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Review

Fungal and oomycete parasites of Chironomidae, Ceratopogonidae and Simuliidae (Culicomorpha, Diptera)



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ABSTRACT

Members of the families Chironomidae (chironomids or non-biting midges), Ceratopogonidae (ceratopogonids or biting midges) and Simuliidae (simuliids or blackflies) are ubiquitous dipterans of the infraorder Culicomorpha. They are extremely diversified in ecological strategies. Their larvae play major roles in aquatic food webs as detritivores or predators, whereas their adults can be general predators (Chironomidae), hemolympagous or hematophagous predators (Ceratopogonidae and Simuliidae) or pollinators. Both larval and adult stages are commonly infected by bacteria, viruses, protists, nematodes, true fungi and oomycetes. These phylogenetically diverse assemblages of microorganisms can simultaneously infect multiple species of chironomids, ceratopogonids and simuliids, and each host may become trophically interrelated with other hosts by sharing their parasites. Here, we review the information on fungal and oomycete parasites of these dipteran groups with special reference to the natural regulation of host populations, the impact of parasitism in food webs, and the potential of these parasites as biocontrol agents.

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1. Introduction

The infraorder Culicomorpha (Diptera) is an ecologically and morphologically rich assemblage of true flies, which appears as a well-supported clade in molecular phylogenies and contains eight phylogenetically related families

(Yeates *et al.*, 2007), including the Chironomidae (commonly called chironomids or non-biting midges), Ceratopogonidae (ceratopogonids or biting midges) and Simuliidae (simuliids or blackflies). The chironomids are represented by 339 genera and 4,147 species widely distributed in terrestrial, freshwater, brackish and marine habitats throughout the

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world, in which they are often dominant in abundance and richness, playing significant roles in nutrient cycling and energy flow (Cranston, 1995; Ferrington, 2008). The 103 genera and ca. of 6,000 species of ceratopogonids are found in a wide range of semi-aquatic (moist) and aquatic habitats (Wagner et al., 2008). Their larvae are mostly detritivores or predators, and adults are pollinators or hemolymphagous predators on other insects, whereas only four genera are hematophagous predators on vertebrates (Borkent and Spinelli, 2007; Wagner et al., 2008). The 72 genera of simuliids are represented by 2,142 species with a worldwide distribution in lotic habitats (Figueiró and Gil-Azevedo, 2010; Adler and Crosskey, 2013; Hernandez-Triana, 2013), where their larvae are filter feeders, scrapers and collector-gatherers (Figueiró and Gil-Azevedo, 2010; McCreadie et al., 2011), transferring soluble organic matter to higher trophic levels (Hernandez-Triana, 2013). Most female simuliids are hematophagous predators on humans and other vertebrates (Amaral-Calvão and Maia-Herzog, 2003; Figueiró and Gil-Azevedo, 2010; Hernandez-Triana, 2013).

Large populations of adults of these dipterans can simultaneously emerge under certain environmental conditions and cause nuisances in urbanized regions. For example, species of *Culicoides* (Ceratopogonidae) are hematophagous on humans, causing allergic reactions with high impact on ecotourism. They can also transmit a great number of protozoa and filarial nematodes to birds and mammals and have global importance as vectors of viruses to wild ruminants and livestock (Mellor et al., 2000; Borkent and Spinelli, 2007; Ansari et al., 2011). *Culicoides* biting midges are widely distributed throughout the world and are vectors of internationally important livestock viruses, including Bluetongue virus, African horse sickness virus, Akabane virus and Epizootic haemorrhagic disease virus (Mellor et al., 2000). Strong evidence also indicates that ceratopogonids of the genus *Forcipomyia* are vectors of *Leishmania* spp. (Dougall et al., 2011). Simuliids cause problems for tourism and agropecuary industry by transmitting the nematode *Onchocerca volvulus* that causes onchocerciasis, river blindness (Amaral-Calvão and Maia-Herzog, 2003; Figueiró and Gil-Azevedo, 2010). They also are ultimate hosts of *Leucocytozoon* spp., which parasitize wild and domesticated birds (Skovmand et al., 2007; Ortego and Cordero, 2009). Some reports suggest changes in the distribution and substantial increases in population sizes of chironomids and ceratopogonids with climate change (Wittmann and Baylis, 2000; Mika et al., 2008).

