RESEARCH ARTICLE



Observations on the habitat and feeding behaviour of the hypogean genus *Eukoenenia* (Palpigradi, Eukoeneniidae) in the Western Italian Alps

Valentina Balestra^{1,2,3}, Enrico Lana^{2,3}, Stefano Vanin^{4,5}

I Department of Environment, Land and Infrastructure Engineering, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy 2 Biologia Sotterranea Piemonte – Gruppo di Ricerca, clo Bossea cave, Frabosa Soprana (CN), Italy 3 S.O. Bossea C.A.I – Underground Karst Laboratory of Bossea cave, Frabosa Soprana (CN), Italy 4 Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy 5 National Research Council, Institute for the Study of Anthropic Impact and Sustainability in the Marine Environment (CNR-IAS), Via de Marini 6, 16149, Genova, Italy

Corresponding author: Valentina Balestra (valentina.balestra@polito.it)

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Abstract

The order Palpigradi includes species characterized by millimetric size and a characteristic flagellum with bristles at the end of the opisthosoma. They represent one of the less well-known and obscure arachnid orders. In this paper, observations were made on the ecology and feeding behavior of species belonging to the genus *Eukoenenia* Börner, 1901, from the Western Italian Alps. Direct observations and photographic documentation of 141 individuals in their cave habitat, allowed the recording of data on the physical and trophic conditions such as the presence/absence of trophic resources, temperature and relative humidity, of the underground environment in which they were found. Results showed that the species of this taxon are not as rare as previously reported and that their presence is mainly influenced by temperature, relative humidity, trophic resources and the presence of two speleothems: rimstone dams and rafts. The combination of our observations as well as data previously published highlights that the taxon can have predatory and saprophagous feeding behavior depending on the availability of the food resources. This work represents the starting point for a further investigation of the taxon.

Keywords

Arachnida, microwhip scorpions, subterranean biology, caves

Introduction

Palpigradi is the last described arachnid order and, to date, one of the lesser known and studied. The order includes small size species characterized by the whip-like flagellum with bristles at the end of the opisthosoma. The world fauna of living palpigrads consists of more than 100 described species divided in two families: Eukoeneniidae and Prokoeneniidae (Harvey 2002; Giribet et al. 2014).

The first scientific investigation of the species in this order began in 1885 in Sicily (Italy), where the zoologist Giovanni Battista Grassi (Grassi and Calandruccio 1885) discovered a "mysterious species" of arachnid that he described as *Koenenia mirabilis* Grassi & Calandruccio, 1885, now *Eukoenenia mirabilis* (Grassi & Calandruccio, 1885). Silvestri (1905) described for the first time the anatomy of the species and he was followed by other authors but many aspects of the biology of the order, including food selection and reproduction, are still unknown (Condé 1996; Smrž et al. 2013; Christian et al. 2014; Parimuchová et al. 2021). Molecular phylogeny of the taxon was performed only by Giribet et al. (2014) that demonstrated the monophily of the order and of the family of Eukoeneniidae.

The elective habitats of the species in the genus *Eukoenenia* are interstitials (Howarth 1983; Mammola 2019) and the species can be ecologically classified in soiland cave-dwelling (Mammola et al. 2021b). The cave-dwelling species have been reported walking on the cave walls and ground (Condé 1996), speleothems (Souza and Ferreira 2010), decomposing wood (Balestra et al. 2019), sand banks near water stream (Souza et al. 2020) and on the surface of water pools (Christian et al. 2014; Balestra et al. 2019).

There is currently little known about the feeding of palpigrades. An observation of *Eukoenenia* hunting springtail provides evidence of a predatory habit (Lukić 2012), however, the presence of Cyanobacteria in their gut indicates an alternative food source (Smrž et al. 2013) and scavenging is also another possibility. A recent study based on molecular analysis (NGS) on the gut-content of *Eukoenenia spelaea* (Peyerimhoff, 1902) in Ardovská Cave, Slovakia, supports carnivory behaviour (Parimuchová et al. 2021).

Because there is minimal data available about the biology of the species of this genus, a continuous survey was carried out within 20 caves in Piedmont and Liguria regions (Northern Italy) in order to describe and better understand some aspects of their biology: nutrition, microhabitat and distribution. In particular, the following questions have been addressed: i) Is the paucity of records of this taxon related with bias in sampling or is it related with the low density of the populations? ii) What kind of environmental/ecological parameters affect the presence and the distribution of palpigrades in caves? iii) What is the trophic role of the taxon (potential predatory species, saprophagous species or both)?

Materials and methods

Study area

South Western Italian Alps are rich in natural caves of diverse origins and at different altitudes. The range of cave temperature is varied, with cold caves yielding temperatures lower than 8 °C, mainly at high altitude, and more mesophilic caves with average temperature at around 9–13 °C. This area is also rich in artificial cavities of interest for hypogean life study (Fig. 1).



Figure 1. Location of the sampling (maps used for the plate retrieved from https://d-maps.com/carte.php?num_car=2232&lang=en, https://d-maps.com/carte.php?num_car=5894&lang=en, https://d-maps.com/carte.php?num_car=8273&lang=en and modified).

In this area, six hypogean species of the *E. spelaea* species-complex have been reported (Balestra et al. 2019; Christian et al. 2014; Isaia et al. 2011; Pantini and Isaia 2019): *E. spelaea, E. strinatii* Condé, 1977, *E. bonadonai* (Condé, 1979), *E. roscia* Christian, 2014, *E. lanai* Christian, 2014, and an undescribed species of *Eukoenenia*. (E. Christian in litteris).

