Scale-worm Systematics
(Annelida, Polychaeta)

Doctoral Thesis

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2013

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ISBN 91-89677-53-6

Printed by Kompendiet, Aidla Trading AB, Gothenburg,Sweden

Dissertation abstract

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Scale-worm Systematics
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Paper I. Scale-worms are segmented worms. They can be found in all marine benthic habitats, including about 1200 species and over 200 genera. There has been little known about the phylogeny of this group and this study is aiming at achieving one. 56 terminal taxa are examined, including 8 out-group taxa. Nuclear markers (18SrRNA, 28SrRNA) and mitochondrial markers (16SrRNA, COI) for the molecular analysis and 24 morphological characters were combined in the analysis. The data are analyzed with Bayesian analyses, maximum likelihood and parsimony. The combined data confirm that scale-worms is a monophyletic group. However, the scale-less pisionids and *Palmyra aurifera* Savigny in Lamarck, 1818, also nest within the scale-worms. In pisionids the lack of elytra represent a secondary loss but the case with *P. aurifera* is unresolved. There are multiple equally parsimonious pathways one can use to explain this. Only with the case of loss of elytra in *P. aurifera*, the scales represent a clear-cut synapomorphy (a shared derived state) for scale-worms. The phylogenetic result render some taxonomic changes on family and sub-family level.

Paper II. More taxonomy is dealt with in a re-description of *Bylgides sarsi* (Kinberg in Malmgren, 1865) based on syntypes and fresh material from the Baltic Sea and the use of the phylogenetic results from the phylogeny discussed above.

Paper III. *Harmothoe imbricata* (Linnaeus, 1767), has been reported as a colour-polymorphic species. Hitherto no genetic studies have confirmed this assumption leaving a possibility of cryptic species. 57 individuals representing 10 different colour morphs from Svalbard, Norway and Sweden were investigated. Based on two molecular markers it turns out that *H. imbricata* indeed is polymorphic and the only differences in allele frequencies is explained by distance.

Paper IV. In systematics it is vital to have vouchers. Vouchers enable others to examine the taxonomic identity assigned to a sample by the author of a study. Vouchers are specimens, tissues or preparations. In order to identify different kinds of vouchers, a terminology is suggested with the value for taxonomic verification.

Keywords: Scale-worms, systematics, phylogeny, taxonomy, cryptic species, voucher

Svensk sammanfatting

Erika Norlinder, 2013
Skalmasksystematik
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Papper I. Skalmaskar utgör en grupp segmenterade marina maskar. De finns i alla marina bentiska habitat, från strandkanten ner till de stora havsdjupen. Gruppen innefattar ungefär 1200 arter indelade i över 200 släkten. Släktskapet inom gruppen skalmaskar, har varit näst intill okänt. Den här studien belyser den släktskapen. 56 taxa skalmaskstaxa inklusive 8 utgruppstaxa inkluderades i analysen som använde både nukleära markörer (18SrRNA, 28SrRNA), mitokondriella markörer (16SrRNA, COI) och 24 morfologiska karaktärer. Både separat och kombinerat data analyserades. Bayesiansk analys, maximum liklihood och parsimoni användes i analyserna. Analyserna visar att skalmaskarna utgör en monofyletisk grupp. Några taxa utan skal (elytror), lägger sig också väl inom skalmaskgruppen, pisionider och *Palmyra aurifera* Savigny in Lamarck, 1818. Avsaknad av skal inom pisioniderna grundas på en sekundär förlust av den karaktärer. I fallet med *P. aurifera*, ligger frågan öppen. I nuläget finns det flera lösningar på den. Det är enbart i det fall där *P. aurifera* har en sekundär förlust av skal, som den karaktären blir en entydig synapomorfi (karaktär som härleds från en gemensam anfader). Lösningen är beroende av hur tricotomin löser upp sig basalt i trädet. Resultaten från studien skapar endel taxonomiska ändringar på familj- och underfamiljnivå.

Papper II. Mer taxonomi behandlas i en återbeskrivning av *Bylgides sarsi* (Kinberg in Malmgren, 1865). Den baseras på syntyper och nytt material från Östersjön och resultat från släktskapsträdet i papper I.

Papper III. *Harmothoe imbricata* (Linnaeus, 1767) har rapporterats vara färgpolymorf. Tills nu har inga genetiska studier genomförts för att ge stöd för det, vilket gör att det skulle kunna röra sig om en kryptisk art. 57 individer med 10 olika färgmorfer från Svalbard, Norge och Sverige användes i en molekylär analys med två markörer (COI and ITS1–5.8SrRNA–TIS2). Det visade sig att *H. imbricata* är färgpolymorf. Den skillnad på allelfrekvens som upptäcktes, kunde förklaras med skillnad i avstånd.

Papper IV. I systematisk forskning är det av stor vikt att deponera vouchers (verifiering) av de organismer man jobbar med. Det gör att andra forskare kan verifiera den taxonomiska identiteten som författaren använt till en studie. En voucher kan bestå av arter, underarter, lokala populationer eller cellkulturer. En terminologi läggs fram som ska framhålla värdet på den taxonomiska verifieringen.

List of papers

This thesis is based on the following papers:

I. Norlinder, E., Wiklund, H., Nygren, A., Pleijel, F., 2012. Phylogeny of scale-worms (Aphroditiformia, Annelida), assesed fron 18SrRNA, 28SrRNA 16SrRNA, mitochondrial cytochrome c oxidase subunit I (COI), and morphology. *Molecular Phylogenetics and Evolution,* 65, 490–500.

II. Norlinder, E., Pleijel, F. Redescription and generic affinity of *Bylgides sarsi* (Kinberg in Malmgren, 1865)*,* (Polynoidae, Aciculata, Annelida). Manuscript.

III. Nygren, A., Norlinder, E., Panova, M., Pleijel, F., 2011. Colour polymorphism in the polychaete *Harmothoe imbricata* (Linnaeus, 1767). *Marine Biology Research*, 7, 54– 62.

IV. Pleijel, F., Jondelius, U., Norlinder, E., Nygren, A., Oxelman, B., Schander, C., Sundberg, P., Thollesson, M., (2008). Phylogenies without roots? A plea for the use of vouchers in molecular phylogenetic studies. *Molecular Phylogenetics and Evolution,* 48, 369–371.

Table of contents

Summary of included papers

Paper I presents a phylogeny of scale-worms based on morphological and molecular data. The position of different families, taxonomic issues relating to families and subfamilies, and the root of the scale-worm tree is the focus.

Paper II treats the distribution, morphology and generic affinity of *Bylgides sarsi* (Kinberg in Malmgren, 1865). We select a lectotype and re-descibe *B. sarsi* based on syntypes and newly collected material from the Baltic Sea. Aims to assess the generic affinity are based on morphological and molecular data from Paper I.

Paper III investigates whether the colour polymorphic *Harmothoe imbricata* (Linnaeus, 1767) consists of several, cryptic species or a single species with varying colouration. We investigate this by comparing the different colour morphs and geographical distribution using a haplotype network based on two molecular markers.

Paper IV advocates the use of vouchers in molecular studies. The vouchers enable research of the actual biological unit used in the molecular analysis. Specimens from this thesis are deposited at the Swedish Muséum of Natural History.

