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Trichoptera larvae and gregarines: Host-parasite relationships

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Abstract

The presence of eugregarines (Apicomplexa, Conoidasida, Eugregarinorida) in the larval midgut of several Trichoptera families found in Italy has been well established. Literature and our data indicate that gregarine infestation is influenced by the trichopteran diet and, except for a few cases, there is not a species-specific relationship. In this paper an updated list of Italian Trichoptera species hosting gregarines as well as data on the biology, morphology and ultrastructure of the corresponding parasites are presented. The host-parasite interaction was also investigated showing the involvement of the host diet in the onset of parasitosis and the influence of the host habitat on the degree of infestation.

Gregarine infestation was massive in species inhabiting springs, particularly noticeable in close environments like troughs ("trocchi") which are man-modified springs for drinking water for cattle. Larvae of *Drusus improvisus* found in the Tiber river springs were heavily infested and the parasite density at every season and the degree of infestation at each larval stage are reported.

Key words: caddisflies, eugregarines, Apicomplexa, Conoidasida, Eugregarinorida

Introduction

Eugregarines (Apicomplexa, Conoidasida, Eugregarinorida) are monoxenous parasites of invertebrates. Septate eugregarines (Septatina), are intestinal parasites found in many insects including larval Trichoptera. These unicellular organisms range from 30 µm to 2 mm in length and the body is divided by a septum into an anterior protomerite and a posterior deutomerite containing the nucleus (Fig. 1). The sporozoite has an apical complex, the epimerite, which is lost in the mature trophozoite.

The host is infested by oral ingestion of oocytes (spores), each of which releases 8 sporozoites in the host midgut. Sporozoites penetrate the midgut cells, becoming trophozoites which feed, mature and subsequently detach from the midgut cells, losing their epimerite. Mature trophozoites become gamonts which eventually become paired in a process called syzygy (side-to-side, head-to-tail, or head-to-head) and secrete a gamontocyst wall. Inside the gamontocyst, the nucleous of each gamont divides into hundreds of gametic nuclei. Gametes from each partner fuse, forming zygotes. Each zygote, surrounded by an oocyst wall, undergoes meiosis (sporogony) forming 8 sporozoites. Eventually, each gamontocyst contains hundreds of oocysts, each of which contains 8 sporozoites.

Moretti and Corallini (1969) first reported gregarine infestation in Italian Trichoptera and research has been conducted since then in our Department at the University of Perugia (Bicchierai & Corallini-Sorcetti 1999; Cianficconi *et al*. 1994, 1996, 1998, 1999, 2000, 2008; Corallini 1981, 1984, 1997, 2011; Corallini-Sorcetti & Bicchierai 2002; Corallini & Bicchierai 2007, 2011; Corallini & Marchetti 2003; Corallini & Moretti 1988, 1991; Moretti & Corallini-Sorcetti 1981; Moretti & Corallini 1991).

The present paper is an updated review of the Italian Trichoptera species hosting gregarines, and aims to highlight the biology, morphology and ultrastructure of the parasites and the major aspects of their host-parasite relationship. Data are updated with new gregarine findings and recent developments in the systematics of Trichoptera (Malicky 2005) and Eugregarinae (Clopton 2002) and with recent findings on the type of peritrophic membrane and presence of goblet cells in the larval midgut (Corallini 2003, 2007, 2011).

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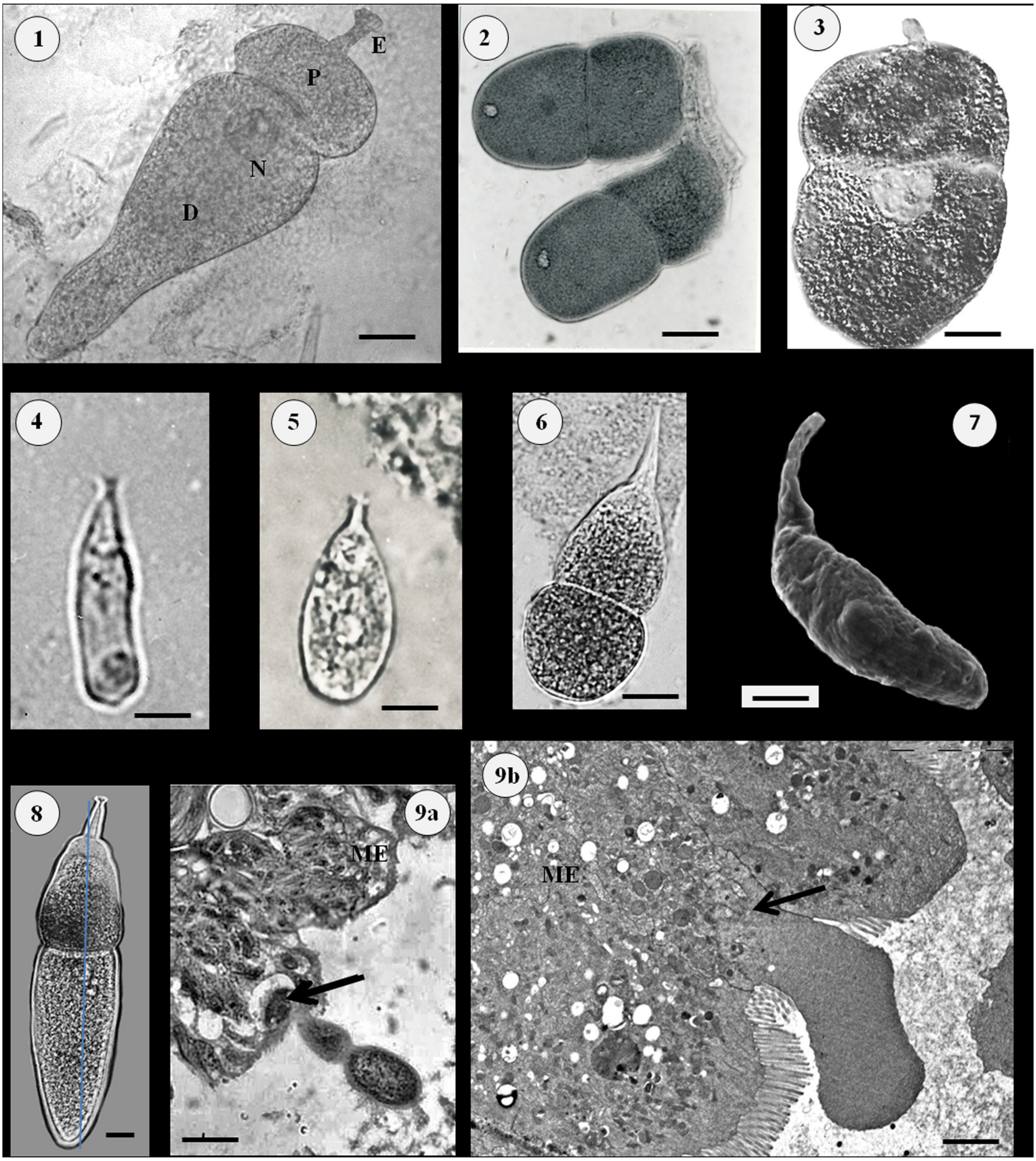

FIGURES 1–9. 1, septate gregarine. D = deutomerite, E = epimerite, N = nucleus, P = protomerite; light microscope; bar = 41 µm. **2**, *Pomania morettii* trophozoite; light microscope; bar = 105 µm. **3**, *Globulocephalus hydropsyches* trophozoite; phase contrast microscope; bar = 41 µm. **4**, *Gregarina fontinalis* trophozoite; light microscope; bar = 12 µm. **5**, *Gregarina pusilla* trophozoite; light microscope; bar = 10 µm. **6**, *Phileocephalus heeri* trophozoite; light microscope; bar = 23 µm. **7**, *Phileocehalus heeri* trophozoite; SEM; bar = 25 µm. **8**, *Asterophora tiaroides* trophozoite; light microscope; bar = 10 µm. **9**, *Pileopcephalus heeri* trophozoite; ME = midgut epithelium, epimerite (arrow); a: light microscope, bar = 25 µm; b: TEM, bar = 2.5 µm.

