Zootaxa 3794 (1): 052–086
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3794.1.2
http://zoobank.org/urn:lsid:zoobank.org:pub:AE0A99F2-1D71-46CB-89E2-08ED4C010E17

Differentiation among cave populations of the *Eukoenenia spelaea* species-complex (Arachnida: Palpigradi) in the southwestern Alps

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Abstract

Current knowledge of the taxonomy and distribution of European cave palpigrades largely rests upon incidental records. Samples seldom comprise more than one or two specimens, and many regions have only been unevenly explored, if at all. The present study is the first to investigate morphological variability within, and differentiation between, subterranean palpigrade populations in a small, geographically coherent area. It is based on relatively abundant material from six localities in the SW Alps (Piedmont, Italy) and two non-Italian comparative localities. Discrete and continuous characters reveal, on the one hand, a close grouping within the *Eukoenenia spelaea* species-complex, and, on the other hand, a remarkable short-range endemism. The occurrence of five distinct morphologies in a SW Alpine area of just 2000 square kilometres or so indicates that subterranean biodiversity parallels the rich above-ground biodiversity there. The Cottian Alps in the North harbour *E. roscia* Christian **sp. n.**, *E. lanai* Christian **sp. n.** and the widespread *E. spelaea*. Two caves in the Maritime and Ligurian Alps are populated by *E. bonadonai*, which was originally described from the Provence Alps, while the Bossea cave in the Ligurian Alps remains the only known locality for *E. strinatii*. We evaluate the traditionally employed morphological characters and those recently proposed for use in *Eukoenenia* systematics. Further, we provide data on the formerly undescribed females of *E. strinatii* and *E. bonadonai*, and provide comprehensive pictorial information on the SW Alpine cave palpigrades to set new benchmarks for the taxonomy of the *E. spelaea* species-complex.

Key words: Palpigrade, taxonomy, morphology, morphometry, Principal Component Analysis, new species, subterranean, troglobiotic, Piedmont, Italy

 Accepted by M. Judson: 6 Mar. 2014; published: 5 May 2014

Introduction

Palpigradi is the last discovered branch of the arachnid tree and has persistently posed a challenge to zoologists. Over 120 years have passed since the description of the first known species, *Eukoenenia mirabilis* (Grassi & Calandruccio, 1885), yet vast fields of palpigrade biology, including nourishment and reproduction, still remain obscure (Condé 1996). No better is the status of our understanding at all levels of systematics. The sister-group of the Palpigradi is controversial, as discussed by Coddington *et al*. (2004), and hypotheses of suprageneric relationships do not go beyond the discrimination of two families. The alpha taxonomy of the group suffers from the scarcity of samples large enough for an assessment of morphological variation. Attempts to exploit molecular information are under way, but have not produced substantial results so far.

Currently about 100 species-level taxa of the order Palpigradi are known (Harvey 2003, 2013). Only a few have been studied on the basis of rich material, such as *Prokoenenia wheeleri* (Rucker, 1901) in the classic publication of Rucker (1903) and the tropical soil dwelling *E. janetscheki* Condé, 1993 in Condé (1997). For a number of predominantly cave-dwelling species, information comes solely from the holotype. Until a recent inventory of the subterranean arachnids of Piedmont, Italy (Isaia *et al*. 2011), two troglobiotic *Eukoenenia* species of the southwestern Alps, namely *E. strinatii* Condé, 1977 and *E. bonadonai* Condé, 1979, were among such "one-specimen taxa".

For the present study of morphological variation within and between *Eukoenenia* cave populations in the southwestern Alps we had abundant material at our disposal, at least by palpigrade standards. Several authors have reported on variation among a handful of individuals from a particular population: see Mayoral & Barranco (2013) and the case studies reviewed therein. Montaño-Moreno (2012) included 23 specimens of *E. hanseni* (Silvestri, 1913) and the new species *E. chilanga*, from two populations in Mexico (240 km apart), in a Principal Component Analysis (PCA). Our investigation, however, is the first to compare the morphological spectra of several populations scattered over an area of hardly 2000 square kilometres. Since preliminary examination revealed that these populations belonged to the complex surrounding *E. spelaea* (Peyerimhoff, 1902), we take the opportunity to evaluate single characters with respect to their utility for discriminating between closely related populations and to analyse a set of continuous variables by PCA to assess morphological distances or overlaps among the populations.

Material and methods

Sampling. Between 2006 and 2013, two of us (MI, MP) and Enrico Lana, partly assisted by other Italian biospeleologists, hand-collected more than 40 *Eukoenenia* specimens (Fig. 1), including fragments and mouldy cadavers, in six caves in the Province of Cuneo, Piemonte, Italy. The caves (we use this term collectively for large subterranean cavities) were situated south of the river Varaita in the mountains that form the interior of the southern West-Alpine arc. The greatest distance between any two caves was about 53 km as the crow flies (Fig. 2).

In the text we refer to each cave and its *Eukoenenia* population with a single designation. The list below gives the designation, the full name, the cadastre number (if available) and the municipality, followed by the Alpine sector (termed according to Marazzi 2001), the altitude at the cave entrance, the geographic coordinates, a brief description, and the palpigrades used for the present study. Information on the caves was partly extracted from Lana *et al.* (2008) and Lana (2013).

Fornaci: Grotta delle Fornaci o di Rossana [1010 Pi/CN], Rossana; Alpi Cozie, 554 m a.s.l., 44°32′02″N, 07°25′52″E. A 195 m long horizontal cave with a small stream, developed in crystalline limestone, below an active quarry at the bottom of the Varaita valley. 3 females (16 Feb. 2012, 15 Aug. 2012, 3 March 2013), 1 male (16 Feb. 2012).

Partigiano: Buco del Partigiano [Pi/CN], Roccabruna; Alpi Cozie, 1170 m a.s.l., 44°30′32″N, 07°17′40″E. A small entrance leads into a talus cave of 15 × 7 m with a drip pool; in non-soluble micaschistic gneiss. 1 female (21 July 2012), 1 juv. female (fragment; 11 Sep. 2011).

Monfieis: Miniera superiore di Monfieis [Pi/CN], Demonte; Alpi Cozie, 1750 m a.s.l., 44°21′44″N, 07°16′00″E. An abandoned multi-level coal mine in Carboniferous-Permian phyllite of 473 m in length, that was in operation from 1870 to 1945. 1 female, 2 males (all 12 Sep. 2010).

Litrôn: Barôn Litrôn (or Maissa 6) [1214 Pi/CN], Valdieri; Alpi Marittime, 1050 m a.s.l., 44°15′47″N, 07°24′37″E. A subterranean "cave-mine" of copper and manganese dug at the end of the 19th century in a karstic

FIGURE 1. Habitus of cave palpigrades of the *Eukoenenia spelaea* complex. From above: *E. spelaea* from Ardovská, Slovakia; *E. strinatii* from Bossea; and *E. lanai* **sp. n.** from Monfieis. Photographs courtesy of Ľubomír Kováč (top) and Enrico Lana.

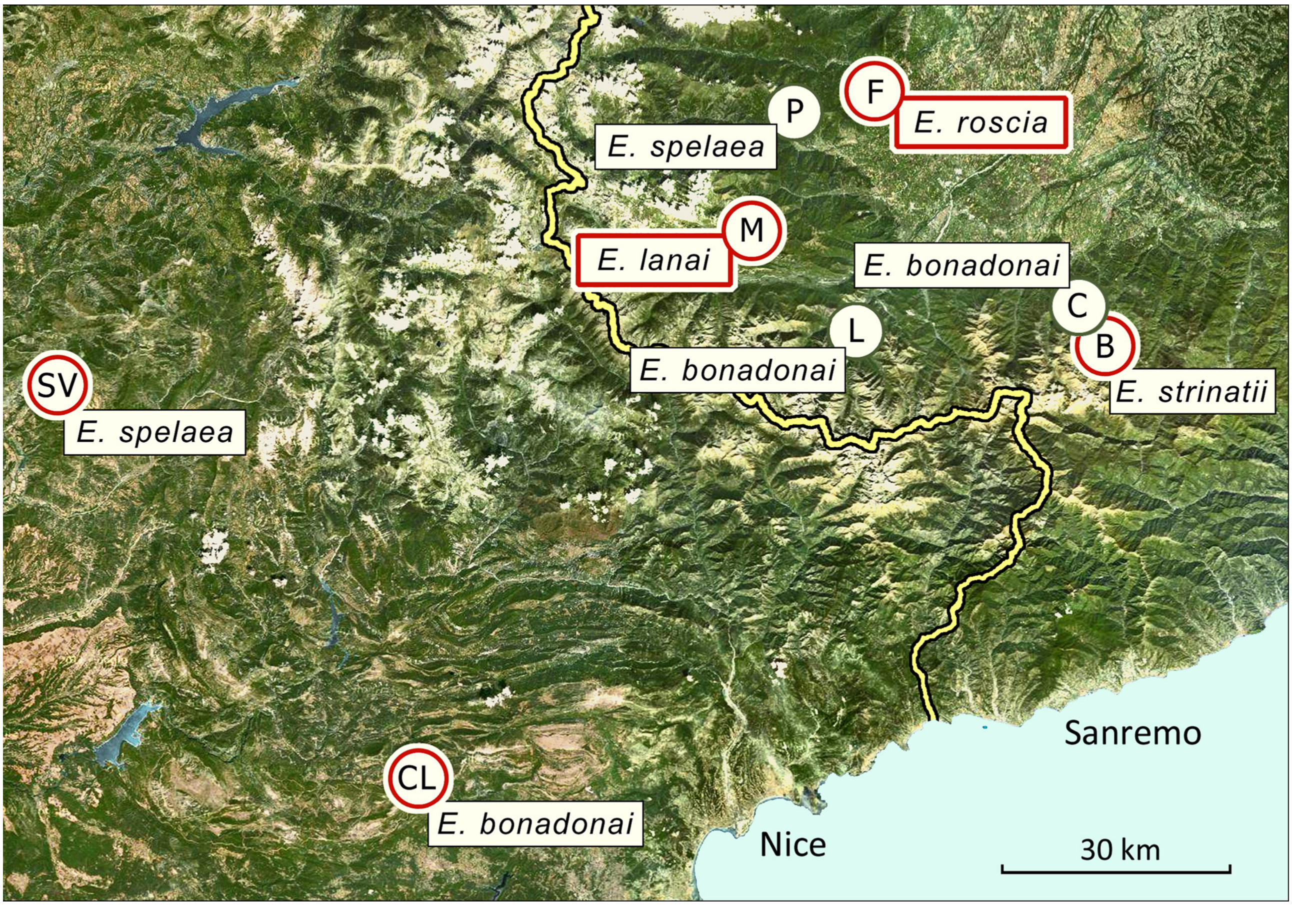

FIGURE 2. Study area and cave populations of palpigrades. B = Bossea, C = Caudano, F = Fornaci, L = Litrôn, M = Monfieis, P = Partigiano. Not sampled for this study: CL = Grotte de la Clue and SV = Grotte de Saint-Vincent-de-Mélan. Type localities and new species are highlighted. Base map © 2012 Google, Cne/Spot Image, Tele Atlas.

area of Upper Cretaceous limestone, forming a 861 m long system. 4 females (3 Dec. 2006 (2), 23 Oct. 2008, 19 March 2009), 2 males (14 May 2009, 24 April 2010), 1 larva (5 June 2012).

Caudano: Grotte del Caudano [121-122 Pi/CN], Frabosa Sottana; Alpi Liguri, 780 m a.s.l., 44°17′40″N, 07°47′25″E). A 3200 m long cave in Middle Triassic limestone, partly run as a show cave. 9 females (8 Nov. 2009 (2), 5 Jan. 2010, 7 April 2011, 17 Oct. 2011, 8 Nov. 2011, 22 Dec. 2011 (2), 22 June 2012), 1 male (22 June 2012), 1 juv. female (5 Jan. 2010).

Bossea: Grotta di Bossea [108 Pi/CN], Frabosa Soprana; Alpi Liguri, 836 m a.s.l., 44°14′31″N, 07°50′27″E. A 2800 m long cave in Middle Triassic limestone, partly run as a show cave. Type locality of *E. strinatii*. 7 females (16 Dec. 2009, 20 Dec. 2009, 21 Dec. 2009, 17 April 2010, 18 Aug. 2010, 11 Jan. 2011) , 4 males (16 Dec. 2009, 17 April 2010, 11 Jan. 2011 (2)), 1 juvenile (mouldy cadaver; 11 Jan. 2011), 1 larva (17 April 2010).

Localities of comparative specimens (not shown on map in Fig. 2):

Ardovská: Ardovská jaskyňa, south of Rožňava, Slovakia; Slovenský kras (Western Carpathians), 314 m a.s.l., 48°31′20″N, 20°25′23″E. A 1600-m long cave developed in Wetterstein limestone of the Silická plateau. 1 female leg. L. Kováč, 5 May 2008, from the population published as *E. spelaea* by Kováč (1999).

Griffen: Griffner Tropfsteinhöhle, Griffen, Austria; Norische Alpen, 490 m a.s.l., 46°42′15″N, 14°43′48″E. A show cave with a passage length of about 200 m in the crystalline limestone of an isolated hill. 1 female, leg. Ch. Komposch, 19 May 2005, published as *E. spelaea vagvoelgyii* by Christian & Komposch (2006). (The assignment to ssp. *vagvoelgyii* appears doubtful in the light of the present investigation.)

Although not sampled for this study, the following type localities of palpigrade species in the French part of the southwestern Alps are also shown on the map in Fig. 2:

Grotte de Saint-Vincent-de-Mélan, near Digne; Alpes de Provence, 1450 m a.s.l., type locality of *E. spelaea* (according to Condé 1956).

Grotte de la Clue, near Séranon; Alpes de Provence, 1170 m a.s.l., type locality of *E. bonadonai* (the identity of the cave is dubious).

Imaging and measuring. Palpigrades from the Italian localities were fixed and stored in 70% ethanol, cleared in Marc André I, and subsequently mounted individually on slides using the water soluble Marc André II medium (Massoud 1967). We studied the specimens under a Nikon E 600 microscope with phase contrast and DIC optics and a measuring eyepiece. Photographs—usually a focus series for image stacking—were taken with a Nikon 1 camera and edited with Photoshop CS5. Auto-blending of the stacks often produced inconsistent images; in these cases we used the "paste in place" function to assemble sharp details from several exposures of the series. Thus it was possible to depict significant structures free of distracting setae and dirt particles. Such images are clearly not impartial photographs, nor do they carry any depth-of-field information. However, the advantage over camera lucida drawings is that details are shown just as they appear under the microscope.

We treated the distal articles of the legs as oblique-ended cylinders. The length of the article (IV bta in Fig. 3 for example) is the longest axis-parallel straight line on the surface of the cylinder. The insertion distance of a seta (see example of d*esd* in Fig. 3) is the length of an axis-parallel line between the most proximal contact point of the seta and the bottom of the cylinder. Since the base of the cylinder is inclined to the axis, setae may have different insertion distances even if they appear inserted at the same level, i.e. on the same imaginary plane perpendicular to the long axis.

Most setae (written in Italics) are termed according to common usage (e.g. Condé 1977). The basal segment of the chelicera has a proximal series of setae, $p_{1\text{-}6}$, and a distal series of aligned setae, $d_{1\text{-}3}$ (terminology according to Christian & Christophoryová 2013). Abbreviations: L = body length; B = length of propeltidium; P = pedipalp; ta = (telo)tarsus; bta = basitarsus; ti = tibia; cx = coxa; I-IV = first to fourth (pair of) leg(s); a = width of the basitarsus immediately distal of the insertion of seta *r*; d*r*, d*grt*, d*esd* = insertion distance of the respective seta; LatO *l* = longest blade of the lateral organ; Prop *l* = longest seta of the propeltidium; Metap = metapeltidium; III cx *c l* = longest thick seta on the coxa of the third leg; III cx *l* = longest seta on the coxa of the third leg; St VI *a l* = longest seta *a* on the sixth sternite; Seg XI *l* = longest seta on the eleventh opisthosomal segment. All measurements are given in micrometers.

Character evaluation. Discrete characters such as trunk chaetotaxy were determined for 40 individuals from Italy (25 females, 10 males, 3 juveniles, 2 larvae) and the 2 non-Italian females. Morphometric data were collected from 29 individuals from Italy (1–9 per locality: 17 females, 10 males, 2 larvae) and the 2 non-Italian females (Tables 1 & 2). The remaining specimens were poorly preserved or overly dirt-encrusted. No male was available from Partigiano or the comparative populations. Since some of the 29 adults used for statistical analysis were damaged, we determined the full set of 42 morphometric measurements and 10 indices for only 79% of the individuals (80% of the males, 79% of females). 15 (36%) of the measurements were obtained in all specimens, most (16 = 38%) other measurements had 2 missing values (range: 1–5 missing values). The highly unequal representation of populations and sexes in the material additionally hindered a thorough statistical treatment. Therefore we used chiefly graphical methods for data analysis.

To render a multivariate analysis possible in spite of missing values, we used multiple imputation to create and analyse five multiply imputed datasets under the fully conditional specification (Buuren 2012). These calculations were performed with the mice 2.13 package (Buuren & Groothuis-Oudshoorn 2011). Since procedures suggested for parameter and model optimization (Buuren 2012) did not improve the results, we used the default settings of the package. We checked for any differences of averages and frequency distributions between original and imputed data, but found none. Furthermore, we did not find any convergence problems of the mice algorithm, and therefore considered the imputation appropriate.

We ran a correlation-based PCA on the measurement imputed data. A scree plot (not shown) indicated that ~80% of the variability was adequately represented by the first two components, so we limited our interpretation to these. All calculations and plots were produced in R 2.15.2 (R Core Team 2012).

Deposition. The type specimens of the new taxa are deposited in the Arachnida collection of the Museum of Natural History, Vienna. The female from Griffen has been returned to the collector. The remaining material is in the collection of the first author.

TABLE 1. Measurements and indices of palpigrade individuals from SW Alpine caves and from two external comparative populations (A, G) of the *Eukoenenia spelaea* species-complex. Abbreviations for species: *bon.* = *E. bonadonai*, *strin.* = *E. strinatii*, *spel.* = *E. spelaea*. Abbreviations for caves: L = Litrôn, B = Bossea, A = Ardovská, G = Griffen, F = Fornaci, P = Partigiano, M = Monfieis. For morphological abbreviations see Material and Methods.