Scaling of scale-worms

This thesis is about scales. Scales may have different meanings when working with systematics of scale-worms. The most obvious meaning of the word is the morphological character that has held this group together ever since they were introduced to science by Linnaeus in 1758. Possession of scales has been considered a character unique to and shared by all members of the group of the Aphroditiformia (Annelida, Polychaeta). Scales, or elytra, are situated on the dorsal side of the worms and are often easily spotted, when present, because, as it has turned out, some scale-worms actually lack scales.

Another meaning of the word scale is at what scale we study a phylogenetic tree. One might look at the tree of life on a scale including the whole metazoan branch, which would include many representatives from the multi-cellular life as in Hejnol *et al.* (2009) (Fig.1). Based on almost a hundred taxa and over a thousand genes, Annelida (segmented worms) is placed as sister group to Mollusca and Kryptrochozoa (Brachiopoda, Nemertea).

Zooming in on the Annelida part of the metazoan tree as in Bleidorn *et al.* (2003), Rousset *et al.* (2006) and Struck *et al.* (2011), where focus lies on trying to find resolution within the annelid tree by adding more molecular data. The most recent study by Struck *et al.* 2011, re-establish the long gone taxon names Errantia and Sedentaria among annelids, taxa based on their errant or sedentary life-styles, and placed the scale-worms within the Errantia. I have focused on the scale that includes the scale-worms as a whole group, on a species scale as well as a within species scale.

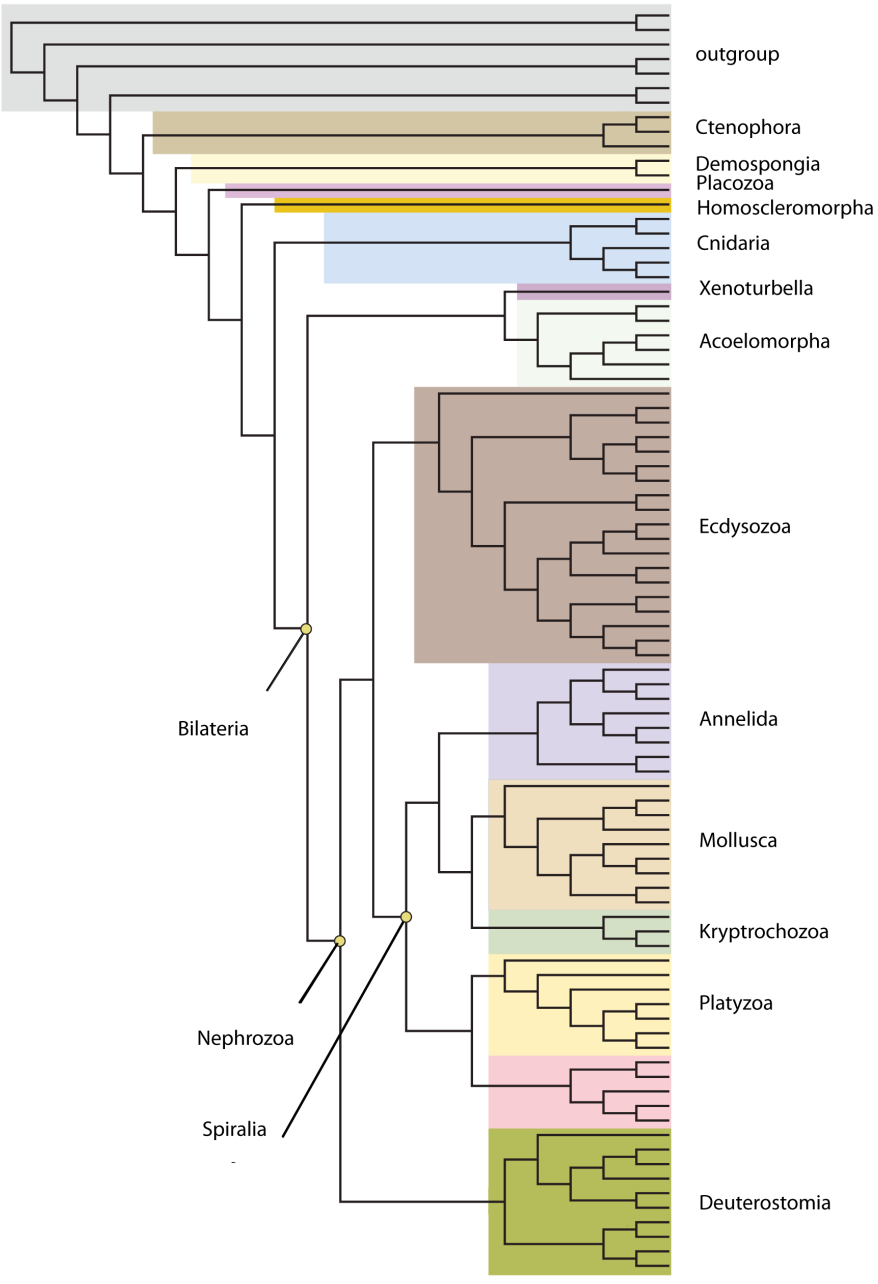


Fig.1. Metazoan tree. The most likely tree from maximum likelihood based on 1487 genes. Modified from Hejnol *et al.* (2009). The Annelida is the sister group to Mollusca, Kryptrochozoa (Brachiopoda, Nemertea).

Scale-worms

Annelids are segmented worms with well-known representatives such as earthworms and leeches (Clitellata). Polychaetes are a less known group which also belongs to the annelids. They are mainly marine worms and the total number of species is estimated to around 9 000 (Rouse and Pleijel, 2001). The polychaetes have lateral outgrowths called parapodia and provided with many bristles, thereof the Greek name: poly = many, chaeta = bristle. The Aphroditiformia, or scale-worms, constitute about 1 200 polychaete species divided into over 200 genera. They are traditionally separated into the families Acoetidae, Aphroditidae, Eulepethidae, Pholoidae, Polynoidae and Sigalionidae (e.g., Beesley *et al*., 2001; Rouse and Pleijel, 2001). From the result in this thesis there are some taxonomical changes regarding the families. From the phylogentic analysis in Paper I, we now recognise the families Acoetidae, Aphroditidae, Eulepethidae, Iphionidae, Polynoidae, Pholoidae and Sigalionidae. A representative from each of these families can be seen in Fig. 2.. Scale-worms are dorso-ventrally flattened animals, but there is considerable variation in body shape, ranging from elliptic to vermiform. The number of segments and coloration varies, from a few fixed to many continually added, from almost transparent to striking with various patterns, especially within the family Polynoidae. These characters are used for species identification. Colouration often provide informative characters when looking at live specimen, but is difficult with fixed ones, where the colour is faded or lost. When elytra are available they might be useful for species identification since they posses different shapes and structures *e.g.,* micro- and macrotubercles and different patterns and coloration. The shape of the prostomium, its appendages and how they are positioned, and the presence and position of eyes are also used. The parapodia and particularly the chaetae are diverse in their shapes and with different ornamentations, and they might be split or not, simple or compound, and having spines or hairy structures (Fig. 3).

Scale-worms are abundant in all marine habitats, from the intertidal to the deep-sea. They live both on soft and hard bottoms and also include pelagic species; most are free-living but some construct mud-tubes with spinning glands. Since the techniques of sampling in the deep-seas are now improved,

new scale-worm taxa are continuously being described from a variety of deep-sea habitats such as hydrothermal vent areas, cold seeps and whale falls.

