

# 6 Placozoa

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## 6.1 HISTORY OF THE MODEL

More than a century ago, the simplest of all metazoan animals was discovered in a seawater aquarium and described as *Trichoplax adhaerens* (Schulze 1883). This tiny, flattened animal lacked any kind of symmetry, mouth, gut, nervous system and extra-cellular matrix and immediately stimulated inspiring discussions on the ancestral morphology of a hypothetical “urmetazoon” (for overview, see Schierwater and DeSalle 2007; Schierwater et al. 2016; Schierwater and DeSalle 2018 and references therein). For more than half a century, this important animal was completely ignored, however, because of a wrong claim that *Trichoplax* was a larva form of a hydrozoan (see Ender and Schierwater 2003; Schierwater 2005 and references therein). It was the very tedious and precise work of the German zoologist Karl Gottlieb Grell which led to the erection of its own phylum for *Trichoplax* in 1971 (Grell 1971). Just recently, two more placozoan species were described, *Hoilungia hongkongensis* and *Polyplacotoma mediterranea* (Eitel et al. 2018; Osigus et al. 2019). Genetic data suggest the presence of even more—at least several dozen—placozoan species, which might be morphologically indistinguishable, that is, cryptic species (Eitel and Schierwater 2010). A yet-undescribed species, represented by the haplotype H2 (see e.g. Kamm et al. 2018), seems to be the most robust placozoan species for culturing and manipulations in the laboratory, and we use it, for example, for gravity research on earth and in space. Most people prefer to work with the original species, *Trichoplax adhaerens*, which has been the best-studied species, since it harbors the first characterized genome (Srivastava et al. 2008).

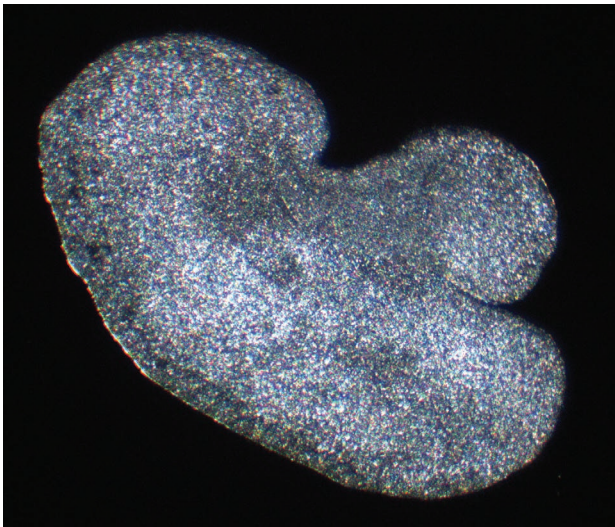
Placozoans diverged early in metazoan history, and their morphology fits nicely into almost any of the existing urmetazoan hypotheses, no matter if we derive placozoans from an early benthic gallerteroid stage or any pelagic placula or planula stage (for overview, see Syed and Schierwater 2002; Schierwater et al. 2009 and references therein). In addition,

the *Trichoplax* genome resembles the best living surrogate for a metazoan ancestor genome (Srivastava et al. 2008), and almost all major gene families known from humans are already present in *Trichoplax*. Thus, it comes as no surprise that from comparative morphology to cell physiology and molecular development to cancer research, *Trichoplax* has now been used as a basic model system to answer complex questions. From the very beginning of placozoan research and also from modern integration of molecular data, many evolutionary biologists have seen compelling evidence for an early branching position of placozoans at the very root of the metazoan tree of life (e.g. Schierwater et al. 2009; Schierwater et al. 2016 for references). However, a variety of molecular trees suggests Porifera as the earliest branching metazoans (e.g. Philippe et al. 2009; Pick et al. 2010; Simion et al. 2017).

When we have been sending placozoan cultures to different laboratories worldwide, we have mostly sent benign *Trichoplax adhaerens* (the original Grell culture-strain originating from the Red Sea, haplotype H1 (Figure 6.1); see Schierwater 2005 for details) or the yet-unnamed haplotype H2 (see e.g. Eitel and Schierwater 2010; Schleicherova et al. 2017; Kamm et al. 2018). For some literature on *T. adhaerens*, it is unclear, however, which species or haplotype was actually studied. This is because of the existence of an estimated number of at least two dozen cryptic placozoan species, which under the microscope all look identical to *T. adhaerens* (e.g. Voigt et al. 2004; Eitel and Schierwater 2010; Eitel et al. 2013).

