Systematics of the Chaetognatha under the light of molecular data, using duplicated 18S ribosomal DNA

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While the phylogenetic position of Chaetognatha has became central to the question of early bilaterian evolution, the internal system-atics of the phylum are still not clear. The phylogenetic relationships of the chaetognaths were investigated using newly obtained small subunit ribosomal RNA nuclear 18S (SSU rRNA) sequences from 16 species together with 3 sequences available in GenBank. As previ-ously shown with the large subunit ribosomal RNA 28S gene, two classes of Chaetognatha SSU rRNA gene can be identiWed, suggesting a duplication of the whole ribosomal cluster; allowing the rooting of one class of genes by another in phylogenetic analyses. Maximum Parsimony, Maximum Likelihood and Bayesian analyses of the molecular data, and statistical tests showed (1) that there are three main monophyletic groups: Sagittidae/Krohnittidae, Spadellidae/Pterosagittidae, and Eukrohniidae/Heterokrohniidae, (2) that the group of Aphragmophora without Pterosagittidae (Sagittidae/Krohnittidae) is monophyletic, (3) the Spadellidae/Pterosagittidae and Eukrohnii-dae/Heterokrohniidae families are very likely clustered, (4) the Krohnittidae and Pterosagittidae groups should no longer be considered as families as they are included in other groups designated as families, (5) suborder Ctenodontina is not monophyletic and the Flabell-odontina should no longer be considered as a suborder, and (6) the Syngonata/Chorismogonata and the Monophragmophora/Biphragmophora hypotheses are rejected. Such conclusions are considered in the light of morphological characters, several of which are shown to be prone to homoplasy.

Keywords: Chaetognatha; Systematics; Phylogeny; *Sagitta*; *Spadella*; *Eukrohnia*; *Krohnitta*; *Pterosagitta*; 18S rDNA

1. Introduction

Chaetognaths constitute a small marine phylum of approximately 120 nominal species. They have been known to zoologists since at least the 18th century (Slabber, 1778). In the last few decades, their relationships within the metazoans have been strongly debated because of embryological and morphological features shared with the two main branches of Bilateria, the deuterostomes and the protostomes (see Hyman, 1959; Nielsen, 2001). Classical phylogenetic molecular markers such as small subunit ribosomal RNA nuclear 18S (SSU rRNA) sequences or intermediate filaments did not help convincingly to define the Chaetognatha affinities, due to the long-branch attraction artefact (Erber et al., 1998; Halanych, 1996; Mallatt and Winchell, 2002; Telford and Holland, 1993; Wadah and Satoh, 1994). Finally, while a *Hox* gene survey suggested a basal position among the Bilateria (Papillon et al., 2003), the analyses of the mitochondrial genomes of *Spadella cephaloptera* (Papillon et al., 2004) and *Paraspadella gotoi* (Helfenbein et al., 2004) supported close relationships with the protostomes.

Chaetognaths, commonly named arrow worms owing to their shape and high swimming velocity, are found in coastal and open waters. Most species are planktonic

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although a few are benthic. Chaetognaths are not metameric and display a very simple body plan divided into three regions: head, trunk, and tail. Their body bears a tail fin and one or two pairs of lateral fins, and is built around a hydroskeleton, which together with four longitudinal muscles plays a major role in locomotion (Duvert and Salat, 1979). The main internal organs, with the exception of nervous system and muscles, are the gut and ovaries in the trunk, and the testes in the tail segment. All extant species display this almost invariant organization, and this homogeneity has led to great difficulties in resolving an internal classification of the phylum.

Ritter-Zahony (1911), as well as Hyman (1959), recognized 6 genera: *Sagitta, Pterosagitta, Spadella, Eukrohnia, Heterokrohnia* and *Krohnitta*. This classification was followed until Tokioka (1965a) proposed a new systematics of Chaetognatha (Fig. 1A). The class Sagittoidea (extant species) was divided into two orders: the Phragmophora (presence of a transverse musculature, namely the phragmes, and of various kinds of glandular structures on the body surface) and the Aphragmophora (absence of a transverse musculature, and few glandular structures). Two families composed the Phragmophora: Spadellidae (genus *Spadella*) and Eukrohniidae (genera *Eukrohnia*, *Heterokrohnia*, and *Bathyspadella*). Tokioka suggested two suborders for the Aphragmophora: Flabellodontina and Ctenodontina, owing to the number of set of teeth and shape of teeth and hooks. The first suborder (Flabellodontina) only comprised the Krohnittidae family (*Krohnitta*), because of highly specialized features (only an anterior teeth-row, teeth stouter than in Ctenodontina and arranged in a fan shape and hooks curved abruptly), while the families Pterosagittidae (*Pterosagitta*) and Sagittidae (nine genera) belonged to the second suborder (Ctenodontina). In a following work, Tokioka (1965b) suggested that the Aphragmophora was not a natural group, and that the Ctenodontina were closer to the Phragmophora than to the Flabellodontina. In approximately the same way as Alvariño (1963), he also decided to split the genus *Sagitta*, described by Ritter-Zahony (1911), into nine new genera and gathered them into the Sagittidae. Bieri (1991a) followed this classification, and even proposed new genera of Sagittidae, to make more homogenous groupings. However, as with Salvini-Plawen (1986), Bieri's systematic system omitted the Aphragmophora suborders Ctenodontina and Flabellodontina of Tokioka.

Following the discovery of several new deep benthoplanktonic chaetognaths, another slight modification of Tokioka's hypothesis was proposed by Casanova (1985) (Fig. 1B). In this new classification, the Phragmophora was split into two orders: the Monophragmophora (Spadellidae and Eukrohniidae, with transverse muscles in trunk only) and the Biphragmophora (the new Heterokrohniidae family, with transverse muscles in both trunk and tail). Each of these orders belonged to new subclasses of the Sagittoidea: the Syngonata (with ducts between the genital glands in trunk and tail) included the Biphragmophora, and the Chorismogonata (without such ducts) contained the Monophragmophora and Aphragmophora (Casanova, 1985).

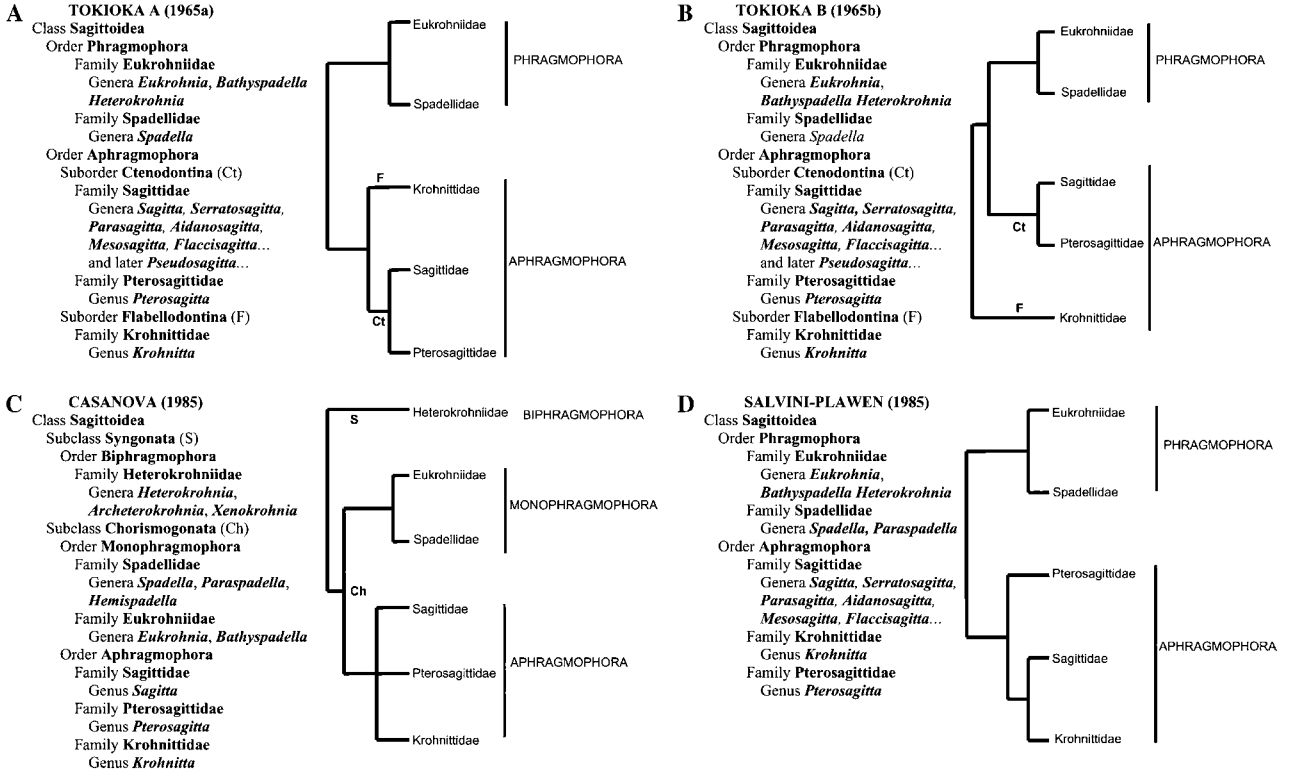

Fig. 1. Main hypotheses of chaetognaths systematics based on morphological criteria. (A) Tokioka A (Tokioka, 1965a), (B) Tokioka B (Tokioka, 1965b), (C) Casanova (1985), (D) Salvini-Plawen (1986). Ch, Chorismogonata; Ct, Ctenodontina; F, Flabellodontina; S, Syngonata.

