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Parasitic infestation in a female of *Micropterna nycterobia* McLachlan 1875 (Trichoptera, Limnephilidae) found in Monte Cucco cave (central Italy)

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Abstract

In this study, parasitosis due to *Ophryoglena* sp. (Ciliophora, Oligohymenophorea) in a *Micropterna nycterobia* (Trichoptera, Limnephilidae) female is described. The trichopteran was found on 31 October 2002 in a cave, named "Grotta di Monte Cucco," in Central Italy. The specimen, observed *in vivo* under the stereomicroscope, showed that its abdomen was packed with the ciliate. This parasite had fed on gonads, muscles and fat body of the host, thus confirming the role of *Ophryoglena* as a parasitic spayer. Different stages of development of the parasite were also detected. The interaction of the parasite with host tissues was investigated by means of light and electron microscopy.

Key words: Stenophylacini species, symbionts, hystophagous ciliates

Introduction

Trichoptera may host symbionts throughout their life cycle. Gregarines, microsporidia, peritrichs, trematodes, rotifers, nematodes, Gordiacea, and the wasp *Agriotypus armatus* Curtis 1832 are frequently found in the aquatic stages, mite larvae are found mainly on adults.

This paper reports a particular case of massive infestation due to the ciliated protozoan *Ophryoglena* sp. in a female of *Micropterna nycterobia* McLachlan 1875 found in the "Caves of Monte Cucco," located in the Umbrian-Marches Apennines (Fig.1).

Research on Trichoptera found in these caves has been undertaken by Moretti and collaborators since 1946. The study deals with the presence and the turnover of the trichopterans inhabiting the caves, which are Stenophylacini species belonging to the genera *Mesophylax, Micropterna* and. *Stenophylax* (Moretti *et al*. 1965). Spring and early summer are the periods of maximum entrance flow of Trichoptera into the caves. Most individuals mate in summer (Moretti *et al*. 1966, 1967). Trichoptera are able to survive by licking the water film that permeates the cave walls and tiny drops of water often cover their bodies. They leave the cave and go back outside in late October and early November. The period these insects spend inside the caves seems to have an important role in overcoming the summer drought period. Moretti *et al*. (1972) and Bouvet (1971) demonstrated that the maturation of the gonads in Stenophylacini females undergoes a hypogean summer diapause. When Trichoptera females enter the caves and mate, their ovaries are still immature and the maturation process takes place in early autumn. Novak & Sehnal (1964) suggested that diapause helps survival during the temporary shortage of water, allowing females to lay their eggs in the fall when it rains.

Moretti and collaborators noticed that a high number of Trichoptera were preyed upon by bats [*Rhinolophus ferrumequinum* (Schreber 1774)], Orthoptera (*Dolichopoda laetitiae* Minozzi 1920) and spiders [*Meta menardi* (Latreille 1804), *Nesticus hermita* (Clerck 1757)]. In fact, there were remains of sclerified body parts and fragments of wings trapped in cobwebs. Mite larvae have been identified between thoracic and abdominal segments and necrophilous springtails also have been found on adult remains (Moretti *et al*. 1967; Bani 1984).

