Molecular Phylogeny of the Model Annelid *Ophryotrocha*

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Abstract. Annelids of the genus *Ophryotrocha* are small opportunistic worms commonly found in polluted and nutrient-rich habitats such as harbors. Within this small group of about 40 described taxa a large variety of reproductive strategies are found, ranging from gonochoristic broadcast spawners to sequential hermaphroditic brooders. Many of the species have a short generation time and are easily maintained as laboratory cultures. Thus they have become a popular system for exploring a variety of biological questions including developmental genetics, ethology, and sexual selection. Despite considerable behavioral, reproductive, and karyological studies, a phylogenetic framework is lacking because most taxa are morphologically similar. In this study we use 16S mitochondrial gene sequence data to infer the phylogeny of *Ophryotrocha* strains commonly used in the laboratory. The resulting mtDNA topologies are generally well resolved and support a genetic split between hermaphroditic and gonochoristic species. Although the ancestral state could not be unambiguously identified, a change in reproductive strategy (*i.e.,* hermaphroditism and gonochorism) occurred once within *Ophryotrocha*. Additionally, we show that sequential hermaphroditism evolved from a simultaneous hermaphroditic ancestor, and that characters previously used in phylogenetic reconstruction (*i.e.,* jaw morphology and shape of egg mass) are homoplasic within the group.

Introduction

Marine annelids belonging to the group *Ophryotrocha* have been used as a laboratory system for much of this century (*e.g.,* Bergh, 1895; Bergmann, 1903; Meek,

1912; Huth, 1933; Hartmann and Lewinski, 1940; Bacci and La Greca, 1953; Bacci, 1965; Åkesson, 1972; Sella, 1988; Vitturi *et al.,* 2000), not least because they are easy to maintain in cultures and have short generation times. *Ophryotrocha* has traditionally been treated as a genus within the eunicimorph family Dorvilleidae (*e.g.,* Fauchald, 1977; Eibye-Jacobson and Kristensen, 1994), but inclusion within the Dorvilleidae has been challenged (Orensanz, 1990). Many ecological (*e.g.,* Åkesson, 1977; Berglund, 1991; Cassai and Prevedelli, 1999), ethological (*e.g.,* Sella, 1991), developmental (*e.g.,* Åkesson, 1967, 1973; Zavarzina and Tzetlin, 1991), and toxicological (*e.g.,* Åkesson, 1970, 1975) studies have been conducted on these worms. Charnov (1982) and Gambi *et al.* (1997) among others, argue that *Ophryotrocha* is a near ideal group for studies of the evolution of sex strategies, since all known forms (gonochorism, sequential and simultaneous hermaphroditism) are represented within a few closely related species.

Since *Ophryotrocha* was first described (Claparède and Mecznikow, 1869), more than 40 species have been added to the group, most of which are reported from shallow, nutrient-rich waters such as harbors (*e.g.,* La Greca and Bacci, 1962; Åkesson, 1976; Paavo *et al.,* 2000). Recent contributions have also shown a considerable diversity in the deep sea (Jumars, 1974; Blake, 1985; Hilbig and Blake, 1991; Lu and Fauchald, 2000). To the best of our knowledge, the Appendix lists all the described species of *Ophryotrocha* with their type locality. Only species from shallow, temperate or tropical waters, however, have been successfully cultured in the laboratory (at present ~20 distinct forms). Among the cultured forms, some of which are yet to be formally described,

most taxa are morphologically identical; but each is believed to be a distinct species because species crosses failed to produce viable offspring in breeding experiments (Åkesson, 1978, 1984, and unpubl.).

Even though the taxonomical and descriptive morphological literature is extensive (*e.g.,* La Greca and Bacci, 1962; Pfannenstiel, 1972, 1975; Josefsson, 1975; Åkesson, 1978; Oug, 1978, 1990; Blake, 1985; Ockelmann and Åkesson, 1990; Hilbig and Blake, 1991; Lu and Fauchald, 2000; Paavo *et al.,* 2000), there has been but a single study (Pleijel and Eide, 1996) focusing on the phylogenetic history of a broader selection of *Ophryotrocha* species. That study, based on an analysis of 25 morphological characters and 7 electrophoretic protein loci scored for 20 *Ophrytrocha* taxa, described two major clades and suggested that simultaneous hermaphroditism is the pleisiomorphic condition for the group as a whole. Basal branching in Pleijel and Eide's tree (1996, Fig. 1B), however, was poorly supported, as depicted by their highly collapsed strict consensus tree. One of the clades found by Pleijel and Eijde (1996) is congruent with the gonochoristic *labronica* group previously suggested by Åkesson (1984). On the basis of morphological features, such as similarities in jaw apparatuses and egg-mass morphology, Åkesson (1984) and Ockelmann and Åkesson (1990) also recognized the hermaphroditic "*gracilis*" and "*hartmanni*" groups. These taxa form a grade at the base of the most parsimonious tree favored by Pleijel and Eide (1996, Fig. 1A), and they consequently suggested that their similarities are sympleisiomorphic.

Chromosome number and karyological characters were used in an analysis of nine species by Robotti and collaborators (1991). The resulting topology suggested that forms with equal number of chromosomes constitute monophyletic groups. The data also indicated that the distance between *O. robusta* and *O. hartmanni* (2n = 10) is larger than the distances between members of the two other groups (2n = 6 and 2n = 8). Further, on the basis of work by Colombera and Lazzaretto-Colombera (1978) suggesting that karyotypes evolved towards reduced chromosome numbers, Robotti *et al.* (1991) proposed that *O. robusta* occupies an ancestral position. The same conclusion was reached by Vitturi and collaborators (2000) in a study of karyotypes of 10 *Ophryotrocha* species.