Scale-worms are highly diverse in life history traits, and many are associated with hosts, including echinoderms, cnidarians, other polychaetes, bivalves and decapods (Rouse and Pleijel, 2001; Martin and Britayev, 1998). Most scale-worms have separate sexes, although hermaphroditism does occur (Wirén, 1907; Rouse and Pleijel, 2005). Sperm ultra-structure indicates both external and internal fertilization, and larval brooding is known from various members (Rouse and Pleijel, 2001; 2005). Judging from the jaw-structures, most scale-worms seem to be predatory (Fauchald and Jumars, 1979).

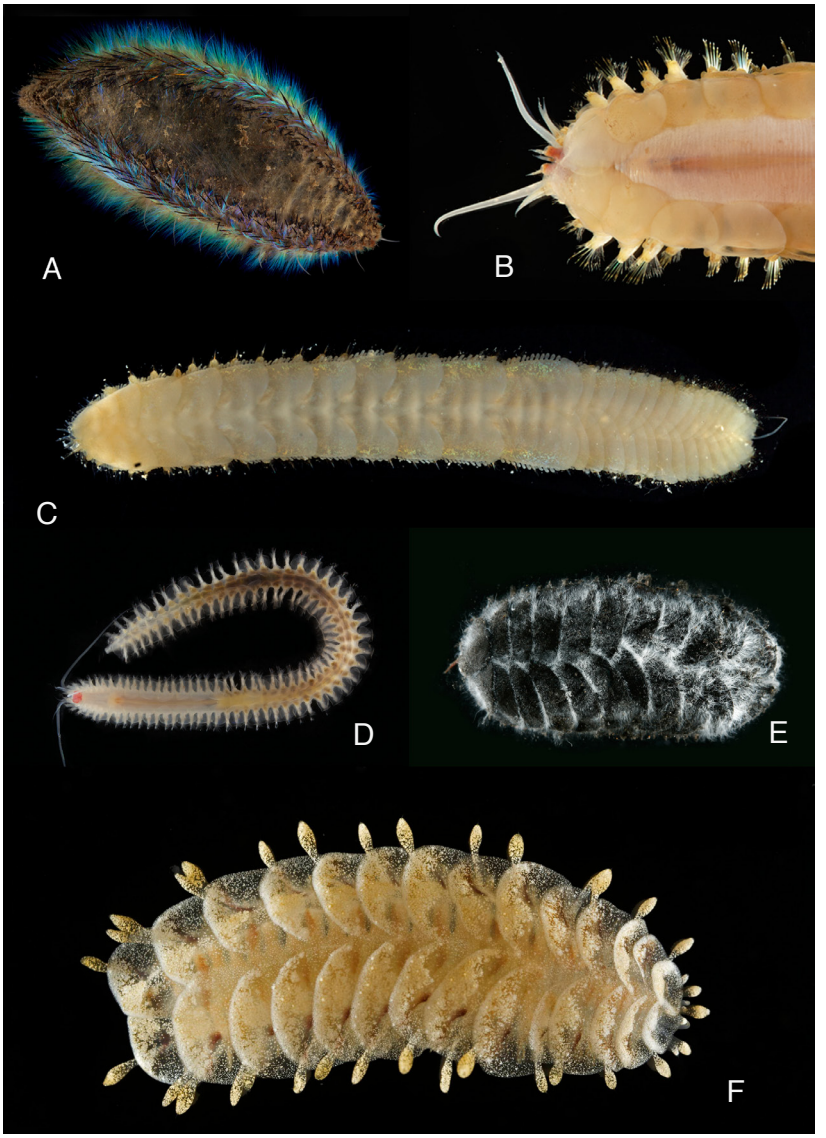


Fig.2. Scale-worms from different families. A. *Aphrodita aculeata* (Aphroditidae). B. *Panthalis oerstedi* (Acoetidae). C. *Grubeulepis mexicana* (Eulepethidae). D. *Neoleanira tetragona* (Sigalionidae). E. *Thermiphione* sp. (Iphionidae). F. *Gastrolepidia clavigera* (Polynoidae).

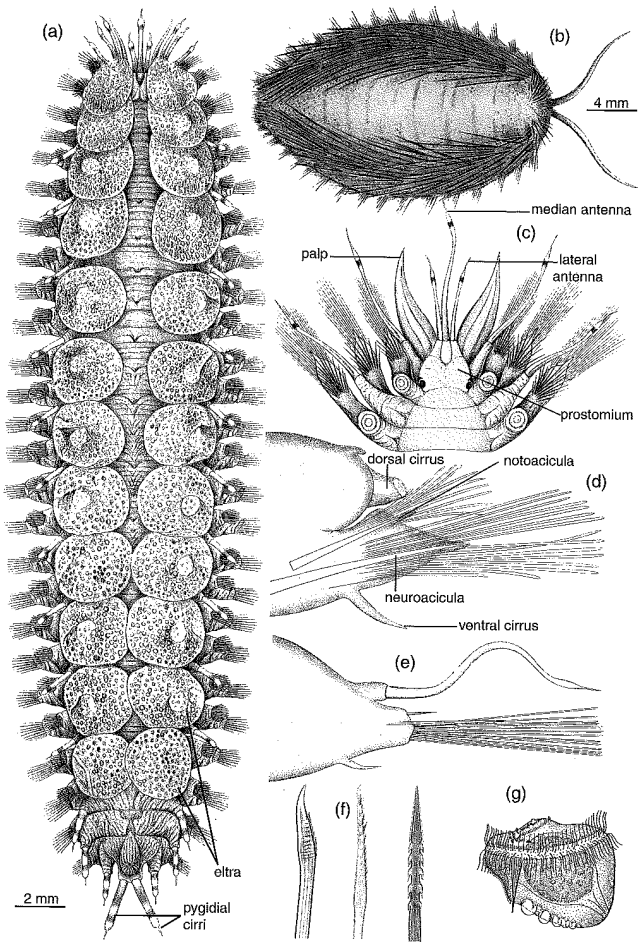


Fig.3. Scale-worm anatomy. (a) *Lepidonotus wahlbergi* (Polynoidae). Entire animal, dorsal view. (b) *Laetmonice aphroditoides* (Aphroditidae). Entire animal, dorsal view. (c) *Lepidonotus iphionides* (Polynoidae). Anterior end, dorsal view. (d) *Robertianella synopthalma* (Polynoidae). Anterior parapodium. (e) *Admetella longipedata* (Polynoidae). Anterior parapodium. (f) left, *Lepidonotus wahlbergi.* Polynoid neurochaeta; centre, *Lepidonotus iphionides.* Polynoid neurochaeta; right, *Laetmonice producta.* harpoon-shaped aphroditid notochaeta. (g) *Halosydna brevisetosa* (Polynoidae). Trochophore. (From Rouse and Pleijel, 2001).

Scales

When working with scale-worms, scales themselves trigger ones curiosity. It is not clear if the dorsal cirri and the scales are homologous. The digitiform dorsal cirri are usually situated only on segments without scales which is an argument for homology between the two structures, meaning that the scales would represent modified dorsal cirri. However, in many taxa the non-elytra bearing segments also possess dorsal tubercles that are situated at the same position as the elytra. The dorsal cirri instead are found distally on the parapodia, which instead would indicate a homology between elytra and dorsal tubercles. Pseudo-elytra are a special kind of elytra found within the family Eulepetidae. They occur on the posterior segments and differ both in size and in how they are attached to the cirriophores (Rouse and Pleijel 2001). The elytra are attached to the dorsum with an elythrophore by a hollow muscular stalk. This is an extension of the body wall and of the body cavity (Heffernan, 1990).

