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Comparison of articulate brachiopod nuclear and mitochondrial gene trees leads to a clade-based redefinition of protostomes (Protostomozoa) and deuterostomes (Deuterostomozoa)

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Nuclear and mtDNA sequences from selected short-looped terebratuloid (terebratulacean) articulate brachiopods yield congruent and genetically independent phylogenetic reconstructions by parsimony, neighbour-joining and maximum likelihood methods, suggesting that both sources of data are reliable guides to brachiopod species phylogeny. The present-day genealogical relationships and geographical distributions of the tested terebratuloid brachiopods are consistent with a tethyan dispersal and subsequent radiation. Concordance of nuclear and mitochondrial gene phylogenies reinforces previous indications that articulate brachiopods, inarticulate brachiopods, phoronids and ectoprocts cluster with other organisms generally regarded as protostomes. Since ontogeny and morphology in brachiopods, ectoprocts and phoronids depart in important respects from those features supposedly diagnostic of protostomes, this demonstrates that the operational definition of protostomy by the usual ontological characters must be misleading or unreliable. New, molecular, operational definitions are proposed to replace the traditional criteria for the recognition of protostomes and deuterostomes, and the clade-based terms 'Protostomozoa' and 'Deuterostomozoa' are proposed to replace the existing terms 'Protostomia' and 'Deuterostomia'.

Keywords: brachiopod; protostome; phoronid; deuterostome; ectoproct; Protostomozoa

1. INTRODUCTION

We recently reported analyses of DNA sequences representing the nuclear-encoded small subunit ribosomal RNAs (hereafter nDNA) from a taxonomically representative selection of articulate and inarticulate brachiopods, together with homologous sequences from a number of phoronids and a few (taxonomically unrepresentative) ectoprocts (Cohen & Gawthrop 1996; Cohen & Gawthrop 1997; Cohen *et al*. 1998). One important conclusion of these studies was that, with minor exceptions, the resulting brachiopod nDNA gene tree is broadly concordant with the largely shell morphology-based taxonomy of the group (Williams 1997), although for some clades, branch-lengths were short and support indices were relatively low. We also confirmed that brachiopods, phoronids and ectoprocts cluster with undoubted protostomes such as molluscs and annelids, not with deuterostomes. This result is contrary to much zoological opinion about these lophophorates (e.g. Brusca & Brusca 1990; Nielsen 1991, 1995; Eernisse *et al*. 1992), but consistent with earlier conclusions from molecular analyses (Field *et al*. 1988; Halanych *et al*. 1995). (Pterobranchs, a fourth lophophorate group, do cluster amongst deuterostomes (Halanych 1995)). Because acceptance of the protostome affinity of most lophophorates has far-reaching implications, it needs to be confirmed by independent, molecular evidence. In this paper we provide such evidence, using trees based on a segment of the small subunit ribosomal RNA gene from mitochondrial DNA (mtDNA) of selected articulate brachiopods and both protostome and deuterostome outgroups. The new data confirm both the nDNA phylogeny and the protostome affinity of articulate brachiopods and, by inference, of inarticulate brachiopods, phoronids and ectoprocts. That these lophophorate phyla are genealogically allied to undoubted protostomes implies that traditional morphological criteria for the diagnosis of protostomes and deuterostomes are unreliable when applied to lophophorates. Yet protostomes and deuterostomes appear to be genuine, apparently monophyletic, supra-phylum aggregates. We therefore propose molecular, clade-based diagnoses and new names for these groups.

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Table 1. *Specimens and mtDNA sequences*

(Details of nDNA sequences have been reported (Cohen *et al*. 1998). Taxon description abbreviations: (ingroups) AB=articulate brachiopods; lophophore support (loop) types: C=cancellothyrid; R=rhynchonellid; S=short. (Outgroups) P=protostome; D=deuterostome.)