Eggs, larvae, pupae and adults of chironomids, ceratopogonids and simuliids can be parasitized by bacteria, viruses, protists, nematodes, true fungi and oomycetes (Wirth, 1977; Frana et al., 2001; Ansari et al., 2010a, 2011; Zídková et al., 2010; Campanini et al., 2012, Table 1). Therefore, we believe that their populations may be regulated by an assemblage of parasites that may act together rather than only one kind of microbial agent acting alone. In this manuscript, we review the information on fungal and oomycete parasites of the three dipteran families giving particular emphasis to the natural

regulation of host population sizes and the impact of these parasites in food webs.

2. Parasites vs pathogens and the concept of multiple pathosystems

The terms parasite and pathogen are often used interchangeably in disease ecology to describe organisms that live in or on and obtain resources from a host, usually to the host's detriment. However, a parasite can be defined as an organism living on or in, and obtaining its nutrients from another living organism. A pathogen is a parasite able to cause disease in a particular host or range of hosts under normal conditions of host resistance and rarely living in close association with their host without causing disease (Onstad et al., 2006; Kirk et al., 2008). For simplification, we will use the term parasite throughout this review.

As defined by Zadoks (1990), multiple pathosystems involve two or more pathogenic species coexisting in the same population of hosts and interacting to alter the population size and impact the general health of the hosts. Herein, we include under this concept a range of parasites infecting multiple hosts that inhabit similar ecological niches. Interactions among different hosts may take place by multiple infections with the same parasite species or with different parasite species from one or from diverse taxonomical groups. For instance, each host, primary or alternate, infected by a single or multiple species, can be interpreted as a subsystem which interacts with other subsystems (i.e. other infected hosts). Each host may, therefore, be interrelated by sharing their parasites via a reciprocal contagion. This concept provides a framework for understanding the complexity of host-parasite interactions from a micro to macro scale of analysis. In the case of chironomids, ceratopogonids and simuliids, multiple pathosystems involve multiple populations of these hosts in all stages of their life cycle, from eggs, larvae, pupae to adults, which can be potentially infected by multiple species of virus, bacteria, protists, insects, mermithid nematodes, fungi and oomycetes, and may transmit them to other hosts (Wirth, 1977; Frana et al., 2001; Ansari et al., 2010a, 2011; Zídková et al., 2010; Campanini et al., 2012).

3. Parasites of chironomids, ceratopogonids and simuliids

As previously stated, here we will only consider information on fungal and oomycete parasites which belong to the kingdoms Fungi and Straminipila, respectively. Although these groups are phylogenetically unrelated, they have adapted to similar habitats and niches and have traditionally been studied by mycologists. Several species within the Microsporidia, Blastocladiomycota, Entomophthoromycota and Ascomycota (Fungi) and some species within the Oomycota (Straminipila) are parasites of at least one life stage of chironomids, ceratopogonids and simuliids (Table 1).

Table 1 – Fungal and oomycete parasites of Chironomids, Ceratopogonids and Simuliids. The species of parasites are grouped according to the host family (Chironomidae, Ceratopogonidae and Simuliidae) and listed in alphabetical order within each phyla. A: adults, E: eggs, I: imagoes, L: larvae, P: pupae.