Species	***bon.***	***strin.***	***spel.***	***spel.***		***E. roscia***		***spel.***		***E. lanai***				***E. bonadonai***		
Cave	**L**	**B**	**A**	**G**	**F**	**F**	**F**	**P**	**M**	**M**	**M**	**L**	**L**	**L**	**L**	**L**
Individual	larva	larva	♀	♀	♀01	♀02	♂03	♀04	♀05	♂06	♂07	♀08	♀09	♀10	♂11	♂12
L	1050	1210	1540	1660	1830	1960	1835	1650	2170	2125	2100	1750	1900	1970	—	1735
B	271	277	350	408	418	434	426	394	476	469	456	416	431	441	—	416
P ta3	56	56	64	68	78	80	83	78	84	85	90	89	88	87	—	80
P ta2	52	44	49	52	59	62	59	49	71	70	72	65	63	63	—	61
P ta1	35	38	35	35	41	43	42	36	50	48	53	40	41	46	—	43
P bta2	56	52	74	71	93	94	96	80	107	106	113	92	87	91	—	89
P bta1	48	47	67	68	84	84	85	67	100	100	102	86	79	84	—	83
P ti	105	103	159	154	199	203	199	166	227	222	237	189	184	194	—	192
I ta3	103	113	134	132	179	178	181	153	186	199	192	171	164	164	—	163
I ta2	43	42	51	49	63	63	59	50	70	71	74	60	53	64	—	58
I ta1	21	27	36	34	44	43	50	33	50	53	53	39	42	45	—	42
I bta4	50	50	65	60	86	82	88	61	89	94	97	75	72	76	—	74
I bta3	54	57	76	68	100	102	103	74	110	111	118	86	89	92	—	91
(I) a	31	31	25	32	31	29	30	32	36	36	37	34	35	35	—	34
(I) *r*	53	52	80	75	91	92	97	72	96	99	99	87	86	89	—	88
(I) d*r*	26	29	36	35	42	44	47	40	53	55	62	45	43	46	—	45
(I) *grt*	52	56	76	74	88	89	101	79	92	106	107	79	85	83	—	79
(I) d*grt*	15	13	11	14	16	15	18	12	21	21	20	15	18	18	—	16
I bta1+2	89	94	139	136	181	186	184	137	204	198	192	164	164	170	—	170
I ti	110	111	178	168	243	242	245	176	257	257	277	209	211	224	—	222
II ti	61	69	96	91	122	134	129	100	134	128	140	136	106	113	—	113
III ti	66	65	108	97	139	136	137	106	154	151	156	123	123	129	—	131
IV ta2	54	58	82	77	92	90	99	85	100	101	101	98	81	95	82	99
IV ta1	51	42	63	60	78	76	79	64	84	95	85	77	74	76	65	74
IV bta	73	79	135	123	165	170	169	124	177	194	202	154	154	159	136	157

……*continued on the next page*

TABLE 1. (Continued)

Species	*bon.*	*strin.*	*spel.*	*spel.*	*E. roscia*			*spel.*	*E. lanai*			*E. bonadonai*				
Cave	**L**	**B**	**A**	**G**	**F**	**F**	**F**	**P**	**M**	**M**	**M**	**L**	**L**	**L**	**L**	**L**
(IV) a	24	24	30	27	31	26	31	30	36	35	34	32	29	30	28	32
(IV) *r*	45	41	74	73	86	77	82	69	83	89	91	81	81	80	77	83
(IV) d*r*	33	47	67	63	84	94	88	70	98	107	113	83	84	86	69	81
(IV) *esd*	52	48	82	78	103	102	105	81	109	109	109	96	97	102	88	103
(IV) d*esd*	31	28	48	42	59	60	59	47	74	75	84	56	58	63	56	56
(IV) *grt*	—	—	79	75	96	81	91	69	—	96	107	75	86	85	87	89
(IV) d*grt*	—	—	39	29	57	60	53	30	57	53	66	40	47	44	38	40
IV ti	116	112	185	169	227	232	228	174	242	252	267	213	202	214	182	217
LatO *l*	30	36	37	38	42	44	43	35	49	45	49	43	43	43	—	45
Prop *l*	29	27	39	36	50	54	54	38	53	57	55	50	53	49	—	44
Metap t_1	39	33	51	50	76	82	79	57	74	85	85	74	79	75	—	72
Metap t_2	70	62	96	88	127	135	134	101	132	142	145	110	124	125	—	117
Metap t_3	41	49	62	63	84	100	92	66	85	98	98	81	—	79	—	77
III cx *c l*	24	35	45	46	65	64	64	44	65	74	71	63	64	62	55	57
III cx *l*	116	113	163	161	197	193	196	160	214	222	229	184	197	194	179	190
St VI *a l*	23	36	45	56	64	65	70	52	75	80	83	57	58	56	47	53
Seg XI *l*	69	57	102	108	123	135	124	106	139	149	149	118	131	121	121	118
I bta3 / a	1.74	1.84	3.04	2.13	3.23	3.52	3.43	2.31	3.06	3.08	3.19	2.53	2.54	2.63	—	2.68
I bta3 / *r*	1.02	1.10	0.95	0.91	1.10	1.11	1.06	1.03	1.15	1.12	1.19	0.99	1.03	1.03	—	1.03
I bta3 / d*r*	2.08	1.97	2.11	1.94	2.38	2.32	2.19	1.85	2.08	2.02	1.90	1.91	2.07	2.00	—	2.02
I bta3 / ti	0.49	0.51	0.43	0.40	0.41	0.42	0.42	0.42	0.43	0.43	0.43	0.41	0.42	0.41	—	0.41
I bta3 / B	0.20	0.21	0.22	0.17	0.24	0.24	0.24	0.19	0.23	0.24	0.26	0.21	0.21	0.21	—	0.22
IV bta / a	3.04	3.29	4.50	4.56	5.32	6.54	5.45	4.13	4.92	5.54	5.94	4.81	5.31	5.30	4.86	4.91
IV bta / *r*	1.62	1.93	1.82	1.68	1.92	2.21	2.06	1.80	2.13	2.18	2.22	1.90	1.90	1.99	1.77	1.89
IV bta / d*r*	2.21	1.68	2.01	1.95	1.96	1.81	1.92	1.77	1.81	1.81	1.79	1.86	1.83	1.85	1.97	1.94
IV bta / ti	0.63	0.71	0.73	0.73	0.73	0.73	0.74	0.71	0.73	0.77	0.76	0.72	0.76	0.74	0.75	0.72
IV bta / B	0.27	0.29	0.39	0.30	0.39	0.39	0.40	0.31	0.37	0.41	0.44	0.37	0.36	0.36	—	0.38

TABLE 2. Measurements and indices of palpigrade individuals of the *Eukoenenia spelaea* species-complex from two SW Alpine caves. Abbreviations for caves: C = Caudano, B = Bossea. For morphological abbreviations see Material and Methods.

Species			***E. bonadonai***								***E. strinatii***				
Cave	**C**	**C**	**C**	**C**	**C**	**C**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**
Individual	♀13	♀14	♀15	♀16	♀17	♂18	♀19	♀20	♀21	♀22	♀23	♂24	♂25	♂26	♂27
L	1630	1600	1680	1690	1670	1705	2040	1980	1875	1580	—	2010	2025	—	1980
B	415	406	408	411	403	408	454	446	453	430	—	437	451	—	441
P ta3	80	78	78	75	76	76	89	90	89	86	—	81	81	—	84
P ta2	61	58	62	60	60	62	64	71	64	62	—	67	64	—	64
P ta1	44	41	42	44	43	42	47	48	44	44	—	48	49	—	44
P bta2	90	85	89	84	88	86	106	103	107	103	—	105	106	—	104
P bta1	81	80	77	78	76	81	93	94	89	90	—	88	91	—	95
P ti	186	176	171	176	184	173	212	206	209	210	—	207	216	—	217
I ta3	168	166	154	156	169	165	181	188	184	190	193	176	179	—	189
I ta2	59	57	58	63	58	61	64	67	64	64	62	63	68	—	64
I ta1	41	37	38	39	43	48	45	48	42	42	42	44	47	—	46
I bta4	71	68	74	68	64	68	90	91	87	85	89	87	85	—	91
I bta3	86	84	84	82	86	83	118	124	115	111	108	114	107	—	113
(I) a	34	35	34	33	33	31	35	35	33	32	32	37	34	—	32
(I) *r*	88	89	80	88	82	81	95	93	98	90	92	99	92	—	100
(I) d*r*	45	36	41	38	41	41	65	66	62	59	54	63	58	—	65
(I) *grt*	85	79	75	79	80	80	84	87	81	81	80	90	86	—	74
(I) d*grt*	18	16	18	15	17	18	12	13	16	8	20	13	11	—	21
I bta1+2	163	164	151	156	162	163	193	198	197	193	186	197	197	—	202
I ti	209	206	204	204	209	206	255	254	251	247	232	247	255	—	257
II ti	116	111	111	106	112	115	136	136	132	129	116	131	131	—	132
III ti	136	134	126	121	125	128	153	151	151	145	141	156	156	—	159
IV ta2	89	86	84	89	89	86	100	106	95	98	96	103	98	104	101
IV ta1	74	74	68	70	71	72	79	78	70	76	70	81	83	81	80
IV bta	151	149	146	144	147	148	183	193	181	183	176	194	189	194	200
(IV) a	29	30	28	26	30	29	32	31	30	30	28	32	29	33	33

……continued on the next page

TABLE 2. (Continued)

Species			*E. bonadonai*								*E. strinatii*				
Cave	**C**	**C**	**C**	**C**	**C**	**C**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**	**B**
(IV) *r*	81	78	74	72	75	74	89	86	81	81	86	95	82	84	89
(IV) d*r*	84	78	81	83	81	88	103	117	110	105	104	110	109	113	105
(IV) *esd*	92	93	90	95	99	95	103	97	96	98	96	99	105	106	109
(IV) d*esd*	56	53	59	53	55	58	86	94	83	75	81	91	78	93	89
(IV) *grt*	83	85	79	82	93	90	100	95	87	98	85	108	97	98	103
(IV) d*grt*	42	39	45	40	37	41	49	68	53	50	49	65	43	63	21
IV ti	214	204	205	202	205	206	247	242	239	237	228	249	255	241	253
LatO *l*	43	44	47	42	48	44	51	48	48	53	49	49	50	—	53
Prop *l*	43	43	42	39	42	42	43	42	38	39	—	42	41	—	37
Metap t_1	74	70	65	68	—	70	70	67	65	66	—	64	72	—	68
Metap t_2	126	117	116	116	—	119	126	128	126	123	—	127	126	—	134
Metap t_3	79	79	74	74	—	77	89	80	81	86	—	91	85	—	88
III cx *c l*	61	58	59	55	58	62	65	64	64	64	65	66	64	—	66
III cx *l*	192	189	184	176	198	180	204	207	207	207	194	199	209	—	214
St VI *a l*	58	54	53	50	61	47	65	65	63	68	66	69	68	61	67
Seg XI *l*	129	125	115	116	—	115	127	132	125	136	—	131	134	134	146
I bta3 / a	2.53	2.40	2.47	2.48	2.61	2.68	3.37	3.54	3.48	3.47	3.38	3.08	3.15	—	3.53
I bta3 / *r*	0.98	0.94	1.05	0.93	1.05	1.02	1.24	1.33	1.17	1.23	1.17	1.15	1.16	—	1.13
I bta3 / d*r*	1.91	2.33	2.05	2.16	2.10	2.02	1.82	1.88	1.85	1.88	2.00	1.81	1.84	—	1.74
I bta3 / ti	0.41	0.41	0.41	0.40	0.41	0.40	0.46	0.49	0.46	0.45	0.47	0.46	0.42	—	0.44
I bta3 / B	0.21	0.21	0.21	0.20	0.21	0.20	0.26	0.28	0.25	0.26	—	0.26	0.24	—	0.26
IV bta / a	5.21	4.97	5.21	5.54	4.90	5.10	5.72	6.23	6.03	6.10	6.29	6.06	6.52	5.88	6.06
IV bta / *r*	1.86	1.91	1.97	2.00	1.96	2.00	2.06	2.24	2.23	2.26	2.05	2.04	2.30	2.31	2.25
IV bta / d*r*	1.80	1.91	1.80	1.73	1.81	1.68	1.78	1.65	1.65	1.74	1.69	1.76	1.73	1.72	1.90
IV bta / ti	0.71	0.73	0.71	0.71	0.72	0.72	0.74	0.80	0.76	0.77	0.77	0.78	0.74	0.80	0.79
IV bta / B	0.36	0.37	0.36	0.35	0.36	0.36	0.40	0.43	0.40	0.43	—	0.44	0.42	—	0.45

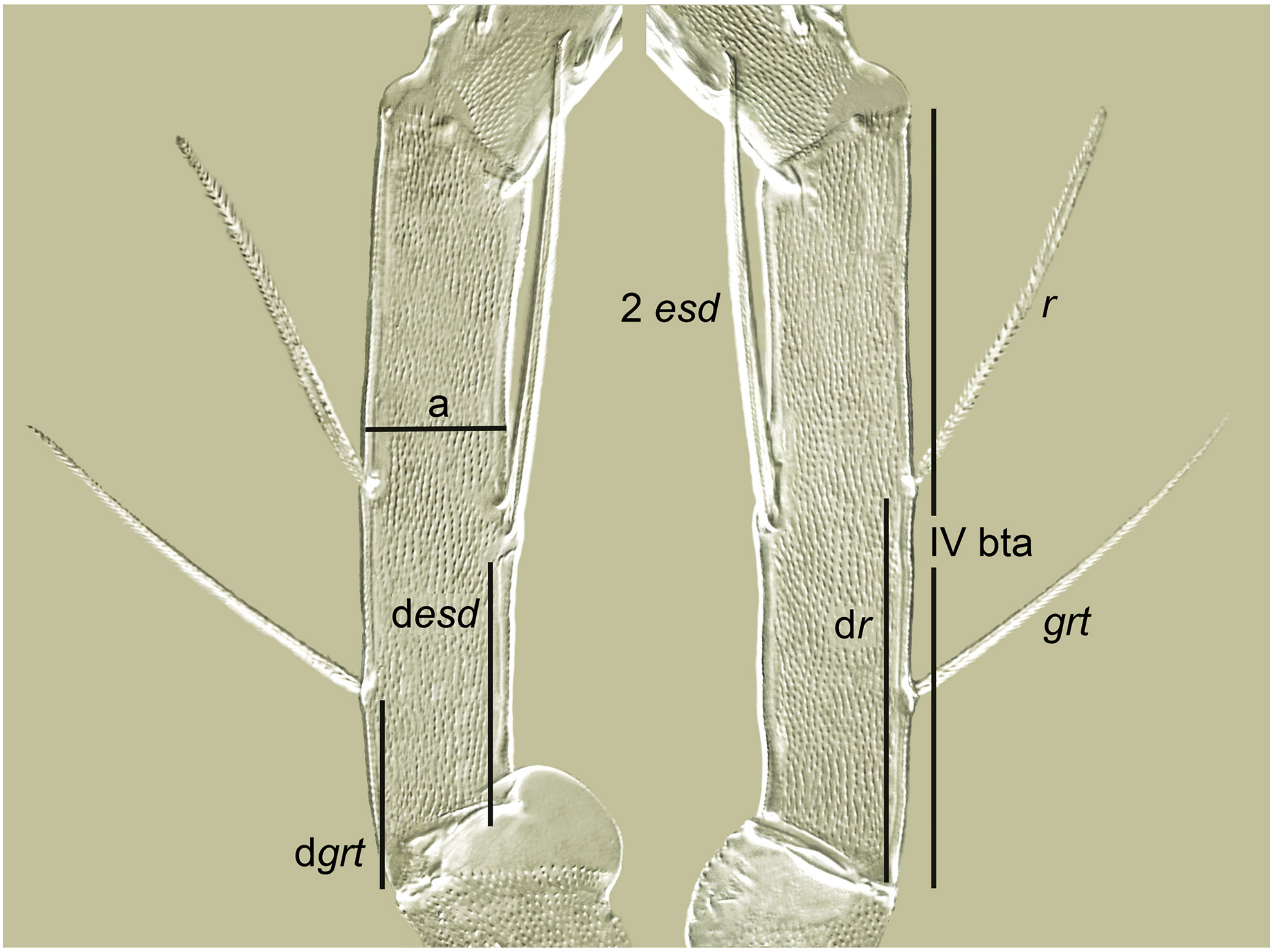

FIGURE 3. Basitarsi of the fourth pair of legs of a *E. bonadonai* ♂ from Litrôn, showing setal terminology and measurements (see Material and Methods). Scale: IV bta = 157 µm.

Adult morphology: similarities and differences between the populations

Unless otherwise stated, all our palpigrades share the particular phenotypic character in common. Continuous variables are compiled in Tables 1 & 2. Species assignments are discussed in the final section. For clarity, we employ names of new taxa that are formally described in the systematics section below.

General appearance. Midsized to large palpigrades of troglomorphic habitus which is less pronounced in the Partigiano and the non-Italian individuals, i.e. in the populations assigned to *E. spelaea*. A narrowing between the opisthosomal segments VIII and IX is always clearly visible. Living animals show a yellowish or reddish hue, particularly on the opisthosoma (Fig. 1). The pubescence is almost uniformly short; certain body parts are densely pubescent, while others are nearly glabrous.

Chelicerae. First segment with a proximal series of 6 setae (setae p_4 and p_6 thickened and densely barbed), a distal series of 3 aligned setae (d_3 very strong and barbed, more than twice the length of d_1), and 1 apical seta. Hand with 1 ventral and 6 dorsal setae. Each finger has 8 teeth (Fig. 4).

Frontal organ. Mounting often causes deformation of the frontal organ. The length of the blades is more reliably determined than the total length. No population-specific differences in relative length, shape and reticulation of the consistently lanceolate blades are evident.

Lateral organ. No variation in shape and reticulation of the pointed blades is evident (Fig. 5), but mounting artefacts are common. In relation to propeltidium length, the blades are slightly shorter in *E. spelaea* from Partigiano and Griffen. The number of blades varies among the populations (Fig. 6).