The pattern of segmental occurrence of scales varies along the worm. Up to segment 23 there is a consistency with the alternation between the dorsal cirri and elytra in most scale-worms. After segment 23 the scales are present on every third segment as in the Polynoidae or on every segment as in Sigalionidae. Some taxa lack scales from segment 23, and yet others have no scales at all like *Palmyra aurifera* Savigny in Lamarck, 1818, and the pisionids within the Sigalionidae. The elytra may serve different functions. Pettibone (1953), suggested that the elytra may be involved in respiration and sensory perception. The enhanced circulation of water due to the movement of the elytra probably contribute to facilitate respiration. The sensory role is supported by numerous complex papillae with sensory-structures similar to those of the cilia on the palps. In addition the elytra possess a well-developed neuronal system (Heffernan, 1990). In some pholoids, especially among interstitial taxa, the development of the embryos and juveniles occur in the elytra (viviparity) or under the elytra (brood-care) (Pettibone, 1992). In *Harmothoe imbricata* the scales are bioluminescent, probably to startle predators and perhaps for communication between individuals. The bioluminescence originates in a protein called polynoidin, and seems to be induced by the

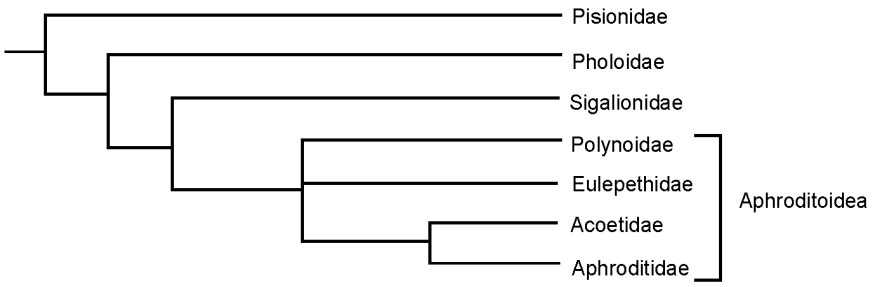
destruction of the electrochemical coupling between the body and the elytra when the latter are detached (Plyusheva and Martin, 2009). After detachment the elytra can re-generate in 10-15 days. The lower surface of the elytra has a layer of luminescent cells or photocytes (modified epidermal cells), lacking in non-luminescent species.

Paper I

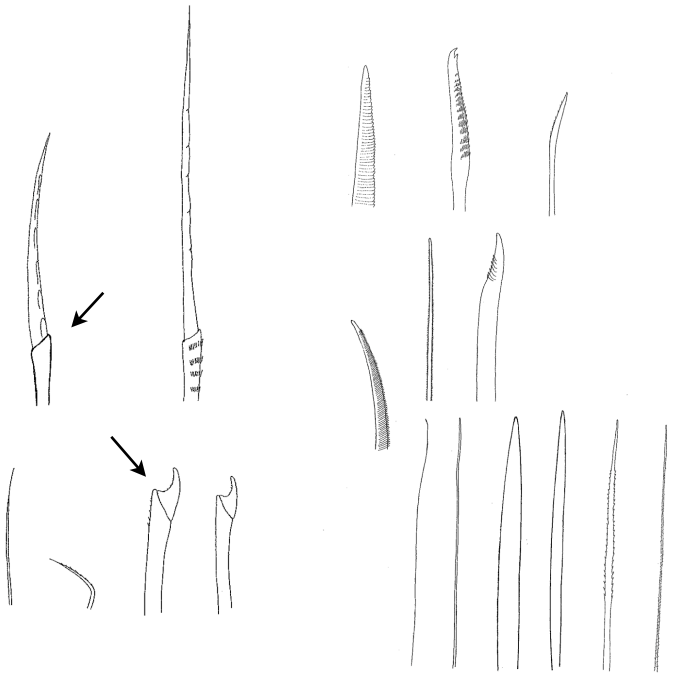
Systematics of scale-worms

Systematics is the broad scale study of biological diversity and its origin. It aims at understanding the evolutionary relatedness of biological entities, their phylogeny. The idea of systematics is based on Darwin's theories of common ancestry of all species, with the explanation that all species evolve through natural selection (Darwin, 1859). Darwin had a tree-like view of evolution, with lineages being split up and becoming more and more different with time. The underlying concept of his theory was that of homology, where a character would share common descent. Today we know that natural selection is not the only process involved in the evolutionary process. Genetic drift is a phenomenon where the change, mutation, in allele frequency occur by chance. After several generations the "new" allele frequency becomes fixed within the population and the ancestral allele is ultimately lost from the population.

Scales have for a long time been the homology of the scale-worms. Earlier cladistic works were based on morphology only, as in Rouse and Pleijel (2001). In their study the Acoetidae, Aphroditidae, Eulepethidae and Polynoidae constitute the Aphroditoidea, based an the presence of simple nerochaetae (Fig. 4).



a



b c

Fig.4.a) A tree based on morphology from Rouse and Pleijel, 2001. The Aphroditoidea have simple neurochaetae. b) Examples of compound neurochaeta with hinges (arrows) c) Examples of simple neurochaeta.

In 2005 two studies, by Wiklund *et al.* and Struck *et al.* investigated the phylogenetic relationships within the scale-worms, using morphological and molecular data, the latter based on molecular data only. Wiklund *et al.* (2005) placed the Aphroditidae basally in the tree and the Eulepethidae in a trichotomy with Polynoidae and Acoetidae. They also concluded that *Pisione* and Pholoidae are nested within the Sigalionidae (Fig. 5). The result were however ambiguous for the Eulepethidae, probably due to a limited set of data. An intriguing result from these studies is the position of the pisionids (usually treated as a separate family positioned outside the scale-worms) and *Palmyra aurifera* (by many earlier authors referred to the Chrysopetalidae, also outside the scale-worms), which both lack scales, but were situated within the scale-worm tree.

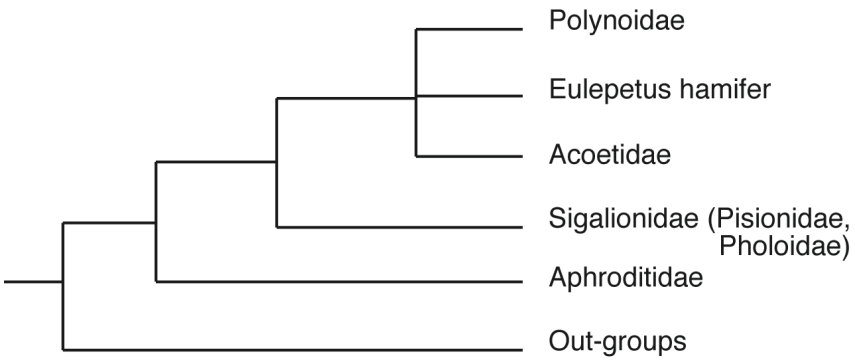


Fig.5. Scale-worm phylogeny based on 18S/CO1/Morphology and Bayesian analysis, Maximum likelihood and parsimony. Modified from Wiklund *et al.* 2005.

