When molecules support morphology: Phylogenetic reconstruction of the family Onuphidae (Eunicida, Annelida) based on 16S rDNA and 18S rDNA ☆

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ABSTRACT

Onuphid polychaetes are tubicolous marine worms commonly reported worldwide from intertidal areas to hadal depths. They often dominate in benthic communities and have economic importance in aquaculture and recreational fishing. Here we report the phylogeny of the family Onuphidae based on the combined analyses of nuclear (18S rDNA) and mitochondrial (16S rDNA) genes. Results of Bayesian and Maximum Likelihood analyses supported the monophyly of Onuphidae and its traditional subdivision into two monophyletic subfamilies: Onuphinae and Hyalinoeciinae. Ten of 22 recognized genera were monophyletic with strong node support; four more genera included in this study were either monotypic or represented by a single species. None of the genera appeared para- or polyphyletic and this indicates a strong congruence between the traditional morphology-based systematics of the family and the newly obtained molecular-based phylogenetic reconstructions. Intergeneric relationships within Hyalinoeciinae were not resolved. Two strongly supported monophyletic groups of genera were recovered within Onuphinae: ((Onuphis, Aponuphis), Diopatra, Paradiopatra) and (Hirsutonuphis, (Paxtonia, (Kinbergonuphis, Mooreonuphis))). A previously accepted hypothesis on the subdivision of Onuphinae into the Onuphis group of genera and the Diopatra group of genera was largely rejected.

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1. Introduction

Annelids of the family Onuphidae Kinberg, 1865 are tubicolous sediment dwellers inhabiting various marine biotopes worldwide. They are very common in the intertidal zone (Fauchald, 1980; Paxton, 1986a, 1993) and additionally represent the fourth most diverse polychaete family in the deep sea (Paterson et al., 2009). Onuphids may reach extremely high densities in some habitats. Hawaiian *Diopatra dexiognatha* Paxton and Bailey-Brock, 1986 forms mounds on the reef flat built of the densely aggregated vertical tubes, up to 21,800 ind./m², which provide specific tridimensional environment for the rich local community (Bailye-Brock, 1984). *Hyalinoecia* Malmgren, 1866 species with tubes up to 20 cm long dominate in various slope communities (Zühlke et al., 2001; Neumann et al., 2008; Quiroga et al., 2009) with up to 905 ± 250 ind. per 100 m² (Hecker, 1994), showing remarkably high values of polychaete density and biomass in the deep-sea habitats. Being abundant in the intertidal zone, onuphids are widely harvested as bait sustaining local fisheries in southeastern Australia (Paxton, 1979), Mediterranean (Dağli et al., 2005; Gambi et al., 1994) and Portuguese coasts (Cunha et al., 2005) and are even commercially produced in aquaculture to feed farmed fish and to be used as bait for recreational fishing (Safarik et al., 2006).

A pair of distinct frontal lips projecting on the anterior margin of the prostomium represents a synapomorphy of the family (Paxton, 1986a) (Fig. 1A and B). Three dorsal antennae and a pair of dorsolateral palps with multi-ringed basal ceratophores are

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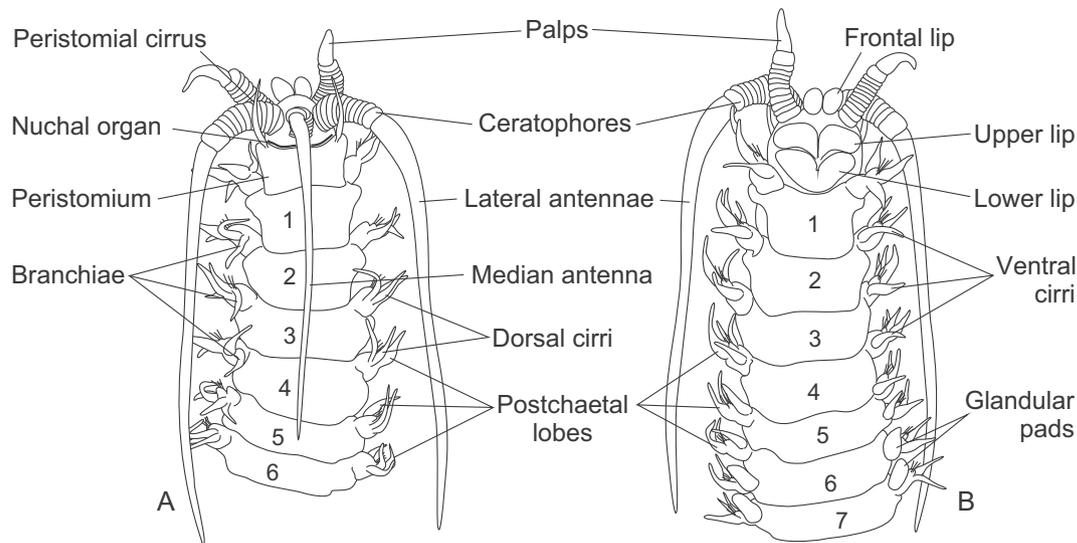
Fig. 1. Scheme of *Onuphis* anterior part of the body. A, dorsal view; B, ventral view. Numerals represent chaetigers (segments with parapodia and chaetae).

always well developed (Fig. 1A and B). All onuphids have paired nuchal organs – dorsal ciliated grooves located at the posterior margin of the prostomium (Fig. 1A); many species have eyes. Peristomium is a single ring with usually a pair of cirri on the dorsal side (Fig. 1A); sometimes cirri may be absent. The whole body can be divided into two regions based on the morphology of parapodia and distribution of the different types of chaetae. The anterior two to eight segments bear modified parapodia equipped with so-called pseudocompound or simple falcigers. These parapodia can be prolonged and/or enlarged with reduced or expanded prechaetal lobes. Alternatively, they may be similar in size to the parapodia of the following region, but if so they are possessing digitiform ventral cirri (Fig. 1B). The following region bears segments with ventral cirri transformed into ventral glandular pads used in tube constructing (Fig. 1B). Onuphids are characterized by an outstanding diversity of tube morphology and composition of building materials. The tubes can be organic, totally secreted by their inhabitants (Pautard and Zola, 1967) or covered by mud, sand or various external particles such as shells of foraminiferans and bivalves (Orensanz, 1990; Paxton, 1986a), small stones or plant debris (Myers, 1972). They can be cylindrical of flattened, having appendages (Budaeva and Fauchald, 2011) or brooding chambers (Hartman, 1967), several times longer than the worm or relatively short.

Onuphids demonstrate a great diversity of lifestyles which have been presumably evolved as a result of the specialization of the anterior part of the body and also in the diversification of tube shapes and composition. For instance, Australian intertidal beach worms *Australonuphis* Paxton, 1979 are large, motile omnivores reaching up to 3 m in length. They build temporary mucous tubes in the sand beaches and actively move in the sediment, using the enlarged muscular parapodia, in search of large food objects (Paxton, 1979). Japanese *Longibrachium* Paxton, 1986a inhabit permanent vertical tubes in sandy bottom and use their extended anterior parapodia with extremely long and recurved falcigers in ambush capture of animal prey from the tube opening (Nishi and Kato, 2009). Species of *Hyalinoecia* and *Nothria* Malmgren, 1866 are believed to be epibenthic motile scavengers or predators crawling on the surface of the sea bottom and carrying their lightweight or flattened tubes (Dayton and Hessler, 1972). Eastern Pacific *Diopatra ornata* Moore, 1911 was shown to be a sessile herbivore living in vertical tubes protruding from the sediment surface and feeding on captured kelp drifting in the water column (Fauchald

and Jumars, 1979). Other *Diopatra* Audouin and Milne Edwards, 1833 species were proposed to be sessile omnivores using their vertical complex ornamented tubes as a substrate populated by various small-sized marine invertebrates "farmed" by the worms as a food source (Mangum et al., 1968; Mangum and Cox, 1971; Myers, 1970, 1972).

