TRICHOPLAX ADHAERENS: DISCOVERED AS A MISSING LINK, FORGOTTEN AS A HYDROZOAN, RE-DISCOVERED AS A KEY TO METAZOAN EVOLUTION

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TRICHOPLAX ADHAERENS
PLACOZOA
METAZOAN EVOLUTION
DIPLOBLASTS
PLACULA HYPOTHESIS
BILATEROGASTRAEA HYPOTHESIS
GALLERTOID HYPOTHESIS

ABSTRACT. – *Trichoplax adhaerens* is more simply organized than any living metazoan. After its original description by FE Schulze 1883, it attracted particular attention because it possibly possessed the basic and ancestral state of metazoan organization. The interest of zoologists and evolutionary biologists suddenly vanished for more than half a century when *Trichoplax* was claimed to be an aberrant hydrozoan planula larva. Recently, *Trichoplax* has been rediscovered as a key species for unraveling early metazoan evolution. Hox genes and whole genome sequencing promise insights into the genetics underlying the origin and development of basal metazoan phyla. We here review the history of research on *Trichoplax*, and provide a modern interpretation of special *Trichoplax* features in an evolutionary context.

TRICHOPLAX ADHAERENS
PLACOZOAIRES
ÉVOLUTION DES MÉTAZOAIRES
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HYPOTHÈSE DE LA PLACULA
HYPOTHÈSE DE LA
BILATEROGASTRAEA
HYPOTHÈSE DES GALLERTOIDES

RÉSUMÉ. – L'organisation de *Trichoplax adhaerens* est plus simple que celle des autres métazoaires vivants. Décrit par F E Schulze en 1883, cet organisme attirait l'attention des scientifiques qui pensaient d'abord que son organisation pourrait représenter un état basal et ancestral pour les métazoaires. Mais par la suite les zoologistes et les biologistes de l'évolution délaissèrent ce modèle, et durant plus d'un demi-siècle, *Trichoplax* passa pour une larve planula aberrante d'un Hydrozoaire. Récemment cet organisme a été redécouvert en tant qu'espèce clé pour la compréhension de l'évolution précoce des métazoaires. Les gènes Hox et le séquençage complet du génome devraient permettre d'aborder la génétique sous-jacente qui pourrait élucider l'origine et le développement des grands embranchements de métazoaires. Nous passons en revue l'histoire des recherches consacrées à *Trichoplax* et proposons une interprétation moderne des caractères particuliers de *Trichoplax* placés dans un contexte évolutif.

I. The discovery of Trichoplax adhaerens – Schulze's original description and Bütschli's "Placula-Hypothesis"

In 1883, the German zoologist Franz Eilhard Schulze published as a short communication the description of a new species, *Trichoplax adhaerens* (the "sticky hairy plate"; Greek *trich*=hair, *plax*=plate): a flattened, crawling marine animal of up to a few millimeters in size (Fig. 1). Schulze found these organisms settling on the glass sides of seawater aquaria at the University of Graz (Austria), recognizing their amoeba-like movements

and continual shape changes. These were new features for metazoan animals.

Schulze's histological analysis of *Trichoplax*, based on microtome sections and various staining procedures, revealed a three-layered sandwich organization of the animal, with morphologically different upper and lower epithelia. The epithelia enclose an inner, connective-tissue-like union of cells. A more detailed description was published by Schulze in 1891, and most of his results are still valid today (Fig. 2). The upper epithelium consists of a thin squamous layer while the lower epithelium consists of relatively large columnar cells and smaller gland cells. The lower epithelium is spe-
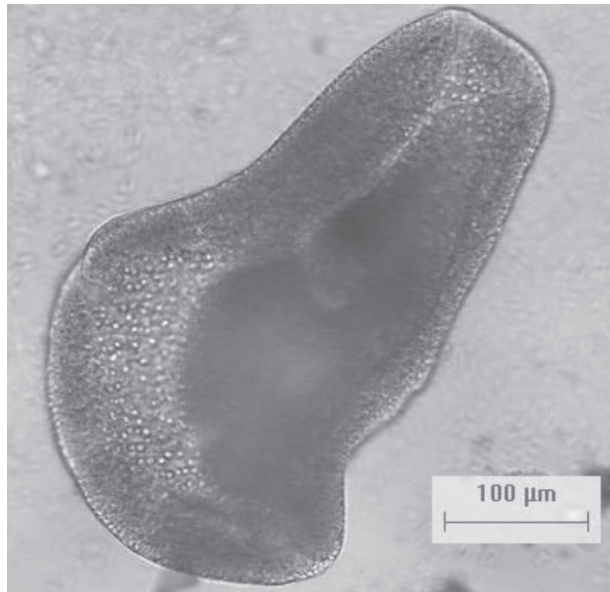
Fig. 1. – Photograph of living specimen of *Trichoplax adhaerens*. The animal shown is changing from an infolded (upper and right side) to its preferred flattened form (left side). The "shiny spheres" of the upper epithelium (cf. Fig. 2) are mainly visible on the left, where the body is already flattened.

cialized for extracellular digestion (the animal creeps over small food particles). The only obvious specialized structures of the upper epithelium are large lipid droplets, which were named "Glanzkugeln" ("shiny spheres") by Schulze (1891). Probably, the latter are degenerate cells that serve as a nutritive reservoir (but see also below, IV). Apparently degenerate cells are regularly found also in the lower epithelium. Cells of both epithelia are monociliated; the animal moves by ciliary walking. The interior cells are connected by cytoplasmatic extensions and form a three-dimensional meshwork. Schulze (1883, 1891) already noted that contractions of these cells cause the shape-changes of the animal. He named these contractile cells "Faserzellen" ("fibre cells").

Since only four somatic cell types are found in *Trichoplax*, and since noncellular structures such as a basal lamina and extracellular matrix (=ECM) are lacking, the animal is the most simply organized metazoan animal known. Moreover, because of the non-fixed outer shape, no axes of symmetry are present, and only a top-bottom polarity is seen (Fig. 2). Summarizing his first results, Schulze (1883) concluded that *Trichoplax* does not fit into any of the bauplan patterns of sponges, coelenterates (ctenophores and cnidarians), or the
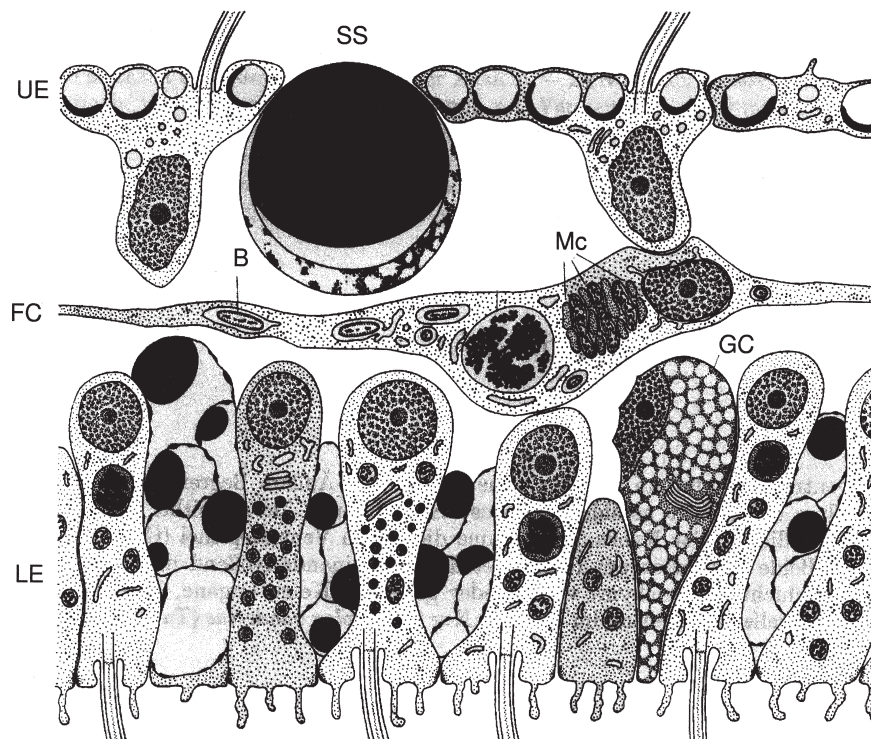

