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

Valentina Balestra¹ ² ³, Enrico Lana² ³, Stefano Vanin⁴ ⁵

¹ 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, c/o 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)

Academic editor: R.L. Ferreira | Received 26 September 2021 | Accepted 7 January 2022 | Published 18 January 2022

Citation: Balestra V, Lana E, Vanin S (2022) Observations on the habitat and feeding behaviour of the hypogean genus *Eukoenenia* (Palpigradi, Eukoeneniidae) in the Western Italian Alps. Subterranean Biology 42: 23–41. https://doi.org/10.3897/subtbiol.42.75784

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 soil-and 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 Table 1 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 Monfies 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 °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...
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.

| Cavity name                      | Cavity type | Id       | Geographical coordinates WGS84 | Municipality/ area | Alps   | Altitude (m a.s.l.) | Linear cave development (m) | Geology                                      | Sampling areas | Distance from the entrance (m) |
|---------------------------------|-------------|----------|-------------------------------|-------------------|--------|--------------------|-----------------------------|---------------------------------------------|----------------|-----------------------------|
| Buco del Partigiano Cave        | Cave        | PI1315   | 44.506897 N, 7.2932269 E       | Roccabruna        | Cottian| 1170               | 13                          | Dora-Maira units              | PAR1           | 0–50                        |
| Buranco di Bardinetto Cave      | Cave        | L1364–1364 | 44.1994345 N, 8.12489 E       | Bardinetto        | Liguri | 770                | 2060                        | Limestones and dolostones of the Brianzonesi units | BUR1           | 100–200                     |
|                                 |             |          |                               |                   |        |                    |                             | 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           | Cave        | PI108    | 44.241548 N, 7.8398498 E      | Frabosa Soprana   | Ligurian | 836             | 2800                        | Marbles and dolomitic marbles of the Brianzonesi Units | BOS1           | 400–500                     |
|                                 |             |          |                               |                   |        |                    |                             | BOS2                          |                | 500–600                     |
|                                 |             |          |                               |                   |        |                    |                             | BOS3                          |                | 500–600                     |
|                                 |             |          |                               |                   |        |                    |                             | BOS4                          |                | 500–600                     |
|                                 |             |          |                               |                   |        |                    |                             | BOS5                          |                | 400–500                     |
|                                 |             |          |                               |                   |        |                    |                             | BOS6                          |                | 400–500                     |
|                                 |             |          |                               |                   |        |                    |                             | BOS7                          |                | 200–300                     |
| Grotta Rio dei Corvi Cave       | Cave        | PI884    | 44.3012409 N, 7.9935906 E     | Lisio             | Ligurian | 800             | 300                          | Piedmontese zone              | RDC1           | 100–200                     |
| Grotta del Baraccone Cave       | Cave        | PI309    | 44.2733643 N, 8.0867885 E     | Bagnasco          | Ligurian | 1040            | 39                          | Dolomites of the Piemontesi Units | BAR1           | 0–50                        |
| Grotta di Rio Borgoassozo Cave  | Cave        | PI695    | 44.1219794 N, 7.8294941 E     | Ormea             | Ligurian | 870             | 90                          | Limestones and dolostones of the Brianzonesi units | RBS1           | 0–50                        |
|                                 |             |          |                               |                   |        |                    |                             | RBS2                          |                | 0–50                        |
|                                 |             |          |                               |                   |        |                    |                             | RBS3                          |                | 0–50                        |
| Grotta dei Dossi Cave           | Cave        | PI106    | 44.3404645 N, 7.7429119 E     | Villanova Mondovi | Ligurian | 626             | 580                          | Dolomites of the Piemontesi Units | DOS1           | 0–50                        |
|                                 |             |          |                               |                   |        |                    |                             | DOS2                          |                | 0–50                        |
|                                 |             |          |                               |                   |        |                    |                             | DOS3                          |                | 0–50                        |
| Grotta "Barón Litrón" Cave     | Cave        | PI1214   | 44.2616808 N, 7.4093819 E     | Valdieri          | Marittime | 1050            | 861                          | Limestones of the SubBrianzonesi Units | BAL1           | 100–200                     |
| Grotta del Caudano Cave         | Cave        | PI121–122 | 44.2930025 N, 7.7905788 E     | Frabosa Sottana   | Ligurian | 780             | 3200                        | Marbles and dolomitic marbles of the Brianzonesi Units | CAU1           | 400–500                     |
|                                 |             |          |                               |                   |        |                    |                             | CAU2                          |                | 500–600                     |
| Grotta delle Vene Cave          | Cave        | PI103    | 44.1514836 N, 7.7513324 E     | Upega             | Ligurian | 1558            | 6285                        | Limestones and dolostones of the Brianzonesi units | VEN1           | 500–600                     |
| Grotta di Rossana Cave          | Cave        | PI1010   | 44.5325001 N, 7.4306342 E     | Rossana           | Cozie   | 554             | 195                          | Dolomites of the Piemontesi Units | ROS1           | 100–200                     |
| Miniera superiore di Monfiesi    | Mine        | CAPI7046 | 44.3604152 N, 7.2658630 E     | Demonte           | Cozie   | 1750            | 474                          | Brianzonesi zone              | MIN1           | 0–50                        |
| Cavity name                          | Cavity type | Id            | Geographical coordinates WGS84 | Municipality/area | Alps       | Altitude (m a.s.l.) | Linear cave development (m) | Geology                                         | Sampling areas | Distance from the entrance (m) |
|-------------------------------------|-------------|---------------|------------------------------|------------------|------------|---------------------|------------------------------|----------------------------------|----------------|-------------------------------|
| Grotta della Taramburla Cave        | Cave        | PI204–227–228–284 | 44.1177137 N, 7.9699005 E   | Caprauna         | Ligurian   | 860                 | 2570                         | Limestones and dolostones of the Brianzoni units | TAR1 TAR2 TAR3     | 0–50 50–100 50–100             |
| Garb del Dighea                    | Cave        | PI126         | 44.1293344 N, 7.9333236 E   | Ormea            | Ligurian   | 1590                | 143                          | Limestones and dolostones of the Brianzoni units | DIG1 DIG2 DIG3    | 50–100 50–100                  |
| Grotta di Costacalda Cave          | Cave        | PI3613        | 44.2402139 N, 7.8485798 E   | Roburent         | Ligurian   | 1037                | 1701                         | Marbles and dolomitic marbles of the Brianzoni Units | GCC1 GCC2 GCC3    | 400–500 50–100 200–300         |
| Grotta dell’Orso di Ponte di Nava  | Cave        | PI118         | 44.1190364 N, 7.8753066 E   | Ormea            | Ligurian   | 810                 | 705                          | Limestones and dolostones of the Brianzoni units | PDN1 PDN2 PDN3    | 0–50 100–200 100–200           |
| Arma Cornarea                      | Cave        | LI252         | 44.11901 N, 7.8101901 E     | Piancavallo-Armase | Ligurian   | 1038                | 120                          | Brianzoni-Ligurian zone          | COR1             | 100–200                       |
| Grotta della Mottera Cave          | Cave        | PI242         | 44.1997424 N, 7.8350138 E   | Ormea            | Ligurian   | 1325                | 2279                         | Brianzoni-Ligurian zone          | MOT1             | 100–200                       |
| Carsena di Piaggia Bella Cave      | Cave        | PI160         | 44.1673208 N, 7.7060822 E   | Briga Alta       | Marittime  | 2163                | 4300                         | Brianzoni-Ligurian zone          | CPB1              | 200–300                       |
Habitat and feeding behaviour of the hypogean genus *Eukoenenia* (Palpigradi)

