Mosquito and sand fly gregarines of the genus *Ascogregarina* and *Psychodiella* (Apicomplexa: Eugregarinorida, Aseptatorina) – Overview of their taxonomy, life cycle, host specificity and pathogenicity

Lucie Lantova a,⁎, Petr Volf b

a Institute of Histology and Embryology, First Faculty of Medicine, Charles University in Prague, Albertov 4, 128 00 Prague 2, Czech Republic

b Department of Parasitology, Faculty of Science, Charles University in Prague, Vinicna 7, 128 44 Prague 2, Czech Republic

ARTICLE INFO

Article history:
Received 30 January 2014
Received in revised form 16 April 2014
Accepted 24 April 2014
Available online xxxx

Keywords:
Ascogregarina
Psychodiella
Coevolution
Host specificity
Pathogenicity

ABSTRACT

Mosquitoes and sand flies are important blood-sucking vectors of human diseases such as malaria or leishmaniasis. Nevertheless, these insects also carry their own parasites, such as gregarines; these monoxenous pathogens are found exclusively in invertebrates, and some of them have been considered useful in biological control. Mosquito and sand fly gregarines originally belonging to a single genus *Ascogregarina* were recently divided into two genera, *Ascogregarina* comprising parasites of mosquitoes, bat flies, hump-backed flies and fleas and *Psychodiella* parasitizing sand flies. Currently, nine mosquito *Ascogregarina* and five *Psychodiella* species are described. These gregarines go through an extraordinarily interesting life cycle; the mosquito and sand fly larvae become infected by oocysts, the development continues transstadially through the larval and pupal stages to adults and is followed by transmission to the offspring by specific mechanisms. In adult mosquitoes, ascogregarines develop in the Malpighian tubules, and oocysts are defecated, while in the sand flies, the gregarines are located in the body cavity, their oocysts are injected into the accessory glands of females and released during oviposition. These life history differences are strongly supported by phylogenetical study of SSU rDNA proving disparate position of *Ascogregarina* and *Psychodiella* gregarines. This work reviews the current knowledge about *Ascogregarina* and *Psychodiella* gregarines parasitizing mosquitoes and sand flies, respectively. It gives a comprehensive insight into their taxonomy, life cycle, host specificity and pathogenicity, showing a very close relationship of gregarines with their hosts, which suggests a long and strong parasite-host coevolution.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

1. Introduction

Mosquitoes (Diptera: Culicidae) and sand flies (Diptera: Psychodidae) are blood-sucking insects and important vectors of human pathogens. Apart from a number of pathogenic viruses, mosquitoes transmit *Plasmodium* (Apicomplexa: Haemosporida) and Filarioidea (Nematoda: Spirurida), while sand flies are the vectors of *Leishmania* (Euglenozoa: Trypanosomatida) and *Bartonella* (Proteobacteria). As pointed out by Warburg (1991), the life cycle of these insects comprising water and terrestrial larval stages, respectively, can facilitate growth and persistence of various entomopathogens. In mosquitoes, these include mites (e.g., Kirkhoff et al., 2013), nematodes (reviewed by Petersen, 1980), protists (reviewed by Clark, 1980), fungi (reviewed by Scholte et al., 2004), microsporidia (reviewed by Andreadis, 2007), bacteria (reviewed by Minard et al., 2013) and viruses (reviewed by Becnel and White, 2007). Similarly, sand flies suffer from the presence of a wide range of pathogens (reviewed by Warburg et al., 1991) such as mites (e.g., Lewis and Macfarlane, 1981), nematodes (e.g., Secundio et al., 2002), protists (e.g., McConnell and Correa, 1964), fungi (e.g., Akhoundi et al., 2012), microsporidia (e.g., Matos et al., 2006), bacteria (e.g., Gouveia et al., 2008) and viruses (e.g., Warburg and Pimenta, 1985).

Mosquitoes and sand flies also serve as hosts of gregarines (Apicomplexa) of the genus *Ascogregarina* Ward, Levine and Craig, 1982 and *Psychodiella* Votypka, Lantova and Volf, 2009, respectively. Phylum Apicomplexa encompasses very important human pathogens such as *Cryptosporidium*, *Toxoplasma* or *Plasmodium*, while gregarines are parasites exclusively of invertebrates. This might give the impression that they would not be of much importance to humans; however, especially the neogregarines are generally considered useful in biological control of insect pests. Furthermore,
sand fly gregarines are known to seriously harm laboratory-reared colonies; therefore, they negatively affect the research on vector-borne infections.

Even though Ascogregarina and Psychodiella are affecting important vectors of human diseases, they have not been given the attention they deserve, and information available about them is currently insufficient. This applies particularly for the sand fly gregarines. Before 2010, there had been only three described species of Psychodiella, the host specificity and pathogenicity of these parasites had only been evaluated experimentally in a single study, and closer descriptions of the life cycle and fine structure had been available only about one species.

This work brings a comprehensive overview of all the described species of Ascogregarina and Psychodiella from mosquitoes and sand flies, including their taxonomy, life cycle, pathogenicity and host specificity features. It brings evidence that both species of these parasites have a very close relationship with their hosts, suggesting a long coevolution.

2. Taxonomy

Members of the genus Ascogregarina (syn. Monocystis von Stein, 1848, Lankestia Mingazzini, 1891 and Ascocystis Grasse, 1953) and Psychodiella are aseptate eugregarines (Eugregarinorida: Aseptatorina), and together, they have been recently included in a new family Ascogregarinidae (Desportes, 2013). The genus Ascogregarina comprises species parasitizing mostly Diptera, particularly mosquitoes, bat flies (Nycteribiidae) and hump-backed flies (Phoridae) but also fleas (Siphonaptera). The genus Psychodiella is found only in sand flies. The terminology and history of the final designation of these parasites is complex. Originally, both these groups were included in a single genus Ascogregarina. The type species of this genus is Ascogregarina culicis (Ross, 1898), originally described as Gregarina culicis Ross (1895) or Gregarina culicis (Ross, 1898) and later renamed as Lankestia culicis (Wenyon, 1911). The first described sand fly gregarine was originally named Monocystis mackiei by Shortt and Swaminath (1927) or Lankestia phlebotomi mackiei by Missiroli (1932). Grasse (1953) proposed a new name Ascocystis for gregarines of the genus Lankestia parasitizing insects, and he renamed La. culicis as Ascocystis culicis. Similarly, Ormieres (1965) and Tuzet and Rioux (1966) renamed La. ph. mackiei as Ascocystis mackiei, and later Scorza and Carnevali (1981) brought morphological evidence placing sand fly gregarines of the genus Monocystis into the genus Ascogregarina. Levine (1977) and Ormieres (1965) accepted Ascocystis from Grasse (1953) for parasites of Diptera and restricted Lankestia to parasites of ascidians. However, the name Ascocytsis is a synonym for Ascocytsis Bather, 1889 used for fossil crinoid echinoderm; therefore, Ward et al. (2007) reclassified the genus Ascogregarina, divided it into two and established a new genus accommodating sand fly gregarines – Psychodiella. The status of the taxonomy of mosquito and sand fly gregarines of the former genus Ascogregarina with nine described mosquito gregarine species and five described sand fly gregarine species is presented in Table 1.

Information about molecular taxonomy of sand fly and mosquito gregarines is currently lacking. Genes for SSU rRNA were sequenced for Ascogregarina armigerei (Lien and Levine, 1980), Ascogregarina sp. from Ochlerotatus japonicus japonicus, As. culicis and Ascogregarina taiwanensis (Lien and Levine, 1980) by Roychoudhury et al. (2007a), and these authors showed close relationship of ascogregarines and cryptosporidians. Partial sequences of SSU rDNA and 28S rDNA and sequences of ITS1, 5.8S rDNA and ITS2 of Ascogregarina barretti (Vavra, 1969), As. culicis and As. taiwanensis were submitted by Morales et al. (2005). Furthermore, there are directly submitted sequences of actin gene, partial SSU rDNA and ITS1, 5.8S rDNA, ITS2, 26S rDNA and SS rDNA of As. taiwanensis. Templeton et al. (2010) accomplished a whole-genome-sequence survey for As. taiwanensis. Less information is available about Psychodiella gregarines; there are only two studies revealing the sequences of SSU rDNA of Psychodiella sergenti Lantova, Volf and Votyypka 2010, Psychodiella chagasi (Adler and Mayrink, 1961) and Psychodiella tobbi Lantova, Volf and Votyypka, 2010 (Lantova et al., 2010; Votyypka et al., 2009).

