Received: 22 March 2021 | Revised: 21 July 2021 | Accepted: 16 August 2021

DOI: 10.1002/bies.202100080

BioEssays

REVIEW ESSAY

Prospects & Overviews

The enigmatic Placozoa part 1: Exploring evolutionary controversies and poor ecological knowledge

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Abstract

The placozoan *Trichoplax adhaerens* is a tiny hairy plate and more simply organized than any other living metazoan. After its original description by F.E. Schulze in 1883, it attracted attention as a potential model for the ancestral state of metazoan organization, the "Urmetazoon". *Trichoplax* lacks any kind of symmetry, organs, nerve cells, muscle cells, basal lamina, and extracellular matrix. Furthermore, the placozoan genome is the smallest (not secondarily reduced) genome of all metazoan genomes. It harbors a remarkably rich diversity of genes and has been considered the best living surrogate for a metazoan ancestor genome. The phylum Placozoa presently harbors three formally described species, while several dozen "cryptic" species are yet awaiting their description. The phylogenetic position of placozoans has recently become a contested arena for modern phylogenetic analyses and view-driven claims. *Trichoplax* offers unique prospects for understanding the minimal requirements of metazoan animal organization and their corresponding malfunctions.

KEYWORDS
endosymbiosis, global distribution, marine model system, Placozoa, systematics, Trichoplax, urmetazoon

INTRODUCTION

The first placozoan species, *Trichoplax adhaerens* (Figure 1), was discovered in 1883 by the German zoologist Franz Eilhard Schulze in a seawater aquarium at the Zoological Institute in Graz, Austria.[1] The species name is derived from the Greek "thrix" ('hair') and "plax" ('plate') and the Latin "adhaerens" (sticking). *Trichoplax* is therefore affectionately known as the "sticky hairy plate", a bauplan that is by far the simplest of all animals (except for some secondarily reduced parasites). The sandwich-like placozoan bauplan is made up of two epithelia and non-epithelial fiber cells: A lower epithelium faces the substrate, an upper epithelium faces the open water, and a layer of so-called "fiber cells" is embedded in between the epithelia.[2] After studying *Trichoplax* in some detail, Karl Gottlieb Grell found that this animal cannot be grouped into any of the existing phyla and he erected the new phylum "Placozoa" for *Trichoplax*.[3] For almost half a century Placozoa was the only monotypic animal phylum, and just recently two more placozoan species were described.[4,5] Originally, placozoans were thought to only occur in warm subtropical and tropical ocean waters, but we now predict that they also occur in moderately cool waters and that the distribution range stretches as far as 55°N.[6]

Placozoa lack any kind of permanent symmetry (but see also[7] and[8]) but show a clear top-bottom polarity.[1] They have no discrete

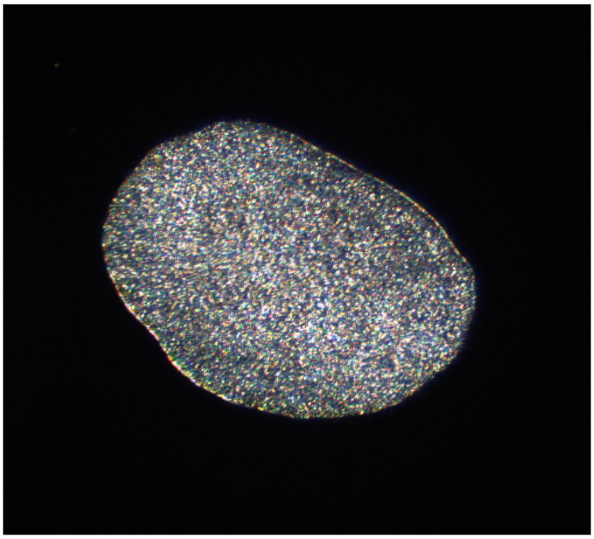


FIGURE 1 Life specimen of the placozoan *Trichoplax adhaerens* (haplotype H1; “Grell clone”). Photo by Hans-Jürgen Osigus

organs, no defined nerve or muscle cells, no extracellular matrix nor a basal membrane.[1,9] Therefore, the nine so far identified somatic cell types perform all functions of nutrition uptake, stimulus perception, locomotion, and reproduction.[1,9–12] However, the presence of additional (sub-) cell types is to be expected, and the precise distribution of already described cell types within the animal body requires further specification.[12,13] Especially the recently reported localization of gland cells in the upper epithelium[11] calls for a revision of some traditional concepts of placozoan morphology. Some recent studies also analyzed how placozoan cells communicate (and thus coordinate) locally or over long distances (see e.g.[14,15] and references therein). In the absence of a nervous system, several signaling molecules were found to play a fundamental role in animal locomotion and/or body shape changes. The identified ligands range from neuropeptide-like proteins to the gas nitric oxide and to amino acids like D-/L-aspartate, glycine and L-glutamate.[13,16–18] Other studies analyzed the ion channel and receptor repertoire found in placozoans.[14,19–26] Without doubt, placozoans are also an important model system to study metazoan signal transduction and coordinated behavior that precedes the evolution of synapses and a nervous system.

Trichoplax and other placozoans represent novel model organisms with tremendous potential for many areas of biological and biomedical research. The steadily growing interest in placozoans comes as no surprise as they (i) show the simplest bauplan of all metazoan animals, (ii) possess the smallest nuclear animal genome, (iii) harbor among the largest mitochondrial genomes, (iv) carry representatives of all major regulatory gene families known from humans, and (v) are highly amenable to experimental manipulations. What can we learn from these tiny masters of morphological simplicity? As a rule of thumb, we here suggest “learn the fundamentals before you analyze the byzantine complexity”. As one interesting example we will use cancer research on placozoans in space at the end of this review (see part 2, topics VIII and IX).