| Parasites | Host | Stage | Reference |
|---|--|-------|---|
| Chironomids (Chironomidae) | | | |
| Blastocladiomycota | | | |
| <i>Catenaria uncinata</i> W. Martin | <i>Chironomus attenuatus</i> | E | Martin, 1975a, 1978, 1981b, 1991 |
| <i>Ca. spinosa</i> W. Martin | <i>Glyptotendipes lobiferus</i> , <i>Endochironomus nigricans</i> , <i>Chironomus</i> sp. | | |
| <i>Coelomyces chironomii</i> Rasin | <i>Chironomus plumosus</i> | L | McCauley, 1976, Weiser, 1976, Whisler, 1985 Weiser and Vávra, 1964 |
| <i>Co. beirnei</i> Weiser and McCauley | <i>Chironomus parapluosus</i> <i>Tanytarsus</i> sp., <i>Cladotanytarsus</i> sp., <i>Psectrocladius</i> sp. | L | Weiser and McCauley, 1971 |
| <i>Co. tuzetiae</i> Manier, Rioux, F. Coste and Maurand | <i>Orthocladius</i> sp., <i>Cricotopus</i> sp. | L | Manier et al., 1970 |
| Oomycota | | | |
| <i>Aphanomyces sexualis</i> W. Martin | <i>Glyptotendipes lobiferus</i> | E | Martin, 1975b, 1977, 1981a,b, 1991, 2000 |
| <i>Couchia amphora</i> W. Martin | <i>Polypedium simulans</i> | E | |
| <i>C. circumplexa</i> W. Martin | <i>Endochironomus nigricans</i> , <i>Pentaneura carnea</i> , <i>Tendipes decorus</i> | | |
| <i>C. limnophila</i> W. Martin | <i>Glyptotendipes lobiferus</i> | | |
| <i>Crypticola clavulifera</i> Humber, Frances and A. W. Sweeney | <i>Chironomus tepperi</i> | L | Frances, 1991 |
| <i>Cr. entomophaga</i> (W. W. Martin) M. W. Dick | <i>Chironomus attenuatus</i> , <i>Endochironomus nigricans</i> , <i>Glyptotendipes lobiferus</i> | E | Martin, 1977, |
| <i>Lagenidium giganteum</i> Couch | <i>Chironomus tentans</i> | L | Nestrud and Anderson, 1994 |
| <i>Leptolegnia chapmanii</i> R.L. Seym. | Unidentified | L | López Lastra et al., 2004 |
| Ascomycota | | | |
| <i>Culicinomyces</i> sp. | <i>Chironomus</i> sp. | L | Sweeney, 1975 |
| <i>Cu. clavispurus</i> Couch, Rommey and B. Rao | <i>Chironomus</i> sp. | | Couch et al., 1974, Cooper, 1984 |
| | <i>Chironomus nubeculosus</i> | L | Unkles et al., 2004 |
| Entomophthoromycota | | | |
| <i>Entomophthora culicis</i> (A. Braun) Fresen. | <i>Chironomus decorus</i> | A | Kramer, 1982 |
| | <i>Cricotopus similis</i> | A | Kramer, 1983 |
| Microsporidia | | | |
| <i>Amblyospora</i> sp. | <i>Eukiefferiella</i> sp. | L | García and Lange, 1986 |
| Ceratopogonids (Ceratopogonidae) | | | |
| Oomycota | | | |
| <i>Lagenidium giganteum</i> Couch | <i>Culicoides molestus</i> | L | Wright and Easton, 1996 |
| | <i>Forcipomyia marksae</i> | L | Frances et al., 1989 |
| <i>Crypticola clavulifera</i> Humber, Frances and A. W. Sweeney | <i>Forcipomyia marksae</i> | L | Frances et al., 1989 |
| Ascomycota | | | |
| <i>Beauveria bassiana</i> (Bals.-Criv.) Vuill. | <i>Culicoides nubeculosus</i> | A/L | Ansari et al., 2010a, 2011 |
| <i>Culicinomyces clavispurus</i> Couch, Romney and B. Rao | <i>Bezzia</i> spp., <i>Culicoides nubeculosus</i> , <i>Dasyhelea</i> spp. | A/L | Sweeney, 1975, Knight, 1980, Unkles et al., 2004 |
| <i>Isaria fumosorosea</i> Wize | <i>Culicoides nubeculosus</i> | A/L | Ansari et al., 2010a, 2011 |
| <i>Lecanicillium longisporum</i> (Petch) Zare and Gams. | <i>Culicoides nubeculosus</i> | A | Ansari et al., 2010a, 2011 |
| <i>Metarhizium anisopliae</i> (Metschn.) Sorokin | <i>Culicoides nubeculosus</i> | A | Ansari et al., 2010a, 2011 |
| Microsporidia | | | |
| <i>Nosema</i> sp. | <i>Culicoides</i> spp. | L | Levchenko and Issi, 1973, Kline et al., 1985 |
| Simuliids (Simuliidae) | | | |
| Blastocladiomycota | | | |
| <i>Coelomyxidium</i> sp. | <i>Simulium pertinax</i> | L | Ginarte et al., 2003 |

(continued on next page)

Table 1 (continued)

