## ДИСКУССИИ

# THE ORIGIN AND EARLY EVOLUTION OF NEODERMATA (PLATYHELMINTHES): 1. ON THE POSSIBLE TURBELLARIAN ROOTS OF THE GROUP — MORPHOLOGICAL APPROACH

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Submitted 29.01.2018

A comparative analysis of molecular based phylogenies of Platyhelminthes and morphological data is provided. Some widespread mistakes in literature (the confusion of Cercomeromorpha hypothesis by Bychowsky and Cercomorpha hypothesis by Janicki, the inclusion of turbellarians PNUK into the family Genostomatidae, etc) are revealed. Synapomorphies for Neodermata proposed by different authors are critically assessed. The ultrastructure of flame bulbs in Neodermata is shown to be a plesiomorphic character rather than a synapomorphy. The morphological analysis proves that Neodermata evolved from the turbellarians close to the early Neoophora. Only the following synapomorphies of Neodermata do not give rise to doubt: 1) the neodermis; 2) the appearance of ciliated larvae; 3) the collar receptors with dense collar inserted into the membrane at the apical level. Other features may be synapomorphies as well as plesiomorphies as well as homoplasies.

*Key words*: Platyhelminthes, «Turbellaria», Neodermata, phylogeny, synapomorphy, plesiomorphy, molecular phylogeny, comparative morphology.

# ПРОИСХОЖДЕНИЕ И РАННЯЯ ЭВОЛЮЦИЯ NEODERMATA (PLATYHELMINTHES): 1. К ВОПРОСУ О ВОЗМОЖНЫХ ТУРБЕЛЛЯРНЫХ КОРНЯХ NEODERMATA — МОРФОЛОГИЧЕСКИЙ ПОДХОД

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Поступила 29.01.2018

Представлен сравнительный анализ филогений Platyhelminthes, основанный на молекулярных исследованиях и морфологических данных. Выявлены некоторые ши-

роко распространенные ошибки в литературе (смешение гипотезы Cercomeromorpha Быховского и гипотезы Cercomorpha Яницкого, включение турбеллярий PNUK в сем. Genostomatidae и т. д.). Критически проанализированы синапоморфии Neodermata, предложенные различными авторами. Показано, что ультраструктура цирто-цитов является плезиоморфным признаком, а не синапоморфным. Морфологический анализ показывает, что Neodermata произошли от турбеллярий, близких к ранним Neoophora. Только следующие синапоморфии Neodermata не вызывают сомнений: 1) неодермис; 2) возникновение ресничной личинки; 3) коллар-рецепторы с электронно-плотным колларом, прикрепленным к мембране на апикальном уровне. Остальные характеристики могут быть как синапоморфиями, так и плезиоморфиями или гомоплазиями.

*Ключевые слова*: Platyhelminthes, «Turbellaria», Neodermata, филогения, синапоморфия, плезиоморфия, молекулярная филогения, сравнительная морфология.

## INTRODUCTION

The phylum Platyhelminthes was long considered to be comprised of several groups of primitive worms: Turbellaria, most of them free-living, and three groups of obligate-parasitic worms, Monogenea, Cestoda (Eucestoda + Cestodaria), and Trematoda (Digenea + Aspidogastrea). Ehlers (1985) established the monophyly of the obligate-parasitic groups of Platyhelminthes with evidence mainly derived from ultrastructural characters and united them in the group Neodermata. Later the monophyly of Neodermata has been confirmed in a number of molecular studies and now of that there is no doubt. In the last decades the evolution and phylogeny of Platyhelminthes and especially of Neodermata have been debated extensively. Traditional phylogeny based on morphology implied that «Turbellaria» was the most primitive group among the Platyhelminthes. Among the obligate-parasitic groups Monogenea was considered as the primary group originated from «Turbellaria», possibly from Rhabdocoela. Bychowsky (1937, 1957) assumed the common origin of Monogenea and Cestoda, and erected a monophyletic group Cercomeromorphae. There is a widespread mistake in the literature: the confusion of the Cercomeromorphae hypothesis by Bychowsky and Cercomorpha hypothesis by Janicki (1921). These are two different hypotheses based on different supposed homologies. Janicki considered the posterior attachment disc of Monogenea, the larval tails of Cestoda, and tails of cercaria of Digenea as homological structures, while Bychowsky homologized the attachment disc of Monogenea (opisthaptor), the part of the oncosphere (the first life cycle stage of Eucestoda), the posterior organ of Gyrocotylida, and caudal part of lycophora larva of Amphilinida, all of them bearing hooks.

Traditionally the evolution of Trematoda has always been considered separately from Monogenea and Cestoda, which is the early trematodes, acquired a parasitic way of life independently from other groups and their primary hosts were mollusks Gastropoda. The hermaphroditic generation (marita) in their very complex life cycle appeared later and evolved independently from other neodermatan groups.