With this two-part review, we will introduce the reader to the following topics in the first part: I. diversity and distribution, II. endosymbiosis, III. life-cycle, IV. original vs. secondary simplicity, and V. systematic position. In part 2 we will discuss VI. mitochondrial genome evolution, VII. genomics, VIII. *Trichoplax* as a model organism for biomedical research, and IX. gravitational biology and gravity sensing.

UNEXPECTED DIVERSITY AND GLOBAL DISTRIBUTION PATTERNS

The phylum Placozoa seems to be much more diverse than initially presumed. Since 1883 *T. adhaerens* has been the only formally described and accepted species within the phylum, which therefore has been the only monotypic animal phylum for almost one and a half century.[27] Due to practical aspects, the only morphologically described species *T. adhaerens* (*sensu* Schulze) has been equated in retrospect to the so-called “Grell” clone, which has been identified as the mitochondrial 16S rDNA haplotype H1.[28,29] In brief, the placozoan “haplotype-concept” is based on a diagnostic 16S rDNA fragment, and each placozoan specimen which does not possess a 100% identical nucleotide sequence in this locus compared to all other already known placozoan 16S haplotypes is assigned to a new consecutive number H# (see[29]), that is, haplotype numbers are purely descriptive numbers not mirroring genetic relationships. Only recently, two more 16S haplotypes have been raised to the rank of a species, that is, *Hoilungia hongkongensis* (haplotype H13[4]) and *Polyplacotoma mediterranea* (haplotype H0[5]). However, more than another 20 genetically well separated placozoan haplotypes are likewise awaiting their taxonomic classification.[30,31] Since diagnostic morphological or ecological differences are scarce in this phylum, the description of new placozoan species is difficult and requires the application of innovative species concepts.[4,5] Although the vast majority of placozoan 16S haplotypes is currently not assigned to a taxonomic rank, there is nevertheless a comprehensive and well-established provisional higher classification system in Placozoa.[29,30,32] Since 2010, this classification system comprises seven well-separated 16S clades of so far undefined taxonomic ranks. Rather surprising, despite intensive sampling efforts, no new 16S clade has been described for more than 10 years in Placozoa, and only one species (i.e., *Polyplacotoma mediterranea*) currently does not fit into the provisional placozoan “clade-system” (Figure 2). A recent study on placozoan mitogenomics[31] stressed the limitations of the short diagnostic 16S fragment for higher systematic approaches in placozoans and suggests the usage of mitochondrial protein sequence data for the illumination of inter-clade relationships. In addition, we must also include whole nuclear genome data if we strive for a higher-level placozoan taxonomy.[4] These data will also be helpful for defining species boundaries and thus estimating the total number of placozoan species crawling in the oceans.

Ecological modeling as well as so far conducted field sampling suggests that placozoans are out there in all oceans between 55°N and 44°S degrees latitude, that is, from Northern Scotland to New Zealand[6,30] (Figure 3). However, field sampling of placozoans in gen-