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: ±2% (5 to 95% RH), ±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 thermohygrometer probe, all parameters were recorded without close human presence. 
PpH 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 Italian Alps caves.

| Taxa              | Cave            | Sampling area | dd/mm/yy | Alive or dead | Find surface          | Trophic resource | T (°C) | UR% | pH |
|-------------------|-----------------|---------------|----------|---------------|-----------------------|------------------|--------|-----|----|
| *Eukoenenia* sp. nov. | Grotta Della Taramburla TAR2 | 23/04/2017 | dead     | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Della Taramburla TAR1 | 23/04/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Della Taramburla TAR1 | 23/04/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Della Taramburla TAR1 | 24/12/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Della Taramburla TAR1 | 31/12/2017 | alive    | water surface | yes                   | 11.7             | 100    | 7   | 7  |
|                   | Grotta Della Taramburla TAR1 | 31/12/2017 | alive    | speleothem   | yes                   | 11.7             | 100    | 7   | 7  |
|                   | Grotta Della Taramburla TAR1 | 31/12/2017 | alive    | water surface | yes                   | –                | –      | –   | 7  |
|                   | Grotta Della Taramburla TAR1 | 30/09/2018 | alive    | water surface | no                    | 13.6             | 91.1   | 7   | 7  |
| Garb del Dighea   | DIG2            | 11/05/2018   | alive    | water surface | no                    | 9.4              | 89.8   | 7   | 7  |
| *Eukoenenia* strinatii | Grotta Di Bossea BOS5 | 20/12/2009 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS6 | 21/12/2009 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 18/08/2010 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 15/01/2011 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS1 | 11/03/2011 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS1 | 12/03/2011 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS1 | 16/08/2011 | alive    | water surface | 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 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 13/08/2012 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS6 | 20/02/2013 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 19/09/2013 | alive    | 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 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 26/09/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS1 | 17/12/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS1 | 17/12/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS2 | 17/12/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS2 | 17/12/2017 | alive    | near water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS2 | 17/12/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS2 | 17/12/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 26/12/2017 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS3 | 09/01/2018 | dead     | water surface | –                     | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 09/01/2018 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS3 | 13/01/2018 | alive    | water surface | yes                   | 11.9             | 81.5   | 7   | 7  |
|                   | Grotta Di Bossea BOS1 | 13/01/2018 | alive    | water surface | yes                   | 9.4              | 92.9   | 7   | 7  |
|                   | Grotta Di Bossea BOS3 | 20/01/2018 | alive    | water surface | yes                   | –                | –      | –   | –  |
|                   | Grotta Di Bossea BOS5 | 20/01/2018 | alive    | water surface | yes                   | –                | –      | –   | –  |
Habitat and feeding behaviour of the hypogean genus *Eukoenenia* (Palpigradi)