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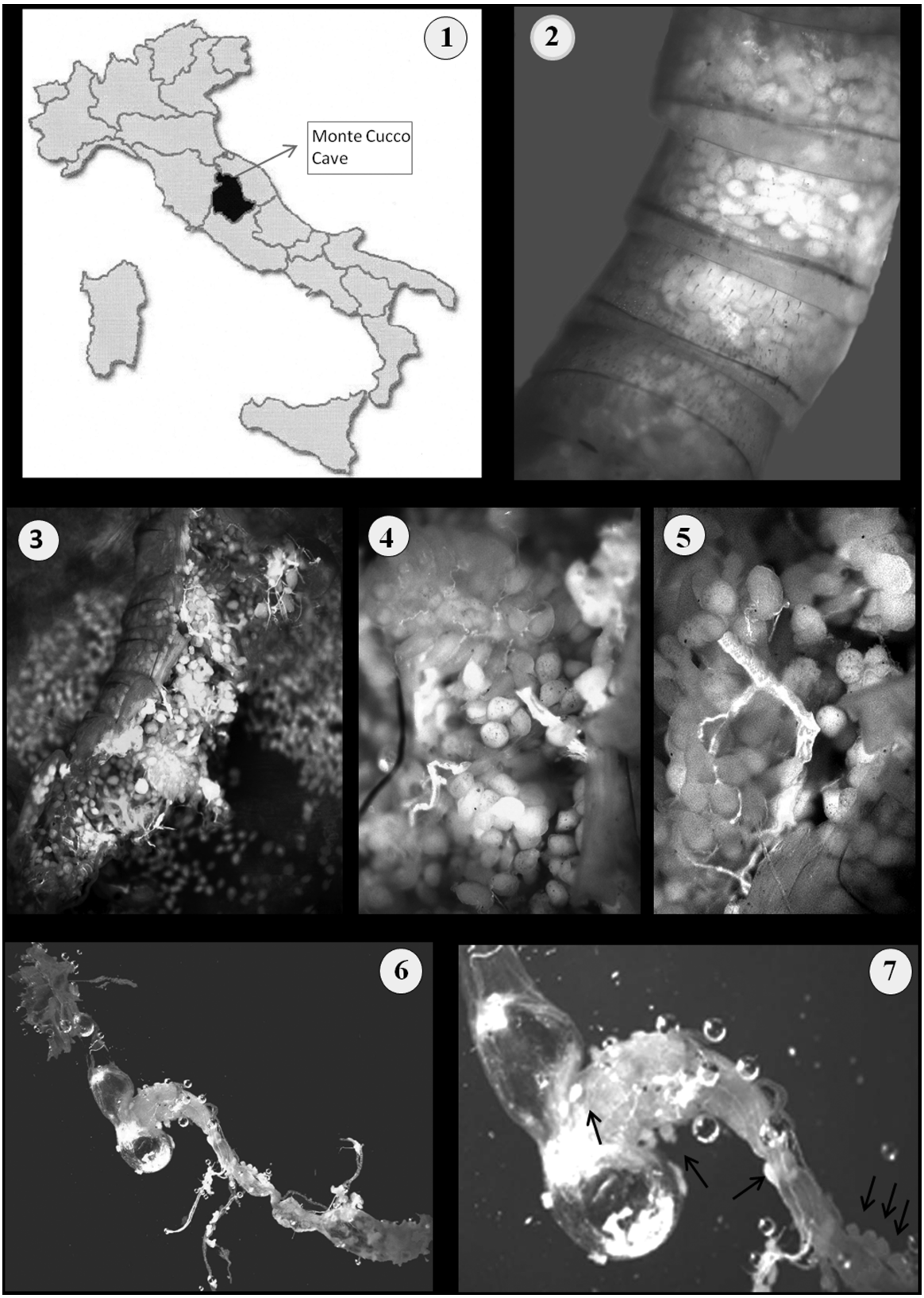
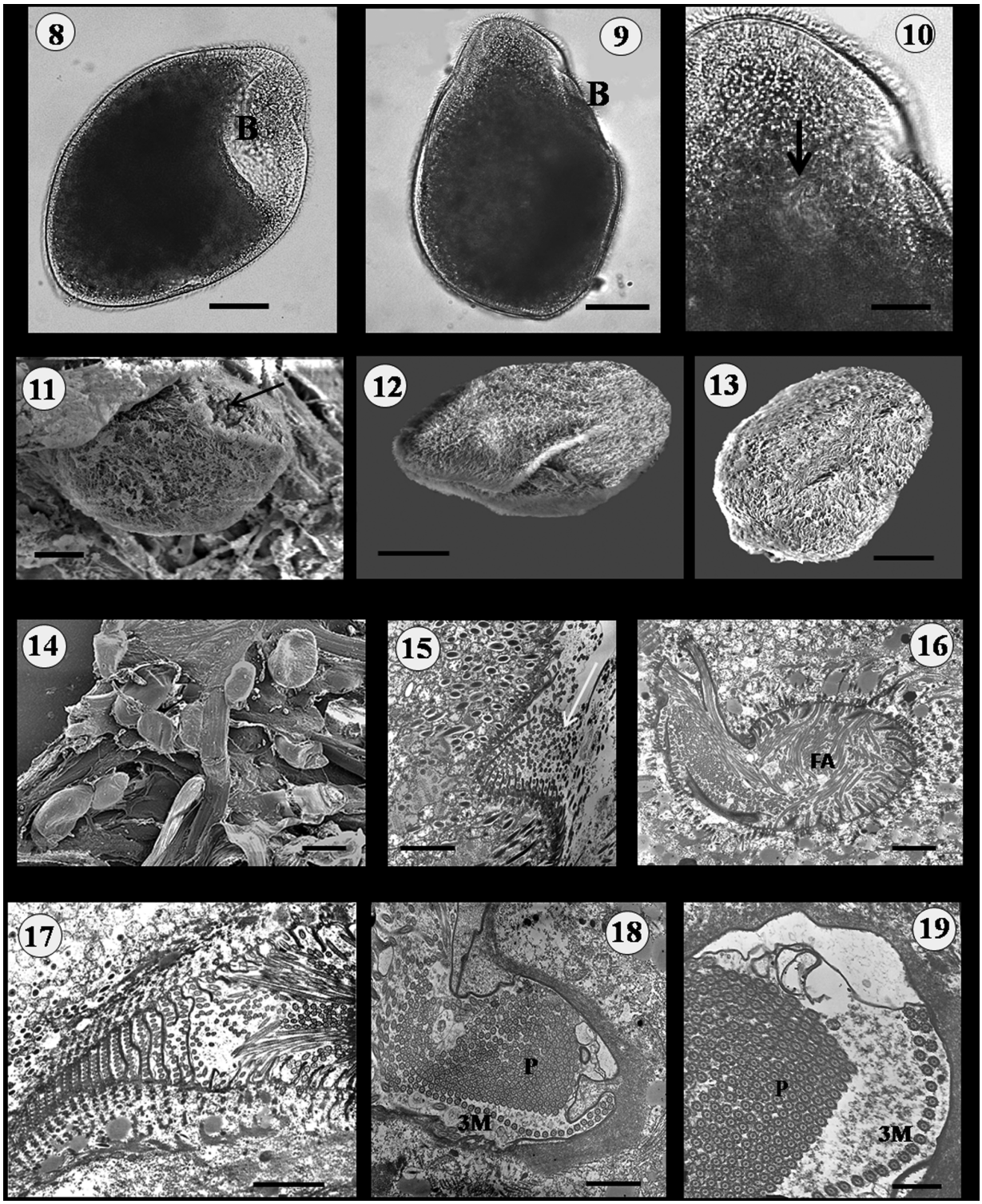