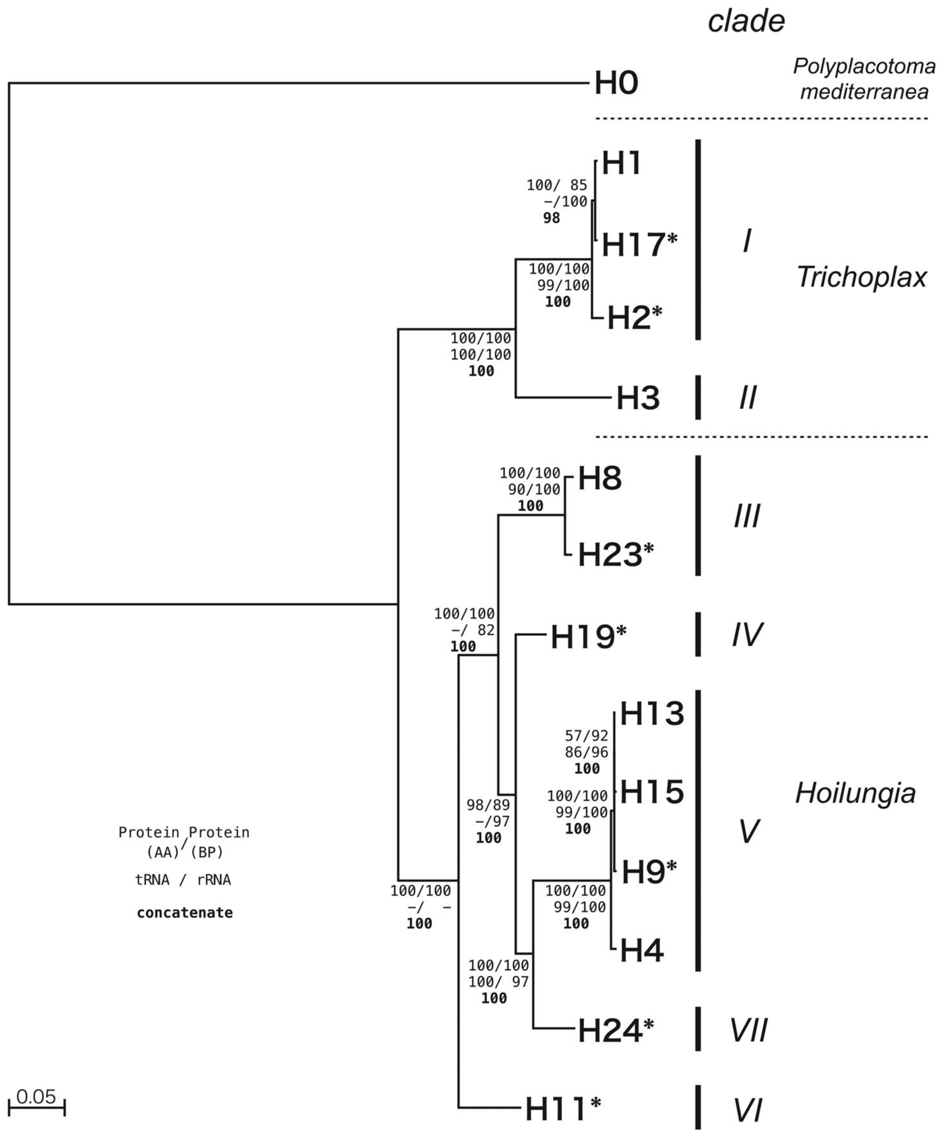


FIGURE 2 Placozoan phylogeny of currently known clades. The phylogenetic tree is based on complete mitochondrial genome data, which robustly resolve relationships. It must be noted that single marker genes like the 16S rRNA fail to provide robust topologies. Image taken from[31]

eral is a difficult and time-consuming matter, mostly due to the microscopic size of the animals. Although there are some well-established sampling methods,[33,34] none of these methods are free of sampling bias and they also only partially allow a comprehensive long-term monitoring of placozoans in their natural habitat. These limitations might explain the somewhat surprising results of a recently published field study on placozoan diversity, which resampled several already known haplotypes, but did not find any new haplotype.[34] It also seems that the genetically most divergent haplotypes show lower population densities in the field and thus are more often overlooked.

The majority of placozoan field sampling has so far been conducted in the 0 to 5 m water depth range,[30,35] but future sampling efforts should also target all vertical areas down to deep sea habitats. The simple feeding mode (biofilm grazers) as well as the most simple body plan without any complex morphological or physiological structures could allow for life even under extreme environmental conditions in the deep sea. Some placozoans tolerate temperatures below 10°C (see[30] and references therein) and we have to expect a broader distribution of placozoans, that is, outside the currently reported geographical distribution range (Figure 3). In addition, placozoans have also been found in areas with comparatively low salinity (i.e., 20‰ in mangroves[4]), and we cannot exclude that some placozoan species might tolerate even lower salinities.

Little is known about the ecology of placozoans. Accidental observations revealed a prey-predator relationship between placozoans and some worm-like gastropods.[33,36,37] Information on other interactions with marine organisms or on the preferred diet in the field is widely missing.[33] With respect to the feeding behavior of placozoans, several studies reported coordinated grazing of multiple individuals, and directed movement towards a food source.[38–40] Beside the regular uptake of nutrition by the lower epithelium,[41,42] a sporadic

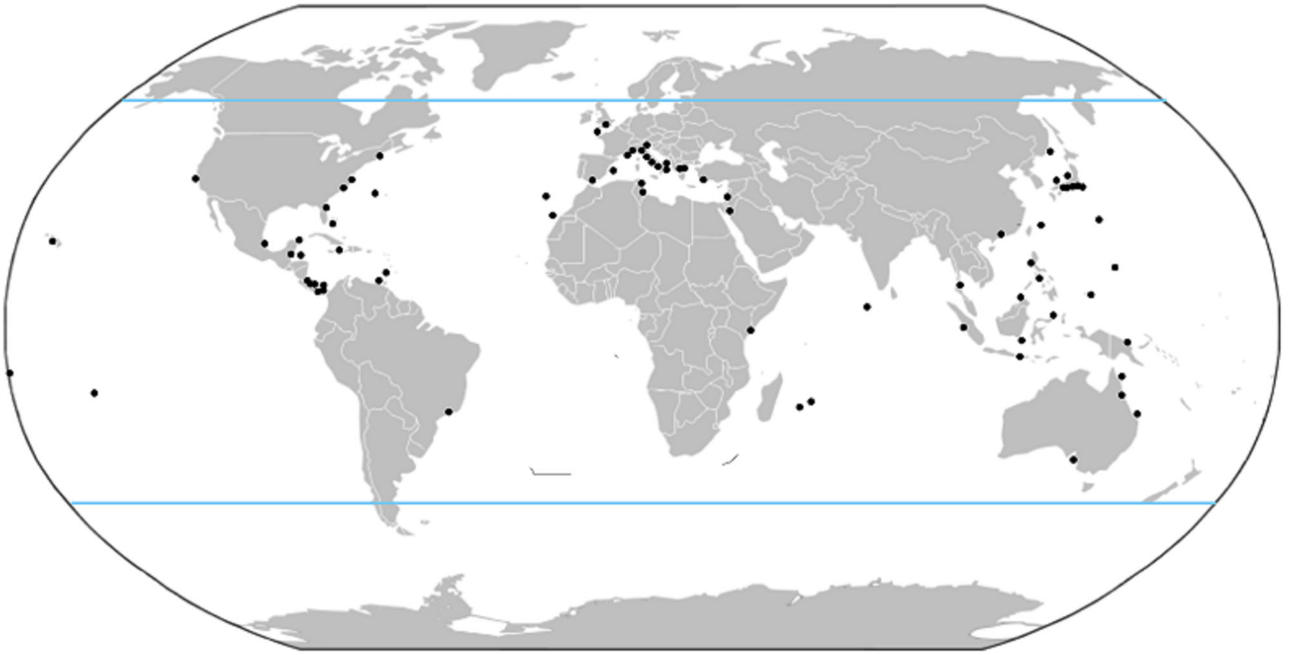


FIGURE 3 Global distribution of placozoans. Previously published placozoan records are shown as black dots. Inferred northern and southern distribution boundaries of placozoans, respectively, are illustrated by blue lines. Data taken from[5,6,35,52,70] and references therein

uptake of nutrition by the upper epithelium has also been reported.[43] Based on observations in the laboratory, vital and healthy placozoans can survive starvation periods of 2 to 3 days before signs of body degradation become visible. Besides this, our knowledge on placozoan bioenergetics and metabolism is speculation and mainly deduced from their gene repertoire.