| Parasites | Host | Stage | Reference |
|--|--|-------|---|
| <i>C. simulii</i> Debaisieux | <i>Cnephia</i> sp., <i>Odagmia</i> sp., <i>Simulium argyreatum</i> , <i>S. japonicum</i> , <i>S. nikkoense</i> , <i>S. notiale</i> , <i>S. tuberosum</i> , <i>S. vandalicum</i> , <i>S. venustum</i> , <i>S. vernum</i> , <i>S. vittatum</i> , <i>S. bonaerense</i> , <i>S. limay</i> , <i>S. rubiginosum</i> , <i>Gigantodax chilense</i> , <i>G. fulvescens</i> , <i>G. rufidulum</i> , <i>Cnesia dissimilis</i> | L/P/I | Levchenko et al., 1974, Garris and Noblet, 1975, Weiser, 1978, Yakushkina and Dubitskii, 1980, López Lastra and García, 1990, Adler et al., 1996, Adler et al., 2005, Kim, 2011 |
| Entomophthoromycota | | | |
| <i>Entomophthora culicis</i> (A. Braun) Fresen. | <i>Simulium venustum</i> , <i>S. vittatum</i> | A | Shemanchuk and Humber, 1978 |
| <i>E. simulii</i> S. Keller | <i>Simulium lineatum</i> | A | Keller, 2002 |
| <i>Erynia conica</i> (Nowak.) Remaudiere and Hennebert. | <i>Simulium</i> sp. | A | Hywel-Jones and Webster, 1986 |
| <i>E. curvispora</i> (Nowak.) Remaud. and Hennebert | <i>Simulium decorum</i> | A | Kramer, 1983 |
| Microsporidia | | | |
| <i>Amblyospora bracteata</i> (Strickland) J.J. García | <i>Odagmia ornata</i> , <i>Simulium corbis</i> , <i>S. equinum</i> , <i>S. latipes</i> , <i>S. venustum</i> , <i>S. vittatum</i> , <i>S. bonaerense</i> , <i>S. rubiginosum</i> , <i>S. wolffhuegeli</i> , <i>S. perflavum</i> , <i>S. huairayacu</i> , <i>S. stelliferum</i> , <i>S. dureti</i> , <i>S. limay</i> , <i>Simulium</i> sp., <i>Cnesia dissimilis</i> , <i>Gigantodax bonorinorum</i> , <i>G. chilense</i> , <i>G. rufescens</i> , <i>G. fulvescens</i> , <i>G. rufidulum</i> | L | García, 1992, Adler et al., 1996, 2000 |
| <i>Caudospora alaskensis</i> Jamnback | <i>Prosimulium alpestre</i> | L | Jamnback, 1970 |
| <i>C. brevicauda</i> Jamnback | <i>Cnephia mutata</i> | L | Ebsary and Bennett, 1975 |
| <i>C. nasiae</i> Jamnback | <i>Simulium adersi</i> | L | Jamnback, 1970 |
| <i>C. palustris</i> Adler, Becnel and Moser | <i>Cnephia ornithophilia</i> , <i>Stegopterna mutata</i> | L | Adler et al., 2000 |
| <i>C. pennsylvanica</i> Beaudoin and Wills | <i>Prosimulium magnum</i> | L | Beaudoin and Wills, 1965 |
| <i>C. simulii</i> Weiser | <i>Prosimulium fuscum</i> , <i>P. magnum</i> , <i>P. mixtum</i> , <i>P. multidentatum</i> , <i>Simulium latipes</i> | L | Ebsary and Bennett, 1975 |
| <i>Janacekia debaisieuxi</i> (Jírovec) J.I.R. Larsson | <i>Odagmia ornata</i> , <i>Simulium argyreatum</i> , <i>S. bezzi</i> , <i>S. conundrum</i> , <i>S. hematophilum</i> , <i>S. nikkoense</i> , <i>S. vandalicum</i> | L | Boemare and Maurand, 1976, Weiser and Žizka, 1975, Weiser, 1978, Adler et al., 1996, 2005 |
| <i>Helmichia simuliae</i> J.J. García | <i>Cnesia dissimilis</i> | L | García, 1990a |
| <i>Hyalinocysta expilatoria</i> Larsson | <i>Odagmia ornata</i> | L | Larsson, 1983 |
| <i>Nosema stricklandi</i> Jírovec | <i>Simulium ornatum</i> | L | Jírovec, 1943 |
| <i>Octospora simulii</i> Debaisieux | <i>Simulium</i> sp. | L | Debaisieux, 1926 |
| <i>Pegmatheca simulii</i> E.I. Hazard and Oldacre | <i>Simulium tuberosum</i> | L | Hazard and Oldacre, 1975 |
| <i>Pleistophora debaisieuxi</i> Jírovec | <i>Simulium vittatum</i> | A | Undeen, 1981 |
| <i>P. multispora</i> Debaisieux and Gastaldi | <i>Simulium argyreatum</i> , <i>S. ornatum</i> | L | Gassouma, 1972, Weiser, 1978 |
| <i>Polydispyrenia simulii</i> (M.L. Lutz and Splendore) E.U. Canning and E.I. Hazard | <i>Odagmia ornata</i> , <i>Simulium argyreatum</i> , <i>S. pertinax</i> , <i>S. taiwanicum</i> , <i>S. tuberosum</i> , <i>S. venustum</i> , <i>S. vittatum</i> , <i>S. bonaerense</i> , <i>S. delponteianum</i> , <i>S. rubiginosum</i> , <i>S. wolffhuegeli</i> , <i>S. perflavum</i> , <i>S. pertinax</i> , <i>S. limay</i> , <i>S. huairayacu</i> , <i>S. romanai</i> | L | Ebsary and Bennett, 1975, Gassouma, 1972, Weiser, 1978, Castello Branco, 1991, 1994, 1999, García, 1990b, Adler et al., 1996 |
| <i>Ringueletium pillosa</i> J.J. Garcia | <i>Gigantodax rufidulum</i> , <i>G. chilense</i> , <i>G. antarcticum</i> , <i>G. fulvescens</i> , <i>G. rufescens</i> | L | García, 1990c |
| <i>Spherospora andinae</i> J.J. Garcia | <i>Gigantodax rufidulum</i> , <i>G. chilense</i> | L | García, 1991 |
| <i>Thelohania fibrata</i> Strickland | <i>Simulium argyreatum</i> , <i>S. molestum</i> | L | Weiser, 1978, Adler et al., 2005 |
| <i>Weiseria laurenti</i> Doby and Saguez | <i>Prosimulium inflatum</i> | L | Doby and Saguez, 1964 |
| <i>W. sommermanae</i> Jamnback | <i>Gymnopais</i> sp., <i>Prosimulium inflatum</i> | L | Jamnback, 1970 |