Materials and methods

Samplings were made in both lentic and lotic waters in all Italian regions, included islands, with special regard to Central Italy.

Light microscopy: Larval guts of the studied Trichoptera species were dissected and fixed for two hours in Bouin's fluid. *In toto* preparations and sections 2–3 µm thick, stained with Mayer's haemalum-eosin, were observed and photographed with a Leitz Laborlux S light microscope.

Semi-thin sections, about 1 µm thick, of the material prepared for transmission electron microscopy were stained in 1% methylene blue in 1% borax, observed and photographed with a Leitz Dialux 22 phase contrast microscope.

Transmission electron microscopy (TEM): Each larval gut was dissected and fixed for 2h at 4°C in glutaraldehyde 2.5% in 0.1M cacodylate buffer (pH 7.2), rinsed in the buffer, post-fixed for one hour in 1% osmium tetroxide in the same buffer and dehydrated in a graded ethanol series. The material was 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.

S*canning electron microscopy* (SEM): Each larval gut was fixed, post-fixed and dehydrated as for TEM. Critical point drying was performed in a Balzers CPD 020 apparatus with carbon dioxide. Specimens were mounted on stubs, coated with gold-palladium and examined with a Philips SEM 505 scanning electron microscope.

Results

In Trichoptera, gregarines infest only larvae and have never been found in pupae or adults. The species under study belong to 18 Trichoptera families found in Italy. Only Ptilocolepidae and Thremmatidae were not investigated for lack of larval material. Larval guts were examined to highlight both the presence of gregarine infestation and the larval diet. Gregarines are always localized in the midgut. In Table 1, the examined Trichoptera species with corresponding parasite species, when present, the larval diet, and some characteristics of the midgut structure are reported. Examination of Table 1 shows that 12 of 18 families, 30 of 47 genera and 72 of 108 species were infested by gregarines. As regards to larval diet, 87% of carnivorous larvae, 87% of omnivorous larva, 63% of herbivorous larvae, and 42% of herbivorous-detritivorous larvae were infested. Detritivorous larvae were not infested.

TABLE I: List of the examined Trichoptera species, with reported larval diet (C = carnivorous; He = herbivorous; O = omnivorous; Dt = detritivorous), presence/absence of midgut goblet cells (GC, unspecified = unknown), type of peritrophic membrane (PM: I = synthesized and secreted by entire midgut; II = synthesized by specialized cells close to the *cardiac*; unspecified = unknown); infesting eugregarines

TRICHOPTERA	Diet	Goblet Cells	PM	EUGREGARINORIDA
RHYACOPHILIDAE				
Rhyacophila dorsalis acutidens McLachlan 1879	C			*Asterophora mucronata* Lèger 1892
Rhyacophila dorsalis persimilis McLachlan 1879	C			*Asterophora mucronata*
Rhyacophila foliacea Moretti 1981	C			*Asterophora mucronata*
Rhyacophila hartigi Malicky 1971	C			*Pileocephalus heerii* (von Köllicher 1845) Schneider 1887
Rhyacophila intermedia McLachlan 1868	C			
Rhyacophila italica Moretti 1981	C	without GC	I	*Asterophora mucronata*
Rhyacophila pallida Mosely 1930	C			*Pileocephalus heerii*
Rhyacophila pubescens FJ Pictet 1834	C			*Asterophora mucronata*
Rhyacophila rougemonti McLachlan 1880	C			*Asterophora mucronata*

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TABLE 1. (Continued)

TRICHOPTERA	Diet	Goblet Cells	PM	EUGREGARINORIDA
Rhyacophila simulatrix McLachlan 1879	C			*Asterophora mucronata*
Rhyacophila torrentium FJ Pictet 1834	C			*Asterophora capitata* Baudoin 1967
Rhyacophila trifasciata Mosely 1930	C			*Pileocephalus heerii*
Rhyacophila tristis FJ Pictet 1834	C			*Asterophora mucronata*
Rhyacophila vulgaris FJ Pictet 1834	C			*Pileocephalus heerii*; *Asterophora elegans* Lèger 1892
				Asterophora mucronata
GLOSSOSOMATIDAE				
Glossosoma conforme Neboiss 1963	He, Dt			*Pileocephalus glyphotaeli* Stein 1960
Catagapetus nigrans McLachlan 1884	He, Dt	without GC		*Gregarina pusilla* Baudoin 1967
Agapetus cyrnensis Mosely 1930	He, Dt			
HYDROPTILIDAE				
Hydroptila aegyptia Ulmer 1963	He	without GC		
Hydroptila angulata Mosely 1922	He			
Hydroptila sparsa Curtis 1834	He			
PHILOPOTAMIDAE				
Wormaldia mediana McLachlan 1878	Dt	with GC	II	
Wormaldia occipitalis (FJ Pictet 1834)	Dt	with GC		
Wormaldia variegata Mosely 1830	Dt			
Philopotamus ludificatus McLachlan 1878	Dt			
Philopotamus montanus (Donovan 1813)	Dt	with GC		
Chimarra marginata (Linnaeus 1767)	Dt			
ECNOMIDAE				
Ecnomus tenellus (Rambur 1842)	O	with GC	I	*Gregarina lunata* Geus 1969; *Pileocephalus heerii*; *Asterophora tiaroides* Baudoin 1967; *Ancyrophora* sp.1, *Ancyrophora* sp.2
POLYCENTROPODIDAE				
Holocentropus picicornis (Stephens 1836)	C	with GC		*Pileocephalus heerii*
Polycentropus flavomaculatus (FJ Pictet 1834)	C			*Pileocephalus agilis* Geus 1969; *Pileocephalus heerii*
Polycentropus morettii Malicky 1977	C	with GC	II	*Pileocephalus heerii*
Polycentropus mortoni Mosely 1930	C			*Pileocephalus agilis*; *Pileocephalus heerii*
Plectrocnemia geniculata McLachlan1871	C	with GC		
Plectrocnemia geniculata factiosa Moretti 1991	C	with GC	II	*Eugregarinide undetermined*

...Continued on next page

TABLE 1. (Continued)

