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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 2n=16 in *R. montsenyensis*, with only biarmed chromosomes. The diploid number was found to be 2n=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 2n=16, con sólo cromosomas de dos brazos. El número diploide hallado en *R. cadinensis* ha sido 2n=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 Cadí-Moixeró 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 (2n=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) (Šťáhlavský & Král 2004) and *Lasiochernes* Beier, 1932 (Chernetidae) (Šťáhlavský *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 Šťáhlavský & Král (2004). The gonads were dissected and hypotonized in 0.075 M KCl for 10– 15 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°C). The chromosome preparations were stained with a 5% Giemsa solution in Sörensen phosphate buffer (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, 4σ and 1φ of *Roncus cadinensis* and 3σ and 4φ 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

ξ	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 *eb*. 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 *eb*. 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 *eb-esb*. One or a few microsetae are also found slightly or clearly distant of *eb*, 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 *sb*, 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. 2°, Spain, Catalonia, Lleida province, Ribera d'Urgellet, N42°19'17" E01°23'39", altitude 670 m, collected 11 Sept. 2006, lgt. F. Šťáhlavský, 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 (ξ : σ 1.19×, 0.67/0.57, φ 1.16×, 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; ξ : σ 3.39×, 0.69/0.20; φ 3.45×, 0.77/0.22. Patella smooth. Chela, ξ : σ 3.34×, 1.17/0.35, φ 3.06×, 1.30/ 0.42. Ratio femur length/chelal finger 1.04–1.12×, finger/hand 1.06–1.21×. Two or three (seldom four) microsetae below trichobothria *eb* and *esb*. 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 *ib* and *ist* about 1.5× distance *ist–it* (ξ : 1.56×), distance (ξ) *sb–st* 1.13× *b–sb*.

Roncus montsenyensis sp. nov.

(figs 2-17)

Type material. Male holotype: Spain, Catalonia, Barcelona province, Montseny Natural Park, N41° 45'6.14" E02°26'34.30", altitude 1137m, in beech leaf-litter, 9 Sept. 2006, lgt. F. Šťáhlavský, L. Bouzek & V. Opatová; deposited in DEUA. Paratypes: 2 σ and 3 \circ with the same collection data as holotype, 1 \circ deposited in DEUA, 1 σ in MHNG, 1 \circ in MNHNP, 1 \circ in MCNB, 1 σ in MNCNM.

Diagnosis. Epigean *Roncus* of typical roncoid facies, small-medium in size. Carapace longer than broad (ξ : \circ 1.20×, 0.67/0.55; \circ 1.21×, 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; ξ : \circ 3.94×, 0.72/0.18; \circ 3.75×, 0.71/0.19. Patella smooth. Chela, ξ : \circ 3.62×, 1.25/0.35; \circ 3.34×, 1.21/0.36. Ratio length finger/hand, ξ : \circ 1.25×, \circ 1.19×. Five, seldom four, microsetae between trichobothria *eb* and *esb*. Distance between trichobothria *ib* and *ist* about 2.00×distance *ist–it* (ξ : 1.85×), distance (ξ) *sb–st* 0.95× distance *b–sb*. 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.

	♂ holotype		2 ♂ paratypes		3 ^o 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	2.60	0.70	254 262	0.71-0.72	2.05.2.42	0.64-0.69
Chela Chela/aaraa	3.69	1.26/0.34	3.54-3.62	1.24–1.26/0.35	3.25-3.42	1.1/-1.28/0.36-0.3/
Chera/carapace	1.87		1.89-1.91		1.78-1.80	
Femur/Carapace	1.07		1.10		1.02 - 1.08	
Femur/natella	1.02		1.01		1.02-1.08	
Patella/hand	1.27		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/basi-	1.70		1.65		1.65 - 1.78	
tarsus						
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/basi-	1.67		1.70–1.84		1.65–1.84	
tarsus						

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

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) mm long, situated 0.068 (0.055–0.068) 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(9– 11):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 5–7 (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) mm long and 0.018 (0.015–0.019) 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).

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 *ib* and *ist* 2.02 (1.64–2.08) times longer than between *ist* and *it*; 5 (5, only one male 4) microsetae below trichobothria eb and esb and one (1–2) slightly distal of eb (fig. 12); one lyrifissure at level of trichobothria eb, ib and distal of it. 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-sb; one sensillum (p_2) close to dental margin, above or slightly proximad of trichobothrium sb, another sensillum (p_1) distad of sb (more or less close to trichobothrium, but always distal); two ventral glandular pores between trichobothria b and sb (gp, fig. 10); one lyrifissure basal of trichobothrium b, one between b and sb and one slightly basal, level with or even distad of *sb*.