| Taxa                | Cave                  | Sampling area | dd/mm/yy  | Alive or dead | Find surface      | Trophic resource | T (°C) | UR%  | pH |
|---------------------|-----------------------|---------------|-----------|---------------|-------------------|------------------|--------|------|----|
| *Eukoenenia*        | **strinatii**         | **BOS1**      | 03/05/2018| alive         | water surface     | yes              | 10.3   | 90.1 | 7  |
|                     |                       | **BOS1**      | 27/05/2018| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **BOS5**      | 02/06/2018| alive         | water surface     | yes              | 9.3    | 96   | 7  |
|                     |                       | **BOS5**      | 02/06/2018| alive         | water surface     | yes              | 9.3    | 96   | 7  |
|                     |                       | **BOS5**      | 02/06/2018| dead         | water surface     | yes              | 9.3    | 96   | 7  |
|                     |                       | **BOS5**      | 26/06/2018| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **BOS5**      | 26/06/2018| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **BOS5**      | 30/06/2018| alive         | water surface     | yes              | 9.6    | 92.5 | 7  |
|                     |                       | **BOS5**      | 30/06/2018| alive         | water surface     | yes              | 10     | 94.1 | 7  |
|                     |                       | **BOS5**      | 30/06/2018| alive         | water surface     | yes              | 9      | 94.3 | 7  |
|                     |                       | **BOS5**      | 30/06/2018| dead         | water surface     | yes              | 9      | 94.3 | 7  |
|                     |                       | **BOS4**      | 11/07/2018| alive         | water surface     | no               | 11     | 85.6 | 7  |
|                     |                       | **BOS5**      | 11/07/2018| dead         | water surface     | –                | –      | –    |    |
|                     |                       | **BOS5**      | 11/07/2018| alive         | water surface     | no               | 11.2   | 83.6 | 7  |
|                     |                       | **BOS5**      | 11/07/2018| alive         | water surface     | yes              | 10.1   | 89.9 | 7  |
|                     |                       | **BOS5**      | 11/07/2018| alive         | water surface     | yes              | 10.1   | 89.9 | 7  |
|                     |                       | **BOS5**      | 11/07/2018| dead         | water surface     | –                | –      | –    |    |
|                     |                       | **BOS5**      | 11/07/2018| alive         | water surface     | yes              | 10.2   | 93.4 | 7  |
|                     |                       | **BOS5**      | 11/07/2018| alive         | water surface     | yes              | 10.2   | 93.4 | 7  |
|                     |                       | **BOS5**      | 29/08/2018| alive         | water surface     | yes              | 10.5   | 90.4 | 7  |
|                     |                       | **BOS5**      | 29/08/2018| alive         | water surface     | yes              | 10.5   | 90.4 | 7  |
|                     |                       | **BOS6**      | 29/08/2018| alive         | water surface     | yes              | 10.1   | 92.2 | 7  |
|                     |                       | **BOS6**      | 29/08/2018| dead         | water surface     | –                | –      | –    |    |
|                     |                       | **BOS6**      | 18/09/2018| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **BOS6**      | 18/09/2018| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **BOS6**      | 27/01/2019| alive         | water surface     | yes              | 9.5    | 94.5 | 7  |
|                     |                       | **BOS5**      | 27/01/2019| dead         | water surface     | –                | –      | –    |    |
|                     |                       | **BOS6**      | 06/10/2019| alive         | near water surface| yes              | 9.6    | 92   | 7  |
|                     |                       | **BOS6**      | 06/10/2019| dead         | water surface     | –                | –      | –    |    |
|                     |                       | **BOS6**      | 06/10/2019| alive         | water surface     | yes              | 9.6    | 92   | 7  |
|                     |                       | **BOS6**      | 06/10/2019| alive         | water surface     | yes              | 9.6    | 92   | 7  |
