# A new Roncus species (Pseudoscorpiones: Neobisiidae) from Montseny Natural Park (Catalonia, Spain), with remarks on karyology 

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#### Abstract

Roncus montsenyensis sp. nov. is described from Montseny Natural Park (Catalonia, Spain). The new species is geographically and morphologically close to Roncus cadinensis Zaragoza, 2007, but can be separated from it by palpal morphometrics, the chelal microsetae pattern and karyology. The diploid number was found to be $2 \mathrm{n}=16$ in $R$. montsenyensis, with only biarmed chromosomes. The diploid number was found to be $2 \mathrm{n}=38$ in $R$. cadinensis, with a predominance of acrocentric chromosomes. Both species possess the XY sex chromosome system and the X and Y chromosomes are only weakly differentiated.


Key words: Pseudoscorpiones, Neobisiidae, Roncus, new species, karyotype, Catalonia, Spain

## Resumen

Roncus montsenyensis sp. nov. es descrita del Parque Natural del Montseny (Cataluña, España). La nueva especie es geográfica y morfológicamente cercana a Roncus cadinensis Zaragoza, 2007, pero se diferencia de aquella por la morfometría del palpo, el modelo de posición de las microsedas de la quela y la cariología. El número diploide encontrado en $R$. montsenyensis ha sido $2 \mathrm{n}=16$, con sólo cromosomas de dos brazos. El número diploide hallado en $R$. cadinensis ha sido $2 \mathrm{n}=38$, con predominancia de cromosomas acrocéntricos. Ambas especies poseen el sistema de cromosomas sexuales XY y los cromosomas X e Y están sólo levemente diferenciados.

## Introduction

The presence of a new Roncus L. Koch, 1873 species from Montseny Natural Park that is taxonomically and geographically close (about 120 km ) to Roncus cadinensis Zaragoza, 2007 (Zaragoza et al. 2007) from CadíMoixeró Natural Park (both in province of Barcelona, Catalonia, Spain), confirms that Roncus populations in Northeast Spain can present high endemicity. It also provides support for the view that previous records of the genus from that region are in need of a thorough revision (Henderickx \& Zaragoza 2005).

Karyotypes have been described to date for six species of Roncus, all from northwestern Italy. In spite of this limited information, cytogenetic analysis of four epigean (Troiano 1990) and two hypogean (Troiano 1997) species demonstrated the great interspecific variability in the diploid numbers ( $2 \mathrm{n}=22-52$ ), chromosome morphology and even the sex chromosome system (X0 and XY). Troiano (1990, 1997) also showed that karyology may play an important role in the taxonomy of the morphologically very uniform genus Roncus and help to determine the relationships between the species. Later karyological studies support this hypothesis
for other pseudoscorpions, especially in the genera Chthonius C.L. Koch, 1843 (Chthoniidae) (Štáhlavský \& Král 2004) and Lasiochernes Beier, 1932 (Chernetidae) (Štáhlavský et al. 2005). In the genus Roncus it was also found that centric fusions are probably the most frequent type of differentiation of the karyotypes (Troiano 1990). Here we provide further confirmation of the importance of karyology in the taxonomy of pseudoscorpions.

## Material and methods

Specimens for morphological study were dissected and examined as temporary glycerine mounts in cavity slides. Specimens are preserved individually in $70 \%$ ethanol inside glass vials; dissected appendages are kept in glass microvials inside each vial. Microscopical examination was carried out with a Zeiss Axiolab light microscope, which was also used to take measurements of the appendages, with an ocular micrometer, and drawings, with the aid of a drawing tube. SEM photographs were taken with a JEOL JSM-840 microscope. The measurements were taken using Chamberlin's (1931) reference points; the lengths of the chela and its palm include the pedicel; all measurements are in millimetres. The ratios given are the length/width or length/ depth index of an article. Terminology generally follows Chamberlin (1931) and Harvey (1992); that of the faces of appendages and for chelal spot-sensilla follows Judson (2007).


FIGURE 1. Maps showing location of the locus typicus (red star) of Roncus montsenyensis sp. nov., with a photograph of the beech forest where samples were collected.

The chromosome preparations were made from gonads using the technique described in detail for pseudoscorpions by Štáhlavský \& Král (2004). The gonads were dissected and hypotonized in 0.075 M KCl for 1015 minutes and then fixed in fresh fixative ( 1 part glacial acetic acid, 3 parts ethanol) for at least 20 minutes. The fixed material was then removed to a drop of $60 \%$ acetic acid on a clean microscope slide and suspended using a needle. The drop of suspended tissue was then agitated on the slide with a tungsten needle until evaporation on a warm histological plate (temperature $40-45^{\circ} \mathrm{C}$ ). The chromosome preparations were stained with a 5\% Giemsa solution in Sörensen phosphate buffer ( $\mathrm{pH}=6.8$ ) for 35 minutes.