The molecular studies of flatworm phylogeny led to the new understanding of evolution of the group and position of Platyhelminthes on the tree of Metazoa. Acoelomorpha and Nemertodermatida were excluded from Platyhelminthes and are now generally considered to be the basal bilaterians and occupying

the pivotal position on the metazoan tree (Ruiz-Trillo et al., 1999, 2002; Jonde-lius et al., 2002; Telford et al., 2000; Jondelius et al., 2002; Telford, 2008). The problematic position of Xenoturbellida (Bourlat et al., 2006; Perseke et al., 2007; Telford, 2008; Cannon et al., 2016) might be tested further. Catenulida is usually considered in molecular studies as the most primitive group of turbella-rians albeit with dubious status on the base of differences in their mitochondrial genome (Telford et al., 2000).

The Platyhelminthes *sensu stricto* (Catenulida + Rhabditophora) as redefi-ned by Larsson and Jondelius (2008) appeared to be a member of Lophotrocho-zoa (review: Littlewood, 2006; Giribet, 2008; Hejnol et al., 2009; Cannon et al., 2016). The interrelationships of the groups comprising Platyhelminthes varied in different molecular reconstructions of phylogeny and are susceptible to poor sampling, types of genes used, choice of outgroup and mathematical methods employed. More than two decades of genomic studies of platyhelminths phylo-geny have not produced a consistent tree of the group. The most prominent problems in current phylogeny of Platyhelminthes are the question about the turbellarian roots of Neodermata, the problem of monophyly or paraphyly of Monogenea and the origin and relationships between ectoparasitic Monogenea and the endoparasitic taxa of Neodermata — the Cestoda and the Trematoda. Baguna and Ruitort (2004, 180) pointed out that «Morphologically based trees conflicted with molecular analyses». Careful comparative analysis of morpholo-gy of «Turbellaria» and Neodermata permits to pinpoint differences between morphological and molecular phylogenies. The aim of this study is to analyze and compare the molecular trees from the viewpoint of morphological data. The absence of consensus between different molecular trees asserts that this appro-ach may be prone to inaccuracies and lacks the monopoly on the truth. In this work the articles of Brooks and co-authors (Brooks, 1989a, b; Brooks et al., 1993, 2000, 2002) are not considered because their analyses are based on nume-rous characters not always homologous. The critical analyses of dozens of cha-racters they use necessitate a special article. Partially such analyses have been given by Pearson (1992) and Rohde (1996).

Until recently the molecular phylogenetic trees could not reveal a modern group of «Turbellaria» that would be close to the ancestor of Neodermata and authors concluded that the sister-group to Neodermata consists of a large clade of neoophoran turbellarians. The last molecular computed trees resolved the free-living turbellarian *Bothrioplana semperi* Braun, 1851 as the closest to the turbellarian root of Neodermata (Laumer, Giribet, 2014; Laumer et al., 2015; Littlewood, Waeschenbach, 2015; Egger et al., 2015). These studies led to simi-lar conclusions as the foregoing morphological analysis (Kornakova, 2010, 2013). *Bothrioplana semperi* shares some morphological characters with both Proseriata and Tricladida (Kornakova, 2013). Previously *B. semperi* has been included in only one molecular study of common phylogeny of Platyhelminthes (Baguna, Ruitort, 2004) and the authors obtained the same result. The molecular study of Proseriata has resolved Bothrioplanidae as a group close to Tricladida (Curini-Galetti, 2001). The most recent works have shown Bothrioplanidae as a group close to Proseriata (Laumer, Giribet, 2014) or close to Tricladida (Egger et al., 2015; Laumer et al., 2015) and the sister group to Neodermata. The incon-sistency of the results of molecular studies reflects the conjoin morphology of the group. Surprisingly, in the study by Laumer et al. (2015) Trematoda has

been resolved as a basal group of Neodermata and the clade Cercomeromorpha has been supported. Both viewpoints were previously rejected in all molecular studies of flatworm phylogeny after the study of Lockyer et al. (2003). In other recent studies (Laumer, Giribet, 2014; Egger et al., 2015) Monogenea has a basal position among Neodermata on computed trees.

At the same time these studies engendered a problem of monophyly vs. paraphyly of Monogenea. Monophyly of Monogenea was supported in the works of Olson and Littlewood (2002) and unresolved in the previous article of the same authors (Olson, Littlewood, 2001), Lockyer et al. (2003), Baguna and Ruitort (2004), and Laumer et al. (2015). The morphological analysis corroborating the monophyly of the group has been given by Boeger and Kritsky (1993, 2001). Other studies have failed to support the monophyly of Monogenea. In the studies by Mollaret et al. (1997, 2000), Litvatis and Rohde (1999), Littlewood et al. (1999a, b), Perkins et al. (2010), Laumer and Giribet (2014) Monogenea has been resolved as paraphyletic group. Morphologically this point of view was supported by Justine (1998) who demonstrated the differences between Monopisthocotylea and Polyopisthocotylea in spermatozoan morphology and spermiogenesis, by Whittington et al. (2000) on the base of morphological differences of the larval stages (oncomiracidia), and in the detailed morphological analysis by Euzet and Combes (2003). At the moment the hypothesis of the paraphyly of Monogenea seems to be better justified.

The monophyly of Neodermata implies the origin from the common ancestor. If we accept the paraphyly of Monogenea, we must take a step back on the evolutionary tree of Neodermata. From the viewpoint of monophyly of Neodermata the paraphyly of Monopisthocotylea and Polyopisthocotylea implies that both groups originated from a bit earlier extinct group of pro-monogeneans, in which the synapomorphic characters of Neodermata have evolved.

