7. General Discussion

The main focus of this thesis is to investigate the relationships within and between sedentary polychaete taxa using molecular markers. Despite the tradition of using 18S sequence data for molecular systematic studies of metazoan relationships (e.g. Field et al., 1988; Aguinaldo et al., 1997) as well as for unraveling clitellate phylogeny (Apakupakul et al., 1999; Trontelj et al., 1999; Martin et al., 2000; Martin, 2001; Siddall et al., 2001; Erséus & Källersjö, 2004), this gene was used only rarely within the field of polychaete systematics (see chapter 1). Thus it is obvious that hitherto the inference of polychaete phylogeny suffered from an insufficient taxon sampling, which is particularly true for sedentary taxa.

Taxon sampling is a crucial step in every phylogenetic study and refers to the process of selecting representative taxa for a phylogenetic analysis (Rosenberg & Kumar, 2001). An insufficient taxon sampling is supposed to represent a major source of error in phylogenetic analyses (see references in Rosenberg & Kumar, 2001). Simulation studies have demonstrated that adding taxa to phylogenetic analyses may improve the phylogenetic signal, and that it is likely to reduce the long branch effect (Hillis, 1996; Graybeal, 1998). It is assumed that on the basis of a broader taxon sampling the resolution of polychaete relationships can be improved.

Within the field of polychaete systematics there is a controversial discussion about the monophyly of the Polychaeta, the monophyly of the polychaete families and the relationship between polychaete families (see Fauchald & Rouse, 1997 and Rouse & Fauchald, 1997 for references). Another question regards the possible inclusion of enigmatic taxa like the Echiura, Myzostomida, or Sipuncula in the Annelida (Halanych et al., 2002). In this chapter I will discuss the results of the analyses of 18S rRNA gene sequence data (chapter 2-4) and its contribution to the above mentioned controversies. Furthermore, the ingroup relationships of many polychaete families are only poorly understood and cladistic analyses often suffer from the poverty of informative morphological characters which could be used. For the Syllidae Nygren & Sundberg (2003) have shown that increasing the taxon sampling together with combined analyses of two genes can significantly improve the resolution as well as the support of polychaete ingroup relationships. Thus, this seems to represent a promising approach for the reconstruction of the phylogeny of other polychaete families as well. In the present study I investigate the ingroup relationships of two sedentary polychaete taxa, the orbiniids and arenicolids. The results of the combined analyses of the phylogenetic relationships of Orbiniidae (18S + 16S, chapter 5) and Arenicolidae (18S + 28S + 16S, chapter 6) are treated below.

7.1 Monophyly of the Polychaeta and the position of the Clitellata

The quest about the monophyly of the Polychaeta is intimately linked to the quest about the position of the Clitellata (Westheide, 1997; Westheide et al., 1999; Purschke, 2002a). The traditional classification of the Annelida with the Polychaeta and Clitellata as highest ranked sister taxa can be found in most textbooks (e.g. Ax, 1999). The monophyly of the Clitellata is supported by a vast number of morphological autapomorphies (Purschke, 2002a) as well as through results of molecular analyses using different genes (Winnepenninckx et al., 1998; Kojima, 1998; Brown et al., 1999; McHugh, 2000; Struck et al., 2002a). The monophyly of the Polychaeta is controversially discussed. The cladistic analysis by Rouse & Fauchald (1997) recovered the Polychaeta as a monophyletic taxon, but this was only weakly supported by the presence of nuchal organs. The alternative hypothesis, which places the Clitellata as a polychaete ingroup which implies the paraphyly of the latter group, is favored by many authors (Purschke *et al*., 1997; Westheide, 1997; McHugh, 2000; Martin, 2001; Struck *et al*., 2002a). This hypothesis is also supported by the results of my study (see chapter 2-4). In all three analyses the Clitellata are recovered as monophyletic group that are highly derived within clades comprising polychaetes. While the monophyly of the Clitellata is well supported by bootstrap values and bayesian posterior probabilities, sister group relationships of the Clitellata to annelid taxa are highly dependent on the taxon sampling. Furthermore, none of the hypotheses gain significant support. These results are in congruence to other molecular analyses (McHugh, 2000; Martin, 2001; Struck *et al*., 2002a) and support the proposition of Rouse & Fauchald (1997), that no obvious sister group for Clitellata within Polychaeta can be identified. Nevertheless, at least a paraphyletic status of the Polycheta and the assumption of a reduction of the nuchal organs in the clitellate stem line must be concluded from the results of the molecular analyses. Further on, no evidence is found for a close relationship between Clitellata and *Hrabeiella periglandulata* (chapter 2 & 3), which has been suggested by Purschke (2003) based on ultrastructural data.