|                     |                       | **BOS6**      | 06/10/2019| alive         | water surface     | yes              | 9.2    | 95.5 | 7  |
|                     |                       | **BOS6**      | 06/10/2019| alive         | water surface     | yes              | 9.2    | 95.5 | 7  |
|                     |                       | **BOS6**      | 06/10/2019| alive         | water surface     | no               | 9.2    | 96.1 | 7  |
|                     |                       | **RDC1**      | 05/01/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **RDC1**      | 05/01/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **RDC1**      | 15/03/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **RDC1**      | 15/03/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **RBS1**      | 01/05/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **RBS2**      | 03/06/2018| alive         | water surface     | no               | 10.8   | 88.7 | 7  |
|                     |                       | **RBS3**      | 03/06/2018| alive         | water surface     | yes              | 10.9   | 87.7 | 7  |
|                     |                       | **RBS3**      | 03/06/2018| alive         | water surface     | no               | 10.9   | 87.7 | 7  |
|                     |                       | **BAR1**      | 11/03/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **DOS1**      | 30/12/2017| dead         | water surface     | –                | 12.2   | 87.3 | 7  |
|                     |                       | **DOS2**      | 30/12/2017| dead         | water surface     | –                | 12.3   | 83.8 | 7  |
|                     |                       | **DOS3**      | 30/12/2017| alive         | water surface     | yes              | 13.3   | 81   | 7  |
|                     |                       | **DOS3**      | 30/12/2017| alive         | water surface     | yes              | 13.3   | 81   | 7  |
|                     |                       | **VEN1**      | 30/12/2016| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **VEN1**      | 28/05/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **CAU1**      | 14/02/2010| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **CAU1**      | 26/02/2012| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **CAU1**      | 11/01/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **CAU2**      | 26/02/2017| dead         | water surface     | –                | –      | –    |    |
|                     |                       | **CAU2**      | 26/02/2017| alive         | water surface     | yes              | –      | –    |    |
|                     |                       | **BAL1**      | 13/04/2003| alive         | Wet wood          | yes              | –      | –    |    |
**Taxa** Cave Sampling area dd/mm/yy Alive or dead Find surface Trophic resource T (°C) UR% pH

**Eukoenenia bonadonai**
- Grotta Baròn Litron BAL1 03/12/2006 alive water surface no – – –
- Grotta Baròn Litron BAL1 17/01/2012 alive speleothem no – – –
- Grotta Baròn Litron BAL1 11/10/2016 alive water surface yes – – –

**Eukoenenia spelaea**
- Buco Del Partigiano PAR1 11/09/2011 alive under stone no – – –
- 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 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 roscia**
- Grotta Di Rossana ROS1 16/02/2012 alive speleothem no – – –
- 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 lanai**
- Miniera Sup. Di Monfieis MIN1 12/09/2010 alive speleothem yes – – –
- Miniera Sup. Di Monfieis MIN1 12/09/2010 alive water surface yes – – –
- Miniera Sup. Di Monfieis MIN1 12/09/2010 alive speleothem yes – – –

**Eukoenenia sp. 1**
- Grotta Di Costacalda GCC1 03/05/2018 alive water surface no – – –
- Grotta Di Costacalda GCC2 11/05/2018 alive water surface no – – –
- Grotta Di Costacalda GCC3 27/05/2018 alive water surface yes – – –

**Eukoenenia sp. 2**
- Grotta Dell’Orso Di Ponte Di Nava PDN1 03/06/2018 alive water surface yes 10.9 87.6 7
- Grotta Dell’Orso Di Ponte Di Nava PDN2 03/06/2018 alive water surface yes 10 98.3 7

**Eukoenenia sp. 3**
- Arma Cornarea COR1 04/01/2018 alive water surface yes – – –
- Arma Cornarea COR1 04/01/2018 alive water surface yes – – –
- Arma Cornarea COR1 20/04/2019 alive water surface yes 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.
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 which 13 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 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*.

