004; 2♂: 4.v.2005; 2♂: 22.iv.2006).

Chromosome preparations

The chromosome preparations were made from gonads using the spreading technique described in Šťáhlavský & Král (2004). Briefly, the gonads were dissected and placed in a hypotonic solution of 0.075 M KCl for 15 min and then fixed in a fresh solution of glacial acetic acid : ethanol (1 : 3) for at least 20 min. The fixed material was then transferred to a drop of 60% acetic acid on a clean microscope slide using a tungsten needle. The slide was placed on a warm histological plate (temperature 40–45°C) and the drop then spread on the slide with the aid of a needle until it evaporated. The chromosome preparations were stained in a 5% Giemsa solution in Sörensen phosphate buffer (4.75 g $Na_2 HPO_4 \cdot 12 H_2O$ and 4.54 g KH_2PO_4 in 1,000 ml H_2O, pH = 6.8) for 35 min.

The chromosomes were photographed using a Jenaval microscope and Kodak Technical Panfilm and then digitized using a Nikon Super Coolscan 5000 ED scanner. The chromosome morphology was classified according to Levan et al. (1964) and the measurements of the chromosomes were taken from photographs using the software ImageJ 1.45 (http://imagej.nih.gov/ij) with the Levan plugin (Sakamoto & Zacaro, 2009). The sister metaphase II cells with well-marked centromeres were used to obtain measurements of chromosomal arms and assemble the karyograms. At least ten measurements were used for the characterization of the karyotype in all species. We calculated the relative chromosome length as a percentage of the total length of the diploid set, including the sex chromosome.

RESULTS

Roncus alpinus

The male diploid complement comprises 43 chromosomes in all four populations (Fig. 1a). The male karyotype of specimens from Castelo Toblino comprises two metacentric (Nos 12, 20), two submetacentric (Nos 16, 21), six subtelocentric (Nos 2, 5, 9, 14, 17, 19) and eleven telocentric pairs of autosomes. The autosomes gradually decrease in size from 3.76% to 1.10% of the diploid set. The sex chromosome X is metacentric (arm ratio 1.26) and it is the smallest chromosome within the karyotype (0.79% of the diploid set). Similar characteristics were also recorded for the specimens of *R. alpinus* collected from other Italian localities – Egna and Aprica.

The odd diploid number of males indicates that the sex chromosome system is X0. In meiosis from leptotene the sex chromosome is clearly visible as a knob showing positive heteropycnosis (Fig. 3a). Starting from the diplotene phase, the structure of the X chromosome is isopycnotic and during diplotene easily recognized as a small univalent (Fig. 3b). In the following metaphase II (Fig. 1a) and anaphase II (Fig. 3c) the X chromosome is still isopycnotic. Low chiasma frequency was recorded in this species. Predominance of bivalents with one chiasma (81% cases) was observed at diplotene (N = 59). Rarely, one or two bivalents with two chiasmata were observed. The mean chiasma frequency was 21.31 ± 0.68 SD per cell, which equates to 1.02 ± 0.03 SD per bivalent.

Roncus lubricus

The male diploid complement of specimens of *R. lubricus* from all three locations comprised 45 chromosomes. The karyotype based on sister plates of metaphase II from the Czech Republic (Fig. 1b) includes two pairs of metacentric (No. 18, 22), one pair of submetacentric

Fig. 1. Male karyotypes of *Roncus* (based on sister metaphase II plates). (a) *R. alpinus* (2n = 43, X0); (b) *R. lubricus* (2n = 45, X0). Bars = 10 µm.

(No. 20), seven pairs of subtelocentric (No. 1, 2, 11, 12, 14, 17, 21) and twelve pairs of acrocentric autosomes (all other pairs). The autosomes of pair No. 1 are the largest elements in the karyotype. Their size is 5.03% of the diploid set. The following autosomes (pairs No. 2 – No. 20) gradually decrease in size from 3.70% to 1.16% of the diploid set. The last two pairs of autosomes are small (0.83% and 0.67%). The sex chromosome X is metacentric (arm ratio 1.61) and is the smallest element in the karyotype (0.6% of the diploid set). A similar characteristic was recorded for the population from the United Kingdom, over 1,200 km away.