The very complex history of the terminology and the final designation of the genus Psychodiella and Ascogregarina show that the life cycle and morphological characteristics are not sufficient anymore for determining the genera and species of gregarines. Description of new gregarine species should combine both the biological and molecular features, as shown by Lantova et al. (2010), Leander et al. (2003a), Rueckert and Leander (2009) or Votyypka et al. (2009). The lack of taxonomical data about Psychodiella and Ascogregarina calls for more molecular phylogenetic studies and sequencing more genes of more species to reveal the hidden biodiversity among sand fly and mosquito gregarines. Such a complex approach would also support the recent inclusion of Ascogregarina and Psychodiella in a new family Ascogregarinidae (Desportes, 2013) and would clarify the relationship of siphonapteran and brachyceran Ascogregarina species with nematoceran Ascogregarina and Psychodiella species. Furthermore, the close relationship of aseptate eugregarines and neogregarines as demonstrated e.g., by Carreno et al. (1999), Lantova et al. (2010), Leander et al. (2003a,b, 2006) and Votyypka et al. (2009) may raise the question, whether the absence of merogony in some aseptate eugregarines is not only the case of the merogony not being detected.

3. Genus Ascogregarina: the life cycle and species overview

3.1. General life cycle

The life cycle of As. culicis (Fig. 1), the type species of the genus Ascogregarina, is very similar to other mosquito ascogregarines and is used as a typical example. Minor interspecific differences and details are explained for each species separately (see Sections 3.2–3.11). Published measurements of oocysts of all mosquito Ascogregarina species are summarized in Table 2.

The mosquito larvae become infected by ingesting Ascogregarina oocysts. Each spindle-shaped oocyst contains eight sporozoites, which are released in the larval intestine and invade the epithelial cells. Inside the cells, the sporozoites develop into trophozoites, which are later, when the epithelial cell ruptures, released into the gut lumen. Initially, they attach to the epithelial cells of the larval intestine, and during pupation, the gregarines migrate to the Malpighian tubules, the site of sexual development of the parasites in adult mosquitoes; the gamonts pair in syzygies and develop into gametocysts with oocysts inside. The oocysts are released during defecation with faeces into the water and infect freshly hatched larvae. (McCray et al., 1970; Vavra, 1969; Walsh and Callaway, 1969; Wenyon, 1911).
The species of the genus *Ascogregarina* are listed below according to the year of their description.

### 3.2. *Ascogregarina culicis*

*Asc. culicis* was described in India by Ross (1895). Oocysts of this parasite are infective to all larval stages of *Aedes aegypti* (Lin., 1762); when the early larval instars are infected with the gregarine oocysts, the development of both the parasite and the host are synchronized, while the gregarine development stops at the gamont stage, when the oocysts are ingested by the late 4th instar larvae. Similar results were observed also for *As. taiwanensis* in *Aedes albopictus* (Skuse, 1894) (Roychoudhury and Kobayashi, 2006; see Section 3.8).

The sporozoites are 9.5–10 μm long with a tapered posterior end. Their pellicle, according to the authors (Sheffield et al., 1971), consists of an outer and a thicker inner membrane. The anterior part of the sporozoites contains conoid, two apical rings and a polar ring. A “flask-shaped” organelle observed in the anterior part of the sporozoites was suggested to function in the host cell invasion (Sheffield et al., 1971). Trophozoites of *As. culicis* are 170 μm long, gametocysts are 71–125 μm in diameter, and oocysts are 11 μm long and 5 μm wide (Lien and Levine, 1980).

Susceptibility of *Ae. aegypti* to *As. culicis* varies among geographical strains (Reyes-Villanueva et al., 2003; Sulaiman, 1992); furthermore, one strain of *Ae. aegypti* from Trinidad was not susceptible to a Florida strain of *As. culicis* as no oocysts developed, while other Trinidad strains were susceptible (Beier et al., 1995).

Several studies on the prevalence of *As. culicis* in natural *Ae. aegypti* populations showed that it ranges from 0% to 100% and is seasonally and spatially very heterogeneous (Albicocco and Vezzani, 2009; Beier et al., 1995; Blackmore et al., 1995; Dellape et al., 2005; Vezzani and Vwisney, 2006).

According to some authors, *As. culicis* was found also in mosquito species other than *Ae. aegypti*: Kramar (1952) and Ganapatil and Tate (1949) reported it in *Aedes geniculatus* (Olivier, 1791), Ray (1933) in *Ae. albopictus*, Feng (1930) in *Aedes koreicus* and Pillai et al. (1976) in *Aedes polynesiensis* Marks, 1951. Vavra (1969) suggested that only those from *Ae. albopictus* and *Ae. koreicus* could be *As. culicis*. Munstermann and Levine (1983) consider the two gregarines from *Ae. geniculatus* to be *Ascogregarina geniculata* Munstermann and Levine, 1983, and Levine (1985) designated the gregarine from *Ae. polynesiensis* as *Ascogregarina polynesiensis* Levine, 1985 (see Sections 3.9 and 3.10).

### 3.3. *Ascogregarina tripteroidesi*

*Ascogregarina tripteroidesi* (Bhatia, 1938) was found in *Triptero-ides doifeini* (Guengerich, 1913) in Sri Lanka by Guengerich (1914) and later denominated by Bhatia (1938). The whole life cycle was not described; the only known stages are trophozoites, which were

---

**Table 1** Overview of denominated mosquito and sand fly gregarines of the genus *Ascogregarina* and *Psychodiella* (originally both included in the genus *Ascogregarina*).

<table>
<thead>
<tr>
<th>Name</th>
<th>Original name</th>
<th>Host species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascogregarina culicis</em></td>
<td><em>A. r. Ward</em></td>
<td><em>Aedes aegypti</em> (Lin., 1762)</td>
</tr>
<tr>
<td><em>As. tripteroidesi</em></td>
<td><em>L. barretti</em></td>
<td><em>Triptero-ides doifeini</em> (Guengerich, 1913)</td>
</tr>
<tr>
<td><em>As. clarki</em></td>
<td><em>La. clarki</em></td>
<td><em>Ae. rubrosimilis</em> (Say, 1823)</td>
</tr>
<tr>
<td><em>As. armigeri</em></td>
<td><em>A. l. armigeri</em></td>
<td><em>Ar. armigeri</em> (Coquillett, 1898)</td>
</tr>
<tr>
<td><em>As. taiwanensis</em></td>
<td><em>A. s. taiwanensis</em></td>
<td><em>Ae. albopictus</em> (Skuse, 1894)</td>
</tr>
<tr>
<td><em>As. geniculati</em></td>
<td><em>A. geniculati</em></td>
<td><em>Ae. geniculatus</em> (Olivier, 1791)</td>
</tr>
<tr>
<td><em>As. polynesiensis</em></td>
<td><em>A. polynesiensis</em></td>
<td><em>Ae. polynesiensis</em> Marks, 1951</td>
</tr>
<tr>
<td><em>Psychodiella mackiei</em></td>
<td><em>M. chagasi</em></td>
<td><em>Lutzomyia longipalpis</em> (Lutz and Neiva, 1912)</td>
</tr>
<tr>
<td><em>Ps. chagasi</em></td>
<td><em>Ps. saraviae</em></td>
<td><em>L. ichyi</em> (Floh and Abonnenc, 1950)</td>
</tr>
<tr>
<td><em>Ps. saraviae</em></td>
<td><em>Ps. tobbi</em></td>
<td><em>Ph. sergenti Parrot, 1917</em></td>
</tr>
<tr>
<td><em>Ps. tobbi</em></td>
<td><em>Ps. tobbi</em></td>
<td>*Ph. tobbi Adler, Theodor and Lourie, 1930</td>
</tr>
</tbody>
</table>

* Additional possible *Ascogregarina* and *Psychodiella* species that have not been denominated or species not parasitizing sand flies and mosquitoes are listed in the text (see Sections 3.11 and 4.7).
recorded in the body cavity, trachea and anal gills of mosquito larvae.