Molecular analyses not only regard polychaetes as paraphyletic, but also the Annelida (chapter 2-4). These results correspond to the results of former molecular analyses (e.g. McHugh, 2000) and are credited to the effect that the phylogenetic signal of the 18S rRNA gene is too low for the resolution of the basal protostome splitting events (Abouheif et al., 1998; Giribet, 2002).

7.2 Polychaete systematics

As described in chapter 1, the most widely used systematization of the polychaetes is based on the cladistic analysis of Rouse & Fauchald (1997). According to their view, within the Polychaeta, the Scolecida and the Palpata are recognized as highest ranked sister groups. The monophyly of these taxa is only weakly supported and challenged by some authors (e.g. Hausen, 2001).

The monophyly of Scolecida is based on the presence of parapodia with similar rami and the possession of two or more pairs of pygidial cirri (Rouse & Pleijel, 2001). This clade comprises the Arenicolidae, Capitellidae, Cossuridae, Maldanidae, Opheliidae, Orbiniidae, Paraonidae, Questidae, and Scalibregmatidae. The analysis in chapter 3 includes a broad sampling of scolecid taxa including Capitellidae (4 sequences), Arenicolidae (3), Maldanidae (2), Scalibregmatidae (3), Opheliidae (6), *Questa* (1), and Orbiniidae (8). None of the recovered trees support a closer relationship of these taxa and the paraphyly of the Scolecida is strongly suggested. The same holds true for the Palpata (chapter 2-4) and the hypothesis concerning polychaete relationships suggested by Rouse & Fauchald (1997) (Scolecida + (Canalipalpata + Aciculata)) is significantly rejected by the data set analyzed in chapter 4.

However, all in all the relationships between polychaete families are only poorly resolved, i.e. recovered sister group relationships lack from a sufficient support. Exceptions are the well supported Arenicolidae – Maldanidae, and Pisionidae – Polynoidae clades (chapter 4).

7.3 Monophyly of polychaete families

In the traditional classification polychaete annelids have been classified into over 80 families (Fauchald, 1977). Fauchald & Rouse (1997) reviewed 81 of these, briefly discussed the knowledge of internal and external morphological structures, and assessed the evidence for monophyly. For 22 families no available evidence for monophyly could be presented and many other are only weakly supported. In previous molecular analyses most families are only represented by a single taxon (Kojima, 1998; McHugh, 2000; Rota *et al.*, 2001) and thus no conclusion regarding their monophyly can be drawn. Due to an enhanced taxon sampling of polychaetes which has been used in chapter 2, 3, and 4, evidence for the monophyly of some of the investigated families can be presented. Thus Amphinomidae, Arenicolidae, Capitellidae, Lumbrineridae, Maldanidae, Onuphidae, Phyllodocidae, Polynoidae, Siboglinidae, Spionidae, and Syllidae are supported as monophyletic groups. The Orbiniidae appear paraphyletic with regard to the questids, the former opheliid *Travisia* should be transferred to Scalibregmatidae, and

Ctenodrilus cirratus shows close affinities to the cirratulid genus *Dodecaceria* (chapter 2, 3, and 4). In the following these results are discussed in more detail.

On Ctenodrilidus and Cirratulidae

The first described ctenodrilid, *Ctenodrilus serratus*, was originally included in the rhabdocoel Turbellaria by Schmidt (1857). Since then only a few species have been described in the genera *Ctenodrilus*, *Aphropharynx, Raphidrilus,* and *Raricirrus*. A new genus and species is mentioned in Rota *et al.* (2001), but is still waiting to be described. *Zeppelina* was synonymized with the cirratulid taxon *Dodecaceria* by George and Petersen (1991), whereas Petersen and George (1991) refer the genus *Raricirrus,* originally described in the Cirratulidae but lacking the grooved tentacles characteristic of these, to the Ctenodrilidae. Fauvel (1927) and Day (1967) considered the ctenodrilids to be a part of the Cirratulidae, while Hartmann-Schröder (1971) retains them as a separate drilomorph family and later (1996) together with Parergodrilidae as part of the order Ctenodrilida,. An examination of the nervous system of *Ctenodrilus serratus* by Gelder and Palmer (1976) reinforced the idea that this taxon has close affinities with the Cirratulidae. In the cladistic analysis of Rouse and Fauchald (1997) they form a clade together with Fauveliopsidae, Poebiidae and Sternaspidae. My study (chapter 3) reveals that *Ctenodrilus serratus* clusters together with the cirratulids and groups between the two included *Dodecaceria* species. The Ctenodrilus – Dodecacerias clade gains high support values (chapter 3). As pointed out by George and Petersen (1991), similarities between adult ctenodrilids and juveniles or asexual generates of *Dodecaceria* often caused confusion, being described as species of the ctenodrilid genus *Zeppelina*. The systematic placement of the ctenodrilids as part of the Cirratulidae has a long tradition (Mesnil and Caullery, 1897; Fauvel, 1927; Day, 1967) and is herewith confirmed, contradicting Hartmann-Schröder (1996) and Rouse and Fauchald (1997) who treated them as a separate family outside the Cirratulidae. A possible nesting of Ctenodrilidae within the Cirratulidae was also suggested by Rouse & Pleijel (2003).