Chromosome morphology was classified according to Levan et al. (1964). The karyogram (fig. 18) was assembled from two sister metaphase II plates. Relative chromosome length (below $=\%$ of TCL) was calculated as a percentage of the total chromosome length of the diploid set from ten metaphase I in both species (Table 2). During this stage the position of the centromere was obvious as a prominent knob and the pairing of the chromosomes was easier. In total, $4 \sigma^{x}$ and $1+\frac{+}{}$ of Roncus cadinensis and $3 \sigma^{x}$ and $4+$ of $R$. montsenyensis sp. nov. were karyotyped. The localities and the details of collections are the same as those given below in the taxonomic section.

Abbreviations

| $\xi$ | average measurements. |
| :--- | :--- |
| DEUA | Departamento de Ecología, Universidad de Alicante. |
| MCNB | Museu de Ciències Naturals, Barcelona. |
| MHNG | Muséum d'Histoire naturelle de la Ville de Genève. |
| MNCNM | Museo Nacional de Ciencias Naturales, Madrid. |
| MNHNP | Muséum national d'Histoire naturelle, Paris. |
| PT | pseudotactile seta (similar to tactile seta, but shorter). |
| TS | tactile setae. |

## Systematics

Chelal microsetae pattern and chelal pores
Gardini (1981) proposed a new taxonomic characteristic for Roncus species based in the presence/absence of microsetae proximal to the trichobothrium $e b$. This characteristic was used by the same author (Gardini 1983) in the revised diagnosis of Roncus lubricus L. Koch, 1873, which has microsetae proximal to $e b$. This new characteristic was considered sufficiently important by some authors (e.g. Gardini \& Rizzerio 1985, 1986) to serve as the first premise in separating species in their keys to the genus Roncus. A different chelal microsetae pattern was mentioned in the description of Roncus judsoni (Henderickx \& Zaragoza 2005), which has a group of numerous microsetae lying below trichobothria eb and esb, and this was used as complementary diagnostic feature to separate it from Roncus duboscqi Vachon 1937. The first author has observed that chelal microsetae occur below eb-esb in all examined Roncus specimens and that they are quite constant for each species, with only small variations. Microsetae that are considered to belong to this group are those lying exactly below $e b-e s b$. One or a few microsetae are also found slightly or clearly distant of $e b$, but these are not considered to belong to this group (although they are reported in descriptions or shown in illustrations). These distal microsetae are also occasionally longer than those below eb-esb and seem to belong to a different "group". The chelal microsetae pattern can provide an additional characteristic for distinguishing between close species, providing that the limited variations are taken into account.

Glandular pores have been noted and illustrated on the chelal palm, at the antiaxial face close to the finger base, in descriptions of many Roncus species, as well as micropores on the dorsal face of the pedicel; Hender-
ickx \& Zaragoza (2005). Zaragoza et al. (2007) (and this paper) have also mentioned and illustrated two ventral glandular pores in the movable finger of the chela, usually distal and basal of trichobothrium $s b$, but sometimes both basal. Additional pores have been mentioned on the paraxial face of the movable finger by Judson (2007) for Neobisioidea.

## Genus Roncus L. Koch, 1873

Roncus cadinensis Zaragoza, 2007
Roncus cadinensis Zaragoza in Zaragoza, de Mas \& Ribera 2007: 84-89, figs 21-37, map 8.

Material examined. $20^{\prime}$, Spain, Catalonia, Lleida province, Ribera d'Urgellet, $\mathrm{N} 42^{\circ} 19^{\prime} 17^{\prime \prime} \mathrm{E} 01^{\circ} 23^{\prime} 39^{\prime \prime}$, altitude 670 m, collected 11 Sept. 2006, lgt. F. Štáhlavský, deposited in DEUA. These topotypical specimens conform to the description given by Zaragoza et al. (2007). Because the original description of this species was in Spanish, we here provide a diagnosis in English:

Diagnosis. Roncus species of small size. Carapace longer than broad ( $\xi$ : $0^{x} 1.19 \times, 0.67 / 0.57$, 오 $1.16 \times$, $0.74 / 0.64$ ), as long or slightly shorter than palp femur, epistome short and rounded, chaetotaxy: 4-8-6-6=24. Tergites I-V: 6:10:11:11:12 setae. Anterior process of coxa I short and blunt, medial process without denticles. Palp femur finely granulated and without tubercles at paraxial face, one tubercle at middle of antiaxial face, $\xi$ : o $\circ^{x} 3.39 \times, 0.69 / 0.20$; 우 $3.45 \times, 0.77 / 0.22$. Patella smooth. Chela, $\xi$ : 우 $3.34 \times, 1.17 / 0.35$, 우 $3.06 \times, 1.30 /$ 0.42. Ratio femur length/chelal finger $1.04-1.12 \times$, finger/hand $1.06-1.21 \times$. Two or three (seldom four) microsetae below trichobothria $e b$ and $e s b$. Leg IV claws with a tiny tooth on dorsal side; subterminal setae with three rami. ADDENDA: chelicera without granulation at base of movable finger; distance between trichobothria $i b$ and ist about $1.5 \times$ distance $i s t-i t(\xi: 1.56 \times$ ), distance $(\xi)$ sb-st $1.13 \times b-s b$.

