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Progress in the knowledge of the phylogeny of the Chaetognatha needs both molecular biology and zoology

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Abstract

Many authors have emphasized discrepancies between molecular phylogenies and morphological data, and if so have argued caution in accepting the former. Errors in molecular results are a consequence either of the tree reconstruction methods retained or of a bad knowledge of zoology, many previous papers on the biology of organisms being ignored or disregarded. Since the molecular and cladistic phylogenetic tools used until now have failed to clarify the chaetognaths origins, a reappraisal of the molluscan hypothesis based on the traditional zoology is provided.

Keywords: chaetognatha, zoology, biology, phylogeny

1. Introduction

There are yet too numerous controversial proposals and hypothesis in deep phylogeny constructions. Differences arise concerning methods of tree-building and their most appropriate interpretation so that it has been said that wherever a strong contradiction exists between substantial morphological and molecular evidence, one would hesitate to accept the molecular trees [1, 2, 3, 4, 5]. The chaetognaths always remain as an enigmatic phylum and illustrate this statement.

2. Main features of the phylum

The small marine phylum Chaetognatha comprises actually about 130 to 140 species (length: 2 to 120mm) living in various habitats. For a long time, they were considered as strictly carnivores but different aspects of their feeding biology have led to consider that they feed primarily on dissolved and fine particulate matter, not on prey [6]. Planktonic chaetognaths are the most numerous and have a key role in pelagic food webs. They are very ancient animals, already present in the Lower Cambrian rocks (ca 525 million years) from South China [7]. Their bodyplan is strikingly conserved since then [8]. Their main morphological and anatomical characters are indicated in Figure 1.

3. Hypothesis on the chaetognaths origin

The probable existence of anterior coelomoducts was previously mentioned in a few species [9, 10]. Their existence is now well evidenced [11] and allows the reduction of the number of phyla that have been postulated possibly allied to the chaetognaths (see, e.g., Ghirardelli [12] and all the molecular phylogenies published until now). It is the case for all the phyla devoid of an excretory system, for those provided with protonephridia (flam cells and solenocytes), for those also having particular excretory cells such as the renette cells (Nematoda). So, for instance, numerous reports of a chaetognath-nematode clade using different analyses to test hypotheses on the chaetognaths origins indicate the limits of these methods when they are considered alone, i.e., without the help of morphology and anatomy.

Barthélémy *et al.* [13] have given an evolutionary history of the phylum combining paralog genes analyses and paleontological data. It appears that chaetognaths were among the first predators and yet well represented until the later Carboniferous as previously shown in sediments [8]. After this period, they are absent in fossils, indicating a drastic diminution of their number. Now, they are among the most abundant organisms in zooplankton. Carton [14] explained this gap by a major change in the food web by competition with increased new predators.

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This successful reappearance might be the consequence of a shift in their feeding mode, from carnivory to osmotrophy^[6]. Experiments in view to test chaetognath actin phylogeny evidenced that the cytoplasmic paralogs clustered with the cytoplasmic insect actins, while the muscular proteins are placed basal to all muscular vertebrate actins^[13]. These results agree with previous studies by Duvert^[15] who reported that some actins of the primary muscle of *Sagitta friderici* reacted with those involved in the insect flying muscles, while others reacted with those of rabbits. It is also interesting to note that both arthropod and vertebrate canonical repeats have been found in telomeric-like transcripts^[16]. It appears that chaetognaths share numerous characters with different non-reliable phyla. Indeed, they exhibit both characters of protostomes and deuterostomes and that could suggest that they would have emerged very early, when the separation between protostomes and deuterostomes was not yet clear.