The most comprehensive systematic revision of onuphid polychaetes to date was performed by Paxton (1986a). She revised the generic diagnoses and assigned all known species to 22 genera, including five newly described genera. Since then, the system suggested by Paxton (1986a) has been widely accepted and only subject to minor changes. Orensanz (1990) revised onuphids from the Antarctic and Sub Antarctic waters. He described a number of new species and also suggested the synonymization of *Neonuphis* Kucheruk, 1978 with *Leptoecia* Chamberlin, 1919, which was later supported by Budaeva (2012). A new monotypic genus *Fauchaldonuphis* Paxton, 2005, with prolonged anterior parapodia, has been described from the Mozambique Channel (Paxton, 2005). Budaeva and Fauchald (2011) studied the systematics and phylogeny of the *Diopatra* generic complex *sensu* Paxton (1986a) utilizing morphological characters. As a result of this study, two presumably progenetic genera (*Epidiopatra* Augener, 1918 and *Notonuphis* Kucheruk, 1978) have been synonymized and two more monotypic genera (*Paxtonia* Budaeva and Fauchald, 2011 and *Protodiopatra* Budaeva and Fauchald, 2011) have been erected. Complete or partial revisions were performed for the *Rhamphobrachium* complex of genera (incl. *Rhamphobrachium* Ehlers, 1887, *Brevibrachium* Paxton, 1986a and *Longibrachium* Paxton, 1986a) (Paxton, 1986b); *Australonuphis* (León-González et al., 2008); *Hirsutonuphis* Paxton, 1986a (Estrella-Ruiz et al., 2013; Paxton, 1996); *Paradiopatra* Ehlers, 1887 (Budaeva and Fauchald, 2011; Budaeva and Paxton, 2013). Nonetheless, the majority of the most species-rich genera such as *Diopatra*, *Kinbergonuphis* Fauchald, 1982, *Onuphis* Audouin and Milne Edwards, 1833, *Mooreonuphis* Fauchald, 1982, *Hyalinoecia* and *Nothria* are awaiting further revisions. Currently onuphids comprise approximately 300 described species grouped in 22 accepted genera (Table 1).

Paxton (1986a) proposed the phylogeny of Onuphidae based on 46 morphological characters. The family was divided into two sister subfamilies, Hyalinoeciinae Paxton, 1986a and Onuphinae Kinberg, 1865. Following Paxton (1986a), Hyalinoeciinae is comprised by worms with enlarged anterior parapodia modified for crawling, and subacicular hooks inserted medially in the posterior

Table 1
Accepted genera of Onuphidae with the number of species in each genus.

Genera	Authority	Junior synonyms	Type species	Number of species
Hyalinoeciinae	**Paxton, 1986a**			
Anchinothria	Paxton, 1986a	*Paradiopatra sensu* Pettibone, 1970; Fauchald, 1982	*Diopatra pourtalesii* Ehlers, 1887	12
Hyalinoecia	Malmgren, 1866	*Paronuphis* Ehlers, 1877	*Nereis tubicola* Müller, 1776	~20
Hyalospinifera	Kucheruk, 1979		*Hyalospinifera spinosa* Kucheruk, 1979	1
Leptoecia	Chamberlin, 1919	*Neonuphis* Kucheruk, 1978; *Parhyalinoecia* Hartmann-Schröder, 1975	*Leptoecia abyssorum* Chamberlin, 1919	7
Nothria	Malmgren, 1866	*Northia* Johnston, 1865	*Onuphis conchylega* Sars, 1835	19
Onuphinae	**Kinberg, 1865**			
Americonuphis	Fauchald, 1973		*Diopatra magna* Andrews, 1891	2
Aponuphis	Kucheruk, 1978		*Hyalinoecia bilineata* Baird, 1870	8
Australonuphis	Paxton, 1979	*Americonuphis* Orensanz, 1974	*Americonuphis casamiquelorum* Orensanz, 1974	7
Brevibrachium	Paxton, 1986a		*Rhamphobrachium capense* Day, 1960	4
Diopatra	Audouin and Milne Edwards, 1833	*Epidiopatra* Augener, 1918	*Diopatra amboinensis* Audouin and Milne Edwards, 1833	~55
Fauchaldonuphis	Paxton, 2005		*Diopatra paradoxa* Quatrefages, 1866	1
Hartmanonuphis	Paxton, 1986a		*Onuphis pectinata* Knox and Hicks, 1973	1
Heptaceras	Ehlers, 1868	*Tradopia* Baird, 1870	*Diopatra phyllocirra* Schmarda, 1861	4
Hirsutonuphis	Paxton, 1986a		*Onuphis mariahirsuta* Paxton, 1979	8
Kinbergonuphis	Fauchald, 1982		*Onuphis tenuis* Hansen, 1882	~37
Longibrachium	Paxton, 1986a		*Rhamphobrachium atlanticum* Day, 1973	5
Mooreonuphis	Fauchald, 1982		*Onuphis nebulosa* Moore, 1911	20
Onuphis	Audouin and Milne Edwards, 1833		*Onuphis eremita* Audouin and Milne Edwards, 1833	>40
Paradiopatra	Ehlers, 1887	*Sarsonuphis* Fauchald, 1982; *Notonuphis* Kucheruk, 1978	*Diopatra (Paradiopatra) fragosa* Ehlers, 1887	32
Paxtonia	Budaeva and Fauchald, 2011		*Onuphis amoureuxi* Intes and Le Loeuff, 1975	1
Protodiopatra	Budaeva and Fauchald, 2011		*Nothria willemoesii* McIntosh, 1885	1
Rhamphobrachium	Ehlers, 1887	*Paranorthia* Moore, 1903	*Rhamphobrachium agassizii* Ehlers, 1887	15

parapodia. The subfamily was divided into the *Hyalinoecia* group and the *Nothria* group. The *Hyalinoecia* group (incl. *Hyalinoecia, Leptoecia, Neonuphis,* and *Hyalospinifera* Kucheruk, 1979), so-called quill worms, lack peristomial cirri and maxillae V and build organic quill-like tubes secreted by an inhabitant. The *Nothria* group (incl. *Nothria* and *Anchinothria* Paxton, 1986a) comprises short-bodied epibenthic worms with scoop-shaped pectinate chaetae, normally developed peristomial cirri and flattened tubes covered by various sediment particles.

Paxton (1986a) defined Onuphinae as worms with ventral position of the subacicular hook in unmodified parapodia and divided it into the *Onuphis* group and the *Diopatra* group of genera. The *Onuphis* group (incl. *Australonuphis, Hartmanonuphis* Paxton, 1986a, *Hirsutonuphis, Aponuphis* Kucheruk, 1978, *Kinbergonuphis, Mooreonuphis, Onuphis,* and *Heptaceras* Ehlers, 1868) comprised the worms with lateral position of anterior parapodia, small maxillae V and bi- to tridentate pseudocompound falcigers with short hoods. In addition, the *Australonuphis* complex of three shallow water genera (*Australonuphis, Hartmanonuphis* and *Hirsutonuphis)* was erected within the *Onuphis* group based on the shape of the nuchal organs and the absence of the complete anterior peristomial fold separating the prostomium and the peristomium.