## Roncus montsenyensis sp. nov.

(figs 2-17)

Type material. Male holotype: Spain, Catalonia, Barcelona province, Montseny Natural Park, N41 ${ }^{\circ} 45^{\prime} 6.14$ " E02 ${ }^{\circ} 26^{\prime} 34.30^{\prime \prime}$, altitude 1137 m , in beech leaf-litter, 9 Sept. 2006, lgt. F. Št’áhlavský, L. Bouzek \& V. Opatová; deposited in DEUA. Paratypes: $2 o^{x}$ and $3 \circ$ with the same collection data as holotype, 1 of deposited in DEUA, $1 o^{x}$ in MHNG, $1+$ in MNHNP, $1+$ in MCNB, $1 o^{x}$ in MNCNM.

Diagnosis. Epigean Roncus of typical roncoid facies, small-medium in size. Carapace longer than broad ( $\xi$ : $o^{x} 1.20 \times, 0.67 / 0.55$; 오 $1.21 \times, 0.67 / 0.55$ ); epistome moderately prominent, isosceles triangle shape, apex rounded; chaetotaxy: 4-8-6-6=24. Chelicera without granulation at base of movable finger. Tergites I-V: 6-7:7-11:11:10-11:11. Medial process of coxa I without denticles. Palp femur granulated but without tubercles at the paraxial face, one tiny tubercle at middle of antiaxial face; longer than either chelal finger or carapace; $\xi$ : $\circ^{\star} 3.94 \times, 0.72 / 0.18$; 우 $3.75 \times, 0.71 / 0.19$. Patella smooth. Chela, $\xi$ : ㅇ $3.62 \times, 1.25 / 0.35$; 우 $3.34 \times, 1.21 / 0.36$. Ratio length finger/hand, $\xi: o^{x} 1.25 \times$, 우 $1.19 \times$. Five, seldom four, microsetae between trichobothria $e b$ and $e s b$. Distance between trichobothria ib and ist about $2.00 \times$ distance ist-it ( $\xi: 1.85 \times$ ), distance ( $\xi$ ) sb-st $0.95 \times$ distance $b-s b$. Leg IV claws with a tiny tooth on dorsal side; subterminal setae with three rami.

Etymology. Named in reference the locus typicus of the species, Montseny Natural Park.
Description. Data correspond to the male holotype, with paratype data in parentheses. Measurements and ratios as in Table 1.

Opisthosomal pleura and legs yellowish, tergites slightly sclerotized. Carapace, chelicerae and pedipalps red-brownish.

TABLE 1. Roncus montsenyensis sp. nov.: measurements and ratios.