## THE ANALYSIS OF SYNAPOMORPHIES OF NEODERMATA

To reveal the possible turbellarian roots of Neodermata it is necessary to analyze the synapomorphies of Neodermata. In his monograph Ehlers (1985) formulated the synapomorphies of Neodermata very cautiously. In later years the characters that he revealed and other ultrastructural characters of Neodermata were intensively studied in all groups.

In current literature authors formulate synapomorphies of Neodermata somewhat differently. Thus, Baguna and Ruitort (2004, p. 183) characterize Neodermata as bearing the following features.

1. Neodermis — syncitial, unciliated, insunk epithelium with pericaria having multiple connections with surface layer.

2. Replacement of the larval epidermis by the neodermis.

3. Lack of vertical ciliary rootlets of epidermal cilia.

4. Presence of specific electron-dense collars of sensory receptors.

5. Axonemes of sperm incorporated into the sperm body.

6. Incorporation of a vertebrate host in the life cycle.

Littlewood (2006, p. 7) provides some other characters as synapomorphies of Neodermata.

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1. Multiciliated ectoderm limited to «larval» stages and shed later and replaced by the syncitial neodermis with sub-epidermal pericaria each separately connected to surface layer.

2. Protonephridia with a two-cell weir.

3. Epidermal locomotory cilia with single cranial rootlet.

4. Epithelial sensory receptors with electron-dense collars.

5. Complete incorporation of both axonemes in the sperm body.

6. Two long and one short insertion in nuclear small subunit (SSU) ribosomal DNA (rDNA).

Critical analysis of these definitions in terms of synapomorphies of Neodermata is necessary to reveal real synapomorphies; at the same time it gives opportunity for understanding what evolutionary morphological and ecological events lead to the appearance of Neodermata. Littlewood (2006) correctly noticed that although the appearance of Neodermata was a major single evolutionary event, this does not mean it happened quickly or without intermediate forms.

The definition of neodermis needs elaboration: the tegument develops first, later the insunk pericaria join to the tegument by multiple bridges. Neodermis and the specific for Neodermata electron-dense collars in the receptors are described rather correctly in these definitions and represent the true synapomorphies. It is necessary to specify that electron-dense structures similar to those described in the receptors of Neodermata are widespread among the receptors of «Turbellaria». The principal difference between them is the site of insertion: in collar receptors of Neodermata electron-dense structure is always inserted into the cellular membrane of the receptor from the apical level of the cell while in the receptors of «Turbellaria» the site of insertion is situated always at the level of the septate desmosome. The only known exception is the monociliary receptors in *Bothrioplana semperi*, in which the electron-dense ring lies above the septate junction (Kornakova, Joffe, 1996).

Other characters should be elaborated and analyzed.

Among them the ultrastructure of the flame bulbs must be considered before everything else. Ehlers (1985) described, as a synapomorphy of Neodermata, a flame bulb consisting of two cells: the terminal cell bearing a bundle of axonemes and the proximal canal cell. Each cell gives rise to the row of longitudinal ribs, forming together the filtration apparatus. The terminal cell gives rise to internal ribs while the external ribs derive from the proximal canal cell. The ribs of external row are united by a thin «membrane» of the extracellular matrix.