## 6.2 GEOGRAPHICAL LOCATION

The precise geographical and global distribution of placozoans is difficult to define, since their microscopic size and fluctuating population densities call for time-intensive sampling and microscopy efforts (see Eitel et al. 2013; Voigt and Eitel 2018). Nonetheless, from available records and



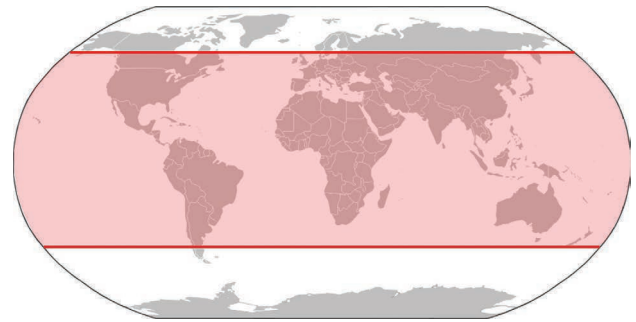
**FIGURE 6.1** Life image of *Trichoplax adhaerens*. The shown animal measures about 3 mm in diameter.

mathematical modelling, we conclude that placozoans are strictly marine (although they show some tolerance to brackish water, Eitel et al. 2013; Eitel et al. 2018) and are found between 55° northern and 44° southern latitude (Figure 6.2) (Paknia and Schierwater 2015). Placozoans live in all marine waters where the lowest water temperature is above 10°C (see Eitel et al. 2013).

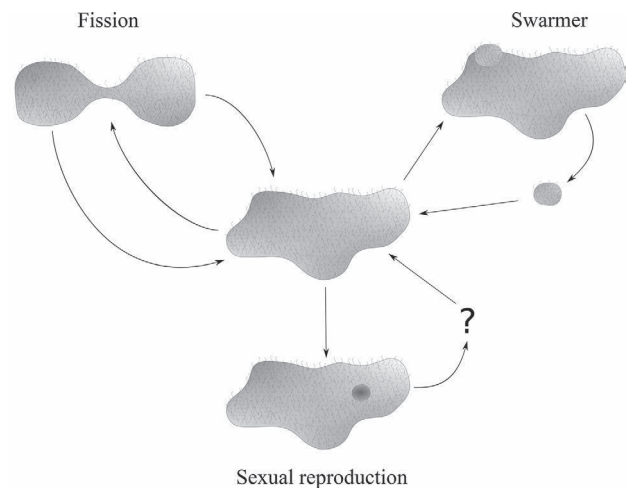
While *Trichoplax adhaerens* (H1) is cosmopolitan and has been repeatedly found in warm oceans (see Eitel et al. 2013), the other two described placozoan species have each been found at one specific location only, but it remains to be seen whether these species are endemic (Eitel et al. 2018; Osigus et al. 2019). In general, there are clear differences between placozoan clades with respect to global distribution patterns (Eitel et al. 2013; Voigt and Eitel 2018). But, as noted before, global sampling records are highly preliminary, and reports are hard to compare because of different sampling and identification methods used. The two main sampling methods, trap-sampling and hard substrate sampling, differ substantially not only with respect to efficiency, but they also collect different life-cycle stages of placozoans (Pearse and Voigt 2007; Eitel and Schierwater 2010; Eitel et al. 2013; Miyazawa and Nakano 2018; Voigt and Eitel 2018): substrate sampling depends on the presence of a natural biofilm and mainly collects feeding adult animals, while trap sampling rather targets the planctonic placozoan swarmer stages. Thus, trap sampling methods in general shift the sampling bias toward placozoan species with higher rates of swarmer formation.

### 6.3 LIFE CYCLE

The complete life cycle of placozoans remains an unresolved mystery since the discovery of the first placozoan specimen in 1883 (Schulze 1883). The typical adult placozoan, that is, the benthic, disc-shaped (in one case ramified, Osigus



**FIGURE 6.2** Inferred geographic distribution of placozoans based on habitat modeling predictions. (From Paknia and Schierwater 2015.)



**FIGURE 6.3** Schematic life cycle of placozoans. Vegetative reproduction in placozoans comprises the process of fission as well as the budding of mobile swarmer stages. Sexual development has only been recorded up to the 128-cell-stage of the embryo. (From Eitel et al. 2011.)

et al. 2019) animal with no symmetry, normally reproduces by vegetative fission (see Figure 6.3), that is, by dividing into two—sometimes three—daughter individuals (Schulze 1883; Schulze 1891). Sometimes the vegetative formation of swarmers from the upper epithelium is seen in laboratory cultures (e.g. Thiemann and Ruthmann 1988). These pelagic swarmers are believed to float in the open water to eventually attach to a new substrate and this way allow dispersal if local conditions become unfavorable or population density calls for a change of location.