|  | $0^{4}$ holotype |  | $20^{x}$ paratypes |  | 3 우 paratypes |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Ratio | Mean | Ratio | Mean | Ratio | Mean |
| Body |  | 1.94 |  | 1.50-1.63 |  | 1.42-2.04 |
| Carapace | 1.19 | 0.67/0.57 | 1.19-1.23 | 0.65-0.67/0.54-0.55 | 1.18-1.26 | 0.65-0.69/0.52-0.58 |
| Chelicera |  |  |  |  |  |  |
| Hand | 1.98 | 0.41/0.21 | 1.94-1.95 | 0.42-0.43/0.21-0.22 | 1.86-1.99 | 0.41-0.44/0.21-0.23 |
| Finger |  | 0.28 |  | 0.28-0.29 |  | 0.28-0.29 |
| Palp |  |  |  |  |  |  |
| Trochanter | 2.26 | 0.41/0.18 | 2.17-2.23 | 0.41-0.42/0.19 | 1.67-2.22 | 0.40-0.42/0.18-0.24 |
| Femur | 4.00 | 0.72/0.18 | 3.84-3.97 | 0.72-0.73/0.18-0.19 | 3.64-3.82 | 0.67-0.75/0.18-0.20 |
| Patella | 2.45 | 0.57/0.23 | 2.38-2.42 | 0.57-0.58/0.24 | 2.26-2.38 | 0.53-0.58/0.23-0.25 |
| Pedicel |  | 0.21 |  | 0.21-0.22 |  | 0.19-0.20 |
| Club | 1.57 | 0.36/0.23 | 1.50-1.52 | 0.36-0.37/0.24 | 1.45-1.53 | 0.34-0.38/0.23-0.25 |
| Club/pedicel | 1.76 |  | 1.70-1.71 |  | 1.74-1.90 |  |
| Hand | 1.66 | 0.57/0.34 | 1.59-1.67 | 0.55-0.59/0.35 | 1.49-1.58 | 0.53-0.59/0.36-0.37 |
| Pedicel |  | 0.09 |  | 0.10-0.11 |  | 0.09-0.10 |
| Finger |  | 0.70 |  | 0.71-0.72 |  | 0.64-0.69 |
| Chela | 3.69 | 1.26/0.34 | 3.54-3.62 | 1.24-1.26/0.35 | 3.25-3.42 | 1.17-1.28/0.36-0.37 |
| Chela/carapace | 1.87 |  | 1.89-1.91 |  | 1.78-1.86 |  |
| Femur/carapace | 1.07 |  | 1.10 |  | 1.02-1.08 |  |
| Femur/finger | 1.02 |  | 1.01 |  | 1.02-1.08 |  |
| Femur/patella | 1.27 |  | 1.23-1.28 |  | 1.27-1.28 |  |
| Patella/hand | 1.00 |  | 0.99-1.03 |  | 0.93-1.03 |  |
| Finger/hand | 1.25 |  | 1.21-1.30 |  | 1.12-1.29 |  |
| Leg I |  |  |  |  |  |  |
| Femur | 3.21 | 0.36/0.11 | 3.18-3.19 | 0.38/0.12 | 3.14-3.35 | 0.37-0.39/0.12 |
| Patella | 2.40 | 0.24/0.10 | 2.51-2.55 | 0.26/0.10 | 2.35-2.65 | 0.23-0.27/0.10 |
| Tibia | 4.27 | 0.32/0.08 | 4.25-4.45 | 0.33-0.35/0.08 | 4.27-4.56 | 0.31-0.34/0.07-0.08 |
| Basitarsus | 2.56 | 0.16/0.06 | 2.68-2.78 | 0.17/0.06 | 2.42-2.56 | 0.15-0.16/0.06-0.07 |
| Telotarsus | 4.95 | 0.27/0.06 | 4.95-5.04 | 0.27-0.29/0.06 | 4.52-4.67 | 0.25-0.28/0.06 |
| Femur/patella | 1.50 |  | 1.46-1.47 |  | 1.45-1.59 |  |
| Telotarsus/basitarsus | 1.70 |  | 1.65 |  | 1.65-1.78 |  |
| Leg IV |  |  |  |  |  |  |
| Femur+patella | 3.03 | 0.65/0.21 | 2.78-2.91 | 0.64/0.22-0.23 | 2.86-3.18 | 0.61-0.68/0.21-0.22 |
| Tibia | 5.09 | 0.59/0.12 | 4.92-5.46 | 0.56-0.60/0.11 | 5.03-5.24 | 0.55-0.60/0.11-0.12 |
| Basitarsus | 2.47 | 0.21/0.09 | 2.28-2.50 | 0.20-0.21/0.08-0.09 | 2.48-2.53 | 0.19-0.22/0.08-0.09 |
| Telotarsus | 4.67 | 0.35/0.08 | 4.61-4.85 | 0.36/0.08 | 4.47-4.61 | 0.34-0.36/0.07-0.08 |
| Telotarsus/basitarsus | 1.67 |  | 1.70-1.84 |  | 1.65-1.84 |  |

Carapace (fig. 2) longer than broad, maximum width at medial third. One pair of reduced eyes with flattened lens $0.075(0.063-0.070) \mathrm{mm}$ long, situated $0.068(0.055-0.068) \mathrm{mm}$ from anterior margin. Epistome (fig. 3) obtuse-angled isosceles triangle shaped (more or less pronounced), apex broadly rounded, 0.020 ( $0.015-0.023$ ) mm long and 0.028 ( $0.022-0.030$ ) mm wide. Chaetotaxy: 24 (23-25) setae, formula: 4:4:10(911):6. Glandular pores present, three (one to four) on each side between anterior and ocular zones. One microlyrifissure close to each eye and one on each side of the posterior zone.

Coxal area. Manducatory process with $3(3-4)$ setae; palpal coxa with 7 (7-9) setae, pedal coxa I with 57 (5-6) setae, II: 5 (6-8), III: 4-5, IV: 7-8 (6-9). Anterior process of coxa I (fig. 13) with short and simple
tooth shape, apically pointed, $0.020(0.015-0.018) \mathrm{mm}$ long and $0.018(0.015-0.019) \mathrm{mm}$ broad; medial process straight, not prominent, without denticles.


FIGURES 2-7. Roncus montsenyensis sp. nov., male holotype. 2. carapace; 3. anterior margin of carapace, showing epistome; 4. left chelicera; 5. Partial view of fingers of left chelicera; 6. distal end of tarsus and apotele of left leg IV, lateral view; 7. left IV, TS: tactile seta. Scale bars (mm): 0.05 (figs 3, 5, 6), 0.10 (fig. 4), 0.20 (figs 2, 7).


FIGURES 8-13. Roncus montsenyensis sp. nov., male holotype. 8. right chela; 9. dorsal view of right palp, without chela; 10. lateral view of right chela, gp: glandular pore; 11. tips of fixed and movable fingers of right chela, lateral view; 12. chelal microsetae pattern below trichobothria eb/esb; 13. Anterior and medial processes of coxa I. Scale bars ( mm): 0.05 (figs $11,12,13$ ), 0.20 (figs $8,9,10$ ).