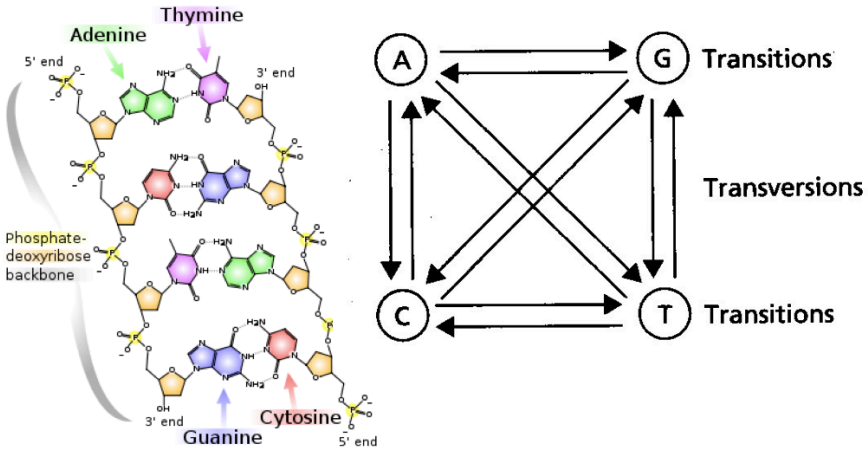
A phylogenetic tree is a graph depicting the ancestor–descendant relationships between organisms or gene sequences. The sequences are situated at the tip of the tree and the branches are the unobservable ancestral sequences. Molecular markers in combination with morphological characters were used in Paper I to assess the phylogeny of the scale-worms. The position

of the different scale-worm families and their positions in the tree in relation to each other and the of root of the scale-worm tree were investigated.

In phylogenetics, monophyletic groups are desired for defining groups of organism. A monophyletic group is a group of organism forming a clade in a tree and consists of species and all its descendants. Monophyletic groups share derived characters with their most recent common ancestor (synapomorphy), which in turn do not share that character with its own ancestor. A synapomorphy is an apomorphy common to several taxa. However, the trait might have gone through a secondary loss and is in that case not longer observable. The ancestral state is plesiomorphic.

The historical events illustrated in the tree are mutations that the sequences have gone through and there are different tree-building approaches to recover this phylogeny. We used Parsimony (PA), maximum likelihood (ML) and Bayesian analysis (BA). PA chooses the tree(s) with the fewest changes and thus assumes that the simplest explanation is the most likely solution. PA is a fast method and is good for slowly mutating genes and morphological studies. PA is however not able to detect hidden substitutions and other processes involved in the history of the sequences.

The nucleotides in a gene-sequence involve the purines, adenine (A) and guanin (G), and the pyrimidines, cytosine (C) and thymin (T). This is important for the applied evolutionary models since the transition-transversion rate often is considered less likely to occur and the models are based on these assumptions (Fig. 5a and 5b).



a b

Figure 5. a) DNA molecule showing adenine, thymine, the purines and guanine, cytosine, the pyrimidines. b) A schematic view of transitions and transversions.

ML and BA incorporate evolutionary models, of which there are several (Fig. 6). The models take into account the different evolutionary events that sequences may go through.

The application of ML to phylogenetics involves searching for the tree that has the highest probability of giving rise to the observed data. ML takes the combination of branch lengths, tree topologies and all the parameters in the evolutionary model into account to optimize that likelihood. Whereas likelihood judges a tree based on how probable it is that evolution would have produced the data, Bayesian inference judges trees based on their posterior probability, the probability that the tree is true, given the data, our evolutionary models, and our prior beliefs (Baum and Smith, 2013).

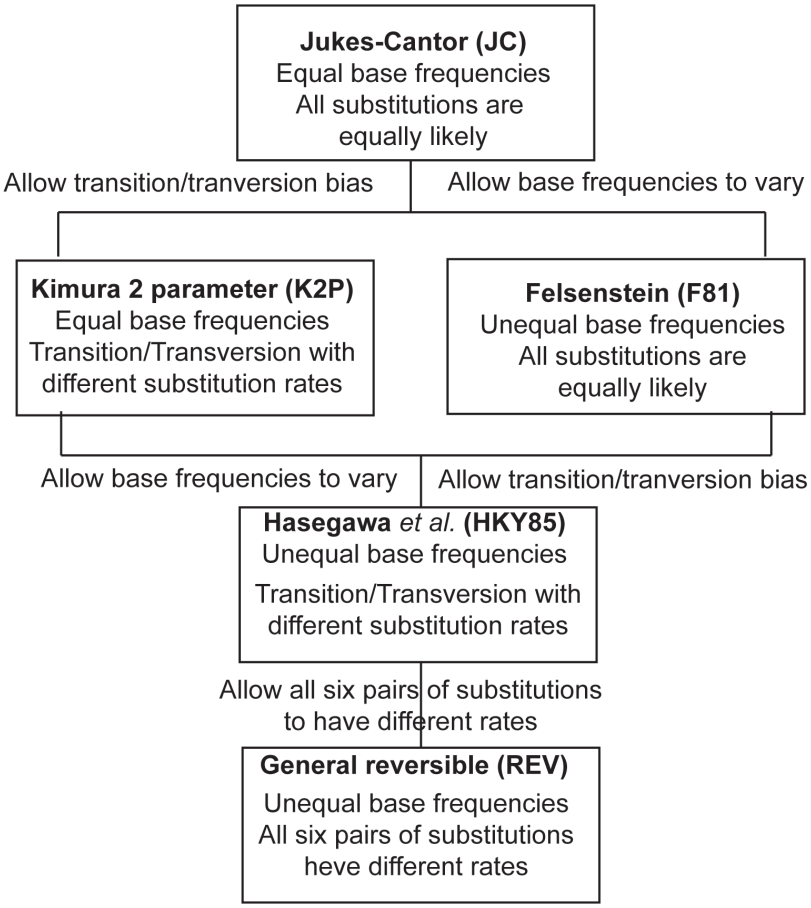


Figure 6. Interrelationships between five models for estimating the number of nucleotide substitutions among pairs of DNA sequences. Modified from Page and Holmes, 1998.

Some genes evolve slower than others and this can be used to investigate events taking place on different time scales. The genes also have different evolutionary history depending on if they are nuclear or mitochondrial. Nuclear genes are inherited from both parents and recombine during meiosis. This means that the genes are being mixed in every generation. In contrast, the mitochondrial genes are inherited from the mother only and do not recombine.

The morphological characters chosen for the study were intended to get a resolution at a basal level of the tree. The outcome of the study has given us new insights into the phylogeny of scale-worms ande has enabled us to retrieve a number of monophyletic groups (Fig. 7). The Polynoidae are monophyletic with Acoetidae as sister-group. The Iphionidae is elevated to family level. This group is mainly found in the deep-seas, and Pettibone (1986) and other authors had previously referred to them as a subfamily, Iphioninae. Another subfamily name that we eliminate is Harmothoinae, which is treated as a junior synonym of Polynoinae. Throughout the history of scale-worm systematics, Pholoidae, Pholoididae, Pisionidae and Sigalionidae are groups that have been discussed back and forth as to where they should be placed. We can confirm that Pholoididae och Pisionidae cluster inside the Sigalionidae, whereas Pholoidae may, or may not be treated as a separate family.