The *Diopatra* group (incl. *Diopatra, Paradiopatra, Epidiopatra, Notonuphis, Americonuphis* Fauchald, 1973, *Rhamphobrachium, Brevibrachium,* and *Longibrachium*) is characterized by the presence of small lateral spines on the shafts of the pseudocompound falcigers in some species. Additionally, two complexes of genera representing the lowest suprageneric division were suggested within the *Diopatra* group. The *Diopatra* complex combining *Diopatra, Epidiopatra* and *Paradiopatra* was defined based on the anterior position of branchiae, the presence of long pointed hoods on the anterior falcigers and the presence of lateral projections on the ceratophores of some species in each genus. The *Diopatra* complex was later revised by Budaeva and Fauchald (2011) who performed a phylogenetic analysis based on morphology that supported its

monophyly with the inclusion of *Notonuphis*. The *Rhamphobrachium* complex comprised three genera (*Rhamphobrachium, Brevibrachium,* and *Longibrachium*) having prolonged anterior parapodia with extremely long recurved falcigers.

Phylogenetic analysis performed by Paxton (1986a) involved genera as terminal taxa and thus did not test monophyly of the examined genera. To date no molecular studies specifically on onuphid phylogeny have been conducted. Few species were involved into various phylogenetic reconstructions of the order Eunicida or polychaetes in general. Though a sister group relationship between Onuphidae and Eunicidae has previously been found (Rousset et al., 2007; Struck et al., 2006; Zanol et al., 2010), the monophyly of the family and all the genera has not been tested.

The present study aims to investigate the phylogenetic relationships of Onuphidae, to test the monophyly of currently accepted subfamilies and genera of onuphids, and to test the hypotheses on the intergeneric relationships within the family previously suggested by Paxton (1986a). For that purpose, a molecular combined analysis of two makers, the nuclear 18S rDNA and partial mitochondrial 16S rDNA, has been performed.

2. Material and methods

2.1. Taxon sampling

We have sampled 31 species of onuphid worms representing 14 of the 22 currently known genera. Sequences for five more species from the sampled genera were obtained from GenBank (Table 2). The taxon sampling in the present study was sufficient to cover the most species-rich genera from all the taxonomic subgroups suggested in the previous studies. Of the eight genera not included in the analysis due to unavailability of material suitable for DNA extraction, four genera (*Hyalospinifera, Hartmanonuphis, Fauchaldonuphis,* and *Protodiopatra*) are monotypic; three genera

Table 2
List of species used in the phylogenetic analyses with geographical locality and GenBank accession numbers.

Species	Locality	Voucher	GenBank accession numbers	
			16S rDNA	18S rDNA
Onuphidae				
Americonuphis magna (Andrews, 1891)	Florida, USA	USNM1205940	KJ027316	KJ027355
Americonuphis magna (Andrews, 1891)	North Carolina, USA	USNM1205939	KJ027317	KJ027356
Aponuphis bilineata (Baird, 1870)	Qawra, Malta		AY838824[a]	AF412795[b]
Aponuphis fauveli (Rioja, 1918)	Gulf of Guinea, Cameroon, 3.291°N, 9.5695°E, 37 m	ZMBN91312	KJ027318	KJ027357
Aponuphis sp.	Banyuls, France, 42.4892°N, 3.1873°E, 70 m	USNM1207008	KJ027319	KJ027358
Australonuphis parateres Paxton, 1979	NSW, Australia	ZMBN91324	KJ027320	KJ027359
Australonuphis teres (Ehlers, 1868)	NSW, Australia	ZMBN91325	KJ027321	KJ027360
Diopatra aciculata Knox and Cameron, 1971	Not reported		AY838826[a]	AY838845[a]
Diopatra sp.1	Gulf of Guinea, Cameroon, 3.8323°N, 9.1958°E, 22 m	ZMBN91326	KJ027322	KJ027361
Diopatra dentata Kinberg, 1865	Adelaide, SA, Australia	USNM1122125	GQ478129[c]	GQ497475[c]
Diopatra ornata Moore, 1911	California, USA, 36.8572°N, 121.8573°W, 30 m	USNM1207005		KJ027364
Diopatra ornata Moore, 1911	San Juan Island, Washington, USA	Not vouchered	KJ027324	KJ027363
Diopatra cf. *ornata* Moore, 1911	California, USA	USNM1122750	GQ478130[c]	GQ497476[c]
Diopatra sp.2	Gulf of Guinea, Nigeria, 4.1482°N, 5.7688°E, 26 m	ZMBN91313	KJ027323	KJ027362
Diopatra sp.3	California, USA, 33.6985°N, 118.3355°W, 61 m	Not vouchered	KJ027325	KJ027365
Diopatra sugokai Izuka, 1907	Sagami Bay, Japan, 35.2878°N, 139.5732°E, 0 m	USNM1205941	KJ027326	KJ027366
Diopatra sugokai Izuka, 1907	Sagami Bay, Japan, 35.2878°N, 139.5732°E, 0 m	USNM1205942	KJ027327	KJ027367
Diopatra tuberculantennata Budaeva and Fauchald, 2008	Carrie Bow Cay, Belize, 16.8027°N, 88.0819°W, 1 m	Isolate 11, not vouchered	KJ027328	KJ027368
Diopatra tuberculantennata Budaeva and Fauchald, 2008	Carrie Bow Cay, Belize, 16.6714°N, 88.199°W, 1 m	Isolate 28, not vouchered	KJ027329	KJ027369
Hirsutonuphis gygis (Paxton, 1979)	NSW, Australia	ZMBN91327	KJ027330	KJ027370
Hyalinoecia longibranchiata McIntosh, 1885	East off New Zealand, 44.45°S, 174.467°E, 701–720 m	ZMBN91328	KJ027331	KJ027371
Hyalinoecia sp.	Massachusetts, USA	USNM1121743	GQ478125[c]	GQ497471[c]
Hyalinoecia tubicola A (Müller, 1776)	Campese, Isola de Giglio, Italy		AY838830[a]	AF412794[b]
Hyalinoecia tubicola B (Müller, 1776)	Bohuslän, Sweden		DQ779618[d]	DQ779654[d]
Hyalinoecia tubicola (Müller, 1776)	Banyuls, France, 42.5167′N, 3.175°E, 66–68 m	USNM1207011	KJ027332	KJ027372
Hyalinoecia tubicola (Müller, 1776)	Bergen, Norway	ZMBN91320	KJ027333	KJ027373
Hyalinoecia sp.1 (Müller, 1776)	Gulf of Guinea, Nigeria, 6.05°N, 4.2167°E, 98 m	ZMBN91314	KJ027334	KJ027374
Kinbergonuphis pulchra (Fauchald, 1980)	Carrie Bow Cay, Belize, 16.8027°N, 88.0819°W, 1 m	Not vouchered	KJ027335	KJ027375
Leptoecia midatlantica Budaeva, 2012	Mid-Atlantic Ridge, 54.2167°N, 36.0681°W, 2615–2604 m	ZMBN91329	KJ027336	KJ027376
Leptoecia midatlantica Budaeva, 2012	Mid-Atlantic Ridge, 54.2174°N, 36.0679°W, 2619–2598 m	ZMBN91330	KJ027337	KJ027377
Leptoecia sp. A	South Atlantic, 36.0102°S, 59.0257°W, 4608 m	Not vouchered	KJ939528	KJ939529
Mooreonuphis dangrigae (Fauchald, 1980)	Carrie Bow Cay, Belize, 16.8027°N, 88.0819°W, 1 m	Not vouchered		KJ027378
Mooreonuphis pallidula (Hartman, 1965)	Massachusetts, USA	Not vouchered	GQ478126[c]	GQ497472[c]
Mooreonuphis pallidula (Hartman, 1965)	Massachusetts, USA, 39.9362°N, 69.5762°W, 253 m	USNM1207007	KJ027338	KJ027379
Mooreonuphis stigmatis (Treadwell, 1922)	San Juan Island, Washington, USA, 0 m	Not vouchered	KJ027339	KJ027380
Nothria sp.	Gulf of Guinea, Gabon, 3.8128°S, 10.6133°E, 69 m	ZMBN91319	KJ027340	KJ027381
Nothria conchylega (Sars, 1835)	Koster area, Sweden		AF321417[e]	AY176295[f]
Nothria conchylega (Sars, 1835)	Møre og Romsdal, Norway, 100 m	ZMBN91321	KJ027341	KJ027383
Nothria conchylega (Sars, 1835)	Kuril islands, Sea of Okhotsk, Russia, 46.9712°N, 152.2017°E, 245 m	ZMBN91331	KJ027342	KJ027384
Nothria conchylega (Sars, 1835)	Trondheim, Norway, 63.4563°N, 10.2561′E, 16–32 m	USNM1207009		KJ027382
Onuphis elegans A (Johnson, 1901)	Not reported		AY838839[a]	AY838854[a]
Onuphis elegans B (Johnson, 1901)	Washington, USA	USNM1121747	GQ478128[c]	GQ497474[c]
Onuphis iridescens (Johnson, 1901)	Bamfield, Canada		HM746715[g]	HM746729[g]
Onuphis cf. *iridescens* (Johnson, 1901)	California, USA	USNM1121744	GQ478127[c]	GQ497473[c]
Onuphis opalina (Verrill, 1873)	Massachusetts, USA, 39.8431°N, 70.5807°W, 776 m	USNM1207006	KJ027343	KJ027385
Onuphis opalina (Verrill, 1873)	Newfoundland Great Bank, Canada, 44.8036 N, 48.9923°W, 635 m	ZMBN91332	KJ027344	KJ027386
Onuphis shirikishinaiensis (Imajima, 1960)	Primorsky Krai, Russia, 42.8396°N, 132.7507°E, 25 m	ZMBN91333	KJ027345	KJ027387
Paradiopatra bihanica (Intes and Le Loeuff, 1975)	Gulf of Guinea, Nigeria, 5.5055°N, 4.7272°E, 105 m	ZMBN91317	KJ027346	KJ027388
Paradiopatra bihanica (Intes and Le Loeuff, 1975)	Gulf of Guinea, Nigeria, 6.0853°N, 4.0388°E, 113 m	ZMBN91318	KJ027347	KJ027389
Paradiopatra bihanica (Intes and Le Loeuff, 1975)	Gulf of Guinea, Nigeria, 5.5055′N, 4.7272′E, 105 m	ZMBN91334	KJ027348	KJ027390
Paradiopatra fauchaldi Buzhinskaya, 1985	Sakhalin, Sea of Okhotsk, Russia, 54.3343°N, 141.9993°E, 25.5 m	Not vouchered		KJ027391
Paradiopatra fiordica (Fauchald, 1974)	Møre og Romsdal, Norway, 644 m	ZMBN91323	KJ027349	KJ027392
Paradiopatra fiordica (Fauchald, 1974)	Møre og Romsdal, Norway	Not vouchered	KJ027350	KJ027393
Paradiopatra quadricuspis (M. Sars in G.O. Sars, 1872)	Trondheim, Norway	USNM1121745	GQ478131[c]	GQ497477[c]
Paradiopatra quadricuspis (M. Sars in G.O. Sars, 1872)	Trondheim, Norway	USNM1207010	KJ027351	KJ027394
Paxtonia amoureuxi (Intes and Le Loeuff, 1975)	Gulf of Guinea, Nigeria, 6.0853°N, 4.0388°E, 113 m	ZMBN91316	KJ027352	KJ027395
Rhamphobrachium brevibrachiatum (Ehlers, 1875)	Møre og Romsdal, Norway, 122 m	ZMBN91322	KJ027353	KJ027396
Rhamphobrachium cf. *agassizii* Ehlers, 1887	Gulf of Guinea, Nigeria, 4.0158°N, 6.9705′E, 65 m	ZMBN91315	KJ027354	
Dorvilleidae				
Dorvillea erucaeformis (Malmgren, 1865)	Not reported		AY838827[a]	AY838846[a]
Pettiboneia urciensis Campoy and San Martin, 1980	Not reported		AY838842[a]	AF412801[b]
Protodorvillea kefersteinii (McIntosh, 1869)	Roscoff, English Channel, France		AY838843[a]	AF412799[b]