Dallot and Ibanez (1972), using multivariable technique analyses based on body appearance characters and morphometry, suggested the existence of three groups: *Sagitta*, *Eukrohnia*, and *Spadella*/*Bathyspadella*. The inclusion of *Sagitta lyra* within the genus *Sagitta* was questioned, and they proposed that *Pterosagitta draco* was a member of the Spadellidae adapted to planktonic life.

The first molecular study of the chaetognath systematics was conducted with a short portion of the large subunit ribosomal RNA 28S (LSU rRNA) gene (Telford and Holland, 1997). The authors concluded that (1) LSU rRNA gene is duplicated in Chaetognatha, (2) the separation into Aphragmophora and Phragmophora is supported, and (3) several genera of the Sagittidae family described by Tokioka (1965a) and Bieri (1991a) are recovered in the molecular analysis. However, this study was limited in terms of genera representation of the entire phylum. Indeed, the genera from the Sagittidae family represented more than 71% of the sequences analyzed, and only three of the six classical families were taken into account: Sagittidae, Eukrohniidae, and Spadellidae.

Since then, no study has been made to further establish a classification of Chaetognatha. In an attempt to corroborate the usefulness of the morphological characters discussed above, and to test the validity of the variously held systematics of the Chaetognatha, 26 new SSU rRNA sequences have been isolated. A parallel application of this sequencing survey was to try to characterize one or several slow evolving SSU rRNA sequence in the phylum, to avoid LBA when comparing to a bilaterian data set of SSU rRNA genes. However, no slow evolving sequence has been found (data not shown).

Nevertheless, this data set has been used to construct a classification of the Chaetognatha. SSU rRNA gene has already provided meaningful systematic data for other groups: platyhelminths (Carranza et al., 1998), Acoela (Hooge et al., 2002), sea urchins (Littlewood and Smith, 1995), arachnids (Giribet et al., 1999), and Porifera (Borchiellini et al., 2001). This work extends the molecular study of the chaetognath systematics to a large representation, as sequences from members of all six chaetognath families are utilized.

2. Materials and methods

2.1. DNA extraction and cloning of ribosomal genes

All specimens were placed in 80% ethanol for preservation. Then, the selected specimens were dried on filter paper and DNA was prepared from adults devoid of alimentary bolus to prevent contamination by ingested prey as described in Papillon et al. (2003).

The SSU rRNA genes from 16 species of chaetognaths (Table 1) were amplified by polymerase chain reaction (PCR). The 25 μl PCR reaction mix contained 100 ng template DNA, 2.5 μl *Taq* DNA polymerase buffer 10×, 1 μl dNTP mix (50 μM), 1.25 μl of each primer (20 μM), and 1 U *Taq* DNA polymerase (Promega). Samples were amplified during 30 cycles under the following regime: 94 °C for 1 min, 57 °C (or 47 °C for some samples) for 1 min, and 72 °C for 2 min. Each PCR fragment was cloned into pGemT-easy vector (Promega) and sequenced by Genome Express (Grenoble, France). The sequences of the first pair of primers used were 18S1 AACCTGGTGATCCTGCCA and 18S2 TGCAGGTTCACCTACAGAA. These are universal SSU rRNA oligonucleotides defined in Borchiellini et al. (2001) spanning a 2000 nucleotide-long region that can be used throughout the metazoans. Using these primers, we were able to amplify SSU rRNA genes from *Parasagitta megalophthalma*, *Sagitta bipunctata*, *Krohnitta pacifica*, *Mesosagitta*

Table 1
List of the species and sequences used in the study

Species	Family	Class		Collector	Localisation
Aidanosagitta neglecta (Aida, 1897)	Sagittidae	I		B. Thomassin	W. Indian Hongoni Bay, Mayotte, 0–10 m, 03.2002
Aidanosagitta crassa[a]	Sagittidae	I			
Eukrohnia bathypelagica (Alvariño, 1962)	Heterokrohniidae	I	II	Y. Perez	E. Atlantic Arcachon, France 700 m, 03.2001
Eukrohnia Fowleri (Ritter-Zahony, 1909)	Heterokrohniidae		II	Y. Perez	E. Atlantic Arcachon, France 700 m, 03.2001
Eukrohnia hamata (Möbius, 1875)	Heterokrohniidae	I		F. Norrbin	N. Atlantic Tromso, Norway, 0–175 m, 01.2001
Flaccisagitta enflata (Grassi, 1881)	Sagittidae	I		B. Thomassin	W. Indian. Hongoni Bay, Mayotte, 0–10 m, 03.2002
Krohnitta pacifica (Aida, 1897)	Krohnittidae	I	II	J.P. Casanova	E. Atlantic Cap vert, 1968
Mesosagitta decipiens (Fowler, 1905)	Sagittidae	I	II	Y. Perez	N. Medit Marseille coast, France, 0–500 m, 03.2001
Parasagitta megalophthalma (Dallot and Ducret, 1969)	Sagittidae	I	II	Y. Perez	N. Medit Marseille coast, France, 0–500 m, 03.2001
Parasagitta setosa (Müller, 1847)	Sagittidae		II	J.P. Casanova	N. Atlantic Brittany coast, France, 1969
Parasagitta elegans[a]	Sagittidae	I			
Paraspadella gotoi[a]	Spadellidae	I			
Pseudosagitta lyra (Krohn, 1853)	Sagittidae	I	II	Y. Perez	N. Medit Marseille coast, France, 0–500 m, 03.2001
Pterosagitta draco (Krohn, 1853)	Pterosagittidae	I	II	J.P. Casanova	E. Atlantic Cap vert, 1968
Sagitta bipunctata (Quoy and Gaimard, 1828)	Sagittidae	I	II	J.P. Casanova	E. Atlantic Cap vert, 1968
Serratosagitta tasmanica (Thomson, 1947)	Sagittidae	I		F. Norrbin	N. Atlantic Tromso, Norway, 0–175 m, 01.2001
Spadella cephaloptera (Busch, 1851)	Spadellidae	I	II	Y. Perez	N. Medit Marseille coast 0–5 m, France, 03.2001
Spadella ledoyeri (Casanova, 1986)	Spadellidae	I	II	C. Lejeusne	N. Medit Marseille coast 15 m, France, 03.2003
Xenokrohnia sorbei (Casanova, 1993)	Heterokrohniidae	I	II	Y. Perez	E. Atlantic Arcachon, France 700 m, 03.2001

[a] Obtained from GenBank.

decipiens, *Flaccisagitta enflata*, *Aidanosagitta neglecta*, *Pseudosagitta lyra*, *Eukrohnia bathypelagica*, *Eukrohnia hamata*, *Xenokrohnia sorbei*, *Spadella ledoyeri*, *Spadella cephaloptera*, and *Pterosagitta draco*. We observed that in two species (*P. lyra* and *K. pacifica*) two very distinct gene classes could be observed: one similar to the other chaetognaths sequences amplified (which we defined as the class I), and one quite different (class II). At this stage, only a class II sequence was isolated from *S. bipunctata*. Comparison of the whole set of sequences allowed us to design new primers (18SCII5′ TCGTCGGGGTCTCATCC and 18SCII3′ AGATACCTCGCAAAATCG) specific to this second class of SSU rRNA. Using these primers in the same reaction as described above, we amplified class II SSU rRNA sequences of 1100 bp from *Mesosagitta decipiens*, *Eukrohnia bathypelagica*, *Spadella cephaloptera*, *Xenokrohnia sorbei*, *Pterosagitta draco, Spadella ledoyeri*, *Eukrohnia fowleri*, *Parasagitta setosa*, and *Parasagitta megalophthalma*. Another pair of primers, specific to the chaetognath SSU rRNA gene (18SC5′ TTGATGAAACTCTGGATAACTC and 18SC3′ GGACCTCTCTACATCGTTCG), were designed to amplify 1500 bp class I SSU rRNA sequences from *Sagitta bipunctata* and *Serratosagitta tasmanica*. Sequences accession numbers are given as supplementary data.