The root of the scale-worm tree remains enigmatic, with Aphroditidae and Eulepethidae as unresolved sister taxa in a polytomy with the rest of the scale-worm families. Since the scale-less *Palmyra aurifera* also belongs within the Aphroditidae we cannot answer the question whether scales is a synapomorphy for the group . That scales of the scale-worm tree remain to be solved.

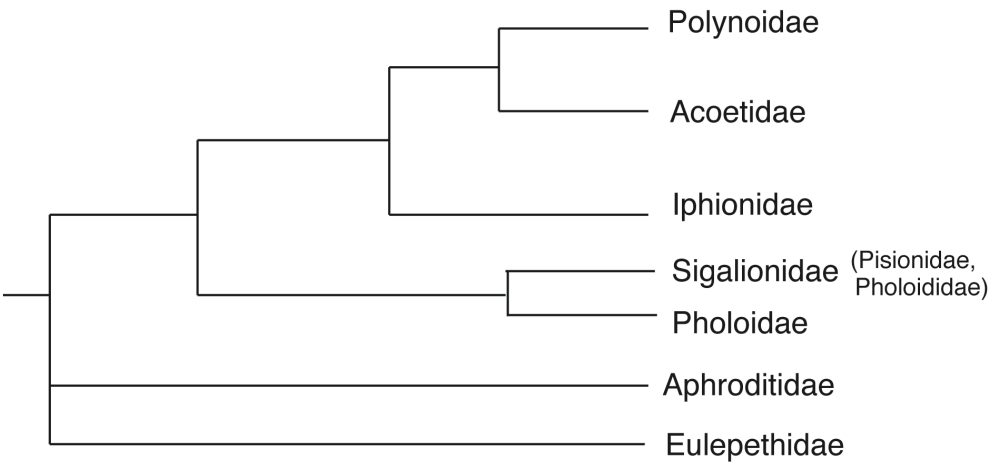


Fig.7**.** Scale-worm phylogeny based on 18SrRNA, 28SrRNA, 16SrRNA and morphology. Modified from Norlinder *et al*, 2012.

Paper II

Why so many names?

Linnaeus's binomial naming in *Systema Naturae, 10th Edition* (1758), is still used in today's zoological nomenclature. Linnaeus used a hierarchical classification to organize the knowledge of the living world. He focused on what he believed to be two basic ranks, genus and species. Genus is above the species rank. A genus can have many species but a species can only be assigned to one genus. In traditional nomenclature, the main role of ranks is to provide a basis for organizing the taxa into a hierarchy. The role of nomenclature is to provide stable tools for scientific communication.

The International Code of Zoological Nomenclature (ICZN) is a set of formal rules to regulate taxon names. However, these rules were set long before the phylogenetic principles were accepted. The rules are based on ranks, even though ranks lack clear evolutionary meaning (Baum and Smith, 2013). There are three features of ICZN that show the emphasis on ranks. All organism must be assigned to the mandatory ranks, species, genus and family.

1. Standardized forms of the names should be applied to the different ranks (Table. 1.).
2. The correct naming of a taxon is under the rule of priority.
3. The correct taxon name (defined with reference to a specimen, a type), is the earliest name published.

Typification of species:

Holotype. A single specimen designated as name-bearer by the original author at the time of publication.

Paratype. Non name-bearing specimen(s) designated at the same time as the holotype by the original author at the time of publication.

Neotype. A name-bearing specimen designated to replace the holotype or other types if these have been lost, destroyed or never were designated.

Syntypes. A group of name-bearing specimens designated or indicated by the author of the name at the time of publication.

Lectotype. One of the syntypes chosen after the original publication to function as name-bearer.

Paralectotype. The remaining specimens from a syntype series when a lectotype has been designated.

Table 1. Some of the main ranks and their endings in the Zoological Code of Nomenclature.

Rank	Zoological ending
Tribe	-ini
Subfamily	-inae
Family	-idae
Order	Not specified
Class	Not specified
Phylum	Not specified

Paper II illuminates the importance of distinct and explicit species-descriptions. It addresses the taxonomical issues regarding the generic affinity and species delineation of *Bylgides sarsi* (Kinberg in Malmgren, 1865). This species is part of the benthic macro-faunal community in the Baltic Sea. It has been monitored and ecologically investigated since the 1920 (Sarvala, 1971, Agrenius *et al.* 2010 Cederwall *et al.* 2011, Villnäs and Norkko 2011)). Both the generic affinity and the species delineation of Kinberg's species is confusing. Quite a lot of taxonomical studies have been made since the original description (e.g.; Théel, 1879; Levinsen, 1883; Augener, 1928; Pettibone, 1993). However, none of these studies have included any explicit phylogentic considerations. In Paper II we aply the phylogentic information given in Paper I on *B. sarsi*. Looking into the tree (Fig. 4, Paper I) it is situated as a sister taxon to *Bylgides elegans* (Théel, 1879), the type-species for the genus *Bylgides*.

This position in the tree gives us the possibility to settle it's generic affinity to *Bylgides*. We conclude that morphology alone is not able to resolve these issues. We redescribe *B. sarsi* and designate a lectotype, since no holotype has ever been designated, together with newly collected specimen from Västervik in the Baltic Sea, which is one of the original localities from Kinberg's material from 1865.

Paper III

Polymorphism and cryptic species

The occurrence of more than one form, or morph, of one or several characters within a species is called polymorphism. Common biological examples of this are sexual dimorphism, different blood types in humans, and mimetic forms of butterflies. Colour polymorphism is common in several major taxa but has rarely been reported in polychaetous annelids (Pleijel *et al.*, 2009). However, this is probably partly a reflection of how most collected specimens are treated. Most studies have been made on preserved animals where colour and pigmentation patterns have disappeared and colour polymorphisms in polychaetes is probably more common than presently known. There have been some reports of this in some sedentary serpulids and sabellids (Dales, 1962; ten Hove, 1970; Føyn and Gjønen, 1954) and in some errant syllids, hesionids and polynoids (Imajima, 1966; Pleijel *et al.*, 2009; Fauvel, 1923). In 2009, Pleijel *et al.,* found five colour morphs of a hesionid genus, *Gyptis* under a jetty in Edithburgh, South Australia. As it turned out these five morphs constituted three genetically different species, where one of the species was polymorphic. In another study by Nygren *et al.* (2005), they present an analysis of the relationships between the shallow water pigmented hesionid polychaete *Nereimyra punctata* Blainville, 1828, and a deep-water, unpigmented form from Norway and Sweden. No morphological differences are observed apart from the pigmentation. The results from that study, based on CO1 parsimony analysis, indicate that specimens belonging to the same form (pigmented or unpigmented) from different areas are more closely related to each other than different forms from the same areas. These studies lift the

importance to separate intraspecific colour polymorphism from colour variation among closely related species, cryptic spieces.

Investigating the presence of cryptic species may have an important role in marine biodiversity. In a study from 1985 by Christie, the polychaete *Chaetozone setosa* Malmgren, 1867, was investigated. This species was described as being cosmopolitan and found in depths ranging from the intertidal to 4436 m. However, the study showed that, from a site spanning 65 km with two intertidal sampling stations and one at 80 m, each had a distinct species. As a very rough estimate, one can expect the number of marine species to increase by an order of magnitude if cryptic species (sibling species) are considered (Knowlton, 1993). To have a closer look into the fine scale on the species level within the scale-worms will certainly prove worthwhile.