Monophyly of Opheliidae and Scalibregmatidae and the position of Travisia

Opheliids are worldwide distributed with more than 150 described species. However, there is no convincing apomorphy known for the Opheliidae (Fauchald and Rouse, 1997), and Rouse and Pleijel (2001) suggested that they might be paraphyletic with regard to Scalibregmatidae. Three distinct morphological groups can be recognized within the Opheliidae: A taxon Opheliinae comprising those genera with distinct body regions (*Euzonus*, *Lobochesis* and *Ophelia*), a taxon Ophelininae including all genera with an anal tube consisting of several reduced segments (*Ammotrypanella*, *Antiobactrum*, *Armandia*, *Ophelina*, *Polyophthalmus,* and *Tachytrypane)* and the

taxon *Travisia*, which closely resembles scalibregmatid species (Bellan *et al*., 1990; Hartmann-Schröder, 1996) and which is closely related to Scalibregmatidae, as mentioned in Blake (2000a). Dauvin and Bellan (1994) studied the systematics of Travisiinae and synonymisized *Dindymenides* and *Kesun* with *Travisia*. They also found that ventral and lateral grooves are generally absent or only poorly developed if present. In contrast, a well developed ventral groove can be found in all other opheliid taxa. All *Travisia* species (except the fusiform species *Travisia hobsonae* and *Travisia fusiformis* (Santos, 1977)) are maggot-shaped and resemble scalibregmatids like *Polyphysia*, while all other opheliids are fusiform or cylindrical in shape (Bellan *et al*., 1990). Storch (1988) pointed out that *Travisia* possesses a stratified epidermis, which is unusual for invertebrates.

Scalibregmatids are worldwide distributed polychaetes with 55 nominal species. According to Fauchald and Rouse (1997) there is no known autapomorphy for this taxon and typical scalibregmatid characters such as the rugose epidermis and segmental annuli can be found in the Opheliidae, too. In their polychaete "meta-tree", Rouse and Pleijel (2001) show scalibregmatids as a sistergroup to a taxon consisting of arenicolids, capitellids, maldanids and opheliids. In traditional classifications scalibregmatids are grouped together with opheliids (Hartmann-Schröder, 1996). Within the Scalibregmatidae Kudenov and Blake (1978) and Blake (1981) distinguish three groups representing different body forms: A group with an arenicoliform body (e.g. *Scalibregma*), a group with a maggot-like body (e.g. *Polyphysia*) and *Scalibregmella,* which has a slender and elongated body.

Summarizing these findings, uncertainties regarding the monophyly of opheliids and scalibregmatids are due to the uncertain placement of *Travisia*, a taxon which closely resembles scalibregmatid species (Bellan *et al*., 1990), and which is tradiotionally classified as a basal opheliid. The present study (chapter 3 and 4) strongly supports a common ancestry of *Travisia* and scalibregmatids, whereas *Travisia* appears as ingroup taxon of Scalibregmatidae. This result is in congruence with the view of Blake (2000b). After transferring *Travisia* to the Scalibregmatidae it is necessary to check wether the presence of lateral grooves and a strong ventral groove support the monophyly of the remaining newly combined Opheliidae (=Opheliidae minus *Travisia*). Monophyly of the remaining Opheliidae, as well as a subdivision in Opheliinae and Ophelininae is strongly supported in the analyses presented in chapter 3.