Fig. 2. – Schematic cross section of *Trichoplax adhaerens* (modified after Grell & Ruthmann 1991). UE = upper epithelium, LE = lower epithelium, FC = contractile fibre cell, GC = gland cell, SS = shiny sphere, Mc = mitochondrial complex, B = (endosymbiotic?) bacterium in endoplasmic reticulum. Note that the interspace between fibre cells and epithelia is free of ECM and that a basal lamina is missing.

vermiform phyla. Consequently, he assumed that *Trichoplax* was an isolated, basal offshoot close to the root of the metazoan phylogenetic tree.

Schulze's original description of *Trichoplax* soon sparked debate on the hypothetical first metazoan ("urmetazoa" or "archimetazoa") between Haeckel, Lankester, Metschnikoff, and other zoologists (for overview see: Gruner 1993). Only one year after Schulze's original description of *Trichoplax adhaerens*, O. Bütschli (University of Heidelberg) published an improved version of Haeckel's "gastraea-hypothesis". While Haeckel's "gastraea", a hypothetical spherical, pelagic organism, invaginates from a pelagic "blastaea" at its posterior pole, Bütschli (1884) tried to derive the gastraea from a flat, benthic-vagile ancestor, the hypothetical "placula" (cf. Fig. 3). According to Bütschli, the first metazoans emerged after colonial flagellates (Protozoa) fused into a benthic, single layered organism with ciliary locomotion. From this stage, the two-layered "placula" developed with an upper "ectoderm" and a lower "entoderm". Gradual invagination of the "entodermal" layer led to a benthic gastraea-like animal. The entodermal invagination finally led to closed gastric cavities or through-guts, as was already described in Haeckel's model.

Bütschli argued that the three-layered *Trichoplax* is a comparatively derived organism, still mirroring the two-layered placula´s mode of life. It is important to note that both Schulze and Bütschli agreed in interpreting the upper epithelium of *Trichoplax* as an ectoderm and the lower epithelium as an entoderm homolog. The question then arises whether the interior fibre cell complex of *Trichoplax* is a mesoderm homolog or not. Both authors hesitated to interpret the fibre cells as a mesoderm homolog because this would have implied a close affinity of *Trichoplax* to the triploblastic phyla. Bütschli (1884, p. 425) therefore saw in the fibre cell layer an analogy to mesodermal structures of triploblasts. Schulze (1883) pointed out that observations on the ontogeny of *Trichoplax* would be required to solve this question. Both researchers were aware of the prin-
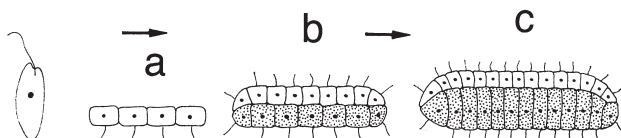
cipal counter-hypothesis that *Trichoplax* might be a secondarily simplified organism, as this was already proposed for some parasitic mesozoans. The latter alternative was regarded as relatively unlikely as there was no evidence for parasitism by *Trichoplax*. After the first morphological descriptions and resulting phylogenetic interpretations it was expected that elucidating the ontogeny and the life cycle of *Trichoplax adhaerens* would be the next crucial step in resolving the phylogenetic position of the Placozoa.

II. The hydrozoan-interpretation – *Trichoplax* is forgotten

In 1890, one year before Schulze's detailed *Trichoplax* description, FC Noll from the Senckenberg Museum in Frankfurt reported observations on the animals' normal mode of vegetative reproduction, which is binary fission of the entire body into two new individuals. Noll wrongly suggested the presence of otoliths in large *Trichoplax* specimens and thus a close relationship to the acoel turbellarians. This idea was supported by L von Graff (1891), an expert on Acoela. Schulze (1891) remained skeptical about this interpretation, mainly because of the arrangement of the inner contractile cells of the animal (which do not resemble a myoepithelium), and also because of the lack of any fixed axes of symmetry. At this time, most zoologists agreed with Schulze (1883) that the functional layer of contractile cells rejects a close relationship to either coelenterates or sponges. Through the end of the 19th century a close relationship of *Trichoplax* to acoel turbellarians or mesozoans was discussed. After FS Monticelli (1893, 1896) described another *Trichoplax*-like animal, *Treptoplax reptans*, both forms were united as Mesenchyma, in reference to the fibre cells, and grouped within the Mesozoa (Delage & Herouard 1899), a phylum that had already become "a dumping ground for a host of multicellular but presumed nonmetazoan organisms" (Brusca & Brusca 1990). True metazoan phyla were seen as showing an invaginating gastrula stage during embryogenesis (e.g., Neresheimer 1912), although this definition had been intensely debated from the very beginning.

The question of the complete life cycle of *Trichoplax* initially yielded a most surprising -- and completely wrong -- answer when the German zoologist Thilo Krumbach observed these animals in a seawater aquarium that was settled by sexual medusae of the hydrozoan *Eleutheria krohni* (Krumbach 1907). As in medusae of other *Eleutheria* species, for example *E. dichotoma*, the eggs of *E. krohni* develop in a brood pouch, which eventually opens to release well-developed planula larvae (Hauenschild 1956, Schierwater 1989,

Fig. 3. – Placula-hypothesis of metazoan evolution according to O Bütschli 1884. Flagellated protozoans unite to form a benthic-vagile, plate-like metazoan organism. The one-layered form (a) evolves to the two-layered "placula" (b). Upper cells of the placula are presumed ectoderm, while the lower cells adapt to nutritive function and thus are presumed entoderm (c). Note that there is no gradual transformation from stage a to b (modified from Gruner 1993).