Table 2 (continued)

Species	Locality	Voucher	GenBank accession numbers	
			16S rDNA	18S rDNA
Eunicidae				
Eunice cariboea Grube, 1856	Carrie Bow Cay, Belize	USNM1120732	GQ478141[c]	GQ497487[c]
Eunice norvegica (Linnaeus, 1767)	Trondheim, Norway	USNM1122749	GQ478147[c]	GQ497493[c]
Eunice valens (Chamberlin, 1919)	Washington, USA	Not vouchered	GQ478139[c]	GQ497485[c]
Lysidice collaris Grube, 1870	Catalonia, Spain	USNM1122617	GQ478170[c]	GQ497516[c]
Lysidice ninetta Audouin and Milne Edwards, 1833	Catalonia, Spain	Not vouchered	GQ478169[c]	GQ497515[c]
Marphysa californica Moore, 1909	California, USA	USNM1122558	GQ478162[c]	GQ497507[c]
Marphysa sanguinea (Montagu, 1815)	Roscoff, France	USNM1122123	GQ478157[c]	GQ497502[c]
Nematonereis unicornis (Grube, 1840)	Catalonia, Spain	USNM1122623	GQ478172[c]	GQ497519[c]
Palola cf. *siciliensis* (Grube, 1840)	Catalonia, Spain	USNM1120744	GQ478168[c]	GQ497514[c]
Palola viridis Gray in Stair, 1847	Kosrae, Micronesia	USNM1084405-9C	GQ478167[c]	GQ497513[c]
Lumbrineridae				
Lumbrineris inflata Moore, 1911	AquaZoo Düsseldorf, Germany		AY838832[a]	AY525622[h]
Lumbrineris latreilli Audouin and Milne Edwards, 1834	Aquarium University of Innsbruck, Austria		AY838833[a]	AY525623[h]
Lumbrineris magnidentata Winsnes, 1981	Bohuslän, Sweden		DQ779621[d]	DQ779657[d]
Lumbrineris zonata Johnson, 1901	Bamfield, Canada		HM746713[g]	HM746727[g]
Ninoe nigripes Verrill, 1873	Not reported		AY838837[a]	AY838852[a]
Oenonidae				
Arabella semimaculata (Moore, 1911)	Not reported		AY838825[a]	AY838844[a]
Drilonereis longa Webster, 1879	Not reported		AY838828[a]	AY838847[a]

USNM, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA; ZMBN, Natural History Collections, University Museum of Bergen, Norway.

[a] Sequences from Struck et al. (2006).
[b] Sequences from Struck et al. (2002).
[c] Sequences from Zanol et al. (2010).
[d] Sequences from Rousset et al. (2007).
[e] Sequences from Dahlgren et al. (2001).
[f] Sequences from Worsaae et al. (2005).
[g] Sequences from Paul et al. (2010).
[h] Sequences from Struck and Purschke (2005).

(*Heptaceras, Brevibrachium,* and *Longibrachium*) have few species with very restricted distribution. *Anchinothria* is the only diverse and widely distributed genus occurring mostly in deep waters (below 500 m) that was not included in the present study due to unavailability of material. Representatives of four eunicidan families: Dorvilleidae (3 genera, 3 species), Eunicidae (5 genera, 10 species), Lumbrineridae (2 genera, 5 species), and Oenonidae (2 genera, 2 species) were selected as outgroup taxa. Voucher specimens are deposited in the Museum of Natural History, Smithsonian Institution, Washington DC, USA (USNM) and the University Museum of Bergen, Norway (ZMBN) (Table 2).