3.4. Ascogregarina barretti

As. barretti was described from Aedes triseriatus (Say, 1823) in Texas by Vavra (1969). Trophozoites develop in the epithelial cells of the larval intestine, after reaching the size of 150–200 \( \mu \text{m} \), they are released from the ruptured cells and appear in the ectoperitrophic space of the intestine as gamonts. These grow up to the length of 310 \( \mu \text{m} \), and during pupation, they enter into the Malpighian tubules as gamonts and their nucleus, the character and size of the oocysts

3.6. Ascogregarina armigerei

As. armigerei was described from Armigeres subalbatus (Coquillett, 1898) in Taiwan by Lien and Levine (1980). The trophozoites measure 135 × 23 \( \mu \text{m} \) and are contractile, the gametocysts are 71.2 \( \mu \text{m} \) in diameter. The character and size of the oocysts (14.5 × 6 \( \mu \text{m} \)) clearly distinguishes between As. culicis, As. armigerei, Ascogregarina tainwanensis (Lien and Levine, 1980) and As. taiwanensis (Lien and Levine, 1980).

3.7. Ascogregarina lanyuensis

As. lanyuensis was described from Aedes alicasidi Huang, 1972 in Taiwan along with two other Ascogregarina species (Lien and Levine, 1980). Trophozoites measure 190 × 26 \( \mu \text{m} \), gametocysts are 89.9 \( \mu \text{m} \) in diameter, and oocysts are 9 \( \mu \text{m} \) long and 6 \( \mu \text{m} \) wide.

3.8. Ascogregarina taiwanensis

As. taiwanensis was described from Ae. albopictus in Taiwan by Lien and Levine (1980). Sporozoites are slender with a three-layered pellicle (recorded also in trophozoites) and possess typical apical complex with conoid, polar rings, rhoptries, subpellicular microtubules and micronemes (Chen et al., 1997a). The amyllopectin granules recorded in the oocysts disappear during the morphogenesis of the sporozoites (Chen et al., 1997a, Chen et al. 1997b) usually found two sporozoites in each epithelial cell. The release of the sporozoites from the oocysts may be triggered by V-ATPase modulated

---

**Table 2**

Overview of published oocyst measurements of mosquito Ascogregarina and sand fly Psychodella species.

<table>
<thead>
<tr>
<th>Gregarine species</th>
<th>Oocyst length</th>
<th>Oocyst width</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascogregarina culicis</td>
<td>6 ( \mu \text{m} )</td>
<td>Not given</td>
<td>Ross (1985)</td>
</tr>
<tr>
<td></td>
<td>10 ( \mu \text{m} )</td>
<td>6 ( \mu \text{m} )</td>
<td>Ray (1933)</td>
</tr>
<tr>
<td></td>
<td>10–12 ( \mu \text{m} )</td>
<td>6–7 ( \mu \text{m} )</td>
<td>Vavra (1969)</td>
</tr>
<tr>
<td></td>
<td>11 ( \mu \text{m} )</td>
<td>5 ( \mu \text{m} )</td>
<td>Lien and Levine (1980)</td>
</tr>
<tr>
<td></td>
<td>11.2 (9.5–12.2) ( \mu \text{m} )</td>
<td>4.9 (4.7–5.1) ( \mu \text{m} )</td>
<td>Delage et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>11.1 (10.6–11.4) ( \mu \text{m} )</td>
<td>5 (4.8–5.5) ( \mu \text{m} )</td>
<td>Vezzani and Wisniewsky (2006)</td>
</tr>
<tr>
<td></td>
<td>8.8 ( \mu \text{m} )</td>
<td>4.22 ( \mu \text{m} )</td>
<td>Roychoudhury et al. (2007a)</td>
</tr>
<tr>
<td>As. tripteroidesi</td>
<td>Not published</td>
<td>Not published</td>
<td>Vavra (1969)</td>
</tr>
<tr>
<td>As. barretti</td>
<td>11 ( \mu \text{m} )</td>
<td>5.4–5.7 ( \mu \text{m} )</td>
<td>Sanders and Poinar (1973)</td>
</tr>
<tr>
<td>As. clarki</td>
<td>11 (10–12) ( \mu \text{m} )</td>
<td>6 (5–6) ( \mu \text{m} )</td>
<td>Lien and Levine (1980)</td>
</tr>
<tr>
<td>As. armigerei</td>
<td>14.5 ( \mu \text{m} )</td>
<td>5.78 ( \mu \text{m} )</td>
<td>Lien and Levine (1980)</td>
</tr>
<tr>
<td>As. tainwanensis</td>
<td>9 ( \mu \text{m} )</td>
<td>5 ( \mu \text{m} )</td>
<td>Roychoudhury et al. (2007a)</td>
</tr>
<tr>
<td></td>
<td>10 ( \mu \text{m} )</td>
<td>5 ( \mu \text{m} )</td>
<td>Lien and Levine (1980)</td>
</tr>
<tr>
<td></td>
<td>9.3 (8.3–9.9) ( \mu \text{m} )</td>
<td>4.6 (4.3–4.9) ( \mu \text{m} )</td>
<td>Garcia et al. (1994)</td>
</tr>
<tr>
<td></td>
<td>8.72 ( \mu \text{m} )</td>
<td>4.97 ( \mu \text{m} )</td>
<td>Chen et al. (1997a)</td>
</tr>
<tr>
<td></td>
<td>9.9 ( \mu \text{m} )</td>
<td>4.85 ( \mu \text{m} )</td>
<td>Munstermann and Levine (1983)</td>
</tr>
<tr>
<td>As. genculati</td>
<td>12 ( \mu \text{m} )</td>
<td>4 ( \mu \text{m} )</td>
<td>Munstermann and Levine (1983)</td>
</tr>
<tr>
<td></td>
<td>9–11 ( \mu \text{m} )</td>
<td>4–6 ( \mu \text{m} )</td>
<td>Kramar (1952)</td>
</tr>
<tr>
<td></td>
<td>13 (12–14) ( \mu \text{m} )</td>
<td>5 ( \mu \text{m} )</td>
<td>Vavra (1969)</td>
</tr>
<tr>
<td>As. polyneisensis</td>
<td>9.32 ( \mu \text{m} )</td>
<td>4.24 ( \mu \text{m} )</td>
<td>Pillai et al. (1976)</td>
</tr>
<tr>
<td>Psychodella mackiei</td>
<td>9.6 ( \mu \text{m} )</td>
<td>5.8 ( \mu \text{m} )</td>
<td>Shortt and Swanninath (1927)</td>
</tr>
<tr>
<td>Ps. chagasi</td>
<td>10.9–11.9 ( \mu \text{m} )</td>
<td>5.8 ( \mu \text{m} )</td>
<td>Adler and Mayrinck (1961)</td>
</tr>
<tr>
<td></td>
<td>10.9 (10–12) ( \mu \text{m} )</td>
<td>6.5 (6–7) ( \mu \text{m} )</td>
<td>Brazil and Ryan (1984)</td>
</tr>
<tr>
<td></td>
<td>12.7 (11.6–14.5) ( \mu \text{m} )</td>
<td>7.5 (7.2–7.8) ( \mu \text{m} )</td>
<td>Ostrowska et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>12.7 (11.6–14.5) ( \mu \text{m} )</td>
<td>7.5 (7.2–7.8) ( \mu \text{m} )</td>
<td>Warburg and Ostrowska (1991)</td>
</tr>
<tr>
<td></td>
<td>12.7 (12–13.3) ( \mu \text{m} )</td>
<td>8.3 (7.3–8.9) ( \mu \text{m} )</td>
<td>Lantova et al. (2010)</td>
</tr>
<tr>
<td>Ps. saravai</td>
<td>12.4 (11.6–13.1) ( \mu \text{m} )</td>
<td>5.8 (5.6–5.9) ( \mu \text{m} )</td>
<td>Ostrowska et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>12.4 ( \mu \text{m} )</td>
<td>5.8 ( \mu \text{m} )</td>
<td>Warburg (1991)</td>
</tr>
<tr>
<td>Ps. sergenti</td>
<td>9.6 (8.7–10.3) ( \mu \text{m} )</td>
<td>6.7 (6.2–7.1) ( \mu \text{m} )</td>
<td>Lantova et al. (2010)</td>
</tr>
<tr>
<td>Ps. tobbi</td>
<td>9.6 (8.8–10.7) ( \mu \text{m} )</td>
<td>7.5 (6.8–8.5) ( \mu \text{m} )</td>
<td>Lantova et al. (2010)</td>
</tr>
</tbody>
</table>
alkalization in the anterior midgut of the mosquito, and V-ATPase may also play a role in the parasite invasion and the formation of the extracellular stages (Huang et al., 2006).