FIGURES 1–7. 1–Political map of Italy, divided by the borders of the regions, with highlighted area of the Umbria region. **2**–*Micropterna nycterobia* McLachlan, 1875 ♀: numerous ciliates occupy the entire abdomen. **3**–Abdomen after dissection; many ciliates were lost during laparotomy. **4**–*Ophryoglena* sp. caused the disappearance of fat bodies, ovaries and large part of the musculature. **5**–Detail of fig.4: tracheae are present between ciliates pressed onto each other occupying the whole abdomen, are clearly visible through the body wall. **6**–*Micropterna nycterobia* McLachlan, 1875. Gut: note the complete disappearance of the muscle layers. **7**–*Micropterna nycterobia* McLachlan, 1875: individuals of *Ophryoglena* sp. (arrows) located externally to the intestinal wall.



FIGURES 8–19. 8–*Ophryoglena* sp.: trophont; B = buccal cavity, Light microscope, Bar = 50µm. **9**–*Ophryoglena* sp.: protomont; B = buccal cavity, Light microscope. Bar = 60 µm. **10**–*Ophryoglena* sp.: protomont; organelle of Lieberkühn (arrow), Light microscope, Bar = 20 µm. **11**–*Ophryoglena* sp. trophont: buccal cavity with food (arrow) SEM, Bar = 40 µm. **12**–*Ophryoglena* sp. protomont: somatic ciliature organized in longitudinal kineties. SEM, Bar= 50 µm. **13**–*Ophryoglena* sp. tomont: rounded shape before encystment. SEM, Bar = 50 µm. **14**–*Ophryoglena* sp: individuals attached to the thorax wall SEM, Bar = 100µm. **15**–*Ophryoglena* sp.: Oral depression (arrow). TEM, Bar = 5 µm. **16**–*Ophryoglena* sp.: filtering structure formed by the cilia of the oral apparatus. FA = filtering apparatus. TEM, Bar = 2 µm. **17**–*Ophryoglena* sp.: rows of cilia in a spiral arranged along the buccal cavity (arrows). TEM, Bar= 4 µm. **18**–*Ophryoglena* sp. Terminal part of the buccal cavity: first and second membranella, spirally wound, form the *peniculus* (P); isolated third membranella (3 M) formed by a single row of cilia.TEM, Bar= 2 µm. **19**–*Ophryoglena* sp.: detail of the terminal part of the buccal cavity. P= *peniculus,* 3M = third membranella. TEM, Bar= 1 µm.

Study area

The Caves of Monte Cucco, among the largest and most important of Central Italy, are part of the park in the northeastern part of Umbria, near Perugia. These caves stand in an area of high seismic activity due to compression caused by the collision between the African and the Eurasian landmasses. Such compression produced rock layer fracturing and pressure conditions which pumped into the fractures hypogenic fluids in the form of highly corrosive thermal hot water loaded with minerals. The dual action of meteoric waters and hypogenic fluids (thermal waters) led to the shaping and excavation of the limestone layers by dissolution, giving rise to the caves. The Caves of Monte Cucco are an underground system which extends for over 30 km at a maximum depth of 923 m.

Materials and methods

Micropterna nycterobia was first examined *in vivo* and then dissected with a stereomicroscope, for light microscopy, specimens of *Ophryoglena* were observed and photographed *in vivo* with a Leitz Laborlux S light microscope.

For transmission electron microscopy (TEM), selected material was fixed for 2 hours in glutaraldehyde 2.5% in 0.1M cacodylate buffer (pH 7.2), rinsed in the buffer, post-fixed for 2 hours in 1% osmium tetroxide in the same buffer, dehydrated in a graded ethanol series, infiltrated with propylene oxide, embedded in an Epon-Araldite mixture, and polymerized at 60°C. Thin sections, obtained with a Reichert Ultracut OM U3 microtome, were placed on formvar coated copper grids, stained with uranyl acetate and lead citrate, and observed with a Philips EM 400 transmission electron microscope.

For scanning electron microscopy (SEM) specimens were fixed and dehydrated using the above described procedures. Samples were mounted on stubs, coated with gold-palladium and examined with a Philips SEM 505 scanning electron microscope.

Results

A *Micropterna nycterobia* female was collected on 31 October 2002 in order to study the midgut ultrastructural morphology after a long fasting period of the trichopteran in the cave.