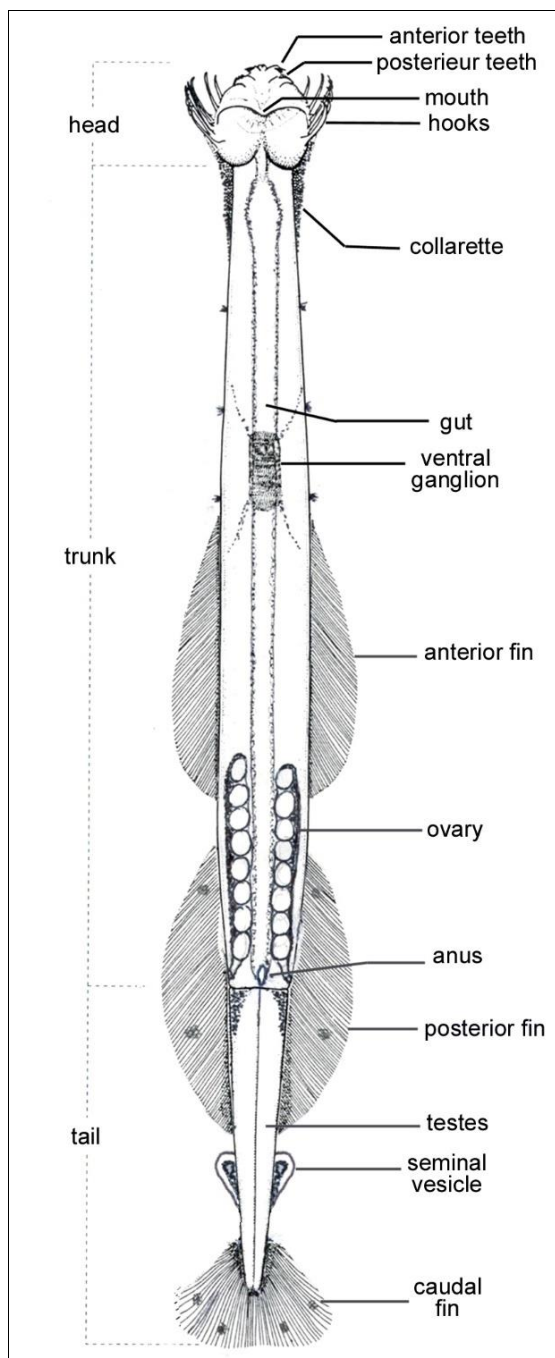


Fig 1: Main diagnostic characters of chaetognaths (*Sagitta* sp.)

The interpretation of chaetognaths as a deep-branching lineage of metazoa is consistent with their presence that can be traced back to the Cambrian radiation. Their grasping spines are among the earliest skeletal animal fossils near the base of the Cambrian^[17], and moreover they have almost the same aspect as their present forms^[8]. These paleontological data fit with phylogenetical analyses by Barthélémy *et al.*^[16] who suggested a deep-branching clade of the retrovirus-like elements found in their genome, which is in agreement with the probable Cambrian origin of the phylum as revealed by fossils. Moreover, based on 28S Ribosomal DNA, Telford & Holland^[18] hypothesized that they emerged early in the metazoan radiation, possibly in the Precambrian. But their place remains questioned as shown by their various and often contradictory positions in the too numerous publications that cannot all be reported here. The first one seems to be that of Telford & Holland^[19] who suggested that they probably arose before the advent of the coelomates. Most of the recent molecular data agree to place the chaetognaths within the protostomes or close to them. Telford^[20] noticed that the difficulty of the correct positioning of the phylum lies in the rapid evolution of the chaetognath genes as compared with other phyla. The species of the genus *Archeterokrohnia* might be good candidates for such a purpose, owing to the persistence of primitive characters^[21-24], implying perhaps a more normal rate of evolution. Telford's remark was later supported by Towers^[25] in her thesis on "*the mystery of chaetognaths*"; she concluded that different molecular phylogenetic approaches revealed that various clades could be formed, depending on the genes and trees reconstruction methods and, among them, chaetognaths sometimes appear grouped with molluscs. More recently, in his thesis, Carton^[14] tested new generation sequencing technology and phylogenomics on Protostomia. He concluded that the chaetognaths are sister to the Lophotrochozoa, as already said by Matus *et al.*^[26] and Kocot *et al.*^[27], and rejected all the other numerous previous topologies he listed. Since, on the one hand, all the numerous molecular data published until now and, on the other hand, cladistic morphological analyses have failed to give a clear undisputed positioning of the chaetognaths among the protostomian metazoans, it is perhaps time to return to the traditional zoology and to expose new arguments strengthening the molluscan hypothesis. The presence of coelomoducts^[9, 10, 11], which confirms unambiguously the protostomian affinities of chaetognaths, justifies this reappraisal.