2.2. Sequence alignments and phylogenetic analysis

The 26 sequences we isolated, together with the three chaetognath SSU rRNA sequences available in GenBank (*Parasagitta elegans* Z19551, *Aidanosagitta crassa* D14363, and *Paraspadella gotoi* D14362), were aligned automatically using Clustal W (Gap initiation penalty: 3, Gap extension penalty: 1, Base match score: 2, Base mismatch penalty: 1) in BioEdit and alignments were refined by eye.

Two data sets were constructed, each comprising representatives from all the classical families. The first data set (data set 1) comprises all the available sequences of class I and II (29 sequences from 19 species, 17 class I sequences, and 12 class II sequences) some longer than others (1761 positions, 264 Parsimony informative sites). The second data set (data set 2) is an alignment of the longest class I and II sequences available (18 sequences from 15 species, 15 class I sequences, and 3 class II sequences) with same length for all sequences (1761 positions, 213 Parsimony informative sites). As mentioned in Section 1, SSU rRNA sequences of the chaetognaths are highly derived compared to other metazoans, and very distant outgroups can cause incorrect rooting of the tree (see Philippe et al., 2005). Given that the two paralogous classes of SSU rRNA genes are far closer to each other than to any other metazoan SSU rRNA sequences, 3 class II sequences were used to determine the position of the root within the 15 longest class I sequences (see Brown and Doolittle, 1995; Gribaldo and Cammarano, 1998; Iwabe et al., 1989; Telford and Holland, 1997). Alignments are available as supplementary material.

The two alignments were analyzed with the Maximum parsimony (MP) method implemented in MEGA version 2.1, gamma model of distances and sites pairwise deletion (Kumar et al., 1994). Clade support was evaluated by bootstrapping (1000 replicates) and with Bremer support values which were obtained in PAUP* using a command file created by AutoDecay (version 4.0, Eriksson, 1999). Maximum likelihood (ML) analysis employed 10,000 quartet puzzling steps and a 8 category gamma rate in Tree-Puzzle version 5.0 (Schmidt et al., 2002). Bayesian analyses with Markov Chain Monte Carlo sampling were also carried out with MrBayes v3.0b4 (Huelsenbeck and Ronquist, 2001). The analysis was run for 500,000 generations, with four simultaneous chains and a burn-in time determined by the time of convergence of the likelihood scores. Clade support was estimated by computing the posterior probabilities of each node across the sampled trees after burn-in. In ML and MrBayes (MB) analyses, several substitution models were tested (HKY, Kimura-2-parameters, Tamura and Nei/F84 and General Time Reversible).

Finally, eight different tree topologies were statistically tested: (1) the Syngonata/Chorismogonata (Casanova, 1985) hypothesis (Fig. 1A), (2) the Tokioka A (Tokioka, 1965a) hypothesis (Fig. 1B), (3) the Tokioka B (Tokioka, 1965b) hypothesis (Fig. 1C), (4) the Salvini-Plawen (1986) hypothesis (Fig. 1D) and four hypotheses derived from the present molecular results: (5) (Spadellidae/Pterosagittidae, Eukrohniidae/Heterokrohniidae, Sagittidae/Krohnittidae), (6) (Spadellidae/Pterosagittidae, (Eukrohniidae/Heterokrohniidae, Sagittidae/Krohnittidae)), (7) (Eukrohniidae/Heterokrohniidae, (Spadellidae/Pterosagittidae, Sagittidae/Krohnittidae)), and (8) ((Spadellidae/Pterosagittidae, Eukrohniidae/Heterokrohniidae), Sagittidae/Krohnittidae). For each hypothesis, an exhaustive search of ML trees in ProtML (Molphy 2.3b3 [Jun Adachi and Masami Hasegawa, 1992–1996]) was performed using the same substitution model as in ML and MB analyses, and the best tree for the eight constraints by the likelihood criterion was retained. Then, to chose among these phylogenetic hypotheses, the eight selected trees were compared using the Approximately Unbiased (AU) test (Shimodaira, 2002, and references therein), as implemented in the CONSEL program (Shimodaira and Hasegawa, 2001), to test whether the difference between the log-likelihood scores (LnL) of the selected trees was statistically significant. This procedure was performed with the two data sets.

3. Results

Using universal and specific primers we amplified SSU rRNA genes from 16 species (Table 1) representing the six classical families of Chaetognatha: Sagittidae, Pterosagittidae, Krohnittidae, Spadellidae, Eukrohniidae, and Heterokrohniidae.

3.1. Paralogous SSU rRNA genes in chaetognaths

We isolated fragments belonging to two distinct classes of SSU rRNA from most of the species studied: class I SSU

rRNA fragments from 14 species, and class II SSU rRNA fragments from 12 species (Table 1). Moreover, class I and II SSU rRNA genes could be isolated even from single individual DNA preparations (in *P. draco* and *E. fowleri* for instance) showing that both classes are present in the chaetognath genome.

3.2. Systematics

Phylogenetic analyses were performed with various substitution models which had no major influence on tree topologies (data not shown); so we only present the trees based on the HKY model, which is the only model available for all the phylogenetic programs used in this study (Molphy, Treepuzzle, and MrBayes). Two data sets of SSU rRNA sequences were used. First, an unrooted analysis was conducted with the data set 1, which comprises all available sequences (29 sequences from 19 species: 17 class I and 12 class II sequences, Fig. 2A). Then, the data set 2 was used because it includes the longest sequences available (18 sequences from 15 species: 15 class I rooted with 3 class II sequences) and, in these analyses, the 3 class II sequences were used as outgroups (Fig. 2B). The phylogenetic analyses based on this data set gave more robust (higher support values) and accurate (only one hypothesis is not rejected in the statistical tests) results than the data set 1.

3.2.1. Phragmophora/Aphragmophora division

From classical definitions, the Phragmophora are represented in this study by the Spadellidae, Eukrohniidae, and Heterokrohniidae families, and the Aphragmophora by the Sagittidae, Pterosagittidae, and Krohnittidae. In most of the phylogenetic analyses of the two SSU rRNA data sets, three monophyletic groups are significantly supported: Sagittidae/Krohnittidae, Spadellidae/Pterosagittidae, and Eukrohniidae/Heterokrohniidae (Fig. 2C). Hence, because a species without phragmes (*P. draco*) lies in a family of Phragmophora (the Spadellidae), the orders Aphragmophora and Phragmophora are not monophyletic. All the species that do not possess phragmes, except *P. draco*, are robustly gathered: 95/1.00/99/11 (respectively, quartet puzzling support in ML, posterior probabilities in MB, bootstrap support in MP and Bremer values) support for class I sequences with data set 1 (Fig. 2A), 57/0.99/78/2 for class II sequences with data set 1 (Fig. 2A), and 94/0.99/96/8 for data set 2 (Fig. 2B). This clade is refered in the rest of the text as "Aphragmophora without *P. draco*."

The group constituted of the classical Phragmophora species and *P. draco* (which we designate as "Phragmophora + *P. draco*") is strongly supported with data set 2 (98/1.00/79/9) but not with data set 1. However, topologies and support values obtained from data set 1 in ML (Fig. 2A), MB, and MP (data not shown) analyses do not exclude the monophyly of this group.

To test different tree topologies, we conducted several statistical analyses. Eight hypotheses were tested with the two data sets (Table 2). With data set 1, while classical hypotheses (1–4) and hypothesis 5 are rejected (p value < 0.05), the test cannot discriminate between hypotheses 6, 7 and 8. Analyses based on data set 2 are more accurate: the AU test strongly supports the monophyly of Phragmophora + *P. draco* (hyp. 8), as all other hypotheses can be rejected (hyp. 1–7).