Hadrys *et al.* 1990). Krumbach (1907) reported that he found *Trichoplax* individuals in exactly those positions where *Eleutheria* planulae had settled before. Krumbach was convinced that *Trichoplax adhaerens* was a deformed larva of *Eleutheria krohni*, although he never observed the supposed metamorphosis. Surely Krumbach's interpretations were influenced by speculations of other authors, who thought of *Trichoplax* as a "paranormal" organism that was unable to complete its life cycle under culture conditions (e.g., Ehlers 1887: 497). Although refused by Schulze (1891), this interpretation persisted in zoological textbooks of that time (e.g., Lankester 1901: 158). Krumbach's 1907 publication led to a corresponding statement on *Trichoplax* in the first installment of Bütschli's "Vorlesungen über vergleichende Anatomie" (1910) and was cited as fact in a reference book (Neresheimer 1912: 827). However, the hydrozoan interpretation was soon criticized by Schubotz (1912) and Schulze (1914). Schubotz compared the histological organization of *Eleutheria krohni* planulae and *Trichoplax*. He noted that the ectoderm of the planula already contains nematocysts, which would have to vanish during any transformation into a *Trichoplax*. Schulze completed Schubotz's argumentation by mentioning some special features of the inner fibre cell layer of *Trichoplax*. Note that Schulze's article of 1914 was the last publication on *Trichoplax* in a zoological journal for more than half a century.

How can an exciting animal like *Trichoplax* be pushed out of scientific research by a shaky larva-hypothesis ? It is an amazing fact that the completely unsupported larva-hypothesis remained in German, French, and Anglo-American textbooks for decades. After World War One, the first German encyclopedia on animal phyla was prepared by the meritorious zoologist W. Kuekenthal, who died one year before the first volumes were published in 1923 (also, F E Schulze died in 1921). The editor who finished the volumes was Thilo Krumbach, a supporter of the larva-hypothesis. Through his hands, volume one of the "Handbuch der Zoologie" contained the Protozoa, Porifera, Coelenterata, and Mesozoa. *Trichoplax* is briefly mentioned in the chapter "Hydroida" (H Broch, Oslo) and the chapter "Mesozoa" (M Hartmann, Berlin-Dahlem). Both authors interpreted *Trichoplax* as a transformed planula of *Eleutheria krohni*. Hartmann (p. 1014) cites Krumbach's paper from 1907 but ignores the replies of Schubotz (1912) and Schulze (1914). It was through this single, pivotal circumstance that Krumbach's larva-hypothesis became widely accepted. In her influential "Invertebrates", LH Hyman (1940) also cites only Krumbach's paper (p. 247) and ignores the other data: "... *Trichoplax* and *Treptoplax*, which have the construction of planulae, were found actually to be modified planulae of Hydroidea" (p. 243).

PP Grassé's "Traité de zoologie IV" (1961: 694) mentions *Trichoplax* in a similar way.

III. The rediscovery of **Trichoplax adhaerens** *and birth of the phylum "Placozoa"*

It is often said that *Trichoplax adhaerens* was rediscovered when the German protozoologist KG Grell (University of Tübingen) found this animal in an algal sample from the Red Sea in 1969. Although it is true that the first electron-microscopical examinations by Grell were decisive for the final falsification of Krumbach's larva-hypothesis, at this time the animal had already found its way back into science.

In July 1961, the cell biologist W Kuhl (University of Frankfurt) found *Trichoplax* in a seawater aquarium containing organisms from the Mediterranean Sea. Although Kuhl's research on *Trichoplax* concentrated on locomotion and regeneration, he and co-workers clearly stated that they had never observed any connection between *Trichoplax* and hydrozoans in the aquarium (Kuhl & Kuhl 1966: 433). At about the same time, *Trichoplax* was also cultured in Moscow, where it inspired Russian researchers to reinforce E Metschnikoff's phagocytella-hypothesis of metazoan evolution (Ivanov 1968, cited in Ivanov 1973, 1988).

When Grell (1971b, 1972, Grell & Benwitz 1974) discovered oogenesis and cleavage processes (after mixing *Trichoplax* clones from different locations) it became clear that *Trichoplax* specimens in culture represent an adult stage. Unfortunately, the embryos regularly died after reaching the 64-cell-stage (cf. Ruthmann *et al.* 1981, Grell 1984), and the further development of *Trichoplax* remains unknown. However, Grell's meticulous research provided sufficient support for placing *Trichoplax adhaerens* in a new phylum, the "Placozoa" (Grell 1971a). The new phylum was named after Bütschli's placula hypothesis, and consequently the Russian researchers around AV Ivanov responded by proposing a phylum "Phagocytellozoa" for *Trichoplax* (Ivanov 1973). Grell's phylum Placozoa has survived to this day and has marked the rediscovery of *Trichoplax adhaerens*.

German and Russian research groups focused mainly on morphological descriptions, while researchers in the US began fieldwork on placozoans (close to nothing was known about the biology of *Trichoplax* in its natural habitat). It quickly became clear that *Trichoplax adhaerens* could be found worldwide in the littoral of subtropical and tropical regions (e.g. Pearse 1989). *Treptoplax reptans* Monticelli 1893 (see II.) has never been found again, and its existence can be doubted.

Detailed electron-microscopical studies by the groups around KG Grell in Tübingen and

A Ruthmann in Bochum confirmed and extended Schulze's (1883, 1891) classical descriptions of *Trichoplax*. No basal lamina could be found, and the interspace between the fibre cells and epithelia was found to be free of any collagenous ECM (e.g. Grell & Benwitz 1971, 1981). For the fibre cells, a syncytial (Buchholz & Ruthmann 1995) and wide-meshed organization (instead of a compact mass; Stiasny 1903) was described. Interestingly, von Graff (1891) and Stiasny (1903) had described unicellular algae in the cell bodies of the fibre cells and interpreted them as symbiotic or commensal zooxanthelles. Wenderoth (1986)

found that algae and other food particles adhere to the slime layer of the upper epithelium and are subsequently phagocytized by the inner fibre cells. Food particles must be pulled through gaps of the upper epithelium, and Wenderoth (1986) called this unique mode of feeding "transepithelial cytophagy". Thus, the incorporated algae are prey. However, there may also be endosymbionts present in *Trichoplax*, as bacteria were regularly found in the endoplasmic reticulum of the fibre cells (Grell & Benwitz 1971).