taxon description	binomial	Glasgow accession numbers	GenBank accession number	reference or collector (locality)
ingroup				
AB, S	*Abyssothyris* sp.	D1181	AF034220	(Cohen *et al*. 1998)
AB, C	*Cancellothyris hedleyi*	D1150	AF034230	(Cohen *et al*. 1998)
AB, S	*Dyscolia* sp.	D1219	AF034221	(Cohen *et al*. 1998)
AB, S	*Gryphus vitreus*	D521, 525	AF034222 AF034223	(Cohen *et al*. 1998)
AB, S	*Liothyrella neozelanica*	DNZ289, 290	AF034227 AF034228	(Cohen *et al*. 1998)
AB, S	*Liothyrella uva*	D930, 1024	AF034225 AF034226	(Cohen *et al*. 1998)
AB, R	*Notosaria nigricans*	DNZ100	AF034235	(Cohen *et al*. 1998)
AB, S	*Stenosarina crosnieri*	D1163	AF034229	(Cohen *et al*. 1998)
AB, C	*Terebratulina retusa*	D677, 678	AF034231 AF034232	(Cohen *et al*. 1998)
AB, C	*Terebratulina septentrionalis*	D163, 164	AF034233 AF034234	G. B. Curry (Bay of Fundy, Newfoundland)
outgroup				
apodan, D	*Scolecomorphus* sp.	T7	—	J. A. Sheps (unpublished)
coelacanth, D	*Latimeria chalumnae*	—	M87534	(Zardoya & Meyer 1997)
frog, D	*Xenopus laevis*	—	M10217	(Stanley 1993)
lungfish, D	*Protopterus annectens*	—	M87535	(Zardoya & Meyer 1996)
centipede, P	*Allotheura* sp.	—	L02376	(Ballard *et al*. 1992)
chiton, P	*Ischnochiton australis*	—	L02388	(Ballard *et al*. 1992)
hemipteran, P	*Magicicada tredecim*	—	X97146	(Simon *et al*. 1996)
scorpion, P	*Liocheles waigiensis*	—	L02397	(Ballard *et al*. 1992)
spider, P	*Tetragnatha mandibulata*	—	U00118	(Croom & Gillespie 1991)

2. MATERIALS AND METHODS

(a) *Specimens and sequences*

Provenance, identification and taxonomy of the animals studied and GenBank accession numbers for the nDNA sequences have been reported (Cohen *et al*. 1998). Details relating to the mtDNA sequences are given in table 1.

(b) *Laboratory procedures*

Procedures for DNA isolation, polymerase chain reaction amplification, DNA sequencing and data-handling were as described (Cohen *et al*. 1998). The sequenced fragment of mtDNA was defined by the 'universal' gene primers L1091 and H1478 which amplify domain three of the mitochondrial small subunit ribosomal RNA gene (Kocher *et al*. 1989). Sequencing was assisted by taxon-specific internal primers.

(c) *Sequence alignment and masking*

The nDNA alignment and excluded sites have been described (Cohen *et al*. 1998). Newly determined mtDNA sequences were aligned manually with outgroup sequences from a published alignment, making use of the associated secondary structure model and conserved motifs (Hickson *et al*. 1996). To exclude ambiguously aligned or misaligned sites, two masks were constructed, guided by a GDE 50% consensus selection mask (Smith *et al*. 1994). Since alignment difficulties were largely confined to phylogenetically distant taxa, one mask, used only for analyses of the ingroup plus the chiton outgroup, excluded as few sites as possible (25 out of 426), leaving 401, of which 101 were parsimony-informative, whereas the second mask, used for analyses of the ingroup plus multiple, more distant, outgroups excluded 100 potentially misaligned sites, leaving 316 of which 160 were parsimony-informative. The mtDNA sequence alignment and masks are available on request from the author for correspondence or from the EMBL alignment database, accession number DS32096. The EMBL alignment database may be accessed from the EBI FTP server by anonymous FTP from `ftp.ebi.ac.uk` in the directory `/pub/databases/embl/align` or from the EBI WWW server: (`URL ftp://ftp.ebi.ac.uk/pub/databases/embl/align/`).