Fungi

Microsporidia (Microsporids)

This phylum contains unicellular eukaryotes that are considered to have evolved within the fungi (Keeling *et al.*, 2000) and are obligate intracellular parasites of arthropods, including insects of the families Chironomidae, Ceratopogonidae, Simuliidae and Culicidae. They are typically chronic parasites, causing prolonged sublethal effects, but occasionally they can rapidly kill their hosts. Their life cycles may include single to multiple spore types, haplois, meiosis, sexual cycles and intermediate hosts (Solter and Becnel, 2007). Microsporids occur more frequently in larvae of simuliids (Table 1), and less frequently in adults (Undeen, 1981). Generally, their prevalence is low (McCreadie *et al.*, 2011) and do not cause a dramatic suppression of their host's population size (Castello Branco, 1999). Only two species have been reported infecting chironomid and ceratopogonid larvae (Table 1), which may cause epizooties under certain conditions (Solter and Becnel, 2007; McCreadie *et al.*, 2011).

Blastocladiomycota

A few members of this group have been reported as parasites of eggs and larvae of Chironomidae and Simuliidae in freshwater (Table 1) but to date no species have been found parasitizing Ceratopogonidae. Species in the genera *Catenaria* and *Coelomomyces* play important roles in the natural regulation of midge populations, showing high levels of infection and mortality of larvae and eggs (Weiser, 1976; Martin, 1981a). Species of *Coelomomyces* are obligate parasites of the larvae of chironomids and simuliids (Federici, 1981; Scholte *et al.*, 2004) (Table 1).

Entomophthoromycota

Almost all species within this phylum are parasitic on arthropods and actively discharge conidia that function as infecting propagules capable of adhering to and penetrating the host tissues (Eilenberg, 2002; Humber, 2012). Despite the great number of entomopathogenic species within this phylum, only a few species infect adults of chironomids, ceratopogonids and simuliids in terrestrial habitats (Table 1). In fresh and brackish waters, *Erynia* (= *Entomophthora*) *aquatica* (Entomophthorales) causes epizooties in larvae and pupae of mosquitoes such as *Aedes* spp. and *Culiseta moristans* (Anderson and Anagnostakis, 1980; Christie, 1996) but it is not known if this species is capable of causing epizooties in immature chironomids and ceratopogonids.

Ascomycota

A large number of entomopathogenic species are assigned to both anamorphic and teleomorphic phases within this phylum (Madelin, 1966, 1968; Campbell *et al.*, 2002; Unkles *et al.*, 2004; Wraight *et al.*, 2007). However, only a few species have been found infecting larvae and adult stages of chironomids and ceratopogonids and there are no records of these species infecting simuliids (Table 1). Strains of *Metarhizium anisopliae* and *Beauveria bassiana* have been used to control a wide range of terrestrial arthropods including pest of agro-forest crops and vectors of humans and animals (Butt *et al.*, 2001; Scholte *et al.*,

2004; Jackson *et al.*, 2010; Ansari *et al.*, 2010a,b, 2011; Medlock *et al.*, 2012). In the case of ceratopogonids, *M. anisopliae* has proved to be effective for killing larvae and adults of *Culicoides nubeculosus* (Ansari *et al.*, 2010a, 2011) while *Culicinomyces clavisporus*, a fungal pathogen of a wide range of mosquito species, proved to kill only *C. nubeculosus* larvae (Unkles *et al.*, 2004). Nevertheless, biological products (=bioinsecticides) are not yet commercially available for controlling these dipterans in field populations. However, *M. anisopliae* var. *anisopliae* strain F52 (Met52 bioinsecticide) is commercially available for the control of midges in agricultural crops.