Similar ultrastructure of the weir of the flame bulb was found in Proseriata. Ehlers (1985) and Ehlers and Sopott-Ehlers (1986, 1987) studied the ultrastructure of flame bulbs in several species of proseriate turbellarians: *Monocelis fusca* Orsted, 1843, *Notocaryoplanella glandulosa* Ax, 1951, *Nematoplana coelogynoporoides* (Meixner, 1938), and *Invenusta paracnida* Karling, 1966. They assumed that the external ribs are derivatives of the proximal canal cell and the internal ones are outgrowths of the terminal cell. Two cytoplasmic cords formed by the canal cell extending along the weir and joined by septate junction were found in *Monocelis* sp. Ehrenberg, 1831 (Rohde et al., 1988), but they were not observed in other proseriates described (Ehlers, 1985; Ehlers, Sopott-Ehlers, 1987). Rohde et al. (1995) noticed that this structure may be absent or very short in some proseriates studied by him.
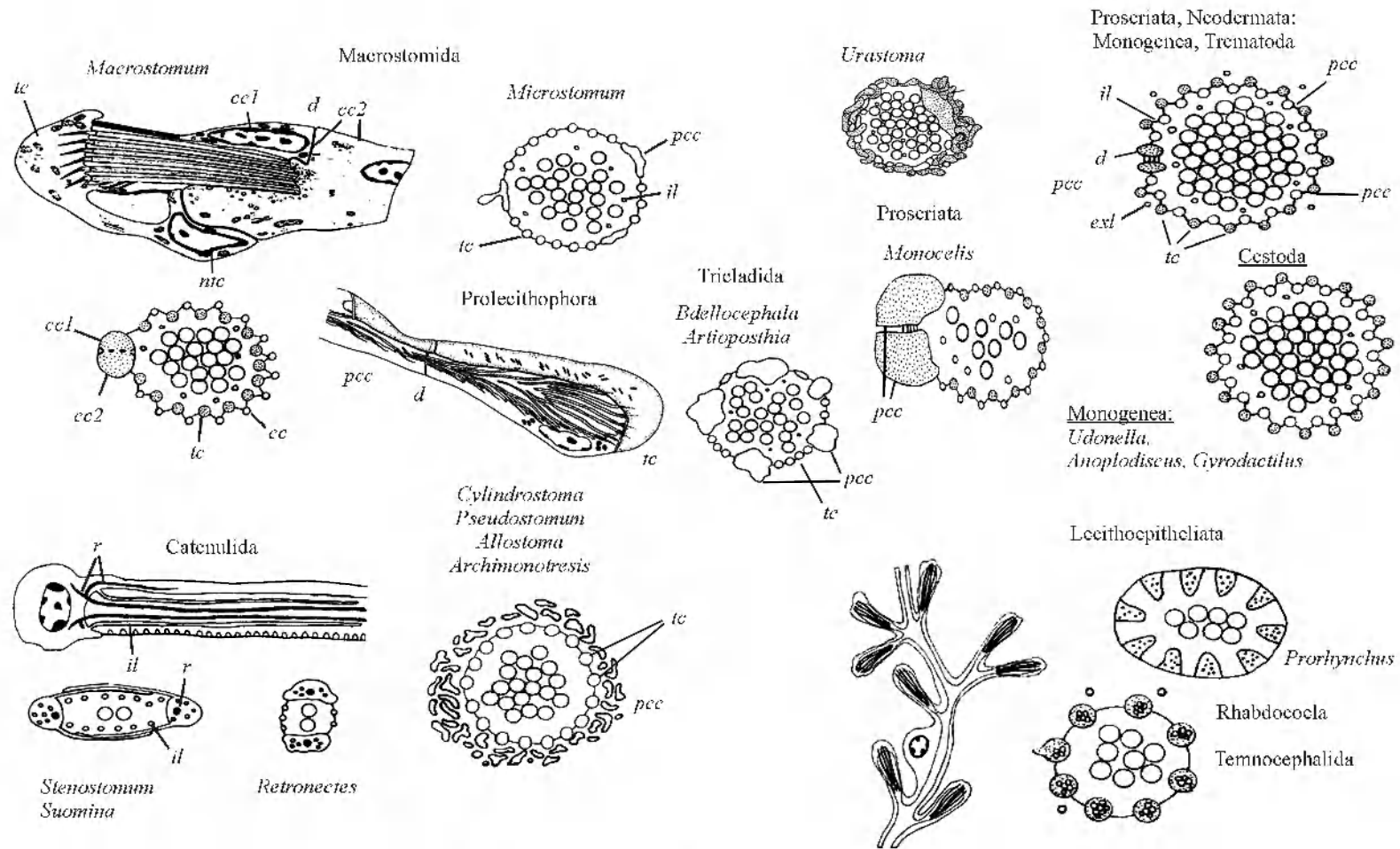
Fig. 1. The scheme of the ultrastructure of flame bulbs in different groups of Platyhelminthes.
cc1 — the first proximal canal cell, cc2 — the second proximal canal cell, cc — ribs derived from canal cells, d — desmosome, exl — external leptotrich, il — internal leptotrich, ntc — the nucleus of the terminal cell, pcc — proximal canal cell, r — rootlets of axonemes, tc — terminal cell.

A contradiction exists in the interpretation of the structure of the flame bulbs in Proseriata. Rohde et al. (1988) observed reversed mode of formation of the weir in *Monocelis* sp.: the inner ribs are the outgrowths of the proximal canal cell while the outer ones are the outgrowths of the terminal cell. Rohde (2001) considered the structure of flame bulbs in all proseriates to be the same. Rohde (1991) wrote that he studied the ultrastructure of flame bulbs in several species of proseriate turbellarians, however he did not described nor illustrated these data. It may be difficult to determine the origin of ribs without full reconstruction by serial ultrathin sections. Nevertheless, in illustrations provided by Ehlers and Sopott-Ehlers, and especially the photographs of the flame bulbs in *N. coelogynoporoides* (Ehlers, 1985; p. 286—287, figs 69, 70) and *I. paracnida* (Ehlers, Sopott-Ehlers, 1987; p. 379, 381—382, figs 1—3) it is possible to retrace the origin of both rows of ribs, which justifies the interpretation by Ehlers. Our own data on ultrastructure of the flame bulbs in *Bothrioplana semperi*, the species supposedly close to ancestors of Proseriata (Kornakova, 2010, 2013), also corroborates such interpretation because the presence of bundles of microfilaments which go from the cytoplasm of the proximal canal cell into each external rib makes it clear what cell each row derives from.

Since Ehlers (1985) published his monograph the ultrastructure of flame bulbs has been studied in representatives of most taxa of Platyhelminthes (fig. 1). It appears that the ultrastructure of flame bulbs is an important phylogenetic character for turbellarian taxa of high rank (order or suborder) (Rohde, 1991, 2001). The flame bulbs of representatives of the high rank taxa studied so far are similar in such characters as the number of cells which contribute to the flame bulb, the mode of formation of filtration apparatus (weir formed by the interdigitating ribs or by the slits in the wall of the terminal cell or by one row of ribs, derived from the terminal cell), the presence or absence of leptotriches, the supporting structures in the cytoplasm of the ribs (microfilaments or microtubules), the presence or absence of filtration membrane.