The development of As. taiwanensis is influenced by the larval age at the time of infection in a similar pattern as in As. culicis (Roychoudhury and Kobayashi, 2006; see Section 3.2). The development of the extracellular trophozoites is conditioned by their migration to the Malpighian tubules; the ones who fail to migrate undergo apoptosis (Chen et al., 2013). At the anterior end of the migrating parasite, a “protruding apparatus” with enhanced actin expression is formed (Chen and Fan-Chiang, 2001). The sexual reproduction of As. taiwanensis in the Malpighian tubules is synchronized with the host metamorphosis; increase of the level of the molting hormone 20-hydroxyecdysone signals migration of the parasite and expedites the formation of gametocytes (Chen, 1999; Chen and Yang, 1996). Gamonts are 234 μm long, gametocytes are 87.5 μm in diameter, and oocysts measure 10 × 5 μm (Lien and Levine, 1980).

The prevalence of this parasite was recorded to be up to 100% by various authors (Blackmore et al., 1995; Dos Passos and Tadei, 2008; Garcia et al., 1994; Munstermann and Wesson, 1990; Reyes-Villanueva et al., 2013). It was found to be the most common parasite of Ae. albopictus in Florida (Fukuda et al., 1997), and in New Orleans, the prevalence was shown to be seasonally heterogeneous (Comiskey et al., 1999a).

3.9. Ascogregarina geniculati

As. geniculati was found in Ae. geniculatus by Ganapati and Tate (1949) in England and by Kramar (1952) in the former Czechoslovakia. It was originally described as As. culicis; however, Munstermann and Levine (1983), who were studying this gregarine in Sardinia, determined it as As. geniculati. The main characteristic that can differentiate this species from other mosquito ascogregarines is the dimension of oocysts that measure 13.5 × 5 μm. Gamonts measure on average 175 × 31 μm, and gametocytes are 77 μm in diameter (Munstermann and Levine, 1983).

3.10. Ascogregarina polynesiensis

As. polynesiensis was found in Ae. polynesiensis in Samoa by Pillai et al. (1976) and was, similarly to As. geniculati, originally described as As. culicis. However, Levine (1985) pointed out that As. culicis differs from the gregarine from Ae. polynesiensis, and that Pillai et al. (1976) examined 325 Ae. aegypti (the natural host of As. culicis) and found only a single infected one, while the prevalence in Ae. polynesiensis was 39.5%. Therefore, this gregarine was renamed to As. polynesiensis (Levine, 1985). Trophozoites have a mean dimension of 65 × 35 μm, gametocytes measure approximately 40 μm, and oocysts measure 9.32 × 4.24 μm (Pillai et al., 1976).

3.11. Other Ascogregarina species

A gregarine similar to As. barretti was found in Indiana in Aedes hendersoni (Rowton et al., 1987); however, the differences in the number of gregarine stages, localization of the trophozoites within the larval gut and the presence of dead gamonts in cross-infections with As. barretti and Ae. triseriatus suggest that the gregarine from Ae. hendersoni is a new species, distinct from As. barretti. This was accepted by Chen (1999).

A gregarine described from O. j. japonicus in Japan by Roychoudhury et al. (2007b) was originally named as “Ascogregarina japonicus”; however, the same authors denominated it as Ascogregarina sp. from O. j. japonicus (Roychoudhury et al., 2007a). The only described stage of this species are the oocysts, which measure on average 10.7 × 5.15 μm (Roychoudhury et al., 2007a).

Other species of the genus Ascogregarina have been recorded from different mosquito species in the USA, Brazil, West Africa, China, Malaysia, Philippines, Italy or France (reviewed by Christophers, 1960; Clark, 1980).

Additionally to those parasitizing mosquitoes, there are three Ascogregarina species parasitizing hosts belonging to other orders or suborders. Ascogregarina cheopis Mourya, Geevarghese and Gokhale, 1996 was described from the flea (Siphonaptera: Pulicidae) Xenopsylla cheopis (Rothschild, 1903) in India by Mourya et al. (1996). Ascogregarina galliardi (Garnham, 1973) was described from the bat fly (Brachycera: Nycteribiidae) Nycteribia dentata Theodor, 1967 by Garnham (1973), and Purruni (1980) identified and described Ascogregarina brachyceri (Purruni, 1980) in the hump-backed fly (Brachycera: Phoridae) Megaselia subnitida Lundbeck, 1920 in India.

4. Genus Psychodiella: the life cycle and species overview

4.1. General life cycle

The life cycle of Ps. chagasi (Fig. 2), the type species of the genus Psychodiella, is used as a typical example. Details and interspecific differences are explained for each species separately (see Sections 4.2-4.7). Published measurements of oocysts of all Psychodiella species are summarized in Table 2.

The first instar larvae are infected by swallowing spindle-shaped oocysts. Eight sporozoites released from these oocysts reside in the larval midgut, attach to the epithelial cells and develop into trophozoites. Later, gamonts can be found mostly in the larval gut lumen, where the gregarines undergo sexual development from the formation of syzygy to the production of...
ocysts. In the adults, the gregarines are located in the body cavity forming syzygy and gametocysts with oocysts inside. The gametocysts attach to the accessory glands of females, and the oocysts are injected into their lumen. This unique mechanism of vertical transmission was recorded by several authors (Adler and Mayrink, 1961; Coelho and Falcao, 1964; Lantova and Volf, 2012; Lewis et al., 1970; Scorza and Carnevali, 1981) and supports the hypothesis about coevolution of gregarines and sand flies (Ostrovska et al., 1990). During oviposition, contents of the glands including the oocysts are attached to the chorion of eggs and serve as a source of infection for newly hatched larvae (Adler and Mayrink, 1961; Coelho and Falcao, 1964; Warburg and Ostrovska, 1991). This general life cycle is modified in Psychodiella mackiei (Shortt and Swaminath, 1927), where the development of the sporozoites and trophozoites in larvae is intracellular (Shortt and Swaminath, 1927; see Section 4.2).

The species of the genus Psychodiella are listed below according to the year of description.

4.2. Psychodiella mackiei

Ps. mackiei was described by Shortt and Swaminath (1927) from Phlebotomus argentipes Ann. and Brun., 1908 in India and later by Missirioli (1929, 1932) from Phlebotomus papatasi in Italy. It is the only Psychodiella species with known intracellular development. In the larvae, four gregarine stages can be found: ingested oocysts, sporozoites released from these oocysts, intracellular stages (all in the intestine) and adult gregarines in the intestine (often in the ectoperitrophic space) or the body cavity. The sporozoites (leaf-shaped, 4.8 µm long and 1.8 µm wide) are located mostly in the posterior part of the midgut. The authors postulate that most sporozoites do not survive defecation; therefore, only a certain number of them are able to invade the host epithelial cells. The intracellular gregarines (23.4 µm) are initially round and later become triangular with no special organ of attachment. The authors mention that the gregarine can complete its life cycle in the blood meal of the mosquito. The first oocysts are released in the body cavity and the recomposition of the gregarine occurs. The gregarine is able to complete its life cycle in the larva, and oocysts in their faeces are a source of horizontal transmission (Coelho and Falcao, 1964).