The evolutionary relationships among 18 *Ophryotrocha* forms were investigated using the mitochondrial 16S rDNA gene. Due to organismal availability, we have focused on intertidal forms that are easily kept in laboratory cultures (Åkesson, 1970, 1975). The present paper builds on the work of Pleijel and Eide (1996) to reconstruct the phylogeny of this model annelid system so that knowledge gained about *Ophryotrocha* may be assessed in a comparative context.

Materials and Methods

Taxa

Eighteen *Ophryotrocha* cultures representing intertidal and shallow-water forms were selected for this study (Table 1). All of these terminal taxa were readily available because they are maintained as laboratory cultures by B.Å. and represent the best-studied members of *Ophryotrocha.* Nine of the eighteen strains included are not formally described as species. Six are referred to by the name under which they will be described, followed by "nom. nud." to indicate their present status as *nomina nuda, i.e.,* names not available. The remaining three strains are referred to by the location where they were originally collected. The informally named taxa are not to be regarded as descriptions *sensu* International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature, 1985).

Outgroup terminals were chosen on the basis of recent analyses of eunicimorph and annelid phylogeny (Paxton, 1986; Orensanz, 1990; Eibye-Jacobson and Kristensen, 1994; Rouse and Fauchald, 1997). These taxa include *Hyalinoecia tubicola* (Müller, 1776), *Nothria conchylega* (Sars, 1835), *Eunice pennata* (Müller, 1776), *Dorvillea albomaculata* Åkesson and Rice, 1992, and *Dinophilus gyrociliatus* Schmidt, 1857. Specimens of *H. tubicola, N. conchylega,* and *E. pennata* were collected by epibentic dredge, while *Dorvillea albomaculata* and *Dinophilus gyrociliatus* were acquired from laboratory cultures (BÅ). Locality details are provided in Table 1.

Data collection

The worms were taken from cultures and placed in 70% ethanol. Voucher specimens from the same cultures were fixed in 5% formaldehyde for 1 h and subsequently transferred to 70% ethanol. The voucher specimens are deposited in the Zoological Museum, Copenhagen (ZMUC) and designated the numbers given in Table 1. DNA was extracted by either employing a Chelex protocol (Sundberg and Andersson, 1995) or a standard chloroform/phenol protocol (Doyle and Dickson, 1987). An approximately 400-bp fragment of the mitochondrial large subunit ribosomal RNA gene was amplified with the universal primers 16Sar-L (5′-cgcctgtttatcaaaaacat-3′) and 16Sbr-H (5′-ccggtctgaact-cagatcacgt-3′) according to standard protocols (Palumbi, 1996). The amplification profile was 40 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 45 s with an initial single denaturing step at 95 °C for 2 min, and a final single extension step at 72 °C for 7 min. After spin-column purification (QiaGen, Inc.), the PCR products were sequenced in both directions on a PharmaciaBiotech ALF-Express automated sequencer using the TermoSequenase kit (AmershamPharmacia) and Cy-5 labeled 16Sar-L and 16Sbr-H

Table 1

Collection data and GenBank accession numbers for Ophryotrocha *taxa examined*

Taxon	Collection site	Coll. year	GenBank Accession Nr	ZMUK Voucher Nr
Dorvillea albomaculata * Åkesson and Rice, 1992	Tarifa, Spain	1990	AF380115	N/A
Dinophilus gyrociliatus * Schmidt, 1857	Xiamen, China	1995	AF380116	N/A
Hyalinoecia tubicola (Müller, 1776)	Koster area, Sweden	1997	AF321416	N/A
Nothria conchylega (Sars, 1835)	Koster area, Sweden	1997	AF321417	N/A
Eunice pennata (Müller, 1776)	Koster area, Sweden	1997	AF321418	N/A
O. adherens Paavo *et al.,* 2000	Kyrenia, Cyprus	1971	AF321419	ZMUC-POL-1110
O. alborana nom. nud.	Algeciras, Spain	1978	AF321420	ZMUC-POL-1111
O. costlowi Åkesson, 1978	Duke, NC, USA	1974	AF321421	ZMUC-POL-1112
O. diadema Åkesson, 1976	L.A. harbor, USA	1972	AF321422	ZMUC-POL-1113
O. gracilis Huth, 1934	Helgoland, Germany	1988	AF321423	ZMUC-POL-1114
O. hartmanni Huth, 1933	Malaga, Spain	1990	AF321424	ZMUC-POL-1115
O. japonica nom. nud.	L.A. harbor, USA	1989	AF321433	ZMUC-POL-1116
O. labronica La Greca and Bacci, 1962	Naples, Italy	1965	AF321425	ZMUC-POL-1117
O. macrovifera nom. nud.	Cyprus	1972	AF321426	ZMUC-POL-1118
O. notoglandulata Pfannenstiel, 1972	Misaki, Japan	1961	AF321427	ZMUC-POL-1119
O. obscura nom. nud.	Pet store, Sweden	1978	AF321436	ZMUC-POL-1120
O. permanni nom. nud.	Indian River, Florida	1991	AF321428	ZMUC-POL-1121
O. puerilis Claparède and Mecznikow, 1869	Malaga, Spain	1990	AF321429	ZMUC-POL-1122
O. robusta nom. nud.	Malaga, Spain	1990	AF321430	ZMUC-POL-1123
O. socialis Ockelmann and Åkesson, 1990	Helsingör, Denmark	1986	AF321431	ZMUC-POL-1124
Eilat-Hurghada	Red Sea	1996	AF321432	ZMUC-POL-1125
Qingdao	Qingdao, China	1995	AF321434	ZMUC-POL-1126
Sanya sp. 2	South Hainan, China	1995	AF321435	ZMUC-POL-1127

* Sequenced by Arne Nygren

primers in accordance with the manufacturer's protocols. GenBank accession numbers are given in Table 1.