The ability for transepithelial cytophagy indicates a relatively loose arrangement of the epithelia.
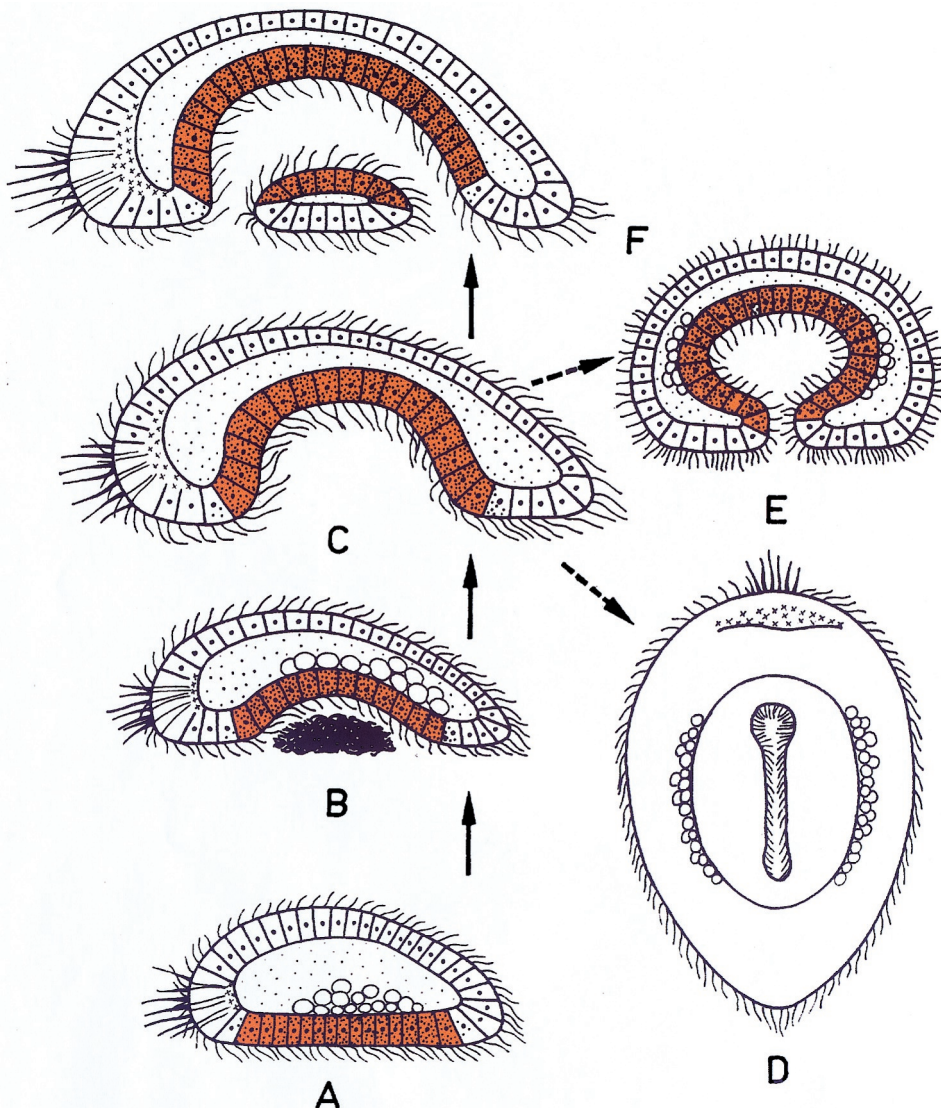

Fig. 4. – Bilaterogastraea-hypothesis of metazoan evolution according to G Jägersten 1955/59: A "benthoblastaea"-stage gives rise to the "bilaterogastraea" with a through-gut. A-benthoblastaea showing polarity, specialized lower epithelium and gonads, B- digesting benthoblastaea, C, E -benthoblastea changes to bilaterogastraea by developing an inner gastric cavity (D: transverse section showing "oral slit"), F: a through-gut develops by partial closing of the "oral slit". Note that the vagile benthoblastaea had already developed an A/P-axis and bilateral symmetry, inferring that a placozoan-construction without such axes of symmetry would have to be derived from an earlier stage. In the given scenario, the presumed entoderm of the benthoblastaea (orange color) would be homologous to the nutritive lower epithelium of *Trichoplax*.

Only two types of epithelial cell-cell connections are present in *Trichoplax*, belt and septate desmosomes (Ruthmann *et al.* 1986, Ruthmann 2000). Connections between the epithelia and the fibre cells remain unknown. It seems likely that these connections get continually rearranged. Studies on isolated fibre cells (Thiemann & Ruthmann 1989) revealed their ability to build up cytoplasmatic extensions by microtubuli-assembly. Those extensions are probably mediated by an actinomyosin system (Ruthmann 2000). Since isolated fibre cells live for hours in seawater, Grell & Ruthmann (1991) suggest that the interspace between epithelia and fibre cells may not be very different from the seawater medium. The loose arrangement of the epithelia and the lack of an underlying basal lamina support this idea.

IV. Trichoplax adhaerens, *the "Archimetazoon"?*

With the beginning of the molecular revolution in biology, molecular phylogenetic approaches have been used in an attempt to unravel the phylogenetic position of the Placozoa. Ironically some of the modern molecular analyses moved the phylogenetic view on *Trichoplax* back to the turn of the century, and Krumbach's larva hypothesis almost was rejuvenated in a "phylogenetic" version. Based on DNA sequence analyses of ribosomal genes it was proposed that the phylogenetic position of *Trichoplax* could fall within or very close to Cnidaria, and therefore placozoans could potentially be derived from a neotenic planula larva (e.g. Bridge 1994, Aleshin *et al.* 1995, Collins 1998). Later, limitations of rRNA sequence based phylogenies were noted and so far rRNA sequences have failed to unambiguously resolve evolutionary relationships among the diploblast phyla (perhaps due to the age of these groups; cf. Adoutte *et al.* 2000). The use of new molecular markers, such as mtDNA and rRNA molecular morphology, however, has supported the hypothesis that Placozoa are not very closely related to Cnidaria (Ender &

Schierwater 2002). For example, the secondary structure of the 16S rRNA molecule is substantially more complex in *Trichoplax* than what is known in any cnidarian. At present, molecular systematics has not resolved the issue of the phylogenetic position of the Placozoa. The sum of evidence available to date suggests a basal position for *Trichoplax* within Metazoa (e.g. Schierwater & DeSalle 2001, Syed & Schierwater 2002, Ender & Schierwater 2002). Resolving this issue is clearly of key importance to our understanding of the origin of the Metazoa.

Identification of the earliest diverging lineage of metazoans will be of decisive value for reconstructing the hypothetical urmetazoan bauplan, and thus early character evolution within Metazoa. In a previous paper we reconstructed the placozoan bauplan as derived from hypothetical "gallertoid" precursors (compare Fig. 5) and we argued in detail against the evolutionary possibility of a gradual transformation of a neotenic cnidarian planula larva into a placozoan (Syed & Schierwater 2002). Although planulae occur in a great variety of forms and creeping planulae exist (e.g. Tardent 1978), two main morphological problems contradict the larva-hypothesis. Essentially, planulae are composed of an outer ectoblastema and an inner endoblastema with both blastemas connected by a thin mesogloea. If one takes such a bilayered construction as a placozoan ancestor, one must explain how the interior contractile fibre cells of placozoans emerged. Furthermore, if one regards the "nutritive" endoblastema of a planula as a homolog to the "nutritive" lower epithelium of *Trichoplax*, it becomes difficult to explain why and how the inner cell layer was exposed to the outside. For these and other reasons (cf. Syed & Schierwater 2002) the "phylogenetic" larva-hypothesis seems as unrealistic as Krumbach's (1907) "ontogenetic" precursor.