The investigated cavities are listed in Table1 and reported in Fig. 1. Their entrances are located at altitudes between 554 and 2163 m a.s.l. Two of them, Grotta della Mottera and Carsena di Piaggia Bella, are cold caves while the others 18 have an average temperature ranging between 9 and 13 °C. Miniera superiore di Monfieis is an artificial cavity.

The majority of the data reported in this paper were collected from the Bossea cave, the first Italian show cave, that is composed by different environments, and from four other cavities where *Eukoenenia strinatii* have been already reported (Balestra et al. 2019).

Sampling and observation method

Pre-evaluations based on literature research, biogeographic and field observations allowed the selection of 20 different caves where biotic and abiotic factors that can influence palpigrade life as temperature (T $^{\circ}$ C), relative humidity (RH%), light intensity (LI lux), type of substrate, speleothems, presence, and abundance of decomposing organic matter and presence of water and fractures were monitored.

Random observations of each cave were performed in different periods of the year, with a minimum number of 2 days per cave in two different seasons. One up to six observation areas were defined for each cave at different distances from the cave entrance (ranging from few meters from the entrance to great depths) depending on the cave structure and the presence of peculiar microhabitat (Table 1). These areas were coded according to the cave name (three letters) and a number, corresponding with the inner sampling sites. The observations for each cave area lasted 20 minutes. Visual encountered surveys, supported with the acquisition of macrophotographs were performed. This is a non-invasive method, however, it required a priori biological knowledge of the taxa observed for the identification and it does not allow always a correct determination at a specific level of the taxa. Palpigrades are tiny arachnids and often close related species have very uniform general morphology, consequently, specimens for species identification were collected manually, using a metal spatula made by one of the authors (E.L.) which is more effective in collecting palpigrades, and placed directly in 70-96% ethyl alcohol in sampling tubes. Identification was performed by specialists, as listed in the acknowledgments.

Macrophotography has several advantages, such as highlighting details not visible at naked eye or reviewing behaviour of the observed individuals, however, also different disadvantages, especially in cave where bringing photographic equipment can be complex due to habitat impediments (see Balestra et al. 2021; Mammola et al. 2021a). Photographs of the specimens were taken using a Canon EOS 550D and 760D reflex cameras equipped with MP-E 65 mm Macro lenses and MT-24EX Macro flash and Canon EOS 70D reflex camera equipped with EF 100 mm Macro lens 1:2.8 USM

Cavity name	Cavity type	Ы	Geographical coordinates WGS84	Municipa- lity/ area	Alps	Altitude (m a.s.l.)	Linear cave development (m)	Geology	Sampling areas	Distance from the entrance (m)
Buco del Partigiano	Cave	PI1315	44.506897 N, 7.2932269 E	Roccabruna	Cottian	1170	13	Dora-Maira units	PAR1	0-50
Buranco di Bardineto	Cave	LI364–1364	44.1994345 N, 8.12489 E	Bardineto	Liguri	770	2060	Limestones and dolostones of	BUR1	100-200
)			the Brianzonesi units	BUR2	100 - 200
									BUR3	100 - 200
									BUR4	200-300
Grotta occidentale del Bandito	Cave	PI1003	44.290002 N, 7.427431 E	Valdieri	Marittime	714	690	Sub-Brianzonesi units	BAN1	0-50
Grotta di Bossea	Cave	PI108	44.241548 N, 7.8398498 E	Frabosa	Ligurian	836	2800	Marbles and dolomitic marbles	BOS1	400-500
				Soprana				of the Brianzonesi Units	BOS2	500-600
									BOS3	500-600
									BOS4	500-600
									BOS5	400-500
									BOS6	400-500
									BOS7	200–300
Grotta Rio dei Corvi	Cave	P1884	44.3012409 N, 7.9939506 E	Lisio	Ligurian	800	300	Piedmontese zone	RDC1	100 - 200
Grotta del Baraccone	Cave	PI309	44.2733643 N, 8.0867885 E	Bagnasco	Ligurian	1040	39	Dolomites of the Piemontesi	BAR1	0-50
								Units		
Grotta di Rio Borgosozzo	Cave	P1695	44.1219794 N, 7.8294941 E	Ormea	Ligurian	870	90	Limestones and dolostones of	RBS1	0-50
								the Brianzonesi units	RBS2	0-50
									RBS3	0-50
Grotta dei Dossi	Cave	PI106	44.3404645 N, 7.7429119 E	Villanova	Ligurian	626	580	Dolomites of the Piemontesi	DOS1	0-50
				Mondovì				Units	DOS2	0-50
									DOS3	050
Grotta "Barôn Litrôn"	Cave	PI1214	44.2616808 N, 7.4093819 E	Valdieri	Marittime	1050	861	Limestones of the Subbrianzo-	BAL1	100-200
								nesi Units		
Grotta del Caudano	Cave	PI121-122	44.2930025 N, 7.7905788 E	Frabosa	Ligurian	780	3200	Marbles and dolomitic marbles	CAU1	400–500
				Sottana				of the Brianzonesi Units	CAU2	500-600
Grotta delle Vene	Cave	PI103	44.1514836 N, 7.7513324 E	Upega	Ligurian	1558	6285	Limestones and dolostones of	VEN1	500-600
								the Brianzonesi units		
Grotta di Rossana	Cave	PI1010	44.5325001 N, 7.4306342 E	Rossana	Cozie	554	195	Dolostones of the Piemontesi Units	ROS1	100–200
Miniera suneriore di Monfieis	Mine	CAPI7046	44.3604152 N. 7.2658630 E	Demonte	Cozie	1750	474	Brianzonese zone	MIN1	0-50
						> \				>

Table 1. Examined caves and sampling areas. The identification code of the cave in the Piedmont and Ligurian cadastre caves is shown in the column Id.