In pupae, only gamonts were found (Coelho and Falcao, 1964), and the relocation from the midgut to the body cavity occurs during pupation (Shortt and Swaminath, 1927; Warburg and Ostrovska, 1991). In males, Adler and Mayrink (1961) recorded only gamonts, while Coelho and Falcao (1964) found all developmental stages of Ps. chagasi (gamonts, syzygy, gametocysts and oocysts) in the body cavity and a few gamonts also in the intestine.

In females, Adler and Mayrink (1961) found syzygy, gametocysts and oocysts of Ps. chagasi 2–4 days post blood meal, while Warburg and Ostrovska (1991) found them 72 h post blood meal. The smallest gamonts are 30 µm in diameter (those over 60 µm have a lenticular-like projection), and adult round or oval gamonts which are ready to undergo syzygy measure 72–120 µm in diameter. Their nucleus (28 µm) has a distinctive nucleolus (8–10 µm) (Adler and Mayrink, 1961). According to various authors, gametocysts range from 72 to 150 µm in diameter (Adler and Mayrink, 1961; Lantova et al., 2010; Warburg and Ostrovska, 1991). According to Adler and Mayrink (1961), oocysts measure 10.9–11.9 × 5.8 µm. The cytoplasm of the developing oocyst contains amylopectin granules, and all the life cycle stages apart from the gametocysts have a diffuse actin-like protein in the cytoplasm suggesting the involvement of actin in the movement (Warburg and Ostrovska, 1991).

The gametocysts are attached to the accessory glands of females. Injection of the oocysts into the accessory glands is enhanced by plasmacyte humoral encapsulation; the encapsulated gametocysts and the internal pressure of the developing oocysts results in the rupture of the gametocysts at the site where they are attached to the accessory glands, releasing the oocysts into the gland lumen (Warburg and Ostrovska, 1989). The majority of the oocysts in the accessory glands are uninucleate, and the nuclear divisions occur after 24 h in a moist chamber (Adler and Mayrink, 1961). There are two modes of vertical transmission of this gregarine; the larvae ingest either the oocysts released from the accessory glands on the exochorion of eggs or the ones in the body cavity of dead sand flies.

The prevalence of Ps. chagasi in wild-caught L. longipalpis in Brazil was recorded by Adler and Mayrink (1961) as 10%, while the infection rate of laboratory-reared colonies varies between 86% and 100% (Dougherty and Ward, 1991; Warburg and Ostrovska, 1989; Wu and Tesh, 1989).

In Brazil, gregarines identified as Ps. chagasi were recorded from several sand fly species: Lutzomyia townsendi (Scorza and Carnevali, 1981), Lutzomyia evandroi (Brazil and Ryan, 1984), Lutzomyia sallesi (Coelho and Falcao, 1964), Lutzomyia sordelli (Oliveira et al., 1991 in Brazil et al., 2002) and Lutzomyia cruzi (Brazil et al., 2002).

4.3. Psychodiella chagasi

Ps. chagasi, originally named M. chagasi, was described from Lutzomyia longipalpis (Lutz and Neiva, 1912) females in Brazil by Adler and Mayrink (1961), who gave several morphological and life cycle features that differentiate this parasite from Ps. mackiei. In the 1st instar larvae, the sporozoites and young trophozoites are attached to the larval epithelium through an osmophilic contact zone; they are never located intracellularly (Warburg and Ostrovska, 1991). The sporozoites are surrounded by a two-layered pellicle with subpellicular microtubules and possess an apical complex with conoid. The oval gamonts (60–90 µm), on the other hand, have a three-layered pellicle forming longitudinal epicytic folds (Warburg and Ostrovska, 1991). They were found in the ectoperitrophic space of the intestine of the 3rd and 4th instar larvae by Warburg and Ostrovska (1991) and in the ectoperitrophic space of the 1st, 2nd and 3rd and in the intestinal lumen of the 4th instar larvae (Coelho and Falcao, 1964). The gregarine is able to complete its life cycle in the larva, and oocysts in their faeces are a source of horizontal transmission (Coelho and Falcao, 1964).

In pupae, only gamonts were found (Coelho and Falcao, 1964), and the relocation from the midgut to the body cavity occurs during pupation (Shortt and Swaminath, 1927; Warburg and Ostrovska, 1991). In males, Adler and Mayrink (1961) recorded only gamonts, while Coelho and Falcao (1964) found all the developmental stages of Ps. chagasi (gamonts, syzygy, gametocysts and oocysts) in the body cavity and a few gamonts also in the intestine.

In females, Adler and Mayrink (1961) found syzygy, gametocysts and oocysts of Ps. chagasi 2–4 days post blood meal, while Warburg and Ostrovska (1991) found them 72 h post blood meal. The smallest gamonts are 30 µm in diameter (those over 60 µm have a lenticular-like projection), and adult round or oval gamonts which are ready to undergo syzygy measure 72–120 µm in diameter. Their nucleus (28 µm) has a distinctive nucleolus (8–10 µm) (Adler and Mayrink, 1961). According to various authors, gametocysts range from 72 to 150 µm in diameter (Adler and Mayrink, 1961; Lantova et al., 2010; Warburg and Ostrovska, 1991). According to Adler and Mayrink (1961), oocysts measure 10.9–11.9 × 5.8 µm. The cytoplasm of the developing oocyst contains amylopectin granules, and all the life cycle stages apart from the gametocysts have a diffuse actin-like protein in the cytoplasm suggesting the involvement of actin in the movement (Warburg and Ostrovska, 1991).

The gametocysts are attached to the accessory glands of females. Injection of the oocysts into the accessory glands is enhanced by plasmacyte humoral encapsulation; the encapsulated gametocysts and the internal pressure of the developing oocysts results in the rupture of the gametocysts at the site where they are attached to the accessory glands, releasing the oocysts into the gland lumen (Warburg and Ostrovska, 1989). The majority of the oocysts in the accessory glands are uninucleate, and the nuclear divisions occur after 24 h in a moist chamber (Adler and Mayrink, 1961). There are two modes of vertical transmission of this gregarine; the larvae ingest either the oocysts released from the accessory glands on the exochorion of eggs or the ones in the body cavity of dead sand flies.

The prevalence of Ps. chagasi in wild-caught L. longipalpis in Brazil was recorded by Adler and Mayrink (1961) as 10%, while the infection rate of laboratory-reared colonies varies between 86% and 100% (Dougherty and Ward, 1991; Warburg and Ostrovska, 1989; Wu and Tesh, 1989).

In Brazil, gregarines identified as Ps. chagasi were recorded from several sand fly species: Lutzomyia townsendi (Scorza and Carnevali, 1981), Lutzomyia evandroi (Brazil and Ryan, 1984), Lutzomyia sallesi (Coelho and Falcao, 1964), Lutzomyia sordelli (Oliveira et al., 1991 in Brazil et al., 2002) and Lutzomyia cruzi (Brazil et al., 2002).

4.4. Psychodiella saraviae

Psychodiella saraviae (Ostrovska, Warburg and Montoya-Lerma, 1990) was described from females of Lutzymia lichi (Floh and Abonnenc, 1950) in Colombia (Ostrovska et al., 1990). Two or three gametocysts were attached to the accessory glands of each infected
female. Oocysts (12.4 × 5.8 μm) are located in the gametocytes, in the glands or on the egg surface. The differences in the size and shape of the oocysts (those of Ps. saraviae have thinner walls and narrower midsections than those of Ps. chagasi) give evidence that Ps. saraviae is a different species (Ostrovská et al., 1990). The prevalence of this parasite in Colombia was 1.9% to 3.3% (Ostrovská et al., 1990).

Oliveira et al. (1991) in Brazil et al. (2002) found a gregarine in Lutzomyia schreiberi, which they consider to be Ps. saraviae.

4.5. Psychodiella sergenti

Ps. sergenti was described from Phlebotomus sergenti Parrot, 1917 laboratory-reared colony originating from Turkey (Lantova et al., 2010). This gregarine was distinguished from Ps. chagasi and Ps. tobbi (see Section 4.6) due to the differences in their life cycles and the size and morphology of their life stages, due to their high host specificity in cross-infections (see Section 5) and due to the differences in the sequences of SSU rDNA. The prevalence of Ps. sergenti in wild-caught sand flies was 15.6% (Lantova et al., 2010), while in the colony, it reached 100% (Lantova, unpublished).