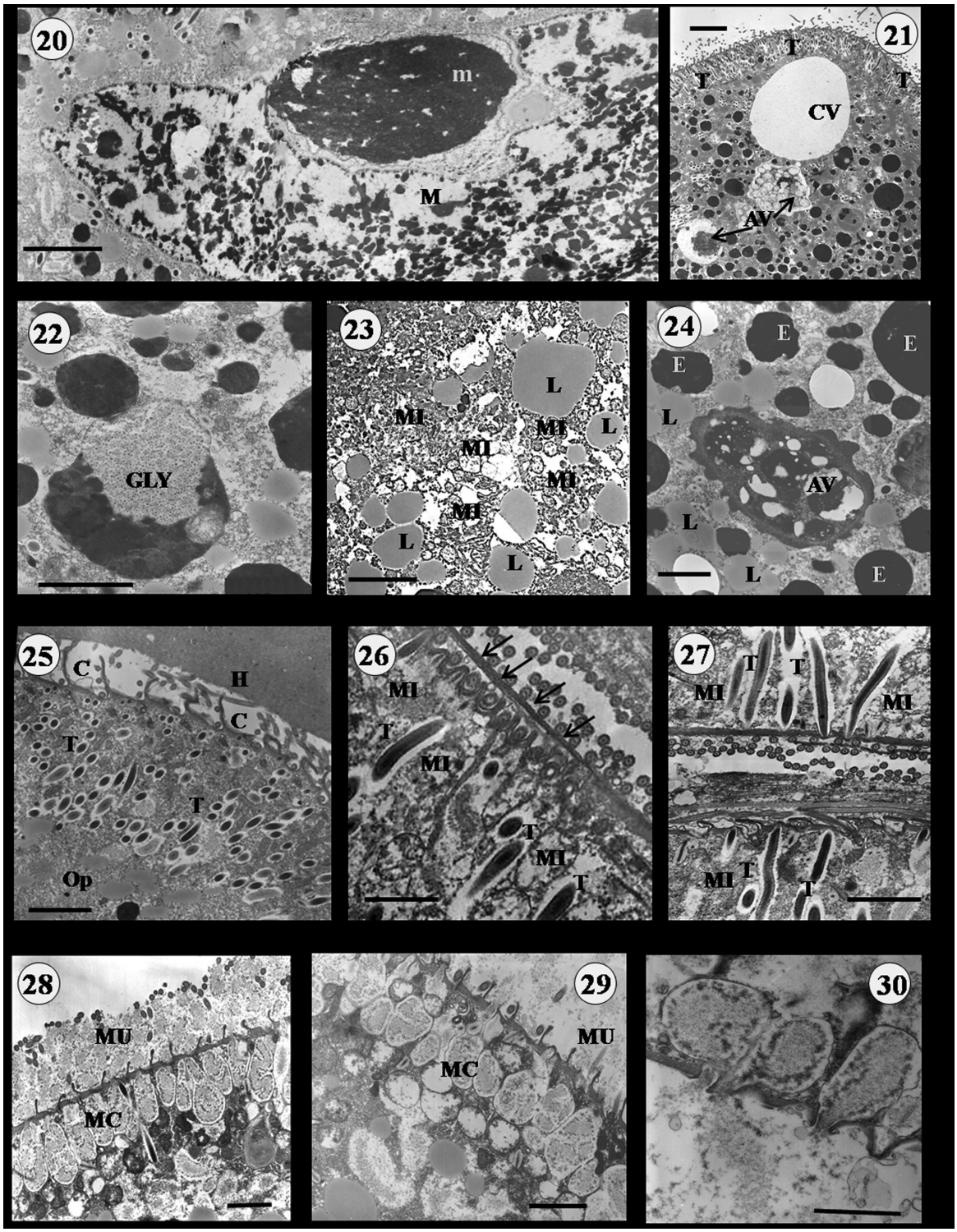
At the time of capture the trichopteran was in good living condition. The presence of numerous small rounded bodies, piled on each other, were clearly seen through the body wall and occupied the whole abdomen (Fig. 2). After dissection of the specimen, about 300 individuals filling the abdomen were counted (Fig. 3). This number could be higher because many individuals were lost during laparotomy.