Figure 4. Percentage of specimens found on different microhabitats.
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).

**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).
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.

Acknowledgments

Authors are very grateful to Erhard Christian for the determination of the samples and useful information. We cordially thank Loris Galli, Massimo Meregalli and Maysa Sousa for helpful comments, Bartolomeo Vigna and Jo De Waele for geological suggestions, Michelangelo Chesta, Sara Longo, Marco Marovino, Nicoletta Pastorino, Fabrizio Falco, Marco Isaia, Mariagrazia Morando, Massimo Sciandra, Denise Trombin, Arianna Paschetto, Pier Mauro Giachino, Speleo Club Tanaro, Gruppo Speleologico Valli Pinerolesi, Gruppo Speleologico Savonese DLF and Gruppo Speleologico Piemontese for helping us. We acknowledge Liguria Region authorities for providing the necessary permits to collect hypogean Palpigradi and the DISTAV-University of Genova for the thermohygrometer use. Authors are grateful to Hanna Moore (Cranfield University) for the English revision of the manuscript and for helpful comments.

This work was possible with the contribution of the Scholarship of the National Congress of Biospeleology (Cagliari 7–9 April 2017), won for the project “Study of distribution and behavior of hypogean palpigrades (genus Eukoenenia) of Piedmont” by V.B. and E.L., and with the contribution of the association Biologia Sotterranea Piemonte – Gruppo di Ricerca.

References

Balestra V, Lana E, Carbone C, De Waele J, Manenti R, Galli L (2021) Don’t forget the vertical dimension: assessment of distributional dynamics of cave-dwelling invertebrates in both ground and parietal microhabitats. Subterranean Biology 40: 43–63. https://doi.org/10.3897/subtbiol.40.71805

Balestra V, Lana E, Casale A (2019) Note sulle specie ipogee del genere Eukoenenia in Piemonte: distribuzione, habitat, comportamento (Arachnida, Palpigradi: Eukoeneniidae). Bollettino della Società Entomologica Italiana 151: 13–23. https://doi.org/10.4081/BollettinoSEI.2019.13

Brignoli M (1976) Su di un Palpigrado di una grotta piemontese (Arachnida, Palpigrada). Fragmenta Entomologica XII: 63–67.

Christian E, Isaia M, Paschetta M, Bruckner A (2014) Differentiation among cave populations of the Eukoenenia spelaea species-complex (Arachnida: Palpigradi) in the southwestern Alps. Zootaxa 3794: 52–86. http://dx.doi.org/10.11646/zootaxa.3794.1.2