Orbiniid monophyly and the phylogenetic position of Questidae
As shown in chapter 2-5, a clade consisting of the analyzed orbiniid taxa and *Questa* is

well supported and some evidence is given, that the latter taxon does belong inside the Orbiniidae (chapter 5). Since their discovery by Hartman (1966) questid relationships are an open discussion. This family comprises a group of interstitial polychaetes which superficially resemble marine oligochaetes. The presence of nuchal organs, the prostomial position of the supraoesophageal ganglia and the absence of an acrosomic tube in the spermatozoa are typical polychaete characters (Jamieson & Webb, 1984; Rouse & Fauchald, 1997; Giere & Erséus, 1998). However, their gonads are limited to a few body segments (Giere & Rieser, 1981), which is typical for oligochaetes. Furthermore, some authors (Almeida *et al.*, 2003) regard their papillate glandular epidermis, which forms a cocoon, as a homologous structure to the clitellum of the Clitellata. The results of the analyses in chapter 2-4 contradict this view of a close questid – clitellate relationship. Morphologically, the presence of camerated chaetae in both taxa supports the monophyly of such a clade (chapter 5).

A closer relationship between orbiniids and questids was also suggested by Rouse & Fauchald (1997), who grouped them together with the Paraonidae.

7.4 Annelids and allies

Pogonophorans, echiurids, and myzostomids have been previously regarded as separate phyla in the classification of traditional zoological textbooks (Brusca & Brusca, 1990). The phylogenetic position of these marine worms is a matter of controversy (Halanych et al., 2002). Whereas for the Pogonophora morphological and molecular evidence is presented that they can be regarded as derived polychaetes (Bartolomaeus, 1995; McHugh, 1997; Rouse & Fauchald, 1997; Kojima, 1998), the phylogenetic position of the Echiura and Myzostomida remains ambiguous.

Position of the Echiura
Echiura include about 160 species, which inhabit burrows in soft marine sediments (Halanych et al., 2002). As Nielsen (2000) pointed out, the Echiura resemble annelids in anatomy and embryology, with the exception that they show no trace of segmentation. In the analyses of Rouse and Fauchald (1995, 1997) and Rouse (1999) they are treated as sister taxon of the Articulata (Annelida + Arthropoda). Molecular analyses instead place them as derived polychaetes (McHugh, 1997, 1999; Brown *et al.*, 1999). As shown in chapter 2-4, strong evidence is given that Echiura represent a polychaete ingroup taxon with a close affinity to the Capitellidae. This relationship is well supported through bootstrap values and Bayesian probabilities. This view is congruent with the findings of Hessling and Westheide (2002) that Echiura show serially repeated

units in their nervous system which correspond to typical metameric ganglia of the Annelida. The placement as derived polychaetes favors the hypothesis of a secondary loss of segmentation in Echiura. Up to now no morphological synapomorphies of a possible common ancestry of Capitellidae and Echiura have been found.

Position of Myzostomida

Myzostomida are marine worms associated with Echinoderms (Grygier, 2000). As host-specific symbionts (or parasites) they show a highly derived anatomy in their adult morphology (Eeckhaut *et al*., 2000). While many authors regard them as derived annelids (Nielsen, 2000) or polychaetes (Rouse and Fauchald, 1997), recent cladistic analyses of morphological and molecular data support the hypothesis that Myzostomida are not nested within annelids (Haszprunar, 1996; Eeckhaut *et al*., 2000; Zrzavy *et al*., 2001). Zrzavy *et al*. (2001) propose that Myzostomida are the sistergroup of the Cycliophora; in Eeckhaut *et al*. (2000) they are closely related to Plathelminthes, and Haszprunar (1996) favors a sistergroup relationship to a taxon consisting of sipunculids, clitellates and polychaetes. A close relationship to acanthocephalans is proposed by Mattei and Marchand (1987) on the basis of ultrastructural sperm cell similarities. Since some of the above-mentioned possible myzostomid sistergroups have not been included, not all of the above mentioned hypotheses can be tested, but the results of the ML analyses in chapter 3 and 4 show some support for the idea that Myzostomida are aberrant polychaetes. This view is congruent with the results of Müller & Westheide (2000) on the nervous system of *Myzostoma cirriferum*, which shares several structures with the typical polychaete nervous system.

7.5 Ingroup relationships of polychaete families

Phylogeny of the Orbiniidae

The Orbiniidae comprise a group of world-wide distributed deposit feeding polychaetes; approximately 150 species have been described in 18 genera. Traditionally the Orbiniidae are classified in two groups, Orbiniinae and Protoariciinae (Hartman, 1957). All Protoariciinae are small and slender and possess two peristomial rings, whereas most of the Orbiniinae are medium to big sized species with only one peristomial ring. An alternative hypothesis is that probably many of the taxa currently assigned to Protoariciinae are actually juvenils of species already described in Orbiniinae. A cladistic analysis of the ingroup relationships of Orbiniidae (Blake, 2000c) reveals that the newly described deep-sea species *Methanoaricia dendrobranchiata*, which has been found in methane cold seeps in the Gulf of Mexico, could represent the sister group of

all other orbiniids.