The life cycle of Ps. sergenti possesses a unique feature, similar to the one recorded in a single study of an undescribed gregarine parasite in Lutzomyia vexatrix occidentis by Ayala (1971) (see Section 4.7). In adult Ph. sergenti, the sexual development (formation of syzygies, gametocytes and oocysts) occurs exclusively in blood-fed females, while in males and unfed females, only gamonts were recorded (Lantova and Volf, 2012; Lantova et al., 2010). Contrastingy, e.g., Ps. chagasi (Coelho and Falcao, 1964) or Ps. tobbi (Lantova et al., 2010; see Section 4.6) develop into syzygies, gametocytes and oocysts also in males and unfed females. Ayala (1973) and Lantova and Volf (2012) hypothesized that the hormonal changes influenced by a blood meal intake trigger the sexual cycle of the gregarines in Ph. sergenti and L. v. occidentis, respectively.

Lantova et al. (2011) determined the number of oocysts commonly produced in a laboratory-reared colony of Ph. sergenti as 15,158 oocysts per one Ph. sergenti female. The oocysts are located on the chorion of eggs, in contact with the exochorion longitudinal sculpturing ridges, suggesting that the process of the attachment of the oocysts is connected to the formation of exochorion (Lantova and Volf, 2012). Sporozoites, located in the ectoperitrophic space of the 1st instar larval midgut, are never intracellular and have a three-layered pellicle, distinctive mucron, long conoid, numerous micronemes and a polar ring. In the 4th instar larvae, the gamonts (sometimes syzygies and gametocytes) are located either in the ectoperitrophic space (in younger larvae) or in the lumen of the intestine (in older larvae, after defecation prior to pupation). In blood-fed females, the gametocytes attach to the accessory glands, and the oocysts are injected into their lumen. In the males and females fed exclusively on sugar, only gamonts were found (Lantova and Volf, 2012). On average, oocysts measure 9.6 × 6.7 μm, gamonts measure 114.6 μm, and gametocytes are 128.2 μm in diameter (Lantova et al., 2010). The number of gregarine parasites able to develop in a sand fly is limited and not correlative to the infection dose; in the 4th instar larvae, a ten-time higher infection dose led to roughly 10 times more gamonts, while in adults, the intensity of infection achieved by a ten-time higher infection dose was only doubled (Lantova et al., 2011). The authors suggest that this decrease in gregarine numbers in adult sand flies shows that the pupal stage is the most critical period for the survival of Ps. sergenti.

4.6. Psychodiella tobbi

Ps. tobbi was described from Phlebotomus tobbi Adler, Theodor and Lourie, 1930 laboratory-reared colony originating from Turkey (Lantova et al., 2010). In the 4th instar larvae, the gamonts and gametocytes are mostly located in the ectoperitrophic space (in younger individuals) or the body cavity (in older individuals, shortly before pupation). In the adults, the gamonts and gametocytes occur in the body cavity, and the oocysts are located also in the accessory glands. The round or oval gametocytes measure on average 123.6 μm, gametocytes measure 137.2 μm, and broad spindle-shaped oocysts measure 9.6 × 7.5 μm. The prevalence in the wild-caught sand flies was 16.8% (Lantova et al., 2010).

4.7. Other Psychodiella species

A gregarine found in L. v. occidentis in California (Ayala, 1971) differs from Ps. chagasi in several morphological and life cycle characteristics suggesting that it is a new species; gamonts are pear-shaped (74–180 × 58–95 μm) or spherical (68–180 μm), with a light nucleus (24–26 μm) and nucleolus (8.5 μm), gametocytes are spherical (110–190 μm), and oocysts are spindle-shaped (10.5 × 6 μm) (Ayala, 1971).

A number of studies recorded unidentified gregarine species from Lutzomyia shannoni in Belize (Garnham and Lewis, 1959), Lutzomyia cruciata in Belize (Lewis, 1965), Lutzomyia flaviscutellata in Brazil (Lewis et al., 1970), six species of Lutzomyia sp. in Brazil (Mayrink et al., 1979), ten species of Phlebotomus sp. in Panama (McConnell and Correa, 1964) and Lutzomyia apache in Wyoming (Reeves et al., 2008).

5. Host specificity

The most studied mosquito gregarine in terms of its host specificity is As. taiwanensis. This parasite infected 100% of Ae. aegypti and Aedes taeniorhynchus larvae (by oocysts recovered from Ae. taeniorhynchus adults); however, it was not infective to Culex and Anopheles species (Garcia et al., 1994). Munstermann and Wesson (1990) found As. taiwanensis in Aedes eparctius and Culex restuans, and after experimental infections, oocysts were recovered from Ae. aegypti and Aedes atropalpus. Lien and Levine (1980) were also able to recover As. taiwanensis oocysts from its unnatural host, experimentally infected Ae. alcastis. Furthermore, this species infected sabethine mosquito Wyeomyia smithii, and although the infection rates were low, gametocytes were recovered from one female (Reeves and McCullough, 2002). A recent study also showed that As. taiwanensis completes the life cycle and remains infectious in O.j. japonicus (Erthal et al., 2012). The low host specificity of several other mosquito ascogregarines was shown too: As. barretti developed in Ae. geniculosus (Rowton and Munstermann, 1984) and Ae. hensondii (Copeland and Craig, 1992), and Spencer and Olson (1982) were able to infect Ae. eparctius with As. culicis.

Contrary to the above presented information, several authors showed that mosquito ascogregarines are fairly host specific. During cross-infections of Ae. sierrensis with As. culicis and Ae. aegypti with As. clarki, neither of the two gregarines produced oocysts in their unnatural hosts (Sanders and Poinar, 1973). Lien and Levine (1980) used oocysts of As. armigerei, As. culicis, As. lanuyensis and As. taiwanensis to infect Ae. aegypti, Ae. albopictus, Ae. alcastis and Ar. subalbatus. Even though the trophozoites of all ascogregarines were recorded in all but one mosquito species (with lower infection rates), the oocysts were, apart from their natural hosts, recovered only from Ae. alcastis for As. taiwanensis and As. armigerei.

In general, the host specificity studies of mosquito ascogregarines gave contradictory results; some authors found these parasite to be fairly host specific, while others did not, and this applies, in some cases when assessing a single species. For example, the results of Lien and Levine (1980) suggesting high host specificity of As. lanuyensis (see above) contradict those of Jacques and Beier (1982), who showed that this parasite completed...
its life cycle in ten experimentally infected mosquito species. In addition, the host specificity of \textit{As. geniculata} is questionable; the gregarine was able to develop oocysts in \textit{Ae. sierrensis}, \textit{Ae. aegypti} and \textit{Ae. triseriatus} (Munstermann and Levine, 1983), while, according to Kramar (1952), it could not infect \textit{Aedes communis}, \textit{Aedes cantans} and \textit{Culex pipiens}. It is appropriate to point out the wide host specificity across various host orders of the genus \textit{Ascogregarina}, as, apart from nematoceran mosquitoes, two species were described from brachyceran flies and one from fleas (see Section 3.11).

As the host specificity of some species of mosquito ascogregarines is unclear, methods that would differentiate between individual species were studied. Several morphological features, particularly the character of pigmentation and shape, distinguishing between gamonts of \textit{As. culicis} and \textit{As. taiwanensis} were found (Reyes-Villanueva et al., 2001). Oocysts of \textit{As. armigerei}, \textit{As. culicis}, \textit{As. taiwanensis} and \textit{Ascogregarina} sp. from \textit{O. j. japonicus} were compared under the scanning electron microscope, showing that they differ mainly in the length and structure of their surface (Roychoudhury et al., 2007a). \textit{As. barretti} and \textit{As. geniculata} were differentiated by isoenzyme electrophoresis (Rowton and Munstermann, 1984); different migration rates were observed for isocitrate dehydrogenase, lactate dehydrogenase and malate dehydrogenase, and the authors find this method reliable for distinguishing between the two ascogregarines. A species specific PCR method for \textit{As. culicis} and \textit{As. taiwanensis} based on amplification of ribosomal ITS1 and ITS2 regions was developed by Morales et al. (2005); the PCR products differed by at least 100 bp. In addition, the authors found a diagnostic PCR method for the presence of ascogregarines in mosquitoes.

