

# The aphelids, intracellular parasitoids of algae, consume the host cytoplasm “from the inside”

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## Summary

In this discussion, we are clarifying the interface between the aphelid trophont and the host in different life cycle stages from the invasion until zoospore maturation. As a result, we consider that the aphelid trophont does not consume host cytoplasm from outside, as it has been suggested earlier, but from inside, i.e., in the same manner as *Rozella*.

**Key words:** Apicomplexa, Microsporidia, parasitoid/host interface, rozellids, ultrastructure

## Introduction

The aphelids and rozellids (genus *Rozella*) belong to the superphylum Opisthosporidia and represent the parasitoids of algae and zoosporic fungi with a phagotrophic intracellular stage (Karpov et al., 2014a). They both have a life cycle somewhat similar to that of chytrids, but after zoospore encystment on the host surface they form a penetration tube into the host. Then, the growing vacuole in the cyst appears and pushes out the parasitoid through the penetration tube into the host where its trophont consumes the host cytoplasm by phagocytosis, like amoeba, grows and finally replaces the host cell totally becoming the multinuclear plasmodium. Plasmodium then divides into uniflagellate zoospores, which release the host envelope.

Previous investigations of phagocytic process have shown the differences between rozellids and aphelids: “In contrast to *Rozella*, however, the unwalled protoplasts of aphelids do not directly enter into the host cytoplasm. With aphelids, the mode of infection only requires penetration of the host cell wall. As a consequence, the aphelid endoparasite does not have to cross the host plasma membrane (PM) in the infection process (Karpov et al., 2014a, 2014b) as does *Rozella*. Whereas *Rozella* consumes its host cytoplasm from the inside (Powell et al., 2017), aphelids consume host cytoplasm from the outside (Karpov et al., 2014a, 2014b)” – page 115 in Powell and Letcher (2019). This statement has been used recently in the review by Timofeev et al. (2020) to illustrate differences between the aphelids and rozellids.

Here, we would like to comment on the interface of rozellid and aphelid trophonts in their hosts and clarify some points based on a deeper analysis of trophont's behaviour in aphelids.

### Remarks on the *Rozella* trophont/host interface

Excellent comparative ultrastructural studies of trophont/host interplay of *Rozella allomycis* (Held 1973; Powell et al., 2017; Powell and Letcher, 2019), *R. polyphagi* (Powell, 1984), and *R. rhizoclosmatii* (Letcher et al., 2017), which were summarized in the recent paper by M. Powell and P. Letcher (2019), revealed a variation in the number of membranes interfacing the parasitoid and host cytoplasm. The trophont within the host is always delimited by its own PM, but the number of the host membranes around the parasitoid trophic stage varies from 0 to 2.

According to A. Held (1973), at the early stages after penetration, the trophont of *R. allomycis* delimited by the host membrane, but in the later stages this host membrane is absent. The chytrid host of *R. polyphagi* (indefinite stage of development) does not produce a membrane around the parasitoid, which is surrounded by scattered patches of the host smooth and rough endoplasmic reticulum (Powell, 1984), probably, remnants of the former membrane envelope.

The interface between *R. allomycis* and its blastoclad host and *R. rhizoclosmatii* and its chytrid host consisted of three membranes: one belongs to the parasitoid and two represent the smooth ER of the host (Letcher et al., 2017b; Powell et al., 2017).

M. Powell and P. Letcher (2019) assume: “If indeed the surrounding host PM breaks down as the parasite fills the compartment, it is possible that the one-membrane stage is a transition stage between the two and three-membrane interface stages we observed”. Then the host envelopes a parasite consecutively with one and two endomembranes (Powell and Letcher 2019).

To check this point, we referred to the well-studied intracellular parasites of other eukaryotes.

In microsporidia, the closest relatives of *Rozella*, the discharged (everted) polar tube of microsporidian spores interacts with the host cell forming an invagination in the host PM, the so-called “invasion synapse”. In the invasion synapse, either the penetration tube may inject the sporoplasm into the host cell through the host PM with its local destruction, or the sporoplasm itself may interact with the host PM initiating the phagocytosis. The

first process results in that the microsporidian parasite initially lies directly within the host cell cytoplasm, the second one – in a phagosome vacuole derived from the host PM (Cali et al., 2017; Han et al., 2020). Afterwards, microsporidia develop in the host cell in direct contact with the host cell cytoplasm or isolated by membranes which may be produced by either a parasite, or the host, or both, the parasite and the host. Such differences of the parasite development in the host cell may appear even in closely related microsporidia (Cali et al., 2017; Sokolova et al., 2013, 2014). Thus, the number and the origin of membranes of the interface between microsporidia and their host vary essentially even in closely related species, like in rozellids.