Analysis

The sequences were aligned with Clustal X (Thompson *et al.,* 1994) and proofread by eye. Regions that could not be unambiguously aligned were excluded from the analysis. The alignment is deposited at TreeBase and available at http://phylogeny.harvard.edu/treebase or from TD. Neighbor-joining (NJ), parsimony, and maximum-likelihood (ML) analyses were conducted with the PAUP*4.0b2 software package (Swofford, 2000). PAUP* was further used for parameter estimations for the ML searches. For NJ, a Kishino-Hasegawa (1989) likelihood test found no significant differences between trees generated under the Jukes-Cantor, Kimura-2-parameter, Tamura-Nei, and general-time-reversible (GTR) models (see Swofford *et al.,* 1996, for a brief description of models). Parsimony trees were inferred from an unweighted character matrix (*i.e.,* the transition/transversion ratio was assumed to be 1 with the heuristic search option using the tree-bisection-reconnection (TBR) branch-swapping algorithm and 100 random-sequence addition replicates. To reduce the computation time of the ML search, the most parsimonious tree was used as

starting tree in the ML heuristic search. The model parameters were estimated from a likelihood analysis of the most parsimonious tree and included a nucleotide model with six substitution types (a GTR model), and amongsites rate heterogeneity used a gamma distribution with shape parameter of 0.30. A GTR model was chosen since it is the most general of the ones mentioned above, all of which are special cases of a GTR (Swofford *et al.,* 1996). Nucleotide frequencies were set to empirical values. Bootstrap analyses for both ML and parsimony employed 1000 iterations.

The four characters—(1) sex strategy, (2) jaw morphology, (3) egg-mass morphology, and (4) diploid number of chromosomes—were chosen in part on the basis of previous efforts to estimate *Ophryotrocha* relationships (*e.g.,* Åkesson, 1984; Robotti *et al.,* 1991; Pleijel and Eide, 1996; Vitturi *et al.,* 2000). MacClade 3.06 (Maddison and Maddison, 1992) was used to manipulate the molecular data, and to map sex strategy and morphological and karyological character state changes on the mtDNA topology. Character states were scored following Pleijel and Eide (1996) except for strains from Eilat-Hurghada, Sanya sp. 2, and Qingdao, which were obtained from the same cultures as the specimens sampled for sequencing. The diploid number of chromosomes is not known for these three forms.

Table 2

Pairwise molecular distances (absolute number of unambiguously aligned substitutions above diagonal and Jukes Cantor distances below); Sanya sp. 2 and O. obscura *nom. nud. are distinguished in three positions located in the excluded regions of the alignment*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	*Dorvillea albomaculata*		73	60	69	75	69	75	77	75	74	74	81	74	75	79	79	69	65	72	74	83	79	79
2	*Dinophilus gyrociliatus*	0.32		68	65	79	77	89	79	91	88	80	88	76	83	81	75	77	67	77	80	90	82	82
3	*Eunice pennata*	0.25	0.30		36	46	73	79	88	70	79	72	92	84	80	93	75	70	72	82	75	94	86	86
4	*Hyalinoecia tubicola*	0.30	0.28	0.14		40	75	78	84	70	74	78	89	83	76	87	77	74	72	80	76	88	81	81
5	*Notria conchylega*	0.33	0.35	0.19	0.16		80	77	82	73	83	78	86	81	79	84	84	78	75	76	78	88	81	81
6	*O. adherens*	0.30	0.35	0.33	0.34	0.37		67	68	63	41	41	70	63	69	66	57	60	23	62	71	65	67	67
7	*O. alborana* nom. nud.	0.33	0.42	0.36	0.35	0.35	0.29		73	44	77	71	72	67	71	73	70	67	66	69	72	61	73	73
8	*O. costlowi*	0.34	0.35	0.42	0.39	0.37	0.30	0.32		66	75	80	21	25	29	16	67	48	65	33	29	71	19	19
9	*O. diadema*	0.33	0.43	0.31	0.31	0.32	0.27	0.18	0.29		62	62	71	69	58	69	58	59	65	69	57	51	67	67
10	*O. gracilis*	0.33	0.41	0.36	0.33	0.38	0.16	0.35	0.33	0.26		37	76	76	75	77	60	67	42	76	76	72	77	77
11	*O. hartmanni*	0.33	0.36	0.32	0.35	0.35	0.16	0.31	0.36	0.26	0.15		78	70	77	78	61	68	42	74	76	73	76	76
12	*O. labronica*	0.37	0.41	0.44	0.42	0.40	0.31	0.32	0.08	0.31	0.34	0.35		25	31	15	68	50	67	31	32	77	11	11
13	*O. macrovifera* nom. nud.	0.33	0.34	0.39	0.38	0.37	0.27	0.29	0.10	0.30	0.34	0.31	0.10		32	27	67	52	59	24	32	75	27	27
14	*O. notoglandulata*	0.33	0.38	0.37	0.34	0.36	0.30	0.31	0.11	0.24	0.33	0.35	0.12	0.12		28	66	44	66	34	8	69	28	28
15	*O. permanni* nom. nud.	0.36	0.37	0.45	0.4	0.39	0.29	0.32	0.06	0.30	0.35	0.35	0.06	0.10	0.11		71	47	66	29	31	70	13	13
16	*O. puerilis*	0.36	0.33	0.34	0.35	0.39	0.24	0.31	0.29	0.24	0.25	0.26	0.30	0.29	0.29	0.31		62	54	68	66	73	66	66
17	*O. robusta* nom. nud.	0.30	0.34	0.31	0.33	0.35	0.25	0.29	0.2	0.25	0.29	0.30	0.20	0.21	0.18	0.19	0.26		59	51	43	65	50	50
18	*O. socialis*	0.28	0.29	0.32	0.32	0.34	0.09	0.29	0.28	0.28	0.17	0.17	0.29	0.25	0.29	0.29	0.22	0.25		62	67	66	67	67
19	Eilat-Hurghada	0.32	0.34	0.38	0.36	0.34	0.26	0.30	0.13	0.30	0.34	0.33	0.12	0.09	0.13	0.11	0.30	0.21	0.26		32	71	32	32
20	*O. japonica* nom. nud.	0.33	0.36	0.34	0.34	0.35	0.31	0.32	0.11	0.24	0.34	0.34	0.12	0.12	0.03	0.12	0.28	0.17	0.29	0.12		74	29	29
21	Qingdao	0.38	0.42	0.46	0.41	0.41	0.28	0.26	0.31	0.21	0.32	0.32	0.35	0.33	0.30	0.31	0.32	0.28	0.29	0.31	0.33		77	77
22	Sanya sp. 2	0.36	0.37	0.40	0.37	0.37	0.29	0.32	0.07	0.29	0.35	0.34	0.04	0.10	0.11	0.05	0.29	0.20	0.29	0.12	0.11	0.35		0
23	*O. obscura* nom. nud.	0.36	0.37	0.40	0.37	0.37	0.29	0.32	0.07	0.29	0.35	0.34	0.04	0.10	0.11	0.05	0.29	0.20	0.29	0.12	0.11	0.35	0.00	