TRICHOPTERA	Diet	Goblet Cells	PM	EUGREGARINORIDA
PSYCHOMYIIDAE				
Tinodes antonioi Botosaneanu & Taticchi-Viganò 1974	He, Dt			*Gregarina pusilla*
Tinodes maclachlani Kimmins 1966	He, Dt			*Gregarina pusilla*
Tinodes waeneri (Linnaeus 1758)	He, Dt	with GC		*Gregarina lunata*; *Gregarina pusilla*
HYDROPSYCHIDAE				
Diplectrona magna Mosely 1930	O			Eugregarinide undetermined
Cheumatopsyche lepida (FJ Pictet 1834)	C			*Pileocephalus heerii*; *Asterophora caliciphormis* Baudoin 1967; *Asterophora hydropsyches* Baudoin 1967; *Asterophora mucronata*
Hydropsyche angustipennis (Curtis 1834)	O			
Hydropsyche fumata Tobias 1972	O			*Asterophora caliciphormis*; *Globulocephalus hydopsyches* Baudoin 1965
Hydropsyche instabilis (Curtis 1834)	O			*Asterophora hydropsyches*; *Globulocephalus hydopsyches*
Hydropsyche klefbecki Tjeder 1946	O			*Asterophora hydropsyches*; *Globulocephalus hydopsyches*
Hydropsyche modesta Navás 1925	O			*Asterophora hydropsyches*; *Globulocephalus hydopsyches*
Hydropsyche morettii De Pietro 1996	O	without GC		*Asterophora hydropsyches*; *Globulocephalus hydopsyches*
Hydropsyche pellucidula (Curtis 1834)	O	without GC	II	*Asterophora hydropsyches*; *Globulocephalus hydopsyches*
Hydropsyche sattleri Tobias 1972	O			*Asterophora caliciphormis*; *Globulocephalus hydopsyches*
PHRYGANEIDAE				
Agrypnia varia (Fabricius 1793)	C	without GC		*Pileocephalus heerii*; *Ancyrophora* sp.
BRACHICENTRIDAE				
Brachycentrus montanus Klápalek 1892	He, Dt			
Micrasema minimum McLachlan 1876	He, Dt			
Micrasema togatum (Hagen 1864)	He, Dt			
GOERIDAE				
Silonella aurata (Hagen 1864)	He, Dt			
Silo mediterraneus McLachlan 1884	He, Dt	without GC	I	
LEPIDOSTOMATIDAE				
Lepidostoma basale (Kolenati 1848)	He	with GC		*Pileocephalus agilis*
Lepidostoma hirtum (Fabricius 1775)	He			
Crunoecia sp.	He			
LIMNEPHILIDAE				

...Continued on next page

TABLE 1. (Continued)

TRICHOPTERA	Diet	Goblet Cells	PM	EUGREGARINORIDA
Apatania volscorum Moretti, Cianficcon, & Papagno 1988	He	without GC	I	*Pileocephalus chinensis* Schneider 1875; *Pileocephalus heerii*
Drusus aprutiensis Moretti 1981	He			*Gregarina fontinalis* Zwetkow 1929
Drusus camerinus Moretti 1981	He			*Gregarina fontinalis*; *Gregarina pusilla*
Drusus improvisus (McLachlan 1884)	He	without GC	I	*Gregarina fontinalis*; *Gregarina pusilla*; *Pileocephalus lanceatus* Baudoin 1967
Leptodrusus budtzi (Ulmer 1913)	He			*Gregarina limnophili* Zwetkow 1929; *Gregarina stenophylacis* Zwetkow 1929
Anabolia lombarda Ris 1897	He			*Gregarina anaboliae* Zwetkow 1929
Glyphotaelius pellucidus (Retzius 1783)	He			
Grammotaulius nigropunctatus (Retzius 1783)	He	without GC	I	*Pileocephalus glyphotaeli*
Limnephilus bipunctatus Curtis 1834	He			*Gregarina pusilla*
Limnephilus flavicornis (Fabricius 1787)	He			*Gregarina limnophili*; *Gregarina stenophylacis*
				Pileocephalus chinensis
Limnephilus flavospinosus (Stein 1874)	He			
Limnephilus hirsutus (FJ Pictet 1834)	He			
Limnephilus lunatus Curtis 1834	He		I	*Gregarina limnophili*; *Pileocephalus agilis* Geus 1969
				Pileocephalus glyphotaeli; *Pileocephalus chinensis*
Limnephilus rhombicus (Linnaeus 1758)	He	without GC	I	*Gregarina limnophili*; *Gregarina stenophylacis*; *Pileocephalus agilis*
Limnephilus rhombicus reseri Malicky 1985	He			*Gregarina limnophili*; *Gregarina sericostomae* Baudoin 1966
Chaetopteryx gessneri McLachlan 1876	He	without GC	I	*Gregarina limnophili*
Allogamus ausoniae Moretti 1991	O	without GC	I	*Gregarina limnophili*; *Gregarina stenophylacis*
Allogamus auricollis (FJ Pictet 1834)	O			*Gregarina sericostomae*; *Gregarina stenophylacis*
Allogamus illiesorum Botosaneanu 1980	O			*Gregarina limnophili*; *Gregarina stenophylacis*
				Pileocephalus chinensis; *Leidyana vierlingi* Geus 1969
Allogamus hilaris (McLachlan 1876)	O			Eugregarinorida undetermined
Allogamus uncatus (Brauer 1857)	O			*Gregarina limnophili*; *Gregarina sericostomae*
Halesus appenninus Moretti & Spinelli-Batta 1979	O	without GC	I	*Leidyana vierlingi*
Halesus calabrus Moretti & Spinelli-Batta 1979	O			
Halesus nurag Malicky 1974	O			*Gregarina limnophili*; *Gregarina stenophylacis*
				Pileocephalus chinensis
Halesus radiatus (Curtis 1834)	O			*Gregarina limnophili*; *Gregarina sericostomae*; *Gregarina stenophylacis*
Halesus rubricollis (FJ Pictet 1834)	O			
Melampophylax melampus (McLachlan 1876)	He			

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TABLE 1. (Continued)

TRICHOPTERA	Diet	Goblet Cells	PM	EUGREGARINORIDA
Mesophylax aspersus (Rambur 1842)	He		I	*Pileocephalus chinensis*
Mesophylax sardous Moretti & Gianotti 1964	He			
Micropterna sequax (McLachlan 1875)	O	without GC		*Gregarina stenophylacis*; *Pileocephalus agilis*; *Leidyana vierlingi*
Micropterna wageneri Malicky 1981	O			*Gregarina fontinalis*; *Gregarina limnophili*; *Pileocephalus agilis*; *Pileocephalus chinensis*
Potamophylax cingulatus alpinus Tobias 1994	O			*Pileocephalus chinensis*
Potamophylax cingulatus inermis Moretti & Cianficconi 1994	O		I	*Gregarina stenophylacis*; *Pileocephalus agilis; Pomania morettii* Corallini-Sorcetti 1981
	O			
Potamophylax cingulatus gambaricus Malicky 1971	O	without GC		*Gregarina limnophili*; *Gregarina sericostomae*; *Gregarina stenophylacis; Pileocephalus agilis*; *Pileocephalus caudatus* Corallini 1994; *Pileocephalus lanceatus; Pileocephalus schyphoides* Baudoin 1967; *Pomania cianficconii* Corallini 1984
Potamophylax nigricornis (FJ Pictet 1834)	O			*Gregarina stenophylacis*
Stenophylax mitis McLachlan 1875	O			*Gregarina stenophylacis*
Stenophylax permistus McLachlan 1895	O		I	*Pileocephalus agilis*; *Asterophora tiaroides*
SERICOSTOMATIDAE				
Sericostoma cianficconiae Moretti 1978	He		I	*Gregarina sericostomae*
Sericostoma italicum Moretti 1978	He			*Gregarina sericostomae*
Sericostoma pedemontanum McLachlan 1876	He	with GC		*Gregarina sericostomae*
Sericostoma personatum (Spence 1826)	He			*Gregarina sericostomae*
Sericostoma siculum McLachlan 1876	He			*Gregarina sericostomae*
ODONTOCERIDAE				
Odontocerum albicorne (Scopoli 1763)	O	without GC	I	*Gregarina sericostomae*; *Pileocephalus chinensis* Schneider 1875; *Pileocephalus heerii*; *Asterophora tiaroides*
HELICOPSYCHIDAE				
Helicopsyche sperata McLachlan 1876	He, Dt	without GC		
BERAEIDAE				
Beraea maura (Curtis 1834)	He			
Beraeodes minutus (Linnaeus 1761)	He	without GC		
LEPTOCERIDAE				
Triaenodes ochreellus lefkas Malicky 1974	He	with GC		