If we regard the Placozoa as a phylum of animals whose simple organization is primary, the nutritive lower epithelium and the interior fibre cells of *Trichoplax* require particular discussion.

Fig. 5a, b. − Gallertoid-hypothesis of metazoan evolution according to Bonik *et al.* 1976.
a) pelagic gallertoid (=blastaea-like form with inner contractile cells and jelly-like ECM) develops inner canal systems, later giving rise to sponges, coelenterates and bilaterians (modified after Gutmann 1989). a.1.- early gallertoid (e=epithelium, c=contractile cell, g=gelatinous ECM), a.2., a.3.- infolding of grooves and canals, a.4.- superficial canals can be displaced into the interior of the body.
b) flattened, benthic gallertoids give rise to placozoan constructions, and probably some of the Ediacaran "problematica" (modified from Syed & Schierwater 2002). b.1. - flattened, benthic gallertoid b.2., b.3.- specialization of the lower epithelium and reduction of ECM. b.4. - recent *Trichoplax adhaerens* showing different (optimized) modes of locomotion, nutrition and reproduction. Note that the nutritive epithelium of *Trichoplax* (yellow color) evolves independently from the presumed entoderm of the other phyla (red color). Swarmers are shown in blue. Details can be found in Syed & Schierwater 2002.
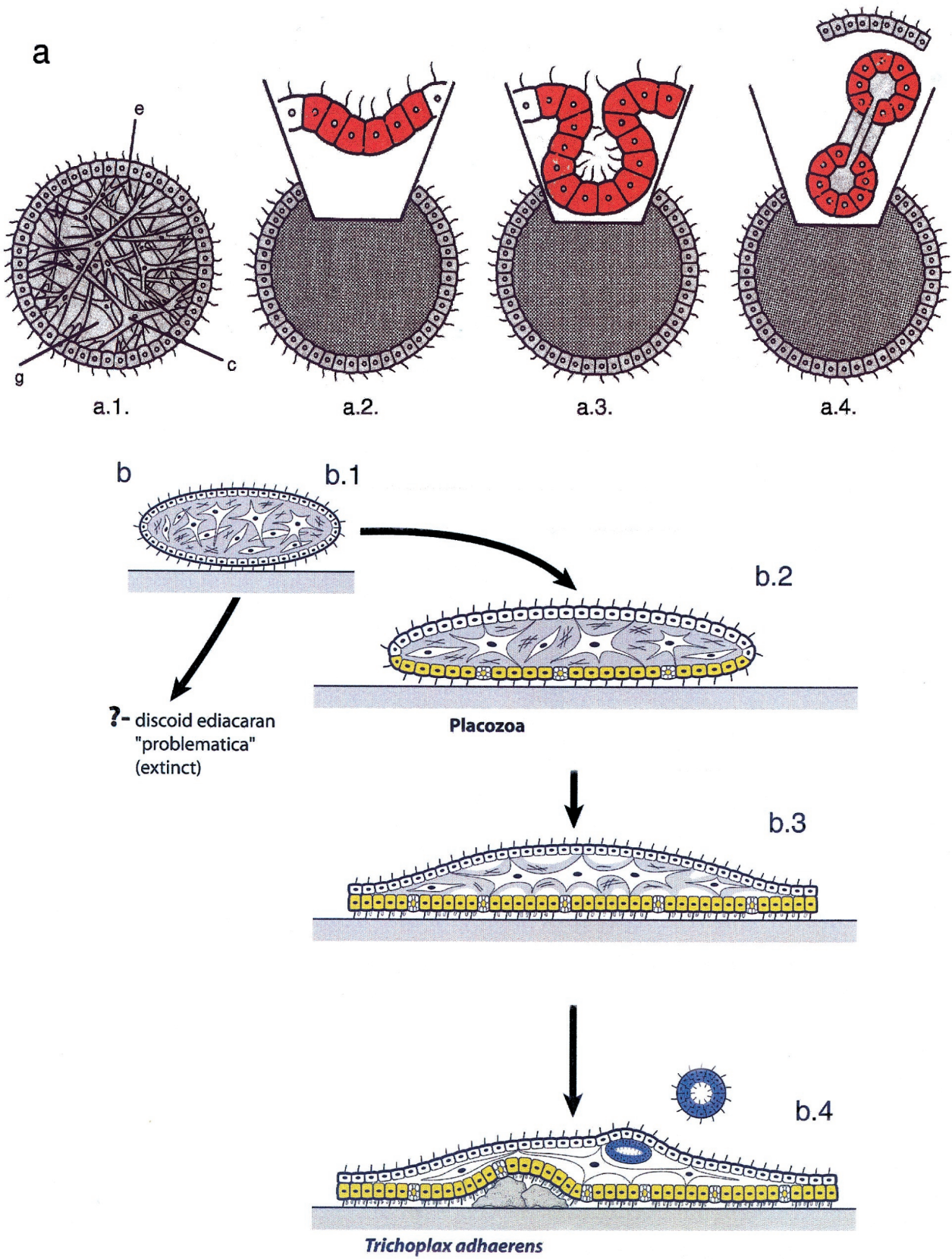
The specialized lower ("nutritive") epithelium: Precursor of the Entoderm?

As Bartolomaeus (1993) and Ax (1995) pointed out, the term "entoderm" has always been used in a topographic sense, i.e. it has only been applied to inner nutritive cell layers. Nonetheless, in a cladistic analysis, Ax (1995) considered the nutritive epithelium of *Trichoplax* as entoderm, implying homology. As a consequence – if one assumes a basal position for *Trichoplax*, the possibility we are addressing here – one faces the hypothetical evolutionary scenario of a *Trichoplax*-like, benthic animal, which gradually had its lower, nutritive epithelium displaced into the interior of the body. This is the case in both the placula hypothesis (Fig. 3) and the so-called benthoblastea/ bilaterogastraea hypothesis of Jägersten (1955, 1959; cf. Fig. 4). Both are modified versions of Haeckel's blastaea-gastraea hypothesis and propose benthic vagile forms as the first metazoans. Considering the nutritive epithelium of *Trichoplax* to be an independently evolved character instead of an entoderm homolog has some explanatory advantages. Specifically, if the earliest lineages of metazoans are derived from a small pelagic metazoan, adaptation of the lower epithelium in the line leading to the recent *Trichoplax* and an infolding of inner canal systems in the line leading to the other metazoan phyla appear reasonable (cf. Fig. 5).

At present we are left with two plausible evolutionary scenarios:

1. a benthic-vagile, flattened urmetazoan with an adapted lower epithelium (=homology scenario, as it is assumed in the placula/benthoblastaea/ bilaterogastraea hypothesis, Fig. 4); 2. a pelagic urmetazoan of variable outer shape (depending on the arrangement of inner contractile elements): flat individuals adapt to benthic vagile life, while the remaining pelagic forms develop inner canal systems for ventilation and filtration/digesting (=analogy scenario, as it is assumed in the gallertoid hypothesis, Fig. 5).