Condé B (1977) Nouveaux palpigrades du muséum de Genève. Revue Suisse de Zoologie 84: 665–674. https://doi.org/10.5962/bhl.part.91415
Condé B (1984) Les Palpigrades: quelques aspects morpho-biologiques. Revue Arachnologique 5: 133–143.
Condé B (1996) Les Palpigrades, 1885–1995: acquisitions et lacunes. Revue Suisse de Zoologie, hors série 1: 87–106.
Ferreira RL, Souza MFVR (2012) 4. Notes on the behavior of the advances troglobite Eukoenenia maquinensis Souza and Ferreira 2010 (Palpigradi: Eukoeneniidae) and its conservation status. Speleobiology Notes 4, 1–7.
Ficetola GF, Canedoli C, Stoch F (2019) The Racovitzan impediment and the hidden biodiversity of unexplored environments. Conservation Biology 33: 214–216. https://doi.org/10.1111/cobi.13179
Giribet G, McIntyre E, Christian E, Espinasa L, Ferreira RL, Francke ŐF, Harvey MS, Isaia M, Kováč Ž, McCutchen L (2014) The first phylogenetic analysis of Palpigradi (Arachnida)—the most enigmatic arthropod order. Invertebrate Systematics 28: 350–360. https://doi.org/10.1071/IS13057
Grassi B, Calandruccio S (1885) Intorno ad un nuovo Aracnide Artrogastro (Koenenia mirabilis [sic]) che crediamo rappresentante d’un nuovo ordine (Microteliphonida). Il Naturalista Siciliano 4: 127–133; 162–169.
Harvey MS (2002) The neglected cousins: what do we know about the smaller arachnid orders? The Journal of Arachnology 30: 357–372. https://doi.org/10.1636/0161-8202(2002)030[0357:TNCWDW]2.0.CO;2
Hill C, Forti P (1997) Cave Minerals of the World (2nd Edn.). Mineralogical Society of America, 463 pp.
Howarth FG (1983) Ecology of cave arthropods. Annual review of entomology 28: 365–389. https://doi.org/10.1146/annurev.en.28.010183.002053
Isaia M, Paschetta M, Lana E, Pantini P, Schönhöfer AL, Christian E, Badino G (2011) Aracnidi sotterranei delle Alpi Occidentali italiane. [Subterranean Arachnids of the Western Italian Alps]. Monografie 47. Museo Regionale di Scienze Naturali, Torino, 325 pp.
Lana E, Casale A, Grafitti G (2016) Note sulla biologia del genere Eukoenenia con particolare riferimento alle specie ipogee di Palpigradi del Piemonte e della Sardegna. In: Biospeleology Congress. Cagliari, 24 pp.
Lukič M (2012) Collembola in caves.
Mammola S (2019) Finding answers in the dark: caves as models in ecology fifty years after Poulson and White. Ecography 42: 1331–1351. https://doi.org/10.1111/ecog.03905
Mammola S, Lunghi E, Bilandžija H, Cardoso P, Grimm V, Schmidt SI, Hesselberg T, Martínez A (2021a) Collecting eco-evolutionary data in the dark: Impediments to subterranean research and how to overcome them. Ecology and evolution 11: 5911–5926. https://doi.org/10.1002/ece3.7556
Mammola S, Souza MFVR, Isaia M, Ferreira RL (2021b) Global distribution of micro-whip scorpions (Arachnida: Palpigradi), Journal of Biogeography 48(6): 1518–1529. https://doi.org/10.1111/jbi.14094
Millot J (1942) Sur l’anatomie et l’histophysiologie de Koenenia mirabilis Grassi (Arachnida Palpigradi). Revue Française d’Entomologie 9: 33–51.
Habitat and feeding behaviour of the hypogean genus *Eukoenenia* (Palpigradi)

Morisi A (1992) La grotta di Bossea (108 Pi/CN): cent’anni di biospeleologia. “Ambiente carsico e umano in Val Corsaglia”. C.A.I., Comitato Scientifico Ligure-Piemontese-Valdostano, Bossea, 65–90 pp.

Pantini P, Isaia M (2019) Araneae. it: the online catalog of Italian spiders, with addenda on other arachnid orders occurring in Italy (Arachnida: Araneae, Opiliones, Palpigradi, Pseudoscorpionida, Scorpiones, Solifugae). Fragmenta Entomologica 51: 127–152. https://doi.org/10.4081/fe.2019.374

Parimuchová A, Dušátková LP, Kováč Ľ, Macháčková T, Slabý O, Pekár S (2021) The food web in a subterranean ecosystem is driven by intraguild predation. Scientific reports 11: 1–11. https://doi.org/10.1038/s41598-021-84521-1

Silvestri F (1905) Note Arachnologiche I-III. I Specie italiane del genere Koenenia con descrizione delle femmine giovani e del maschio della K. mirabilis. Redia 2: 239-253.

Smrž J, Kováč Ľ, Mikeš J, Lukešová A (2013) Microwhip scorpions (Palpigradi) feed on heterotrophic Cyanobacteria in Slovak caves—a curiosity among Arachnida. PLoS One 8: e75989. https://doi.org/10.1371/journal.pone.0075989

Souza MF, Mayoral J, Ferreira RL (2020) A new troglobitic palpigrade from Central Brazil, with notes on a new opisthosomal character (Arachnida: Palpigradi). Zootaxa 4869(4): 493–514. https://doi.org/10.11646/zootaxa.4869.4.2

Souza MFV, Ferreira RL (2010) *Eukoenenia* (Palpigradi: Eukoeneniidae) in Brazilian caves with the first troglobiotic palpigrade from South America. The Journal of Arachnology 38: 415–424. https://doi.org/10.1636/Ha09-112.1

Wheeler WM (1900) A singular arachnid (*Koenenia mirabilis* Grassi) occurring in Texas. The American Naturalist 34: 837–850. https://doi.org/10.1086/277805