3.2.2. Sagittidae and Krohnittidae families

Our data set contains 10 sequences representing 7 of the 9 genera of Sagittidae proposed by Tokioka (1965a): *Flaccisagitta* (*F. enflata*), *Aidanosagitta* (*A. neglecta* and *A. crassa*), *Mesosagitta* (*M. decipiens*), *Pseudosagitta* (*P. lyra*), *Parasagitta* (*P. elegans*, *P. setosa*, and *P. megalophthalma*), *Sagitta sensus stricto* (*S. bipunctata*), and *Serratosagitta* (*S. tasmanica*). In the molecular analyses presented here, the Sagittidae is a paraphyletic assemblage from which *K. pacifica* is derived. This Sagittidae/Krohnittidae group corresponds to the "Aphragmophora without *P. draco*" clade described above, and shows therefore high support values.

The genus *Parasagitta* seems to be a natural group comprised of *P. megalophthalma*, *P. elegans*, and *P. setosa* (51/0.98/88/2, 96/0.99/92/2, 98/1.00/93/7, respectively, for class I and class II sequences with data set 1 and with data set 2). *S. bipunctata, S. tasmanica* (only in data set 1), *K. pacifica*, and *Parasagitta* form a monophyletic group with undetermined internal relationships (90/1.00/69/0, 71/1.00/99/11, 98/1.00/90/4). Relationships between the other Sagittidae species are less clear. Monophyly of the genus *Aidanosagitta* (*A. crassa* and *A. elegans*, only class I sequences) is not recovered. However, the grouping of *A. crassa*, *A. neglecta*, *F. enflata*, and *M. decipiens* is relatively well supported (87/1.00/76/1, 78/1.00/92/7, respectively, with class I sequences with data set 1 and with data set 2). Within the Sagittidae, the most obscure affinities are those of *P. lyra*, which is either in an undetermined position or belongs to the *Aidanosagitta*/*Flaccisagitta*/*Mesosagitta* group (87/0.96/59/1, 88/0.99/82/2 respectively with class I sequences with data set 1 and with data set 2). When assessing the Sagittidae internal relationships, the closest outgroup sequences are the Phragmophora sequences. Therefore, we rooted the Sagittidae with the Phragmophora SSU rRNA sequences by exclusion of all class II sequences. This gave similar Sagittidae topology (data not shown).

3.2.3. Spadellidae and pterosagittidae families

The Spadellidae appears as a paraphyletic group in all the phylogenetic analyses reported here, because of the presence of *P. draco* (83/1.00/−/−, 87/1.00/100/6, 99/1.00/68/1). Within this surprising assemblage, the grouping of *S. cephaloptera*, *S. ledoyeri*, and *P. draco* is well supported (58/1.00/100/30, 87/1.00/100/6, 100/1.00/100/31), with *P. Draco* deriving from the paraphyletic genus *Spadella* (74/1.00/99/9, 95/1.00/97/3, 100/1.00/99/13).

3.2.4. Eukrohniidae and Heterokrohniidae families

Three Eukrohniidae species are included in the analysis (*E. hamata, E. fowleri*, and *E. bathypelagica*), and are always sister taxa (84/1.00/100/7, 76/1.00/98/7, 100/1.00/100/

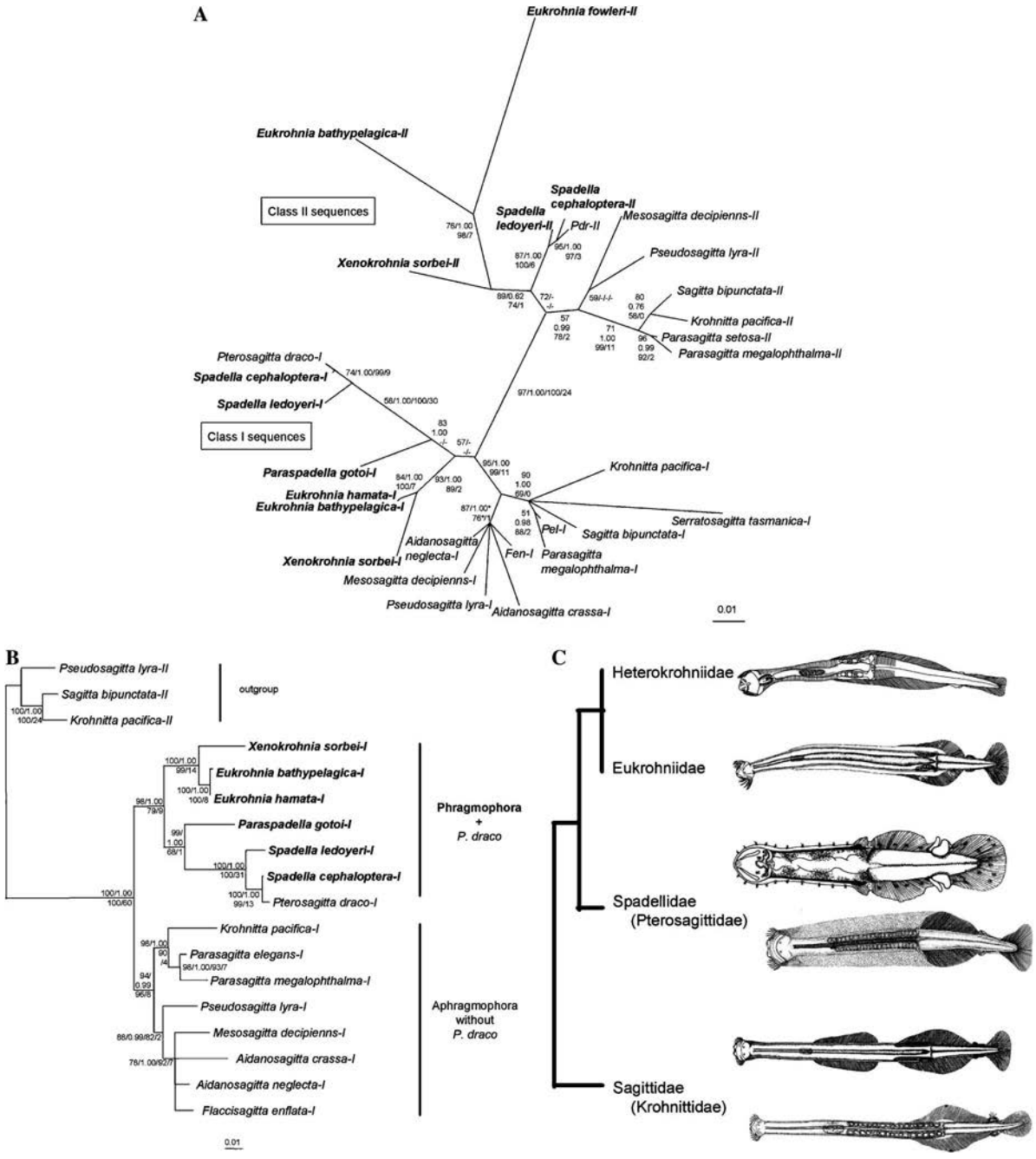

Fig. 2. Phylogenetic analyses of SSU rRNA sequences from 19 species of chaetognaths with Maximum Likelihood (ML), Bayesian (MB), and Maximum Parsimony (MP) methods. (A and B) The trees presented are the ML reconstruction. The ML and MB analyses were conducted using the HKY model of evolution. For each branch, the first two numbers (often above the branches) represent percentage of quartet puzzling replicates for ML and posterior probabilities for MB. The last two numbers (often below the branches) are bootstrap and Bremer support values for MP. Boldface type indicates Phragmophora species. I, class I sequence; II, class II sequence. (A) Unrooted analyses of the data set 1: all the available sequences of class I and II (29 sequences from 19 species, 17 class I sequences, and 12 class II sequences) some longer than others (1761 positions, 264 Parsimony informative sites). *, support value for the same clade but without *P. Lyra* (the values for the clade including *P. Lyra* are, respectively, 0.96 and 59 for MB and MP); Fen, *Flaccisagita enflata*; Pdr, *Pterosagitta draco*; Pel, *Parasagitta elegans*. (B) Analyses of the data set 2: an alignment of the longest class I and II sequences available (18 sequences from 15 species, 15 class I sequences, and 3 class II sequences) with same length for all sequences (1761 positions, 213 Parsimony informative sites). The three class II sequences are used as outgroups. (C) Chaetognaths systematics presented here, on the basis of molecular data. Pictures of the specimens from top to bottom: *Xenokrohnia sorbei* (Heterokrohniidae; Casanova, 1993), *Eukrohnia bathypelagica* (Eukrohniidae; Alvarino, 1967), *Paraspadella gotoi* (Spadellidae; Casanova 1990), *Pterosagitta draco* (Pterosagittidae; Alvarino, 1967), *Parasagitta elegans* (Sagittidae; Alvarino, 1967), *Krohnitta pacifica* (Krohnittidae; Alvarino, 1967).