(m) Cave P1204-227- 44.1177137 N, 7.9699005 E Caprauma Ligurian 860 2570 228-284 228-284 44.1293344 N, 7.9333236 E Ormea Ligurian 860 2570 Cave P1126 44.1293344 N, 7.9333236 E Ormea Ligurian 1590 143 Cave P13613 44.2402139 N, 7.8485798 E Roburent Ligurian 1037 1701 cave P13613 44.1190364 N, 7.8753066 E Ormea Ligurian 810 705 cdi Nava Cave P1118 44.11901 N, 7.8101901 E Piancavallo- Ligurian 1038 120 Cave L1252 44.11901 N, 7.8101901 E Piancavallo- Ligurian 1038 120 Cave P1242 44.1997424 N, 7.8350138 E Ormea Ligurian 1325 22779 cave P1160 44.1672208 N, 7.7060822 E Brian Alta Marticine 2163 43000	Cavity name	Cavity type	Ы	Geographical coordinates WGS84 Municipa- lity/ area	Municipa- lity/ area	Alps		Altitude Linear cave (m a.s.l.) development	Geology	Sampling Distance areas from the	Distance from the
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Cave L1252 44.11901 N, 7.8101901 E Piancavallo- Ligurian 1038 120 Armasse trtera Cave P1242 44.1997424 N, 7.8350138 E Ormea Ligurian 1325 22279 sia Bella Cave P1160 44.1673208 N.7.7060822 E Brita Alta Maritrine 2163 43000									the Brianzonesi units	PDN2	100 - 200
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Cave P1242 44.1997424 N, 7.8350138 E Ormea Ligurian 1325 22279 Ila Cave P1160 44.1673208 N, 7.7060822 E Briea Alta Marittime 2163 43000					Armasse						
Cave P1160 44.1673208 N. 7.7060822 E Briga Alta Marittime 2163 43000	Grotta della Mottera	Cave	P1242	44.1997424 N, 7.8350138 E	Ormea	Ligurian	1325	22279	Brianzonese-Ligurian zone	MOT1	100-200
	Carsena di Piaggia Bella	Cave	P1160	44.1673208 N, 7.7060822 E	Briga Alta	Marittime	2163	43000	Brianzonese-Ligurian zone	CPB1	200-300

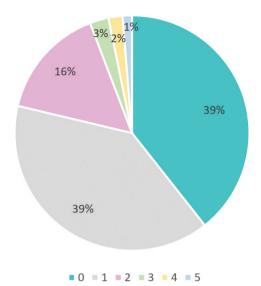


Figure 2. Number of specimens observed for sampling site.

and integrated flash. For the environmental photos, a Canon EOS 70D reflex camera equipped with EFS 18–55 mm lens and a Canon Power Shot D30 camera were used.

Environmental parameters were recorded in the presence of living animals. An HD 2101.1 Delta Ohm Thermohygrometer with a combined probe HP 472AC %RH and temperature Pt100 were used for environmental temperature and relative humidity measurements (Thermohygrometer HD 2101.1 Delta Ohm: Temperature: -200 / +650 °C, Relative humidity: 0.0 / 100.0%. HP 472AC %RH and temperature Pt100 combined probe: Area of use: -20 / +80 °C, 0 / 100% RH; accuracy: $\pm 2\%$ (5 to 95% RH), $\pm 3\%$ (95 to 99% RH), ± 0.3 °C (-20 to + 80 °C)). Due to the use of a 2.5 m long extension for the termohygrometer probe, all parameters were recorded without close human presence. pH was measured using litmus paper (Vetrotecnica 08.3000.00 pH 1 / 11).

Results

Survey and records

114 surveys in caves were performed finding at least one *Eukoenenia* specimen in 65.79% of the cases. No specimens were observed in cold caves (Grotta della Mottera and Carsena di Piaggia Bella) or in Grotta occidentale del Bandito, despite a previous record from this cave (Brignoli 1976). A total of 143 individuals in 17 different caves of the Western Italian Alps were observed (Table 2). 110 individuals were observed in the last 4 years of monitoring. The number of specimens observed in a sampling site in a day ranged from 0 to 5 (Fig. 2), with an average of 0.93 individuals/sampling site/day, and the number of specimens in a cave ranged from 0 to 8 per day.

Table 2. Observational records of *Eukoenenia* species under natural conditions in the western ItalianAlps caves.