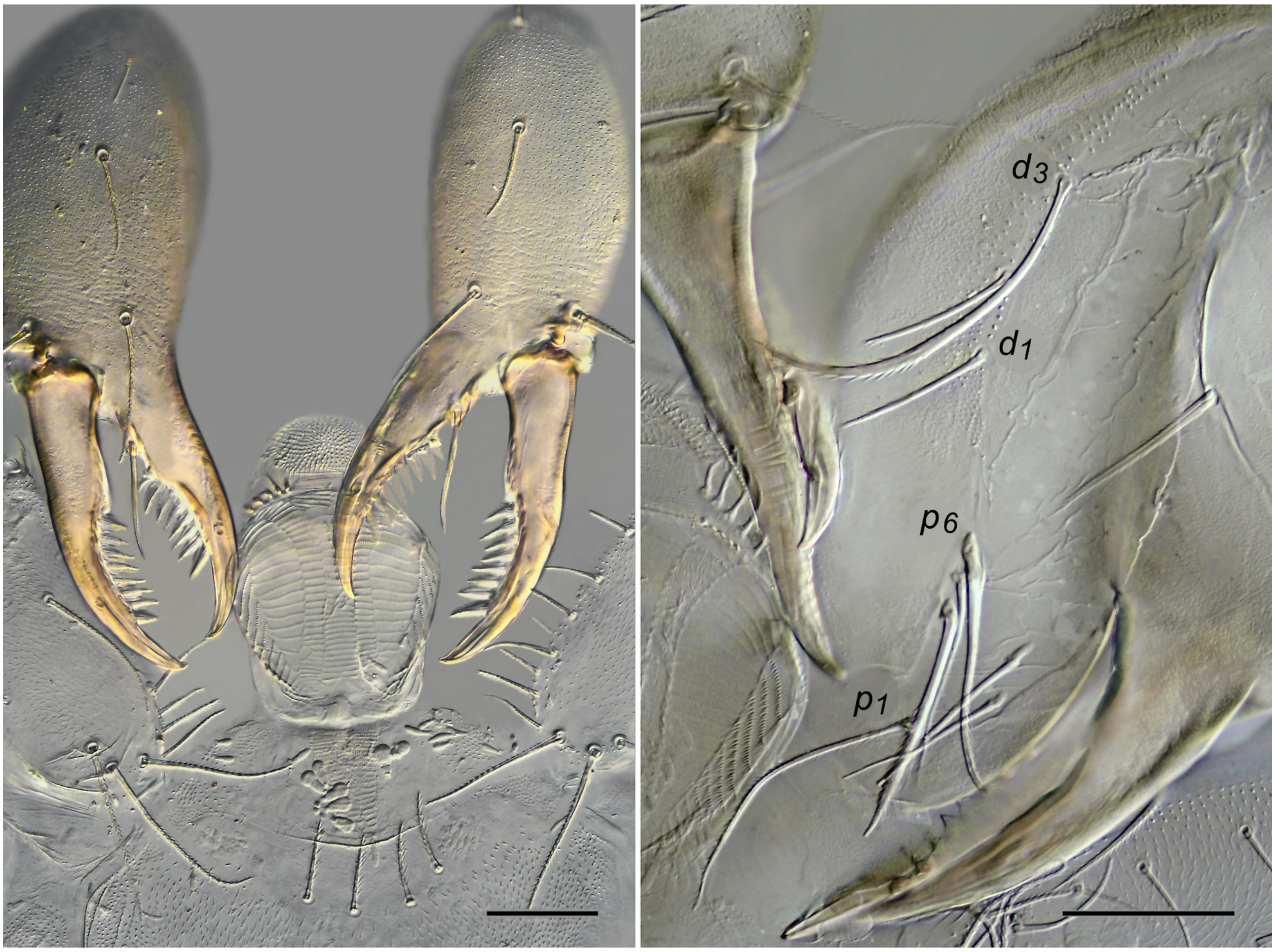

FIGURE 4. Left: chelicerae, mouth cone, parts of the pedipalp coxae, and deuto-tritosternum. This *E. bonadonai* ♂ from Caudano is exceptional in having only four setae on the deuto-tritosternum. Right: first cheliceral article of a *E. bonadonai* ♀ from Litrôn. Six setae in proximal series *p* (p_4 and p_6 thick) and three setae in distal series *d*; apical seta of the article not shown. Scale bars: 50 µm.

Mouth cone. The labrum is evenly rounded and densely pubescent. Five short setae are aligned on either side of the oral fissure. The labium shows the usual cuticular ornament (Fig. 4).

Deuto-tritosternum. On the deutosternal area a submedian strip of parallel, transverse ridges is visible. It ends at a bent edge that possibly marks the border between deuto- and tritosternum (Fig. 4). Behind this structure are usually 5 setae, arranged in a wide V-shape in a glabrous field. Numerical variants are not infrequent. *E. roscia* (Fornaci): three of four individuals with 6; *E. bonadonai* (Caudano): one of seven with 6, another one with 4 (Fig. 4); *E. strinatii* (Bossea): one of eight with 3 setae.

Propeltidium. All our specimens have 10 pairs of setae on the dorsal shield. The shortest setae are in submedian position, the longest ones laterally in the posterior half. In a teratological case (*E. strinatii*) an additional seta has a very strange shape (Fig. 7).

Metapeltidium. The 3+3 setae of the metapeltidium are of different lengths: always $t_2 >> t_3 > t_1$.

Coxal chaetotaxy. It is virtually impossible to bring all appendages of an entire palpigrade specimen into optimal viewing positions under the cover glass. Some rolling of the coxae around the longitudinal axes is inevitable, and since every torsion changes the aspect of the setal arrangement, it is pointless to prescribe a standard view. Therefore Fig. 8 assembles arbitrary projections. The diagram is based on the length, the approximate shape and the insertion distance of the setae.

Recent descriptions and revisions of *Eukoenenia* species (Barranco & Mayoral 2007, Barranco & Harvey 2008, Souza & Ferreira 2010, 2011a, 2011b, Ferreira *et al.* 2011, Mayoral & Barranco 2013) reveal differences in the number of ordinary and, when distinguished, thick setae on the coxae of the pedipalp and legs. All palpigrades under study, including the specimens from Slovakia and Austria, are equipped with ordinary and thick setae as shown in Fig. 8. The pedipalp coxa carries 19 setae; even the longest ones, near the base, are of moderate size compared to the macrosetae of cx I–III. The coxae of legs I–IV bear 15–14–13–9 setae respectively, including 0–4–4–1 thick setae. The thick coxal setae are inserted along a straight line on cx II and cx III (Fig. 9). They are cylindrical, rather than conical, and carry at the blunt tip an obliquely projecting spine that is stronger than the barbs on the shaft, as do certain setae on other podomeres. Thick coxal setae thus resemble *a*-setae of the opisthosomal sternites IV–VI. On cx I–III, the setae differ strikingly in size: cx I has 1 macroseta and 3 microsetae; cx II has 2 contiguous macrosetae of similar length; cx III has 2 macrosetae (the distinctly longer one is the longest seta of the entire body), and 1 microseta near the base.

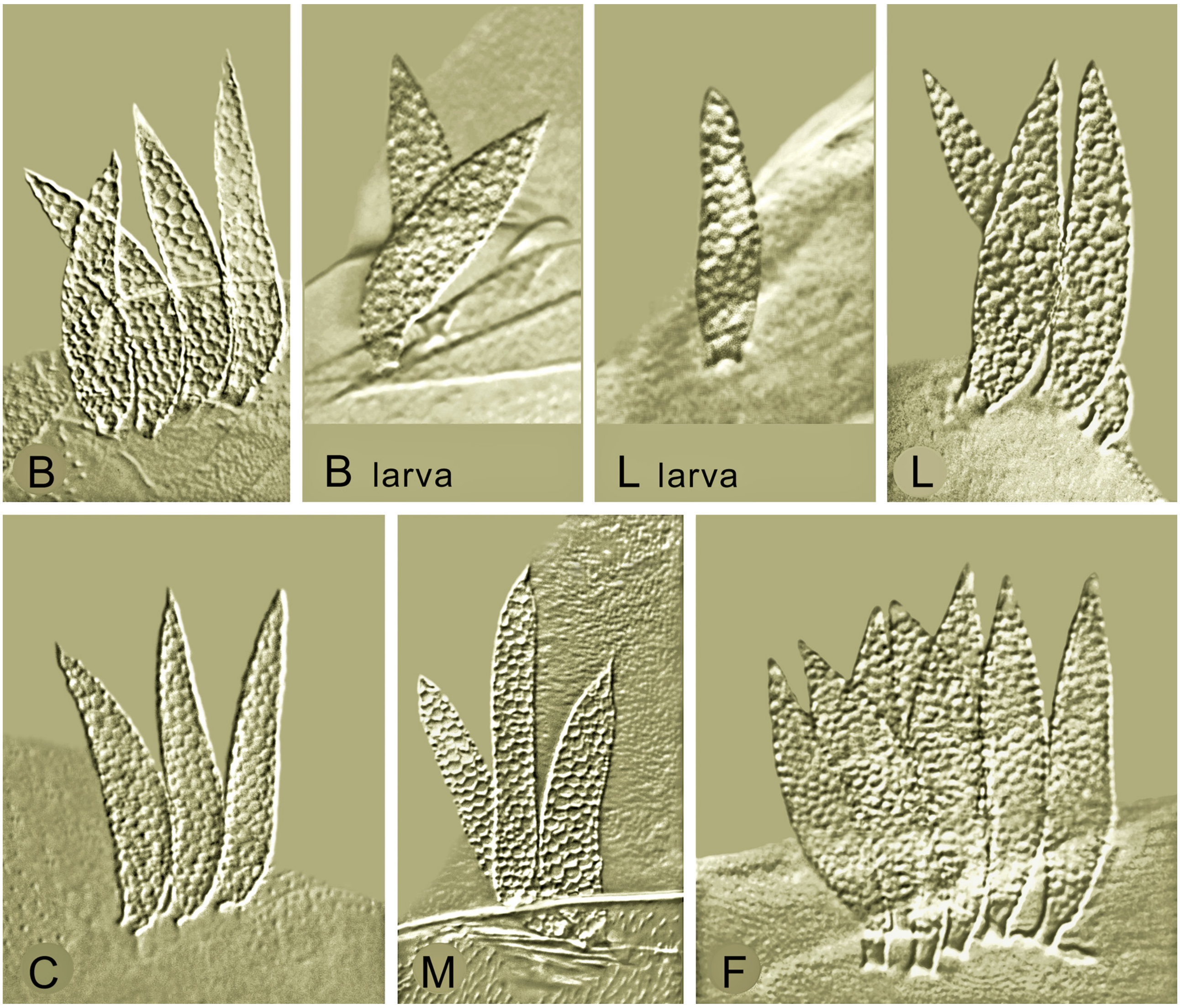

FIGURE 5. Lateral organ. B: Bossea ♀ (*E. strinatii*), L: Litrôn ♂ (*E. bonadonai*), C: Caudano ♀ (*E. bonadonai*), M: Monfieis ♀ (*E. lanai* **sp. n.**), F: Fornaci ♀ (*E. roscia* **sp. n.**). Images enlarged to same size to facilitate comparison. The blades are 40–50 µm long.

Modified setae on pedipalp and foreleg. The pedipalp has, near the tip of ta3, a curved seta (*cs*) with a conspicuous smooth spine that branches off quite a distance above the base. Christian *et al.* (2012) referred to it as a "long forked seta", and indeed it looks like a large and very asymmetric forked seta (*fs*). However, a narrower definition of a forked seta appears more appropriate. In the sense of the term adopted here (*Gabelhaare*: Börner 1901; *bifurcated hairs*: Rucker 1903), a forked seta bifurcates near the base into a barbed and a smooth branch of similar length. According to this definition, there is only one *fs* on P ta3. It is inserted between *cs* and a seta with a distinct basal spine. The proximal half of P ta3 bears a rod seta (*rs*).

These types of modified setae are also present on ta3 of leg I (Fig. 10). Four forked setae are arranged as 1+1+2 (from proximal to distal). The most proximal one, termed fs_1, can be inserted from slightly behind to slightly in front of *rs*, even within a population. The length ratio fs_1/rs, however, is rather constant within populations and varies between populations. In 5 of the 6 Italian populations fs_1 and *rs* are approximately of the same length, as in the Griffen female. Only in *E. lanai* from Monfieis is the *rs* twice the length of fs_1, as in the Ardovská female. Also variable is the position of fs_2; it is most often, but not always, inserted closer to the macroseta *m* (which always has a small basal spine) than to *cs*, without obvious differences between the populations. The remaining forked setae of the last tarsal article, fs_3 and fs_4, are situated in close proximity to *cs*, which has no basal spine.

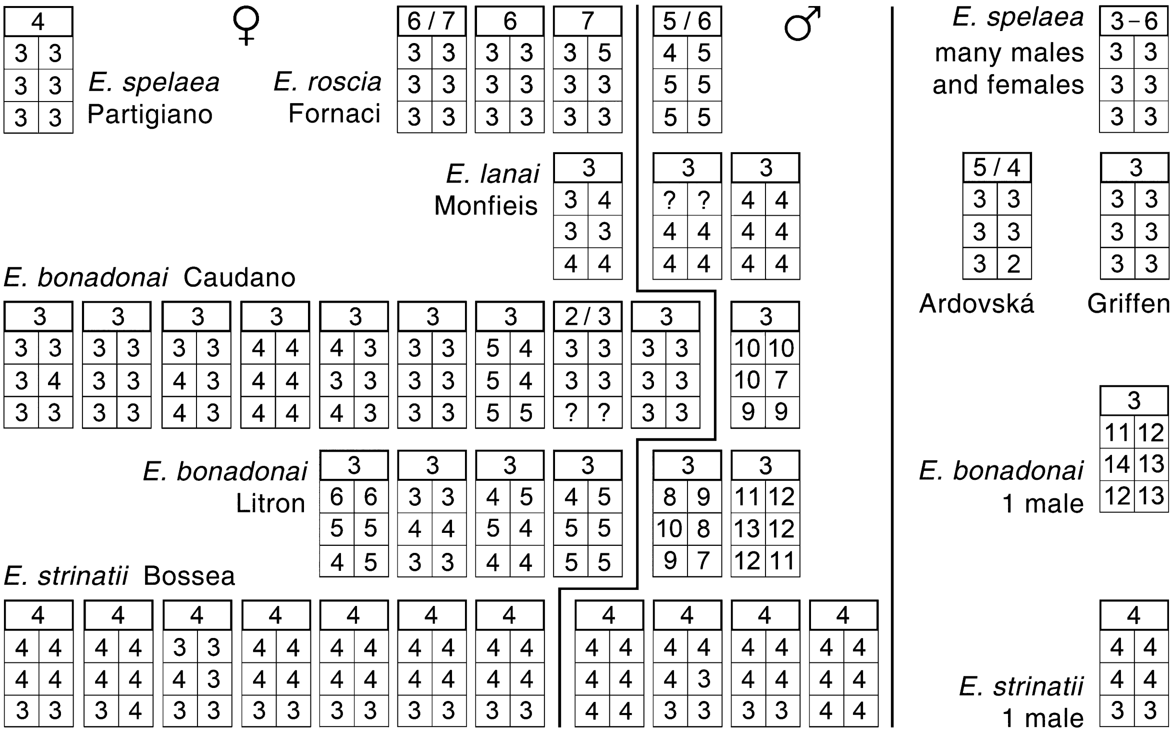

FIGURE 6. Lateral organ and opisthosomal chaetotaxy of *Eukoenenia* specimens from the six Italian cave populations. The right column shows the values for published specimens and the two comparative females (Ardovská, Griffen). The top line of each box gives the number of blades in the lateral organs (one digit, if symmetric), the three lines below give the numbers of setae *a* on sternites IV–VI (left | right).

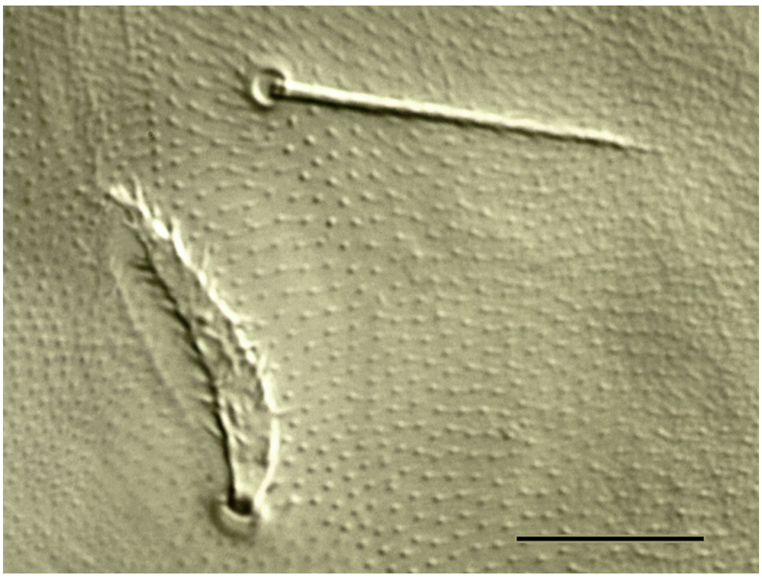

FIGURE 7. Propeltidium. Normal (above) and teratological seta of a *E. strinatii* ♀ from Bossea. Scale bar: 20 µm.

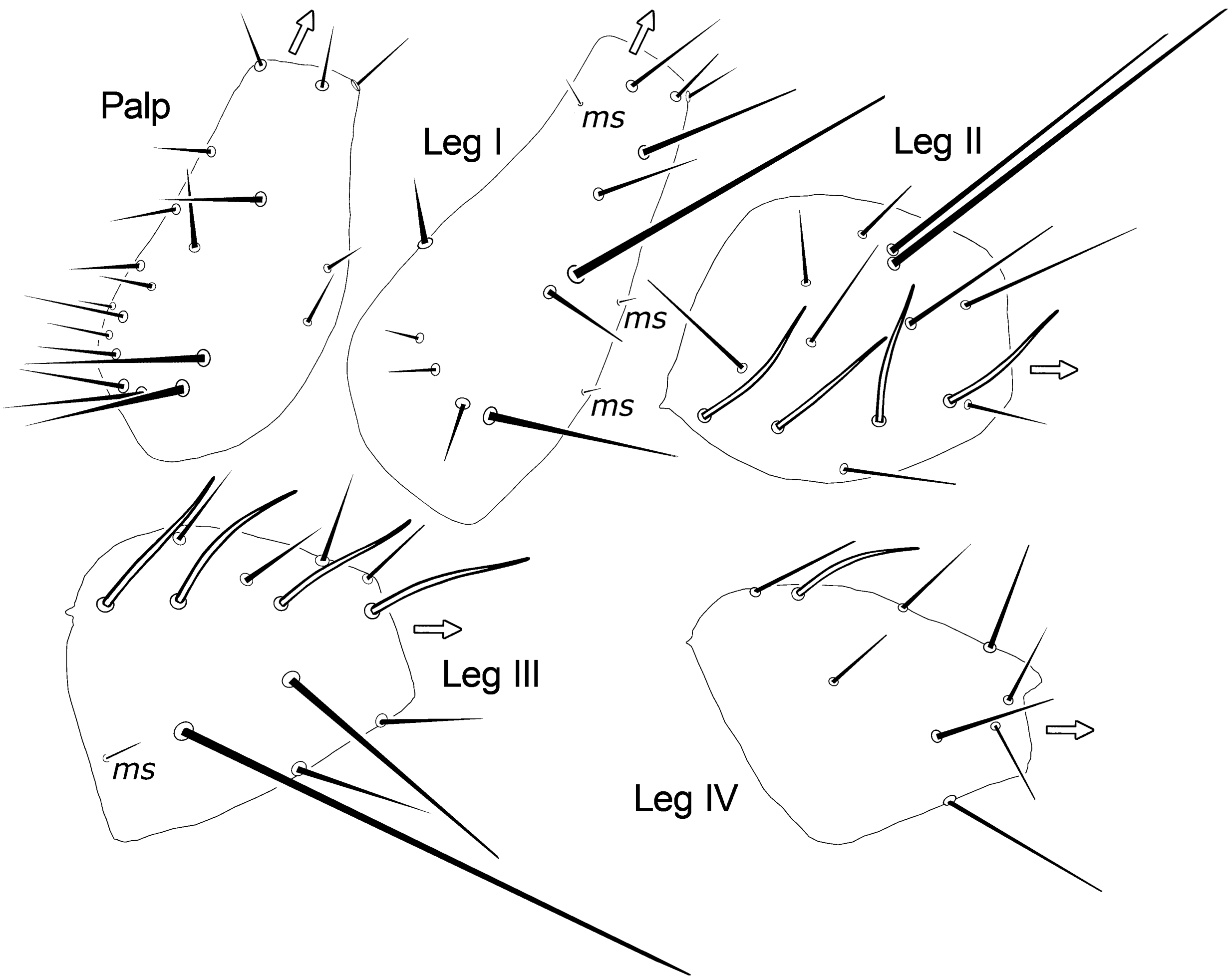

FIGURE 8. Diagram of coxal chaetotaxy in the *E. spelaea* complex. Ordinary setae in solid black, thick setae as contours, *ms* = microseta. Arrows point distad.