Among the Neoophora, the flame bulb with ultrastructure similar to the one typical for Proseriata has not been found apart from this order and Neodermata, and two exceptions, which will be analyzed hereafter.

The flame bulbs with similar ultrastructure of the weir (two parallel rows of ribs) appeared in turbellarians belonging to a more primitive group, Macrostomida, in representatives of genus *Macrostomum* Schmidt, 1848 (Rohde, Watson, 1991). These flame bulbs differ from proseriate ones in the order of formation of the weir: internal row is derived from the canal cell, while external row is an outgrowth of the terminal cell. Another difference is that more than one canal cell may contribute to the weir formation in macrostomids. Among the modern turbellarians, Macrostomida is the closest group to the ancestor of neoophoran turbellarians. It is fair to assume that the inversion of the order of the ribs took place in evolution, and proseriate type of flame bulb originated from the flame bulb in macrostomid species. The ultrastructure of the weir in *Macrostomum tuba* Graff, 1882 and *M. spirale* Ax, 1956 shows the possibility of such inversion: in these species the terminal parts of internal ribs deriving from the canal cells turn towards the exterior and at the level of the base of the flame shift the position to the outside of the ribs extending from the terminal cell (Kunert, 1988; Watson et al., 1991).

The absence of flame bulbs with ultrastructure similar to those in Proseriata among other modern groups of neoophoran turbellarians implies that this structure has been inherited by Neodermata from the ancestor. Given that the ultrastructure of flame bulbs is important phylogenetic character it is possible to conclude that Neodermata derived from the ancestor belonging to the most primitive Neoophora, an extinct group close to Proseriata. In molecular studies Proseriata were included in the large clade of neoophoran turbellarians considered as the possible ancestor of Neodermata (Rohde et al., 1995; Littlewood et al., 1999a; Noren, Jondelius, 2002; Baguna, Ruitort, 2004; Riutort et al., 2012). Consequently, the ultrastructure of flame bulb in Neodermata represents plesiomorphic rather than apomorphic character.

Another character, which Neodermata and Proseriata share, is the mode of the embryological development of epidermis in these turbellarians and in the larvae of Neodermata (Tyler, Tyler, 1997).

The reduction of the vertical rootlet of cilia in ciliated epithelial cells of neodermatan larvae is not a proven synapomorphy for Neodermata. The reduction of the vertical rootlet of cilia of the epithelial cells has also been traced in some parasitic turbellarians (Rohde, 1994) and even in some free-living Proseriata species (Bedini, Papi, 1974). Thus, this character may be a synapomorphy as well as a plesiomorphy.

## ANALYSIS OF DIFFERENCES BETWEEN THE PROCESS OF SPERMIOGENESIS IN «TURBELLARIA» AND NEODERMATA

The next character, incorporation of axonemes into the sperm body, is formulated incorrectly, both in the list by Baguñà and Ruitort (2004, p. 183) and in that by Littlewood (2006, p. 7). In fact, their definition represents a corrupt and scaled down version of the original definition by Justine (1991, p. 1440): «Axonemes are incorporated into the sperm body by proximo-distal fusion».

Spermatozoa with incorporated axonemes are widespread among turbellarians, especially parasitic species (L'Hardy, 1988; Justine, 1991, 2001). The difference between «Turbellaria» and Neodermata is not the incorporation of axonemes, but the process of spermiogenesis and spermatozoa polarity (fig. 2). This character has been introduced as synapomorphy of Neodermata by Justine (1991). During turbellarian-type spermiogenesis the nucleus occupies the proximal position in relation to the cytophore and retains this position during spermiogenesis. Basal bodies of axonemes lie more distally near the nucleus and during shaft formation they move to the distal end of the spermatozoon. In neodermatan-type spermiogenesis the nucleus at the initial stage of spermiogenesis is situated in the proximal part of early spermatid, basal bodies lie near the nucleus. During spermiogenesis the basal bodies retain the proximal position while the nucleus migrates to the distal end of spermatid. The essence of difference is the reversal of polarity during neodermatan-type spermiogenesis. As a result, we see absolutely morphologically identical spermatozoa with incorporated axonemes in Neodermata and in «Turbellaria». However, these similar structures have been formed by different processes, and the nucleus-containing part of the sperm cell in turbellarians represents the proximal end relatively to the cytophore, while in the spermatozoa of Neodermata the nucleus lies in its distal
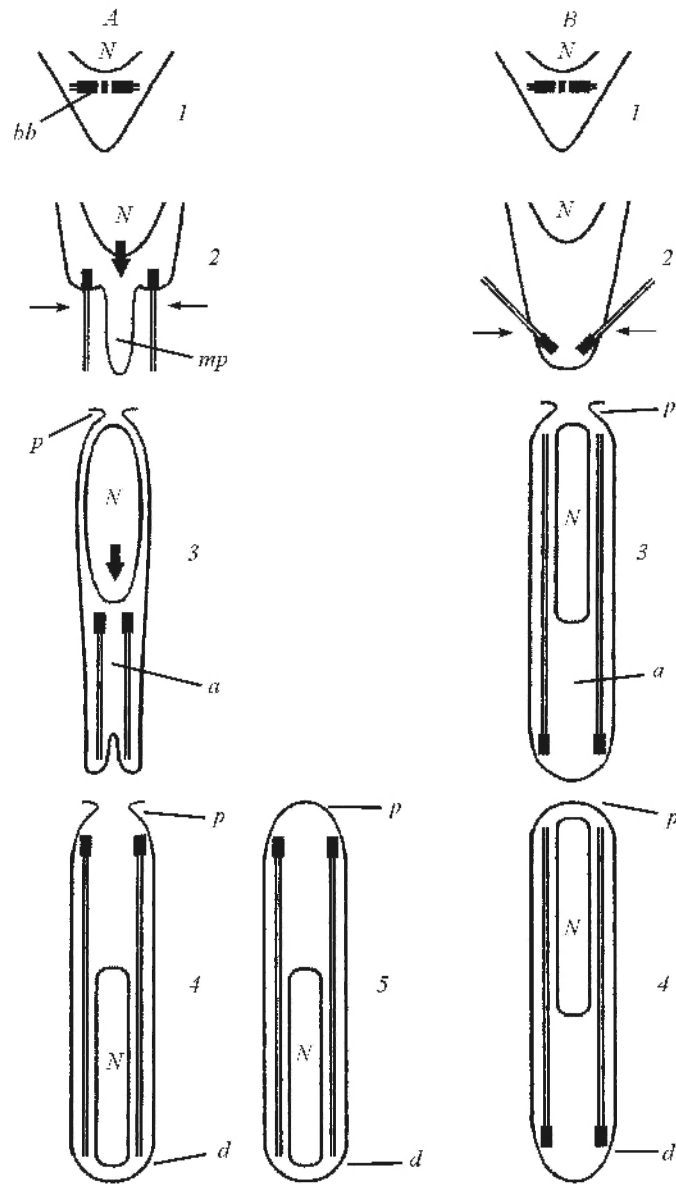
Fig. 2. The scheme of the spermiogenesis in Neodermata (*A*) and «Turbellaria» (*B*).