Invasion stages of Apicomplexa enter the host cell by producing the invagination of the host PM, e.g. *Plasmodium* in red blood cells, *Toxoplasma* in different cells of warm-blooded vertebrates (Frenal et al., 2017; Bisio and Soldati-Favre, 2019; Burrell et al., 2020). The process of host cell invasion by apicomplexans is very fast, it may take 5–10 seconds for sporozoites (Nichols and O'Connor, 1981; Chobotar et al., 1993). In some cases, interruption of the host PM as a result of rapid invasion (e.g., *Eimeria* spp.) with the subsequent formation of a new parasitophorous vacuole membrane around the parasite is also possible (Scholtysek, 1975, 1979; Burrell et al., 2020).

Thus, a temporary and local break of the host PM during parasite penetration occurs in different intracellular parasites, including close relatives of the rozellids. During this very short time the break can be repaired by the new membrane of parasitophorous vacuole originated either from the parasite (*Toxoplasma gondii*), or from the host endomembranes (*Rozella allomycis*), or both (microsporidians).

In the case of *Rozella*, break of the host PM was not shown; so, we can propose two ways: 1) host PM invagination around the parasitoid and its replacement with an endomembrane, which can be broken at later stages (*R. allomycis* – Held, 1973, Powell and Letcher, 2019; *R. rhizoclosmatii* – Letcher et al., 2017b; *R. polyphagi* – Powell, 1984), or 2) replacement with two membranes of ER cisternae (*R. allomycis* – Powell et al., 2017; Powell and Letcher, 2019).

### Aphelid trophont/host interface

In the first EM studies of the aphelids, the early stages of their penetration into the host have been

shown (Gromov and Mamkaeva, 1970a (*Amoebophilidium chlorellavorum*); 1970b (*Am. protococcarum*); 1975 (*Aphelidium chlorococcalium*); Schnepf et al., 1971 (*Aphelidium* sp.)). In all cases, the newly entered amoeba was delimited by its own PM and the host membrane (Schnepf et al., 1971). It is not clear if the host membrane was a PM or a newly built endomembrane. At the later trophic stages including a multinuclear plasmodium, the two membrane interface retains (Schweikert and Schnepf, 1997 (*Pseudaphelidium drebesi*)) but in some parts of plasmodium the host membrane disintegrates (Schnepf et al., 1971).

The trophic stages of the aphelids were studied in details for *Aph. melosirae* (Karpov et al., 2014b), *Paraphelidium tribonematis* (Karpov et al., 2017), and *Aph. insulamus* (Karpov et al., 2020). In general, all these data confirm the statement: the trophic stage of parasitoids is surrounded by a host PM starting from the penetration.

Here we illustrate the aphelid/alga interface at different trophic stages of the parasitoid *P. tribonematis* (Fig. 1).

At some sections, this host PM is visible at the parasitoid posterior and both sides, but seems to be absent in the frontal zone (Fig. 1, A). A similar image was published by Schnepf et al. (1971 – Fig. 5). Obviously, these two images fixed a moment of parasitoid shoot from the cyst into the host: the parasitoid is on the way from the cyst as a part of its cytoplasm is still in the penetration tube, while the main cyst contents are already in the host center. Thus, we can suggest, that the fast trophont movement can break the host PM, which then can be repaired or replaced by endomembranes of the host. At the Fig. 1 (A) the smooth ER cisterna is present in the vicinity of the parasitoid anterior, and can be, probably, a source for reparation the break in the host PM.

Young and developing trophonts (Fig. 1, B, C) have two membrane interface, i.e., the trophont having its own PM lies in the parasitophorous vacuole produced by the host. It is clearly visible during food vacuole formation (Fig. 1, C): the cytoplasm of aphelid pseudopodium is separated from the host cytoplasm by two endomembranes. The two membrane interface retains in the phagotrophic vacuole of *Paraphelidium* (Fig. 1, C, D) and of the other aphelids as well (Karpov et al., 2014b). Thus, the aphelid parasitoid consumes the host cytoplasm and its organelles being limited by its own PM and by the membrane of parasitophorous vacuole, i.e. “from inside” the host.