The hypothesis of a molluscan origin of chaetognaths was lengthily developed by Günther^[28] who considered the chaetognaths as a class of the phylum Mollusca! A long time after, these possible relationships were again mentioned by some authors. Deurs^[29] pointed out the original filiform spermatozooids of the chaetognaths which ultrastructure looks like that of numerous arthropods and gasteropods, and is thus very different from that of deuterostomes to which they were yet said to belong at this date. Green & Bergquist^[30] noted the particular position of the phylum, based on Duvert *et al.*'s^[31] results on the muscles ultrastructure, and wrote: "*Within the Deuterostomia the Chaetognatha are reported to have both a double-septum septate junction, otherwise characteristic of mollusc and arthropod tissues, occurring in the same tissue*". Rehkämper & Welsch^[32] reported that the morphology of the nervous system and the fine structure of the cerebral ganglion of *Sagitta* is nearer to that of

protostomes, molluscs and arthropods than to the deuterostomes. Casanova [22, 33] advanced the same hypothesis of a molluscan origin, saying that this was not contradictory to their deuterostome-like development since, among the molluscs, the cephalopods exhibit this same feature. He based his demonstration on the discovery in the deep north-african Atlantic of the head of an unknown species of gymnosome belonging to, or more probably allied to, the genus *Cephalobrachia* (Fig. 2A, B). Another young specimen was found later in the same area and used for semi-thin sections through the head (Fig. 2C, D). The numerous similarities concern notably the cephalic armature and appendages of chaetognaths that may be very closely paralleled by those of the gymnosomes. Indeed, the larger one has six paired ranks of 12 to 15 hollow hooks clasped into a hook-sack (or hood). To our knowledge, no other marine animals have hollow hooks contained in sacks (Fig. 2C). Moreover, many years ago, Bone *et al.* [34] have described the structure of the hooks of *Sagitta setosa* as two concentric tubes connected by coarse oblique fibrils (Fig. 2E). The cavity is filled with processes of cells whose nuclei are at the base of the hooks that are linked to the head by anchor cells. According to the authors, this concentric tube design is not found in other animal groups that possess chitinous teeth and jaws (*e.g.*, copepods or cephalopods). Interestingly, the hooks of the young gymnosome (Fig. 2C, D) look like those of *S. setosa*: their wall is made of two thin concentric tubes connected by small bridges of dense material (chitine?); their cavity contains cell material and there are anchor cells at their base. If five ranks had disappeared, the disposition would be then similar to that observed in chaetognaths, and more especially in the species of *Archeterokrohnia* where the number of hooks is the highest, varying from 12 to 18. This hypothesis has recently received strong support with the discovery of two new genera of multi-jawed Cambrian chaetognaths, *Ankalodous sericus* [35] and *Capinatator praetermissa* [36], with respectively 5 and 3 paired ranks of hollow hooks. Moreover, in *C.*

praetermissa, their length regularly decreases from the first to the third rank, giving the impression that the two latter are disappearing. In the same manner, the three paired ranks of lateral teeth of the radula of the gymnosome might correspond to the vestibular organs and to the anterior and posterior teeth of the chaetognaths. The gymnosome also bears a median rank of radular teeth that does not exist in the chaetognaths, but a single transient tooth has been observed in young specimens of *Heterokrohnia murina* and *H. davidi* [33]. Moreover, in the gymnosome as well as in these young chaetognaths, the shape of the median teeth is roughly the same, looking like more or less elongated blades, whereas in the two taxa the lateral radular teeth and anterior and posterior teeth respectively are generally spine shaped and different from the median ones. In addition, the gymnosome is provided with a pair of short tentacles, also found in *Spadella cephaloptera*, and with a pair of mouth palps having the same position and shape, bent almost at right angle, as those described in *Archeterokrohnia palpifera* [37, 33]. If the hollow hooks may be considered as an adaptation to the planktonic life in view to lighten the body, and then as a convergence between the two taxa, the hood is less useful in the chaetognaths contrarily to the gymnosome where the hooks are much more numerous and might hold up the locomotion if spread as fans. In the same manner, the tentacles and palps cannot be regarded as convergence between the two taxa since each of them is only present in a single species of chaetognath without an apparent role. They must rather be considered as reminiscent of earliest ancestors, a hypothesis strengthened by the fact that these species are provided with the primitive transverse musculature.