Straminipila

Oomycota

Eight species in this phylum have been reported infecting eggs and larvae of chironomids and ceratopogonids but there are no reports of infection of simuliids to date (Table 1). In general, *Couchia* spp. and *Crypticola entomophaga* cause high levels of host infection while *Aphanomyces sexualis*, on the contrary, usually causes low levels of host infection (Martin, 1975a, b, 1981b, 1991, 2000). *Leptolegnia chapmanii* is highly virulent to larvae of *Aedes*, *Anopheles*, *Culex* and *Ochlerotatus* but results in low pathogenicity to chironomids (López Lastra *et al.*, 1999, 2004). *Lagenidium giganteum* is a facultative parasite of mosquito larvae which has been successfully used to control these insects (Kerwin *et al.*, 1994; Sur *et al.*, 2001; Vandergheynst *et al.*, 2007; Skovmand *et al.*, 2007) and also infects larvae of *Chironomus tentans*, *Culicoides molestus* and *Forcipomyia marksae* (Frances *et al.*, 1989; Nestrud and Anderson, 1994; Wright and Easton, 1996, Table 1).

4. Host population dynamics and roles of parasitism in food webs

Chironomids, ceratopogonids and simuliids play various roles in a wide array of aquatic and terrestrial ecosystems, either as a saprotrophs or parasites. As shown in Fig 1 section A, adult females of most ceratopogonids and some chironomids (species in the genera *Austrochlus* and *Archaeochlus*) are ectoparasites of other insects and vertebrates (Steffan, 1967; Tokeshi, 1995; Borkent and Spinelli, 2007; Azar and Nel, 2012). They suck hemolymph of both pest insects and their natural enemies, naturally controlling their population sizes or making them more vulnerable to diseases (Borkent and Spinelli, 2007). Species of the ceratopogonid *Forcipomyia*, for example, suck hemolymph from many different groups of arthropods that are common crop pests, such as caterpillars, larvae of sawflies, crane flies and spiders or natural enemies of crop pest's insects, such as lacewings (Borkent and Spinelli, 2007). Under certain conditions, ceratopogonid populations might decrease due to infection by fungal and oomycete parasites, and therefore populations of crop pest insects might increase directly (e.g. by decreasing ceratopogonid populations that feed on pest insects) or indirectly (e.g. by decreasing ceratopogonid populations which feed on natural enemies of insect pests).

Adults of many species of ceratopogonids (especially from the genus *Forcipomyia*) and some chironomids can also be