Tergal chaetotaxy I-X: 6:7:11:11:11:11:11:11:11(4 PT):9(4TS) (6-7:7-11:11:10-11:11:11:11-12:1011:11:11). Segment XI with 13 setae, 6 of them tactile setae. Anal cone with 2 dorsal and 2 ventral setae. Male genital area with 15 (13-14) setae on sternite II; sternite III with 13 (13-14) setae, 5 (4-5) of them along posterior margin of genital opening; genital opening with $2+2$ internal setae. Female genital opening with $8-10$ microsetae on sternite II and 12-13 on sternite III. Chaetotaxy of sternites IV-X: 8:13:13:13:13:13:?(sternite X fragmented) (9-10:13-14:13-14:13-15:13-14:13-14:11-13).

Chelicera (figs 4-5) with 6 (6-7) setae on hand and one seta on movable finger, $0.66 \times(0.60-0.68)$ from base. The spinneret a flattened hyaline tubercle (slightly lower in males than in females) with 4 silk ducts. Fixed finger with $4(4-7)$ apical protuberances and 14 (15-18) medium and small size teeth; movable finger with $3(2-5)$ apical protuberances and $12(9-12)$ teeth, $4(2-3)$ of large size. Flagellum with 8 denticulate blades, length of proximal blade about one third that of the others; serrula exterior with 26 (23-27) blades, serrula interior with 24 (18-23) blades.

Palps (figs 8-12, 14-17), trochanter with one small (small or medium) tubercle and one small protuberance at antiaxial face; with tiny dorsal denticulation in distal half; paraxial face of femur completely granulated, one tiny (almost indistinguishable) tubercle at middle of antiaxial face, one glandular pore mediodistally. Patella smooth, two micropores at base of pedicel, some lyrifissures as drawn in fig. 9. Chela (figs 8,10,16): paraxial face of hand granulated at base of fixed finger, one pore at antiaxial face close to finger base, with significant protuberance (gp, fig. 10); one micropore at base of dorsal face of pedicel. Fixed finger with 61 (54-63) teeth up to the level of trichobothrium ib; nodus ramosus (fig. 11) at level of 5th (4th) distal tooth; distance between trichobothria $i b$ and ist 2.02 (1.64-2.08) times longer than between ist and $i t ; 5$ (5, only one male 4) microsetae below trichobothria $e b$ and $e s b$ and one (1-2) slightly distal of $e b$ (fig. 12); one lyrifissure at level of trichobothria $e b, i b$ and distal of $i t$. One sensillum near the tip of both fingers. Movable finger with 59 (54-64) teeth up, extending further than teeth of fixed finger, ending just distad of trichobothrium $b$; distance between trichobothria sb and st 0.92 ( $0.87-0.97$, one female 1.05 ) shorter than distance $b-s b$; one sensillum $\left(p_{2}\right)$ close to dental margin, above or slightly proximad of trichobothrium $s b$, another sensillum $\left(p_{1}\right)$ distad of $s b$ (more or less close to trichobothrium, but always distal); two ventral glandular pores between trichobothria $b$ and $s b$ (gp, fig. 10); one lyrifissure basal of trichobothrium $b$, one between $b$ and $s b$ and one slightly basal, level with or even distad of $s b$.

Claws of legs I and IV (fig. 6) with a tiny tooth at middle of dorsal side. Leg IV (fig. 7) tibia TS ratio 0.51 ( $0.53-0.59$ ), basitarsus TS ratio 0.18 ( $0.16-0.23$ ), telotarsus TS ratio: 0.31 ( $0.35-0.39$ ); subterminal setae (fig. 6) 0.063 ( $0.063-0.071$ ) mm long, with three rami, the longest $[\mathrm{L}=0.030(0.030-0.043)]$ and the next longest $[L=0.019(0.018-0.026)]$ with some spinules, the shortest $[L=0.008(0.05-0.009$, apically broken in some specimens)] smooth.

Distribution. The new species has thus far been found only at the Montseny Natural Park (Barcelona province, Catalonia, Spain) and is probably endemic to that area.