We know from observations in the laboratory and also from population genetics that placozoans do also reproduce sexually in the field (e.g. Eitel et al. 2011; Signorovitch et al. 2005; Kamm et al. 2018), and eggs or early embryo stages have sporadically been seen in laboratory cultures. However, a complete sexual reproductive cycle has never been reported in all the decades the animals have been kept in culture under laboratory conditions. Although eggs and early cleavage stages have been observed, the latter are cytologically anomalous and die at the 128-cell stage at the latest (Eitel et al. 2011 and references therein); neither meiosis,

fertilization nor confirmed sperm cells have ever been documented. Observation of a fertilization membrane (Eitel et al. 2011) and genetic evidence for outcrossing, however, tell us that bisexual reproduction must occur in placozoans (e.g. Kamm et al. 2018). No adult sexual animals have ever been collected from the field (see also Voigt and Eitel 2018), and it remains unclear if fertilized eggs develop directly into adult placozoans or whether there is a larva or other additional life cycle stage in placozoans. We do not know if placozoans are hermaphroditic, but the genetic data do not support the idea that placozoans are using self-fertilization (Kamm et al. 2018). We have no reason to assume any derived mode of reproduction, like haploid or diploid parthenogenesis, to be present in placozoans.

#### 6.4 EMBRYOGENESIS

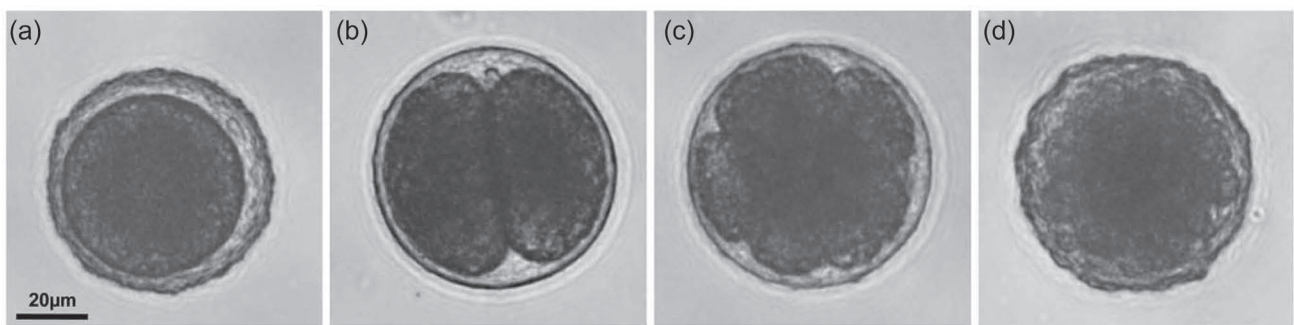
As stated, only early embryogenesis has been seen in placozoans (Figure 6.4). Oocytes are built in the lower epithelium and then move into the intermediate fiber cell layer for further development, where fiber cells provide nutrition for the oocytes (Grell and Benwitz 1974; Eitel et al. 2011). One single mother animal can build up to nine oocytes simultaneously, while oocyte formation and maturation go along with the degeneration of the mother animal (Eitel et al. 2011). After an unknown fertilization process, a fertilization membrane appears around the fertilized egg (Grell and Benwitz 1974; Eitel et al. 2011). The subsequent total and equal cleavages of embryonic cells proceed to the 128-cell stage before the embryos die under laboratory conditions (Eitel et al. 2011).

#### 6.5 ANATOMY

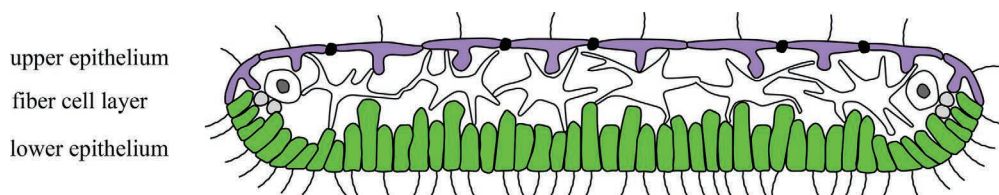
The general morphology of placozoans has been well known since the original description by Schulze (Schulze 1883; Schulze 1891) and the works of Karl Gottlieb Grell (e.g. Grell and Benwitz 1971). The precise ultrastructure of these organisms is still under investigation (e.g. Smith et al. 2014; Romanova et al. 2021). The general placozoan bauplan (see Figure 6.5) can be described as a three-layered disc, with an upper epithelium facing the open water, a lower (feeding) epithelium facing the substrate (see e.g. Smith et al. 2015) and a fiber cell layer (which has nothing to with an epithelium) in between.