Results

The data set consisted of 23 terminal taxa and 485 nucleotide positions. Of the 282 nucleotide positions that could be unambiguously aligned, 55.0% (155 positions) were variable and 45.7% (129 positions) were parsimony informative. Table 2 shows the Jukes-Cantor distances (below diagonal) and absolute distances (above diagonal) for the alignment. Figure 1A shows the highest scoring likelihood tree recovered (Ln likelihood = −2741.47651). More terminal branches were generally well resolved, but support for basal nodes was low (see bootstrap tree, Fig. 1B).

In the ML tree, two large clades emerge. The first, for brevity here called A, is further divided into two clades, A1 and A2. Clade A1 consists of *O. hartmanni, O. gracilis, O. adherens, O. socialis,* and *O. puerilis;* A2 comprises *O. alborana, O. diadema,* and "Qingdao." The second major clade, here called B, includes *O. japonica* nom. nud., *O. notoglandalata, O. costlowi, O. labronica,* Sanya sp. 2, *O. obscura* nom. nud., *O. permanni, O. macrovifera* nom. nud., Eilat-Hurghada, and *O. robusta.* The parsimony and NJ trees (Fig. 2A) differ from the ML tree in the placement of the root. Instead of a monophyletic A group, as in the ML tree, A constitutes a grade where the two clades A1′ (excluding *O. puerilis*) and A2, together with *O. puerilis,* lead to B (Fig. 2A). The general topologies are, however, similar in the parsimony, NJ, and ML trees, and most of the nodes are supported over the 50% bootstrap level (Fig. 1B).

In an attempt to assess support for basal nodes, an additional analysis was performed on an alignment of ingroup taxa only (aligning and ML procedures as described above). Inclusion of more distant taxa in an alignment may reduce the nucleotide positions that can be unambiguously aligned, and a more restricted selection of taxa could potentially increase phylogenetic signal by allowing for a "better" alignment (Halanych *et al.,* 1998). The analysis, of the 18 *Ophryotrocha* ingroup taxa only, did reveal higher bootstrap support for internal branches of the tree. This restricted analysis, however, gave lower support or alternative hypotheses for some of the more recent clades. More recent clades in B are less well resolved than in the original analyses, but *O. japonica* nom. nud. and *O. notoglandalata* form a strongly supported monophyletic group (Fig. 2B).

Figure 3 shows an arbitrarily chosen most parsimonious reconstruction (transformation optimization by ACCTRAN) for each of the four characters mapped on the ML tree (Fig. 1A). Evidence of transformation polarity is given from outgroup analysis of mtDNA data, and traced characters are accordingly not scored for outgroup taxa.