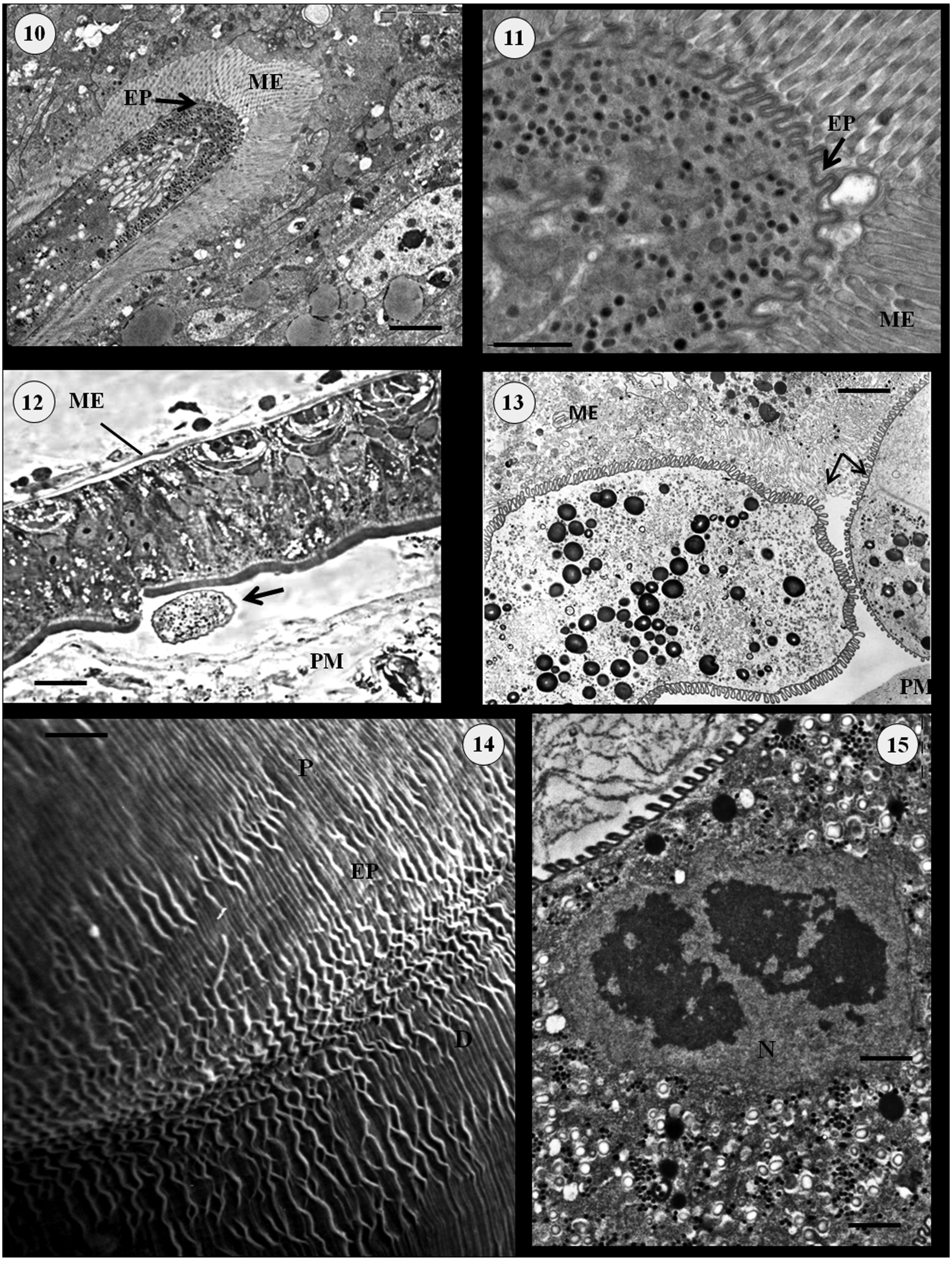
...Continued on next page

TABLE 1. (Continued)

TRICHOPTERA	**Diet**	**Goblet Cells**	**PM**	**EUGREGARINORIDA**
Mystacides azurea (Linnaeus 1761)	He			*Gregarina mystacidarum* Frantzius 1848
Mystacides longicornis (Linnaeus 1758)	He	with GC		
Ceraclea fulva (Rambur 1842)	C	with GC	I	
Leptocerus tineiformis Curtis 1834	He			
Oecetis furva (Rambur 1842)	O			

TABLE 2. List of the eugregarines infesting the examined Trichoptera larvae

EUGREGARINORIDA
GREGARINIDAE Labbé 1899
Gregarina anaboliae Zwetkow 1929
Gregarina fontinalis Zwetkow 1929
Gregarina limnophili Zwetkow 1929
Gregarina lunata Geus 1969
Gregarina mystacidarum Frantzius 1848
Gregarina pusilla Baudoin 1967
Gregarina sericostomae Baudoin 1966
Gregarina stenophylacis Zwetkow 1929
LEIDYANIDAE Kudo 1954
Leidyana vierlingi Geus 1969
ACTINOCEPHALIDAE Léger 1892
ACTINOCEPHALINAE Léger 1899
Asterophora caliciphormis Baudoin 1967
Asterophora capitata Baudoin 1967
Asterophora elegans Léger 1892
Asterophora hydropsyches Baudoin 1967
Asterophora mucronata Léger 1892
Asterophora tiaroides Baudoin 1967
Pileocephalus agilis Geus 1969
Pileocephalus caudatus Corallini 1994
Pileocephalus chinensis Schneider 1875
Pileocephalus glyphotaeli Stein 1960
Pileocephalus heerii (von Köllicher 1845) Schneider 1887
Pileocephalus lanceatus Baudoin 1967
Pileocephalus schyphoides Baudoin 1967
Pomania morettii Corallini-Sorcetti 1981
Pomania cianficconii Corallini 1984
Globulocephalus hydopsyches Baudoin 1965
ACANTHOSPORINAE Léger 1892
Ancyrophora sp.
Ancyrophora sp. 1
Ancyrophora sp. 2
Eugregarinorida undetermined



FIGURES 10–15. 10, *Ancyrophora* sp. 2 trophozoite. EP = epicyte folds; MV = midgut cell microvilli; TEM; bar = 4 µm. **11**, *Ancyrophora* sp. 2 trophozoite: Detail of epimerite connection with the host midgut cell, EP = epicyte folds, MV = midgut cell microvilli; TEM; bar = 1 µm. **12**, *Gregarina limnophili* gamont (arrow) in the space between the midgut epithelium (ME) and the peritrophic membrane (PM); phase contrast microscope; bar = 100 µm. **13**, *Gregarina limnophili* gamont (arrow) in the space between the midgut epithelium (ME) and the peritrophic membrane (PM); TEM; bar = 10 µm. **14**, epicyte folds (EP) in *Pomania moretti*, D = deutomerite, P = protomerite; SEM; bar = 4 µm. **15**, *Asterophora mucronata* nucleous (N); TEM; bar = 1 µm.