A most remarkable and exclusive (and likely plesiomorphic) feature of the Placozoa is the lack of an extra-cellular matrix (ECM) and a basal lamina between the inner fiber cells and the enclosing epithelia (e.g. Smith et al. 2014). The reader must be aware that some textbooks (e.g. Brusca and Brusca 1990) and other publications falsely state the existence of an ECM. The interspace between the fiber cells and the epithelial cells is filled by a liquid, and both epithelia appear to be to some extent permeable for aqueous solutions (Ruthmann et al. 1986; but see also Smith and Reese 2016). The cells of the upper and lower epithelium are connected by adherens junctions, and neither tight nor septate or gap junctions have been found in *Trichoplax* (Ruthmann et al. 1986; Smith and Reese 2016).

So far, nine distinct somatic cell types have been identified in placozoans: upper and lower epithelial cells, sphere cells, crystal cells, three types of gland cells, lipophil cells and fiber cells (Schulze 1883; Smith et al. 2014; Mayorova



**FIGURE 6.4** Early embryonic development in placozoans. A zygote is shown in (a), while (b) to (d) show embryos at the 2-, 8- and 64-cell stage, respectively. (From Eitel et al. 2011.)



**FIGURE 6.5** General anatomy of *Trichoplax adhaerens* shown as a synthesis of recent studies on the placozoan ultrastructure. The three-layered placozoan bauplan consists of an upper epithelium, a lower epithelium and a layer of fiber cells sandwiched between the two epithelia. (From Jakob et al. 2004; Guidi et al. 2011; Smith et al. 2014; and Eitel et al. 2018.)

et al. 2019; Romanova et al. 2021). The upper epithelium (consisting only of upper epithelial cells, some gland cells and sphere cells; Mayorova et al. 2019; Romanova et al. 2021) mainly has a protective function (Jackson and Buss 2009), whereas the lower epithelium (consisting of lower epithelial cells, lipophil cells and gland cells) is involved in digestion and nutrition uptake (e.g. Mayorova et al. 2019). The syncytial fiber cell layer between the two epithelia is involved in body contraction and signal transduction processes (Smith et al. 2014, Romanova et al. 2021 and references therein). The crystal cells are located at the edge of the animal and are likely involved in gravity perception (Mayorova et al. 2018). Also located close to the margin of the animal body are small undifferentiated cells, which have been regarded as omnipotent “stem” cells (Jakob et al. 2004). From comparative morphology, it is obvious that the lower epithelium resembles the entoderm and the upper epithelium the ectoderm of other metazoans (Bütschli 1884). The different lower epithelial cells use pinocytosis to take up food particles (Ruthmann et al. 1986). For this, the epithelial cells are covered with slime/mucus, allowing them to catch small food particles (Wenderoth 1986). The mucus of the lower epithelium is also involved in adhesion, movement and gliding (Mayorova et al. 2019). The upper epithelium shows lower differentiation, with the so-called ‘shiny spheres’ (“Glanz-kugeln”; Schulze 1891; Jackson and Buss 2009), which are lipid droplets within the sphere cells (Romanova et al. 2021), as well as sporadically occurring gland cells (Mayorova et al. 2019).

## 6.6 GENOMIC DATA

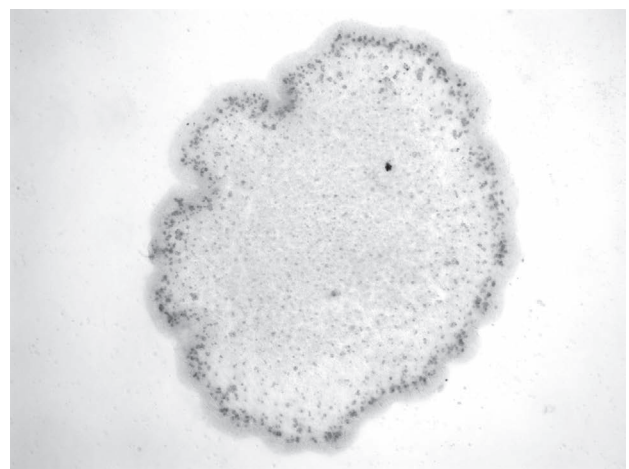
In the last 15 years, three high-quality draft genomes have been published (Srivastava et al. 2008; Eitel et al. 2018; Kamm et al. 2018), in addition to a further three genomes of lower coverage (Laumer et al. 2018). With the genome of the haplotype H2, an additional—yet formally undescribed—*Trichoplax* species becomes available as a favorable model system (Kamm et al. 2018), which shows much higher robustness in laboratory cultures compared to other placozoans. From the available genome data, we can deduce that placozoan genomes range in size from 87–95 megabases and contain approximately 12,000 protein coding genes (Srivastava et al. 2008; Eitel et al. 2018; Kamm et al. 2018; Laumer et al. 2018). Based on the amount of conserved synteny to other metazoans like vertebrates and anthozoans (Srivastava et al. 2008), placozoans thus harbor the smallest not secondarily reduced metazoan genomes. Different placozoan species can be discriminated by a significant amount of gene sequence divergence, and less related species also show substantial differences in their gene’s chromosomal arrangement (Srivastava et al. 2008; Eitel et al. 2018; Kamm et al. 2018; Laumer et al. 2018).