Remarks. Amongst the Roncus species from Spain with a roncoid form [in the sense used by Zaragoza et al. (2007) for the species of the genus with epigean appearance, in contrast to the term parablothroid adopted by Gardini (1982a) for species with a cave-dwelling appearance], R. montsenyensis sp. nov. resembles $R$. neotropicus Redikorzev, 1937 and $R$. judsoni Henderickx \& Zaragoza, 2005 in having a male palpal femur ratio of about 4.0. However, the latter species differ in having the femur distinctly longer (about 1.00 mm in both), and the palpal microsetae below trichobothria eb-esb are more numerous in $R$. judsoni ( $8-10$ microsetae). Compared with species with short palpal articles, $R$. montsenyensis sp. nov. is easily distinguished from $R$. lubricus L. Koch, 1873 (as defined by Gardini 1983) by the absence of a microsetae group proximal to trichobothrium eb (present in R. lubricus); moreover R. lubricus is probably absent from Spain (Gardini 1983, 2000; Henderickx \& Zaragoza 2005; Zaragoza 2007). The differences between the new species and R. cadinensis Zaragoza, 2007 (in Zaragoza et al., 2007), also from Barcelona province, are subtle but consistent: palp trochanter tubercle more pronounced in R. cadinensis, palp smaller and more robust in R. cadinensis ( $\xi$ : femur $\sigma^{\star}$ trichobothria ib and ist $1.85 \times$ distance between ist and it (versus average of 1.56 in $R$. cadinensis), distance ( $\xi$ ) $s b-s t 1.13 \times$ distance $b-s b$ in $R$. cadinensis (versus $0.95 \times$ in $R$. montsenyensis); finally $R$. cadinensis bears only three microsetae below $e b$ and esb, as opposed to five in $R$. montsenyensis, which emphasizes the importance of this characteristic.


FIGURES 14-17. Roncus montsenyensis sp. nov., male paratype. SEM photographs. 14. right palp, dorsal view; 15. fingers of the right chela, partial dorsal view; 16. left chela, lateral-external view; 17. chelal microsetae pattern below trichobothria $e b$ and esb (some setae distorted due to movement during scanning). Scale bars in mm .

In comparison with the French species of the genus and following Gardini's keys (1982b, 1991), the new species is readily distinguished from Roncus duboscqi Vachon, 1937 by the palpal femur measurements-
 ilar differences generally apply to the rest of palp and leg articles. The same is true for Roncus euchirus (Simon, 1879), which also differs in having a large and pointed epistome: 0.035-0.045 mm long (Gardini 1982b), versus $0.015-0.023 \mathrm{~mm}$ in the new species.

Among the mainland Italian species, based on the keys of Gardini \& Rizzerio $(1985,1986)$ and Gardini (1991, 1992, 1993), the new species belongs to the group of species with 6 setae on the posterior carapacial margin, a palpal chela (with pedicel) $1.05-1.54 \mathrm{~mm}$ long, and lacking tubercles on the antiaxial face of the palpal femur. Within this group, R. montsenyensis resembles R. binaghii Gardini, 1991 and R. caprai Gardini, 1993 in having the palpal femur less that 0.80 mm long, but it differs from both in lacking denticles on the medial process of coxa I. Moreover R. montsenyensis has the palpal patella smooth, whereas in $R$. caprai it bears evident granulation.

The new species presents more affinities with species from Sardinia. Following Gardini \& Rizzerio's (1987) key, R. montsenyensis can be compared with the species with ratio finger/hand of the chela $0.90-1.35$, palp femur without tubercles at the antiaxial face, patella smooth, femur length $0.52-0.78 \mathrm{~mm}$ and chela length $0.86-1.30 \mathrm{~mm}$. Roncus abditus (J.C. Chamberlin, 1930) is easily distinguished from the new species by the lower ratios and measurements of the palpal articles ( $\xi$ : femur o ${ }^{*} 3.34 \times, 0.54 / 0.16$, 우 $3.11 \times, 0.54 / 0.17$; chela $o^{x} 3.25 \times, 0.91 / 0.28$, 오 $3.02 \times, 0.94 / 0.31$; finger $o^{x}$ and $+\frac{+}{}$ about 0.50 ). Roncus caralitanus Gardini, 1981 differs from $R$. montsenyensis by the shape of the carapacial epistome (well developed and pointed in the Sardinian species and short and rounded in the Catalonian species; this distinction is also valid for $R$. cadinensis although it was not mentioned in the description of the latter); carapace almost as long as broad in $R$. caralitanus and clearly longer in the new species; palps less slender in the Sardinian species (femur o ${ }^{\star} 3.48-3.52 \times$, 우 $3.54 \times$; chela $o^{x} 2.88-3.12 \times$, 우 $2.95 \times$ ); and Gardini’s figure (1981: fig. 7) shows only one microseta between $e b$ and esb. R. caralitanus has been recorded from the Balearic Islands, Sicily and Sardinia, but according to Gardini (2000) it is probably endemic to Sardinia, the records from the other islands being misidentifications. $R$. montsenyensis shows more similarity to Roncus grafittii Gardini, 1982 (Gardini 1982a), a troglophilous species with some troglomorphic adaptations (eyes reduced to spots and slender palps). Both species present similar morphometric values for some article of the palps and legs, but, in addition to the reduction of the eyes, some clear differences can be observed: the segments of the pedipalp and legs tend to be more slender in the Sardinian species (e.g. palp femur 오 $4.27 \times$, palp patella o ${ }^{*} 2.50 \times$, 오 $2.54 \times$; leg I tibia ơ $5.07 \times$, 우 $5.14 \times$, telotarsus leg I ơ오 $5.40 \times$, tibia leg IV $\circ^{\star 7} 7.26 \times$, 우 $5.81 \times$, telotarsus leg IV ox $5.33 \times$ ), reflecting modifications to cave life; subterminal setae of telotarsus IV with two rami in $R$. grafittii, but clearly with three rami in $R$. montsenyensis; movable cheliceral finger with dorsal-basal granulation in $R$. grafittii (completely smooth in the new species); distance between trichobothria ib and ist $1.85 \times(\xi)$ longer than ist-it in $R$. montsenyensis and $1.57 \times(\xi)$ in $R$. grafittii, average distance $s b-s t 0.95 \times$ distance $b-s b$ in the new species and $1.86 \times$ in the Sardinian species [ratio for $R$. grafittii calculated from Gardini’s figures (1982a: figs 14,15,17)]; chelal microsetae pattern below $e b$ and $e s b$ reduced to two microsetae in the Sardinian species (observed in micrograph of male holotype kindly sent by Dr Giulio Gardini).