ENDOSYMBIOSIS IN PLACOZOA—A RESEARCH FIELD WITH POTENTIAL

Endosymbionts in placozoans have first been detected in the 1970s by the pioneers in placozoan research, Karl Grell and Gertrud Benwitz.[44–48] In these and later works[49] endosymbiontic bacteria have been found inside the animals' fiber cells and also in developing oocytes.[49] It has been assumed that host and symbiont form a stable relationship and that the bacteria are propagated to the next generation both by vegetative and sexual reproduction. The first molecular insights into the nature of the placozoan endosymbionts were derived from the *T. adhaerens* genome project.[50] Analyses following the publication of the draft genome revealed the presence of several genome fragments of a bacterium that phylogenetically groups within the family Midichloriacea of the order Rickettsiales.[51] The study of Driscoll et al.[51] pointed to a potentially single endosymbiont in *Trichoplax*, but the limited data left several questions open: (1) What is the principle nature of the relationship between host and symbiont, that is, is the bacterium parasitic, pathogenic or endosymbiotic? (2) How stable is the relationship, is it obligate or facultative? (3) Do all placozoan species carry endosymbionts? (4) Are there correlations between host and endosymbiont phylogenies?

State-of-the-art and future directions

Three recent studies addressed these questions by analyzing near complete genome drafts of Rickettsiales bacteria obtained from two different strains of the placozoan host *Trichoplax* sp. H2 and, surprisingly, from one cnidarian host.[52–54] The placozoan strain in Kamm et al.[54] was the same that was used for the recently published host genome assembly (H2 Panama, Atlantic Ocean),[55] while the placozoan host in Gruber-Vodicka et al.[52] was sampled in the Pacific Ocean, Hawaii. The study of Klinges et al.[53] indirectly contributed to this question by investigating the genome of a bacterium that is frequently associated with stony corals (e.g., *Acropora)*. This bacterium turned out to be closely related to the endosymbiont found in *T. adhaerens*. For clarification, Gruber-Vodicka et al.[52] proposed a new *Candidatus* taxon and named their midichloriacean endosymbiont *Candidatus Grellia incantans*. Likewise, Klinges et al.[53] erected a *Candidatus* taxon and named the *Acropora* endosymbiont *Candidatus Aquarickettsia rhoweri*. The study of Gruber-Vodicka et al.[52] also found a second, less abundant and unrelated bacterium, named *Ruthmannia eludens*, in the host's lower epithelial cells which is not subject of the further discussion.

All three above studies have shown that the endosymbiotic bacteria possess a typical Rickettsia-like genome with reduced size (around 1.3 MB) and gene content, and because of their limited metabolic capacities they require a host for survival. For example, the bacteria possess a very limited capacity to synthesize amino acids. Although they are capable of producing their own ATP, all possess the ATP/ADP symporter Tlc1 that exchanges host ATP with bacterial ADP, which is a typical rickettsial trait of energy parasitism. On the other hand, the endosymbionts were found to be capable of producing several important co-factors, like riboflavin, which are potentially beneficial for the

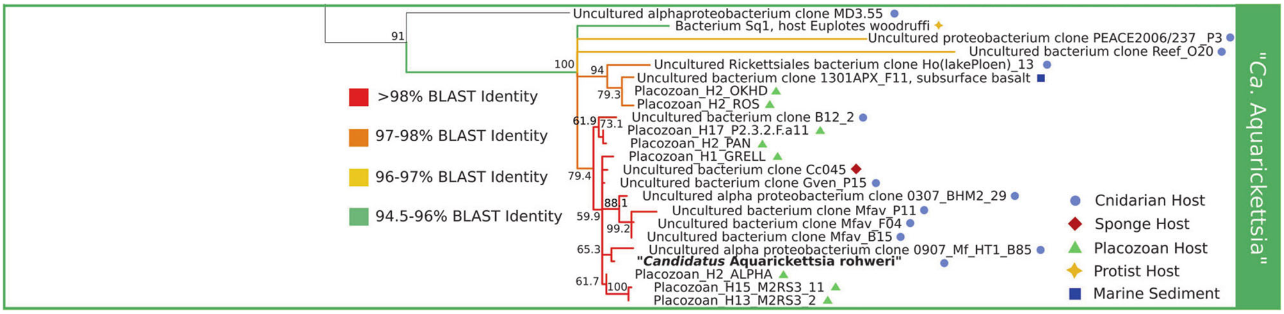


FIGURE 4 16S rRNA phylogeny of the proposed endosymbiont genus "*Candidatus* Aquarickettsia." Endosymbiont relationships only sometimes reflect the phylogenetic relationships of their host. For example, the placozoan haplotypes H2 Panama (H2_PAN) and H17, as well as H13 and H15, are closely related, and so are their endosymbionts. On the other hand, some H2 strains like H2_OKHD, H2_ROS, and the H2 strain from Hawaii[52] harbor endosymbionts that segregate with the "uncultured bacterium clone 1301APX_F11". That the endosymbionts of H2 "Panama" and *Trichoplax adhaerens* (H1) are more closely related to each other than to the endosymbiont in H2 "Hawaii" has been detailed in Kamm et al.[54] These exceptions highlight the still poorly understood complexity of host-endosymbiont relationships in marine Metazoa. Image taken from Klinges et al.[53]

host. In some cases, the host may even complement missing bacterial genes of a particular pathway. Surprisingly, only the placozoan endosymbiont of the "Panama" H2 strain and *A. rhoweri* of *Acropora* were found to be capable of producing the essential amino acids lysine and threonine and potentially providing it to their hosts,[53,54] while *Grellia* of the Hawaiian H2 strain is missing the necessary genes.[52]