Discussion

Ophryotrocha phylogeny was investigated by employing ML, NJ, and parsimony analyses of 16S rDNA data. One alignment of these data included the ingroup and five outgroup taxa and resulted in poor support for basal branching patterns (Fig. 1B). The second data set was limited to the 18 *Ophryotrocha* terminals (*i.e.,* the ingroup) and produced better supported and nearly identical topologies under ML
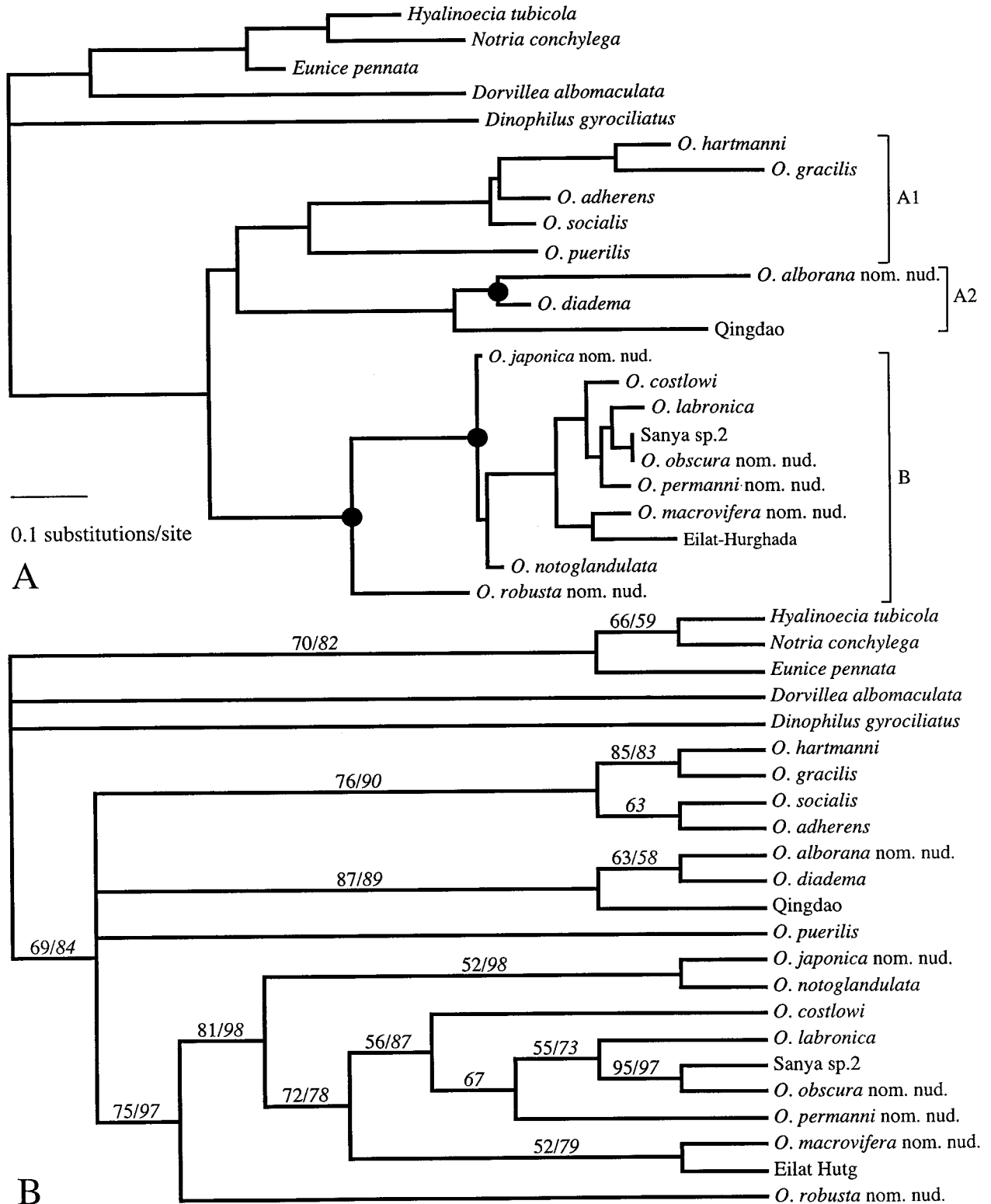
Figure 1. (A) Best maximum likelihood tree. −ln = 2741.47651. Highlighted nodes indicate clades congruent with morphological analysis by Pleijel and Eide (1996). (B) 1000 bootstrap consensus. Numbers in upright type are maximum likelihood and in italic are parsimony support values.
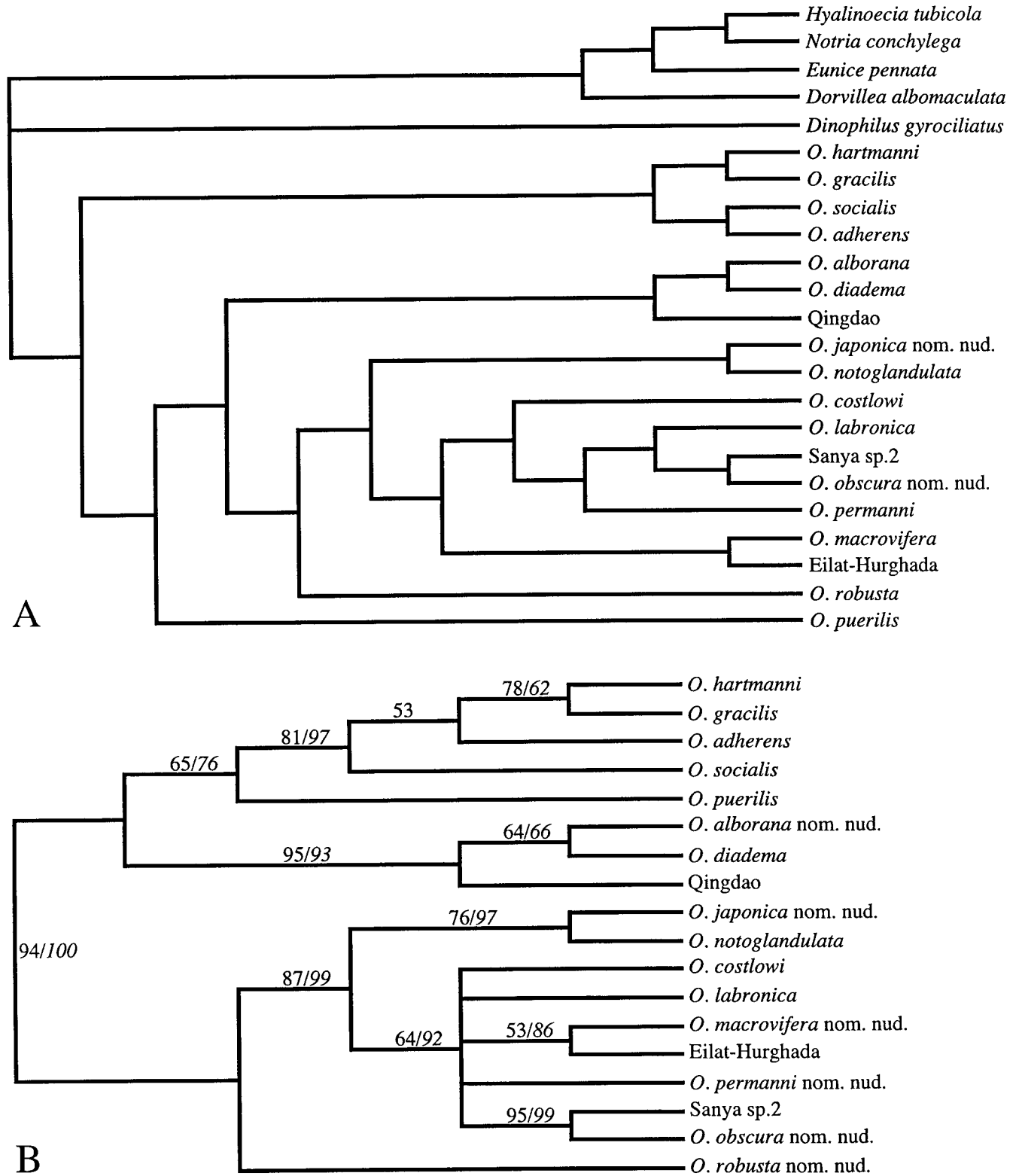