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 [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. neo-tropicus* 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* (ξ : femur \checkmark

 $3.39\times$, 0.69/0.20, $93.45\times$, 0.77/0.22; chela $3.34\times$, 1.17/0.35, $93.06\times$, 1.30/0.42); average distance between trichobothria *ib* and *ist* 1.85× distance between *ist* and *it* (versus average of 1.56 in *R. cadinensis*), distance (ξ) *sb–st* 1.13× distance *b–sb* in *R. cadinensis* (versus 0.95× in *R. montsenyensis*); finally *R. cadinensis* bears only three microsetae below *eb* 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 *eb* 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— $4.24-4.30 \times (0.89/0.21)$ in \circ \circ of the French species, $4.00 \times$ or lower (0.72/0.18) in *R. montsenyensis*—and similar 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 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 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. montsenvensis 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 mm and chela length 0.86–1.30 mm. Roncus abditus (J.C. Chamberlin, 1930) is easily distinguished from the new species by the lower ratios and measurements of the palpal articles (ξ : femur $3.34 \times$, 0.54/0.16, $9.3.11 \times$, 0.54/0.17; chela ♂ 3.25×, 0.91/0.28, ♀ 3.02×, 0.94/0.31; finger ♂ and ♀ about 0.50). Roncus caralitanus Gardini, 1981 differs from R. montsenvensis 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 $3.48-3.52\times$, 23.54×; chela \triangleleft 2.88–3.12×, $\stackrel{\circ}{}$ 2.95×); and Gardini's figure (1981: fig. 7) shows only one microseta between *eb* 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. montsenvensis 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 $\stackrel{\circ}{\downarrow}$ 4.27×, palp patella $\stackrel{\circ}{\neg}$ 2.50×, $\stackrel{\circ}{\downarrow}$ 2.54×; leg I tibia $\stackrel{\circ}{\neg}$ 5.07×, $\stackrel{\circ}{\downarrow}$ 5.14×, telotarsus leg I $\triangleleft \varphi$ 5.40×, tibia leg IV \triangleleft 7.26×, φ 5.81×, telotarsus leg IV \triangleleft 5.33×), reflecting modifications to cave life; subterminal setae of telotarsus IV with two rami in R. grafittii, but clearly with three rami in R. montsenvensis; 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 sb-st 0.95× distance b-sb in the new species and 1.86× in the Sardinian species [ratio for R. grafittii calculated from Gardini's figures (1982a: figs 14,15,17)]; chelal microsetae pattern below eb and esb 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 19-21) 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 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. mont-senyensis*; **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); **21.** 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 μm.

Roncus cadinensis Zaragoza, 2007

The male metaphase I includes 19 bivalents (2n=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.

Pair No.	Roncus montsen	yensis	Roncus cadinens	Roncus cadinensis		
	% TCL	AR	% 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			2.0	1.5		
12			1.9	7.5		
13			1.9	10.2		
14			1.9	10.9		
15			1.5	8.2		
16			1.2	8.8		
17			1.0	8.4		
18			0.8	8.9		
Х	5.7	1.6	7.1	1.4		
Y	5.2	1.4	6.5	1.3		

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).

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× 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×, 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 (2n=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.

Acknowledgements

Thanks are due to Dr Eduardo Seva (Departamento de Ecología, Universidad de Alicante) and Dr Antonio Escarré (Departamento de Ciencias de Mar y Biología Aplicada, Universidad de Alicante), for SEM facilities. To Andrés Amorós (Servicio Técnico de Investigación, Universidad de Alicante) for his help taking SEM photographs. Dr Giulio Gardini (Università degli Studi di Genova) kindly provided unpublished data on the chelal microsetae pattern in *Roncus grafittii*. We are indebted to Dr Mark Judson (MNHN) for his helpful comments on the manuscript and a revision of the English prior to submission. We wish to thank Dr Giulio Gardini, Dr James C. Cokendolpher (Texas Tech) and an anonymous referee for their thoughtful comments on the manuscript. The second author is also grateful to Lukáš Bouzek and Věra Opatová for their help and patience during the collecting in the field. The karyological part of this study was financed by three funds: GAUK B/BIO/197/2006, GAČR 206/07/P161 and MSM 0021620828.

References

- Beier, M. (1963) Ordnung Pseudoscorpionidea (Afterskorpione). *Bestimmungsbücher zur Bodenfauna Europas*. Vol. 1 Akademie-Verlag, Berlin, 313 pp.
- Chamberlin, J.C. (1931) The arachnid order Chelonethida. *Stanford University Publications, Biological Sciences*, 7 (1), 1–284.
- Ćurčić, B.P.M. (1988) *Cave-dwelling pseudoscorpions of the Dinaric Karst*. Slovenska Akademija Znanosti in Umetnosti, Ljubljana, 191 pp.
- Gardini, G. (1981) *Roncus caralitanus* n. sp. della Sardegna meridionale (Pseudoscorpionida Neobisiidae) (Pseudoscorpioni d'Italia XIII). *Bollettino della Società Entomologica Italiana*, 113, 129–135.
- Gardini, G. (1982a) Pseudoscorpioni cavernicoli Sardi. II. Neobisiidae e Chernetidae, con considerazioni sui Neobisiinae cavernicoli. (Pseudoscorpioni d'Italia XII). *Fragmenta Entomologica*, 16, 89–115.
- Gardini, G. (1982b) Compléments à la description de *Roncus euchirus* (Simon, 1879) (Pseudoscorpionida, Neobisiidae). *Revue Arachnologique*, 4, 151–155.