P**aper III** addresses the issue whether *Harmothoe imbricata* (Linnaeus, 1767), a common polynoid, is subject to color polymorphism or cryptic speciation. This species has been reported as a colour polymorphic species for a long time, but up until this study no genetic studies have been performed to confirm this assumption. The samples in this study were ranging from Svalbard to the Swedish west-coast. *Harmothoe imbricata* has a distribution in the Arctic, the North Pacific, the North Atlantic and the Mediterranean Sea and it can be found on a variety of different substrates. It can be either free-living or together with hermit crabs or tubiculous polychaetes. The depth of its distribution varies from intertidal banks of blue mussels to 300 m depth.

We use the mitochondrial genetical marker CO1 and nuclear ITS1–5.8SrRNA–ITS2 and we identified ten different colour morphs. The results show that *Harmothoe imbricata* is clearly a single species based on a minimum spanning haplotype network from the CO1 data. The variation in the ITS region was very limited.

Paper IV

The importance of vouchers

The use of molecular data have brought a revolution to systematics and phylogenetics. **Paper IV** concerns the importance of depositing vouchers. The increase of available molecular data in accessible repositories, such as GenBank, has awakened concerns as to the taxonomic origin of the data. Unfortunately there are a large number of sequences in GenBank that are incorrectly labelled. If they are not corrected they will continue to be associated with the wrong taxa, thus influencing the results from the scientists using those sequences. **Paper IV** is a plea to journals and organisations hosting public data repositories to require vouchers deposited in publicly accessible collections. In order to reflect the value in the taxonomic identification of the study species, a terminology is also suggested with a decreasing value for taxonomic verification. A voucher can be a whole specimen, tissues, preparations or of some other kind that enables others to examine its taxonomic identity.

Voucher terminology:

Hologenophore. Parts of the study organism is used for molecular studies and other parts from the same specimen are deposited as voucher.

Isogenophore. A different organism with clonal relationship to the study organism is deposited as voucher.

Progenophore. A sexual parent or offspring to the study organism, or a sexually produced full sibling, is deposited as voucher.

Paragenophore. An organism considered to be of the same taxonomic unit, collected at the same time and place as the study organism is deposited as voucher.

Syngenophore: Another organism identified as belonging to the same taxonomic operational unit as the study organism, collected from another locality and another location as the organism used for the molecular study, is deposited as voucher.

When working with molecular phylogenetics the vouchers connects the experimental data (sequences) to the designated operational taxonomic unit. This unit may be of different sorts e.g. species, subspecies, strains, local populations, demes or cultures.

The future of systematics lies in the use of molecular data that will be easy to retrieve from public repositories. However, without a voucher enabling a verification of the taxonomic unit, the information is at the mercy of the provider. We need to be able to go back to the source of the information. Vouchers are the only means of ensuring the quality of the results and are as important as the requirements of keeping laboratory journals and records in many disciplines of science. The voucher should be listed together with the sequences at the repository. The important role of museums and other public institutions has to be lifted. They need to be adequately funded in order to have the means to take care of the different kind of vouchers, preserved in different ways. Without proper use of vouchers, we might end up in a situation where we cannot trust the taxonomic identity of molecular data in our public repositories.

Difficulties in phylogenetic analysis

There are two steps to consider when building a morphological matrix. **Character encoding** is the limit of the character and the alternative states for each character. For example, when you decide to score the position of the median antenna in scale-worms, using two states, dorsal and anterior, you have encoded one character (position of median antenna). **Character scoring** is when you look at each taxa and assign the state for each encoded character. The choices of character encodings are built on the knowledge of the organism and characters chosen should vary in an informative way between taxa.

The delimitation of character states is sometimes tricky, since many morphological characters are continuous and there might be variation within taxa. This poses some problems since phylogenetic analysis need discrete characters. This division of continuous characters are subjectively chosen by the scientist and might have an impact on the results. While subjectivity is something that generally makes scientists uncomfortable, this fact does however not invalidate morphology as a source of phylogenetic data (Baum and Smith, 2013). Questionmarks in the morphological matrix might pose some other problems.

1. The character can´t be observed and it is not possible to say whether it exists or not. The head might be missing due to poor sampling or be old and deteriorated.
2. The character is not applicable. The shape of the eyes might be a character in the matrix, but the taxa lack eyes.
3. The character is polymorph, which could be a problem with a multi-state character. However this obstacle can be solved by soft-ware programs.

Molecular data are intuitively easier to score in matrix with the four possible scores A, T, C, G, but it does come with its own peculiarities. A change from A–T might seem simple, but there might have been an A–C–T change which we can't see. The evolutionary models we choose to work with in Maximum likelihood and Bayesian analysis provide means to deal with this. The use of one model of evolution or another may change the results of the analysis (Posada and Crandall, 2001). This choice is made by soft-ware that provide a suggestion for the best model that should be used for the data. The subjective choice of character encoding in morphological data is thus moved into the a choice suggested by computer soft-ware. This is also a subjective choice, but not as coupled to a specific knowledge of a scientist. The ability for another scientist to repeat the study is facilitated. Your input data are sequences, which can´t be as subjectively chosen as the morphological once. Sequences can be found on *e.g.* GenBank and anyone can use them without having to even look at the organism. The nucleotides are there in a specific

order. To score a morphological matrix a more in depth knowledge about the specific organisms is need, and that takes time and not as easily repeated by someone not specifically working on that group of organisms.

Other challenges with molecular data is that the DNA sequences must be aligned so that homologous sites can be compared. Changes in some sequences might be non-independent due to preservation of function. The structure of ribosomal RNA molecule for example includes short sequences whose bases must pair to form 'stems', so changes in those sequences are not independent. The more different the sequences are, the harder they are to align when you also take insertions, deletions and possible inversions, into account.

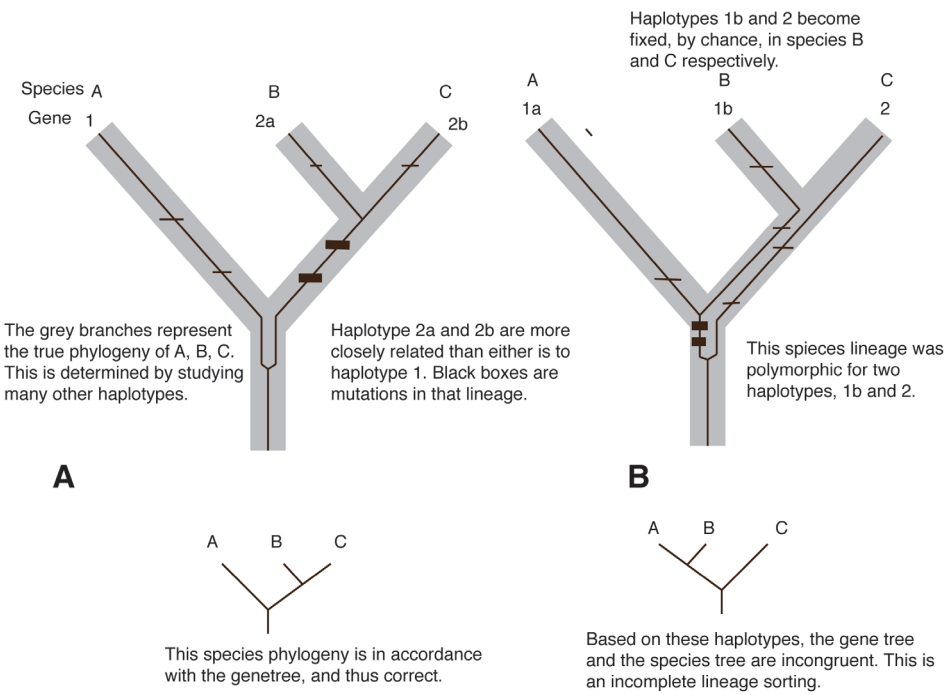


Fig. 8. A) An illustration where the gene tree and species tree are congruent, a case of lineage sorting. B) An example of incomplete lineage sorting, where the gene tree and species tree are incongruent. Modified from Futuyma, 2009.