Infesting gregarines are reported in Table 2. One sample of Gregarinidae is still undetermined and the other 34 species belong to 5 families and 7 genera.

There is not a high parasitic specificity between trichopterans and gregarines except for two gregarine genera (*Pomania* and *Globulocephalus)* which are restricted to particular trichopteran genera. Species of the genus *Pomania* (Fig. 2) infest *Potamophylax* larvae. *Globulocephalus hydropsyches* Baudoin 1965 (Fig. 3) infests *Hydropsyche* larvae. The genus *Sericostoma* is infested only by *Gregarina sericostomae* which, on the other hand, can infest other Trichoptera genera like *Limnephilus*, *Allogamus*, *Potamophylax*, and *Odontocerum*.

The larval diet has a strong influence on the gregarine-trichopteran relashionship. Species of the genus *Gregarina* (Figs 4, 5) each have a simple epimerite and mainly infests vegetarian larvae with a more distended mesenteron and a slower peristalsis. Species of the genera *Pileocephalus* (Figs 6, 7) and *Asterophora* (Fig. 8), which have more complex epimerites, mainly infest omnivorous or carnivorous larvae. Nevertheless, species of *Pileocephalus* may occur in vegetarian larvae like *Drusus improvisus* (McLachlan 1884) and *Apatania volscorum* Moretti, Cianficconi & Papagno 1988. Under the light microscope and under TEM it was possible to observe the elongated epimerite of *Pileocephalus heeri* (Köllicher 1845) Schneider 1887 trophozoites embedded in the host midgut cells (Fig. 9 a,b). The genus *Ancyrophora*, with a robust epimerite, parasitizes *Ecnomus tenellus* (Rambur 1842) frequently, if not in high numbers, and rarely infests *Agrypnia* spp. Observation under TEM showed that the epicyte folds of *Ancyrophora* spp. trophozoites are tightly attached to *E. tenellus* midgut cell microvilli in order to resist the peristaltic movement of the midgut (Figs 10, 11).

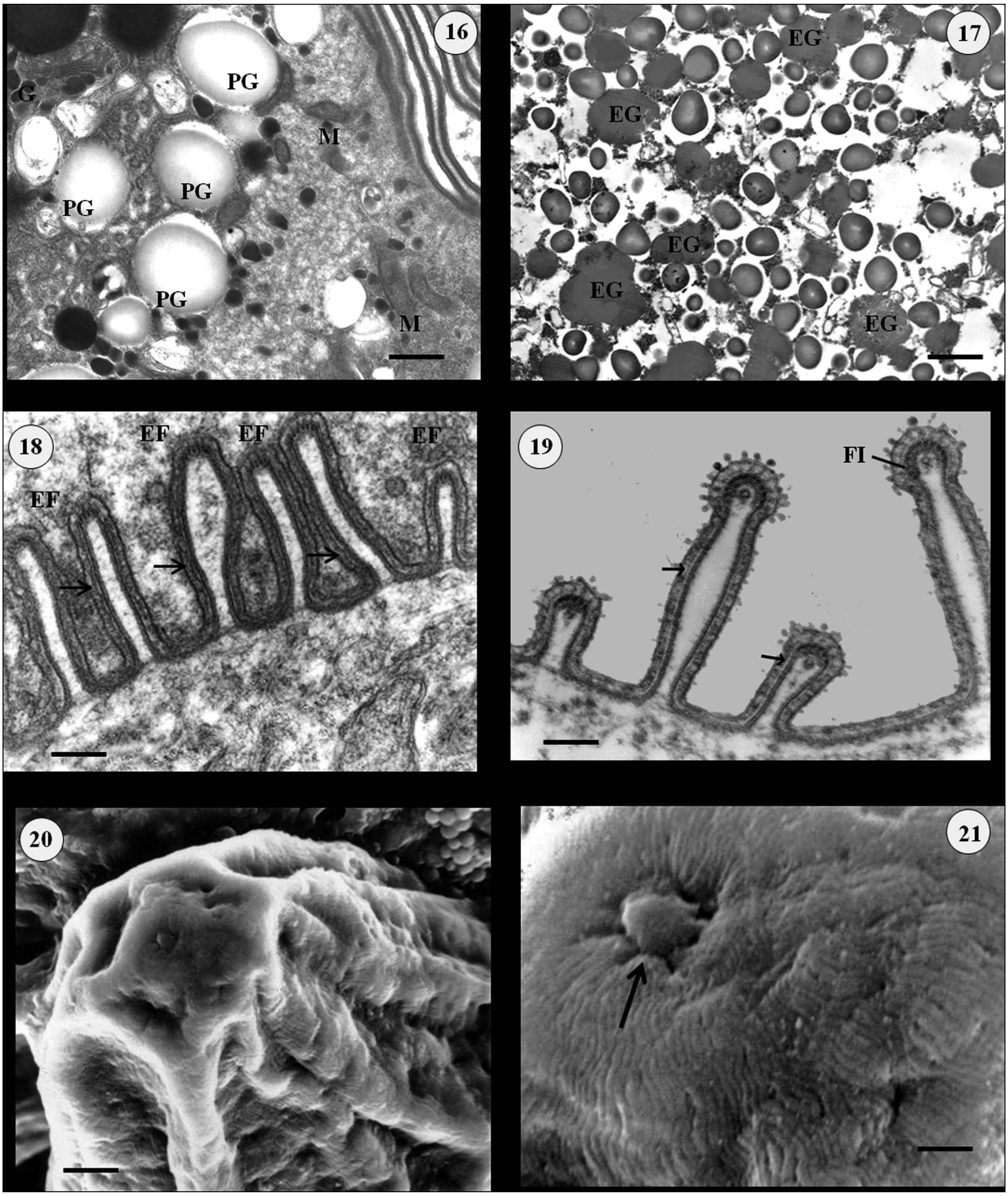
The genus *Gregarina* is widespread and is found in various orders of insects, including Trichoptera. *Gregarina* is the most abundant genus in trichopteran larvae, which often show massive infestations, and was found in Glossosomatidae, Psychomyiidae, Ecnomidae, Limnephilidae, Sericostomatidae, Odontoceridae, and Leptoceridae. The genus *Gregarina* may coexist with the genera *Pileocephalus*, *Asterophora*, *Ancyrophora*, *Leidyana*, and *Pomania*. Sometimes more than one species of *Gregarina* coexist in the same host. The genus *Asterophora* was found in Hydropsychidae, Ecnomidae, Odontoceridae and most species of *Rhyacophila*. In *Hydropsyche* larvae, *Asterophora hydropsyches* Baudoin 1967 may coexist with *G. hydropsyches*. Species of the genus *Pileocephalus* are found in Rhyacophilidae, Glossosomatidae, Ecnomidae, Polycentropodidae, Hydropsychidae, Lepidostomatidae, Limnephilidae, and Odontoceridae. The species *Leidyana vierlingi* Geus 1969 was found in Limnephilidae. Species of the genus *Pomania* were found in two *Potamophylax* subspecies endemic to Italy.