- Gardini, G. (1983) Redescription of *Roncus lubricus* L. Koch, 1873, type-species of the genus *Roncus* L. Koch, 1873 (Pseudoscorpionida, Neobisiidae). *Bulletin of the British Arachnological Society*, 6, 78–82.
- Gardini, G. (1991) Tre nuove specie di *Roncus* L. Koch, 1873 della Liguria occidentale (Pseudoscorpionida Neobisiidae). *Memorie della Società Entomologica Italiana*, 70, 313–334.
- Gardini, G. (1992) *Roncus belluatii*, nuova specie eutroglofila del Toiranese, Liguria occidentale (Pseudoscorpionida, Neobisiidae). *Fragmenta Entomologica*, 23, 201–211.
- Gardini, G. (1993) Roncus caprai n. sp. della Liguria orientale e note su R. gestroi Beier, 1930. Memorie della Società Entomologica Italiana, 71, 409–425.
- Gardini, G. (2000) Catalogo degli Pseudoscorpioni d'Italia. Fragmenta Entomologica, Roma, 32 (Suppl.), 181 pp.
- Gardini, G. & Rizzerio, R. (1985) Materiali per una revisione del genere *Roncus* L. Koch, 1873. I. Ridescrizione dei tipi di alcune specie italiane non cavernicole (Pseudoscorpionida, Neobisiidae). *Fragmenta Entomologica*, 18, 47–79.
- Gardini, G. & Rizzerio, R. (1986) Materiali per una revisione del genere *Roncus* L. Koch, 1873. II. Ridescrizione dei tipi delle specie parablothroidi alpine e appenniniche. *Fragmenta Entomologica*, 19, 1–56.
- Gardini, G. & Rizzerio, R (1987) *Roncus zoiai*, nuova specie cavernicola del Monte Albo, Sardegna Nord-Orientale (Pseudoscorpionida, Neobisiidae). *Fragmenta Entomologica*, **1**9 (2), 283–292.
- Harvey, M.S. (1992) The phylogeny and classification of the Pseudoscorpionida (Chelicerata: Arachnida). *Invertebrate Taxonomy*, 6, 1373–1435.
- Henderickx, H. & Zaragoza, J.A. (2005) Notes on *Roncus* (Pseudoscorpiones: Neobisiidae) from the Eastern Pyrenees: new synonymy and description of a new species. *Revista Ibérica de Aracnología*, 11, 47–59.
- Judson, M.L.I. (2007) A new and endangered species of the pseudoscorpion genus *Lagynochthonius* from a cave in Vietnam, with notes on chelal morphology and the composition of the Tyrannochthoniini (Arachnida, Chelonethi, Chthoniidae). *Zootaxa*, 1627, 53–68.
- Levan, A.K., Fredga, K. & Sandberg, A.A. (1964) Nomenclature for centromeric position on chromosomes. *Hereditas*, 52, 201–220.
- Mahnert, V. (1977) Spanische Höhlenpseudoskorpione. Miscelanea Zoologica, 4, 61-104.
- Šťáhlavský, F., Henderickx, H. & Král, J. (2005) Karyotype study on pseudoscorpions of the genus *Lasiochernes* Beier (Pseudoscorpiones: Chernetidae). *Folia biologica* (*Kraków*), 53, 69–74.
- Šťáhlavský, F. & Král, J. (2004) Karyotype analysis and achiasmatic meiosis in pseudoscorpions of the family Chthoniidae (Arachnida: Pseudoscorpiones). *Hereditas*, 140, 49–60.
- Troiano, G. (1990) Karyotype and male meiosis of four species of *Roncus* L. Koch, 1873 (Pseudoscorpionida, Neobisiidae). *Bollettino di Zoologia*, 57, 1–9.
- Troiano, G. (1997) Further studies on caryology of the pseudoscorpions of the gen. *Roncus*: the karyotype of *Roncus* gestroi and *Roncus belluatii*. *Caryologia*, 50, 271–279.
- Vachon, M. (1964) *Roncus (R.) barbei* nouvelle espèce de Pseudoscorpion Neobisiidae des cavernes du Lot-et-Garonne, France. *Bulletin du Muséum National d'Histoire Naturelle, Paris,* (2) 36, 72–79.
- Zaragoza, J.A. (2007) Catálogo de los pseudoescorpiones de la Península Ibérica e Islas Baleares (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología*, 13, 3–91.
- Zaragoza, J.A. (in press). On the status of the subspecies of *Roncocreagris galeonuda* (Pseudoscorpiones: Neobisiidae): importance of the chelal microsetae pattern. Remarks on the genus *Roncocreagris* Mahnert. *Revista Ibérica de Arac-nología*, 15.
- Zaragoza, J.A., De Mas, E. & Ribera, C. (2007) Pseudoescorpiones del Parque Natural del Cadí-Moixeró (Pirineo Catalán): Estudio ecológico, faunístico y taxonómico (Arachnida: Pseudoscorpiones). *Revista Ibérica de Aracnología*, 14, 69–95.