The articles ta2, bta2 and bta1 of leg I each carry a single *fs*. *E. spelaea* from Partigiano and Griffen (but not from Ardovská!) and *E. roscia* from Fornaci (Fig. 11) have also one *fs* on I bta4, a feature that seems to be randomly distributed in *Eukoenenia* species throughout the world. Since a *fs* on I bta4 has also been found in species of other palpigrade genera (e.g. *Koeneniodes spiniger* Condé, 1984), its presence is probably the plesiomorphic state. Remarkably, this character does not vary within the populations.

The 7 trichobothria of leg I are arranged as usual with *Eukoenenia*.

Third basitarsus of the foreleg. I bta3 carries 3 setae, namely from base to tip *grt*, *r*, and one microseta. Visual inspection (Fig. 12) and indices (I bta3/a and I bta3/B: Fig. 25) reveal differences in the shape of this article. In relation to propeltidium length, I bta3 is longest in *E. strinatii*, *E. roscia* and *E. lanai* (in *E. strinatii* also in relation to tibia and seta *r*). I bta3 is most slender in *E. strinatii* and *E. roscia*. At the other end of the spectrum, *E. spelaea* from Partigiano and Griffen and *E. bonadonai* (Litrôn, Caudano) show a relatively short and wide I bta3. In Partigiano and the comparative specimens (all *E. spelaea*) the tip of seta *r* comes closest to the insertion of the trichobothrium on I bta4.

Basitarsus of leg IV. The character states (Figs 13 and 24) parallel those of I bta3. Intra-population variability of the insertion distances of the 4 setae (*grt*, *r* and 2 *esd*) is considerable (see IV bta/d*r* in Fig. 25). *E. strinatii*, *E. roscia* and *E. lanai* have a long and slender IV bta. In Partigiano and the comparative specimens (all *E. spelaea*) this article is conspicuously wide. *E. bonadonai* from Litrôn and Caudano are very much alike, lying in the middle of the spectrum.

Opisthosomal chaetotaxy. The setation of the anterior tergites is almost uniformly t_1, t_3, *s* on II, and t_1, t_2, t_3, *s* on III–VI. Seta t_2, which is inserted slightly behind the line t_1–t_3, is absent in a few individuals: one *E. roscia* female from Fornaci (III left & right and IV right), one *E. bonadonai* female from Caudano (III right and V left & right) and two *E. strinatii* females from Bossea (both III right). The instability of t_2 is noteworthy, since Condé (1974) distinguished *E. spelaea hauseri* on the lack of this seta on tergites III–VI. This subspecies occupies the southeastern end of the species range. It is known from caves between the Gulf of Trieste, the Kočevje region in Slovenia and the Croatian Lika.

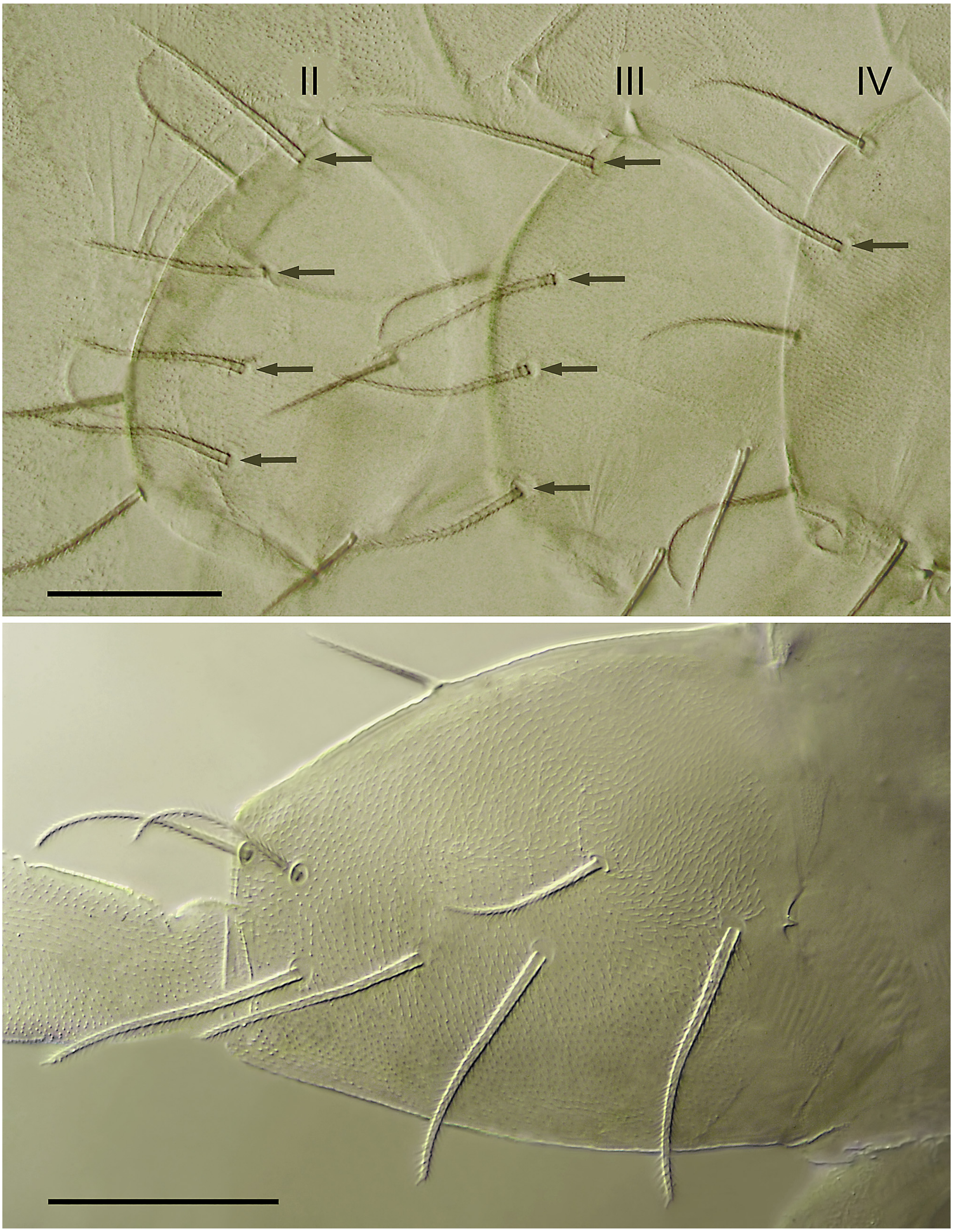

FIGURE 9. Above: thick setae (arrows) on the coxae of the legs II–IV of a *E. roscia* **sp. n.** ♀ from Fornaci. Below: coxa of leg III of a *E. strinatii* ♀ from Bossea; the four aligned thick setae and some ordinary setae are in focus. Scale bars: 50 µm.

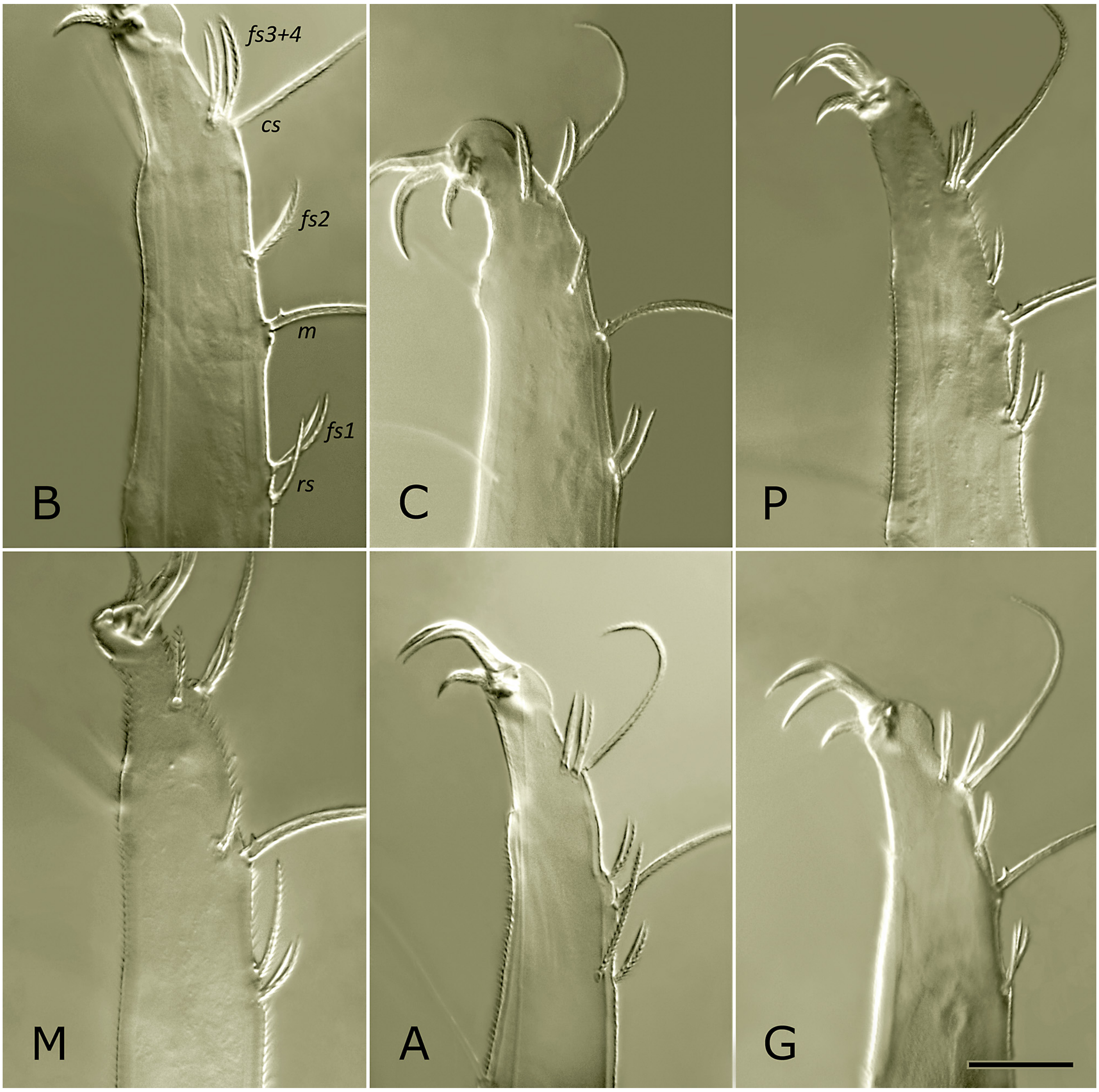

FIGURE 10. Tip of leg I. Modified setae: *rs* = rod seta; *fs* = forked seta; *m* = macroseta; *cs* = curved seta; the remaining setae are out of focus. B: Bossea ♀ (*E. strinatii*), C: Caudano ♀ (*E. bonadonai*), P: Partigiano ♀ (*E. spelaea*), M: Monfieis ♀ (*E. lanai* **sp. n.**), A: Ardovská ♀ (*E. spelaea*), G: Griffen ♀ (*E. spelaea*). Scale bar: 20 µm.

Compared to the tergites, the chaetotaxy of the opisthosomal sternites IV–VI shows much more variation (Figs 6 and 14). Each half-sternite of the female carries 3–6 setae *a* (that of the male even more in certain populations), followed by s_1 and s_2. Seta s_2 is lacking in one *E. bonadonai* female from Caudano (VI right) and three *E. strinatii* females from Bossea (IV left; IV left & right and V left; IV left & right). The instability of the setae *s* corresponds with Condé's (1972) observation on a female of *E. spelaea* from Austria. Compared to the females, the few available males of *E. roscia* (Fornaci) and *E. lanai* (Monfieis) have a slightly higher number of setae *a*. The numerical increase of setae *a* in *E. bonadonai* males from Caudano and Litrôn (Fig. 14 C and L) is such that it leads to a striking sexual dimorphism. The relative length of setae *a* differs among the populations, as shown by the ratio (St VI *a l*)/B. The values or averages are 0.129 (*E. spelaea* from Ardovská), 0.132 (*E. spelaea* from Partigiano, *E. bonadonai* from Litrôn and Caudano), 0.137 (*E. spelaea* from Griffen), 0.150 (*E. strinatii* from Bossea), 0.156 (*E. roscia* from Fornaci), and 0.170 (*E. lanai* from Monfieis). Apart from Partigiano, which is represented by a single specimen of *E. spelaea*, the Italian populations are heterogeneous in terms of sternite IV–VI chaetotaxy; they include individuals with more than 3+3 setae *a* on one or more of these sternites. A pair of submedian circular structures—probably gland orifices—is always visible behind the line a_1–a_1 on sternites IV–VI.

The number of setae on segments VII–XI is given in Table 3. Due to considerable intra-population variability, the only pattern to emerge is a numerical reduction of setae in the more southern populations of Litrôn and Caudano (*E. bonadonai*) and Bossea (*E. strinatii*). Remarkable is the perfect match of Partigiano and Griffen. These two females conform, in the setation of VIII–XI, to the numbers given by Condé (1956) for the male type specimen of *E. spelaea*.

TABLE 3. Number of setae on opisthosomal segments VII–XI. C = Caudano; other abbreviations of caves as in Table 1.

	VII	VIII	IX	X	XI		VII	VIII	IX	X	XI
E. spel. ♀ A	11	14	11	12	10	*E. bon.* ♀ C	14	12	9	11	9
E. spel. ♀ G	15	15	12	12	10	*E. bon.* ♀ C	?	?	11	12	9
E. roscia ♀ F	16	16	12	12	10	*E. bon.* ♀ C	13	13	10	11	9
E. roscia ♀ F	15	16	12	11	10	*E. bon.* ♀ C	12	12	9	11	9
E. roscia ♂ F	13	16	12	12	10	*E. bon.* ♀ C	14	12	9	11	9
E. spel. ♀ P	15	15	12	12	10	*E. bon.* ♂ C	?	14	11	12	9
E. lanai ♀ M	13	16	12	12	9	*E. strin.* ♀ B	10	14	10	12	9
E. lanai ♂ M	13	14	12	12	10	*E. strin.* ♀ B	12	13	10	12	9
E. lanai ♂ M	17	16	12	11	10	*E. strin.* ♀ B	11	12	10	11	9
E. bon. ♀ L	?	14	12	11	9	*E. strin.* ♀ B	10	13	9	12	10
E. bon. ♀ L	13	13	11	11	9	*E. strin.* ♀ B	?	?	11	11	9
E. bon. ♀ L	12	13	12	11	9	*E. strin.* ♂ B	10	12	9	12	8
E. bon. ♂ L	14	16	12	11	10	*E. strin.* ♂ B	?	?	10	11	9
E. bon. ♂ L	14	16	12	11	9						

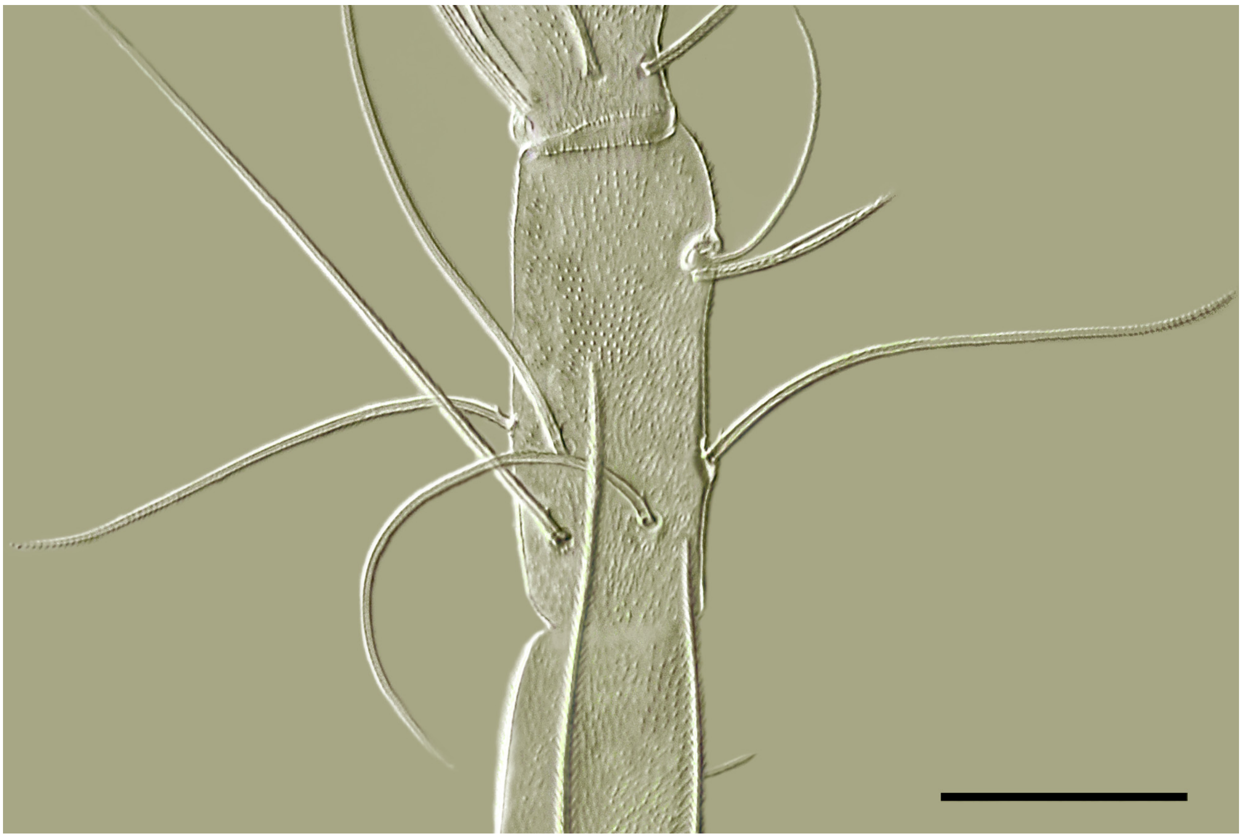

FIGURE 11. Chaetotaxy of I bta4 of the Fornaci ♂ (*E. roscia* **sp. n.**): 5 macrosetae, 1 forked seta, 1 trichobothrium. Scale bar: 20 µm.