The diploid number of chromosomes is 45 in males and 46 in females (Fig. 3d). Additionally, one univalent was found in late metaphase I (Fig. 3f) and a different number of chromosomes in the cells of the sister metaphase II (22 and 23). The combination of these factors indicates that the males of this species have an X0 sex chromosome system. As in the previous species, the X chromosome is positively heteropycnotic during leptotene, zygotene (Fig. 3e) and pachytene, but starts to be isopycnotic from diplotene. We recorded a low chiasma frequency despite almost half of the observed diplotene, diakinesis and metaphase I included one or two bivalents with two chiasmata (Fig. 3f). The mean chiasma frequency was 22.55 ± 0.69 SD per cell, which is equivalent to 1.02 ± 0.03 SD per bivalent (total N = 36).

Roncus transsilvanicus

The diploid chromosome number is 43 in males (Fig. 2a) and 44 in females (Fig. 3g). The male karyotype based on sister plates of metaphase II of specimens from the Retezat Mts contains one pair of metacentric (No. 10), one pair of submetacentric (No. 6), five pairs of subtelocentric (Nos 9, 14, 16, 20, 21) and 14 pairs of acrocentric (Nos 6, 23, 26, 32, 33, 34) autosomes (Fig. 2a). Autosomes gradually decreased in size from 3.80% to 1.15% of the diploid set. This species has an X0 sex chromosome system. The sex chromosome X is metacentric (arm ratio 1.60) and is the smallest element in the karyotype (1.43% of the diploid set). The X chromosome is positively heteropycnotic during early prophase (leptotene, zygotene, pachytene) (e.g. Fig. 3h) but starts to become isopycnotic at diplotene (Fig. 3i) and stays this way until the end of meiosis. A similar low chiasma frequency as in *R. alpinus* was recorded. Bivalents with one chiasma (81% cases, total N = 69) also predominate in *R. transsilvanicus*. A bivalent with two chiasmata was rarely