Figure 2. (A) Parsimony and neighbor-joining tree topology. (B) Unrooted bootstrap tree from alignment of ingroup taxa only and drawn to represent the rooting suggested in the original maximum likelihood analysis. Bootstrap values for maximum likelihood in upright type and for parsimony in italic type.
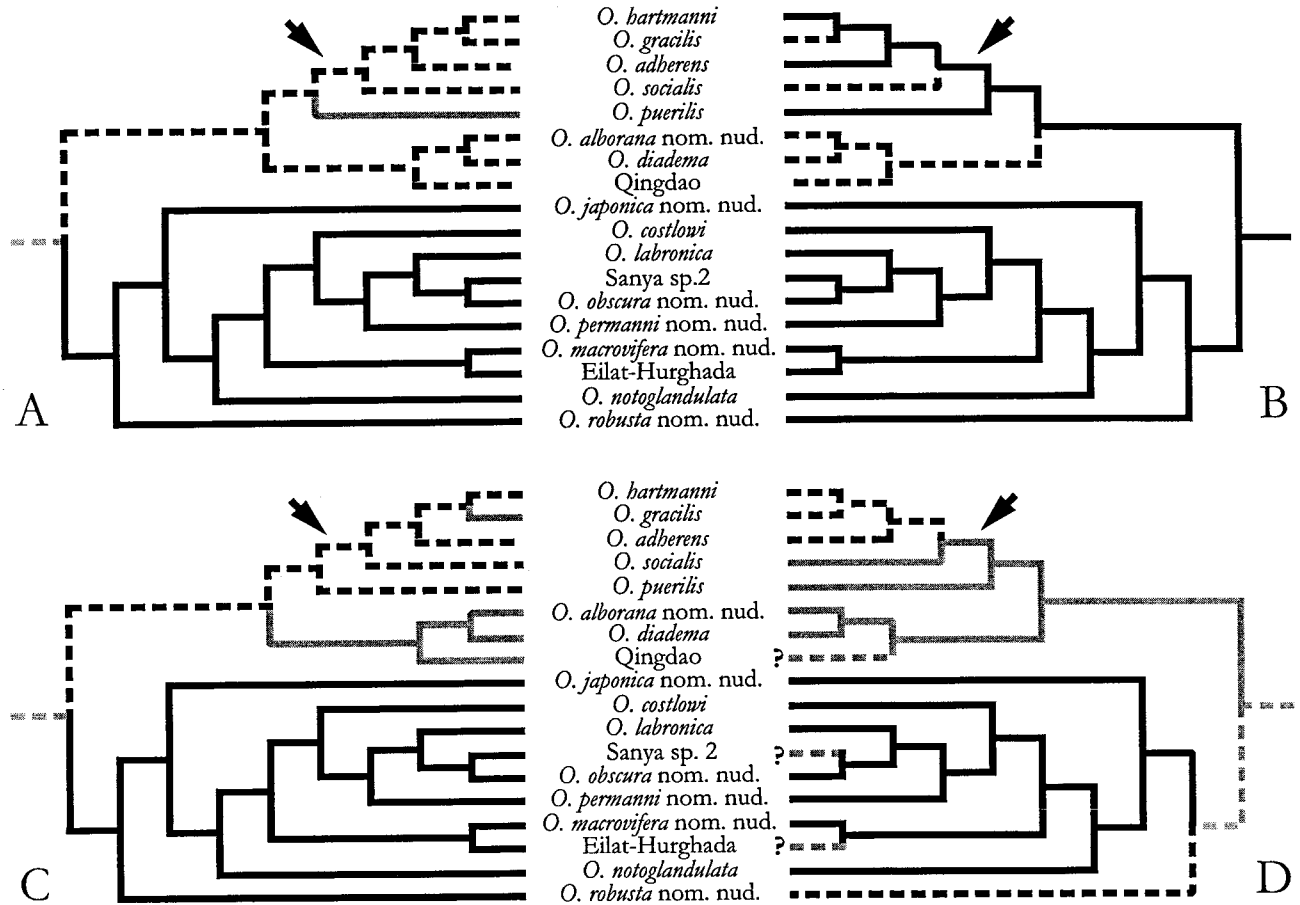
Figure 3. Character transformation hypothesis. Alternative rooting as suggested by parsimony analysis is indicated by an arrow. Dashed gray line depicts equivocal or unknown taxon state. (A) Sex strategy. Full line = gonochorism; dashed line = simultaneous hermaphroditism; gray line = sequential hermaphroditism. (B) Jaws. Full line = P and K-type of jaws; dashed line = presence of P-type only. (C) Shape of egg mass. Full line = tubular; gray line = fusiform; dashed line = irregular. (D) Diploid number of chromosomes. Full line = 6; gray line = 8; dashed line = 10.