Other arguments may be put forward to support these probable relationships. The separation of an ovotestis into testes and ovaries, as it seems to result in chaetognaths [21], also exists in two species of heterobranch molluscs to which the gymnosomes belong. According to Haszprunar [38], this phenomenon is unique in this group.

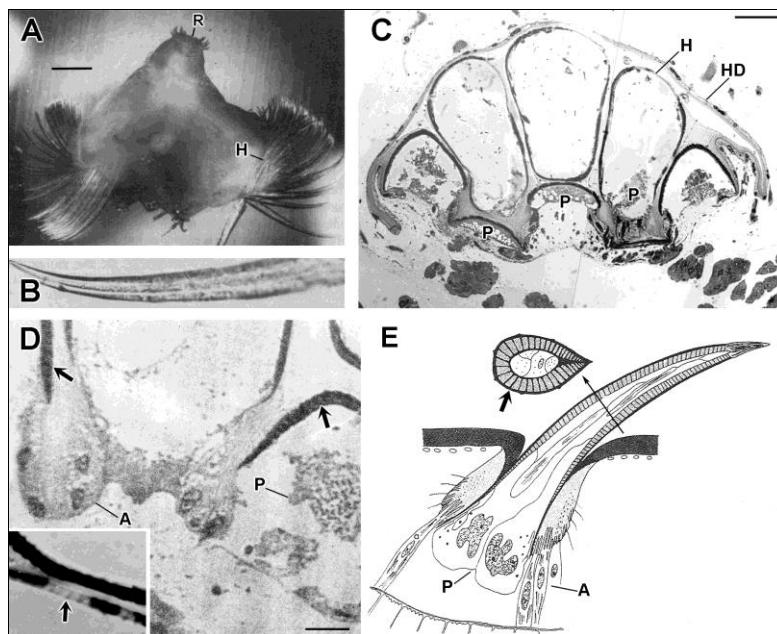


Fig 2: A: Head of a deep unknown gymnosome in dorsal view looking like that of a chaetognath; B: detail of a hook; C: semi-thin transversal section in the head of a young specimen; D: transmission electron micrographies of a section in the hook area (detail of the hook wall in insert); E: schematic diagram of organisation of hook of chaetognaths (modified from Bone *et al.* [34]). Note the astonishing resemblance between them, and notably the structure of the hook wall (arrows in D and E). A: anchor cells; H: hook; HD: hood; P: cells of the pulp cavity; R: radula. Scale bars: A, 600 μ m; C, 25 μ m; D, 7 μ m.

It leads to a simplification of the genital apparatus (disappearance of penis, oviduct opening independently of the genital orifice, seminal receptacle isolated) that might foreshadow that of *Archeterokrohnia* and *Heterokrohnia* [22, 23]. In most of the *Sagitta* species, eggs are shed freely into the water. But in two species there are original similarities with the Gastropoda. In *Pterosagitta draco*, eggs are clumped and shed in pelagic gelatinous masses in which they hatch as it occurs in the pteropod *Cavolinia inflexa* [39]. To our knowledge, this is probably unique in marine plankton. In *S. hispida*, they are laid as a double string from each gonopore and then coated by an adhesive material on the substratum. These laying features are known in many opisthobranchs which lay fertilized eggs in jelly-like mucopolysaccharide masses or strings produced by the secretory section of the oviduct [40].

The ventral secretory gland of *Xenokrohnia sorbei* is curious and its role remains enigmatic; the hypothesis of a role in feeding was put forward [41]. It is roughly horseshoe-shaped and situated on the outside of the body wall where it is partly deep-rooted in the median ventral line of the neck (Fig. 3A, B). At this level, the gut looks like a narrow oesophagus that does not exist in other chaetognaths. The gland has apparently no connection with the body cavity. Each of its two branches is made of large secretory cells which delimit grooves where the secretion is discharged. A short median lamella of secretory cells is inserted between the two branches. This position and aspect strikingly resemble the ventral groove of the neomenian aplacophoran molluscs (Fig. 3C), considered to house the foot of these molluscs appended in the groove [42]. Moreover, the other aplacophorans (Caudofauveata) exhibit a cuticular oral shield as that existing in the

chaetognaths. According to Fischer-Piette & Franc [43], the oral shield of these aplacophorans would be homologous of the ventral groove of the former, but this view is contested [44, 45]. Moreover, a multi-layered epidermis, considered as an originality of chaetognaths among the invertebrates, is reported in one aplacophoran [43].