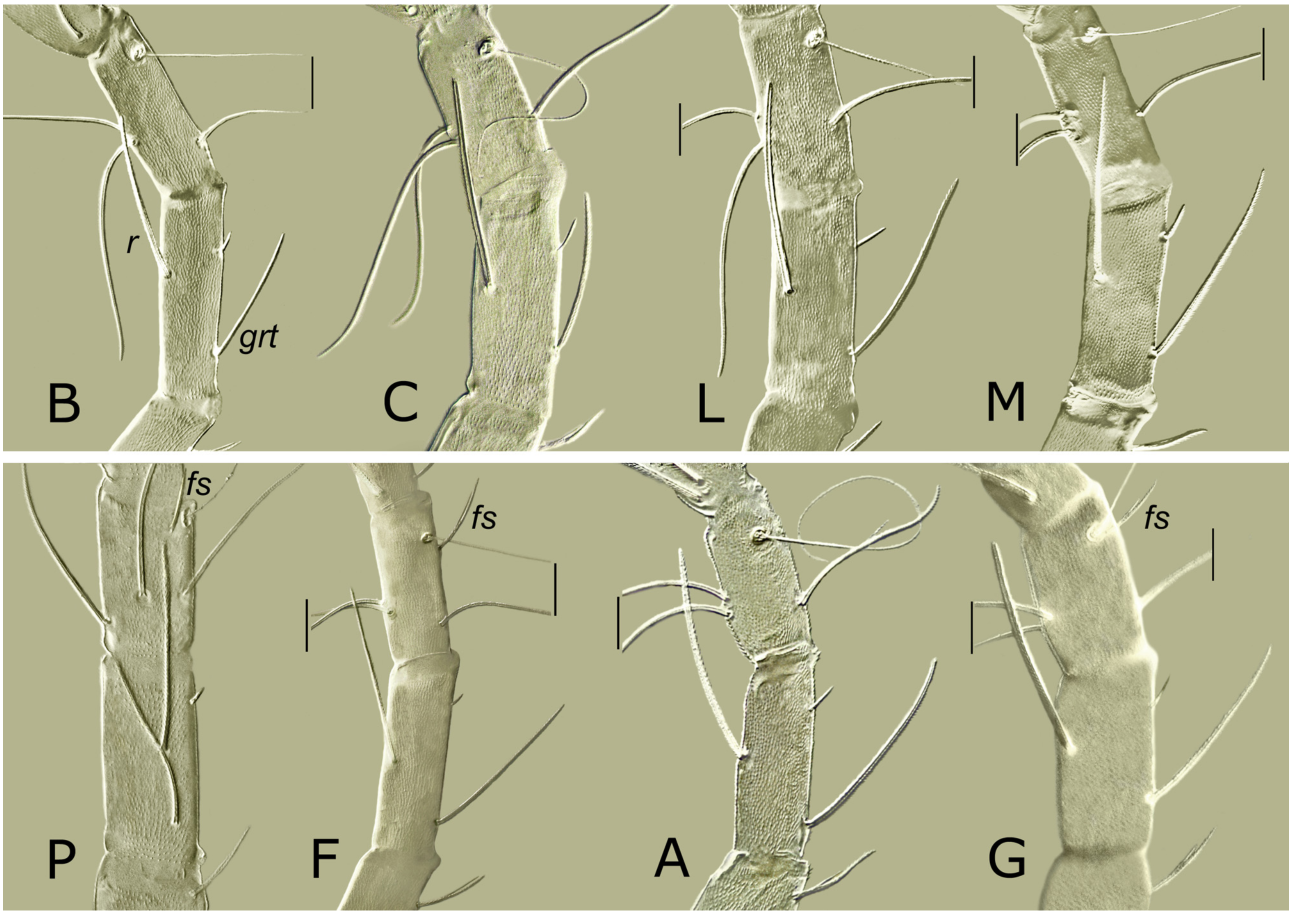

FIGURE 12. Third and fourth basitarsus of leg I. For better comparison of the shape, the third basitarsi are all enlarged to the same length. B: Bossea ♂ (*E. strinatii*), C: Caudano ♂ (*E. bonadonai*), L: Litrôn ♂ (*E. bonadonai*), M: Monfieis ♂ (*E. lanai* **sp. n.**), P: Partigiano ♀ (*E. spelaea*), F: Fornaci ♀ (*E. roscia* **sp. n.**), A: Ardovská ♀ (*E. spelaea*), G: Griffen ♀ (*E. spelaea*).

Genital region of female. The shape of the genital lobes is uniform in uncompressed females, but mounting often leads to considerable deformation (compare Fig. 15 to the compressed lobes in Fig. 16). The rounded-rhombic first lobe has a small U-shaped indentation apically, the two halves of the second lobe end in blunt triangles (inset in Fig. 15). The general chaetotactic formula is (6)7(8,9)+3/3, i.e., the first lobe usually has 7 setae on each side of the ventral area (but sometimes less or more) plus 3 apical setae $a_{1\text{-}3}$ (a_1 is the shortest), while the second lobe invariably carries 3 setae (*x*, *y*, *z*) on both halves. The 7+3/3 setae (Fig. 16, left) conform to Fig. 5 in Condé (1956). Aberrant chaetotaxy on the ventral area of the first lobe was observed in *E. bonadonai* from Caudano (1 of 9 females with 6 instead of 7 setae on one side, another female with 9 setae in a completely symmetrical arrangement on either side: Fig. 16, right), and in *E. strinatii* from Bossea (3 of 7 females with 8 setae on one side: Fig. 15, another female with 8 setae on both sides; in the latter case the two additional setae were asymmetrically inserted).

Spermatheca. The shape of the spermatheca turned out to be very susceptible to mounting deformation, such that it brings its use as a taxonomic character into question. Note that the background photo and the left inset in Fig. 17 are from the same *E. bonadonai* female (Caudano), taken at different steps of the mounting process.

Genital region of male. The genitalia of our male specimens (Figs 18–20) concur in shape and chaetotaxy. A narrow incision splits the larger part of the first lobe into two halves, each of which has a roundish lateral and an oblong submedian extension. The second lobe furcates into elongate triangles with pointed tips, while each of the two triangular flaps of the third lobe ends in two needle-like processes. The chaetotactic formula 2+9+2f/3/4 indicates that there are, per side, a total of 13 phaneres on the first, 3 setae on the second and 4 on the third lobe. Each half of the first lobe carries 2 setae on the anterior (sternal) area, plus 9 setae on the extensions (3 on the lateral, 6 on the submedian extension), plus 2 fusules (gland spigots) at the apex of the submedian extension. This arrangement conforms to Fig. 1 in Condé (1956). Only in the males from Monfieis (*E. lanai*) are the fusules inserted on dome-shaped bases (Fig. 20).

Flagellum. Our palpigrades from Italy have the usual sequence of flagellar articles: an apical crown of long spikes (Fig. 21) is present only on articles 1, 2, 3, 5, 7 and 9 (basal ring not counted). Hence it is possible to determine the total number even if some articles are missing, provided that a fragment is preserved that reaches from the tip of the flagellum to at least article 9. A few such fragments and photos of live animals, kindly provided by Ľubomír Kováč and Enrico Lana, indicate (11–)14–15 articles (Table 4). The only completely preserved flagellum belongs to a male (B = 416) from Litrôn; its 15 articles have the following lengths (in µm) and numbers of setae in the subterminal whirl (in brackets). 1: 169 (11), 2: 174 (10), 3: 141 (10), 4: 161 (10); 5: 141 (8), 6: 156 (8), 7: 128 (8), 8: 164 (8), 9: 139 (8), 10: 170 (7), 11: 166 (7), 12: 222 (7), 13: 154 (7), 14: 113 (6), 15: 76 (6; no terminal seta). The setae of the subterminal crown are longer than the article.

The flagellum is troglomorphic (Fig. 21) to differing degrees (Fig. 1). It is 1.1–2.0 times the length of the trunk (Table 4).

TABLE 4. Number of flagellar articles and length ratio trunk/flagellum. Data taken from photographs of live animals (courtesy of Ľubomír Kováč and Enrico Lana). Sex could not be determined in two individuals. Abbreviations of caves as in Table 3.

E. spelaea A	15	1:1.1	*E. bonadonai* ♂ L	15	1:1.4
E. spelaea ♀ P	14	1:1.1	*E. bonadonai* C	11	1:1.5
E. lanai ♀ M	14	1:2.0	*E. strinatii* ♂ B	14	1:1.7
E. lanai ♂ M	14	1:1.9	*E. strinatii* ♀ B	15	1:1.6

FIGURE 13. Basitarsus of leg IV. The basitarsi are all enlarged to the same length. Specimens as in Fig. 12.

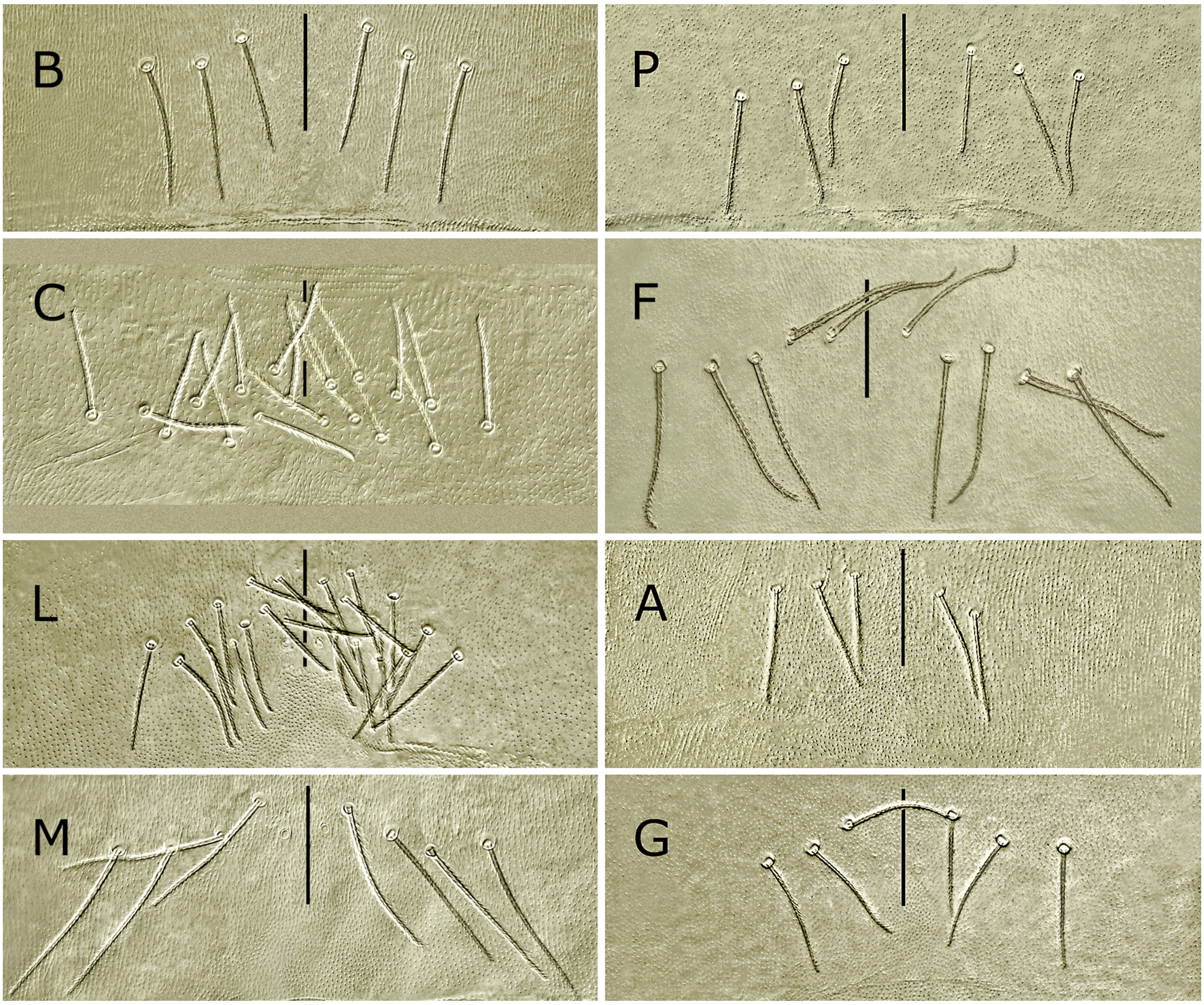

FIGURE 14. Setae *a* on sternite VI. B: Bossea ♂ (*E. strinatii*), C: Caudano ♂ (*E. bonadonai*), L: Litrôn ♂ (*E. bonadonai*), M: Monfieis ♂ (*E. lanai* **sp. n.**), P: Partigiano ♀ (*E. spelaea*), F: Fornaci ♂ (*E. roscia* **sp. n.**), A: Ardovská ♀ (*E. spelaea* with one seta *a* absent as an individual aberration), G: Griffen ♀ (*E. spelaea*). Midline = scale bar: 50 µm.

Adult morphology: morphometric analysis

Strong and linear correlations are evident between almost all morphometric measurements and indices, and the mode of the frequency distribution of the Pearson's correlation coefficients was 0.81 (Fig. 22). Most of the coefficients below 0.4 could be attributed to the correlations of I bta3/d*r* and IV bta d*r* (and to a lesser extent I bta3 a and I bta3 d*grt*) that generally exhibit no relationship to each other and to the other measurements and indices.
With one exception, the median values of the male measurements were 9.0% higher than those of the females (range of median sex differences: -2.0% for I bta3 d*r* to 22.9% for IV bta d*esd*). Likewise, males and females occupied different spaces in the PCA analysis of Mexican *Eukoenenia* species (Montaño-Moreno 2012). We did not remove this sex effect prior to the PCA, because the low sample size did not allow us to check whether it was the same for all populations.

The *Eukoenenia* populations are mostly arranged along the first principal component of the PCA (Fig. 23), which explains 70% of total variance. When plotting PC1 vs. PC2, the specimens of each population cluster together, with no overlap of their plotting areas. The three individuals from Griffen, Ardovská and Partigiano form a clearly distinct cluster, suggesting an entity well differentiated from the others (*E. spelaea*). The Bossea (*E. strinatii*), Fornaci (*E. roscia*), Litrôn (*E. bonadonai*), and Caudano (*E. bonadonai*) clusters form a loose group in the centre of the plot. One *E. bonadonai* specimen from Litrôn is close to the otherwise well defined cluster of *E. strinatii* from Bossea. It appears as an outlier in principal components of higher order (not shown), and may be an aberrant individual.

Figures 24 and 25 visualize the morphometric measurements and indices for the females (males not shown), with the populations being arranged as suggested by the PCA. Compared to the PCA plot, the univariate measurements (Fig. 24) exhibit more overlap. Taken individually, no measurement can sufficiently discriminate between all populations and supposed species, but several measurements support a Griffen-Ardovská-Partigiano group (*E. spelaea*). Several others indicate a close relationship between Caudano and Litrôn (both *E. bonadonai*), and some morphological distance between them and Bossea (*E. strinatii*). However, all populations and supposed species emerge when several measurements are considered simultaneously.

Surprisingly, the discriminative power of morphometric indices (Fig. 25) is no better than that of the original measurements. Only IV bta/a is able to clearly differentiate Griffen-Ardovská-Partigiano (*E. spelaea*) from other populations. However, index values are not necessary to discern the thick basitarsi of these specimens (Fig. 13).

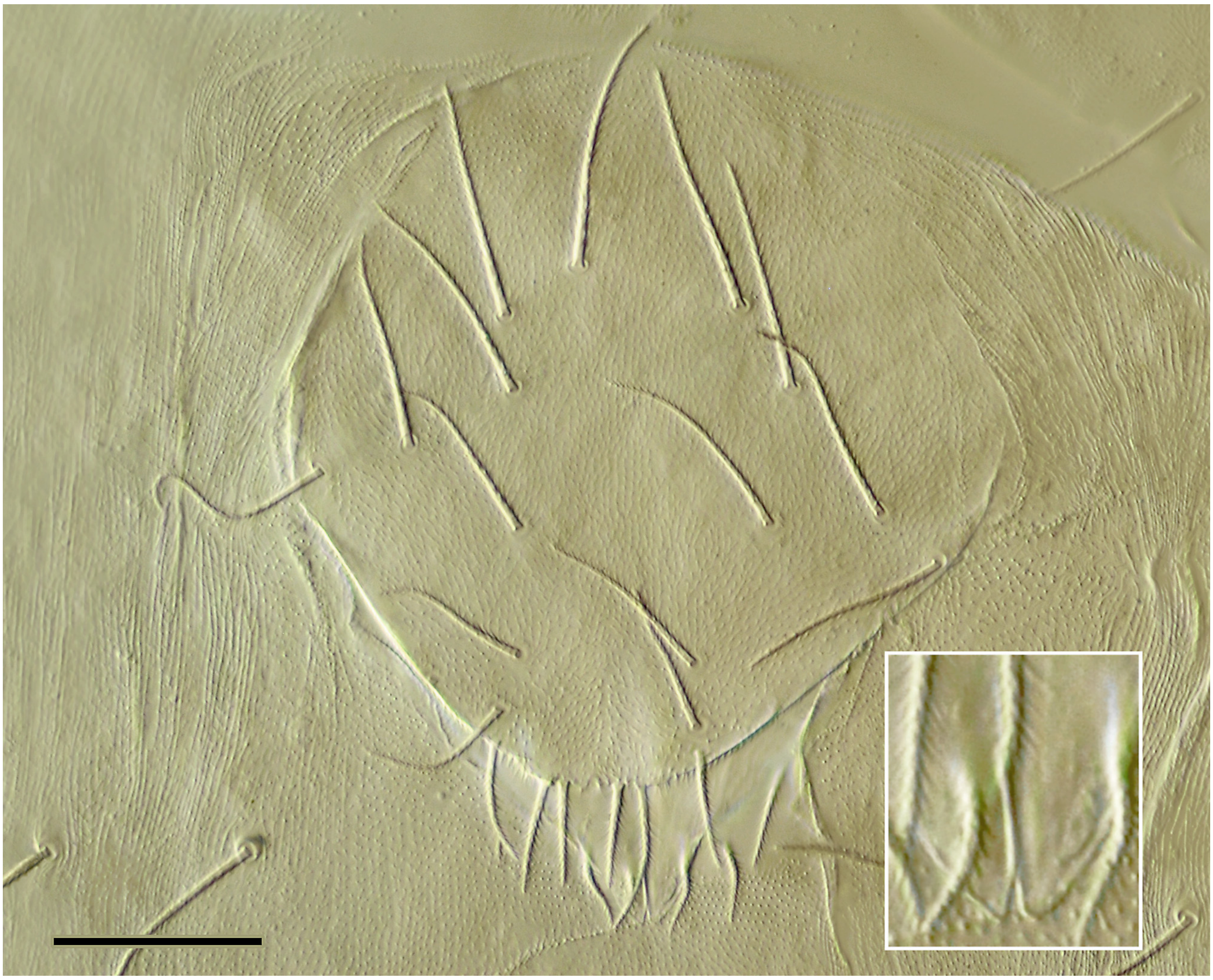

FIGURE 15. Female genitalia. First lobe asymmetrical with 7+8 setae on the ventral area and 3+3 setae in the apical row; second lobe (tip enlarged in the inset) with 3+3 setae. Below, the sternal setae st_1 and st_2 are partly visible. *E. strinatii* ♀ from Bossea. Scale bar: 50 µm.