Taxa	Cave	Sampling	dd/mm/yy	Alive	Find surface	Trophic	Т	UR%	pН
		area		or		resource	(°C)		
				dead					
Eukoenenia	Grotta Della Taramburla	TAR2	23/04/2017		water surface	yes	-	-	-
sp. nov.	Grotta Della Taramburla	TAR1	23/04/2017		water surface	yes	-	-	-
	Grotta Della Taramburla	TAR1	23/04/2017	alive	water surface	yes	-	-	-
	Grotta Della Taramburla	TAR1	24/12/2017		water surface	yes	_	_	_
	Grotta Della Taramburla	TAR1	31/12/2017		water surface	yes	11.7	100	7
	Grotta Della Taramburla	TAR1	31/12/2017		speleothem	yes	11.7	100	7
	Grotta Della Taramburla	TAR3	31/12/2017		water surface	yes	-	-	7
	Grotta Della Taramburla	TAR1	30/09/2018	alive	water surface	no	13.6	91.1	7
	Garb del Dighea	DIG2	11/05/2018	alive	water surface water surface	no	9.4	89.8	7
Fukoenenia	Garb del Dighea	DIG1	11/05/2018		water surface water surface	yes	_	_	_
Euroenenia strinatii	Grotta Di Bossea Grotta Di Bossea	BOS5 BOS6	20/12/2009 21/12/2009		water surface	yes	-		-
struttu	Grotta Di Bossea	BOS5	18/08/2010	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS5	15/01/2011		water surface	yes	_	_	_
	Grotta Di Bossea	BOS1	11/03/2011	alive	water surface	yes	-	_	_
	Grotta Di Bossea	BOS1 BOS1	12/03/2011		water surface	yes	_	_	_
	Grotta Di Bossea	BOS1 BOS1	16/08/2011		water surface	yes yes	_	_	_
	Grotta Di Bossea	BOS5	20/10/2011	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS6	21/12/2011	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS5	26/12/2011	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS5	27/01/2012		water surface	yes	_	_	_
	Grotta Di Bossea	BOS5	13/08/2012		water surface	yes	_	_	_
	Grotta Di Bossea	BOS6	20/02/2013	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS5	19/09/2013		water surface	yes	_	_	_
	Grotta Di Bossea	BOS5	30/04/2014	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS5	17/11/2014	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS7	29/12/2014	alive	water surface	yes	_	_	_
	Grotta Di Bossea	BOS1	15/08/2015	alive	water surface	yes	-	-	_
	Grotta Di Bossea	BOS5	26/06/2016	alive	water surface	yes	-	-	_
	Grotta Di Bossea	BOS5	26/06/2016	alive	speleothem	yes	-	-	_
	Grotta Di Bossea	BOS6	30/08/2016	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS5	10/10/2016	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS6	10/10/2016	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS6	10/10/2016	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS1	19/10/2016	alive	near water surface	yes	-	-	-
	Grotta Di Bossea	BOS1	02/09/2017	dead	water surface	no	-	-	-
	Grotta Di Bossea	BOS1	02/09/2017	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS1	02/09/2017	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS1	13/09/2017	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS1	13/09/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS5	26/09/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS1	17/12/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS1	17/12/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS2	17/12/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS2	17/12/2017		near water surface	yes	-	-	-
	Grotta Di Bossea	BOS2	17/12/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS2	17/12/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS5	26/12/2017		water surface	yes	-	-	-
	Grotta Di Bossea	BOS3	09/01/2018		water surface	-	-	-	-
	Grotta Di Bossea	BOS5	09/01/2018		water surface	yes	-	- 01 5	- 7
	Grotta Di Bossea	BOS3	13/01/2018		water surface	yes	11.9	81.5	7
	Grotta Di Bossea Grotta Di Bossea	BOS1	13/01/2018		water surface	yes	9.4	92.9	7
	Grotta Di Bossea Grotta Di Bossea	BOS3 BOS5	20/01/2018 20/01/2018		water surface water surface	yes	-	-	_
	Giotta Di Dossea	BOS5	20/01/2018	alive	water surrace	yes	-	-	_