and parsimony methods (Fig. 2B). Topologies from the analyses restricted to the ingroup taxa were in overall agreement with the ML and parsimony tree of the first dataset. The problem with alternative hypotheses for placement of the root may be caused by a long branch phenomenon (*e.g.,* Hendy and Penny, 1989) and cannot be conclusively resolved with the data at hand. The parsimony analysis suggests a placement of the root between the (*O. hartmanni, O. gracilis, O. adherens, O. socialis*) clade and the rest of the tree (Fig. 2A), while ML indicates a rooting between the clade of hermaphroditic species and the gonochristic species clade (Fig. 1A). However, contrary to parsimony, ML methods account for branch-length information and should give a better estimate when the model is accurate (Swofford *et al.,* 1996). Therefore, the discussion below focuses on the ML tree that included 18 ingroup and 5 outgroup taxa.

Figure 1A shows considerable congruence with Pleijel and Eide's (1996) results from an analysis employing mor-

phological, sex strategy, and protein data. Clades supported by both sets of data are indicated with the highlighted nodes in the mtDNA tree (Fig. 1A). However, the topologies differ on the internal branching of *Ophryotrocha* and, possibly, on the placement of the root. The mtDNA ML data gives some support for a deep subdivision in two major clades, but no such subdivision is suggested by Pleijel and Eide (1996). Recent fossil evidence further suggests that *Ophryotrocha* is an old lineage (Eriksson and Lindström, 2000).

The evolution of sex strategies is a debated subject (*e.g.,* Ghiselin, 1969; Charnov, 1982; Maynard Smith, 1982; Hurst, 1992). Our analyses, based on species that are sequential (1) or simultaneous (7) hermaphrodites, and gonochorists (10), suggest that, regardless of the placement of the root, the change from one strategy to the other has taken place only once within the group (Fig. 3A). The ancestral state is, given the available data, ambiguous. The sequential hermaphrodite *O. puerilis* is able to switch sex several times

during life, a feature that is rare among metazoans. Using *O. puerilis* as a model, Premoli and Sella (1995) discussed the ecological constraints necessary for an evolution from sequential to simultaneous hermaphroditism. Our data instead suggest that an evolution in the opposite direction, from simultaneous to sequential hermaphrodites, is more probable within *Ophryotrocha.* Such a scenario is also hinted at by A. Berglund, who—according to Premoli and Sella (1995)—commented that *O. puerilis* is "a modified simultaneous hermaphrodite in which a reversible mechanism of temporal inhibition of one of the two sexual phases has evolved." The problem of whether gonochorism or hermaphroditism is the ancestral state was also thoroughly discussed by Sella and Ramella (1999). However, they did not take up a definite position.

In addition to reproduction, jaw morphology has been used to understand *Ophryotrocha* relationships. The hindmost pair of maxillary plates in *Ophryotrocha* species can be of two distinct types. Whereas the P-type has a distal row of fang-like denticles, the K-type plates are distally smooth but often of a robust construction (Hartmann and Huth, 1936). Whereas a P-type jaw is found in larvae and juveniles of all species, the character state for adult worms is either P or K (*e.g.,* Ockelmann and Åkesson, 1990). The terminology emanates from the German words "kompliziert" (K-type) and "primitiv" (P-type). Based on reproduction strategy and jaw morphology, Åkesson (1973, 1984) identified the "*labronica,*" the "*hartmanni,*" and the "*gracilis*" groups within *Ophryotrocha.* The "*labronica*" group of sibling species consists of gonochorists with the K-type of jaws; it is well supported by the analysis presented here and represents clade B in Figure 1A. The "*hartmanni*" and "*gracilis*" groups comprise hermaphroditic species. Species belonging to the former group are distinguished by, among other characters, spawning an entirely soft, irregularly shaped egg mass, and the presence of K-type jaws (Ockelmann and Åkesson, 1990). In contrast, the "*gracilis*" group spawns a fusiform egg mass with a hard protective outer layer, and carries P-type jaws only. The monophyly of the two groups of hermaphrodites, "*gracilis*" and "*hartmanni,*" are, however, not validated by the present analysis. On the contrary, the characters "shape of egg mass" and "type of jaws" are homoplasious in all our trees, irrespective of the phylogenetic optimization used (*i.e.,* ML or parsimony) and the placement of the root (Fig. 3B and C). Therefore, in our trees *O. gracilis* is no longer a member of the "*gracilis*" group despite the close points of similarity in reproductive traits and morphology between this species and other members of the group (Ockelmann and Åkesson, 1990; Pleijel and Eide, 1996). A more extensive study seems to be justified.