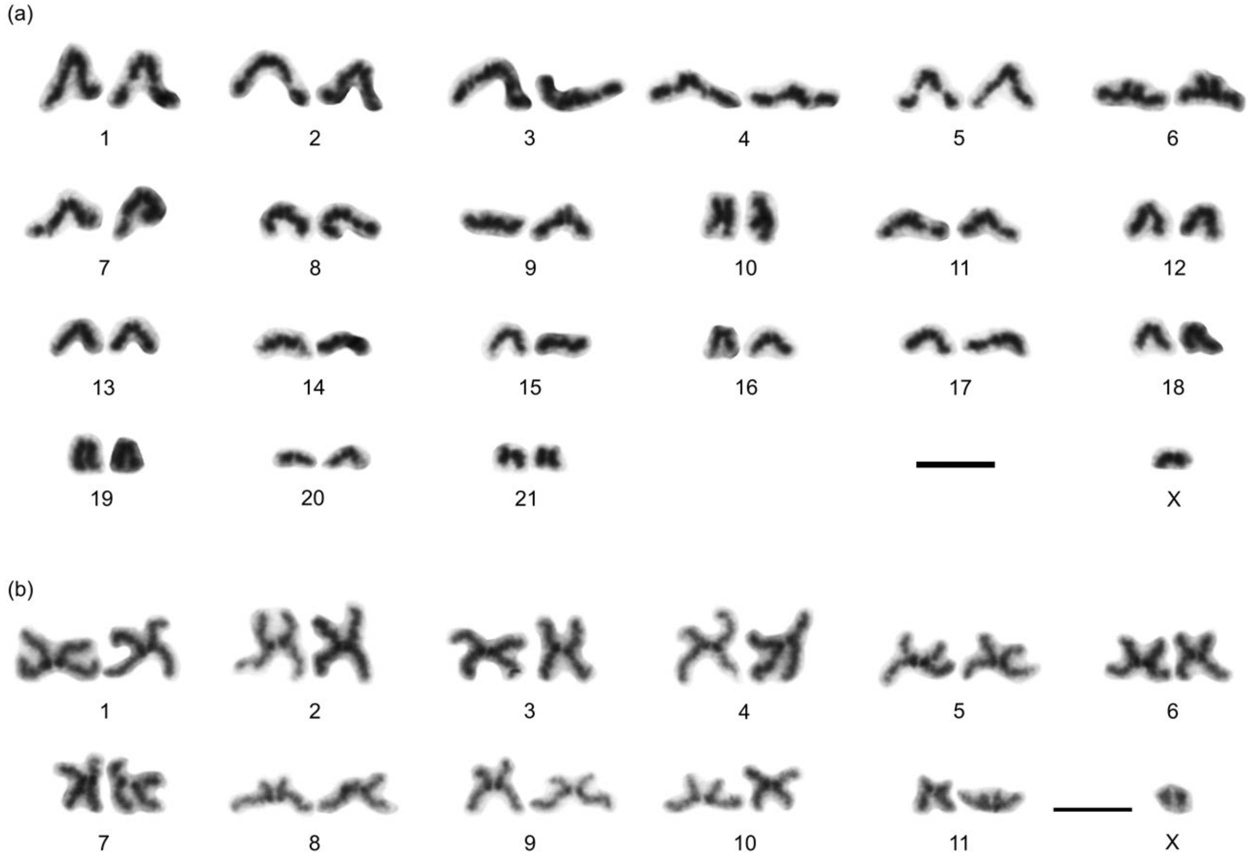

Fig. 2. Male karyotypes of *Roncus* (based on sister metaphase II plates). (a) *R. transsilvanicus* (2n = 43, X0); (b) *Roncus* sp. (2n = 23, X0). Bars = 10 µm.

recorded. The mean chiasma frequency was 21.18 ± 0.39 SD per cell or 1.01 ± 0.02 SD per bivalent.

***Roncus* sp.**

The diploid chromosome complement is 23 chromosomes in males (Fig. 2b) and 24 in females (Fig. 3j). In the karyotype of males there were only biarmed chromosomes: nine pairs of metacentric, two pairs of submetacentric (Nos 8 and 11) autosomes and one metacentric sex chromosome. The length of the chromosomes gradually decreases in size from 6.34% to 3.68% of the total diploid set. Only the last pair of chromosomes (No. 11) is considerably shorter than the previous pairs, forming only 2.46% of the total diploid set. The X chromosome is metacentric (arm ratio 1.3) and is the shortest element of the karyotype, forming only 1.51% of the diploid set. Like all previous species, the X chromosome is positively heteropycnotic during early prophase (leptotene, zygotene and pachytene) (e.g. Fig. 3k) and again starts to become isopycnotic from diplotene to the end of meiosis (Fig. 3l). Despite the marked reduction in the diploid number and larger size of the metacentric chromosomes, a low chiasma frequency was also recorded in this species. The mean chiasma frequency was 11.30 ± 0.72 SD per cell or 1.03 ± 0.07 SD per bivalent (total N = 98).

DISCUSSION AND CONCLUSIONS

Our study provides further information on the cytogenetics of the pseudoscorpion genus *Roncus* (Neobisiidae) based on a study of another four species, which extends the previous work on this genus that was based on eight species (Troiano, 1990, 1997; Zaragoza & Šťáhlavský, 2008). As in the previous study we found differences between the karyotypes that may be useful for future cytotaxonomic studies (Table 1). The great variability in karyotype characteristic of *Roncus* has also been recorded for several other pseudoscorpion families, e.g. Atemnidae, Chthoniidae, Chernetidae, Geogarypidae and Olpiidae (e.g. Šťáhlavský & Král, 2004; Šťáhlavský et al., 2005, 2006, 2009, 2012). In addition, our analysis detected some similar features in the diploid number and morphology of the chromosomes of *R. alpinus*, *R. lubricus* and *R. transsilvanicus*. The karyotypes of these species include some of the highest numbers of chromosomes in the genus *Roncus*, which consist of mainly subtelocentric and acrocentric autosomes. Moreover, all three species have the X0 sex chromosome system with a metacentric X chromosome, which is the smallest element in the karyotypes. This finding contrasts with other species of this genus from Italy (Liguria) (Troiano, 1990, 1997) and Spain (Catalonia) (Zaragoza & Šťáhlavský, 2008). The karyotypes of these species are even more variable. Even species from nearby localities differ significantly in their chromosome number and morphology; furthermore they have different sex chromosome systems (X0 and XY). These major differences, especially in the number and morphology of autosomes, are accounted for in terms