Taxa	Cave	Sampling	dd/mm/yy	Alive	Find surface	Trophic	Т	UR%	pН
		area		or		resource	(°C)		
E I ···	C D'P	DOC1	02/05/2010	dead			10.2	00.1	
Eukoenenia strinatii	Grotta Di Bossea	BOS1	03/05/2018		water surface	yes	10.3	90.1	7
strinatti	Grotta Di Bossea Grotta Di Bossea	BOS1	27/05/2018 02/06/2018	alive	water surface	yes	-	-	- 7
	Grotta Di Bossea Grotta Di Bossea	BOS5 BOS5	02/06/2018	alive alive	water surface water surface	yes	9.3	96 96	7 7
	Grotta Di Bossea	BOS5	02/06/2018	dead	water surface	yes	9.3	96	7
	Grotta Di Bossea Grotta Di Bossea	BOS5 BOS5	26/06/2018	alive	water surface	yes	9.3 -	90	/
	Grotta Di Bossea	BOS5	26/06/2018	alive	water surface	yes	_	_	_
	Grotta Di Bossea Grotta Di Bossea	BOS5 BOS5	30/06/2018	alive	water surface	yes	- 9.6	- 92.5	7
	Grotta Di Bossea	BOS5	30/06/2018	alive	water surface	yes	10	92.9 94.1	7
	Grotta Di Bossea			alive	water surface	yes	9	94.1 94.3	
	Grotta Di Bossea	BOS5 BOS5	30/06/2018 30/06/2018		water surface	yes	9		7 7
						yes		94.3	
	Grotta Di Bossea	BOS4	11/07/2018	alive	water surface	no	11	85.6	7
	Grotta Di Bossea	BOS5	11/07/2018		water surface	-	-	-	-
	Grotta Di Bossea	BOS5	11/07/2018	alive	water surface	no	11.2	83.6	7
	Grotta Di Bossea	BOS5	11/07/2018	alive	water surface	yes	10.1	89.9	7
	Grotta Di Bossea	BOS5	11/07/2018	alive	water surface	yes	10.1	89.9	7
	Grotta Di Bossea	BOS5	11/07/2018		water surface	Yes	_	-	_
	Grotta Di Bossea	BOS6	11/07/2018	alive	water surface	yes	10.2	93.4	7
	Grotta Di Bossea	BOS6	11/07/2018	alive	water surface	yes	10.2	93.4	7
	Grotta Di Bossea	BOS5	29/08/2018	alive	water surface	yes	10.5	90.4	7
	Grotta Di Bossea	BOS5	29/08/2018	alive	water surface	yes	10.5	90.4	7
	Grotta Di Bossea	BOS6	29/08/2018	alive	water surface	yes	10.1	92.2	7
	Grotta Di Bossea	BOS6	29/08/2018	dead	water surface	-	-	-	-
	Grotta Di Bossea	BOS5	18/09/2018	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS6	18/09/2018	alive	water surface	yes	-	-	-
	Grotta Di Bossea	BOS5	27/01/2019	alive	water surface	yes	9.5	94.5	7
	Grotta Di Bossea	BOS5	27/01/2019	dead	water surface	-	-	-	-
	Grotta Di Bossea	BOS6	06/10/2019	alive	near water surface	yes	9.6	92	7
	Grotta Di Bossea	BOS6	06/10/2019	dead	water surface	-	-	-	-
	Grotta Di Bossea	BOS6	06/10/2019	dead	water surface	-	-	-	-
	Grotta Di Bossea	BOS6	06/10/2019	alive	water surface	yes	9.6	92	7
	Grotta Di Bossea	BOS6	06/10/2019	alive	water surface	yes	9.6	92	7
	Grotta Di Bossea	BOS5	06/10/2019	alive	water surface	yes	9.2	95.5	7
	Grotta Di Bossea	BOS5	06/10/2019	alive	water surface	yes	9.2	95.5	7
	Grotta Di Bossea	BOS5	06/10/2019	alive	water surface	no	9.2	96.1	7
	Grotta Rio dei Corvi	RDC1	05/01/2017	alive	water surface	yes	_	_	_
	Grotta Rio dei Corvi	RDC1	05/01/2017	alive	water surface	yes	_	_	_
	Grotta Rio dei Corvi	RDC1	15/03/2017	alive	water surface	yes	_	_	_
	Grotta Rio dei Corvi	RDC1	15/03/2017	alive	water surface	yes	_	_	_
	Grotta di Rio Borgosozzo	RBS1	01/05/2017	alive	water surface	yes	_	_	_
	Grotta di Rio Borgosozzo	RBS2	03/06/2018	alive	water surface	no	10.8	88.7	7
	Grotta di Rio Borgosozzo	RBS3	03/06/2018	alive	water surface	yes	10.9	87.7	7
	Grotta di Rio Borgosozzo	RBS3	03/06/2018	alive	water surface	no	10.9	87.7	7
	Grotta Del Baraccone	BAR1	11/03/2017		water surface	yes	_	_	_
	Grotta Dei Dossi	DOS1	30/12/2017		water surface	_	12.2	87.3	7
	Grotta Dei Dossi	DOS2	30/12/2017		water surface	_	12.3	83.8	7
	Grotta Dei Dossi	DOS3	30/12/2017		water surface	yes	13.3	81	7
	Grotta Dei Dossi	DOS3	30/12/2017		water surface	yes	13.3	81	7
Eukoenenia	Grotta Delle Vene	VEN1	30/12/2016		water surface	yes	-	_	_
bonadonai	Grotta Delle Vene	VEN1	28/05/2017		water surface	yes	_	_	_
/ 100000 / 1444	Grotta Del Caudano	CAU1	14/02/2010		water surface	yes	_	_	_
	Grotta Del Caudano Grotta Del Caudano	CAU1 CAU1	26/02/2010		water surface		-	-	_
	Grotta Del Caudano Grotta Del Caudano	CAU1 CAU1			water surface water surface	yes	-	-	_
	Grotta Del Caudano Grotta Del Caudano		11/01/2017			yes	-	_	_
		CAU1	11/01/2017		water surface	yes	-	-	-
	Grotta Del Caudano	CAU2	26/02/2017		water surface	-	-	-	-
	Grotta Del Caudano	CAU2	26/02/2017		water surface	yes	-	-	-
	Grotta Barôn Litrôn	BAL1	13/04/2003	alive	Wet wood	yes	-	-	-