*A* — spermiogenesis in Revertospermata (Neodermata + *Urastoma*), *B* — spermoigenesis in «Turbellaria» with incorporated axonemes exept turbellarians with revertive spermoigenesis. *A1* — early spermatid, *A2* — spermatid with the median process, *A3* — spermatid with incorporated axonemes, *A4* — spermatid with the nucleus in the distal part of spermatid, *A5* — matured spermium. *B1* — early spermatid, *B2* — spermatid with free axonemes turn to the proximal direction, *B3* — spermatid with incorporated axonemes, *B4* — matured spermium. *bb* — basal bodies, *d* — distal end, *mp* — median process, *N* — nucleus, *p* — proximal end, large arrowhead shows the direction of the migration of the nucleus; small arrowheads indicates free axonemes.

part. Accordingly, the basal bodies are situated at distal end in turbellarian-type sperm and proximal in neodermatan-type sperm. Thus the attribute of Neodermata is the spermoigenesis with the reversion of polarity of the spermatids (termed «Revertospermata» by Kornakova and Joffe, 1999) rather than the incorporation of axonemes.

## THE SYSTEMATIC POSITION OF «TURBELLARIA» REVERTOSPERMATA

The spermiogenesis with the reversion of polarity of spermatids was revealed in several species of parasitic turbellarians belonging to order Fecampiida — representatives of the genus *Kronborgia* Christensen and Kanneworff, 1964, *Notentera ivanovi* Joffe, Selivanova, Kornakova, 1997, and *Urastoma cyprinae* Graff, 1882 (Watson, Rhode,1993a, b; Kornakova, Joffe, 1999; Watson, 1997) and these turbellarians are considered as a possible link between turbellarians and Neodermata (Kornakova, Joffe, 1999). However, all molecular reconstructions of the phylogeny of Platyhelminthes, including our early work (Joffe, Kornakova, 2001) do not support the close relations between these species and Neodermata. On all molecular trees turbellarians with revertive spermiogenesis form one single clade, which, besides the species mentioned above, includes *Ichthyophaga subcutanea* Syromjatnikova, 1949, turbellarian parasitic in fishes, currently classified as a member of the fam. Genostomatidae (Fecampiida). According to the generic names of its members, this clade on molecular trees received the name UNIK (*Urastoma* Dorler, 1900 + *Notentera* + *Ichthyophaga* + *Kronborgia*). In the last studies this clade of turbellarians with revertive spermiogenesis assumed the name PNUK according to the new generic and species name of *Ichthyophaga* — *Piscinquilinus subcutaneous* in accordance with Sluis and Kavakatsu (2005). The spermiogenesis of *P. subcutaneous* has not been studied; nevertheless molecular studies confirm the belonging of this species to PNUK.

Rohde et al. (1994) erected a new class Fecampiida with a single family Fecampiidae for species with revertive spermiogenesis. Later, Noren and Jondelius (2002) basing on molecular analysis amended the diagnosis of Fecampiida adding to it two new families, Urastomidae and Genostomatidae. However, there are two genera in the family Genostomatidae: *Genostoma* and *Piscinquilinus,* but only the latter was included into the phylogenetic analysis by Noren and Jondelius (2002). Thus, *Genostoma* was included into Fecampiida without any molecular or morphological evidence.