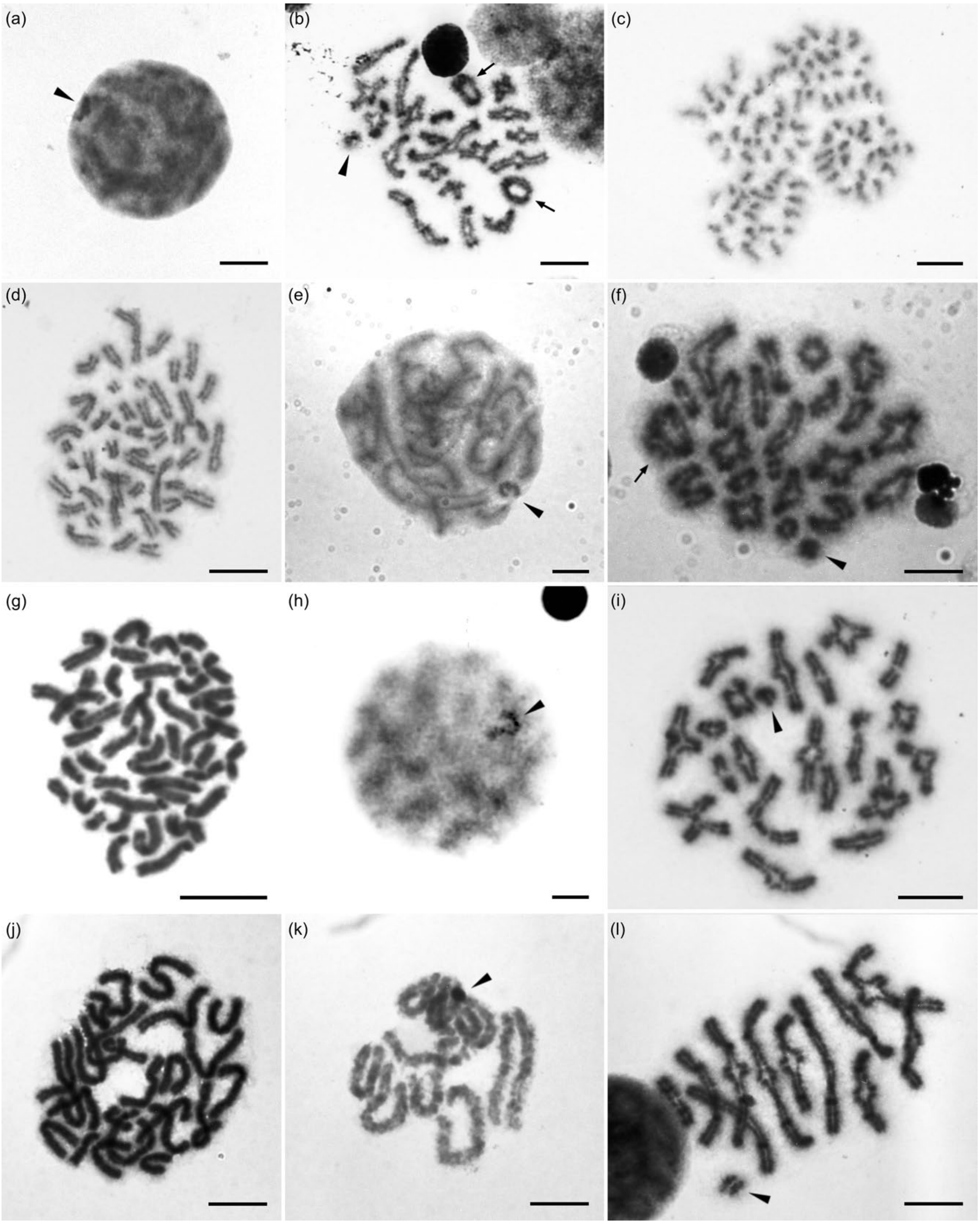

Fig. 3. Chromosome plates of species of *Roncus*. (a)–(c) *R. alpinus*, male. (a) leptotene; (b) diplotene; (c) anaphase II; (d)–(f) *R. lubricus*. (d) female mitotic metaphase; (e) male zygotene; (f) male late metaphase I; (g)–(i) *R. transsilvanicus*. (g) female mitotic metaphase; (h) male leptotene; (i) male late metaphase I; (j)–(l) *Roncus* sp. (j) female mitotic metaphase; (k) male pachytene; (l) male metaphase I. Arrowheads indicate the X chromosome, arrows point to the bivalents with two chiasmata. Bars = 10 µm.