Ultrastructural analyses and copy number ratios of host versus symbiont genome have repeatedly shown that the number of rickettsial bacterial cells in a placozoan is moderate and that they are restricted to the fiber cells.[44,49,51,52,54,55] This indicates that the bacteria are effectively controlled by their placozoan hosts and there is no sign that the bacteria are pathogenic to them. Experimental tests would be needed to validate how and which metabolites are exchanged between host and symbiont and whether the relationship is strictly mutualistic. It seems likely that the nature of the relationship varies with a given environmental context (e.g., food type and availability) and even closely related endosymbionts may differ in their metabolic capacities (see the two different H2 endosymbionts mentioned above). Probably in contrast to placozoan endosymbionts, the related midichloriacean bacteria in scleractinian corals appear to be tolerable parasites under normal conditions but become pathogenic under environmental conditions with high nutrient availability.[53] Here an increase in the bacteria population results in the depletion of host resources and either directly or indirectly (increased susceptibility) leads to coral White Band Disease. Although a comparable phenomenon is not known from Placozoa, the occasionally observed accumulation of degenerated animals and sudden extinction of an entire culture dish under laboratory conditions should be investigated in the context of the dynamics of endosymbiotic bacteria.

If the relationship between host and symbiont is stable and interdependent, the phylogeny of the host should mirror that of the symbiont. In order to test this, one has to compare the tree topologies of the phylogenetic analyses of the three above studies[52–54] since they are based on overlapping datasets. We here follow the taxonomic classification of Klinges et al.[53] since their phylogenetic analysis includes the highest number of 16S sequences from placozoan rickettsial endosymbionts. According to this study, all of the midichloriacean type placozoan endosymbionts (including *Grellia)* fall into the *Candidatus* genus *Aquarickettsia*, together with bacteria that are associated with protists, sponges, cnidarians or whose marker sequences have been obtained from sediment samples (cf.[56]). In several cases the phylogeny of the placozoan host matches that of the respective symbiont, that is, closely related placozoans harbor closely related symbionts. Some examples can be seen in Figure 4[53]: the closely related placozoan haplotypes H2 Panama and H17 or H13 and H15 have almost identical endosymbionts. On the other hand, the endosymbiont of the H2 Panama strain is more closely related to the one in H17 or H1 (*T. adhaerens*) than to *Grellia* in the Hawaiian H2 strain (comparing tree topologies in[52–54]). Even more puzzling, the endosymbiont of the Panama strain is closer related to *A. rhoweri* of *Acropora* than to *Grellia* of the placozoan H2 strain (comparing[52–54]). Our interpretation is that the mutualistic relationships are tight, but that the endosymbionts are interchangeable. A placozoan host strain may eliminate the bacteria under certain conditions and takes up (or gets infected by) a similar bacterium. This also fits to our rare observation that certain placozoan strains harbor no detectable midichloriacean endosymbionts, at least not for a while (unpubl. observations). An alternative scenario could be that related placozoans interbreed and that mitochondria and endosymbionts are differentially propagated to the next generation. Based on the amount of shared alleles of the nuclear genome it has indeed been argued that the Panama H2 strain and *T. adhaerens* (H1 strain Grell) must be the descendants of a hybridization event, that is, are more closely related than indicated by their mitochondrial 16S haplotype.[55]

A MYSTERY: THE LIFE-CYCLE OF PLACOZOANS

The life-cycle of placozoans includes two different modes of vegetative reproduction, binary fission and swarmer budding, and occasionally also bisexual reproduction[1,9,49,57] (Figure 5). Vegetative reproduction

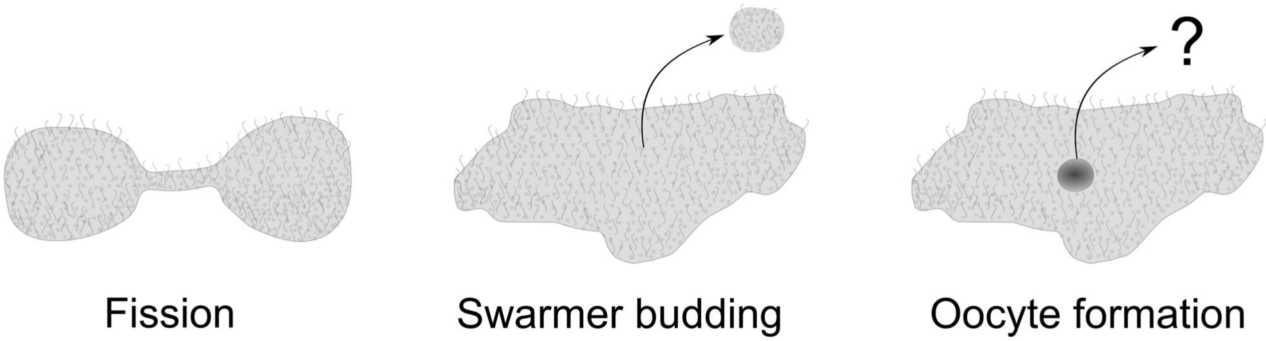


FIGURE 5 The three different modes of reproduction in Placozoa. Vegetative reproduction comprises fission as well as the formation of mobile swarmer stages. In fission the mother animal divides its body in two, sometimes three (see Figure 6) daughter individuals. In swarmer formation mode normally a single pelagic swarmer is formed and released. The complete life-cycle remains unknown, since completion of the sexual reproduction part has never been observed yet. For details see main text