It is noteworthy that the upper and lower epithelia of *Trichoplax* are often misleadingly called "dorsal" and "ventral" epithelia. Since these terms are reserved for bilaterian animals -- and here defined upon the possession of a mouth -- one should avoid these terms in reference to *Trichoplax*. Grell (1971) proposed the functional terms "protective" and "nutritive" for the upper and lower epithelia, respectively. Observations by Vicki Pearse and us support the term "protective" for the upper layer in a more concrete way than originally proposed by Grell. Potential predators usually shrink back when encountering placozoans, perhaps because the shiny spheres (cf. Figs 1, 2) serve as reservoirs of chemical defense substances (V Pearse, pers comm). A biochemical analysis of the shiny spheres would be desirable.

It should further be mentioned that some textbooks incorrectly describe some suprastructures of the lower epithelium of *Trichoplax* as "microvilli" (comp. Fig. 2). These suprastructures are not comparable to typical "entodermal" microvilli, since they consist of a system of fenestrated ledges and folds which likely have an important function in the animals' ability to adhere to flat surfaces (Grell & Benwitz 1981).

In summary, it will be difficult to verify the homology or analogy hypothesis. Potentially, a biochemical characterization of digestive enzymes, which are thought to be produced by the gland cells of the nutritive epithelium, and a molecular characterization of entodermal gene expression would be informative features to investigate.

The interior fibre cells: mesenchyme, parenchyme, or mesoderm?

As discussed above (I), Schulze and Bütschli hesitated to denote the inner fibre cell layer of *Trichoplax* as mesoderm, so as not to imply homology. When the phylum Placozoa was established, the fibre cells were denoted as "mesenchyme-like" by Grell & Benwitz (1971). This more neutral term is still in use (e.g. Buchholz & Ruthmann 1995) and means "connective tissue-like" here (not undifferentiated embryonal tissue, as it is often used). Connective tissue cells are normally embedded in an ECM. Because there is no ECM in placozoans, and also because the fibre cells are clearly involved in the "amoeboid" locomotion of the animal, the term "mesenchyme" appears to be inadequate. Ivanov (1973, 1988) appropriately described the fibre cell system as "differentiated multifunctional tissue" and used the term "parenchyme" for it (also v. Salvini-Plawen 1978).

Given that the diploblast-triploblast distinction may become subject to debate, the question of the putative historical relationship of the fibre cells to typical mesodermal structures of triploblastic phyla may become of interest. Referring to the models outlined above, one can discuss the following theoretical scenarios.

The gallertoid hypothesis (Bonik *et al.* 1976) describes an early differentiation between outer epithelial and inner mesenchymal (in the sense of connective tissue) cells. Deriving a placozoan from this stage implies reduction of ECM and basal lamina (comp. Fig. 5, see also Syed & Schierwater 2002). As a consequence, the mesenchymal cells gradually increase their function in co-ordination of movement. This scenario implies that both placozoan fibre cells and all muscular cell types of other phyla can be derived from contractile mesenchymal cells of early pelagic gallertoids. According to this model both lines diverge very early, which – together with biomechanical reasons – could explain why placozoan fibre cells are diffi-

cult to compare to other muscular cell types or mesodermal tissues, as they are known from more derived phyla.

Because 19th century models of early metazoan evolution mainly focused on the ectoderm-entoderm specialization, they offer less precise statements about connective tissue-like elements. Therefore, Haeckel's classical blastaea-gastraea model was often criticized from a biomechanical point of view, as it is hard to imagine how gradual invagination and forming of inner canals could be stabilized in a hollow sphere (e.g., Bonik *et al.* 1978). Jägersten (1959: 99), whose bilaterogastraea-hypothesis was based on Haeckel's view, addressed this problem by modifying the early benthoblastaea-stages: "It is quite conceivable, and nothing prevents it, that the entire blastocoel was filled with a mesogloea-like substance, containing also somatic cells that had immigrated from the blastoderm. In other words, it is possible that a kind of mesoderm existed even prior to the evolution of the entoderm and the intestine." Thus, Jägersten (1959: 100) describes the term "mesoderm" as follows: "It is now obvious that the mesoderm is not a uniform germ layer in the same sense as the ectoderm and the entoderm. The very fact that it is formed exclusively mesenchymatically in several recent groups, but mainly the circumstance that this way of formation ought to be considered as the original in the metazoans, prevents its interpretation as a strict germ layer." To some extent, this view fits modern definitions of the mesoderm, as they are given by Bartolomaeus (1993) and Ax (1995).

Moreover, it seems clear that the gallertoid-hypothesis and the bilaterogastraea-hypothesis describe nearly the same type of earliest metazoan, from which the Placozoa would have to be derived. The assumption of Jägersten (1955) and Bonik *et al.* (1978) that a mesogloea-like connective tissue served as precursor of the later mesoderm may find support in current molecular studies. Homologs of triploblast mesodermal transcription factors were found to be expressed in the entocodon (an interconnecting structure) of a hydrozoan (Spring *et al.* 2000).

V. Two models of metazoan evolution

At the time when Jägersten (1955, 1959) outlined his bilaterogastraea-model of early metazoan evolution, *Trichoplax* was still assumed to be an aberrant hydrozoan planula and therefore not considered as a distinct phylum. Thus, after Grell established the phylum Placozoa in 1971, the bilaterogastraea-hypothesis was modified by some authors. While Jägersten (1955) derived his bilaterogastraea from a "benthoblastaea" (comp. Fig. 4), Grell (1971) proposed a placula sensu Bütschli (Fig. 3) as precursor of the bilaterogastraea.

Today such modified versions of Jägersten's bilaterogastraea-hypothesis occur in several textbooks (e.g. Siewing 1987, Erben 1990, Ax 1995, Ruthmann 2000). However, there are some critical points in this scenario to note. First, Bütschli's (1884) original model of a one-layered organism that develops a second layer – resulting in precursors of the ecto- and entoderm – looks dubious, because this scenario hides a phylogenetic saltation. The two-layered stage cannot emerge in a gradual way (comp. Fig. 3). Bütschli did not mention this problem. He notes, however, that there is no plausible selective advantage for the newly developed placula.

Bütschli regarded the three-layered *Trichoplax* as derived from the two-layered placula. This is another critical point of the placula-hypothesis, because it is not straightforward how the interior fibre cells could have gradually emerged from a two-layered construction. It might be helpful in this regard to examine the hollow amphiblastula of some sponges, which flatten out after settlement (Gruner 1993). Also, the behavior of *Trichoplax adhaerens* may provide insight on this question. Starving placozoans change from their normal flattened shape to a spherical form, as the interior fibre cells degenerate and lose contact with the epithelia (personal observations). These starving forms are not able to regenerate and soon die (see also Thiemann & Ruthmann 1990). Thus, Jägersten's (1959) improved description of the benthoblastaea (as cited in IV) and the "gallertoid" as proposed by Bonik *et al.* (1976) are among the preferable models of early metazoans.