One reason why we combine molecular and morphological data into the same phylogenetic analysis is the interest in character evolution. A priori we make a decision when we choose taxa to include into our analysis (see above section on character encoding). However, the phylogenetic analysis is dominated by the molecular data set and the tips of the tree is an extrapolation, usually from a single specimen that then comes to represent a whole species or even more inclusive taxa. In morphological analysis many individuals are investigated from collections and the literature, and cover a wider range of what is believed to be that taxon, which is a strength for the morphology-based analysis. In the molecular approach a lot of data is gathered from a single individual. In the morphological approach more individual specimen are examined but with less data, although representing a consensus of the morphological characters.

An accurately estimated gene tree may imply the wrong species phylogeny which might cause problems when interpreting the results from a phylogenetic analysis. This phenomenon can be explained by incomplete sorting of gene lineages. This means that the gene tree and the species tree do not necessarily match. In a case with three species A, B and C, suppose the ancestral species to these carries two different haplotypes of the gene we are studying. By chance haplotype 1 becomes fixed in species A and haplotype 2 is polymorphic then the haplotypes may become fixed, by chance, in such a way that the most closely related species do not inherit the same haplotype. A phylogeny based on these haplotypes might therefor imply an incorrect relationship among the species (Fig 8).

Conclusions and future prospects

In this thesis I have come to the following conclusions:

- Aphroditiformia (scale-worms) is a monophyletic group.
- Polynoidae is the sister group to Iphionidae (elevated to family status from Iphioninae). These two are sisters to the Acoetidae.
- The subfamilies Acholoinae and Harmothoinae are synonymized with Polynoinae.
- Sigalionidae includes the Pholoididae and Pisionidae, which are treated as junior synonyms.
- Pholoidae remains as a separate family, although with uncertainty.
- Aphroditidae and Eulepethidae are situated closest to the root of the scale-worm tree, but without enough support to assess which one is the most basal.
- *Bylgides sarsi* is re-described and typified. A lectotype is selected from the Baltic Sea to settle the type locality.
- *Harmothoe imbricata* is a colour polymorphic species.
- Using vouchers in molecular phylogenetics is a necessity in order to verify the sources of the species (or other taxonomic unit). A terminology for labeling different kinds of vouchers is suggested.

This thesis provides new insights into the evolution of scale-worms. The phylogenetic analyses may serve as a backbone to future analysis and it is safe to say that the last word has not been said about the coarse phylogeny of scale-worms. The family Acoetidae would need more taxa to confirm it's placement in the tree and more representatives of both Aphroditidae and Eulepethidae are needed to shed light on the illusive root of the scale-worm tree. It is vital to continue to explore both the molecular and morphological information to achieve a more in depth knowledge regarding character evolution within scale-worms. The sometimes overwhelming amount of data that are acquired by phylogenomic analysis should not out-compete morphological knowledge. In the end we are not interested in just acquiring long sequences of nucleotides

but to obtain knowledge about the observable biological units and their different characters and their evolutionary history.

A wish for future work with the scale-worms would be to make large revisions of many of the families, based on molecular and morphological data in order to solve some of the confusing taxonomic conundrums that are present among the groups. A way of solving these issues could be to refine the techniques of extracting DNA from formaldehyde preserved specimen. That would enable us to use molecular data from preserved type-material and put them in both a nomenclatural and a phylogenetic context.

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Acknowledgement

See English version below.

Hur ska jag kunna skriva något som inkluderar alla människor jag mött under min tid som doktorand? Alla och envar har gett mig något på sitt sätt. Den enda självklara början är att tacka Fredrik Pleijel. Utan ditt stöd, kunskap, generositet, ditt tålamod och medmänsklighet hade jag inte lyckats få ihop den här avhandlingen. **TACK!**
Arne Nygren, jag uppskattar våra korta, för mig intensiva, stunder på zoologen. Det har varit en skön blandning av alignments, boot-straps och MrBayes, till rytmen av sköna toner från spotify. **TACK!**
Min utsände i London, Helena Wiklund. Det har varit ett privilegium att få jobba med dig. Dina snabba svar och vilja att alltid ställa upp är fantastiskt!. Ibland kanske på din egen bekostnad. Ingen fråga har varit jobbig att ställa till dig och du har avdramatiserat händelser som gjort att jag satt hjärtat i halsgropen. **TACK!**

På tal om halsgrop. Det finns de som hävdar att livet är som en berg-och-dalbana. Visst, jag håller med. Slänger man dessutom i ett par barn, två neurotiska akademiker i vagnen och några extra loopar samtidigt som resultat ska analyseras och fantastiska texter ska skrivas, så har vi en typisk situation för en modern doktorand i sina bästa år! Eller, det kanske bara är jag...Saken är den att jag hade inte kunnat/velat göra det på något annat sätt. Jag gillar berg-och-dalbana! En del dalar har kanske vara lite väl låga och en del svängar något för tvära för att jag skulle hänga med riktigt och det finns stunder jag varit på väg att flyga ut ur vagnen. Men, med hjälp av min familj, vänner och ovan nämnda personer har jag orkat kravla mig tillbaka in i vagnen och stanna där till slutet på den här resan. Och, jag hade inte velat vara utan den! Ok, jag lovade mig själv att inte bli för pretentiös när jag skrev det här, det går så där... Så, kort sagt, det har varit en jävla resa!

Jag kom till Göteborgs Universitet, Tjärnö 2003. Det slog mig nu, tio år sedan! Många av mina närmaste vänner har jag skaffat sedan dess. På Tjärnö, Kristineberg, Botan, Zoologen och runt om i världen. Jag skulle kunna nämna er alla. Alla ni som jag har partajat, bastat, badat, chattat, tjôtat och ältat livets stora och små frågor med. Ni vet vilka ni är!

Så, ingen nämnd, ingen glömd! Jag kan bara hoppas att vår vänskap håller i sig länge än!

In English: Thank you all!

Funding

This thesis was funded by the Swedish Taxonomy Initiative and the Department for Biology and Environmental sciences, University of Gothenburg. Stipends were received from Colliander, Knut och Alice Wallenbergs stiftelse, Letterstedska Föreningen Nordisk Tidskrift, Helge AX:son Johnsons stiftelse, Kungliga Vetenskaps- och Vitterhets-samhället i Göteborg.