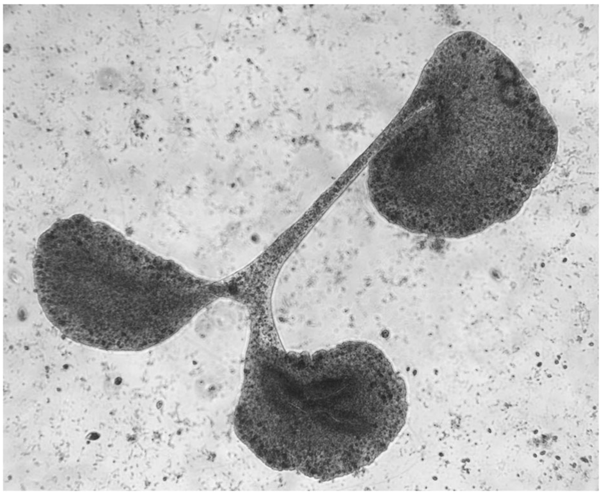


FIGURE 6 Unusual three-daughter fission of a placozoan mother specimen. This is the result of consecutive fissions even if the first fission has not been completed yet (i.e., the mother and the first daughter individual are still connected, while the mother starts the next division). Photo by Hans-Jürgen Osigus

by binary (sometimes also trinary (Figure 6)) fission is the standard mode of reproduction for placozoans in the laboratory. Mean doubling times for a population reproducing by fission can be as short as 1 to 2 days under optimal conditions. Under less optimal conditions the budding of planctonic swarmers is favored. These small (50–100 μm), spheric swarmers are produced in the upper epithelium and either compact or hollow.[57–59] Based on observations in the laboratory, both forms develop directly into a normal adult placozoan. In the field, the swarmers are released into the open water and thus are drifted by chance to a new habitat. The sexual part of the placozoan life-cycle is still widely unknown (see[49] and references therein). Under laboratory conditions, the embryos die at the 128-cell stage in *Trichoplax* sp. H2.[49] For all other placozoan haplotypes, except for H1, H2, and H16,[49] we have never observed sexual reproduction in lab cultures despite various efforts to induce it (Schierwater lab, unpublished data). Also, all attempts to complete the sexual life cycle in H1, H2, or H16 have failed so far[49] indicating that a crucial factor is missing in laboratory cultures. From genetic evidence (i.e., allele sharing patterns) we know that sexual reproduction is rarely but regularly used at least by *Hoilungia* sp. H8 in the field.[60] As of today, not a single placozoan specimen has been collected from the field which harbored oocytes (ref. [35] and own observations).

Our poor knowledge on the sexual life-cycle of placozoans is unfortunate, since it might harbor clues to better understand the sharp controversy between the substantial genetic diversity and the remarkable phenotypic (morphological) stasis.[4] Only for the recently described and morphological remarkably different species, *Polyplacotoma mediterranea*, the genetic distance to all other placozoans is also mirrored by a very distinctive habitus.[5] For all other placozoan haplotypes (species and clades) the discrepancy between morphological and genetic diversity calls for explanations. The most simple explanation for resolving the “morphology vs. genetics” mystery would be to assume the existence of a yet unknown, morphologically different and variable life cycle stage. Such a life cycle stage could derive from sexual reproduction, which we do not understand yet. We can also not exclude the possibility that functional sexual reproduction has been secondarily lost in (some) extant placozoans.

THE SIMPLEST METAZOAN ANIMAL: ORIGINAL OR DERIVED SIMPLICITY?

A global perspective

Phylogeny is largely, but not entirely a guide to determine if placozoans show secondary simplification. For instance, a collagenous extracellular matrix is often considered a shared derived character of the Metazoa and thus a feature of the common ancestor of all animals.[61] Under this assumption placozoans, which lack an extracellular matrix (ECM), would be considered secondarily simplified (see Box 1). Alternatively, the ECM would be an invention of the diploblastic animals after they split off from an early placozoan. Other hypotheses of secondary simplification, like a loss of muscle and nerve cells, could also

BOX 1

The long branch leading to Placozoa combined with short internal branches as observed in phylogenetic analyses (e.g.,[70]) has stimulated discussions of new evolutionary hypotheses. Some of the following aspects have already been discussed in a preprint (https://www.biorxiv.org/content/10.1101/200972v1) but have not made it into a peer-reviewed publication.[70] The scenarios discussed in the following section are sensitive to yet unidentified major placozoan taxa (whether extant or extinct) and life-cycle stages (see main text) and therefore should be taken with caution. It must also be highlighted that even if there had been some degree of secondary morphological simplifications during the course of placozoan evolution (e.g.,[71,72]), this would not affect the outstanding importance of nowadays placozoans as a comparative model system for the early evolution of Metazoa.[27]