Taxa	Cave	Sampling	dd/mm/yy	Alive	Find surface	Trophic	Т	UR%	pН
		area		or		resource	(°C)		
				dead					
Eukoenenia	Grotta Barôn Litrôn	BAL1	03/12/2006	alive	water surface	no	-	-	-
bonadonai	Grotta Barôn Litrôn	BAL1	17/01/2012	alive	speleothem	no	-	-	-
	Grotta Barôn Litrôn	BAL1	11/10/2016	alive	water surface	yes	-	-	-
Eukoenenia	Buco Del Partigiano	PAR1	11/09/2011	alive	under stone	no	-	-	-
spelaea	Buco Del Partigiano	PAR1	21/07/2012	alive	speleothem	no	-	-	-
	Buranco Di Bardineto	BUR1	20/05/2018	alive	water surface	yes	-	-	7
	Buranco Di Bardineto	BUR2	20/05/2018	alive	water surface	yes	-	-	7
	Buranco Di Bardineto	BUR2	20/05/2018	alive	water surface	yes	-	-	7
	Buranco Di Bardineto	BUR3	20/05/2018	alive	water surface	yes	-	-	7
	Buranco Di Bardineto	BUR3	20/05/2018	alive	water surface	yes	-	-	7
	Buranco Di Bardineto	BUR3	20/05/2018	alive	water surface	yes		-	7
	Buranco Di Bardineto	BUR3	20/05/2018	alive	water surface	yes	-	-	7
	Buranco Di Bardineto	BUR4	20/05/2018	alive	water surface	yes	-	-	6
	Buranco Di Bardineto	BUR2	13/10/2019	alive	water surface	yes	13.7	82.8	7
	Buranco Di Bardineto	BUR2	13/10/2019	alive	water surface	yes	12	88.2	7
	Buranco Di Bardineto	BUR3	13/10/2019	alive	water surface	no	12.4	86.7	7
	Buranco Di Bardineto	BUR3	13/10/2019	dead	water surface	-	-	-	_
Eukoenenia	Grotta Di Rossana	ROS1	16/02/2012	alive	speleothem	no	-	-	_
roscia	Grotta Di Rossana	ROS1	16/02/2012	alive	speleothem	no	-	-	_
	Grotta Di Rossana	ROS1	15/08/2012	alive	speleothem	no	-	-	_
	Grotta Di Rossana	ROS1	03/03/2013	alive	speleothem	no	_	_	_
Eukoenenia	Miniera Sup. Di Monfieis	MIN1	12/09/2010	alive	speleothem	no	_	-	_
lanai	Miniera Sup. Di Monfieis	MIN1	12/09/2010	alive	water surface	yes	_	_	_
	Miniera Sup. Di Monfieis	MIN1	12/09/2010	alive	speleothem	yes	_	-	_
Eukoenenia	Grotta Di Costacalda	GCC1	03/05/2018	alive	water surface	no	_	_	_
sp. 1*	Grotta Di Costacalda	GCC2	11/05/2018	alive	water surface	no	_	-	_
	Grotta Di Costacalda	GCC3	27/05/2018	alive	water surface	yes	_	_	_
Eukoenenia	Grotta Dell'orso Di Ponte Di Nava	PDN1	03/06/2018	alive	water surface	yes	10.9	87.6	7
sp. 2**	Grotta Dell'orso Di Ponte Di Nava	PDN2	03/06/2018	alive	water surface	yes	10	98.3	7
Eukoenenia	Arma Cornarea	COR1	04/01/2018	alive	water surface	yes	_	_	_
sp. 3***	Arma Cornarea	COR1	04/01/2018	alive	water surface	yes	_	_	_
	Arma Cornarea	COR1	20/04/2019	alive	water surface	ves	11.7	94.5	7

*Eukoenenia sp.1 – Balestra V. leg., sample damaged during preparation (E. Christian in litt.)

***Eukoenenia* sp.2 – Lana E. & Balestra V. leg., 1 male with a combination of characters that does not conform to any described species of NW Italy: 4 blades in the lat. organ; elevated number of a-setae on sternites IV-VI; tergites IV-VI with s-5t-s., 1 juvenile female, indet. (E. Christian in litt.)

****Eukoenenia* sp.3 – Balestra V. & Marovino M. leg., 1 juvenile female, not determined to species level: Eukoenenia of the group spelaea (E. Christian in litt.)

Environmental/ecological data

Temperature (T °C) and relative humidity (RH%) data related to the presence of 43 living individuals in eight different caves for different species have been recorded (Table 2, Fig. 3A, B). *Eukoenenia* species in Western Italian Alps resulted to be present in a temperature range between 9.0 and 13.7 °C and in a range of RH% between 81 and 100%.

A negative correlation between temperature and relative humidity is observed for the presence of *Eukoenenia strinatii* (r: -0.9285) (Fig.3B). For this species, observation sites at low temperature have a higher RH% compared to the sites at higher temperature. Correlation between the two parameters was not calculated for the other species due to the paucity of data despite a potential trend, shown in Fig. 3A.

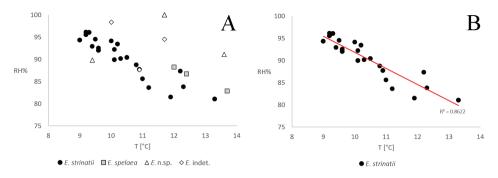


Figure 3. Relation between temperature and relative humidity in the micro-habitat of genus *Eukoenenia* in the Western Italian Alps. **A** *Eukoenenia* species **B** *E. strinatii.*

The pH of the water pools where 52 specimens were observed was close to neutral (pH 7). This result was obtained from 32 sampling sites, of which13 measurements were repetitions from the same site performed in different seasons confirming no seasonal variation (Table 2). The only exception was recorded from the Buranco di Bardineto cave, where the pH was nearly 6 in a single pool which was particularly rich in organic substance.

A total of 143 *Eukoenenia* individuals were observed on different microhabitat with the majority of them from water surfaces: one on wet wood, one under a stone, three near water, ten on the cave ground and 128 on the surface of pools that had calm water or a weak current (Table 2, Fig. 4, 5), especially in the rimstone dams, also called gours (Fig. 5A) – a particular type of speleothems (cave formation) in the form of a stone dam (Hill and Forti 1997). Moreover 16 dead individuals were observed but not

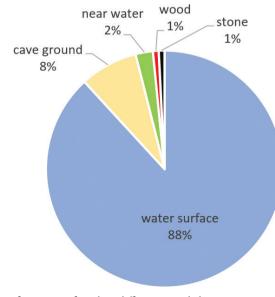


Figure 4. Percentage of specimens found on different microhabitats.