As regards the degree of larval infestation, 80% of *Tinodes maclachlani* Kimmins 1966, 75% of *Rhyacophila foliacea* Moretti 1981, 63% of *E. tenellus*, 58% of *Tinodes waeneri* (Linnaeus 1758), and 56% of *D. improvisus* were infested.

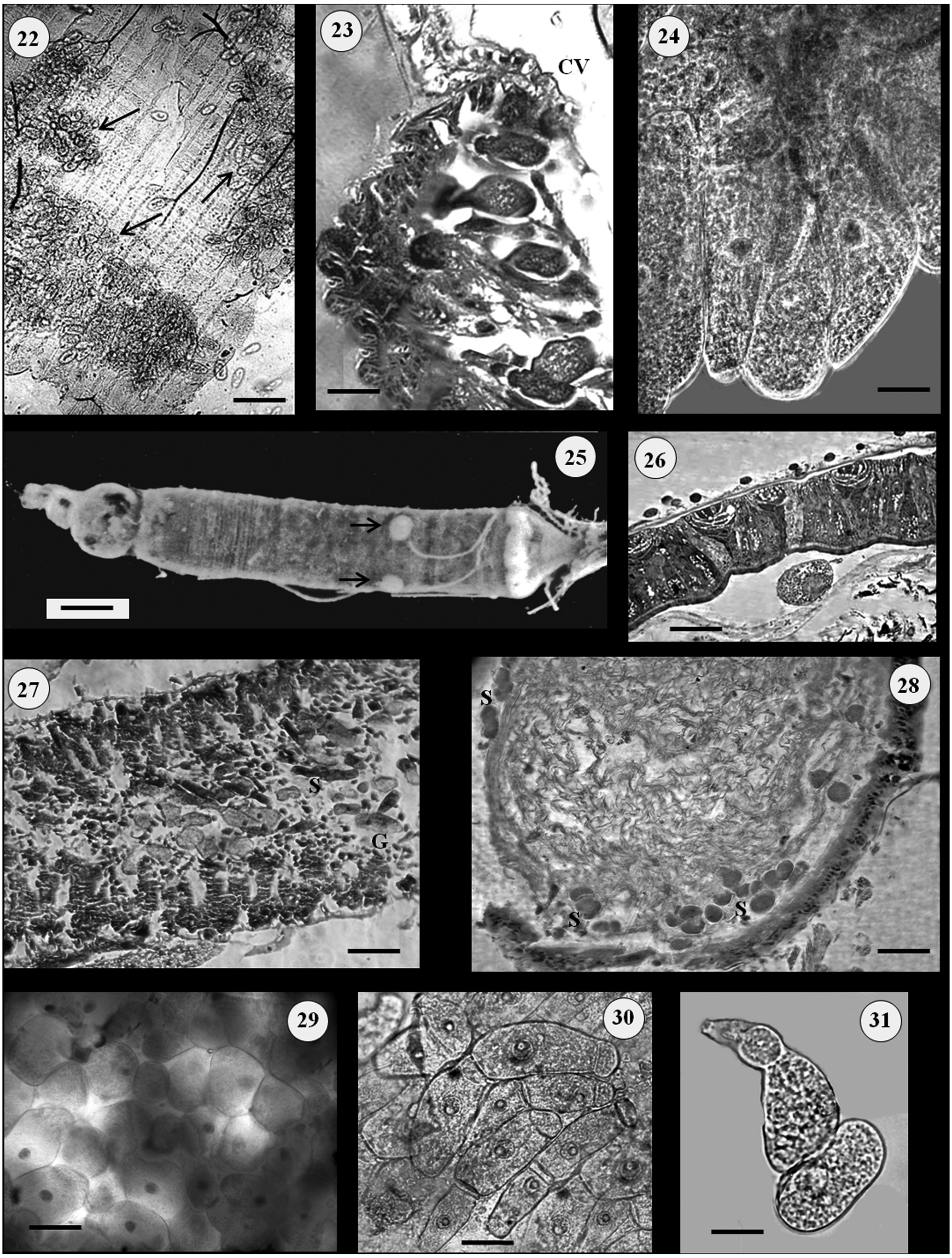
Gregarines are located in the ectoperitrophic space of the midgut, between the periptrophic membrane and the midgut epithelium (Figs 12, 13). Under SEM, gregarine cortex appears arranged in longitudinal epycite folds, which promote the gliding movement of the gregarine (Fig. 14). TEM examination shows the large nucleus, located in the deutomerite, with an evident nucleolus (Fig. 15). In the cytoplasm there are variously sized mytochondria, the Golgi complex, abundant paraglycogen granules (Fig. 16) and electron dense granules (Fig. 17). The epycite folds show a three-membrane-complex associated with cytoskeletal structures (Fig. 18). Between the plasma membrane and the middle membrane there are bundles of intermediate filaments (8−10 nm). A narrow space divides the middle and the inner membrane. Doublets of filaments in the cytoplasm adhere to the inner membrane (Fig. 19).

The immature trophozoite is attached to the midgut cell by means of the epimerite, which varies in shape and complexity within the order Eugregarinorida (Figs 3, 4, 5, 6, 7, 8). Subsequently, the trophozoite detaches from the epimerite. We observed a residual button-like structure left after apical contractility during epimerite detachment in *Pileocephalus lanceatus* Baudoin 1967 (Figs 20, 21). Trophozoites have a different distribution in the larval midgut according to the parasite species. *Gregarina* trophozoites are found along the entire length of the midgut (Fig. 22) while *Asterophora* and *Pileocephalus* (Figs 23, 24) are found mainly in the anterior portion of the midgut. *Pomania* is localized only in the posterior third of the midgut (Fig. 25). Gamonts, prior to the formation of syzygy, are mobile and are found in any portion of the midgut (Fig. 26). Syzygies are found mainly in the central and posterior portion of the midgut (Figs 27, 28). In case of massive infestations the syzygies form a dense layer between the midgut epithelium and the peritrophic membrane in order to enhance the availability of the nutrients. The presence of a large number of syzygies packed together is found mainly in *Allogamus ausoniae* Moretti 1991 (Fig. 29) and *Sericostoma pedemontanum* McLachlan 1876 (Fig. 30).