Immature morphology

During postembryonic development, the body shape changes from the relatively compact habitus of the larva to the troglomorphic appearance of the adult animal (Fig. 26).

Larva. Table 1 includes measurements of the two available larvae from Litrôn and Bossea. The Bossea larva (*E. strinatii*) has 3+3 setulae on the labrum, 2 blades in the lateral organ (Fig. 5), and 1 seta on the deuto-tritosternum. The fingers of the chelicera carry 7 teeth. Chaetotaxy of propeltidium and metapeltidium complete. Coxae II–IV with 3, 3, and 0 thick setae. Five trichobothria are present (no trichobothria on bta1). Forked setae are distributed as in the adult. IV bta has 2 setae (*grt* and 1 *esd* absent). Tergites II–VI with 2+2 setae (probably t_1 and t_3) between the setae *s*. No primordia of genital lobes and no paramedian gland orifices are visible. Sternite II has 2+2 closely paired setae ($st_{1\text{-}2}$), sternite III has 3+3 more distantly inserted setae ($st_{1\text{-}3}$). On sternites IV–VI, only setae a_1 and a_2 are developed, setae *s* are absent. Opisthosomal segments VII–XI have 6 (4 dorsal, 2 ventral), 8, 8, 8, and 8 setae, respectively. The characters of the larva from Litrôn (*E. bonadonai*) correspond perfectly with the above description, except for the presence of a single blade in the lateral organ (Fig. 5).

Juveniles. The three juveniles at hand are more or less damaged, so that we could not determine the opisthosomal chaetotaxy. Mouldy remains of the cadaver or exuvia of a juvenile specimen from Bossea (*E. strinatii*) show 7 cheliceral teeth. This conforms with the fragments of the juvenile female from Partigiano (*E. spelaea*), which exhibits 4+4 setulae on the labrum, 4 blades in the lateral organ, two setae on the deuto-tritosternum, 3–3–0 thick setae on coxae II–IV, and complete sets of trichobothria and *fs* (including the *fs* of I bta4). The best preserved specimen, a juvenile female from Caudano (*E. bonadonai*), measures about 1360 µm, has a 315 µm-long propeltidium, and complies with the discernible characters of the Partigiano specimen, except for the 3 blades in the lateral organ and the absence of *fs* on I bta4. Measurements (in µm) of the pedipalp: ta3 = 64, ta2 = 49, ta1 = 39, bta2 = 66, bta1 = 56, ti = 132. Leg I: ta3 = 133, ta2 = 49, ta1 = 33, bta4 = 59, bta3 = 67, a = 38, *r* = 72, d*r* = 31, *grt* = 66, d*grt* = 13, bta1+2 = 128, ti = 151. Leg IV: ta2 = 77, ta1 = 56, bta = 111, a = 29, *r* = 60, d*r* = 59, *esd* = 69, d*esd* = 42, ril = 5, *grt* = 56, d*grt* = 31, ti = 152. Indices of leg I: bta3/a = 1.76, bta3/*r* = 0.93, bta3/d*r* = 2.16, bta3/ti = 0.44, B/bta3 = 4.70. Indices of leg IV: bta/a = 3.83, bta/*r* = 1.85, bta/d*r* = 1.88, bta/ti = 0.73, B/bta = 2.84. The anterior genital lobe is cleft medio-distally; it carries a proximal pair of long (43 µm) setae, followed by 2+2 somewhat shorter, 1+1 short (16 µm) and 1+1 very short (9 µm) setae. The posterior lobe is composed of two halves, each with 1 relatively long (24 µm) seta (Fig. 27).

FIGURE 16. Female genitalia. Intrapopulation variability of lobe chaetotaxy. One of the Caudano females (*E. bonadonai*) shown has the common number of 7+7 setae on the ventral area of the first lobe, the other female has 9+9. Scale bar: 50 µm.

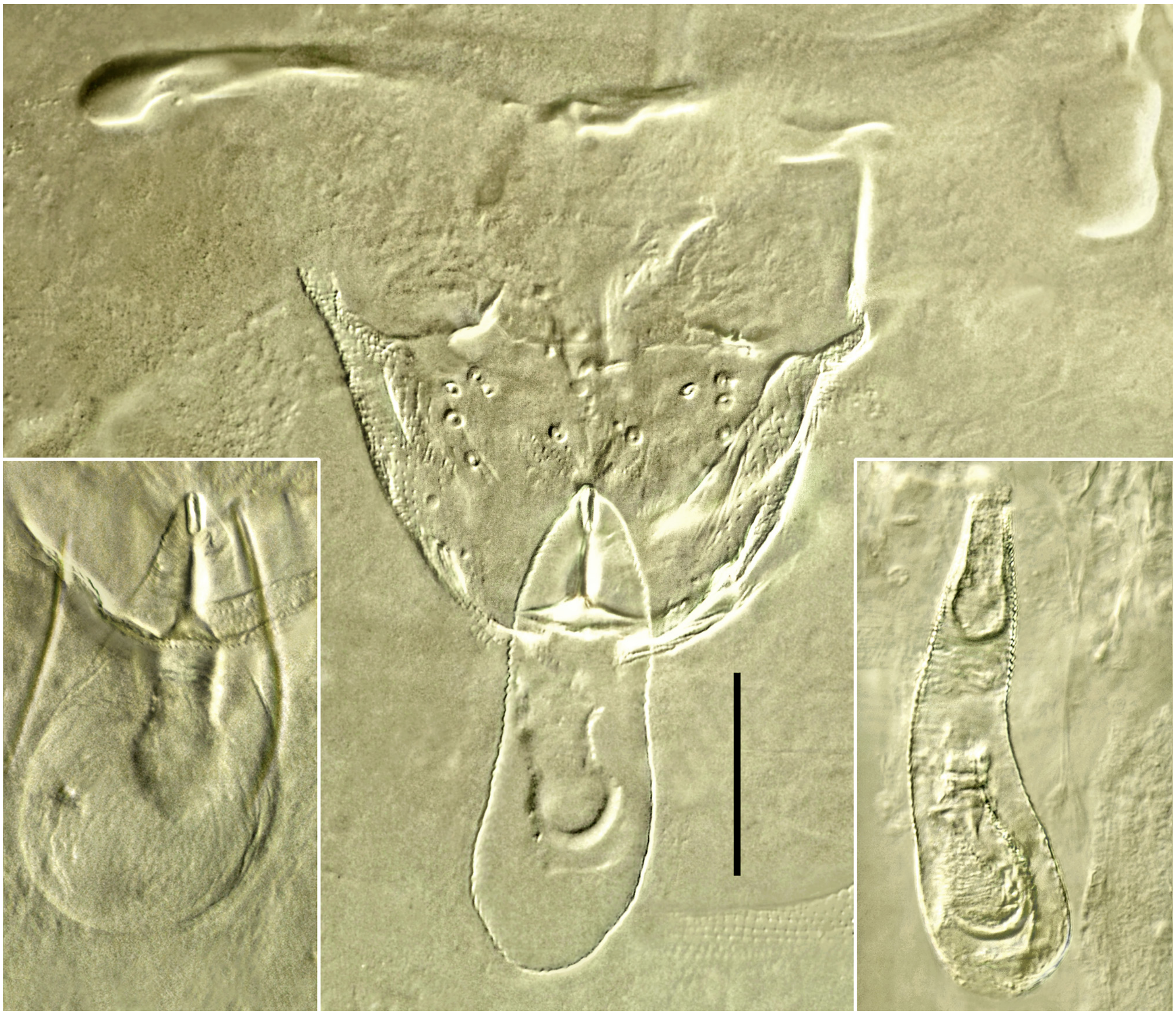

FIGURE 17. Spermatheca. The main image and the left inset are from the same Caudano ♀ in different phases of the mounting procedure. Right inset shows spermatheca of a Litrôn ♀. All *E. bonadonai*. Scale bar: 50 µm.

Hypothesis of phyletic coherence and species assignment

Morphological evidence suggests that all the populations from Italy included in this study are closely related to *Eukoenenia spelaea*. We circumscribe the *E. spelaea* complex by the following adult character states: lateral organ with 3 or more blades; coxae of legs I–IV with 0–4–4–1 thick setae; I ta3 with 4 forked setae; IV bta with 4 setae; metapeltidium with 3 setae; tergite II with 2+2, tergites III-VI usually with 3+3 setae *t* (t_2 absent in *E. spelaea hauseri* Condé, 1974), median seta absent; sternites IV–VI with 3+3 or more setae *a*; female with 3+3 apical setae on the first genital lobe and 3 setae on the second genital lobes; male with 3 setae on the second and 4 on the third genital lobes; opisthosoma with a distinct narrowing between segments VIII and IX.

The populations of this study belong unambiguously to the *E. spelaea* complex. Considering variation within and between the populations, we arrive at the following assignments which will be discussed in the final section of this paper.

Fornaci = *Eukoenenia roscia* Christian, **sp. n.**, described below
Partigiano = *Eukoenenia spelaea* (Peyerimhoff, 1902)
Monfieis = *Eukoenenia lanai* Christian, **sp. n.**, described below

Litrôn	= *Eukoenenia bonadonai* Condé, 1979
Caudano	= *Eukoenenia bonadonai* Condé, 1979
Bossea	= *Eukoenenia strinatii* Condé, 1977

Ardovská and Griffen match the current concept of *E. spelaea*. However, our investigation reveals a morphological difference between the two populations large enough to reject identity at the subspecies level (shape of I bta3: Fig.12; presence / absence of *fs* on I bta4; relative length of fs_1 on I ta3: Fig. 10).

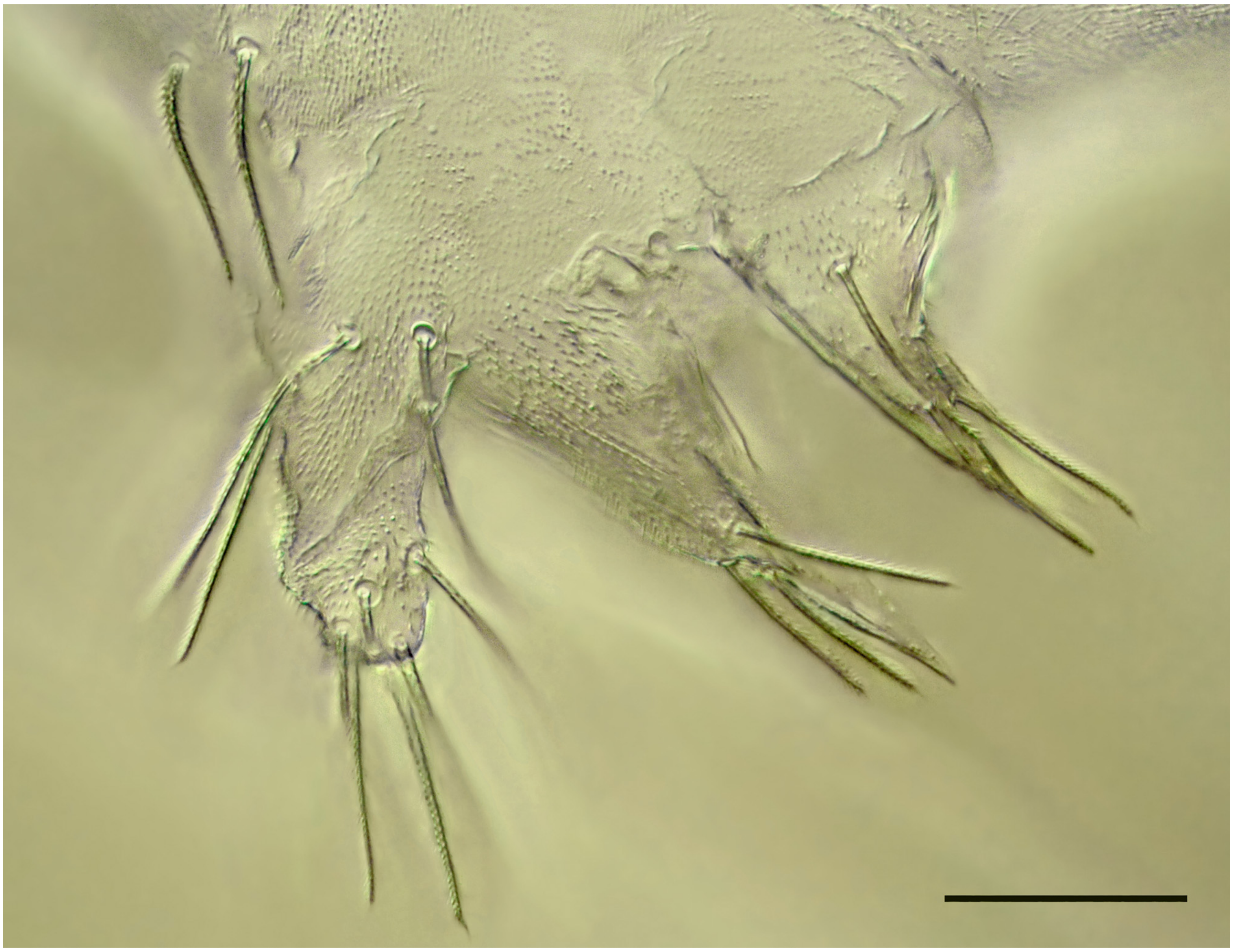

FIGURE 18. Male genitalia in lateral view. The three genital lobes are nearly uncompressed. *E. strinatii* ♂ from Bossea. Scale bar: 50 µm.

Systematics

Descriptions of new taxa

Eukoenenia roscia Christian, species nova

(Figures 2, 5 F, 6, 9, 11, 12 F, 13 F, 14 F, 23–25)

Material examined. Holotype female: Italy, Piemonte, Province of Cueno, Rossana, Grotta delle Fornaci o di Rossana (44°32′02″N, 07°25′52″E, 554 m a.s.l.); 15 August 2012, leg. E. Lana. Two paratypes: 1 male, 1 female; same locality; 16 February 2012, leg. M. Morando & E. Lana. Additional material: 1 female; same locality; 3 March 2013, leg. E. Lana.

Deposition. Museum of Natural History, Vienna, Austria, Arachnological Collection. Acquisition numbers 21.881 (holotype), 21.882 (paratype male), 21.883 (paratype female).

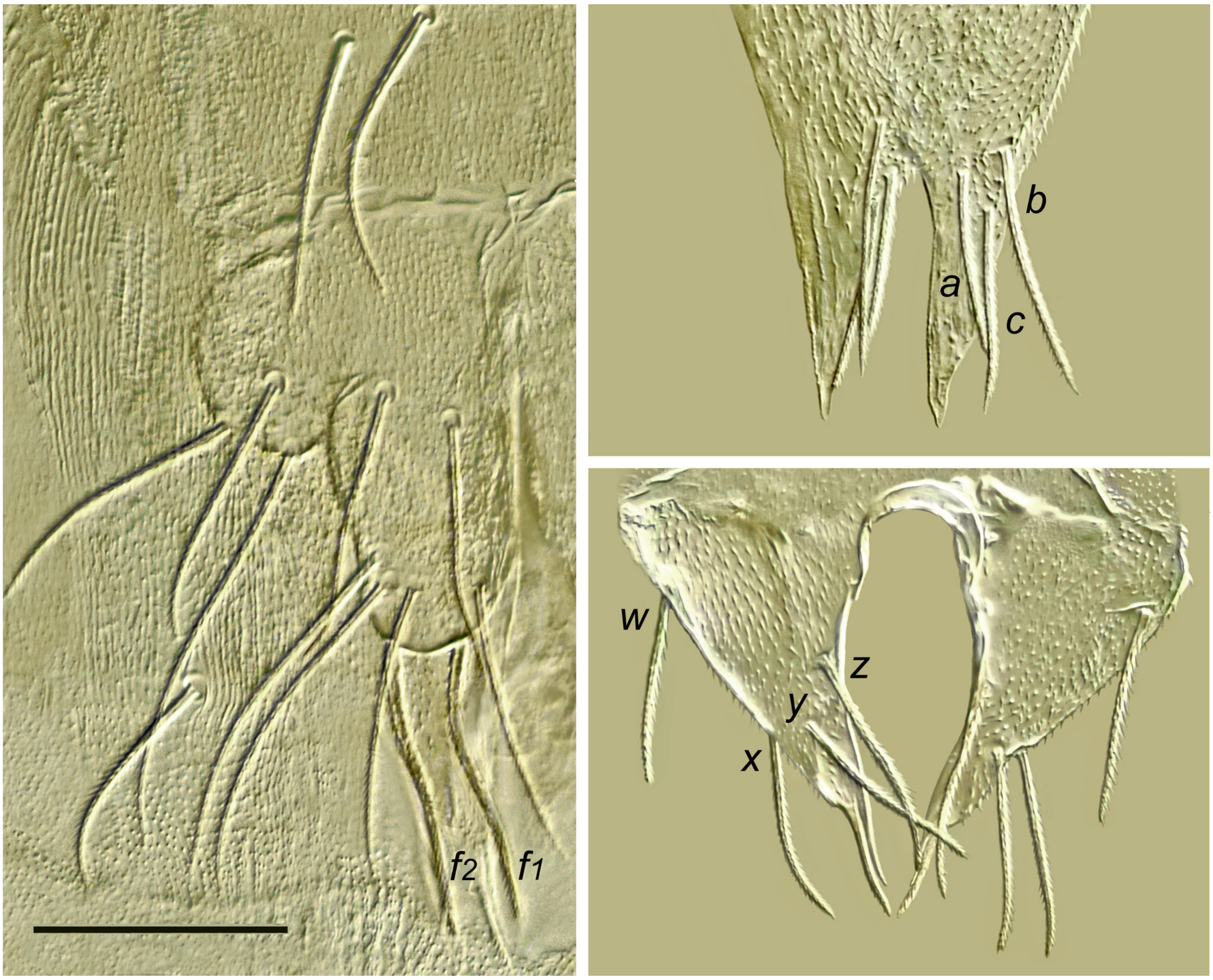

FIGURE 19. Male genitalia in ventral view. One side of the first lobe (left), tips of the second lobe (above), and of the third lobe (below) of a *E. strinatii* ♂ from Bossea. Fusules are marked f_1, f_2. Scale bar: 50 µm.