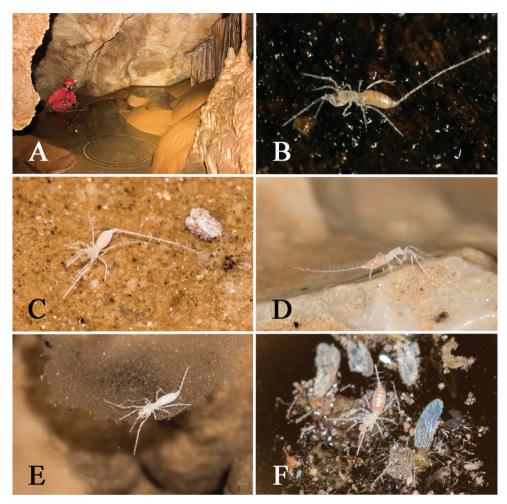


Figure 5. Habitat monitored and different microhabitat where *Eukoenenia* individuals were observed. **A** Rimstone dams (gours) in Buranco di Bardineto cave **B** *E. strinatii* on wood in Bossea cave **C** *E. strinatii* near water in Bossea cave **D** *E. strinatii* on Bossea cave ground **E** *E. strinatii* on raft of crystalline materials in Bossea cave **F** *E. strinatii* on raft of crystalline materials, fungal hyphae and organic remains in Bossea cave. (photos **A, C, D, E, F** by V. Balestra, **B** by E. Lana).

considered for the ecological interpretation of the taxon and it has not been possible to define the way in which they reach the place they were observed.

A significant association between *Eukoenenia* individuals and environments rich in organic matter was observed (Yates' Chi square: 62.41, p < 0.000), in fact, in the rimstone dams, where 112 living *Eukoenenia* individuals were sampled, trophic resources were abundant. Cave rafts, that are mainly calcite crystals, are common on the surface of quiescent waterbody such as the rimstone dams (Hill and Forti 1997) (Fig. 5E) and they often "trap" dead animals, fungal hyphae and organic remains (Fig. 5F).

Eukoenenia individuals were observed in different sampling areas ranging from a few meters to more than 500 m from the entrance. However, due to the sampling method and the structure of the investigated caves, no conclusion can be drawn about the effect of the distance from the entrance to the taxon distribution.

In addition, it is worth mentioning that *E. strinatii* was also observed in 3 sampling areas in the touristic Bossea cave where light, even if not direct or continual, was present.

Observation on trophic role of the taxon and feeding behaviour

As previously mentioned, an association between the presence of living specimens and the presence of organic matter on the water surface was demonstrated in the sampling area. Individuals of Eukoenenia were also observed feeding on different species of dead springtails. In particular, in June 2016, an E. strinatii individual was observed and photographed for the first time feeding on dead springtail (Balestra et al. 2019; Lana et al. 2016) (Fig. 6). The observed specimen (observation made through Canon macro lens, 65 and 100mm, f/2.8) moved on the surface of the water in an unusual way, using the three pairs of hind legs and the palps on the liquid, keeping up the legs of the first pair stretched forward and the flagellum in a vertical position (Fig. 6A–D). The arachnid approached a dead springtail, Pseudosinella alpina Gisin, 1950, and began to suck its internal liquids having damaged the integument of the corpse in the abdomen with the chelicerae; a drop of exudate came out in which Eukoenenia immersed the chelicerae and approached the mouthparts (Fig. 6E-G). The drop of exudate was consumed in about 15 minutes and then the specimen moved away from its meal with a particular walking: with short steps, with the paws of the second and third pair and the palps aligned, the paws of the first pair raise and face forward and the abdomen is raised with the flagellum pointing upwards (Fig. 6H). In addition, in June 2018, in Rio Borgosozzo cave, a specimen of *E. strinatii* that carried a dead springtail, holding it with chelicerae, was photographed (Fig. 7). While moving, it held the same position of the *E. strinatii* as previously described from the Bossea cave after its meal.

In contrast with the feeding activity of *Eukoenenia* on dead springtail specimens and despite the observed proximity between specimens of *Eukoenenia* and living and floating entomobrid Collembola apart from rare phenomena of palpation with the paws of the first pair, no attacks by the palpigrades on the living springtails were observed, recorded and documented.

On the contrary, in one case we observed and documented a potential response of a springtail against *Eukoenenia* (Fig. 8). After several palpations by the palpigrade on two springtails (Fig. 8A, B), *E. strinatii* tried to climb on one of the springtails (Fig. 8C), which in reaction, potentially bit the arachnid in the lower part of the body. The springtail moved to another part of the gour and for a few moments *E. strinatii* remained lying on its side, touching the mesosoma with its chelicerae (Fig. 8D). Later the arachnid got up and started to walk again on the surface of water.

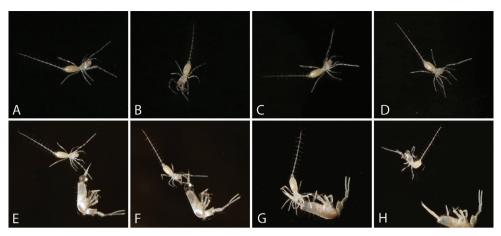


Figure 6. Approach and meal of Eukoenenia strinatii on Pseudosinella alpina (photos by E. Lana).



Figure 7. Meal of *Eukoenenia strinatii* on dead springtail (photo by V. Balestra).

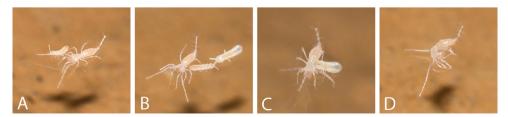


Figure 8. Approach and bite of *Eukoenenia strinatii* and two living Collembola (photos by V. Balestra).