8), corroborating the monophyly of the genus Eukrohnia. Because only one genus of Heterokrohniidae (*Xenokrohnia*) is included in the analysis, the monophyly of this family cannot be assessed. Nevertheless, results show that Heterokrohniidae and Eukrohniidae are sister-groups (93/1.00/89/2, 89/0.62/74/1, 100/1.00/99/14).

Table 2
Tests of significance for the eight competing phylogenetic hypotheses (1–8), with the two data sets

	Phylogenetic hypotheses	AU
Data set 1		
7	**(Eukrohniidae/Heterokrohniidae, (Spadellidae/Pterosagittidae, Sagittidae/Krohnittidae))**	**0.782**
8	**((Spadellidae/Pterosagittidae, Eukrohniidae/Heterokrohniidae), Sagittidae/Krohnittidae)**	**0.303**
6	**(Spadellidae/Pterosagittidae, (Eukrohniidae/Heterokrohniidae, Sagittidae/Krohnittidae))**	**0.094**
5	(Spadellidae/Pterosagittidae, Eukrohniidae/Heterokrohniidae, Sagittidae/Krohnittidae)	0.001
4	Salvini-Plawen	0
2	Tokioka A	0
3	Tokioka B	0
1	Syngonata/Chorismogonata	0
Data set 2		
8	**((Spadellidae/Pterosagittidae, Eukrohniidae/Heterokrohniidae), Sagittidae/Krohnittidae)**	**0.996** ⇐ Best
7	(Eukrohniidae/Heterokrohniidae, (Spadellidae/Pterosagittidae, Sagittidae/Krohnittidae))	0.006
6	(Spadellidae/Pterosagittidae, (Eukrohniidae/Heterokrohniidae, Sagittidae/Krohnittidae))	0
5	(Spadellidae/Pterosagittidae, Eukrohniidae/Heterokrohniidae, Sagittidae/Krohnittidae)	0
4	Salvini-Plawen	0
2	Tokioka A	0
3	Tokioka B	0
1	Syngonata/Chorismogonata	0

The hypotheses (1) Casanova (1985), (2) Tokioka A (Tokioka, 1965a), (3) Tokioka B (Tokioka, 1965b), and (4) Salvini-Plawen (1986) are described in Fig. 1. Data sets are described in the material and methods section. AU, Approximately Unbiased test. Significance level = 5%. Boldface type indicates values above significance level.

4. Discussion

4.1. Paralogous SSU rRNA genes in chaetognaths

Analyses of the SSU rRNA genes show that sequences can be allocated to two different classes (class I and II). In their earlier study, Telford and Holland (1997) also observed two different classes of chaetognath LSU rRNA sequences. The broad distribution within the phylum of this phenomenon and phylogenetic analysis strongly led the authors to suggest that "both classes of [LSU rRNA] gene are present in the genomes of all extant chaetognaths… [and that] each class probably represents a tandem ribosomal gene cluster, homogenized through molecular drive…" (Telford and Holland, 1997).

To our knowledge, the presence of distinct forms of rRNA sequences has only been reported in few other metazoans (*Xenopus* in Fedorof, 1979; platyhelminths in Carranza et al., 1996, 1999; cephalopods in Bonnaud et al., 2002) that did not inherit this condition from their common ancestor. In the Dugesiidae family of Tricladia flatworms, Carranza et al. (1999) showed that the duplication, first detected with the SSU rRNA, is a duplication of the entire ribosomal cluster, as it is likely the case in Chaetognatha. Moreover, it has been suggested that the chaetognaths LSU rRNA and the two classes of Platyhelminths SSU rRNA are expressed and functional (Carranza et al., 1999; Telford and Holland, 1997).

The wide distribution of both SSU rRNA classes across the phylum, the phylogenetic analyses (Fig. 2), and the previously observed LSU rRNA duplication corroborate an ancestral duplication of the whole ribosomal gene cluster, prior to the radiation of extant chaetognaths. Whether the paralogous clusters are tandemly repeated close to each other or located in homologous or non-homologous loci is not known. In platyhelminths, preliminary results with fluorescence in situ hybridization on metaphase chromosome show that type I and type II ribosomal clusters are located in non-homologous loci (Carranza, 1997), and this could decrease the possibility of homogenization with their paralogous relatives during concerted evolution (Carranza et al., 1999).

Interestingly, it has been shown in the chaetognath genus *Eukrohnia* that a similar-sized insertion is present in an identical position in both LSU rRNA classes (Telford and Holland, 1997). This could contradict the expectation that after the ancestral duplication the class I and II LSU rRNA genes evolved at different rates and have not been homogenized (because intragenomic exchange of information did not occur). However, the lack of similarity observed between the insert sequence in class I and class II LSU rRNA genes suggests that these insertions events are more likely due to convergence (see Fig. 1 in Telford and Holland, 1997).

4.2. Systematics

With the data sets 1 and 2 and in all phylogenetic methods conducted here, the main result is that three main clades are recovered (Fig. 2) corresponding to three groups of classical families: Sagittidae/Krohnittidae, Spadellidae/Pterosagittidae, and Eukrohniidae/Heterokrohniidae (Fig. 2C).

4.2.1. Phragmophora/Aphragmophora division

The first issue addressed is the taxonomic division of the phylum into the orders Phragmophora and Aphragmophora. These orders were previously hypothesized by

Tokioka (1965a), and such a division has been followed and supported by many authors (Bieri, 1991a; Dallot and Ibanez, 1972; Telford and Holland, 1997). The phylogenetic analyses reported here show that these two orders are not monophyletic, because a species without a transverse musculature (*P. draco*) is included in the Phragmophora. Phylogenetic and statistical analyses of molecular data suggest that the phylum is divided into two clades: (1) Aphragmophora without *P. draco* and (2) Phragmophora + *P. Draco.*

The presence of a transverse musculature has always been considered as an ancestral state of the phylum by morphologists (Casanova, 1985; Casanova and Duvert, 2002; Tokioka, 1965a). Following this view, the Aphragmophora appears as polyphyletic in the present study. However, as discussed below, molecular data did not help to infer the plesiomorphic state of this character. Hence, the Aphragmophora paraphyly cannot be ruled out.

SSU rRNA study also suggests that the Phragmophora is a paraphyletic group from which *P. draco* derives. However, uncertainty remains on the monophyly of the Phragmophora + *P. draco* group. The enlargement of the taxon sampling with species belonging to the deep benthoplanktonic *Heterokrohnia* and *Archeterokrohnia* should further test this result. The above conclusion also leaves us with a problem of nomenclature, given that the Aphragmophora and Phragmophora terms do not correspond to monophyletic groups but rather refer to a morphological character probably subject to homoplasy.

4.2.2. Sagittidae and Krohnittidae families

The Sagittidae family represents more than 75% of all the extant chaetognath species, and the only morphological synapomorphies described by Tokioka (1965a) are the two pairs of lateral fins and the two sets of paired anterior and posterior teeth. At first, all the members of this family were grouped into a single genus (*Sagitta*). Alvariño (1963), then Tokioka (1965a), and Bieri (1991a) divided the family into various genera. Several criteria were taken into account for these divisions: position and shape of the corona ciliata and of the lateral fins; presence/absence and shape of the intestinal diverticula; trunk/tail length ratio; seminal vesicle position; rayless-zones in the lateral fins; body aspect, etc. Despite the complexity of the distribution of these characters, some of the new genera created by Tokioka (1965a) were recovered in the previous LSU rRNA analysis (Telford and Holland, 1997), namely *Solidosagitta*, *Parasagitta*, *Cæcosagitta*, and *Pseudosagitta*.

In the present study, the Sagittidae is a paraphyletic assemblage from which a *Krohnitta* species (Krohnittidae family) derives. The grouping Sagittidae/Krohnittidae corresponds to the group described earlier as Aphragmophora without *P. draco*, and is highly supported.