The recent history of application of the systematic name Genostomatidae is balled up and full of mistakes. In some articles *Urastoma* and *Piscinquilinus* were included into the family Urastomidae (Littlewood, Olson, 2001) or genus *Piscinquilinus* or both genera were included in the family Genostomatidae (Littlewood, 2006). There is no modern article devoted to the analysis of systematic position of Genostomatidae. In the internet databases summarizing the modern viewpoints on zoological system (for example, http://tupbellaria.umaine.edu/; http://eol.org/; http://www.marinespecies.org) Genostomatidae has the rank of a family in the order Fecampiida or the rank of an order. In these databases the species *P. subcutaneous* is included in Genostomatidae following the descrip-

tion of this species by Syromjatnicova (1949). *Urastoma cyprinae* used to be included in the family Genostomatidae too in Turbellarian Taxonomic Database and Encyclopedia of Life. Both databases include Genostomatida into Revertospermata. This mistaken classification persists in most molecular studies.

The uniting of the genera *Piscinquilinus, Genostoma* and *Urastoma* into the same group lacks the morphological basis. The only phylogenetically sufficient common character of these species is the presence of the oro-genital opening on the caudal end of the body. The type of pharynx, *pharynx plicatus variabilis*, is typical for several orders of lower Neoophora. The representatives of the genus *Genostoma* have the primitive condition of the female genital system — the presence of germovitellaria, similar to *P. subcutaneus*. Such condition arises independently from time to time in different species of Neoophora, for instance, among the forms close to the primitive neoophorans, in free-living turbellarians of the genus *Itaspis* Marcus, 1952 (Proseriata) and in *Puzostoma evelinae* Marcus, 1950 (Prolecithophora) (Marcus, 1950, 1952; Ax, 1961). Thus germovitellaria cannot be regarded as a phylogenetically significant character.

But other significant characters of these species are different. *Urastoma* and *Piscinquilinus* have eyes, *Genostoma* lacks them. In the male genital system of *Genostoma* a copulatory organ is present, in *Urastoma* and *Piscinquilinus* it is absent (Graff, 1882; Syromjatnikova, 1949; Menitskii, 1963). The presence or absence of the male copulatory organ and details of its structure are very important characters for the systematics and taxonomy of Platyhelminthes. The ultrastructure of the flame bulb in *Genostoma* has not been described. Molecular trees always unite *Piscinquilinus* with the turbellarians with revertive spermiogenesis, *Urastoma, Kronborgia* and *Notentera* (Littlewood et al., 1999a, b; Joffe, Kornakova, 2001; Lockyer et al., 2003; Baguñà, Ruitort, 2004). The most essential argument against inclusion of these species into the family Genostomatidae is the ultrastructure of spermiogenesis in *Genostoma kozloffi* Hyra, 1993. It lacks reversion of polarity; instead it has all the features typical for turbellarian spermiogenesis and in some aspects is close to spermiogenesis in Rhabdocoela (Pfistermuller et al., 2002). Such type of spermiogenesis precludes the inclusion of *Genostoma* into Fecampiida. At the same time such type of spermiogenesis undermines the inclusion of *Genostoma* into the order Prolecithophora that has aflagellate spermatozoa (Ehlers, 1988; Jondelius et al., 2001). Now the systematic position of *Genostoma* and the family Genostomatidae must be regarded as taxa *incertae sedis*. In sum, the clade PNUK and its members, *Pisciquilinus* and *Urastoma*, do not belong to the family Genostomatidae and also should be considered as taxa *incertae sedis*.

The morphological arguments presented here are now corroborated by the new molecular evidence: Laumer (2015) in his Ph. D. thesis provided first molecular sequence data (nearly complete 18S and 28S rRNA) from *Genostoma kozloffi*, with which he tested hypotheses on the phylogenetic position of this taxon within Platyhelminthes. These analyses unequivocally positioned *Genostoma* as the sister group of Prolecithophora outside Fecampiida.

## REVERTIVE SPERMIOGENESIS IN TURBELLARIANS PNUK
## AND NEODERMATA — HOMOPLASY OR REAL SYNAPOMORPHY?

To finalize the consideration of spermiogenesis as synapomorphy of Neodermata it is necessary to discuss two questions: 1) could the spermiogenesis with reversion of polarity arise among Platyhelminthes twice; 2) do the molecular phylogenetic trees of Platyhelminthes place the non-neodermatan revertospermatan turbellarians correctly. Theoretically, spermiogenesis with the reversion of polarity could have arisen twice in the evolution of Platyhelminthes. But in the case of PNUK not only migration of the nucleus is the common character of their spermiogenesis with the spermiogenesis in Neodermata. The mature spermatozoa of PNUK and Neodermata lack dense bodies, which are typically present in turbellarian spermatozoa. On molecular trees PNUK is usually grouped with Tricladida. Yet still the dense bodies have been revealed in spermatozoa of Tricladida (Charni et al., 2010) apart from the fact that spermiogenesis in this group is typical for turbellarians. In *Urastoma cyprinae* spermiogenesis occurs not only with the reversion of polarity, but the median process, a structure peculiar for spermatids of Neodermata, develops during spermiogenesis, so this process in this species is absolutely identical to neodermatan spermiogenesis. It seems unlikely that all this complex of characters arose independently and twice. Thus the revertive spermiogenesis represents more than likely plesiomorphy rather than synapomorphy for Neodermata. The spermoigenesis in *Bothrioplana semperi* cannot be studied: *B. semperi* has reduced male organs and is obligatory parthenogenetic (Reisinger, 1940).