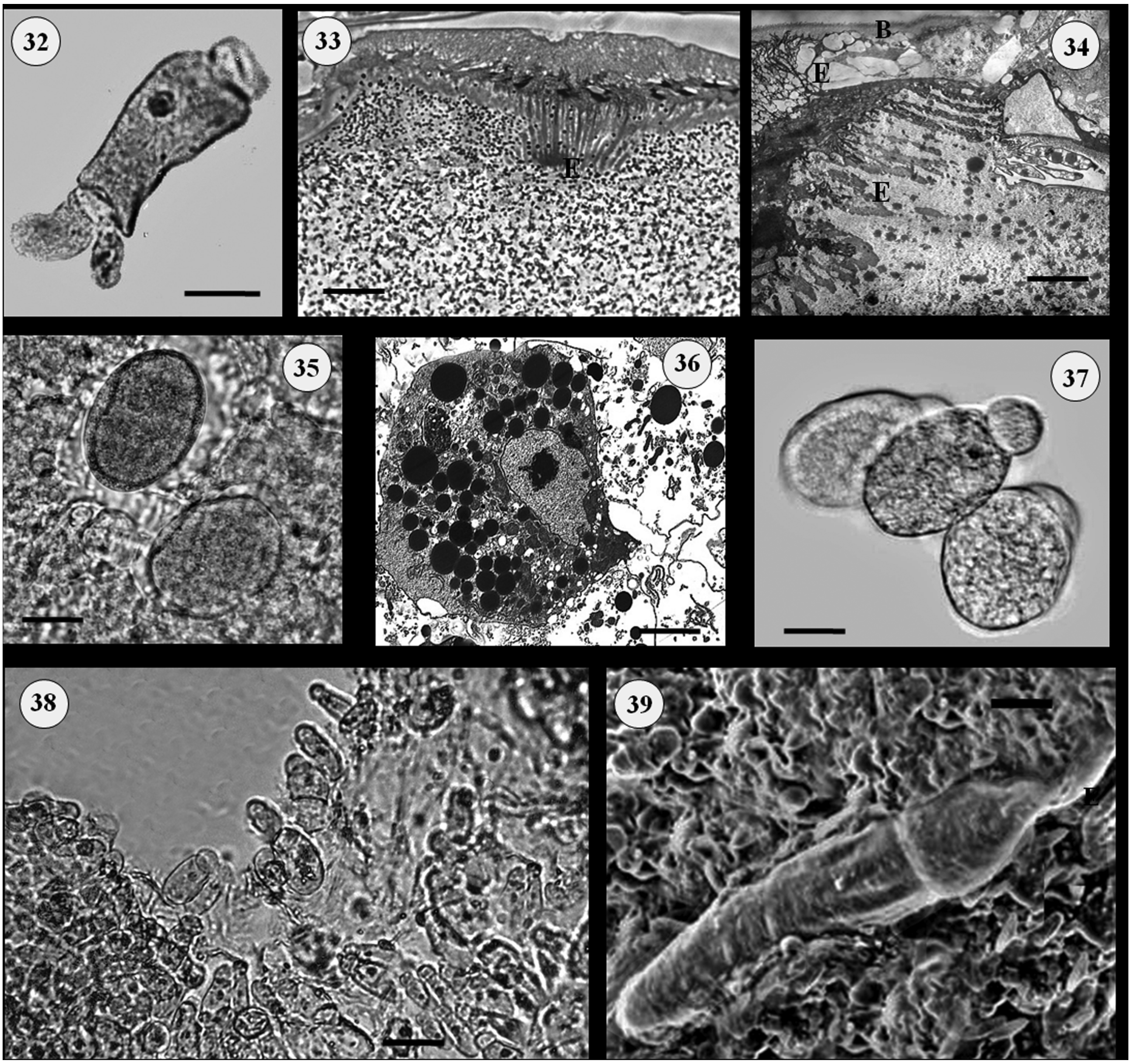
Syzygy is the longest phase of the life cycle of the genus *Gregarina,* also because it may occur when the primite, the first member of the association, is at the early stage of trophozoite (Fig. 31). In *Gregarina*, syzygy often occurs between a primite and two or more smaller satellites (Fig. 32).



FIGURES 16–21. 16, *Asterophora mucronata* cytoplasm showing the Golgi complex (G), mitochondria (M), and paraglycogen granules (PG); TEM; bar = 0.5 µm. **17**, *Asterophora mucronata* cytoplasm with electron-dense granules (EG); TEM; bar = 2 µm. **18**, epycite folds (EF) in *Pileocephalus lanceatus*, showing the three-membrane-complex (arrow); TEM; bar = 0.25 µm. **19**, detail of the apical structure of epycite folds in *Pomania morettii,* FI= intermediate filaments; arrow = three-membrane-complex; TEM; bar = 0.3 µm. **20**, *Pileopcephalus lanceatus* trophozoite after epimerite detachment; SEM; bar = 1.7 µm. **21**, *Pileopcephalus lanceatus* trophozoite: button-like structure (arrow) left after epimerite detachment; SEM; Bar = 0.4 µm.