In the sand fly gregarines, unlike for widely studied mosquito ascogregarines, there are only two studies evaluating host specificity of the genus \textit{Psychodidea} and both demonstrated it to be strict. Seven phlebotomine species were infected with oocysts of \textit{Ps. chagasi}: \textit{Ph. papatasi}, \textit{Ph. argentipes}, \textit{Phlebotomus perniciosus}, \textit{Lutzomyia serrana}, \textit{Lutzomyia abonnencii}, \textit{Lutzomyia columbiana} and \textit{L. longipalpis}. In the Old World sand flies, the trophozoites, and no other gregarine life stages, occurred only in the newly emerged adults of \textit{Ph. papatasi}. In four New World species studied, the trophozoites were found in the adults of \textit{L. columbiana}, \textit{L. serrana} and \textit{L. longipalpis}, while the oocysts were found only in the natural host \textit{L. longipalpis}. Furthermore, different colonies of \textit{L. longipalpis} varied in the susceptibility to \textit{Ps. chagasi} (Wu and Tesh, 1989). The authors argue that the gregarines from a number of New World sand flies identified as \textit{Ps. chagasi} (see Section 4.3) represent several new species.

Strict host specificity was revealed also for \textit{Ps. sergenti}, which was able to fully develop and complete its life cycle only in its natural host \textit{Ph. sergenti}. The oocysts were not recorded in experimentally infected \textit{Ph. papatasi} or \textit{Phlebotomus arabicus}, and they were found only in a single blood-fed \textit{Ph. tobbi} female out of 76 examined (Lantova et al., 2010). Similarly, \textit{Ps. tobbi} was rarely able to produce oocysts in \textit{Ph. perniciosus} females, and it did not complete its life cycle in \textit{Ph. sergenti} (Lantova et al., 2010).

When observing the dynamics of the infections of unnatural host species by either mosquito or sand fly gregarines, we can imply that the bottleneck for the gregarines is the pupal stage. For instance, Erthal et al. (2012) observed 95% of \textit{O. j. japonicus} larvae infected with \textit{As. taiwanensis}, while the prevalence in pupae and adults was only 39% and 30%, respectively. Similar situation in mosquitoes was recorded by Garcia et al. (1994). Lantova et al. (2010) found out that the prevalence of \textit{Psychodidea} gregarines in the larvae of their artificial hosts was roughly four times higher than in the adults.

### 6. Pathogenicity

One of the first records about mosquito ascogregarines being pathogenic brought Barrett (1968); \textit{Ae. aegypti} larvae and pupae infected with \textit{As. culicis} were stunted, and the authors noticed increased mortality. Sulaiman (1992) also observed increased larval mortality (proportional to the infection intensity) and shortened larval development; however, \textit{As. culicis} did not affect the larval development, size, mortality, pupal weight or adult emergence of \textit{Ae. aegypti} in a study of McCray et al. (1970). \textit{As. barretti} reduced the female pupal weight, prolonged the development of males (Beier, 1983) and increased the likelihood of pupal mortality (Siegel et al., 1992). Contrastingly, emergence success (Copeland and Craig, 1992; Walker et al., 1987) or larval developmental time (Walker et al., 1987) were not affected. In addition, Beier (1983) and Copeland and Craig (1992) did not record any increase in the larval mortality of \textit{As. barretti}-infected \textit{Ae. triseriatus}.

Unlike for the immature mosquito stages, ascogregarines do not seem to be very pathogenic to their adult hosts. \textit{As. barretti} does not alter the size of males and females (Walker et al., 1987) or their survival (Beier, 1983); however, the wing length of both sexes is reduced (Siegel et al., 1992). \textit{As. taiwanensis} has a little impact on adults of \textit{Ae. albopictus} (Garcia et al., 1994), and \textit{As. culicis}-infected \textit{Ae. aegypti} do not have decreased survival or fecundity (McCray et al., 1970). An interesting study of Reeves (2004) revealed that the rearing water from \textit{As. taiwanensis}-infected larvae of \textit{Ae. aegypti} is more acceptable for ovipositing female mosquitoes than the water from gregarine-free larvae.

Parasitism by \textit{Ascogregarina} affects the hosts to a larger degree when they are bred in nutrient-deficient conditions; the developmental time of mosquito females is prolonged, the mortality of larvae and blood-fed females is significantly increased, the size of females and males is significantly smaller and the females produce fewer eggs (Comiskey et al., 1999a, 1999b; Walker et al., 1987). On the other hand, the emergence rate, developmental time and the size of males are not affected (Walker et al., 1987). With regard to the crowding effect on the hosts, the sex specific pattern of mosquito reactions to the infection with \textit{Ascogregarina} was supported by Tseng (2004).

The effect of gregarines on mosquitoes can be substantially altered by coinfections of the hosts with other parasites. Fellous and Koella (2010) demonstrated that when \textit{Ae. aegypti} larvae are infected with high doses of \textit{As. culicis} and \textit{Vavraia culicis} (Fungi: Microsporidium) under low food availability conditions, the development of the host is delayed; however, the mortality of immature host stages is affected only mildly, and the wing length is not altered. Furthermore, Fellous and Koella (2009) brought evidence that the coinfection with two parasites does not affect only their host but also the ability of the parasites to produce infective stages, and that these effects are dependent on the infection doses of the parasites and on the food availability.

The level of pathogenicity of mosquito ascogregarines is not influenced only by the sex, coinfection or nutrients, but it can be significantly greater when the gregarine is introduced to an unnatural host. For example, \textit{As. taiwanensis} significantly increases the mortality of its unnatural host \textit{Ae. taeniorynchus}, while the mortality of \textit{Ae. albopictus} is not altered (Garcia et al., 1994). Similarly, \textit{As. barretti} decreases the larval survival, emergence success and female weight of unnatural \textit{Ae. hendersoni}, while the natural host \textit{Ae. triseriatus} is not influenced (Copeland and Craig, 1992). Furthermore, not the natural host \textit{Ae. aegypti} but unnatural host \textit{Ae. epacitus} is negatively affected by simultaneous \textit{As. culicis} infection and methoprene, and the mortality rates are significantly increased with increased methoprene concentrations (Spencer and Olson, 1982).
The pathogenicity of ascogregarines to their dipteran hosts is likely caused by the direct negative impact on the tissues where the parasites develop. The intestinal epithelial cells of the mosquito larvae, the site of infection of the intracellular sporozoites and trophozoites, have enlarged nuclei (Kramar, 1952), and some of them are destroyed by the gregarines (Kramar, 1952; Sanders and Poinar, 1973). The Malpighian tubules of adults, the site of infection of extracellular trophozoites and subsequent developmental stages, are dilated (Wenyon, 1911), and their cells are distorted and damaged (Barrett, 1968; McCray et al., 1970; Sanders and Poinar, 1973). The extent of the damage is proportional to the infection rate, and as few as eight to 25 gametocytes may destroy one third of a single Malpighian tubule (Barrett, 1968).

As cogregarines may play a role in the ability of mosquitoes to invade new areas. When Ae. aegypti and Ae. albopictus coexist in the same habitat, the latter tends to replace Ae. aegypti (Dos Passos and Tadei, 2008). Two complimentary facts connected to the infection with Ascogregarina could explain this phenomenon. (1) When a mosquito species is introduced to a new region, there is a period when it is not infected or infected with a low rate by cogregarines; e.g., Blackmore et al. (1995) showed that parasitism by As. taiwanensis in the newly introduced Ae. albopictus becomes higher only after three years. (2) Newly introduced invasive gregarine-free mosquitoes have competitive advantage over the naturally occurring mosquitoes compared to the invasive gregarine-infected ones; e.g., according to Aliabadi and Juliano (2002), invasive gregarine-free Ae. albopictus larvae reduced the survivorship of Ae. triseriatus, while gregarine-infected Ae. albopictus larvae did not. When considering both above mentioned points, the period of lower gregarine infection shortly after the introduction of a new mosquito species to the area may give it competitive advantage over the naturally occurring mosquito species; therefore enable the expansion of the invasive species.