2.2. DNA extraction, PCR amplification and DNA sequencing

Genomic DNA was extracted from 96% ethanol fixed specimens using three approaches: PROMEGA Wizard® SV Genomic DNA Purification System, QIAGEN BioSprint® 96 Robotic workstation for automation of magnetic-particle purification technology, and universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques (Aljanabi and Martinez, 1997). The complete nuclear gene 18S rDNA (~1800 bp) was amplified in three overlapping fragments using primer pairs: 18e + 18L; 18F509 + 18R; 18F997 + 18D843 (Tables 3 and 4). A fragment of the mitochondrial 16S rDNA (~500 bp) was amplified using 16SarL and 16SONU-R primers (Table 3 and 4). In cases where no good quality amplicons were acquired a set of ambiguous primers with universal sequencing adaptors was used (Table 3). The total volumes of PCR reactions were either 10 µl (0.25 µl Promega GoTaq® Flexi DNA Polymerase (5 U/µl); ddH$_2$O; 5x Colorless buffer; 2 mM MgCl$_2$; 0.2 mM dNTP; 0.5 µM of each primer), or 25 µl (0.2 µl Sileks ColoredTaq DNA Polymerase, (2.5 U/µl); ddH$_2$O; 10x buffer (70 mM Tris–HCl, pH 8.6/25 °C, 16.6 mM (NH$_4$)$_2$SO4); 2.5 mM MgCl$_2$; 0.25 mM dNTP; 0.2 µM of each primer) with 1–2 µl DNA template. PCR thermal conditions are shown in Table 3. PCR products were purified using either ExoSAP-IT® or Ethanol/EDTA/Sodium Acetate Precipitation. Sequencing reactions for both strands of the amplified genes were performed using BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The same primers as for PCR were used for all genes except ONU_16S amplicons, where universal sequencing primers (M13F 5′–GTTGTAAAAC GACGGCCAGT–3′ and M13R 5′–CACAGGAAACAGCTATGACC–3′) were used. Products were sequenced using Applied Biosystems automated sequencer. Sequence contigs were assembled in Sequencher v. 4.5 (Gene Codes, Ann Arbor, Michigan). Fragments of 16S rDNA were sequenced for 40 onuphid specimens; complete or partial 18S rDNA was sequenced for 43 specimens. Fourteen sequences for each of the genetic markers were obtained from GenBank. Four species lacked data for 16S rDNA, one species lacked data for 18S rDNA (Table 4).

2.3. Sequence alignment

Alignment was conducted using the MUSCLE (Edgar, 2004) algorithm implemented in MEGA v. 5.1 (Tamura et al., 2011) with the following settings: −400 gap opening penalty, −50 gap extension penalty. Some of the outgroup taxa had expansion sequences that produced indels in the alignment. We used Gblocks V.0.91b (Castresana, 2000) to eliminate poorly aligned positions in the original alignments. The same parameters were chosen for 16S rDNA and 18S rDNA alignments: minimum number of sequences for a conserved position – 40; minimum number of sequences for a flanking position – 40; maximum number of contiguous non-conserved positions – 8; minimum length of a block – 5; allowed gap positions – with half. The 16S rDNA and 18S rDNA datasets

Table 3
Primer sequences and PCR parameters used for amplification of 18S rDNA and 16S rDNA.

Gene	Sequence (5′–3′)	Direction	Reference	PCR parameters
16S rDNA				
16SarL	CGC CTG TTT ATC AAA AAC AT	Forward	Palumbi et al., 1991	1 cycle: 94 °C/3 min; 40 cycles: 94 °C/30 s,
16S-OnuR	GTC TGA ACT CAG CTC ACG TAG G	Reverse	This study	51 °C/30 s (decreasing 0.2 °C per cycle), 72 °C/70 s; 1 cycle: 72 °C/7 min
Onu_16S_F1_M13	TGT AAA ACG ACG GCC AGT CTG TTT AMC AAA AAC ATH GCC T	Forward	This study	1 cycle: 95 °C/1 min; 40 cycles: 95 °C/15 s,
Onu_16S_R1_M13	CAG GAA ACA GCT ATG ACG CTT ACG CCG GTC TGA ACT CAG	Reverse	This study	52 °C/15 s, 72 °C/30 s; 1 cycle: 72 °C/7 min
18S rDNA				
18e	CTG GTT GAT CCT GCC AGT	Forward	Hillis and Dixon, 1991	1 cycle: 94 °C/3 min; 40 cycles: 94 °C/
18L	GAA TTA CCG CGG CTG CTG GCA CC	Reverse	Hillis and Dixon, 1991	1 min, 42 °C/1 min 30 s, 72 °C/2 min 30 s;
18F509	CCC CGT AAT TGG AAT GAG TAC A	Forward	Struck et al. (2002)	1 cycle: 72 °C/7 min
18R	GTC CCC TTC CGT CAA TTY CTT TAA G	Reverse	Hillis and Dixon, 1991	
18F997	TTC GAA GAC GAT CAG ATA CCG	Forward	Struck et al. (2002)	
18R1843	GA TCC AAG CTT GAT CCT TCT GCA GGT TCA CCT AC	Reverse	Elwood et al. (1985)	

Table 4
Characteristics of analyzed 16S rDNA and 18S rDNA fragments.

Gene	Sequence length (nucleotides)	Missing taxa	Alignment positions	Parsimony informative	Variable sites	Evolution model	Relative rate
16S rDNA	331–528	4	541	317	370	GTR + I + Γ`	4.205
16S rDNA (Gblocks)	305–446	4	446	250	292	GTR + I + Γ`	4.288
18S rDNA	566–1875	1	1939	366	596	GTR + I + Γ`	0.106
18S rDNA (Gblocks)	557–1743	1	1743	349	501	GTR + I + Γ`	0.159

Table 5
Results from (1) Bayesian tree filtering to compute posterior probability (PP) of topological constraints, (2) Bayes Factor testing with stepping-stone sampling, and (3) Shimodaira–Hasegawa tests of Bayesian consensus tree compared to previous hypotheses of phylogenetic relationships among genera.

Test procedure	1	2		3		
Hypothesis	Frequency constrained trees	2 ln BF	Significance	−ln L	Difference −ln L	*P*
H0. The Bayesian consensus tree	–	–	–	18334.68701	0.00266	0.770
H1. *Leptoecia* sister to *Hyalinoecia*	0.22	7.09	Accept	18334.68435	(best)	
H2. *Rhamphobrachium* sister to (*Diopatra, Paradiopatra*)	0.00	8.55	Reject	18406.94482	72.26047	0.000[*]
H3. *Onuphis* sister to (*Hirsutonuphis* (*Kinbergonuphis, Mooreonuphis*))	0.00	8.57	Reject	18447.16873	112.48438	0.000[*]

[*] *P* < 0.05.

were aligned separately and later combined into a single dataset for the analyses.