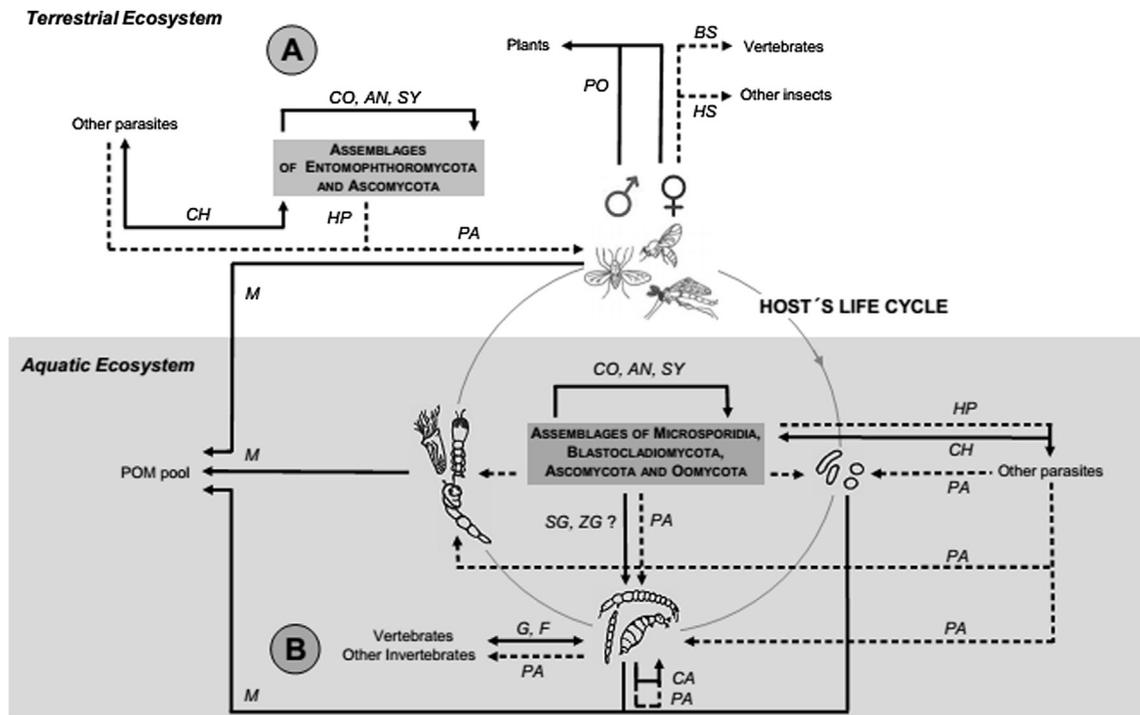


Fig 1 – Trophic links of a generalized terrestrial (section A) and aquatic (section B) food web in which fungal and oomycete parasites of chironomids, ceratopogonids and simuliids are involved. Definition of symbols: AN: antagonism in the host, BS: blood sucking, CA: cannibalism, CH: competition in the host, CO: coexistence in the host, F: feeding, G: grazing, HP: hyperparasitism, HS: hemolymph sucking, M: mortality of hosts, PA: parasitism, PO: pollination, POM: particulate organic matter, SG: spore grazing, SY: synergism in the host, ZG: zoospore grazing. Links involving parasitism are indicated in (–) and saprotrophism are indicated in (—). (?) Indicates potential links in which fungi and oomycete parasites of chironomids, ceratopogonids and simuliids might be involved.

important pollinators of economically important crops, such as cocoa tree, *Theobroma cacao* (Winder, 1978, Fig 1 section A). On the other hand, their larvae are detritivores, grazers of algae (Botts, 1993) and predators of their own larvae (cannibalism; Szadziewski et al., 1997) or of other aquatic insects (Fig 1, section B). In addition, larvae of some chironomid species are known as ectoparasites of other invertebrates, such as bivalves and larvae and pupae of mayflies (Claassen, 1922; Gordon et al., 1978, Fig 1, section B). Immature stages also provide food resource for other invertebrates, fishes and birds (Werner and Pont, 2003; Sánchez et al., 2006; Fagundes et al., 2007), while cadavers contribute to the pool of particulate organic matter in aquatic ecosystems (Fig 1, section B).

Population dynamics of these dipterans can be naturally influenced by (i) physical and chemical factors (e.g. dissolved oxygen concentrations, water velocity, temperature, pH, solid material in suspension, phosphorus, sulfate, calcium and ferrous ions, and electrical conductivity) (Ah et al., 2002; Woodcock et al., 2005; Siqueira et al., 2008; Luoto, 2011); (ii) biotic factors (population density, food supply, competitors, predators, and parasites) (Kim and Merritt, 1987; Crosskey, 1990; Werner and Pont, 2003) and, (iii) genetic factors (intensity of virulence and parasitism and susceptibility of hosts) (Thomas and Blanford, 2003).

Since these groups of dipterans share similar habitats and ecological niches, we hypothesize that they might be affected by common parasites from phylogenetically unrelated groups, such as fungi and oomycetes (Fig 1). Many species of fungi and oomycetes are parasites of these dipterans (Table 1), spores or zoospores being the infective unit. On the other hand, zoospores of Chytridiomycota are efficiently grazed by invertebrates, such as *Daphnia*, and are excellent food resources for their growth (Kagami et al., 2007a,b). Larvae of the ceratopogonid *C. nubeculosus*, can be infected after ingestion of conidiospores of *C. clavisporus*. The conidiospores germinate in the gut larvae, penetrate the gut cuticle, extensively produce hyphal growth throughout the hemocoel (eventually leading to death) and finally emerge through the external cuticle, releasing conidiospores that frequently infect other larvae (Unkles et al., 2004). It is not known if nonmotile spores and zoospores of fungal and oomycete parasites can be used as food resources by larvae of these dipterans (Fig 1, section B).

Members of the Phylum Entomophthoromycota usually infect adults of these dipterans in terrestrial habitats while Microsporidia, Blastocladiomycota and Oomycota are restricted to immature stages in aquatic or semi-aquatic habitats. Species of Ascomycota can parasitize the three

dipteran groups in all stages of the life cycle (Table 1 and Fig 1).

The impact of fungi and oomycetes on the population dynamics of mosquitoes has been extensively investigated (Guzman and Axtell, 1987; Washburn et al., 1988; Scholte et al., 2004, 2005; Andreadis, 2007; Magori and Drake, 2013). Even though mosquitoes, chironomids, ceratopogonids and simuliids share similar habitats and niches and belong to the same infraorder (Culicomorpha), there is no detailed information available on their direct impact on the populations of the last three dipteran groups.