Cytology and karyology have been extensively studied for a variety of species. Diploid numbers of chromosomes are known for 18 species (Åkesson, 1984; Robotti *et al.,*

1991; Shaojie and Knowles, 1992) that have 2n = 6, 2n = 8 or 2n = 10. The genome size (measured as picograms of DNA per cell) in 10 studied forms was discontinuously distributed between 0.4 pg (8 taxa), 0.8 (1 taxon) and 1.16 pg (1 taxon) (Sella *et al.,* 1993; Soldi *et al.,* 1994; Gambi *et al.,* 1997). The apparent discontinuous distribution of genome size (*i.e.,* ≈0.4, ≈0.8, or ≈1.2) was interpreted as an indication that large parts of the genome are acquired simultaneously (Sella *et al.,* 1993). The increments in genome size, however, do not correspond to increased numbers of chromosomes (Gambi *et al.,* 1997). The position of chromosomal nucleolar organizer regions (NOR) has been characterized and found to be highly polymorphic not only within the genus but also within most of the species (Sella *et al.,* 1995; Vitturi *et al.,* 2000). On the basis of inferred low GC contents and only one pair of NOR carrying chromosomes (studied by fluorescent *in situ* hybridization), Vitturi *et al.* (2000) suggested that *O. robusta,* with 2n = 10 and a small genome size (0.4 pg), is pleisiomorphic within the group. A basal position of *O. robusta* within the "*labronica*" group is corroborated by the mtDNA data (Fig. 1). Unfortunately, since *O. rubusta* is the only studied species with this combination of characters, it is impossible to tell if this is an autapomorphy or a sympeisiomorphy.

To summarize, this study presents the first mtDNA gene tree for *Ophryotrocha* species. Examination of the tree, which provides independent data for evaluating the evolution of reproductive strategies, leads us to suggest that hermaphroditism or gonochorism evolved once within studied *Ophryotrocha* taxa and that sequential hermaphroditism evolved from simultaneous hermaphroditism.

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Appendix

Checklist of described *Ophryotrocha* species with original localities

O. adherens Paavo, Bailey-Brock & Åkesson, 2000. Cyprus and Hawai, littoral.

O. akessoni Blake, 1985. Galapagos Rift, East Pacific Basin, deep sea.

O. atlantica Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. baccii Parenti, 1961. Roscoff, France, littoral.

O. bifida Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. claparedii Studer, 1878. Kerguelen, littoral.

O. costlowi Åkesson, 1978. Beaufort, North Carolina, littoral.

O. cosmetandra Oug, 1990. Northern Norway, littoral.

O. diadema Åkesson, 1976. Los Angeles harbor, littoral.

O. dimorphica Zavarzina & Tzetlin, 1986. Peter the Great Bay, littoral.

O. dubia Harmann-Schröder, 1974. North Sea (off Scotland), 68 m.

O. gerlachi Hartmann-Schröder, 1974. North Sea (off Denmark), 52 m.

O. geryonicola (Esmark, 1874). Skagerack, Kattegat, sublittoral.

O. globopalpata Blake & Hilbig, 1990. Juan de Fuca Ridge, deep sea.

O. gracilis Huth, 1933. Helgoland, Germany, littoral.

O. hadalis Jumars, 1974. Aleutian Trench, deep sea.

O. hartmanni Huth, 1933. NE Atlantic, littoral.

O. irinae Tzetlin, 1980. Kandalaksha Bay, White Sea, littoral.

O. kagoshimaensis Miura, 1997. Kagoshima Bay, Japan, 197 m.

O. labidion Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. labronica La Greca & Bacci, 1962. Naples, Italy, littoral.

O. lipscombae Lu & Fauchald, 2000. NW Atlantic, slope depths.

O. littoralis (Levinsen, 1879). Egesminde, Greenland, littoral.

O. lobifera Oug, 1978. West Norway, in mud, 50 m.

O. longidentata Josefson, 1975. Skagerack, Kattegat, 50–100 m.

O. maciolekae Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. maculata Åkesson, 1973. Skagerack, Kattegat, 25 m.

O. mandibulata Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. mediterranea Martin, Abelló & Cartes, 1991. Mediterranean, parasitic, 600–1800 m.

O. minuta Levi, 1954. Roscoff, France, littoral.

O. natans Pfannenstiel, 1975. Red Sea, littoral.

O. notialis (Ehlers, 1908). Southern South America, sublittoral.

O. notoglandulata Pfannenstiel, 1972. Japan, littoral.

O. obtusa Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. pachysoma Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. paralbidion Hilbig & Blake, 1991. NW Atlantic, slope depths.

O. platykephale Blake, 1985. Guayamas Basin, hydrothermal vents, deep sea.

O. puerilis puerilis Claparède & Mecznikow, 1869. Naples, Italy, littoral.

O. puerilis siberti (McIntosh, 1885). Plymouth, England, littoral.

O. scarlatoi Averincev, 1989. Franz Josefs Land, littoral.

O. schubravyi Tzetlin, 1980. Marine aquarium in Moscow, Russia.

O. socialis Ockelmann & Åkesson, 1990. Marine aquarium in Helsingör, Denmark.

O. spatula Fournier & Conlan, 1994. Arctic Canada, littoral.

O. vivipara Banse, 1963. San Juan Archipelago, USA. 22 m.

O. wubaolingi Miura, 1997. Kagoshima Bay, Japan, 200 m.