2.4. Phylogenetic analyses

2.4.1. Bayesian inference (BI)

Substitution models for 16S rDNA and 18S rDNA datasets were selected in ModelGenerator (Keane et al., 2006) based on Akaike Information Criterion. A GTR + I + Γ` substitution model was chosen for both markers. Previous analyses of single gene data from Eunicidae have been proven being unable to recover stable clades at different depths of the tree (Zanol et al., 2010). Therefore we used only a combined dataset for the phylogenetic analyses. Bayesian analysis was performed in MrBayes v. 3.2.1 (Ronquist et al., 2012). Model parameter values for the two partitions were estimated independently using the "unlink" command and site specific rates for the two genes were estimated by setting the prior for "ratepr" to "variable". Two independent and simultaneous runs with flat prior probabilities and four chains were run for 10,000,000 generations. Trees were sampled every 1000th generation. Tracer v. 1.5 (Rambaut and Drummond, 2007) was used to identify the burn-in phase and the first 25% resulting trees were excluded. The remaining trees were summarized into a majority rule consensus tree with posterior probabilities (PP) indicating the support for each clade. Convergence between the runs was verified using the Average Standard Deviation of Split Frequencies (ASDSF) and the Potential Scale Reduction Factor (PSRF), calculated

in MrBayes. Tracer v. 1.5 was used to examine MCMC sampling statistics and parameter estimates and to verify stationarity with plots of log likelihoods. An effective sample size (ESS) higher than 2000 for the log likelihood and all other parameters when the two runs were combined was considered a good mixing and the results of analyses were accepted.

2.4.2. Maximum likelihood (ML)

The same dataset and nucleotide evolution models for partitions were used for phylogeny inference using the maximum-likelihood criterion implemented in GARLI v. 2.0 (Zwickl, 2006). Searches for the best tree were performed in ten independent replications with at least 50,000 generations without topology improvement. Bootstrap (BP) was performed in 1000 iterations, with pseudoreplicate datasets having 1% of alignment columns differing from the original data. Obtained bootstrap values were placed on the best tree with SumTrees v. 3.3.1 from DendroPy Phylogenetic Computing Library Version 3.12.0 (Sukumaran and Holder, 2010).

2.4.3. Testing alternative hypotheses of phylogenetic relationships

Because our Bayesian consensus tree (hypothesis H0) diverged with respect to some of the relationships among genera proposed by Paxton (1986a), we examined the molecular data support for three alternative tree topologies (H1–H3, Table 5) with three different approaches. In the first approach, we used PAUP* v. 4.0 to constrain (monophyletic) topologies congruent with the hypothesis to
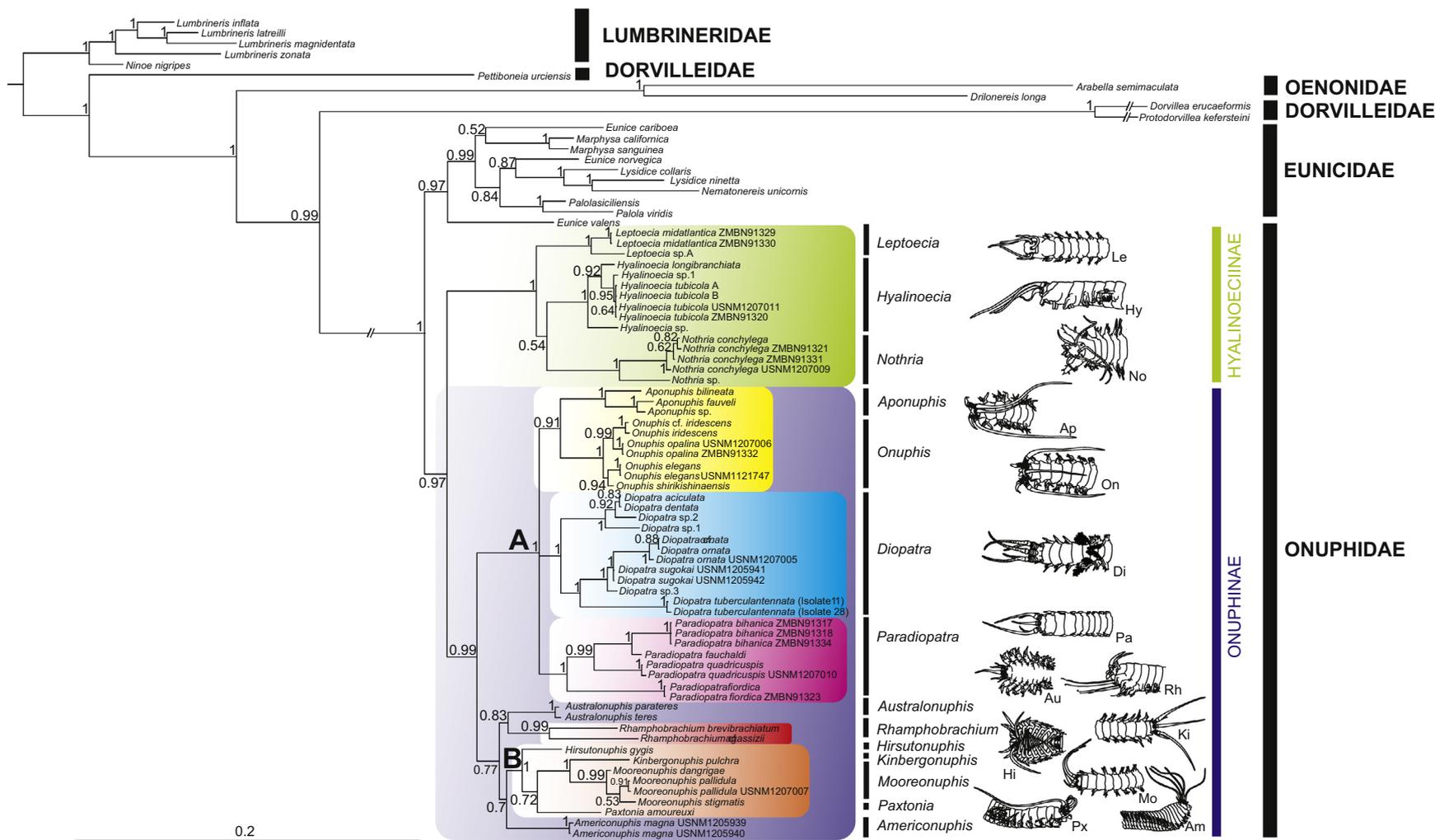
Fig. 2. Consensus tree from the Bayesian analysis of the combined 16S rDNA and 18S rDNA dataset; numbers on nodes indicate Bayesian posterior probabilities; capital letters correspond with the clades discussed in the text. Am – *Americonuphis* (modified from Andrews, 1891); Ap – *Aponuphis*; Au – *Australonuphis* (modified from Rozbaczylo and Castilla, 1981); Di – *Diopatra* (modified from Budaeva and Fauchald, 2008); Hi – *Hirsutonuphis* (modified from Paxton, 1986a); Hy – *Hyalinoecia*; Ki – *Kinbergonuphis*; Le – *Leptoecia*; Mo – *Mooreonuphis*; No – *Nothria* (modified from Budaeva and Paxton, 2013); On – *Onuphis*; Pa – *Paradiopatra* (modified from Budaeva and Fauchald, 2011); Px – *Paxtonia* (modified from Budaeva and Fauchald, 2011); Rh – *Rhamphobrachium* (modified from Imajima, 1999).

be tested and to filter from the pooled set of MCMC trees those trees consistent with the constraint. The frequency of trees consistent with the hypothesis was then computed from the total set of 15,000 post-stationary trees. The frequency represents the posterior probability of the hypothesis being correct. The second approach followed Bergsten et al. (2013) in Bayesian stepping-stone sampling (Baele et al., 2013). In the third approach, we imported the Bayesian consensus tree to Mesquite v. 2.75 (Maddison and Maddison, 2011) and manually modified the internal nodes so as to make it correspond with the topologies of the three alternative hypotheses. The hypotheses tested were: H1 – *Leptoecia* is sister to *Hyalinoecia;* H2 – *Rhamphobrachium* is sister to (*Diopatra, Paradiopatra*); and H3 – *Onuphis* is sister to (*Hirsutonuphis*, (*Kinbergonuphis, Mooreonuphis*)). We used PAUP* v. 4.0 (Swofford, 2002) to perform the one tailed Shimodaira–Hasegawa (SH)-test (Shimodaira and Hasegawa, 1999) with full optimization of the four trees under the GTR + I + Γ model and 1000 bootstrap replicates.