Compared to cnidarians and bilaterians, the complexity of the placozoan gene repertoire is lower (Schierwater et al. 2008; Srivastava et al. 2008; Alie and Manuel 2010; Eitel et al. 2018; Kamm et al. 2018; Kamm et al. 2019).

Most eumetazoan gene families are present, but the expansion of several gene families, for example, homeobox genes, clearly happened after the split off of the Cnidaria (Kamm and Schierwater 2006; Kamm et al. 2006; Ryan et al. 2006; Schierwater et al. 2008). Likewise, the complexity of the gene repertoire related to cell–cell signaling (Srivastava et al. 2008), neuroendocrine function (Srivastava et al. 2008; Alie and Manuel 2010; Varoqueaux et al. 2018) or innate immunity (Kamm et al. 2019) represents a pre-cnidarian stage. On the other hand, placozoan genomes show several examples of phylum-specific gene family expansions (e.g. Eitel et al. 2018; Kamm et al. 2018; Kamm et al. 2019). These examples include genes related to innate immunity and cell death (Kamm et al. 2019) and the large group of G protein-coupled receptors (Kamm et al. 2018). The latter group of cell surface receptors also shows a high diversity within the phylum and may represent more than 6% of all genes in a species (Kamm et al. 2018). Gene duplications within such diverse gene families may thus also be a driver for speciation within the phylum (Eitel et al. 2018).

## 6.7 FUNCTIONAL APPROACHES: TOOLS FOR MOLECULAR AND CELLULAR ANALYSES

The simplicity of the *Trichoplax* model allows the use of the full spectrum of modern molecular methods for mapping and reconstructing fundamental cellular and organismal processes (e.g. von der Chevallerie et al. 2014; Varoqueaux et al. 2018; Popgeorgiev et al. 2020; Moroz et al. 2021 and references therein). New tools such as single-cell transcriptomics have become available and have already been tested in *Trichoplax* (Sebe-Pedros et al. 2018). So have in situ hybridizations (Figure 6.6; see also e.g. DuBuc et al. 2019), as well as RNAi gene silencing (e.g. Jakob et al. 2004), and other modern gene knockout techniques are soon going to be established in placozoans as well.



**FIGURE 6.6** Whole-mount in situ hybridization reveals the typical ring-shaped expression pattern of the ParaHox gene *Trox-2* in *Trichoplax adhaerens*. (Photo by Moritz J. Schmidt and Sonja Johannsmeier.)

At the organismal level, *Trichoplax* allows the use of the *cum grano salis* full spectrum of regeneration, re-aggregation and transplantation techniques (e.g. Schwartz 1984). The size, thickness, transparency and stability of the animals make them preferred objects for traditional and modern techniques of light and high-resolution electron microscopy (e.g. Guidi et al. 2011; Smith et al. 2021). By combining these, that is, the organismal and molecular potential, placozoans offer solid prospects to answer challenging questions.

## 6.8 CHALLENGING QUESTIONS

While some researchers still fight over the phylogenetic position of placozoans, others have realized and accepted the outstanding importance of an early metazoan animal that harbors all the core genes for the regulation of tissue architecture in metazoans. Most regulators are highly conserved (at different levels) between *Trichoplax* and humans, and we can use a simple *Trichoplax* model to learn important details about regulatory interplays in the much more complex worm, fly and mouse models. Thus, it comes as no surprise that the current questions we are asking *Trichoplax* range from “How can symmetry be derived from polarity?” to “What is the basic genetics behind apoptosis?” to “What are the initial genetic malfunctions that start cancer growth?”. And there will be many more to come.

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