This is the first time that a *Krohnitta* is placed within the Sagittidae. Indeed, this genus has always been considered very isolated because of its highly specialized cephalic armature with curved hooks and lanceolated teeth. Moreover, species belonging to this genus possess only one pair of lateral fins beginning above the caudal septum and a slightly different tail fin compared to other families. *Krohnitta* species also present only one set of teeth and are devoid of any collarette or glandular structures on the body surface. Other characteristics defining this genus are: a rayless-zone found on the lateral fins and a short corona ciliata which begins at the level of the neck (defined as type A in Tokioka, 1965b). All these characters explain why morphologists excluded this group from the Sagittidae. However, the recently discovered *Sagitta nairi* displays arrangement, shape, and, to a lesser extent, size of anterior teeth that recall those of the genus *Krohnitta* (Casanova and Nair, 2002).

This new position of *Krohnitta* within the Sagittidae has several implications. First, this is not in agreement with the division of the order Aphragmophora into Flabellodontina and Ctenodontina (Tokioka, 1965a, Figs. 1A and B). Second, this contradicts the rank of family assigned to the Krohnittidae, and rather suggests that they are part of the Sagittidae, and probably close to *Parasagitta*, *Sagitta*, and *Serratosagitta*.

While several relationships between Sagittidae species could be inferred from the SSU rRNA data (mainly the monophyly of the genus *Parasagitta* and its affinities with the *Sagitta*, *Serratosagitta*, and *Krohnitta*), some species relationships are not resolved. These difficulties could be due to a likely recent and rapid evolutionary origin of the family, already proposed on the basis of LSU rRNA analyses (Telford and Holland, 1997), and the relatively small number of Sagittidae studied here.

The monophyly of the genus *Parasagitta* proposed here is in agreement with the LSU rRNA analysis in which *P. setosa* and *P. elegans* sequences were clustered (Telford and Holland, 1997). However, the inclusion of *P. megalophthalma* within the *Parasagitta* contradicts Bieri (1991b) who proposed to place this species into a monospecific genus.

Kinship between *S. tasmanica*, *S. bipunctata* and the *Parasagitta* is in agreement with several morphological characters (Table 3). These species display the same type of very elongate corona ciliata, which begins just behind the brain and stretches backwards onto the dorsal side of the anterior region of the trunk (defined as type C in Tokioka, 1965b), and their lateral fins are wholly set with rays. Moreover, Dallot (1970) already proposed such affinities between *S. bipunctata* and the *Parasagitta* species on the basis of these morphological characters and other ones, such as structure and position of lateral fins, and number of teeth and hooks.

The grouping of *Mesosagitta*, *Aidanosagitta*, and *Flaccisagitta* displays a relatively high support in the work presented here. Some morphological characters are congruent with the association between *Aidanosagitta* and *Mesosagitta*: the corona ciliata begins below the eyes level (defined as the B type in Tokioka, 1965b) and intestinal diverticula are present (Table 3). However, these characters isolate *Flaccisagitta* from the rest of the group: the corona ciliata is short and confined to the head, starting just behind the

Table 3
Distribution of morphological characters in Chaetognatha

Species	Family	Phragme number	Lateral fins[a]	Primary muscles fibers type	Tail/total length (%)	Corona ciliata[b]	Teeth[c]	Ocular type[d]	Intestinal diverticula[e]
Aidanosagitta crassa	Sagittidae	0	2a	**AB**	30	B	**2**	**I**	+
Aidanosagitta neglecta		0	2a	**AB**	30	B	**2**	**I**	+
Flaccisagitta enflata		0	2a	**AB**	17	D	**2**	**I**	–
Mesosagitta neodecipiens		0	2a	**AB**	27	B	**2**	**I**	+
Pseudosagitta lyra		0	2b	**AB**	18	D	**2**	**I**	–
Parasagitta elegans		0	2a	**AB**	25	C	**2**	**I**	+
Parasagitta megalophthalma		0	2a	**AB**	25	C	**2**	**I**	–
Parasagitta setosa		0	2a	**AB**	14	C	**2**	**I**	–
Serratosagitta tasmanica		0	2a	**AB**	27	C	**2**	**I**	–
Sagitta bipunctata		0	2a	**AB**	24	C	**2**	**I**	–
Krohnitta pacifica		0	2c	**AB**	26	**A**	Ant	**I**	+
Paraspadella gotoi	Spadellidae	1	**1a**	A	**48**	**A**	**2**	**I**	–
Spadella ledoyeri		1	**1a**	A	**52**	**A**	**2**	**I**	–
Spadella cephaloptera		1	**1a**	A	**48**	**A**	**2**	**I**	+
Pterosagitta draco		0	**1a**	**AB**	**41**	**A**	**2**	**I**	–
Xenokrohnia sorbei	Heterokrohniidae	**2**	**1b**	**AB**	49	?	**2**	–	–
Eukrohnia fowleri	Eukrohniidae	1	**1b**	**AB**	24	E	Post	**I**	–
Eukrohnia bathypelagica		1	**1b**	**AB**	34	?	Post	E	–
Eukrohnia hamata		1	**1b**	**AB**	24	E	Post	E	–

Boldface type, plesiomorphic state indicated in classical morphological studies.

[a] 1a, one pair of lateral fins beginning at the level of the caudal septum; 1b, one pair of lateral fins always beginning above the caudal septum, approximately at the level of the nervous ventral ganglion; 2a, two pairs of lateral fins; 2b, two pairs of lateral fins linked by a tegumentary bridge; 2c, loss of the anterior pair of lateral fins from the classical two pair state of Sagittidae, the remaining fins beginning above the caudal septum far from the position of the nervous ventral ganglion.

[b] A–D, type of corona ciliata after Tokioka (1965b); E, corona ciliata not described by Tokioka, generally pear shaped, begins at the posterior edge of the brain and ends at the neck region; ?, corona ciliata not observed.

[c] 2, anterior and posterior tooth-rows; Ant, only anterior tooth-row; Post, only posterior tooth-row.

[d] I, inverted type with a pigment cell; E, everted type with ommatidia-like structure; –, no eye.

[e] +, presence; –, absence.

brain and stretching to the neck (defined as the D type in Tokioka, 1965b) and intestinal diverticula are absent (Table 3). On the other hand, *Flaccisagitta* and *Mesosagitta* share distinctive rayless zone on lateral fins while *Aidanosagitta* lateral fins are wholly rayed.

Finally, the isolation of *P. lyra* within the Sagittidae has already been proposed by Dallot and Ibanez (1972) and these authors even suggested that the possibility that *P. lyra* belongs to the Sagittidae could be dubious.

4.2.3. Spadellidae family

This family is characterized by a high ratio between tail length and total body length, i.e., a tail segment equal to the trunk segment (Table 3). Its members have one pair of lateral fins, anterior and posterior rows of teeth, and a short corona ciliata on the neck, wider than long (defined as A type in Tokioka, 1965b). Another characteristic of the Spadellidae is the reduction of the number of the type B fibers in the primary muscles as compared to the other families that possess equally two types of muscle fibers, A and B (Table 3). Bowman and Bieri (1989) separated the original genus *Spadella sensus lato* into two genera, *Spadella* and *Paraspadella*, both included in the Spadellidae. *Spadella* presents clusters of adhesive cells on the ventral side of the body, while *Paraspadella* displays prominent digitate adhesive organs ventrolaterally on the tail segment.

In our analyses, the Spadellidae is a paraphyletic assemblage from which a species without phragmes (*P. draco*) derives. *P. draco* is the only representant of the Pterosagittidae family, which is part of the Aphragmophora and, has been included by Tokioka (1965a) within the Ctenodontina together with the Sagittidae (Figs. 1A and B). *P. draco* presents a massive collarette developed all along the body, one pair of thoroughly rayed lateral fins, beginning at the caudal septum, a ciliary tuft on each lateral side at the level of the ventral ganglion, a corona ciliata confined to the neck region (type A), and a high tail/total length percentage (47%). Relationships between *P. draco* and *Spadella* have been previously mentioned, firstly by Tokioka (1965b): "the existence of a pair of tentacle tufts at the level of the middle of the trunk in *Pterosagitta* […] seems to remind us of various and small sensory apparatus found in the body surface of *Spadella*," and second by Dallot and Ibanez (1972) in their morphometric analysis (taking into account the high tail/total length proportion characteristic to the Spadellidae). In addition, their oval corona ciliata, which is restricted to the neck (type A), and the shape and position of the pair of lateral fins are also reminiscent of those found in the Spadellidae.