## THE POSITION OF TURBELLARIANS PNUK ON MOLECULAR
## PHYLOGENETIC TREES CONFLICTS WITH MORPHOLOGICAL DATA

The morphology of turbellarians PNUK is consistent with presumption that the turbellarian roots of Neodermata are close to early Neoophora (Littlewood et al., 1999a, b; Kornakova, 2010). Karling (1940) supposed that early Neoophora had the posterior oro-genital opening. Indeed, *P. subcutaneous* and *U. cyprinae* have such structure. The Fecampiida lack the digestive system, their genital atriums open at the end of the body. The ultrastructure of the flame bulb in *Kronborgia* has been studied only in larvae. The flame bulbs in *Notentera* are represented by several giant cells having both the excretory and secretory functions and cannot be compared with any type of flame bulbs described so far (Kornakova, 2017). The flame bulb of *Urastoma cyprinae* may be considered similar to the proseriate-neodermatan type — it consists of the same double row of ribs, but they interlace into a basket-like weir (Rohde et al., 1990). Lastly the ultrastructure of the flame bulb in *P. subcutaneous* is absolutely identical to the one in Neodermata (Rohde, 2001). In this respect it is important to point out that the ultrastructure of the flame bulbs in *Bothrioplana semperi* is strongly similar not only to that in Neodermata but to that in *Piscinquilinus subcutaneus*. The peculiarities of the ultrastructure of the flame bulbs in Platyhelminthes are very sensitive to the phylogenetical relations. For example, among Monogenea Monopisthocotylea the genera *Gyrodactylus* von Nordman 1832, *Udonella* Johnson, 1835 and *Anoplodiscus* Sonsino, 1890 bear the marking common gene

(Rohde et al., 1989; Littlewood et al., 1998, 1999a; Litvatis, Rohde, 1999) and form one single clade on molecular trees. The morphology, biology and systematic position of these monogeneans are strongly different. The ultrastructure of flame bulbs in these monogeneans has the peculiar character — the absence of desmosome running along the weir. Such character has never been revealed in flame bulbs of other monogeneans studied thus far. The presence of flame bulbs of proseriate-neodermatan type in turbellarians PNUK and *Bothrioplana semperi* confirms their phylogenetic relationship and denotes the level on flatworm phylogenetic tree where they split off — it is the level at the base of Neoophora.

In molecular estimates of the phylogeny of Platyhelminthes the clade PNUK (former UNIK) forms the cluster with Tricladida (Littlewood et al., 1999a, b; Noren and Jondelius, 1999; Joffe and Kornakova, 2001; Littlewood and Olson, 2001; Baguna and Ruitort, 2004; Laumer, Giribet, 2014b) or with Tricladida + Prolecithophora (Lockyer et al., 2003). All authors of molecular studies of phylogeny admit that «molecular phylogenetic estimates for flatworms have suffered from poor sampling, single gene sampling, long branch attraction and inadequate analysis» (Littlewood, 2006, 2009). The orders Tricladida and Prolecithophora belong to the groups with high rate of nucleotide changes, similar to Fecampiida (Joffe et al., 1995; Joffe, Kornakova, 2001). The clustering of Fecampiidae, *U. cyprinae* and *P. subcutaneous* with orders Tricladida and Prolecithophora in molecular trees is most possibly the result of the effect of «long branch attraction». By contrast, there is no morphological basis for such uniting. Fecampiidae is a well-defined group having the prominent morphological character, the absence of digestive system. Other members of PNUK, *Urastoma* and *Piscinquilinus* do not have sufficient characters similar to Tricladida and Prolecithophora. The only character that is shared by *U. cyprinae* and *P. subcutaneous* with the part of Prolecithophora Combinata, the presence of posterior oro-genital opening, may be explained by the affinity of these species to the early Neoophora (Karling, 1940). All other significant morphological characters of PNUK — the ultrastructure of flame bulbs and the morphology of the excretory system, the revertive spermiogenesis, the morphology of reproductive, especially male, system, the form of the gut and other characters mentioned above collide with incorporation of these species into the orders Tricladida and Prolecithophora. At the same time they do not have characters similar to the typical features of representatives of all other modern orders. In this case turbellarians *Urastoma cyprinae* and *Piscinquilinus subcutaneous* do not belong to any modern order of «Turbellaria» and must be regarded as species *incertae sedis*. At the same time they are possibly close to the roots of Neodermata.

Thus among morphological characters of Neodermata only the following synapomorphies do not give rise to doubt: 1) the neodermis; 2) the appearance of ciliated larvae; 3) the collar receptors with dense collar inserted to the membrane at the apical level. Other features may be synapomorphies as well as plesiomorphies or homoplasies. The consideration of other synapomorphies of Neodermata, which have evolved as a result of obligate parasitism on the vertebrate hosts, would be given in the next article, devoted to the early evolution of Neodermata.

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