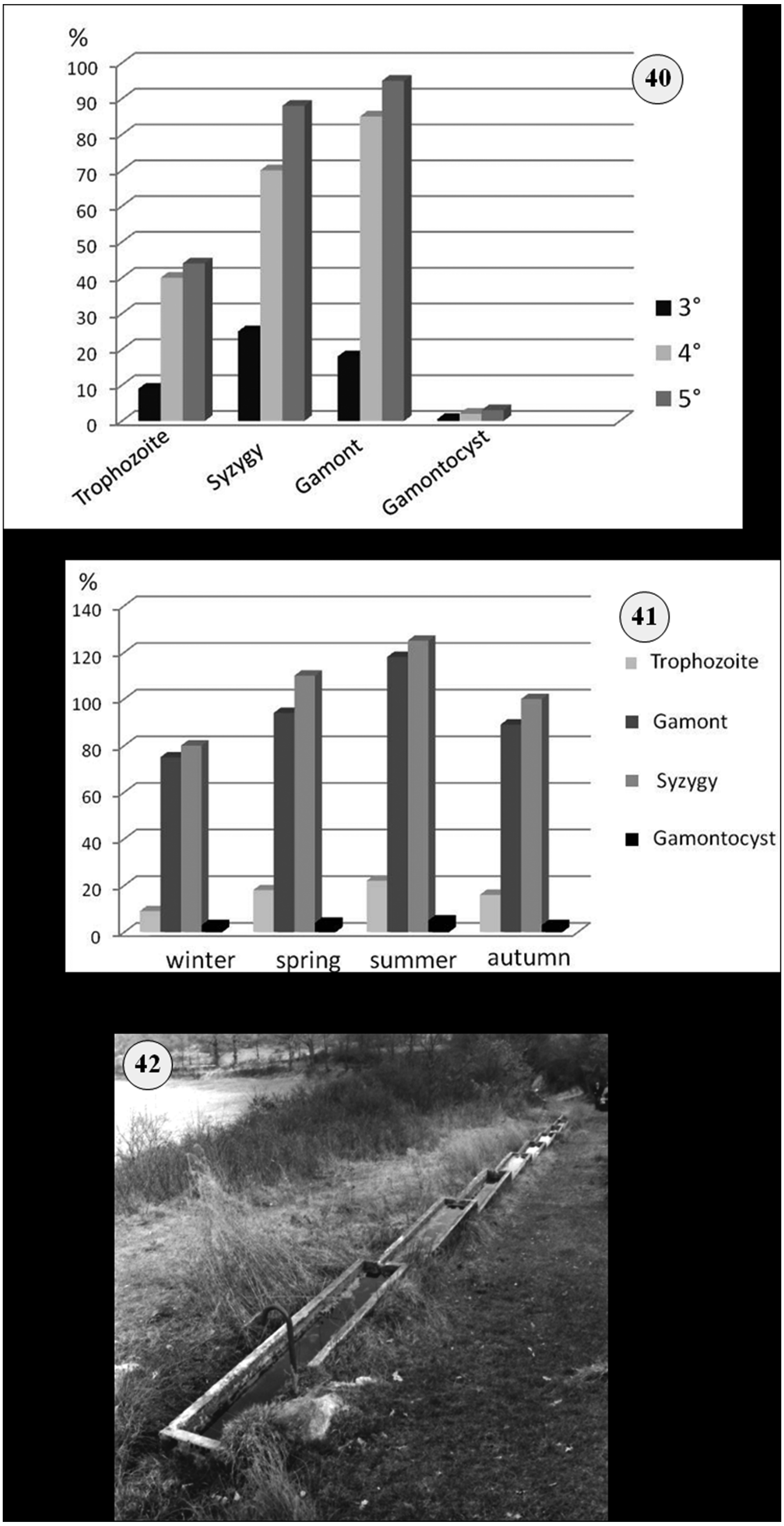


FIGURES 22–31. 22, *Drusus improvisus* midgut wall showing *Gregarina* trophozoites (arrow); light microscope; bar = 150 µm. **23**, *Pileopcephalus lanceatus* trophozoites in the anterior portion of the midgut, showing the cardiac valve (CV) indicating the midgut upper limit; light microscope; bar = 56 µm. **24**, *Pileopcephalus lanceatus* trophozoites pressed together in the anterior midgut; light microscope; bar = 15 µm. **25**, *Potamophylax cingulatus inermis* gut showing two *Pomania morettii* specimens located in the midgut (arrow); light microscope; bar = 1 mm. **26**, *Gregarina stenophylacis* gamont free in the midgut ectoperitrophic space; phase contrast microscope; bar = 80 µm. **27**, *Gregarina fontinalis* gamonts (G) and syzygies (S) in the midgut central portion of *Drusus improvisus*; light microscope; bar = 100 µm. **28**,*Gregarina fontinalis* syzygies (S) in the posterior portion of the midgut of *Drusus improvisus*; light microscope; bar = 150 µm. **29**, *Gregarina limnophili* syzygies packed together in *Allogamus ausoniae*; light microscope; bar = 63 µm. **30**, *Gregarina sericostomae* syzygies forming a compact layer in the ectoperitrophic space of *Sericostoma pedemontanum* midgut; light microscope; bar = 21 µm. **31**, *Gregarina fontinalis* trophozoite in syzygy; light microscope; bar = 15 µm.



FIGURES 32–39. 32, *Gregarina stenophylacis* syzygy with two satellites; light microscope; bar = 48 µm. **33**, *Pomania morettii* epimerite (E) deeply inserted in *Potamophylax cingulatus inermis* midgut epithelium; TEM; bar = 8 µm. **34**, Harmful effect of *Pomania morettii* epimerite (E) which ramifies through the midgut epithelium to the basement membrane (B); TEM; bar = 12 µm. **35**, *Globulocephalis hydropsyches* showing the degeneration of *Hydropsyche pellucidula* midgut cells; light microscope; bar = 40 µm. **36**, *Globulocephalis hydropsyches* within a vacuolized degenerating cell; TEM; bar = 12 µm. **37**, *Gregarina pusilla* gamonts; light microscope; bar = 23 µm. **38**, *Gregarina fontinalis* gamonts and syzygies in the midgut of a *Drusus improvisus* mature larva; light microscope; bar = 102 µm. **39**, *Pileocephalus lanceatus* early trophozoite with a long tapering epimerite (E); SEM; bar = 20 µm.

Most infested larvae are not greatly affected by gregarines as they lack noticeable alterations like abdominal blackening or flaccidity and intestinal alterations. Species of only two of the studied gregarine genera (*Pomania* and, in case of a massive infestation, *Globulocephalus*) cause a harmful effect on the host. We examined *Pomania morettii* Corallini-Sorcetti 1981, found in *Potamophylax cingulatus inermis* Moretti & Cianficconi 1994 (in Moretti *et al*. 1994), and *Pomania cianficconii* Corallini 1984, found in *Potamophylax cingulatus gambaricus* Malicky 1971. These gregarines can be considered harmful parasites as their epimerites cause extensive damage to the midgut cells (Figs 33, 34). *Globulocephalus hydropsyches,* which infests *Hydropsyche* larvae, has intracellular sporozoites and trophozoites. A high number of intracellular phases of *G. hydropsyches* may cause alterations of the midgut epithelium of the host (Figs 35, 36).



FIGURES 40–42. 40, average number of different *Gregarina fontinalis* developmental stages in *Drusus improvisus* 3rd, 4th and 5th larval instars. 41, average number of *Gregarina fontinalis* developmental stages in *Drusus improvisus* mature larvae throughout the year. 42, trough "Centelle."

In the host-parasite relationship, the larval habitat has to be considered. The influence of the trichopteran habitat on the duration and intensity of the gregarine infestation is particularly evident in spring-living larvae. We examined the river Tiber springs and close environments like troughs ("trocchi") which are man-modified springs that provide drinking water for cattle. In the river Tiber springs, infestation of the population of *D. improvisus* was investigated. The spring has a constant annual temperature of 6°C and this implies that all larval stages are present in every season. We found that 56% of *D. improvisus* larvae are infested by three gregarine species: *Gregarina fontinalis* Zwetkow 1929 (Fig. 5), *G. pusilla* Baudoin 1967 (Figs 4, 37) and *P. lanceatus* (Fig. 39). *Gregarina fontinalis* infests the higher number of larvae and numerous gamonts and syzygyies were found in 3rd to 5th larval instars (Figs 38, 40). All developmental stages of *G. fontinalis* were observed throughout the year (Fig. 41).

The examined troughs were located in Umbria, Central Apennines, and were inhabited by a high number (over 200 specimens) of trichopteran larvae. The water flows from the spring into tubes and then slowly into a descending series of basins, allowing the formation of watering troughs for cattle (Fig. 42). Trichopera populations in each trough, although numerous, belong to very few species. In trough «La Spina» there is a monospecific population of *Potamophylax cingulatus gambaricus,* with up to 86% of infested larvae, parasitized by *G. fontinalis*, *G. limnophili* Zwetkow 1929, *P. agilis* Geus 1969, and *Asterophora elegans* Lèger 1892. In trough "dell'Eremita" two trichopteran species were found: *Allogamus ausoniae*, which accounts for 90% of the population and is parasitized by *G. limnophili, P. lanceatus*, and *P. chinensis* Schneider 1875; and *Micropterna* sp.without infestation. In trough «Centelle» three trichopteran species were found. *Sericostoma pedemontanum*, clearly predominant, is parasitized by *G. sericostomae* and *G. limnophili,* with all the larvae in this population infested. The other two species, *A. ausoniae* and *Micropterna* sp., were not infested.

Discussion

Our observations show that gregarines usually have no harmful effects on the trichopteran larvae as reported in literature for other insect orders (Baudoin 1967; Geus 1969), except for two gregarine genera: *Pomania* and *Globulocephalus*, the latter causing midgut cell damage only in case of a massive infestation.

The host-parasite relationship is influenced by the larval diet. Infestation is higher in carnivorous and omnivorus larvae than in herbivorous larvae and their corresponding parasites show differences in their epimerites. Infestation is more likely to occur in larvae with predation, shredding, and filtering feeding behaviours than in detritivorous larvae. The organization of the midgut wall, with special regard to the presence of the goblet cells and the type of peritrophic membrane, do not interfere with gregarine infestation.

It is interesting that larvae inhabiting the river Tiber springs are infested throughout the year. Due to the unchanged environmental conditions in all seasons, the continuous life cycle of *D. improvisus* in the river Tiber springs allows the continuous life cycle of the parasites. The contemporaneous presence of trophozoites, gamonts, syzygies and spores was detected in larvae of all stages.

The highest degree of infestation is found in closed environments like troughs where populations of numerous individuals belonging to few trichopteran species coexist. In trough "Centelle," 100% of *S. pedemontanum* larvae were infested.

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