The anatomical and biological similarities between the two taxa are very numerous. It is difficult to consider that they are only convergences without further investigation. Research on the biochemical nature of the secretion of the ventral secretory gland of *Xenokrohnia sorbei* must be done for comparisons with the foot of aplacophorans. Molluscs are diverse if one observes, for example, the wormlike aplacophorans, considered to be the most primitive extant molluscs, and cephalopods body plans, so that Brusca & Brusca [40] said that "the myriad ways in which evolution has shaped the basic molluscan bauplan provide some of the best lessons in homology and adaptive radiation in the animal kingdom." Thus chaetognaths might be one of these radiations as suggested by Günther [28]. Homologies in the head of the chaetognaths and molluscs are perhaps probable if not certain. The radula is a unique molluscan structure. As strengthened by Nielsen [45], cuticular thickenings looking like teeth and jaws exist in several protostomes (e.g., annelids) but many similar transverse rows of chitinous cuticular teeth only occur in molluscs. The two rows of teeth generally existing in chaetognaths may be considered as a reduced radula. The two lateral plates in the head of chaetognaths would correspond to the "pair of elongate cartilaginous structures" helping, according to Nielsen [45], to protrude the radula through the mouth.

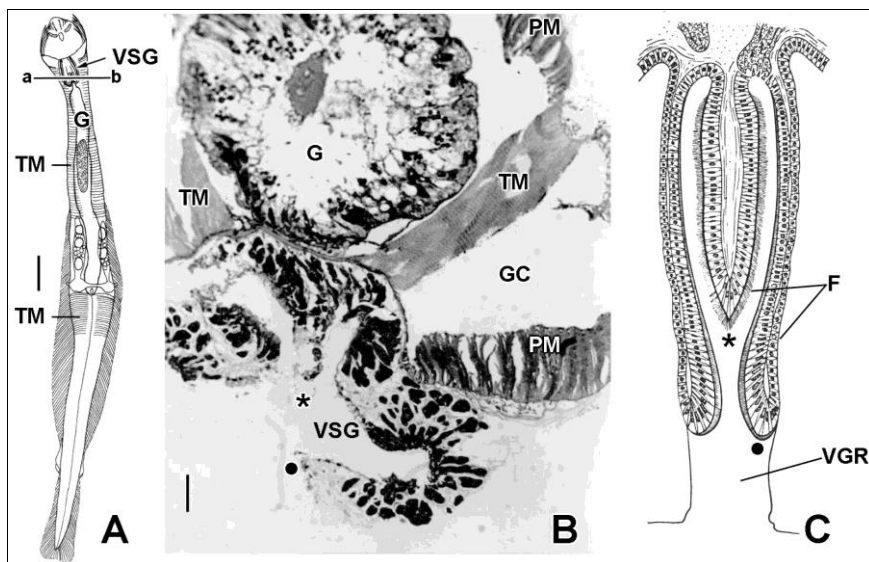


Fig 3: A: *Xenokrohnia sorbei* (ventral view, modified from Casanova [41]); B: semi-thin transversal section in the neck area (line ab in A); C: schematic diagram of a transversal section of the pedal groove of the aplacophoran mollusc *Proneomenia Antarctica* (from Hoffman, in Fischer-Piette & Franc [43]). Note the astonishing similarities between the internal (asterisk) and lateral (black dot) folds of the ventral secretory gland (B) and the foot (C). F: foot; G: gut; GC: general cavity; PM: primary muscle; TM: transverse muscle; VG: ventral ganglion; VGR: ventral groove; VSG: ventral secretory gland. Scale bars: A, 500µm; B, 20µm.

These comparisons might actually be considered a narrative of the chaetognaths evolution. But one must keep in mind that they are based on verifiable facts and that the molluscs are the sole phylum sharing so many characters with the chaetognaths. Until now the modern phylogenetic tools have

failed to solve the problem of the origin of chaetognaths, as already pointed out by Helmkamp *et al.* [46]; this traditional zoological approach needs to be investigated again, in parallel with the continuation and improvement of molecular and cladistic methods.

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