The dissection of the abdomen also showed that the ciliates had caused the disappearance of *M. nycterobia* abdominal muscles, fat bodies and ovaries (Fig. 4), leaving the tracheal system (Fig. 5), thoracic structures, gut and Malpighian tubules intact. In particular, the gut appeared transparent due to complete destruction of the musculature surrounding the digestive system (Fig. 6). The foregut was filled with air, the midgut and the hind gut were empty. Some ciliates were present outside the intestinal wall. The excretory and homeostatic functions accomplished by the Malpighian tubules and gut had not been compromised by the presence of the parasite (Fig. 7).

The *Micropterna nycterobia* parasites were classified as protozoans belonging to the genus *Ophryoglena* Ehrenberg 1831 [Phylum: Ciliophora Copeland 1956; Class: Oligohymenophorea Puytorac *et al*. 1974; Order: Ophryoglenida Canella 1964; Family: Ophryoglenidae Kent 1881].

This *Ophryoglena* sp. measures from 180 to 400 µm, is rounded or pyriform (Fig. 8), the oral cavity is located in the anterior body (Fig. 9) and contains an undulating membrane and three membranelles which are arranged hellically. A refringent body, the organelle of Lieberkühn (Fig. 10), is located near the buccal cavity. The body ciliation is uniform with many kineties running longitudinally down the body.

The life cycle of *Ophryoglena* develops through the stages of theront, trophont, protomont, tomont and tomite, which represent characteristic physiological states: food search, food ingestion, food digestion, food assimilation and reproduction.



FIGURES 20–30. **20**–*Ophryoglena* sp.: micronucleus (m) tightly adjacent to the macronucleus (M). TEM, Bar = 2 µm. **21**–*Ophryoglena* sp.: section showing a contractile vacuole (VC), vesicles, autophagic vacuoles (AV) and numerous tricocists (T) perpendicular to the cell wall TEM, Bar = 5 µm. **22**–*Ophryoglena* sp.: agglomerates of glycogen (GLY) . TEM, Bar= 2 µm. **23**–*Ophryoglena* sp.: cytoplasm with numerous mitochondria (mi) and lipid globules (L). TEM, Bar = 2 µm. **24**–*Ophryoglena* sp.: autophagic vacuoles (AV), lipid globules (L) and elettrondense bodies (E). TEM, Bar= 1 µm. **25**–*Ophryoglena* sp: contact between the cilia of the protozoan and the host's tissues. C = somatic cilium, Op = *Ophryoglena* sp, H = host. TEM, Bar=2 µm. **26**–*Ophryoglena* sp.: cortical region (arrows). Mi = mitochondria, T = tricocyst. TEM, Bar=1 µm **27**–*Ophryoglena* sp.: trichocyst (T) attached on the inner side of the cell membrane of two individuals.; mi = mitochondria, T = trichocyst TEM, Bar= 5 µm. **28**–*Ophryoglena* sp.: precystic stage surrounded by a continuous layer of mucin (MU) and mucocysts (MC) attached on the inner side of the cell membrane TEM, Bar=2 µm. **29**–*Ophryoglena* sp.: the mucocysts (MC) discharge the mucin (MU) through the pellicular membrane. TEM, Bar=2 µm **30**–*Ophryoglena* sp. Detail of mucocysts in secretory phase. TEM, Bar=2 µm.

The more food is available, the faster the parasite reproduces. Examination by light microscopy and SEM showed *Ophryoglena* sp. developmental stages: theront (Fig. 11), trophont (Fig. 12) and tomont (Fig. 13). The presence of a high number of parasites with different morphologies indicates a high reproductive activity in *Ophryoglena* sp. which lasted over time. Some individuals were free in the hemocele and others were attached to host tissues (Fig. 14).