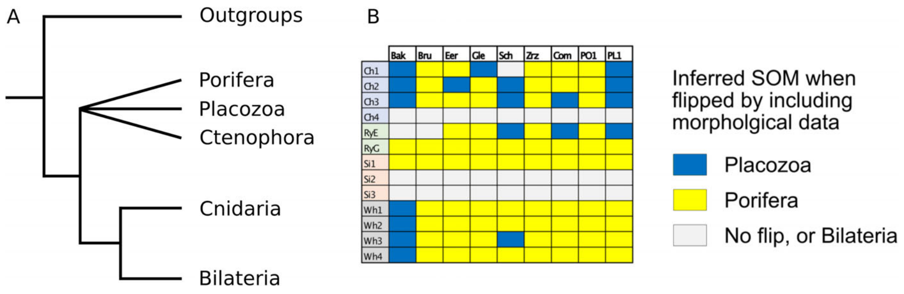
The "ancestral vs. *derived bauplan simplicity" hypotheses*

Molecular systematics have jiggled the Placozoa around into nearly any possible phylogenetic position (see main text) and thus enforced a discussion of ancestral vs. secondarily derived simplicity of the placozoan bauplan. The "derived simplicity" hypothesis postulates a recent genetic bottleneck of the global placozoan diversity with the extinction of all but one placozoan group (cf. https://www.biorxiv.org/content/10.1101/200972v1), implying that the phylum Placozoa originally harbored a variety of diverse and deeply branching taxonomic units over the last 600 million years. Some authors furthermore speculate that extant modern placozoans do not represent the original placozoan bauplan but have lost a basal membrane (BM) and/or extracellular matrix (ECM).[61,73,74] Other speculations even comprise the potential loss of a Hox gene in this context.[75] However, in the absence of any confirmed fossil records and solid phylogenetic trees for early metazoan radiation the way from such speculations to valid scientific hypotheses in sensu stricto seems quite far.

The alternative and traditional hypothesis, that the simplicity of the placozoan bauplan is ancestral, looks very natural from many perspectives, but has been questioned by some molecular analyses. The observed short branch lengths between the different placozoan groups have challenged the view of a long period (several hundred million years) of "morphological stasis" without species and bauplan diversification and suggested that all observed placozoan diversity is quite young.[4,70] The complex placozoan genetic toolkit[4,50,55,76] has been added to the discussion and led to the saying that a conservation of a primitive bauplan over such long periods is "unlikely". On the other hand we know, however, that if there is no competition there is no grounds for radiation. It seems conceivable that for grazers like the small low-density placozoans, a biofilm food source was never limited over longer time periods. Other potential limitations leading to competition between placozoans we also do not know of. If so, then the driving force for radiation, that is, competition for limited resources, is simply not given, and in such a scenario the approved simplest bauplan should outcompete a more complex bauplan.

be addressed if the relationships among the five groups at the base of the metazoan tree of life could be resolved. For this the five principal groups of animals are the bilaterians (or triploblastic animals), cnidarians (corals and medusozoans), ctenophores (comb jellies), poriferans (sponges), and placozoans. If sponges (e.g.,[62]) or placozoans (cf.[63]) are sister to the other four groups, the observed simplicity would most likely be original. Indeed, some simple sponges[64] and particularly placozoans show overall similarity to the non-metazoan holozoans such as *Salpingoeca rosetta*. The situation would become more complicated—or even highly unlikely—if ctenophores were regarded sister to the other four groups.[65] While modern ctenophores are likely a relict of a once-much-larger group, they are fairly sophisticated animals, for example, with muscles, nerves, a mouth, and gut. To derive any of the other diploblast groups from ctenophores would either require modification of evolutionary theory or the assumption that the early ctenophores were much more primitive/simplified, that is, to a degree that they would not be called "ctenophores" anymore (see below). In all such hypotheses games we must also pay attention to the surprisingly strong evidence that many complex features evolved in parallel, that is, independently in many animal groups (e.g.,[66,67]). For instance, a core set of contractile proteins, such as type II myosin heavy chain (MyHC) proteins, characterize vertebrate striated muscles. Surprisingly, MyHC proteins are conserved throughout animals and are even found in some unicellular organisms.[66] Sponges clearly lack muscles, but they express representatives of both MyHC orthology groups in various cell types. Similarly, Dayraud et al.[67] found a ctenophore-specific duplication of the MyHC gene, but only one of the resulting paralogs is associated with muscle cells. Possibly, the common ancestor of metazoan animals possessed the molecular toolkit to build muscles, but only some animals use it to build muscles (placozoans and sponges did not).

The ancestors of placozoans and sponges likely were among the first metazoan animals which evolved in the late Proterozoic. In those bacteria-rich oceans,[68] multicellular organisms that used ciliae for locomotion and feeding conquered a broad ecological niche, which led to the dawn of metazoan animals. Placozoans may have persisted relatively unchanged, while other lineages may have evolved distinct complexity by a combination of sequential and parallel anagenetic evolution using the rich genetic toolkit from the Urmetazoan (cf.[69]). If stem lineages of other major metazoan groups existed at this time, they likely resembled placozoans and simple sponges, as dictated by the ecology of the late Proterozoic. Thus, the bauplan of placozoans and simple sponges most likely is plesiomorphic.