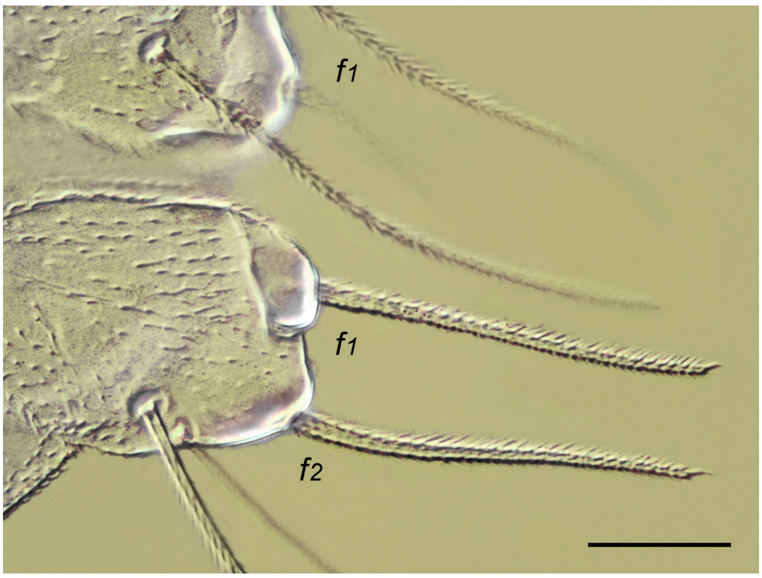

FIGURE 20. Fusules (f_1, f_2) on first genital lobe of a male from Monfieis (*E. lanai* **sp. n.**). The fusules are inserted on dome-shaped bases. Scale bar: 20 µm.

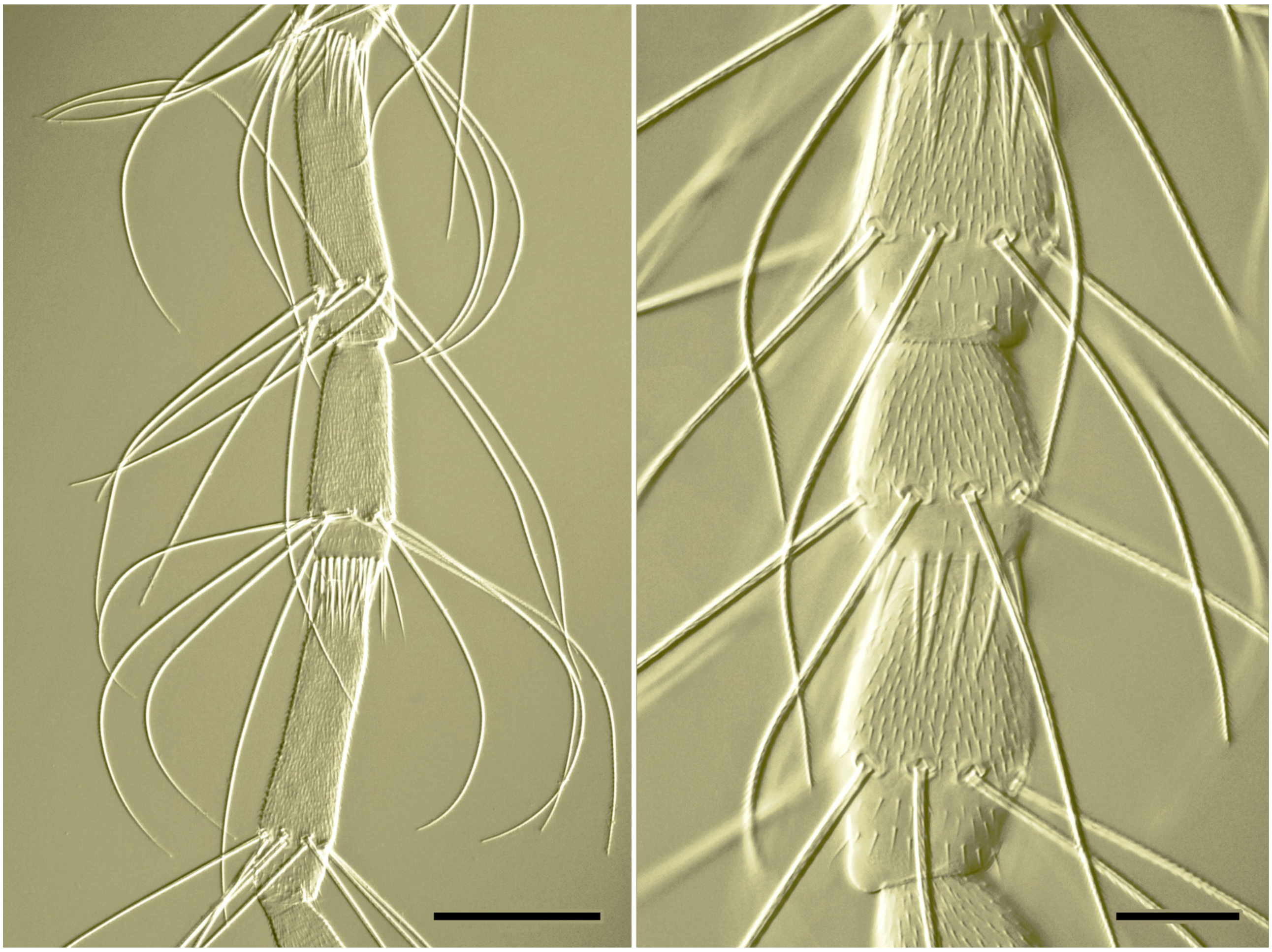

FIGURE 21. Flagellum. Left: articles IV–VI of a Monfieis ♂ (*E. lanai* **sp. n.**; scale bar: 100 µm). Right: the same articles of a *E. mirabilis* ♀ (soil-dwelling) from Bergeggi, Liguria, for comparison (scale bar: 20 µm). Note apical crown of cuticular spines on articles III (at the very top of the images) and V.

Etymology. The Comune di Rossana derives its name from the Roman gens Roscia. The specific epithet *roscia* is used as a noun in apposition.

Diagnosis. A species with all characters of the *E. spelaea* complex, as described above. Body length over 1800 µm; each finger of chelicera with 8 teeth; lateral organ with 5–7 blades; 6 (5) deuto-tritosternal setae; articles I bta3 and IV bta slender; forked seta present on I bta4; proximal forked seta of I ta3 about same length as rod seta; males with more than 3+3 setae *a* on the sternites IV–VI (females often have 3+3); segment XI of opisthosoma with 10 setae.

Description. Morphometric data are given in Table 1 under ♀ 01, ♀ 02 (holotype) and ♂ 03.

General appearance. Body length without flagellum 1830–1960 µm. Shape and pubescence of trunk as in other members of *E. spelaea* complex. Trunk of living individuals orange reddish, particularly opisthosoma.

Prosoma. Frontal organ 39–42 µm long; the two lanceolate, reticulated branches have pointed tips. The 5–7 blades of the lateral organ (Fig. 6) are 42–44 µm long, pointed-lanceolate and finely reticulated (Fig. 5 F). Propeltidium with 10+10 setae, the shortest (30–31 µm) in central position, the longest (50–54 µm) laterally in posterior half. Setae t_1, t_2 and t_3 of metapeltidium 76–82, 127–135 and 84–100 µm long, respectively. Labrum with usual cuticular pattern and 5+5 short setae. Deuto-tritosternum with 6 (3 specimens) or 5 setae (1 specimen) of 43–50 µm. First article of chelicera with a proximal longitudinal series of 6 setae (p_4 and p_6 thick; p_4 sparsely barbed, p_6 partly serrate), a distal series of 3 aligned setae (d_3 strong, smooth near base, sparsely barbed in middle, closely barbed near tip, 2.5× length of d_1 and d_2) and 1 apical seta. Hand of chelicera with 7 setae: 4 in a dorsal line, 1 ventral, 1 close to articulation of movable finger, and 1 on a tubercle of the fixed finger. Fingers with 8 teeth each. Coxal chaetotaxy as described above for *spelaea* group. Near the tip of the pedipalp is a curved seta that looks like a long, strongly asymmetric forked seta; one typical *fs* is inserted somewhat behind. Leg I with 7 trichobothria in usual arrangement and a total of 8 forked setae. Ta3 of leg I has 4 *fs* in distal half, arranged as 1+1+2: fs_1 about same length as nearby *rs*; fs_2 inserted closer to *cs* in the three females, but closer to *m* in the single male. I bta3 very slender, length/width 3.23–3.52; compared to the similarly shaped I bta3 of *E. strinatii*, seta *r* of the new species is longer and more proximally inserted, at 42–46% of article length from base (Fig. 12 F vs. B, and Fig. 24, I bta3/*r* and I bta3/d*r*). I bta4 bears, close to the insertion of the trichobothrium, one *fs* (Fig. 11; this forked seta is also present in *E. spelaea* from Partigiano and Griffen). IV bta slender, length/width 5.32–6.54, with 4 setae, these exhibiting some variation in length and insertion distance, but consistently $r < grt < esd$; tip of *r* does not project beyond distal end of the article (Fig. 13 F).

Opisthosoma. Anterior tergites with t_1, t_3, *s* on segment II, and usually t_1, t_2, t_3, *s* on III–VI. Seta t_2 absent in one female symmetrically on segment III and on the right side of IV. Sternites IV–VI each with a pair of submedian circular structures and lateral setae s_1 and s_2; females can have more than 3+3 submedian setae *a* at least on segment IV, the male has (4–)5 setae *a* on these sternites (Figs 6 & 14 F). The setation of the terminal segments (Table 3) is partly variable, but on segment XI all specimens (N = 4) have 10 setae about as long as the segment. The 2 dorsal setae on the intermediate ring of the flagellum are as long as the 2 ventral ones. Not even fragments of the flagellum are preserved.

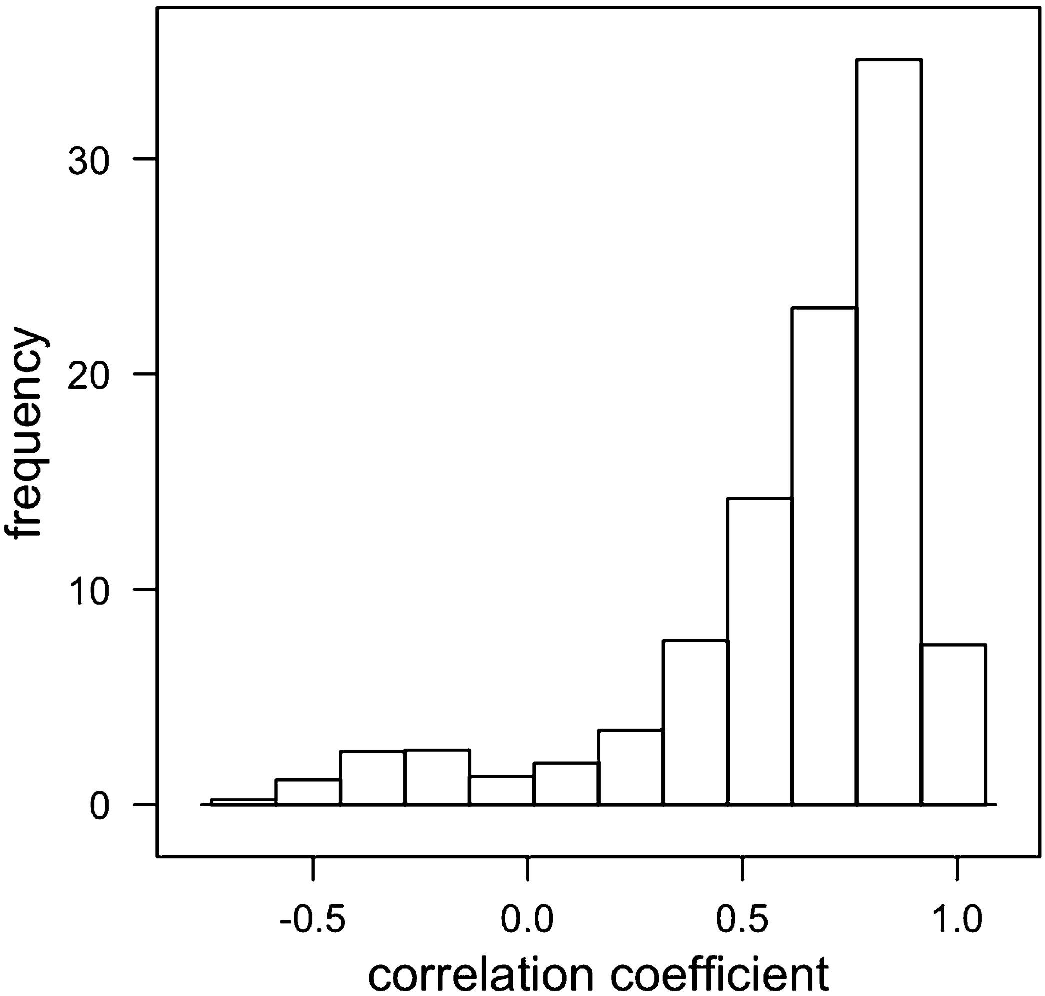

FIGURE 22. Linear correlation among the morphometric measurements and indices: Frequency distribution of the Pearson's correlation coefficients.

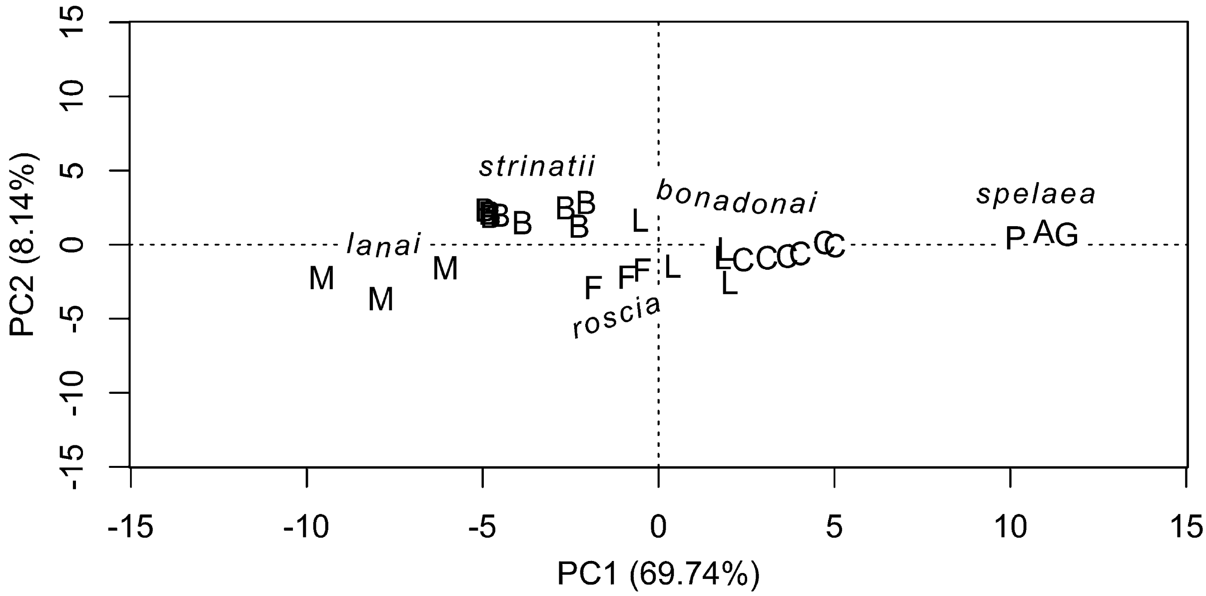

FIGURE 23. Principal component analysis based on measurements of 29 *Eukoenenia* individuals (females and males) from six Italian and two comparative populations (A, G). G = Griffen, A = Ardovská, P = Partigiano, C = Caudano, L = Litrôn, F = Fornaci, B = Bossea, M = Monfieis.

Female genital area. First lobe has an evenly rounded or gently truncate apex, uniform pubescence and 7+3 setae on each side. The 7 setae on ventral area of lobe arranged in 4 transversal rows, 2–3–1–1 from base to tip (as in Fig. 16, left). The innermost of the 3 apical setae measures 23–28 µm; it is always slightly shorter than the others. Each half of second lobe possesses 3 setae. The total formula is thus 7+3/3.

Male genital area. Shape and phaneres of genital area as in *E. strinatii* (compare Figs 18 & 19), chaetotactic formula 2+9+2f/3/4. Base of deeply split first lobe lies behind a row of 2+2 sternal setae. The roundish lateral extension of each half carries 3 setae, the oblong submedian extension has 6 setae and, on the apical bend, 2 fusules (f_1, f_2) that are about the same length as the neighbouring setae. No sockets visible at roots of fusules. Second lobe furcates in its distal half into two triangular, pointed flaps, each of which carries 3 setae (*a*, *b*, *c*) near its base, with *b* being more proximally inserted than *a*. On the third lobe the two flaps branch off closer to the base and converge apically. They are broadly triangular, end in two parallel needles and have 4 setae: *w*, *z*, *y*, *x* (from base to tip).

Eukoenenia lanai Christian, species nova

(Figures 1, 2, 5 M, 6, 10 M, 12 M, 13 M, 14 M, 20, 21, 23–25)

Material examined. Holotype male: Italy, Piemonte, Province of Cueno, Demonte, Miniera superiore di Monfieis (44°21′44″N, 07°16′00″E, 1750 m a.s.l.); 12 September 2010, leg. E. Lana. Two paratypes: 1 female, 1 male; same locality, date, and collector.

Deposition. Museum of Natural History, Vienna, Austria, Arachnological Collection. Acquisition numbers 21.878 (holotype), 21.879 (paratype female), 21.880 (paratype male).

Etymology. The specific epithet is a patronym in honour of Enrico Lana, eminent speleobiologist, photographer and collector of rare subterranean animals.

Diagnosis. A species with all characters of the *E. spelaea* complex as described above. Body length 2100 µm or more; each finger of chelicera with 8 teeth; lateral organ with 3 blades; 5 deuto-tritosternal setae; forked seta absent on I bta4; proximal forked seta on I ta3 only half length of rod seta; some or all of sternites IV–VI with more than 3+3 setae *a*; segment XI of opisthosoma with (9–)10 setae; fusules on first genital lobe of male inserted on dome-shaped bases.

Description. Morphometric data are given in Table 1 under ♀ 05, ♂ 06 and ♂ 07 (holotype).

General appearance. Large *Eukoenenia*, with a body length of 2100–2170 µm. Shape and pubescence of trunk as in other members of the *E. spelaea* complex. Trunk of live individuals yellowish, particularly the opisthosoma.