TEM examination showed a large buccal cavity (Fig.15) and a long cytopharynx where the helical disposition of the membranellae is clearly seen (Figs. 16, 17). The membranellae 1 and 2 are arranged as a screw descending into the infundibulum (Figs. 18, 19). The macronucleus is a flattened ellipsoid in outline. The micronucleus is tightly adjacent to the macronucleus (Fig. 20). There are two contractile vacuoles (Fig. 21) and numerous cytoplasmic inclusions. Agglomerates of glycogen (Fig.22), electron dense bodies (Fig. 24), lipid globules (Figs. 23, 24), gastrioles and autolysosomes are observed. Numerous mitochondria are found at the base of the buccal cavity and near the pellicular system (Figs. 23, 26, 27). TEM images show that the cilia make contact with the host (Fig. 25). The stages of teront and trophont show numerous trichocysts against the body wall (Figs. 25, 26, 27), some being exstruded (Fig. 27) , while protomont and tomont show mucocysts in full secretory activity (Figs. 28, 29, 30). The developing layer of mucin suggests an ongoing process of encystment.

Discussion

The finding of a *M. nycterobia* female stuffed with hundreds of ciliates of the genus *Ophryoglena* is a new report for the hypogean environment. *Ophryoglena* is attracted by fresh tissues. In fact, it is an hematophagous and histophagous ciliate parasite of trematoda, Mollusca [*Dreissena polymorpha* (Pallas 1771)] and Insecta Ephemeroptera.

Parasites in several species of Ephemeroptera have been reported (Arvy & Peters 1973; Gaino & Rebora 2000; Codreanu 1930, 1934, 1972; Codreanu & Codreanu-Balcescu 1979). Ephemeroptera ciliate parasites are parasitic castrators as they are haematophagous and histophagous, feeding on haemolymph and destroying gonads, muscles and fat body. Codreanu (1934) proposed a suggestive hypothesis, according to which the females with the abdomen burdened by ciliates and feeling the deceptive stimulus to lay eggs in water, actually release the parasite and thereby aid the completion of its life-cycle.

Considering the ecdysis time (May) of *M. nycterobia* and its entrance into the cave (June), the caddisfly spent no less than three months in the cave. Hypothesizing that the infestation has occurred in the larval stage, the caddisfly has completed its metamorphosis, despite the massive infestation by *Ophryoglena* that caused body fat, muscle and gonad destruction, and has survived until the time when it left the cave to accomplish oviposition.

Examination of a single specimen of *Micropterna nicterobia* does not allow us to propose any further hypotheses about the host-parasite relationship and their interrelated life cycle, but the parasitic spaying caused by *Ophryoglena* sp. in the studied trichopteran is clearly evident.

References

Arvy, L. & Peters, W.L. (1973) Phorèsies, biocoenoses et thanatocoenoses chez les Ephéméroptères. *In*: Peters, W. & Peters, J. (Eds.), *Proceedings of the 1st International Conference on Ephemeroptera.* J. Brill. Leiden, pp. 254–312.

Bani, M. (1984) *La grotta dei Cinque Laghi. Sez. Biospeleologia* (*Bramante*). Urbania, Città di Castello, 214 pp.

Bouvet, Y. (1971) *Ecologie et biologie des trichoptères cavernicoles*. Thèse pour le Diplôme de Docteur de spécialité – 3° Cycle de Sciences Biologiques, 67 pp.

Canella, M.F. (1964) Contributi alla conoscenza dei Ciliati. IV. Strutture boccali, infraciliatura, filogenesi e sistematica dei Ciliofori. *Annali dell'Università di Ferrara NS 3 Biologia Animale*, 2, 119–188.

Canella, M.F. & Rocchi-Canella, I. (1976) Biologie des *Ophryoglenina* (ciliés hyménostomes histophages). *Annali dell'Università degli Studi di Ferrara*, Nuova Serie, Sect. III, 3 (Suppl. 2), 1–50.

Codreanu, R. (1930) Sur la phase interne du cycle évolutif de deux formes d'*Ophryoglena*, Infusoires endoparasites des larves d'Ephémères. *Comptes Rendus de l'Académie des Sciences de Paris*, 190, 1154–1157.

Codreanu, R. (1934) La présence d'*Ophryoglena*, ciliés endoparasites chez les nymphes de l'Ephémère *Oligoneuria rhenana* Imhoff en France. *Annales de Protistologie*, 4, 181–183.