At the plasmodial stage, the parasitoid can be still delimited with the host endomembrane of parasitophorous vacuole and with remnants of host cytoplasm (Fig. 1, D). When the plasmodium totally replaces the host cytoplasm, it lies freely in an amorphous matrix, which is still delimited by the host PM (Fig. 1, E). The host PM will be broken later, after the formation of zoospores and their movement inside the host cell wall.

These observations totally confirm and add the previous results revealed by Gromov and Mamkaeva (1970a, 1970b; 1975) and Schnepf et al. (1971). To outline the whole process of aphelid penetration and its persistence in the host in regards of parasitoid/host membrane interface we present here a scheme of consecutive stages of parasitoid development (Fig. 2).

In conclusion, we have to consider, that the aphelids and rozellids have the same mode of penetration the host and a similar trophont development in the host in regards of their interface. An obvious difference between these two phagotrophic parasitoids is: *Rozella* trophont contains degraded mitochondria receiving ATP from the host mitochondria (like microsporidians), while the aphelid trophont has normal mitochondria at all stages of life cycle. It supports the idea, that the aphelids are less specialized parasitoids, than the rozellids, what has been shown also by comparison of their metabolic ways (Torruella et al., 2018).

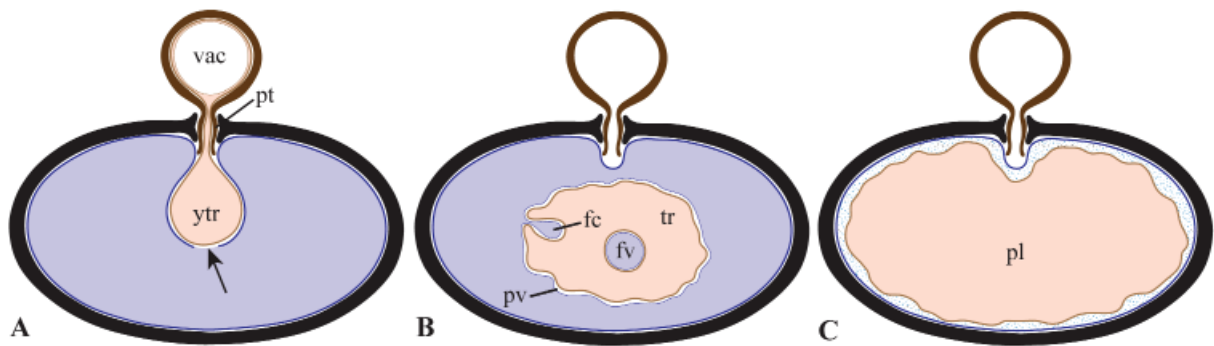
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**Fig. 1.** The interface between different trophic stages of *Paraphelidium* and its host *Tribonema*. A – First seconds after host penetration, when the parasitoid breaks the host PM by its fast movement in the host cytoplasm; B – young trophont in the center of the host cell: two membrane interface; C – food vacuole formation in a developing trophont around the host chloroplast: two membrane interface is clearly visible (*arrows, arrowheads*); D – plasmodial stage of the parasitoid: endomembranes of the host (*him*) and the PM (*arrowheads*) of parasitoid have the same thickness and much thinner than the host PM (*hpm*); E – parasitoid plasmodium lies freely in an amorphous matrix (*am*) of the former host cell delimited by the host PM (*hpm*), *arrowheads* show the parasitoid PM, *arrows* – the host endomembrane at all images. *Abbreviations:* *am* – amorphous matrix, *ch* – chloroplast, *cy* – cyst with parasitoid cytoplasm remnants, *er* – endoplasmic reticulum of the host, *h* – host, *hm* – host mitochondrion, *hpm* – host PM, *p* – parasitoid, *pn* – parasitoid nucleus, *pt* – penetration tube, *w* – host cell wall. Scale bars: A – 1  $\mu\text{m}$ , B-E – 400 nm, D – 200 nm.



**Fig. 2.** Interpretation of parasitoid/host interface during invasion and development of *Aphelidium* in green alga. Host: cell wall is black, cytoplasm is blue, PM is dark blue, amorphous matrix is blue dot pattern. Parasitoid: cyst wall is brown, cytoplasm is pink, PM is dark brown. A – First seconds after invasion of young trophont (ytr) through the penetration tube (pt); invaginated host PM is locally broken (arrow); vac – vacuole; B – amoeboid trophont (tr) in the parasitophorous vacuole (pv) phagocytizes the host cytoplasm forming food cap (fc) and food vacuole (fv); C – multinuclear plasmodium (pl), which totally replaced the host cytoplasm, lies in amorphous matrix surrounded by host PM. Not to scale.

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