3. Results

The combined data set has 2480 aligned positions (16S rDNA with 541 position and 18S rDNA with 1939 position). After applying Gblocks the new 16S rDNA alignment retained 446 positions (82%), 18S rDNA alignment retained 1743 positions (89%). The combined aligned dataset after applying Gblocks is available online at http://purl.org/phylo/treebase/phylows/study/TB2:S15952?x-access-code=c569b467c23099bc2b9ae2bde45d5d46&format=html. Characteristics of the alignments are shown in Table 4. Initial separate tree estimates with each of the two data sets resulted in relatively poor resolution, particularly when including the very divergent 18S sequences in the Oenonidae and Dorvilleidae outgroups. However, the combination of the two data sets with very different evolutionary rates (Table 4) appears well balanced in the sense that the concatenation provides phylogenetic signal from both the older and the more recent evolutionary divergence points of the tree. In the Bayesian run the –ln L estimates reached equilibrium at about 200 K generations. Convergence diagnostic values of 0.003 for ASDSF for the two runs and PSRF = 1.000 for all branches indicated good mixing of the Markov chains and Effective Sample Size values above 2000 for all parameter estimates indicates that the sampling was more than sufficient. There was high congruence between the trees obtained with the Bayesian and Maximum Likelihood approach (Fig. 2, Supplementary material). Regardless of the method used the following clades were obtained. Onuphidae is monophyletic (PP 0.97, BP 0.65) and sister to Eunicidae (PP 1.00, BP 1.00). Two major clades corresponding to two subfamilies can be recognized within onuphids: Hyalinoeciinae (PP 1.00, BP 1.00) and Onuphinae (PP 0.99, BP 0.61). The following genera are monophyletic: *Leptoecia* (PP 1.00, BP 1.00), *Hyalinoecia* (PP 1.00, BP 0.96), *Nothria* (PP 1.00, BP 1.00), *Australonuphis* (PP 1.00, BP 1.00), *Rhamphobrachium* (PP 0.99, BP 0.64), *Aponuphis* (PP 1.00, BP 0.97), *Onuphis* (PP 1.00, BP 0.95), *Diopatra* (PP 1.00, BP 0.79), *Paradiopatra* (PP 1.00, BP 0.96), *Mooreonuphis* (PP 0.99, BP 1.00). *Paxtonia* is a monotypic genus and *Hirsutonuphis*, *Kinbergonuphis* and *Americonuphis* were represented by a single species, thus their monophyly was not tested. No genera appear para- or polyphyletic on Bayesian or ML phylogenetic reconstructions.

Hyalinoeciinae combines three genera *Hyalinoecia*, *Leptoecia* and *Nothria* with a poorly supported clade (*Hyalinoecia*, *Nothria*) (PP 0.54) present in the Bayesian tree. Onuphinae includes a highly supported clade A comprising four genera ((*Aponuphis*, *Onuphis*), *Diopatra*, *Paradiopatra*) (PP 1.00, BP 0.98) and clade B including (*Hirsutonuphis*, (*Paxtonia*, (*Kinbergonuphis*, *Mooreonuphis*))) (PP 1.00, BP 0.59) present in both BI and ML trees. *Aponuphis* is sister to *Onuphis* with low support (PP 0.91, BP 0.56). The genera *Americonuphis*, *Aus-*

tralonuphis, and *Rhamphobrachium* are placed within Onuphinae forming a basal polytomy in the ML analysis or poorly supported clade (PP.0.77) together with the clade B in the Bayesian tree.

Hypotheses testing (Table 5) reveals that based on the frequencies of the constrained topologies in the pool of MCMC trees from the Bayesian analysis, Bayes Factor testing with stepping-stone sampling, and SH-test, two of three tested hypotheses (H2, H3) previously suggested by Paxton (1986a) are rejected. The hypothesis on sister relationships between *Leptoecia* and *Hyalinoecia* (H1) is accepted in stepping-stone sampling procedure and also receives support (22%) in the frequency of the constrained trees pooled from the Bayesian analysis. SH-test shows that there was no significant difference in –ln L values between Bayesian consensus tree (H0) and the tree with rearranged topology with monophyletic (*Leptoecia*, *Hyalinoecia*) (H1) (Table 5).

4. Discussion

4.1. Monophyly of Onuphidae and status of its subfamilies and genera

Onuphidae appears monophyletic and as sister group to a monophyletic Eunicidae in both ML and Bayesian analyses. Short branch lengths within both Onuphidae and Eunicidae were shown by Zanol et al. (2010) with similar results obtained here. Even though relationships between other eunicidan families were beyond the scope of the present study, our results corroborate the multigene-based phylogeny reported by Struck et al. (2006) with polyphyletic Dorvilleidae, and the *Dorvillea* line of Dorvilleidae as sister to a (Onuphidae, Eunicidae) clade.

Our phylogenetic reconstruction based on molecular data clearly supports the systematic reorganization of Onuphidae suggested by Paxton (1986a) based on morphological characters. Two designated subfamilies, Hyalinoeciinae and Onuphinae, are monophyletic. Assignment of all onuphid genera to two subfamilies proposed by Paxton (1986a) is completely supported. Limited species sampling in most of the tested genera prevents from making certain conclusions about their monophyly. Nevertheless ten of 14 analyzed genera form monophyletic groups with high node support, providing strong evidence for their monophyletic status.

Although majority of the annelid families were shown to be monophyletic (Rousset et al., 2007), molecular-based phylogenies at intergeneric level commonly provide conflicting results with traditional systematics based on morphological data. The most species-rich genera are often found to be paraphyletic (Aguado et al., 2012; Bleidorn, 2005; Eklöf et al., 2007; Ravara et al., 2010; Ruta et al., 2006; Zanol et al., 2010) indicating a high level of homoplasy in the evolution of morphological characters and poor distinction between plesiomorphic and apomorphic features. Incongruence between molecular-based reconstructions and morphology-based taxonomic hypotheses often leads to the interpretation of morphology as unreliable evidence for phylogenetic relationships (Lee et al., 2004). In annelid taxa it can be partly explained by the fairly simple external morphology of many bristle worms, with a phenotypical repertoire prone to many homoplastic characters. Also, the progenetic evolution commonly leading to character underdevelopment may be indistinguishable from the secondary loss of characters in adult organisms (Struck, 2006). Unavailability of freshly collected material suitable for molecular studies is the major obstacle for the wide taxon sampling in many annelid families. Until now taxon sampling in published annelid phylogenies at family level remains very scarce covering not more than 10–15% of species diversity (Aguado et al., 2012; Bleidorn, 2005; Eklöf et al., 2007; Ruta et al., 2006; Zanol et al., 2010), which potentially can lead to misinterpretation of phylogenetic signals in poorly sampled groups.

The phylogenetic relationships recovered in this study provide an example in polychaetes of congruence between the traditional taxonomy predating a phylogenetic hypothesis and the molecular-based identification of monophyletic clades. Onuphidae represent a polychaete family with a complex external morphology. The majority of the genera possess one or several synapomorphies, e.g. spiral branchiae in *Diopatra*, spingers in median parapodia of *Mooreonuphis*, auricular prechaetal lobes on the anterior parapodia in combination with scoop-shaped pectinae chaetae in *Nothria*. Many of the morphological characters unique for each genus or for a group of closely related genera are used in feeding (Mangum and Cox, 1971), locomotion, tube construction (Andrade and Liñero, 1993) or reproduction (Hsieh and Simon, 1990) and could have evolved as adaptations to certain environmental conditions. Diversification of life modes in onuphids supported by morphological variability allowed early recognition of the monophyletic groups based on exclusively morphological characters (Paxton, 1986a).

