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Zoology: Invertebrates that Parasitize Invertebrates

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The genome of an orthonectid, a group of highly modified parasitic invertebrates, is drastically reduced and compact, yet it shows the bilaterian gene toolkit. Phylogenetic analyses place the enigmatic orthonectids within Spiralia, although their exact placement remains uncertain.

“The most interesting question to be solved in the history of our parasites is whether these animals have remained normally at the planula-stage, or have retrograded to this primitive stage [...] in consequence of parasitism” A. Giard, 1878 [1]

Invertebrate parasites are fascinating creatures that have inspired many of the most morbid characters in countless science fiction movies. Their life cycles can be extremely complicated, and adults and immature individuals can look radically different, especially when transitioning from free-living aquatic environments to intermediate or definitive hosts. When infecting vertebrates, such invertebrate parasites are often studied due to their potential economic and health impacts. However, when the invertebrates parasitize other invertebrates, they are often neglected. A report in this issue of *Current Biology* of the first genome of *Intoshia linei* [2], an orthonectid parasitizing ribbon worms in the genus *Lineus*, may be able to change our views on the

evolution of these extreme parasites of invertebrates.

Orthonectids, with about 21 known species [3], live inside other invertebrates for most of their life cycle (Figure 1). Their simple morphology led earlier workers to think that they were closely related to ciliated protists or that they represented an intermediate step between protozoans and true animals or metazoans, hence their old name Mesozoa. They are rare parasites of a few marine animals, including acoels, brittle stars, molluscs, flatworms, ribbon worms and ascidians [4]. Orthonectids spend most of their lives as a trophic multinucleated plasmodial stage (a plasmodium is a cytoplasm that contains many nuclei, rather than being divided into individual cells each with a single nucleus) in their unfortunate hosts' tissues. The life cycle of only a few species has been well characterized [5,6]. A few hypertrophied cells develop from the plasmodia into small ciliated worm-like male and female adults (Figure 1A) that escape the host and swim free (Figure 1B). While the name Orthonectida may suggest a straightforward swimming behavior [3], they can exhibit complex movements,

including spinning and bending [2]. These ephemeral adults lack digestive, circulatory or excretory systems, but are full of gametes surrounded by contractile cells of smooth muscle and a layer of ciliated cells. For the longest time, it was thought that they lacked a nervous system, until a recent study described a reduced nervous system using immunohistochemistry [7], which also helped to better characterize its muscular system. Yet both these systems are extremely simple: the whole muscular system consists of four longitudinal and 9–11 circular muscle cells, and its nervous system consists of six serotonin-like cells, and a total of 10–12 cells [7], although there is some variation [8].

When the ca. 1 mm long swimming female meets with a dwarf male in the water, they copulate briefly by uniting their genital pores. The fertilized eggs then develop into minute larvae that exit the female via the genital pore in search of a new victim. Once inside the host, the larva disintegrates, losing the ciliated cells first, and the inner cells scatter within the host's tissues to become new infective cells known as ‘agametes’ to

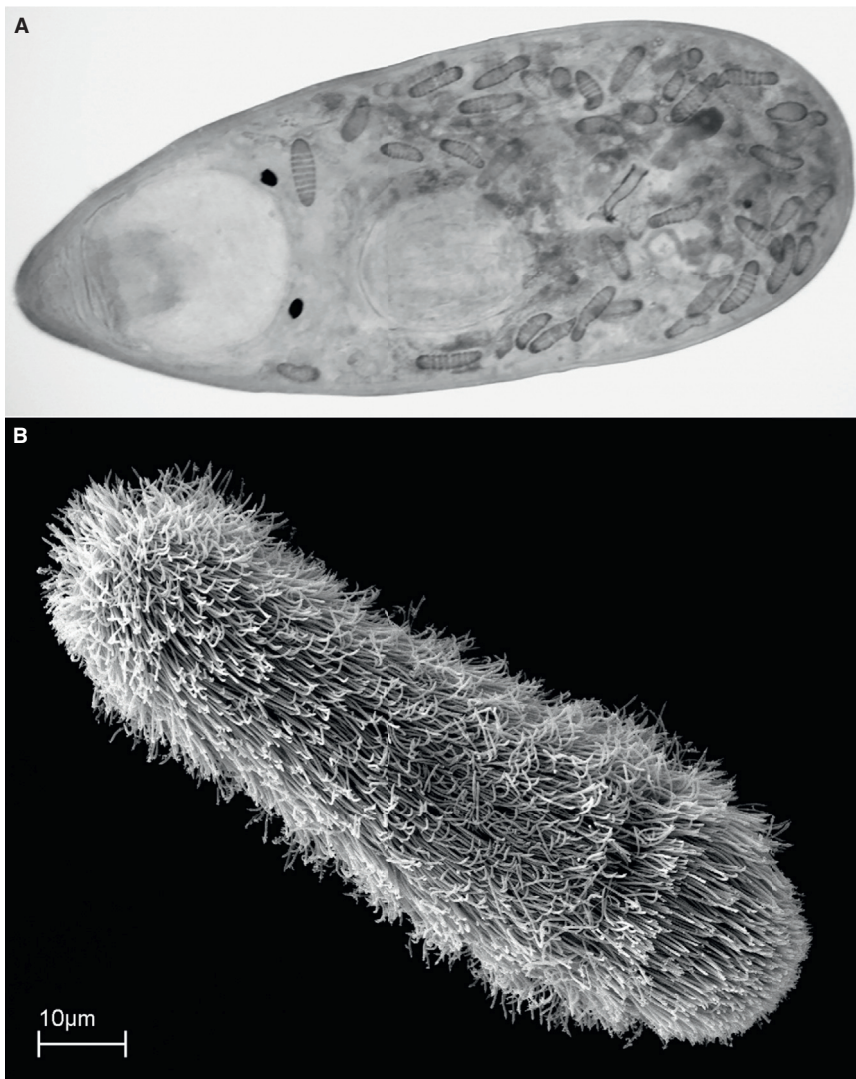


Figure 1. Orthonectids.