The ability of Ascogregarina-infected mosquitoes to transmit parasites and viruses has been also studied. Mourya et al. (2003) showed that Chikungunya virus (Togaviridae) could be vertically transmitted to Ae. aegypti through As. culicis oocysts. On the other hand, Miller and DeFoliart (1979) did not demonstrate that concomitant infection of Ae. triseriatus with As. barretti and La Crosse virus (Bunyaviridae) increases the infection rate of the virus, or that As. barretti is a mechanism for the virus dispersal. Studies evaluating the effects of As. taiwanensis on the development of Dicrofilaria immitis (Nematoda: Spirurida) have come to contradictory conclusions. Comiskey et al. (1999b) found that in high nutrient conditions, D. immitis infective rate in Ae. albopictus females coinfected with As. taiwanensis was significantly higher than in the females infected only with D. immitis. This suggest that As. taiwanensis increases the vector competence of Ae. albopictus for filariae. Contrarily, Beier (1983) did not observe any significant differences in the number of infective D. immitis larvae between gregarine-infected and uninfected mosquitoes.

In contrast to the large number of studies evaluating the effects of ascogregarines on mosquitoes, there are only two works dealing with this topic in sand flies. Wu and Tesh (1989) evaluated the effect of Ps. chagasi on L. longipalpis. The parasite significantly decreased the survival of L. longipalpis females, and the difference was evident from day eight of the experiment. On the 25th day, the survivorship for the control females was 77.1% and for the gregarine-infected females only 49.5%. Ps. chagasi did not significantly affect the fecundity of its host (Wu and Tesh, 1989). Lantova et al. (2011) studied the pathogenic effect of Ps. sergenti on its natural host Ps. sergenti. The gregarine negatively affected the survival of immature sand fly stages, as fewer adults emerged from the infected eggs than did so from the control ones. This effect was even more pronounced under stressful conditions provided by rearing the infected larvae in higher density. The survival of infected sand fly males and females without a blood meal was also significantly decreased. Contrarily, Ps. sergenti had no effect on the mortality of blood-fed females or their fecundity; the infection did not affect the number of ovipositing females or the number of oviposited eggs.

As mentioned above, the intracellular development of Ascogregarina is probably the cause of pathogenicity in the mosquitoes. On the other hand, most Psychodiella gregarines do not develop intracellularly, with the exception of Ps. mackiei; therefore, Lantova et al. (2011) suggest that the main cause of higher mortality of pre-imaginal sand flies could be competition for nutrients and energy between the parasite and its host. Furthermore, the zero effect on blood-fed females indicates that the nutrition could have an impact on adult sand fly response to the Psychodiella infections; the blood meal (as a more nutritious diet) enables the infected females to overcome the negative effect of the parasite.

Several authors have discussed the usefulness of Psychodiella and Ascogregarina parasites in biological control; Barrett (1968) and Sulaiman (1992) consider them potentially useful, while Walker et al. (1987), Wu and Tesh (1989), Siegel et al. (1992) and Tseng (2007) do not. Experiments of Lantova et al. (2011) showed that Ps. sergenti is harmful to its host, and that the effects can be influenced by environmental factors. The authors point out that this effect is very important in the laboratory colonies, where the infection intensity is usually much higher than in natural conditions. However, the authors claim that the potential of Psychodiella gregarines for use in biological control is limited by their strict host specificity and the lack of knowledge about sand fly breeding sites.

7. Parasite-host coevolution

Certain characteristics of Psychodiella and Ascogregarina suggest close relationship of the gregarines with their hosts. In ascogregarines, these aspects were studied e.g., by Roychoudhury et al. (2007a) who showed that, considering the oocyst morphology and nucleotide alignment of SSU rDNA, As. taiwanensis, As. culicis and Ascogregarina sp. from O. j. japonicus are more similar than As. armigerei. The authors suggest that these specific relationships of ascogregarines could be attributed to the different taxonomic positions of their natural hosts. Similarly, in Psychodiella, Lantova et al. (2010) observed that Ps. sergenti and Ps. tobbi are, based on their morphology and SSU rDNA sequences, closer to each other than to Ps. chagasi, which corresponds to the origin of their hosts; Ps. chagasi parasites New World L. longipalpis, while the other two gregarines are found in Old World members of the genus Phlebotomus.

Another feature suggesting close relationship of ascogregarines and mosquitoes is the developmental synchrony of their life cycles. The stage transformation of As. taiwanensis is conditioned by Ae. albopictus metamorphosis and accelerated by a molting hormone 20-hydroxyecdysone (Chen and Yang, 1996). Furthermore, Chen et al. (2013) observed apoptosis of As. taiwanensis trophozoites that did not migrate from the intestine to the Malpighian tubules of Ae. albopictus, and it was speculated that the apoptosis could regulate the intensity of the parasite infection, maintaining the optimal survival rate of both the parasite and the host.

The hypothesis about the coevolution of Psychodiella gregarines and sand flies is supported by several unique features of Psychodiella life cycle. The location of the gregarines in the ectoperitrophic space of the larval intestine (Lantova and Volf, 2012) protects them from being defecated, helping to sustain a certain level of infection during puation. The injection of the oocysts into the accessory gland lumen facilitated by the host immune response (Warburg and Ostrov ska, 1989) is a unique mode of vertical transmission. The attachment of the oocysts to the egg surface is then related...
to the exochorion formation (Lantova and Volf, 2012), and it facilitates the vertical transmission of the parasite. Additionally, in Ps. sergenti, three interesting aspects should be highlighted. This gregarine does not develop sexually in males or unfed females, which is advantageous for the gregarines, as they only invest energy into the sexual development where the vertical transmission is expected – in blood-fed females (Lantova and Volf, 2012). Unfed females die later than males (Lantova et al., 2011), which gives the gregarine another advantage; the longer the female lives, the higher chance they have for a blood meal followed by the egg production and transmission of the parasite. Lastly, the gregarine does not affect the mortality or the fecundity of blood-fed females (Lantova et al., 2011), which gives the gregarines a bigger chance of being transmitted to the offspring.

8. Conclusions

The studies on mosquito and sand fly gregarines revealed very interesting facts considering their taxonomy, when originally one genus Ascogregarina was shown to contain actually two distinct genera. Moreover, the phylogenetic closeness of these gregarines to neogregarines (e.g., Carreno et al., 1999) raised the question about their classification into eugregarines and put in doubt general methods for defining new gregarine species. The pathogenicity but also the host specificity of Ascogregarina and Psychodiella gregarines are very important characteristics. Both these groups are considerably host specific, and their pathogenic effects on their hosts depend on various internal and external conditions. Therefore, although the authors generally lean towards them not being very useful in biological control, they need to be considered in the maintenance of mosquito and sand fly colonies, which is so important for successful research of human pathogens.

The host specificity, life cycle and molecular characteristics of Ascogregarina and Psychodiella support the hypothesis about long and strong coevolutionary association between these parasites and their insect hosts. Even thought they are probably not applicable in biological control, this coevolutionary relationship suggests that there could be an interesting and important connection between the hosts, their gregarine parasites and the human pathogens transmitted by the hosts. Therefore, we trust that the importance and remarkably interesting life features of these parasites will inspire scientists in further studies, bringing new information e.g., about the possible effect of gregarines on the vector competence of mosquitoes and sand flies.

Acknowledgments

We would like to thank Prof. Jiri Vavra and the reviewers for critical reading of the manuscript, Doc. Milena Svobodova and Doc. Jan Votypka for providing useful comments on the manuscript and Ema Klobusovska for assistance in the preparation of the artwork. Lucie Lantova was supported by research projects of the Charles University in Prague UČN 204013 and PRVOUK P2S/LF1/2. Petr Volf was supported by EU grant 2011-261504 EDENext, and the article is catalogued by the EDENext Steering Committee as EDENext 230.

References


Ganapati, P.N., Tate, W., 1949. On the gregarine Lankesteria culicis (Ross), 1898, from the mosquito Aedes (Finlaya) geniculatus (Olivier). Parasitology 39, 291–294.


j.meegid.2014.04.021