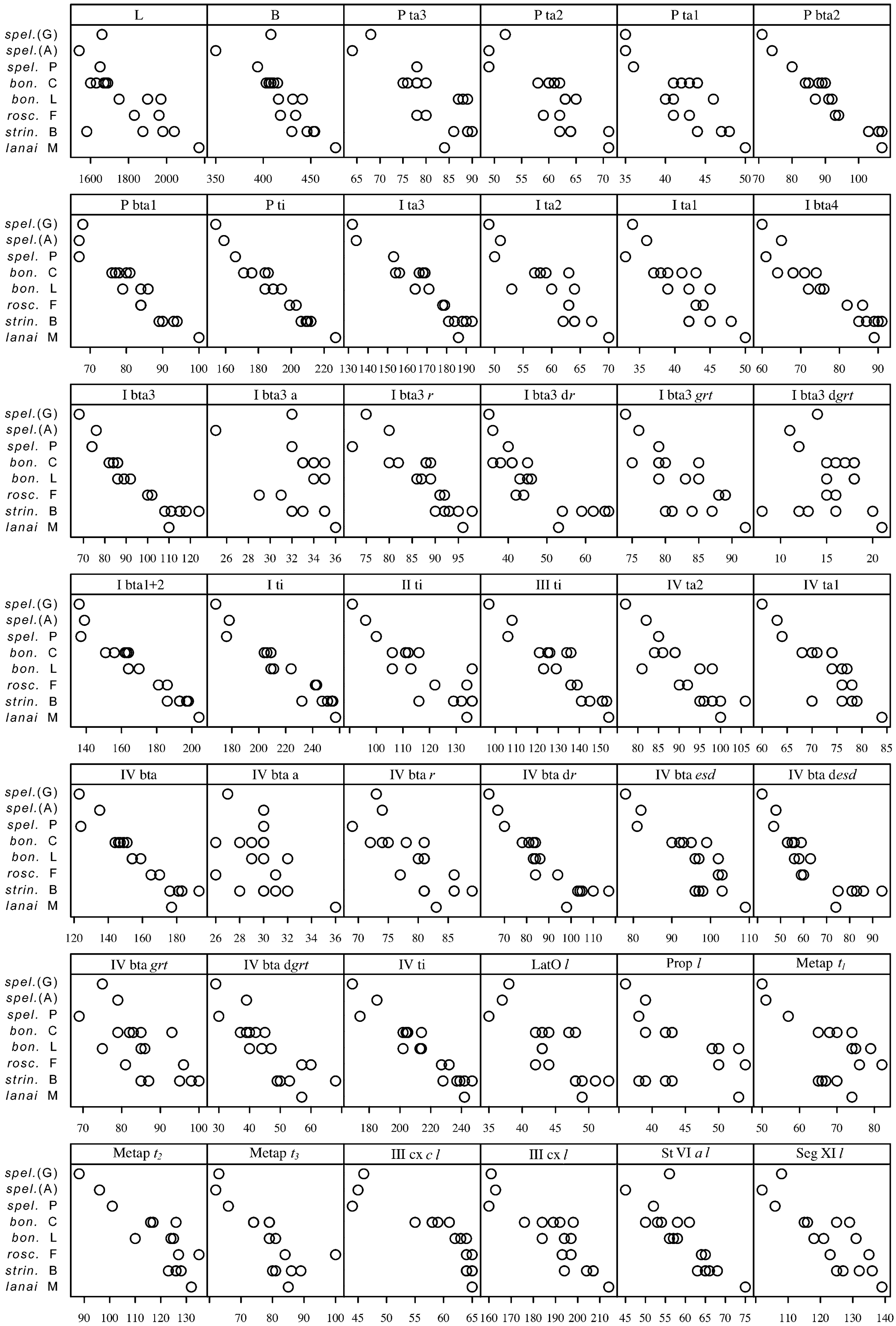

FIGURE 24. Graphic representation of the measurements used for the morphometric analysis (data from Tables 1 & 2, in µm). Only females included. Initials of caves as in Fig. 23 (non-Italian comparative populations in brackets).

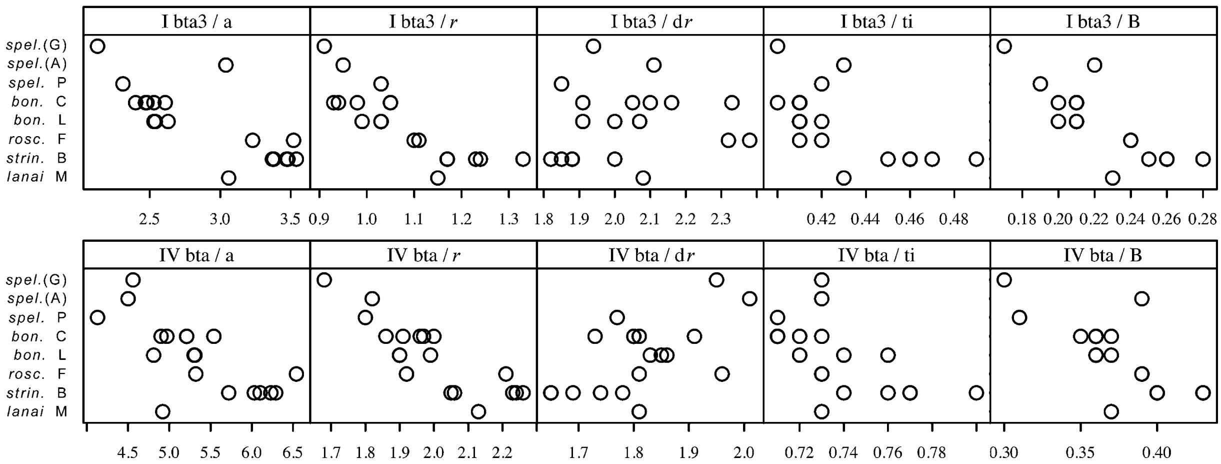

FIGURE 25. Graphic representation of the values of commonly used morphological indices (data from Tables 1 & 2). Only females included. Initials of caves as in Fig. 23.

FIGURE 26. General appearance of a larva (left) and an adult female of *E. strinatii* from Bossea. Specimens enlarged to the same trunk length.

Prosoma. Frontal organ 40 µm long (holotype), with two lanceolate, pointed branches. Lateral organ with 3 reticulated, 45–49 µm-long blades that are parallel-sided with a pointed tip (Fig. 5 M). Propeltidium with 10+10 setae, the shortest (29–31 µm) in central position, the longest (53–57 µm) laterally in posterior half. Setae t_1, t_2 and t_3 of metapeltidium 74–85, 132–145 and 82–98 µm long, respectively. Labrum with usual cuticular pattern and 5+5 short setae. Deuto-tritosternum with 5 setae of 47–54 µm, arranged in a wide V. Chaetotaxy of chelicera and dentition of fingers as in *E. roscia*. Coxal chaetotaxy as described above for *spelaea* group. Curved seta near tip of pedipalp weakly and asymmetrically forked, the typical *fs* is inserted somewhat behind. Leg I with 7 trichobothria in usual arrangement and a total of 7 forked setae. The ta3 of leg I has 4 *fs* in distal half, 1–1–2: fs_1 is only half length of the nearby *rs*; in all specimens fs_2 is inserted much closer to *m* than to *cs* (Fig. 10 M). I bta3 not as slender as in *E. strinatii* and *E. roscia*, length/width 3.06–3.19 (Fig. 12 M); seta *r* inserted at 48–53% of article length. I bta4 without *fs*. IV bta slender, all ratios very similar to those of *E. roscia*; $r < grt < esd$; tip of *r* does not project beyond distal end of article (Fig. 13 M).

Opisthosoma. Anterior tergites with t_1, t_3, *s* on segment II and t_1, t_2, t_3, *s* on III–VI. Sternites IV–VI each with a pair of submedian circular structures and lateral setae s_1 and s_2; more than 3+3 submedian setae *a* on at least one of the sternites IV–VI, the maximum observed (N = 3) was 4 per side (Figs 6 & 14 M). *E. lanai* has by far the relatively longest setae *a* among all populations investigated (see above). The setation of the terminal segments (Table 3) is partly variable; segment VII carries 13–17 setae, segment XI has 9 (female) or 10 setae (the two males). The 2 pairs of setae on the intermediate ring of the flagellum are of equal length. In the holotype, the flagellum (detached, under separate coverslip) is preserved from the third article to the tip (Fig. 21). Length (in µm) and number of setae (in brackets; "+" indicates the presence of an apical crown) are for article 3: 217 (10+), 4: 214 (9), 5: 176 (8+), 6: 212 (8), 7: 171 (8+), 8: 227 (8), 9: 184 (8+), 10: 249 (7), 11: 207 (7), 12: 239 (7), 13: 179 (7), 14: 123 (6 and 1 terminal seta). On article 3, the longest seta measures 277 µm, the longest spine of the apical crown 68 µm. Photographs of two live individuals show 14 flagellar articles and the relatively longest flagellum observed in this study (1.9–2.0× trunk length, Table 4).

Female genital area. First lobe has 7+3 setae on each side; the innermost of the 3 apical setae measures 24 µm, it is somewhat shorter than the others. Each half of second lobe possesses 3 setae. The total formula is 7+3/3.

Male genital area. Chaetotaxy (2+9+2f/3/4) and general shape of genital lobes as in *E. strinatii* (cf. Fig. 19). The fusules, however, are inserted on dome-shaped bases (Fig. 20), a character that separates *E. lanai* from the other species of the present study. In the holotype, the longest seta on the first lobe measures 98 µm, the fusules 60 µm.

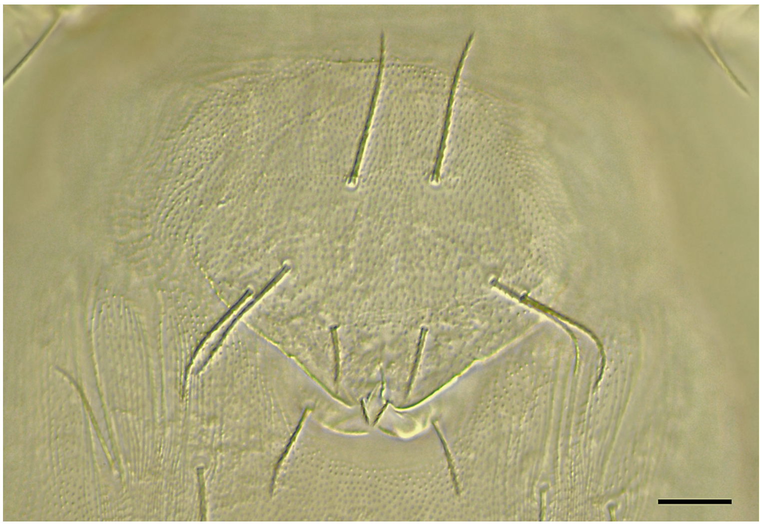

FIGURE 27. Genital area of a juvenile female from Caudano (*E. bonadonai*). Scale bar: 20 µm.

Discussion

The study area in the Alps of SW Piedmont is distinctive, with an exceptionally rich biodiversity (Minelli *et al.* 2006). Botanists regard it as the richest centre of endemism in the Alps (Diadema *et al.* 2005), and the same apparently applies to the subterranean fauna (Isaia *et al.* 2011; Zacharda 2011; Lana 2013). Recent studies (e.g. Lohse *et al.* 2010) support the classical "massif de refuge" hypothesis (Chodat & Pampanini 1902, Holdhaus 1906) which, in its modern interpretation, postulates the existence of definite areas scattered along the borderline of the entire Alpine arc that served as refugia for plant and animal species during glacial periods of the Pleistocene. Médail & Diadema (2009) found a clear spatial congruence between glacial refugia and hotspots of plant biodiversity in the Mediterranean region.

Our area is part of the most prominent Pleistocene refugium of the SW Alps (Schönswetter *et al.* 2005). It lies south of the Holdhaus line, which roughly marks the northern range boundaries of troglobiotic beetles and other subterranean arthropods with similarly limited means of dispersal (Holdhaus 1954, Drees *et al.* 2011). Though the area overlaps the border of maximum Würm glaciation (Casazza *et al.* 2008), subterranean habitats for palpigrades may have existed continuously since late Neogene times. Based on these elements, we had high expectations as to the diversity of cave palpigrades there. What we did not expect was that all populations would belong to the same subgroup of the genus *Eukoenenia*.

The *Eukoenenia spelaea* complex, as defined here, is largely congruent with the "groupe *spelaea*-*vagvoelgyii*" outlined by Condé (1972). It currently comprises *E. spelaea*, *E. strinatii*, *E. bonadonai*, *E. condei* Orghidan, Georgescu & Sârbu, 1982, an unnamed male from the Mačkovica cave near Laze pri Planini, Slovenia (Condé 1976), and the new taxa described in this paper. The next higher level of affinities might include the polytypic, mainly cave-dwelling *E. austriaca* (Hansen, 1926) and its sibling species *E. margaretae* Orghidan, Georgescu & Sârbu, 1982 from Romania (both with 2+2 setae *a* on IV–VI), and the Dinaric troglobiont *E. remyi* Condé, 1974 (with 1+1 setae *a* on IV–VI).

While most of these species appear to be restricted to caves in the strict sense, *E. spelaea* also occurs occasionally in the interstices of scree and gravel deposits (Condé 1984), just as *E. austriaca* does (Christian 1998). *E. spelaea* was described, as the first subterranean palpigrade species, from two caves north of Digne-les-Bains in the Provence Alps (Peyerimhoff 1902), approximately 100 km west of our study area. Condé (1956) supposed the Grotte de Saint-Vincent-de-Mélan to be the type locality. The total range of *E. spelaea*, comprising the four nominal subspecies, extends from France west of the river Rhone across the entire Alpine arc, the western Carpathians and the northern Dinarides (Condé 1996).

The record of *E. spelaea* in Partigiano confirms the occurrence of the species in the Italian part of the SW Alps, since an older record of "*E.* cf. *spelaea*"—pertaining to a juvenile female from the Grotta occidentale del Bandito, a little north of Litrôn (Brignoli 1976)—remains doubtful. Remarkably, the Buco del Partigiano is an opening between blocks of insoluble rock, rather than a cave in the sense of Culver & Pipan (2009), which supports the view that *E. spelaea* is not dependent on deep karst caves. Although we possess only one specimen from this locality, the assignment is well-founded. All characters lie within the known morphological spectrum of *E. spelaea*, and in the PCA the Partigiano female forms a group with the comparative specimens of *E. spelaea* from Austria and Slovakia. This cluster is clearly detached from the other five populations.

Populations from Fornaci, Monfieis, Litrôn, Caudano and Bossea share a number of character states in common. The individuals are larger and more distinctly troglomorphic in terms of elongated limb segments, longer setae on the coxae and segment XI, and a longer flagellum. Most noticeable is a chaetotactic trait that has not been observed in other members of the *E. spelaea* complex: at least some of the individuals have more than 3+3 setae *a* on one or more sternites of the segments IV–VI. Unlike changes in body proportions, the presence of additional setae *a* is probably not an adaptation to cave life; it may be due to common ancestry rather than parallel evolution. The considerable morphological distance revealed by the PCA indicates that none of the five populations is conspecific with *E. spelaea*, although females from Fornaci and Bossea can show the 3+3 pattern on the sternites IV–VI. Condé (1977) was the first to ascribe species rank to one of these populations by describing *E. strinatii* from a single male collected in the Bossea cave. In our topotypic material the number of blades in the lateral organ is constant: *E. strinatii* has 4 blades, which differentiates this species from the morphologically most similar Monfieis population (3 blades). There is some variation in the number of setae *a*, but, in accordance with the holotype, a fourth seta *a* occurs less frequently on sternite VI than on IV–V.

Two of the populations share a conspicuous character, albeit in only one sex: male individuals from Litrôn and Caudano have a greatly increased number of setae *a* on sternites IV–VI, consistent with the holotype of *E. bonadonai* from the Provence Alps (Condé 1979). This feature is not uncommon within the genus. It occurs, just to mention one troglobiotic European species, in *E. bouilloni* Condé, 1980 from southern France. In our material the males of *E. strinatii*, *E. roscia* and *E. lanai* show just a slight tendency toward a numerical increase of setae *a* (Fig. 6). Because the Litrôn and Caudano specimens also match the other characters given in the original description of *E. bonadonai*, and as they cluster closely together in the PCA plot, we assign these two populations to *E. bonadonai.* Apart from the marked sexual dimorphism, *E. bonadonai* differs from *E. strinatii* in the presence of 3 (instead of 4) blades in the lateral organ and in the shape of the articles I bta3 and IV bta (Figs 12 & 13). On the whole, *E. bonadonai* appears less troglomorphic than *E. strinatii*.

More puzzling are the rank and position of the palpigrades from Fornaci and Monfieis. These two populations differ from *E. spelaea* in greater mean body size and in the occurrence of individuals with more than 3 setae *a* on at least one of the sternites IV–VI; from *E. strinatii* in the number of blades in the lateral organ; and from *E. bonadonai* in the much less pronounced sexual dimorphism in the number of setae *a*. The Fornaci and Monfieis populations differ from each other in the number of blades in the lateral organ, in the length ratio fs_1/rs (Monfieis individuals have a very short forked seta compared to the rod seta), in the dome-shaped bases of the fusules on the first genital lobe of the males from Monfieis, and in an ensemble of continuous variables that leads to a clear separation in the PCA plot.

The Fornaci and Monfieis populations are discriminable entities that merit, in our opinion, formal taxonomic names, in order to provide 'landmarks' in the difficult terrain of the *E. spelaea* complex. However, should these populations be most appropriately regarded as species or subspecies? We are aware that any decision is likely to meet with criticism. Describing subspecies implies evolutionary hypotheses that we are unable to defend: we simply have no clue about which existing species taxon or taxa might include the Fornaci and Monfieis populations. Describing them as new species, on the other hand, implies (complete) speciation, which we cannot demonstrate beyond doubt. Our decision to introduce the new species *E. roscia* for the Fornaci population and *E. lanai* for that from Monfieis, was chiefly made to avoid conjectures about their sister taxa.

In any event, we are confronted with a multitude of short-range endemic taxa (Harvey 2002), a fact that has a significant bearing on conservation policies and possibly also on our understanding of evolutionary processes in the subterranean realm. However, the roles of vicariance and dispersal (Culver & Pipan 2009) are difficult to assess in our case, because the signals are partly inconsistent. The distribution of *E. bonadonai* suggests some power of dispersal. Hardly any morphological difference exists between the populations from Italy and the type specimen from France, although the Caudano and Clue caves are 110 km apart. In contrast, the clearly differing *Eukoenenia* populations of Partigiano and Fornaci (10 km apart) indicate low dispersal rates of palpigrades, particularly since the faunas of the two localities are similar overall. The troglobiotic carabid beetle *Doderotrechus casalei*, for example, occurs in both caves (Lana 2013). Bossea and Caudano (7 km apart) harbour different *Eukoenenia* species, in the same way that they have different, short-range endemic spiders of the genus *Troglohyphantes*: *T. pedemontanus* occurs in Bossea and *T. pluto* in Caudano (Isaia *et al.* 2011). On the other hand, the rhagidiid mite *Troglocheles lanai* has been reported from these two and a few other caves, including Litrôn, at a distance of 45 km from Bossea. This extremely troglomorphic mite is likewise a poor disperser, such that Zacharda *et al.* (2011) envisaged cryptic speciation. It is possible that SW Alpine members of the *E. spelaea* complex have speciated (or are continuing to speciate) in a similar, albeit less cryptic, way.

Acknowledgements

Many thanks to those who provided palpigrade specimens or photographs, or helped with collecting in the caves: Alberto Chiarle, Davide Giuliano, Enrico Lana, Stefano Mammola, Mariagrazia Morando, Elena Piano and Giovanni Soldato. Special thanks go to Ľubomír Kováč and Christian Komposch, who provided the comparative material. Also, thanks are due to John Plant for linguistically checking the original manuscript.

Fieldwork of MI and collaborators was supported by CAVELAB "From microclimate to climate change: caves as laboratories for the study of the effects of temperature on ecosystems and biodiversity", an interdisciplinary research project funded by the Compagnia di San Paolo and the University of Turin (2012–2015).

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