When comparing the gallertoid-hypothesis with Jägersten's benthoblastaea-bilaterogastraea scenario, there is one important difference regarding the evolution of placozoans. In Jägersten's model, the early benthic metazoans develop an anterior-posterior polarity before transforming to the bilaterogastraea-stage (cf. Fig. 4). Since *Trichoplax* does not exhibit any indication of an A/P polarity, we prefer the alternative shown in Fig. 5. According to this view, Placozoa would be the oldest extant metazoan group, probably a sister group of some enigmatic discoid "Vendobionta" as they are known from about 600 million year old strata (comp. Fig. 5 and Syed & Schierwater 2002).

VI. Current Research and Conclusions

Trichoplax attracts the attention of modern multi-disciplinary research for at least three good reasons. First, it is the most simply organized metazoan animal; second, it possesses the smallest genome of all known metazoans (Ruthmann & Wenderoth 1975, Ruthmann 1977, Ruthmann *et al.* 1981); and third, it might be relatively basal to all recent metazoan phyla (see above). Thus understanding the genetic control of its development will

redefine the basic, and possibly also the ancestral genetic programming of metazoan organization. With excitement we await the answers to how many genes control the development of a basic metazoan bauplan, how big these genes are, how different they are compared to protists, how regulatory genes in *Trichoplax* have switched or gained new functions in derived animals, and how these genes interact. For example, at present it seems that *Trichoplax* possesses a single Hox gene only (Schierwater & Kuhn 1998), which is substantially smaller and more simply structured than other Hox genes (Schierwater & DeSalle 2001), and which in sharp contrast to those of higher animals has no function with respect to the formation of body polarity (Jakob *et al.* in prep; see also Schierwater & DeSalle 2001). Furthermore, the deduced amino acid sequence of the *Trichoplax* Hox gene, *Trox-2*, looks like a genetic chimera that harbors diagnostic domains from several families of Hox genes (Kuhn *et al.* 1999, Schierwater & DeSalle 2002). At the same time we study the genetics, and particularly the developmental genetics, we need to unravel the complete life cycle, describe the morphological changes during development and reproduction in more detail, and finally resolve the phylogenetic position of *Trichoplax* and the relationships among the diploblastic animals. The answer to the latter seems close, the complete mtDNA genome of *Trichoplax* has been sequenced (Stephen Dellaporta, Bernd Schierwater, and co-workers), and comparative mtDNA genomes from sponges, hydrozoans, scyphozoans, cubozoans, and ctenophores are being sequenced now. At present it is probable that *Trichoplax* is not very closely related to the cnidarians (Ender & Schierwater 2002) and that only the Placozoa or Porifera qualify as candidates for the basal position within recent diploblasts.

Some key answers to the above questions are expected soon, since expression and functional studies on regulatory genes and whole genome sequencing of the *Trichoplax* genome are in progress (*Trichoplax* Consortium 2002). Soon the no-longer hydrozoan *Trichoplax adhaerens* might become what the hydrozoan *Hydra* has been, one of the most promising diploblastic model organisms for development and evolution (Tardent 1988).

LITERATURE

Adoutte A, Balavoine G, Lartillot N *et al.* 2000. The new animal phylogeny. Reliability and implications. *Proc Nat Am Sc* 97 (9): 4453-4456.

Aleshin VV, Vladychenskaya NS, Kedrova OS *et al.* 1995. Phylogeny of invertebrates deduced from 18S rRNA comparisons. *Molecular Biology* 29 (6): 843-855.

Ax P 1995. Das System der Metazoa I. Gustav Fischer, Jena-New York.

Bartolomaeus T 1993. Die Leibeshöhlenverhältnisse und Nephridialorgane der Bilateria-Ultrastruktur, Entwicklung und Evolution. Habilitationsschr Univ Göttingen.

Behrendt G, Ruthmann A 1986. The cytoskeleton of the fiber cells of *Trichoplax adhaerens* (Placozoa). *Zoomorphology* 106: 123-130.

Bonik K, Grasshoff M, Gutmann WF 1976. Die Evolution der Tierkonstruktionen I. *Natur und Museum* 106: 129-143.

Bonik K, Grasshoff M, Gutmann WF 1978. Warum die Gastraea-Theorie Haeckels abgelöst werden muß. *Natur und Museum* 108 (4): 106-117.

Bridge DM 1994. Phylogeny and life cycle evolution in the phylum Cnidaria. PHD-Thesis, Yale University.

Brusca RC, Brusca GJ 1990. Invertebrates. Sinauer Associates, Sunderland, Massachusetts.

Buchholz K, Ruthmann A 1995. The mesenchyme-like layer of the fibre cells of *Trichoplax adhaerens*: A syncytium. *Z Naturforsch* 50c: 282-285.

Bütschli O 1884. Bemerkungen zur Gastraea-Theorie. *Morph Jahrb* 9: 415-427.

Bütschli O 1910. Vorlesungen über vergleichende Anatomie. 1. Lieferung: Einleitung; vergleichende Anatomie der Protozoen, Integument und Skelet der Metazoen. W Engelmann, Leipzig.

Collins AG 1998. Evaluating multiple alternative hypotheses for the origin of Bilateria: An analysis of 18SrRNA molecular evidence. *Proc Nat Acad Sci USA* 95: 15458-15463.

Delage Y, Herouard E 1899. Traité de Zoologie Concrète II: Classe Mesenchymiens-Mesenchymia. Masson, Paris, 1: 9-12.

Ehlers E 1887. Zur Auffassung des *Polyparium ambulans* (Korotneff). *Zeitschr f wiss Zool* 45: 491-498.

Ender A, Schierwater B 2002. Placozoa are not derived cnidarians: Evidence from molecular morphology. *Mol Biol Evol* (in press).

Erben HK 1990. Evolution. Enke, Stuttgart.

Graff L v 1891. Die Organisation der Turbellaria acoela. W Engelmann, Leipzig.

Grassé PP 1961. Traité de Zoologie IV: Plathelminthes, Mésozoaires, Acantocéphales, Némertiens. Masson, Paris.

Grell KG 1971a. *Trichoplax adhaerens* und die Entstehung der Metazoen. *Naturw Rundsch* 24 (4): 160-161.

Grell KG 1971b. Embryonalentwicklung bei *Trichoplax adhaerens* F.E. Schulze. *Naturwiss* 58: 570.

Grell KG 1972. Eibildung und Furchung von *Trichoplax adhaerens* F.E. Schulze (Placozoa). *Z Morph Tiere* 73: 297-314.

Grell KG 1984. Reproduction of Placozoa. *Adv Invertebr Reprod* 3: 541-546.

Grell KG, Benwitz G 1971. Die Ultrastruktur von *Trichoplax adhaerens* F.E. Schulze. *Cytobiologie* 4: 216-240.

Grell KG, Benwitz G 1974. Elektronenmikroskopische Beobachtungen über das Wachstum der Eizelle und die Bildung der "Befruchtungsmembran" von *Trichoplax adhaerens* F.E. Schulze (Placozoa). *Z Morph Tiere* 79: 295-310.