Molecular data are not in agreement with the assignment of Pterosagittidae as a family, since the sole representant of the group within in the genus *Spadella*. More

Spadella sequences would be needed to further test wether the genus is paraphyletic or not. Together with the new position of *K. pacifica* within the Sagittidae, the placement of *P. draco* within the Spadellidae forces the reappraisal of the Ctenodontina/Flabellodontina hypothesis (Tokioka, 1965a, see Figs. 1A and C).

4.2.4. Eukrohniidae and Heterokrohniidae families

Tokioka (1965a) described the Eukrohniidae *sensus lato* (comprising at that time *Eukrohnia*, *Heterokrohnia*, and *Bathyspadella*, Fig. 1A) as pelagic, with short tail segment, vestigial phragmes in the trunk (except for *Heterokrohnia*), and one pair of lateral fins starting well anterior to the caudal septum. In some species, a corona ciliata is described; it is pear shaped and begins at the posterior edge of the brain and ends at the neck region (type E in Table 3) (Kuroda, 1981). Anterior tooth-row (*Eukrohnia*) or both anterior and posterior tooth rows (*Bathyspadella*, not studied here) may be missing in some genera. The Heterokrohniidae family was proposed by Casanova (1985, see Fig. 1C together with his Syngonata/Chorismogonata and Monophragmophora/Biphragmophora hypotheses (see Section 1).

In the phylogenetic trees based on SSU rRNA, the genus *Eukrohnia* is monophyletic and is close to *X. sorbei* (Fig. 2). Hence, the Monophragmophora (Spadellidae/Eukrohniidae) is paraphyletic, and includes the Biphragmophora (the Heterokrohniidae *X. sorbei*), again refuting the Syngonata/Chorismogonata hypothesis by phylogenetic analysis and statistical tests. The question of the monophyly of the Heterokrohniidae will be addressed only when SSU rRNA sequences from *Heterokrohnia* and *Archeterokrohnia* species will be available.

4.3. Morphological characters

The molecular classification presented here (Fig. 2C) forces the re-interpretation of the morphological characters used to establish the classification of Chaetognatha. It appears that several of these characters seem to be either prone to homoplasy, mainly due to ecological pressure, or too variable to be suitable for classification at the order or family levels (Fig. 3, Table 3).

4.3.1. Phragmes

From a morphological point of view, the most fundamental character used to define the Phragmophora and Aphragmophora orders is the presence or absence, respectively, of the transverse musculature, namely the phragmes. At first glance, the distribution of this character seems congruent with the SSU rRNA analysis. However, as already stated, a species without a transverse musculature (*P. draco*) lies in a classical family of Phragmophora (the benthic Spadellidae) showing that this character is subject to homoplasy (Fig. 3A). The observation of phragmes in both trunk and tail of deep bentho-planktonic species (Heterokrohniidae), living in dark and cold deep water traditionally considered not favorable with speciation, led some authors to suggest that the presence of phragmes is the plesiomorphic state of this character (Casanova, 1985; Casanova and Duvert, 2002; Tokioka, 1965a). Within this framework, this structure would have been lost twice independently (parallel evolution) in Sagittidae and *Pterosagitta*, the Aphragmophora group being therefore polyphyletic. Conversely, if the absence of phragmes is considered as the plesiomorphic state of the phylum (Salvini-Plawen, 1986), two evolutionary histories can be hypothesized: (1) several acquisitions of the phragmes in the various Phragmophora lineages (Aphragmophora would then be paraphyletic) or (2) a unique acquisition in the ancestral Phragmophora lineage. This last possibility is more parsimonious and supported by the homology of transverse musculature structures amongst Phragmophora. Hence, during the evolution of the Spadellidae lineage, the loss of phragmes in *Pterosagitta* would be a case of homoplasy by reversion, emphasizing the plasticity of this character. Molecular data cannot discriminate between these hypotheses. Within the Phragmophora + *P. draco* group, there is a correlation between the lifestyle and the absence/presence of phragme. Indeed, all benthic species possess phragmes, whereas *P. draco*, as all other pelagic species of the Sagittidae/Krohnittidae assemblage, does not. The Eukrohniidae, also living in a pelagic environment, possess a vestigial transverse musculature and they could represent a conserved intermediate evolutionary state (Casanova and Duvert, 2002). The plasticity of this character reflects the influence of the environment on morphology particularly when species adapt to a planktonic lifestyle.

4.3.2. Fins

Traditionally, one of the main synapomorphies of Sagittidae is a double pair of lateral fins (anterior and posterior), as opposed to the single pair observed in other families. The molecular analysis, in agreement with previous morphometric studies (Dallot and Ibanez, 1972), isolates *P. lyra* within the Sagittidae. Such a position can be related to the fin morphology. Indeed, a tegumentary bridge connects the anterior and posterior fins, which is an autapomorphy of the genus *Pseudosagitta*. The inclusion of *K. pacifica*, displaying only one pair of lateral fins, within the Sagittidae family also raises some questions about the evolutionary history of this character. One pair of lateral fins is traditionally considered as a plesiomorphic state in all chaetognaths (Table 3). Therefore, *Krohnitta* could be an early off shoot of the *Krohnitta*/*Parasagitta*/*Serratosagitta* group, that retained this ancestral state and the acquisition of the double pair of lateral fins would have occurred several times in Sagittidae. However, the lateral fins of the *Krohnitta* species seem not to be homologous to that of the Eukrohniidae and Spadellidae. Indeed, their shape and positioning along the body are reminiscent of the lateral posterior fins in Sagittidae, suggesting that the anterior pair has been lost in *Krohnitta*. Finally, within the Phragmophora, the unique pair of lateral fins of Eukrohniidae/Heterokrohniidae and Spadellidae can be distinguished on the basis of their position.