of centric fusions or fissions (Troiano, 1990; Zaragoza & Šťáhlavský, 2008). Considering that *R. alpinus*, *R. lubricus* and *R. transsilvanicus* probably represent different phylogenetic lineages (they are from different

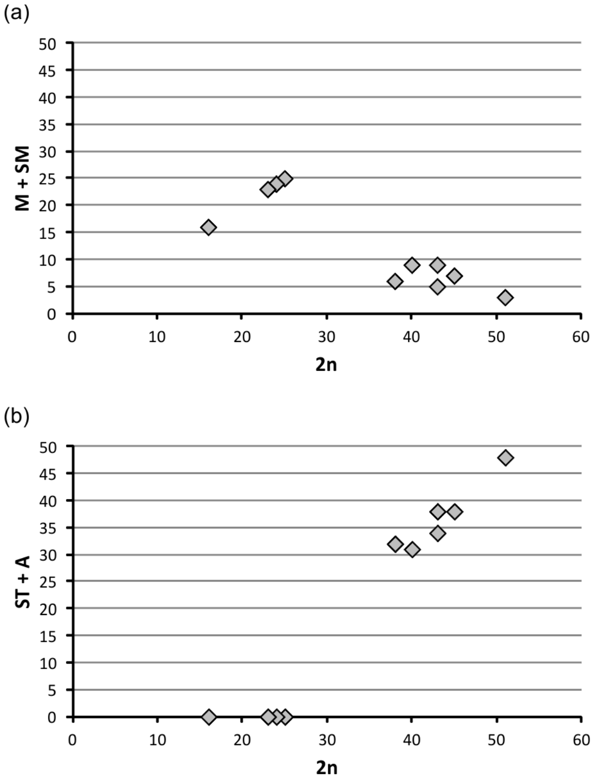

Fig. 4. The relationships between chromosome morphology and the diploid number of species of *Roncus*. (a) Between the ratio of metacentric (M) and submetacentric (SM) chromosomes and the diploid number (2n); (b) between the ratio of subtelocentric (ST) and acrocentric (A) chromosomes and the diploid number (2n). (After Troiano, 1990; Zaragoza & Šťáhlavský, 2008 and present study).

European regions and exhibit different external morphology) (e.g. Beier, 1963), we assume that similar features of the karyotypes of these species reflect an ancestral state of the genus *Roncus*. This hypothesis is also supported by their having the same sex chromosome system X0 in which the size of the X chromosome is similar and same morphology in all three species. The X0 sex chromosome system is generally supposed to be the ancestral one for pseudoscorpions (e.g. Troiano, 1990). This sex system and metacentric morphology of the X chromosome are recorded in Chthoniidae (Šťáhlavský & Král, 2004), one of the most basal lineages of the pseudoscorpions (Murienne et al., 2008) and in other families (e.g. Šťáhlavský et al., 2005, 2006, 2012). The XY sex chromosome system is considered to be a derived state in *R. ingaunus*, *R. tuberculatus* (Troiano, 1990), *R. belluatii* (Troiano, 1997), *R. cadinensis* and *R. montsenyensis* (Zaragoza & Šťáhlavský, 2008), which is also supported by the different size and morphology of the sex chromosomes in these species. The sex chromosomes are nearly homomorphic and both biarmed in *R. inguanus*, *R. cadinensis* and *R. montsenyensis* (Troiano, 1990; Zaragoza & Šťáhlavský, 2008). On the other hand the X is large and metacentric and the Y about a third of the size of X and is acrocentric in *R. tuberculatus* (Troiano, 1990). The main trend in karyotype evolution in the genus *Roncus* is probably in the reduction in the number of chromosomes as a result of centric fusions (Troiano, 1990). This type of chromosomal rearrangement leads to an increase in the number of biarmed chromosomes (metacentric and submetacetric). The reduction in the number of chromosomes (Fig. 4a) leads to full saturation via this type of rearrangement. In contrast, during the reduction in the number of chromosomes, the number of one armed chromosomes (subtelocentric and acrocentric) decreases (Fig. 4b). The results from the current study support this hypothesis. The karyotype of *Roncus* sp. (2n = 23) from the Slovak Republic contains only biarmed chromosomes but this species is morphologically similar to *R. lubricus* (2n = 43) in which there is a predominance of acrocentric chromosomes. However, in order to answer the question, how often has the reduction in the diploid number occurred in the evolution of *Roncus,* an independent phylogenetic analysis is needed.

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