Grell KG, Benwitz G 1981. Ergänzende Untersuchungen zur Ultrastruktur von *Trichoplax adhaerens* F.E. Schulze (Placozoa). *Zoomorphology* 98: 47-67.

Grell KG, Ruthmann A 1991. Placozoa. *In* Microscopic Anatomy of Invertebrates. Edited by FW Harrison & JA Westfall, Wiley-Liss, New York: 13-28.

Gruner HE 1993. Einführung, Protozoa, Placozoa, Porifera. *In* Lehrbuch der speziellen Zoologie Band I. Edited by A Kaestner, Fischer, Jena, 1.

Gutmann WF 1989. Die Evolution hydraulischer Konstruktionen. Waldemar Kramer, Frankfurt.

Hadrys H, Schierwater B, Mrowka W 1990. The feeding behaviour of a semi-sessile hydromedusa and how it is affected by the mode of reproduction. *Anim Behav* 40: 935-944.

Hauenschild C 1956. Experimentelle Untersuchungen über die Entstehung asexueller Klone bei der Hydromeduse *Eleutheria dichotoma*. *Z Naturforsch* 11b: 394-402.

Hyman LH 1940. The invertebrates. Protozoa through Ctenophora. Mc Graw Hill, New York.

Ivanov AV 1973. *Trichoplax adhaerens*, a phagocytella-like animal. *Zoologiceskij Zurnal 52*: 1117-1131 (Russian with English abstract).

Ivanov AV 1988. On the early evolution of the Bilateria. *Fortschr Zool* 36: 349-352.

Jägersten G 1955. On the early evolution of the metazoa. The Bilaterogastraea theory. *Zoologiska Bidrag* 30: 321-354.

Jägersten G 1959. Further remarks on the early phylogeny of metazoa. *Zoologiska Bidrag* 33: 79-108.

Krumbach T 1907. *Trichoplax*, die umgewandelte Planula einer Hydromeduse. *Zool Anz* 31: 450-454.

Kuhl W, Kuhl G 1966. Untersuchungen über das Bewegungsverhalten von *Trichoplax adhaerens* F.E. Schulze. *Zeitschr Ökolog Morph Tiere* 56: 417-435.

Kuhn K, Streit B, Schierwater B 1999. Isolation of Hox genes from the Scyphozoan *Cassiopeia xamachana*: Implications for the early evolution of Hox genes. *J Exp Zool (Mol Dev Evol)* 285: 63-75.

Lankester ER 1901. A treatise on Zoology Part IV: Platyhelmia, Mesozoa and Nemertini. A & C Black, London.

Monticelli FS 1893. *Treptoplax reptans* n.g., n.sp. *Atti dell´ Academia dei Lincei, Rendiconti* (5)II: 39-40.

Monticelli FS 1896. Adelotacta Zoologica. *Mittheil zoolog Station Neapel* 12: 432-462.

Neresheimer E 1912. Mesozoen. *In* Handwörterbuch der Naturwissenschaften. Edited by E Korschelt *et al.* Gustav Fischer, Jena, Band VI: 817-829.

Noll FC 1890. Über das Leben niederer Seetiere. *Ber Senckenb Naturforsch Gesellsch (Abt. Berichte)*: 85-87.

Pearse V 1989. Growth and Behaviour of *Trichoplax adhaerens*: First Record of the Phylum Placozoa in Hawaii. *Pacific Science* 43 (2): 117-121.

Ruthmann A 1977. Cell differentiation, DNA content and chromosomes of *Trichoplax adhaerens* F.E. Schulze. *Cytobiologie* 10: 58-64.

Ruthmann A 2000. Evolution und die Vielfalt des Lebens. Shaker, Aachen.

Ruthmann A, Behrendt G, Wahl R 1986. The ventral epithelium of *Trichoplax adhaerens* (Placozoa). *Zoomorphology* 106: 115-122.

Ruthmann A, Grell KG, Benwitz G 1981. DNA-content and fragmentation of the egg-nucleus of *Trichoplax adhaerens*. *Z Naturforsch* 60: 564-567.

Ruthmann A, Wenderoth H 1975. Der DNA-Gehalt des primitiven Metazoons *Trichoplax adhaerens* F.E. Schulze. *Cytobiologie* 10: 421-431.

Salvini-Plawen L v 1978. On the origin and evolution of the lower Metazoa. *Z zool Syst Evolut Forsch* 16: 40-88.

Schierwater B 1989. Allometric changes during growth and reproduction in *Eleutheria dichotoma* (Hydrozoa, Athecata) and the problem of estimating body size in a microscopic animal. *J Morphol* 200*:* 255-267.

Schierwater B, Kuhn K 1998. Homology of Hox genes and the zootype concept of early metazoan evolution. *Mol Phyl Evol* 9 (3): 375-381.

Schierwater B, Dellaporta S, DeSalle R 2002. Is the evolution of *Cnox-2* Hox/ParaHox genes multicolored and polygenealogical? *Mol Phyl Evol* 24 (3): 374-378.

Schierwater B, DeSalle R 2001. Current Problems with the Invention of the Zootype. *J Exp Zool (Mol Dev Evol)* 291: 169-174.

Schubotz H 1912. Ist *Trichoplax* die umgewandelte Planula einer Hydromeduse? *Zool Anz* 39: 582-585.

Schulze FE 1883. *Trichoplax adhaerens* nov. gen. nov. spec. *Zool Anz* 6: 92-97.

Schulze FE 1891. Über *Trichoplax adhaerens*. *Physik Abh Kgl Akad Wiss Berlin:* 1-23.

Schulze FE 1914. Einige kritische Bemerkungen zu neueren Mitteilungen über *Trichoplax*. *Zool Anz* 64 (1): 33-35.

Siewing R 1987. Evolution. G Fischer, Stuttgart.

Spring J, Yanze N, Middel AM *et al.* 2000. The mesoderm specification factor Twist in the life cycle of jellyfish. *Developmental Biology* 228: 363-375.

Stiasny G 1903. Einige histologische Details über *Trichoplax adhaerens*. *Zeitschr wiss Zool* 75: 430-436.

Syed T, Schierwater B 2002. The Evolution of the Placozoa: A new morphological model. *Senckenbergiana lethaea* 82 (1): 315-324.

Tardent P 1978. Coelenterata, Cnidaria. *In* Morphogenese der Tiere, I Reihe, Edited by F Seidel, Gustav Fischer, Stuttgart: 83-289.

Tardent P 1988. Hydra. Neujahrblatt der Naturforschenden Gesellschaft in Zürich. NGZH, Zürich.

Thiemann M, Ruthmann A 1989. Microfilaments and microtubules in isolated fibre cells of *Trichoplax adhaerens* (Placozoa). *Zoomorphology* 109: 89-96.

Thiemann M, Ruthmann A 1990. Spherical forms of *Trichoplax adhaerens*. *Zoomorphology* 110: 37-45.

Wenderoth H 1986. Transepithelial cytophagy by *Trichoplax adhaerens* F.E.Schulze (Placozoa) feeding on yeast. *Z Naturforsch* 41c: 343-347.