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Ch4	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
RyE	⬜	⬜	🟨	🟨	🟦	🟨	🟦	🟨	🟦
RyG	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Si1	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Si2	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Si3	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜	⬜
Wh1	🟦	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Wh2	🟦	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨
Wh3	🟦	🟨	🟨	🟨	🟦	🟨	🟨	🟨	🟨
Wh4	🟦	🟨	🟨	🟨	🟨	🟨	🟨	🟨	🟨

Inferred SOM when flipped by including morpholgical data

🟦 Placozoa
🟨 Porifera
⬜ No flip, or Bilateria

FIGURE 7 The position of Placozoa within the metazoan tree of life is under dispute. It is the sole use of molecular data which have allowed to generate all possible scenarios, every single one with high support values. The implementation of normally highly outnumbered morphological data, however, can have substantial impact on the outcome of phylogenetic analyses if the morphological data are slightly weighted. (from[63])

THE PHYLOGENETIC POSITION OF PLACOZOA: A ONE-OF-A-KIND BATTLE FIELD

As should be evident from the previous discussion, the placement of the phylum Placozoa in the tree of life is part of one of the more contentious and active areas of phylogenetic research. It is part of what we have called the "sister of all other metazoa" (SOM) problem.[63] There are five major metazoan taxa that are relevant to this question—Bilateria, Cnidaria, Ctenophora, Placozoa and Porifera. The latter four of these "taxa" are described phyla and one—Bilateria—is a complex but monophyletic taxon with tens of phyla. There are 105 possible ways to arrange these five ingroup taxa with one outgroup. It is clear that the five taxa are monophyletic themselves, so the real SOM problem boils down to which of these 105 topologies is supported by the data. A survey of the literature for molecular hypotheses concerning the identity of the sister of all other metazoans indicates that Porifera was the preferred SOM until about 2011 when phylogenomic approaches were initiated. As more and more molecular characters were added to the analyses, the "Ctenophores first" hypothesis grew in preference. The current picture of the literature is rather dichotomous as over the past 5 years (from 2015 to 2020) about 40% of publications prefer Porifera first, 40% prefer Ctenophora first and the remainder present the problem as ambiguous. Since from a comparative zoology and evolutionary point of view, any Ctenophora first scenarios must be analytical artifacts, an interesting question arises here. Does the cumulation of more and more low quality characters (base pairs or amino acid residues) favor stochastic/random outcomes?

Prior to the first molecular studies that address this problem, morphological studies all supported either Porifera or Placozoa as the SOM. As early single gene or small multiple gene studies accumulated, Porifera appeared to be the clear "winner" in the SOM sweepstakes. However, with the advent of phylogenomic scale datasets, also Ctenophora gained support as the SOM in some studies. Another trend to note is that a good proportion of the studies tended to "deresolve" the SOM problem and present an unresolved tree as a hypothesis for the metazoan topology. From a comparative morphology point of view, it is obvious that neither Cnidaria, Ctenophora nor Bilateria are a viable candidate for the SOM. It is clear that the determination of the SOM is highly dependent on the dataset and the methods of analysis (e.g.,[62,65,77,78]). However, a cogent argument can be made that the phylogenomic data will only make sense when the models and methods of analysis take into account compositional heterogeneity.[79] How to model this compositional heterogeneity is a subject of intense debate and several advances have been made on the way. Some researchers prefer to recode the 20 amino acid alphabets into six letter alphabets based on structure and chemical characteristics of the 20 amino acids. This recoding reduces the complexity of the amino acid datasets. Other researchers prefer to impose Bayesian mixture models to correct for the heterogeneity.

Another complicating factor in the determination of the SOM is that most of the recent hypotheses are based entirely on molecular data, with the morphological data entering as an afterthought. Neumann et al.[63] explored the possible role of morphology in the determination of the SOM and showed that relatively slight weighting of the morphological data can result in alternative topologies for the SOM. For instance, any analyses showing Ctenophora as the SOM in a combined analysis can "flip" to Porifera or Placozoa as the SOM with relatively modest weighting of morphology (Figure 7). Since we don't really know how these two categories of data need to be dealt with in phylogenetic analysis, Neumann et al.[63] argued for caution in interpreting what is the SOM for now. It comes as no surprise that Ctenophora first scenarios are best achieved if systematic errors in the analyses are introduced.[80,81]

Nielsen[82] summarized the morphological implications of both Porifera and Ctenophora as the SOM. Only two characters—cells with collar complex and intracellular digestion—could be interpreted as morphological characters uniting Choanoflagellata and Porifera. He also compared the number of shared derived characters (synapomorphies) supporting Porifera as SOM and Ctenophora as SOM. If ctenophores branched off first, Nielsen[82] concludes, there are two options: (1) multiple morphological features that Ctenophora share with Placozoa and Cnidaria need to be lost, or (2) significant independent or convergent evolution (homoplasies) have evolved for a large number of anatomical characters.

Currently, then, Placozoa are enigmatic with respect to their placement in the animal tree of life. Full disclosure prompts us to point out that recent work[70] suggests that placozoans are sister to Cnidaria but we agree with King and Rokas[83] that the uncertainty of the relationships here needs to be "embraced" at this point in time. The grand majority of the 105 hypotheses for these taxa relationships can be whittled away and discarded, leading us to a small number of viable alternatives. The future work in phylogenetics of placozoans promises some novel learned lessons and will help to progress to a sound answer.

CONCLUSIONS

The enigmatic Placozoa have been attracting a lot of attention across disciplines and the momentum keeps going. This is remarkable for various reasons, including (i) the fact that we know very little about the placozoan life-cycle, ecology, and diversity, and (ii) the odd discussions about the phylogenetic position of Placozoa in the tree of life.

ACKNOWLEDGMENTS

The authors thank Kristin Fenske for her help at all different levels of preparing the manuscript. The authors also thank two anonymous reviewers for a wealth of critical and very helpful comments which clearly helped the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors have provided input from their personal experience working with *Trichoplax* and written sections for the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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How to cite this article: Schierwater, B., Osigus, H.-J., Bergmann, T., Blackstone, N. W., Hadrys, H., Hauslage, J., Humbert, P. O., Kamm, K., Kvansakul, M., Wysocki, K., & DeSalle, R. (2021). The enigmatic Placozoa part 1: Exploring evolutionary controversies and poor ecological knowledge. *BioEssays*, *43*, e2100080. https://doi.org/10.1002/bies.202100080