(A) Light microscopy image of a flatworm infected with the plasmodium bearing females and males inside. (B) *Intoshia linei* scanning electron micrograph. Photos courtesy of Georgy S. Slyusarev.

begin their life cycle once more. Their effect on the hosts is poorly understood, but in the case of the bioluminescent brittle stars, it has been shown that the parasite can attenuate the light produced by the host [9].

Animal internal parasites are often a nuisance not just for their unfortunate host but also for the zoologists trying to understand their position in the animal tree of life. Indeed, parasitic animals have been notoriously difficult to place phylogenetically due to their often oversimplified anatomy. Whilst of an uncertain affinity for the longest time, only recently has there been a suggestion that the orthonectid muscular system [7]

compares to those of the smallest annelids and flatworms, and that the ultrastructure of the cuticle and cilia [10] is most similar to that of annelids, thus suggesting a spiralian affinity [8], i.e., a close relationship with the members of the clade Spiralia, which unites animals mostly with a unique type of highly stereotyped egg cleavage called ‘spiral cleavage’ [11].

With the advent of molecular biology, phylogenetic analyses of DNA and RNA have often been used to position such animals in the tree of life. Early studies from two decades ago used sequence data from a single nuclear ribosomal gene, 18S rRNA, and despite

demonstrating a bilaterian affinity of orthonectids — bilaterians are animals with bilateral symmetry and an anterior concentration of ganglia forming a brain — failed to provide a stable home for them [12,13]. Up till now, these two sequences from the same species remained the only molecular data available for this enigmatic animal group. These two studies were influential in dismantling Mesozoa — separating orthonectids from another enigmatic group of animals called ‘dicyemids’ — and in the subsequent recognition of orthonectids as a phylum of their own, as well as inspiring the anatomical studies mentioned earlier. However, subsequent larger phylogenomic analyses of metazoan relationships have ignored orthonectids [14–16] due to the difficulties in obtaining reliable genomic data.

The new study by Mikhailov and colleagues [1] analyzed the genome of the orthonectid *Intoshia linei* in conjunction with other genomes and transcriptomes, unambiguously placing it among spiralian animals, supporting recent morphological studies [7,8,10]. The study was, however, not able to provide an exact placement for the orthonectids within spirilians due to its accelerated rate of evolution, which often causes problems in phylogenetic analyses [17]. Nonetheless, the study provides key tools for understanding this recalcitrant, poorly understood and often ignored metazoan group, but has not yet solved whether it is closest to annelids, flatworms or any other spiralian clade.

The *Intoshia* genome has nonetheless revealed some additional surprises. It is one of the smallest metazoan genomes, encoding about 9,000 genes: only two other parasitic animals have fewer genes; myxozoans, a group of degenerate cnidarians that parasitize fishes, and a plant-parasitic nematode. The orthonectid genome is also amongst the most compact animal genomes, with a low gene count and high average gene density, on par with that of *Caenorhabditis elegans*. Nonetheless, more than a quarter of the genome is constituted by unclassified repetitive elements. Many orthonectid developmental/signaling pathways have reduced numbers of components, showing, for instance, the lowest count of transcription factors

known among animals, a measure often correlating with morphological complexity [18] and thus highlighting *Intoshia*'s simplicity. Unlike in most other animals, developmentally important *Hox* genes are not organized in a cluster and many are missing. Until recently it had been questioned whether orthonectids had a true nervous system [7], despite being able to coordinate muscular movement. It is now known that a simple nervous system exists, and this is also in part reflected in their genome, which contains a large repertoire of neural genes, although certain gene families and pathways have been lost. Therefore, despite being highly simplified, the orthonectid genome still retains elements of the genetic toolkit that characterizes bilaterians.

The new study [1] has not solved all mysteries surrounding orthonectids but provides a solid step forward towards a better understanding of one of the most peculiar clades of animals and the first genetic data in two decades. Future phylogenetic analyses could be directed towards subsets of genes with low evolutionary rates [19] to be able to pinpoint more precisely their position in the tree of life. The available genome will also facilitate much needed evo-devo work in both the plasmodial stage and the worm-like adults. There is little doubt that the future of orthonectid research looks brighter than it ever has before.

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Protein Modification: Bacterial Effectors Rewrite the Rules of Ubiquitylation

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A family of virulence factors from the bacterial pathogen *Legionella pneumophila* has been discovered to modify human Rab GTPases with ubiquitin. Surprisingly, this modification occurs via a non-canonical mechanism that uses nicotinamide adenine dinucleotide as a cofactor.

Humans can be infected by the bacterium *Legionella pneumophila* when macrophages within lung alveoli phagocytose bacteria introduced by contaminated aerosolized water [1,2]. The

bacteria proliferate in the macrophages, and in severe cases infection can lead to Legionnaires' disease, a potentially fatal form of pneumonia [3,4]. The processes of infection and propagation in