Codreanu, R. (1972) Les *Ophryoglena* (Ciliata, Hymenostomata) intra-ovariennes des Ephémères sont-elles des parasites

obligatoires ou facultatifs? *Journal of Protozoology*, 19 (Supplement), 1–63.
Codreanu, R. & Codreanu-Balcescu, D. (1979) Remarques critiques sur les parasites et leurs effects chez les Ephéméroptères. *In*: Pasternak, K. & Sowa, R. (Eds), *Proceedings of the 2st International Conference on Ephemeroptera.* Polska Akademia Nauk, Warszawa, pp. 227–243.
Copeland, A.F. (1956) *The classification of lower organisms*. Pacific Books, Palo Alto, California, 302 pp. http://dx.doi.org/10.5962/bhl.title.4474
Doflein, F. (1901) *Die Protozoen als Parasiten und Krankheitserreger nach biologischen Gesichtspunkten dargestellt*. Fischer, Jena, 274 pp.
Ehrenberg, C.G. (1831) Űber die Entwickelung und Lebensdauer der Infusionsthiere; nebst ferneren Beiträgen zu einer Vergleichung ihrer organischen Systeme. *Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin,* 1831, 1–154.
Gaino, E. & Rebora, M. (2000) *Ophryoglena* sp. (Ciliata: Oligohymenophora) in *Caenis luctuosa* (Ephemeroptera:Caenidae). *Acta Protozoologica*, 39, 225–231.
Kent, W.S. (1881) *A manual of the Infusoria including a description of all known flagellate, ciliate, and tentaculiferous Protozoa. British and foreign and an account of the organization and affinities of the sponges. Vol. 1–3.* David Bogue, London, 472 pp., 903 pp., 215 pp. http://dx.doi.org/10.5962/bhl.title.1243
McLachlan, R. (1875) *A Monographic Revision and Synopsis of the Trichoptera of the European Fauna.* Napier Printers, London, Part 3, 109–144, pls. 12–15.
Moretti, G.P., Cianficconi, F. & Pirisinu, Q. (1972) Annotazioni biologiche sui Tricotteri cavernicoli di un sistema ipogeo della Toscana in rapporto all'idrografia esterna. *Rivista di Idrobiologia,* 11 (1), 17–50.
Moretti, G.P., Gianotti, F.S., Dottorini, C. & Viviani, G.C. (1965) I Tricotteri della grotta di Monte Cucco (Umbria, Perugia). *Rivista di Idrobiologia*, 4 (1–2), 17–26.
Moretti, G.P., Gianotti, F.S., Dottorini, C. & Viviani, G.C. (1966) La distribuzione e l'avvicendamento stagionale dei Tricotteri nella Grotta di Monte Cucco 17U/PG. *Bollettino di Zoologia*, 33 (1), 215–216.
Moretti, G.P., Gianotti, F.S., Dottorini, C. & Viviani, G.C. (1967) La colonizzazione tricotterologica della Grotta di M. Cucco (17U/PG): Avvicendamento, ripartizione, sex-ratio e valutazione delle spoglie. *Memorie della Società Entomologica Italiana*, 46, 201–239.
Novák, K. & Sehnal, F. (1964) Imaginal diapause bei den in periodischen Gewassern lebenden Trichopteren. *Proceedings of the 12th International Congress of Entomology*, *London*, 1964, 434.
Puytorac, P. de, Batisse, A., Bohatier, J., Corliss, J.O., Deroux, G., Didier, P., Dragesco, J., Fryd-Versavel, G., Grain, J., Grolière, C.-A., Hovasse, R., Iftode, F., Laval, M., Rogue, M., Savoie, A. & Tuffrau, M. (1974). Proposition d'une classification du phylum Ciliophora Doflein, 1901. *Comptes Rendus de l'Académie de Sciences, Paris,* 278, 2799–2802.