## Karyology

## Roncus montsenyensis sp. nov.

The male chromosome complement is composed of 16 chromosomes (fig. 18). Analysis of the metaphase I (figs 19, 21) suggests the presence of the XY sex chromosome system. Only biarmed chromosomes were found in the karyotype: 7 pairs of metacentrics and 1 pair of submetacentrics (Table 2). In the karyotype the length of the first pair of chromosomes ( $10.7 \%$ of TCL) is markedly different, whereas the length of the remaining chromosomes gradually decreases from $7.3 \%$ to $4.8 \%$ of TCL. Sex chromosomes X and Y can be distinguish in metaphase I from autosomes by non-pairing part of chromatids at one sex chromosome (figs 1921) and during this stage they pair by terminal (fig. 19) or subterminal (fig. 20) chiasma. Sex chromosomes demonstrate also precocious segregation at the late metaphase I in contrast to autosome bivalents (fig. 21). Both sex chromosomes are metacentrics and they form $5.7 \%$ and $5.2 \%$ of TCL (Table 2). In contrast to the metaphase I, the sex chromosomes can not be distinguished precisely in metaphase II. They are of a similar size and morphology to the autosomes. In metaphase I we found higher chiasma frequency: from two to six of the bivalents possess two chiasmata. Mean chiasma frequency was counted as 11.90 per cell ( 1.49 per bivalent).


FIGURES 18-21. Giemsa stained chromosomes of Roncus montsenyensis and R. cadinensis. 18. karyogram of R. montsenyensis; 19. early metaphase I of $R$. montsenyensis showing five bivalents with two chiasmata, the sex chromosomes pair by terminal chiasma (arrow); 20. the sex chromosomes of $R$. montsenyensis pair by subterminal chiasma (arrow); $\mathbf{2 1 .}$ late metaphase I of $R$. montsenyensis without pairing of the sex chromosomes; 22. metaphase I of $R$. cadinensis. Arrowheads indicate sex chromosomes; asterisks indicate non-pairing part of chromatids at the end of one sex chromosome. Scale bar: $10 \mu \mathrm{~m}$.

## Roncus cadinensis Zaragoza, 2007

The male metaphase I includes 19 bivalents ( $2 \mathrm{n}=38$ ) (fig. 22). The sex chromosome system is XY. In contrast to the previous species, acrocentric chromosomes predominate in the karyotype of R. cadinensis; we detected only one pair of metacentric autosomes, one pair of submetacentric autosomes and metacentric sex chromosomes (Table 2). The length of the autosomes gradually decreases from $3.7 \%$ to $0.8 \%$ of TCL. The sex chromosomes are the largest chromosomes in karyotype, forming $7.1 \%$ and $6.5 \%$ of TCL. The chiasma frequency is lower than in $R$. montsenyensis: average chiasma frequency was 19.30 per cell, which means only 1.02 per bivalent.

## Discussion

The systematics of Roncus species present special difficulties and traditional taxonomy has frequently proved insufficient to clarify the identity of closely related species. The use of palp morphometry (e.g. Beier 1963) is valid for species with clear differences, but it fails for species with similar morphometric ranges. Modern Roncus taxonomy tends to make an integral study of the animal and to pay attention to characteristics that were
not previously used. For example, the chelal microsetae pattern has shown its utility in helping to distinguish species and species groups in Roncus (e.g. Gardini 1981, 1983; Gardini \& Rizzerio 1985, 1986; Henderickx \& Zaragoza 2005). The chelal microsetae pattern has been also studied in another neobisiid genus, Roncocreagris Mahnert, 1974, by Zaragoza (in press), who showed that three different chelal microsetae groups can be distinguished in the subspecies of Roncocreagris galeonuda Beier, 1955; the presence, position, shape and number of microsetae being fairly constant for each species. These microsetae groups, which probably act as sensorial fields, are worthy of study because they provide useful taxonomic characters, at least in neobisiid pseudoscorpions.