Discussions

In the last year, due to the high sampling effort, the level of knowledge about the distribution of the species of the genus *Eukoenenia* in Western Italian Alps has been exponentially increased: 1) more than 100 individuals were observed and documented in the last four years; 2) before 2016 palpigrade specimens were observed only in seven caves, whereas today *Eukoenenia* species are reported from 17 caves.

Therefore, it has been possible to increase the number of sites where *Eukoenenia* species is present, both in term of caves and in term of areas within caves. For example, *Eukoenenia strinatii* was considered endemic in the Bossea cave until 2016, now this species is reported from other four caves and from different areas within the Bossea cave (from seven in 2016 to 10 in 2021) (Balestra et al. 2019; Condé 1977; Morisi 1992).

The data reported here highlights the fact that palpigrades in Western Italian Alps seem to be not so rare: probably the paucity of data on these organisms was due to the difficult access to some habitats (Ficetola et al. 2019; Howarth 1983; Mammola 2019) and to the lack of knowledge of the environmental parameters that define the preferred habitat for this taxon. Moreover, in (Mammola et al. 2021b) a significant species-people correlation effect (number of species/number of researchers studying palpigrades) has been described, showing that the concentration of palpigrade records in some regions of the globe can be influenced by the presence of researchers interested in this group.

As for other species of invertebrates present in the Bossea cave, palpigrades do not seem to be particularly and negatively influenced by tourism. Despite their troglomorphism they tolerate the presence of lights, even if not direct, and probably only for brief periods. The presence of palpigrades in touristic cave was also observed in the Brazilian Maquiné cave, where *E. maquinensis* Souza & Ferreira 2010 is reported (Ferreira and Souza 2012).

Temperature and relative humidity values recorded in this study are typical of Alpine caves, however, the sampling data revealed the absence of *Eukoenenia* in cold caves (T < 8 °C). The most favorable caves for *Eukoenenia* genus seem to be those with mild temperatures and very high relative humidity, or higher temperatures and lower relative humidity. The parameters monitored in Maquiné cave, Brazil, showed higher temperature (23.6 °C to 24.5 °C) but similar RH% (89 to 95%) for *E. maquinensis* (Ferreira and Souza 2012).

Palpigradi living in the hypogean environment of the Western Italian Alps have usually been observed on the surface of pools of rimstone dams. They have rarely been observed on wet wood, near water, on wet stones or on the cave ground. This does not mean that the favored environment of these small arachnids is the water surface of the underground pools, on which they probably can easily float thanks to the surface tension of the liquid or with rafts, but that this possibly reflect higher detectability in this cave habitat. Their main habitats can be those interstitials (Howarth 1983; Mammola 2019) and probably they could be transported in underground pools by water flow during rainy periods, getting trapped on the rafts, or they could voluntarily go into this environment in search of food. In fact, relying on different observations on individuals on the water surface, the presence of trophic resources in 90% of cases makes it reasonable to think that food availability is the main factor influencing *Eukoenenia* specimens to venture on the rafts and in the gours.

In more than 100 direct observations in caves it was never possible to document an attack of palpigrades on a prey. An approach on dead springtails sucking their internal liquids have been observed. If the ingestion of liquid food is their feeding system, as observed in other arachnids, it could be explained why no solid remains were found in the digestive tract of the palpigrades (Condé 1984; Millot 1942). A direct attack on collembola in caves was documented (Lukić 2012) and a specimen of *E. strinatii* that carried a dead springtail, keeping it with chelicerae, was photographed in this work. Moreover, a recent study on gut content of *E. spelaea* has shown the presence of spiders, beetles, mites, springtails, and flies DNA (Parimuchová et al. 2021), supporting carnivory in palpigrades. Wheeler (1900) suggested that palpigrades probably feed on eggs or juvenile stages of bigger arthropods invertebrates, however, our observations on scavenging activity could explain the presence of genetic material of large arthropods in the intestinal tube of palpigrades.

The combination of our observations and the data from other authors support the idea that palpigrades are predator and scavengers depending on the food resources availability, due to the fact that caves are extreme environments with limited trophic resources. Cyanobacteria and Fungi could be an alternative food source or, probably, an accidental consumption due to the ingestion of contaminated prey, as suggested by Parimuchová et al. (2021).

Conclusion

In conclusion the results of this study highlights that:

i. The hypogean palpigrades in the Western Italian Alps seems to be anything but rare and the paucity of records of this taxon is related to sampling bias. A correct knowledge of the preferred habitat and the environmental conditions where they live allows the observation of a relevant number of specimens.

ii. Hypogean palpigrades were observed in distinct regions inside caves, located at different altitudes, including records in areas with artificial lighting for tourist access. The presence of these animals is mainly influenced by temperature, relative humidity, trophic resources and the presence of two speleothems: rimstone dams and rafts. The most favorable cave habitats for *Eukoenenia* genus seem to be those with mild temperatures and very high relative humidity, or higher temperatures and lower relative humidity whereas the taxon was not recorded from cold caves.

iii. Palpigrades are predators and scavengers depending on the food resources availability due to the fact that caves are extreme environment with limited trophic resources.

Caves are special environments that host unique creatures in a world still to be explored and documented. In this study, it was possible to provide a first knowledge on the environmental parameters that can influence the distribution of hypogean palpigrades in Western Italian Alps. Moreover, the feeding behaviour in palpigrades is directly observed and photographed for the first time. Direct observation and photographs can be very useful to elucidate biological aspects of fragile groups that are difficult to keep under laboratory conditions, such as palpigrades. This research can be considered the starting point for future and more detailed studies on this curious genus and other apparently rare hypogean taxa in Italian caves.

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