The analysis of the combined data set in chapter 5 suggests an inclusion of *Methanoaricia dendrobranchiata* in the Orbiniidae with a close relationship to species of *Orbinia* and *Phylo*, rather than being the sister taxon of all other orbiniids. It is noticeable that the phylogenetic analysis of the molecular data (chapter 5) suggests the paraphyly of all genera which have been included with more than one species (*Leitoscoloplos*, *Naineris*, *Orbinia*, *Phylo,* and *Scoloplos*). Thus it is reasonable to conclude that the morphological characters which are presently used for genus diagnosis are not phylogenetically informative enough for cladistic analysis. No support is found for the traditional classification as well as for the hypothesis that taxa of the Protoariciinae represent juveniles of Orbiniinae. Instead, in the case of *Protoaricia oerstedi* strong support for a progenetic origin is given.

Arenicolid relationships

Arenicolids comprise a group of 4 genera in which about 30 nominal species are described (Rouse & Pleijel, 2001). Whereas the biology of many arenicolids is well known, the phylogenetic relationships of these worms are inadequately studied. Gamble & Ashworth (1912) distinguished between caudate (*Abarenicola & Arenicola*) and ecaudate (*Arenicolides & Branchiomaldane*) species and Bartolomaeus & Meyer (1999) proposed an evolutionary scenario in which the ecaudate species represent the basal taxa and the caudate forms are regarded as a derived taxon. A close relationship of Arenicolidae and Maldanidae is generally accepted (Rouse & Fauchald, 1997; Bartolomaeus & Meyer, 1997), but Rouse & Pleijel (2001) suggested that one of the two families might be paraphyletic due to the uncertain placement of Branchiomaldane. As shown in chapter 6, the monophyly of the Maldanidae, as well as of the Arenicolidae is supported by all conducted analyses. In the combined analysis (chapter 6), evidence is given for a closer relationship between the two investigated *Branchiomaldane* species and *Arenicolides ecaudata*. *Branchiomaldane* species differ from all other arenicolids due to the appearance of many characters which are usually also present in other juvenile arenicolids (Bartolomaeus & Meyer, 1999; Nogueira & Rizzo, 2001). In the light of the molecular data the best explanation for these structural and morphological observations is that *Branchiomaldane* evolved by progenesis.

7.6 What can 18S do for polychaete phylogeny?

In the present study, sequence data of the 18S rRNA gene is used to address questions concerning phylogenetic hypotheses of annelid relationships. Sequence data of this gene

can provide promising evidence for the reconstruction of the phylogenetic relationships of taxa whose position, with respect to morphological data, could not be determined satisfactorily yet. Example are Travisia (chapter 3-4), Echiura (chapter 2-4), Ctenodrilus (chapter 3-4), and Branchiomaldane (chapter 6). In the case of Echiura, the controversy about its evolutionary position can be traced back to the ambiguity of the phylogenetic interpretation of important morphological characters due to the possibility of secondary absence of morphological structures as for instance segmentation. Molecular data seems to represent a promising tool for independently testing hypotheses of this kind.

For Branchiomaldane (chapter 6) and Protoaricia (chapter 5), a progenetic origin is supported by the analysis of the molecular data. Progenesis is assumed to have occurred in many annelid taxa (e.g. Westheide, 1987), but in most cases evolutionary scenarios are used as line of argumentation. However, arguing in favour of heterochronic evolution always requires a well supported phylogenetic hypothesis of the relevant taxa as a necessary premise (Fink, 1988) and not vice versa. The use of 18S- as well as of sequence data of other genes has proved to be an appropriate approach for testing such evolutionary scenarios (see also Struck et al., 2002b).

As mentioned above, the monophyly of many of the traditional polychaete families remains doubtful (Fauchald & Rouse, 1997). My results (chapter 2-6) show that the use of 18S sequence data in combination with increasing the taxon sampling is a promising approach for the investigation of questions regarding evolutionary scenarios.

The results of chapter 5 and chapter 6 indicate that the 18S can be used in combination with faster evolving genes to investigate the ingroup relationships of polychaete families.