As shown in Table 1, a single host species can be infected by many species of fungi and oomycetes. In this case, infection by multiple parasitic species in a single host population can potentially influence the colonization of host tissues and subsequent fitness of one another, either directly (e.g., through competition or metabolic products) or indirectly by affecting other aspects of the host health such as the immune system (synergism) (Fig 1). These interactions can therefore highly increase the complexity of food webs through many direct (e.g. mortality of hosts) and indirect effects (e.g. competition between zoospores while locating a suitable host, delay in the development of the larval stage).

Abiotic factors add complexity to the system by regulating both parasite and host physiology and population sizes, increasing or decreasing the virulence and the susceptibility of the host to infection. Martin (1984) observed that zoosporic fungi and oomycetes, particularly species of *Catenaria*, will affect the size of chironomid populations depending on temperature fluctuations, rainfall, light and velocity of running water. In the case of *Coelomomyces* spp., regulation of the host populations is even more complex because primary and alternate hosts are involved (Apperson et al., 1992). For example, *C. chironomi* causes epizootics and persists in chironomid larval populations over several years, resulting in high prevalence and mortality rates (Weiser, 1976; Apperson et al., 1992).

Most of the interactions at different trophic levels in which fungal and oomycete parasites are involved are currently unknown or still remain poorly understood. The understanding of how each pathosystem interacts with other pathosystems and influence each trophic level is required to elucidate these pathways of energy fluxes in food webs.

5. Conclusions and future considerations

The information presented in this review shows that chironomids, ceratopogonids and simuliids are common hosts for a diverse assemblage of fungi and oomycetes. Multiple parasites can infect one individual or many individual hosts within a population. Each individual host infected by a diverse assemblage of these microbes can be interpreted as a multiple pathosystem in which the host not only interacts with its parasites but also with other hosts. In addition, multiple parasites frequently encountered in the same host might interact directly through interspecific relationships (e.g. competition for nutrients and space, by producing antagonistic compounds or direct physical interference) or indirectly by influencing the host immune system and making it either less or more susceptible to other

parasites. Synergism between several parasites might also occur, resulting in increased host susceptibility. However, whether these interactions are antagonistic, synergistic or neutral remains poorly understood.

Chironomids, ceratopogonids and simuliids are among the most difficult insects to control by the use of chemicals. Consequently, biological control is a promising alternative. Generally, only a single pathogen or parasite is considered as microbial control agent (MCA). Theoretically, the association of two or more MCAs could provide the best results in terms of controlling populations of these dipterans, especially if microbial assemblages can be applied to control their different stages of life cycle (eggs, larvae, pupae and adults). For example, the combined application of entomopathogenic nematodes and fungi proved to be the most effective strategy to combat target insects in integrated pest management systems (Ansari et al., 2004, 2006, 2008, 2010b, Ansari and Butt, 2013). We believe that a deeper understanding of the functioning of multiple pathosystems will be of great relevance to advancing the development of multisystem biocontrol technologies, which in some instances, could completely eliminate or at least substantially reduce the necessity of chemical pesticides. One aspect largely neglected is the impact of parasites of chironomids, ceratopogonids and simuliids on non-target organisms. A few laboratory-based studies have shown that *L. giganteum*, a non-specific parasite commonly used to control mosquitoes in the field (Vandergheynst et al., 2007; Skovmand et al., 2007), is capable of causing high infectivity in non-target organisms such as other dipterans (e.g. Chironomidae), cladocerans and fishes, but only at high zoospore concentrations (Nestrud and Anderson, 1994). This zoosporic parasite has not been yet tested for the control of other dipterans. Similar results were observed for *B. bassiana* and *M. anisopliae* with shrimps and fishes (Genthner et al., 1994, 1998; Middaugh and Genthner, 1994; Genthner and Middaugh, 1995). Nevertheless, the ecological impact and consequences of using fungal and/or oomycete parasites as biocontrol agents in food webs is in most cases unknown. This is particularly relevant in the case of introducing non-native, non-host specific fungi and oomycetes as biocontrol agents into novel ecosystems where they have never been present.

Clearly, the complex interactions that can take place between target organisms and MCAs need to be considered during the development of commercial products. Also, the impact of the application of these microorganisms on non-target organisms requires further and more comprehensive investigations. We hope that this review will stimulate further research in this field in order to gain a better understanding of the impact of these parasites within food webs leading to improved strategies for the biological control of these dipterans.

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