4.2. Intergeneric relationships

The most striking differences between the morphology-based phylogeny suggested by Paxton (1986a) and our results based on molecular data can be found in the intergeneric relationships within the two subfamilies leading to re-evaluation of the hypotheses on the homology of several morphological characters.

Nothria and *Hyalinoecia* groups (*sensu* Paxton, 1986a) were not resolved in the tree searches (Fig. 2, Supplementary material). Even though *Hyalinoecia* and *Leptoecia* share a number of morphological characters previously interpreted as synapomorphies such as quill-like tubes and enlarged anterior parapodia (Paxton, 1986a), the hypothesis on their sister relationships was rejected by two of our hypothesis tests (Table 5, H1). Interestingly, however, the stepping-stone sampling procedure resulted in $2 \ln BF = 7$, which is strong support according to the scale of Kass and Raftery (1995) (Table 5). Also, the H1 hypothesis had a marginally better likelihood than the Bayesian consensus tree, but the difference was not significant according to the SH-test (Table 5). Additional taxon sampling and analysis of other genetic markers are required for resolving the relationships within Hyalinoeciinae.

None of the subgroupings within Onuphinae proposed by Paxton (1986a), were supported by molecular data. A strongly supported clade A comprising four genera, ((*Aponuphis*, *Onuphis*), *Diopatra*, *Paradiopatra*) was recovered within Onuphinae (Fig. 2, Supplementary material). An association between *Onuphis*, *Diopatra* and *Paradiopatra* was also reported by Zanol et al. (2010) based on the analysis of three genetic markers in five onuphid species.

Our results provide weak support for the sister relationships between *Onuphis* and *Aponuphis*. These genera appear to be very similar in external morphology and can be distinguished from each other by the absence of the peristomial cirri in the latter. *Onuphis* and *Aponuphis* had not been previously considered as sister genera (e.g., Paxton, 1986a) despite the presence of several morphological similarities such as long multiringed palpophores, conical projection of the frontal margin of the prostomium; appearance of branchiae on the anteriormost segments and the presence of developed postchaetal lobes in the anterior part of the body.

The close relationship between *Diopatra* and *Paradiopatra* suggested by Paxton (1986a) and by Budaeva and Fauchald (2011) was not supported by our results although both genera share the presence of long to moderately long pointed hoods on anterior falcigers and branchiae occurring only in the midbody region. The presence of lateral spines on the anterior falcigers of *Diopatra*,

Paradiopatra and *Rhamphobrachium* was considered as a synapomorphy supporting the monophyly of the *Diopatra* group (incl. the *Diopatra* complex and the *Rhamphobrachium* complex) (Paxton, 1986a). Hypothesis testing rejected the idea that *Rhamphobrachium* alone is sister to *Diopatra* and *Paradiopatra* combined (Table 5, H2). While such spines are similar in shape and topology in *Paradiopatra* and *Diopatra*, they are considerably larger and, in some cases, articulated in *Rhamphobrachium* species. Thus, they appear to be not homologous but rather the result of parallel evolution. The monophyly of the *Rhamphobrachium* complex was not tested since we analyzed the representatives of only one genus from the complex. *Onuphis*, *Kinbergonuphis* and *Mooreonuphis* have traditionally been considered closely related genera based on their minute size, lateral position of anterior parapodia and the presence of both bi- and tridentate anterior falcigers with short hoods (Fauchald, 1982; Paxton, 1986a). Paxton (1986a) placed them together with *Heptaceras* as basal genera within Onuphinae with the derived clade combining *Australonuphis* complex of genera (incl. *Australonuphis*, *Hirsutonuphis*, and *Hartmanonuphis*). The basal position of *Mooreonuphis* within Onuphinae was also suggested by Zanol et al. (2010) based on molecular data. However Zanol et al.'s (2010) study focused on the phylogeny of the family Eunicidae and included only a few representatives of onuphids. Restricted taxon sampling possibly resulted in low support of the position of *Mooreonuphis* and could lead to its misplacement. Our results rejected the hypothesis of sister relationships between *Onuphis* and a (*Hirsutonuphis*, (*Kinbergonuphis*, *Mooreonuphis*)) clade (Table 5, H3). *Mooreonuphis* is a derived genus and sister to *Kinbergonuphis* with *Paxtonia* basal in relation to them (Fig. 2, clade B). *Kinbergonuphis* and *Mooreonuphis* share the presence of large tridentate hooks inserted medially in the transitional parapodia. This character has not been reported for all species in both genera. Among the species analyzed in the present paper, large median hooks were present in *Kinbergonuphis pulchra* (Fauchald, 1980) and *Mooreonuphis stigmatis* (Treadwell, 1922) and absent in
M. dangrigae (Fauchald, 1980) and *M. pallidula* (Hartman, 1965).

The monophyly of the *Australonuphis* complex of genera *sensu* Paxton (1986a) comprising shallow water beach worms *Australonuphis*, *Hirsutonuphis* and *Hartmanonuphis* is not supported by our results. *Hirsutonuphis* is a basal genus in the clade B (Fig. 2) also including (*Paxtonia*, (*Mooreonuphis*, *Kinbergonuphis*)) while *Australonuphis* is combined with *Rhamphobrachium* in the Bayesian tree or placed as basal polytomy in Onuphinae in the ML tree. The lack of the complete anterior peristomial fold separating the prostomium from the peristomium was suggested as a synapomorphy for the *Australonuphis* complex (Paxton, 1986a). Our results show that it is a homoplastic character that has evolved at least twice within Onuphinae, possibly as an adaptation to the infaunal life style. Monophyly of the (*Australonuphis*, *Rhamphobrachium*) clade (PP 0.83, node absent in the ML tree) was not strongly supported statistically. However both genera share the presence of compound or pseudocompound ventral limbate chaetae, so-called spingers, in the midbody parapodia. Similar chaetae have been reported in *Mooreonuphis*, indicating that it is a homoplastic character evolved independently at least twice within Onuphinae. *Paxtonia*, a genus described by Budaeva and Fauchald (2011) as a member of the *Diopatra* complex, is sister to the (*Kinbergonuphis*, *Mooreonuphis*) clade. This supports the validity of the genus, which was previously assigned to *Paradiopatra* (Paxton, 1986a), and indicates that long pointed hoods on anterior falcigers found in *Paradiopatra*, *Diopatra*, *Paxtonia* (Budaeva and Fauchald, 2011) and possibly *Hyalospinifera* (Kucheruk, 1979) have evolved independently several times within Onuphidae.

5. Conclusions

Onuphidae is monophyletic with a sister family Eunicidae. Subfamilies and genera of Onuphidae proposed by Paxton (1986a) are strongly supported by molecular data. The hypotheses on the intergeneric relationships based on morphological data are largely rejected or not resolved using the available genetic data. The accepted division of Onuphinae into the *Diopatra* group of genera and the *Onuphis* group of genera (Paxton, 1986a) was not supported by our results. Several morphological characters such as the long pointed hoods and lateral spines on the anterior falcigers, the absence of the complete peristomial fold, and the presence of compound spinigers previously treated as exclusive synapomorphies defining groups and complexes of genera within Onuphinae appear to be homoplastic.

Acknowledgments

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2015.10.011.

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