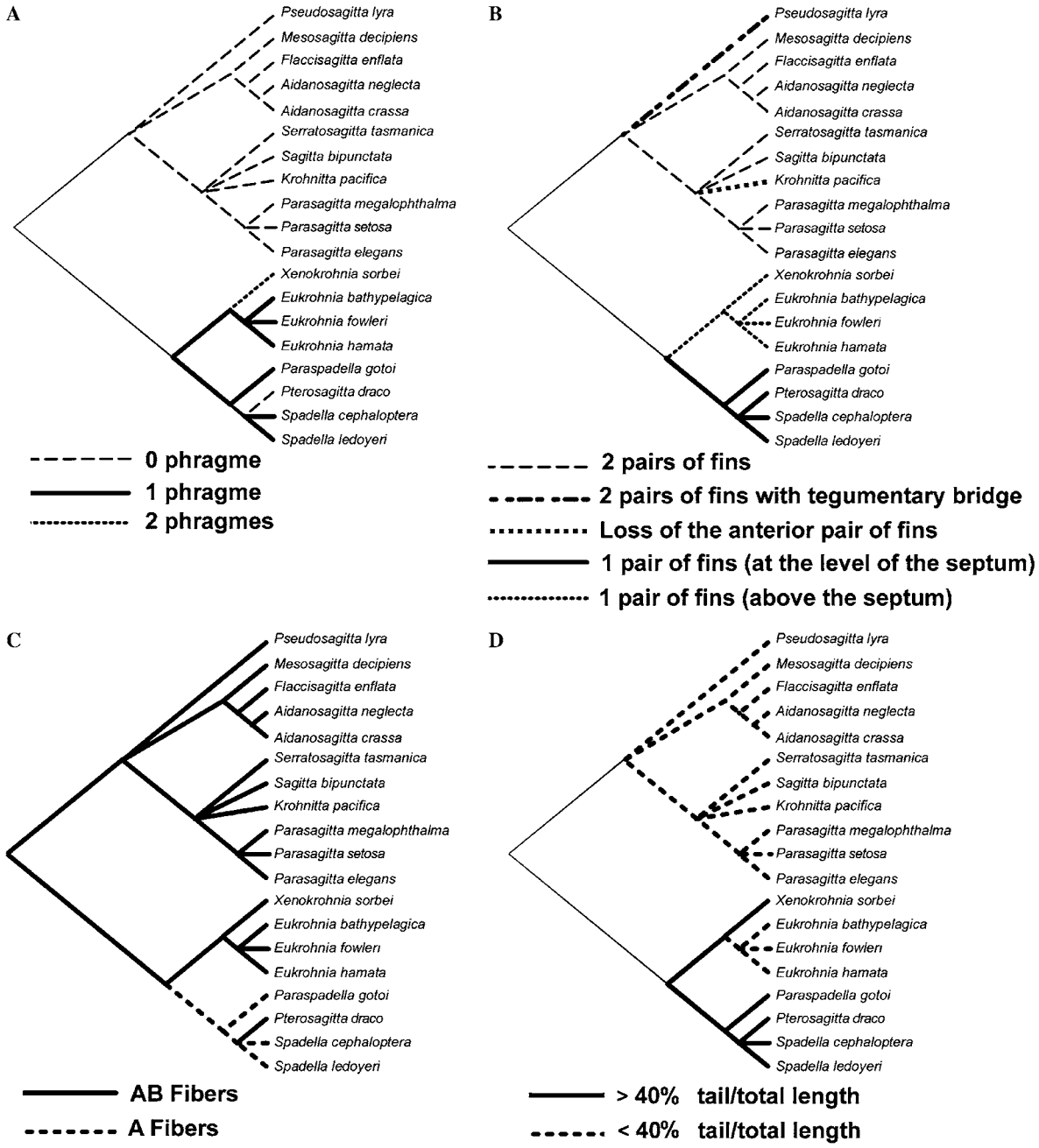

Fig. 3. Evolution of four morphological characters as reconstructed on the topology resulting from the analysis of the SSU rRNA data (Fig. 1C). (A) phragmes number, (B) type of lateral fins, (C) type of fibers in primary muscles, and (D) tail length/total length percentage. Thin branches indicate unknown character state. Only polarization of the character C can be inferred from molecular topology.

Hence, this general observation of the phylum allows the characterization of five distinct states instead of two (Fig. 3B, Table 3): (1) one pair of lateral fins beginning at the level of the caudal septum (Spadellidae and *P. draco*; type 1a), (2) one pair of lateral fins always beginning largely above the caudal septum, approximately at the level of the nervous ventral ganglion (Eukrohniidae and Heterokrohniidae; type 1b), (3) two pairs of lateral fins (Sagittidae except *Pseudosagitta*; type 2a), (4) two pairs of lateral fins linked by a tegumentary bridge (*Pseudosagitta*; type 2b), and (5) loss of the anterior pair of lateral fins from the classical two pairs-state of Sagittidae, the remaining fins beginning above the caudal septum far from the location of the nervous ventral ganglion (*Krohnitta*; type 2c).

Morphology of fins has an obvious influence on the efficiency of movement and buoyancy in aquatic animals. It is then expected that a correlation exists between fin morphology and benthic or pelagic lifestyle. In a pelagic environment, species have to maintain in the water column, and this is favored by a high relative fin surface (surface/volume ratio). This is true for most of pelagic species studied here. The Eukrohniidae members possess one elongated pair of lateral fins. Sagittidae display two pairs of fins, or two pairs connected by a tegumentary bridge (it has been proposed

that this structure is involved in buoyancy [Kapp, 1991]). Another pelagic genus studied here is *Krohnitta*, which displays only one pair of fins, but with significantly increased size. The Heterokrohniidae family has a benthoplanktonic lifestyle (close to the substratum, but staying in the water column) reflected by an intermediate surface/volume ratio. Within the phylum, Spadellidae is the only family that shows a strictly benthic ecology and they possess only one reduced pair of lateral fins. *P. draco* also possesses a reduced pair of fins, but displays a very peculiar adaptation to the pelagic lifestyle: a voluminous collarette, composed of largely vacuolated cells, embeds the greatest part of its body and would likely reduce its specific gravity to reach a better buoyancy (Perez et al., 2001). Therefore, the morphology of lateral fins displays a diversity more probably linked to the species ecology than to their phylogenetic relationships, and this character cannot be polarized with molecular data.

4.3.3. Muscular fibers

The nature of the muscular fibers has also been used to establish the chaetognath evolutionary history (Casanova and Duvert, 2002). All the planktonic chaetognaths examined in this study, including *P. draco*, display A and B fibers in their primary muscles while the number of B type fibers is very reduced in the benthic *Spadella* and *Paraspadella* (Casanova and Duvert, 2002, Fig. 3C, Table 3). The phylogenetic reassignment of *P. draco* within the Spadellidae suggests that this character is either influenced by the mode of life (reacquisition of B fibers in *Pterosagitta*), or, less parsimoniously, conserved in *P. draco* and lost in *Paraspadella* and the two *Spadella* lineages. Nevertheless, since a few B fibers can sometimes be observed in the primary muscles of *S. cephaloptera* (Casanova and Duvert, 2002) and that both type of fibers are observed in the three main clades identified in the molecular analyses, the results presented are congruent with the Casanova and Duvert (2002) proposal that (1) the presence of A and B fibers is ancestral to the phylum and that (2) the benthic members of the Spadellidae have lost most of the B fibers during evolution. We therefore propose that B fibers have been reacquired in the *P. Draco* lineage, in correlation to its pelagic mode of life.

4.3.4. Tail/total length percentage

As for the morphological features discussed above, the tail proportion also appears linked to the lifestyle. Indeed, in benthic (*Spadella* and *Paraspadella*) and some benthoplanktonic (*Archeterokrohnia* and *Xenokrohnia*) species, the tail/total length percentage is higher (close to 50%) than in the rest of the chaetognaths species (17–34%), all of them being planktonic (Fig. 3D, Table 3). For the planktonic mode of life, a relatively longer trunk than tail (yielding a lower tail/total length percentage) could provide better buoyancy, possibly because a long trunk segment can compensate the high density of the tail segment at maturity that contains the testes (Kapp, 1991). The planktonic *P. draco* presents a high tail/total length percentage but slightly smaller (41%) than the Spadellidae. This could be further evidence that *P. draco* is a derived Spadellidae adapted to the planktonic mode of life. Once more, the molecular topology does not help to infer the ancestral state of this character.

4.3.5. Other morphological characters

Other morphological characters can be discussed in the light of the present SSU rRNA analyses (Table 3). It appears that these characters can be useful for phylogenetic considerations only to determining synapomorphies of genera, or small groups of genera. This is the case with the teeth where the loss of the posterior pair is specific to *Krohnitta*, while the loss of the anterior pair is characteristic of *Eukrohnia*. In a similar way, only some *Eukrohnia* species possess the everted ocular type. Concerning the shape of the corona ciliata, the C type has been shown to be specific to the related genera *Parasagitta*, *Serratosagitta*, and *Sagitta.* The A type displayed by the Spadellidae family is considered as the ancestral form (Tokioka, 1965b). This suggest that *Krohnitta* has retained the A type, and could support a basal position of the genus in the *Krohnitta*/*Parasagitta*/*Serratosagitta*/*Sagitta* group. Because of a lack of phylogenetic resolution in the other Sagittidae species, the clarification of the heterogenic distribution of the corona ciliata types (D and B) in these groups requires a larger data set. Another homoplastic character is the presence or absence of the intestinal diverticula. The distribution of this character is stochastic and can be related neither to the molecular classification nor to the ecology. Such a situation is a reminder of the distribution of the cytological characteristics of the muscles in the phylum (Casanova and Duvert, 2002). One of the authors' conclusions from this last study is that the distribution of these characters (except for the primary muscles fibers type) is reminiscent of mosaic evolution, i.e., correlated neither to phylogeny nor to ecology.

5. Conclusions

In conclusion, the molecular analysis of chaetognath systematics (summarized in Fig. 2C) corroborates only some of the polarities of morphological characters determined by morphologists (Fig. 3, Table 3): the presence of two pairs of tooth-rows, ocular inverted type and primary muscles containing both A and B fiber types. Polarity of the other characters cannot be determined due to the lack of external group of the phylum for comparisons, limited data set, or homoplasy. Altogether this greatly reduces the number of suitable characters available to solve the internal relationships of Chaetognatha, and stresses the need for other types of characters (like supplementary ultrastructural or molecular data).

SSU rRNA genes analyses show that Chaetognatha is very likely constituted of two clades: (Sagittidea + *K. Pacifica* = Aphragmophora without *P. draco*) and (Spadellidae + *P. draco*, Eukrohniidae/Heterokrohniidae =

Phragmophora + *P. draco*) (Fig. 1C). However, even though this study is the broadest ever conducted for the phylum, our data set is still limited and some genera absent from the analysis (*Archeterokrohnia, Heterokrohnia, Hemispadella*, *Bathyspadella*, *Calispadella*, *Bathybelos* other Sagittidae genera) will be of importance (1) to test more severely the monophyly of the (Phragmophora + *P. draco*) group, (2) to test the monophyly of the Heterokrohniidae family, and (3) to give more details on the Sagittidae relationships.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2005.12.004.

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