TABLE 2. Roncus montsenyensis and R. cadinensis. Relative chromosome length (\% TCL), expressed as a percentage of total chromosome length of the diploid set and arm ratio (AR) (measurement based on metaphase I).

| Pair No. | Roncus montsenyensis <br> $\% \mathrm{TCL}$ | AR | Roncus cadinensis <br> $\% \mathrm{TCL}$ | AR |
| :--- | :--- | :--- | :--- | :--- |
| 1 | 10.7 | 1.2 | 3.7 | 17.3 |
| 2 | 7.3 | 1.2 | 3.6 | 22.9 |
| 3 | 5.8 | 2.2 | 3.6 | 1.9 |
| 4 | 5.5 | 1.3 | 3.4 | 22.6 |
| 5 | 5.5 | 1.3 | 3.2 | 13.7 |
| 6 | 5.1 | 1.2 | 3.2 | 22.9 |
| 7 | 4.8 | 1.2 | 2.9 | 14.8 |
| 8 |  |  | 2.8 | 16.9 |
| 9 |  |  | 2.5 | 13.2 |
| 10 |  |  | 2.3 | 12.1 |
| 11 |  |  | 1.0 | 1.5 |
| 12 |  |  | 1.9 | 7.5 |
| 13 |  |  | 1.9 | 10.2 |
| 14 |  | 1.6 | 1.5 | 10.9 |
| 15 |  | 1.4 | 1.2 | 8.2 |
| 16 |  |  | 1.0 | 8.8 |
| 17 |  |  | 0.8 | 8.4 |
| 18 |  |  | 6.5 | 8.9 |
| X |  |  | 1.4 |  |
| Y |  |  | 1.3 |  |

Modern descriptions of Neobisiidae usually provide details of the legs, including ratios and measurements of legs I and IV, the form of the claws and subterminal setae, and the positions of tactile setae on leg IV. Ratios of pedal articles offer important information about the way of life of the animal. In particular, cave-dwelling species generally have an enlarged body and appendages, in contrast to strictly epigean species, and intermediate states can be observed in species with troglophilic tendencies. This pattern is particularly reflected in the form of the tibia and telotarsus of legs I and IV. The tibia leg IV can be considered as an index for evaluating the degree of adaptation to cave life, in conjunction of course with other characteristics, such as eye reduction and enlarged palps. We estimate that in Roncus an average ratio of about $6.0 \times$ or higher for the tibia of leg IV suggests a troglophilic or troglobitic condition of the species, as can be inferred from a large number of descriptions (e.g. Ćurčić 1988; Gardini 1982a, 1982b, 1991, 1993; Gardini \& Rizzerio 1985, 1986, 1987; Mahnert 1977; Vachon 1964). This data seems particularly useful in Roncus species with palp femur ratio of about $4.0 \times$, revealing cave-dwelling tendencies [ $R$. montsenyensis (epigean), R. grafittii (troglophilic)].

Our study on two Roncus species from northeastern Spain (Catalonia) confirms the importance of karyological characters in the recognition of morphologically similar species. Comparable results have already been obtained for this genus in northwestern Italy (Liguria) (Troiano 1990, 1997), where it was found that several
different phyletic lines probably exist. Karyotypes of six species there differ not only in the diploid numbers ( $2 \mathrm{n}=22-52$ ) and morphology of chromosomes, but also in the sex chromosome systems (X0 and XY). This allowed Troiano (1997) to confirm the suppositions of Gardini (1992) about the principal lineages in Ligurian populations of Roncus. In the present study we found a similarly large variability in diploid numbers and morphology of chromosomes, but not a difference in the sex chromosome system. The sex chromosomes X and Y are only weakly differentiated in both of the Spanish species, and they are similar in both size and morphology (metacentric). The species studied from Catalonia therefore probably belong to a single phyletic line. Within this line, the karyotype of R. montsenyensis, with presence of only biarmed chromosomes, was probably derived by centric fusions from karyotype of $R$. cadinensis, with predominance of one-armed chromosomes. These chromosomal rearrangements are also very frequent in Italian species (Troiano 1990). Although there are some similarities between $R$. cadinensis and $R$. binaghii in external morphology (especially in morphometry), the karyotypes of these two species are completely different in terms of diploid numbers and the morphology of the chromosomes. The diploid chromosome number of $R$. cadinensis is 38, with a predominance of acrocentric chromosomes, whereas the karyotype of R. binaghii consists of 25 mainly metacentric chromosomes (Troiano 1990). Even more important is the difference in the sex chromosome system between these species, because the XY sex chromosome system in $R$. cadinensis can not be readily derived from the X0 system seen in $R$. binaghii. These two species probably belong to different evolutionary branches of the genus Roncus. The precise reconstruction of the evolution of the whole genus Roncus will need further karyology analysis and detailed description of the external morphology of all species, as well as molecular analyses in the future.

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