(d) *Phylogenetic analysis*

The nDNA alignment was tested for adequacy of phylogenetic information-content using Paup* (Swofford 1997) by plotting the distribution of 10 000 random trees, with calculation of g_1 (Hillis & Huelsenbeck 1992) and by the proportion of unresolved maximum likelihood quartets reported by PUZZLE 3.1 (Strimmer & von Haeseler 1996). Maximum parsimony (MP) and weighted parsimony (WP; equivalent to successive approximation (Farris 1969)) analyses of the nDNA alignment were made using the exhaustive search procedure. For WP, characters were reweighted according to the best fit of the rescaled consistency index (RCI, baseweight=1). Three cycles of reweighting–searching led to stable results. WP bootstrap resampling consensus trees were obtained by branch-and-bound searches with furthest taxon addition. Jackknife replication was performed with default and $1/e$ (Jac) resampling (Farris *et al*. 1996; Swofford 1997), but since the results were similar to those of bootstrapping, they are not reported. Procedures for phylogenetic analysis of mtDNA sequences were similar to those used for nDNA, except that branch-and-bound or heuristic searches were used, depending on the numbers of taxa. The use of exhaustive and branch-and-bound searches ensured that the most parsimonious trees were found. Neighbour-joining (NJ) trees and NJ bootstrap consensus trees were constructed using both Kimura two-parameter (Kimura 1980) and LogDet (Lockhart *et al*. 1994) distances. Maximum

likelihood (ML) analyses was performed in Paup* using heuristic search with the 2ST (HKY) and 6ST models, with all available parameters estimated from the data, and ML bootstrap trees were obtained by NJ analysis with ML distances. Similar results were obtained using quartet puzzling with the HKY substitution model (Strimmer & von Haeseler 1996), and results with other models did not differ appreciably (not shown).

(e) *Secondary structure modelling*

For comparison with published secondary structure models, regions containing a well-defined complementary helical structure and intervening bulge and/or terminal loops were excised and complementary clamp sequences were added. The minimum-energy folded configurations of these regions were then determined using MULFOLD (Jaeger *et al*. 1989*a*,*b*; Zuker 1989; Zuker *et al*. 1991). Output ct files were visualized and converted to graphics files using loop-D-loop (Gilbert 1992).

3. RESULTS

(a) *Sequence reliability, alignment parameters and secondary structures*

Relevant parameters of the nDNA sequences have been reported (Cohen *et al*. 1998). Reliability of the newly determined mtDNA sequences is indicated by hierarchical concordance between sequences from conspecific, congeneric and confamilial groups, and by the presence (not shown) of conserved motifs and secondary structure diagnostic of domain three of mitochondrial small subunit ribosomal RNA gene sequences (Hickson *et al*. 1996). All sequences except those from *Liothyrella neozelanica* were completely determined from both strands, with multiple redundancy. The two exceptional sequences were less perfect near their ends and included a small number of undetermined sites. Mean (range) base composition of the mtDNA sequences from 16 articulate brachiopods did not differ (heterogeneity χ^2, $p>0.05$). A: 0.348 (0.333–0.361); C: 0.278 (0.250–0.296); G: 0.191 (0.175–0.206); T: 0.179 (0.154–0.193). Similarly, base compositions were stationary (heterogeneity χ^2, $p>0.05$) within the arthropod and deuterostome outgroups, between brachiopods and the deuterostome outgroups, and between brachiopods and some of the protostome outgroups. Other outgroup and ingroup–outgroup comparisons showed significant differences in base composition. These differences imply that LogDet and ML trees could be more reliable than those based on parsimony or other distances. However, since the resulting trees did not differ materially, base composition differences were not sufficient to cause false clustering.

The presence of strong non-random structure in the mtDNA ingroup plus chiton outgroup alignment was indicated by $g_1=-0.81$ ($p<0.01$) and by the low frequency of unresolved ML quartets (0–7% depending on numbers of taxa). Slight saturation of transition substitutions was seen in a plot of ingroup plus chiton outgroup Kimura pairwise distances, and strong saturation was evident when multiple, taxonomically distant outgroups were included (not shown).

(b) *Phylogenetic reconstructions of brachiopod phylogeny*

(i) *nDNA sequences*

The nDNA alignment contained sequences from ten taxa. These were a chiton, the closest lophotrochozoan protostome taxon for which both nDNA and mtDNA sequence was available (Halanych *et al*. 1995; Cohen *et al*. 1998), the rhynchonellid brachiopod *Notosaria* (a local outgroup), and eight sequences from morphologically short-looped terebratuloids. Two of these eight sequences (*Cancellothyris* and *Terebratulina*) were from genera which are grouped together on good morphological grounds in the superfamily Cancellothyridoidea (Cooper 1973; Williams 1997). From morphology, higher-level relationships of the other five genera are less clear. After removing parsimony-uninformative sites and seven potentially misaligned sites, 44 informative sites remained. An MP exhaustive search found four trees of length 67 steps (consistency index (CI) = 0.806, retention index (RI) = 0.79), differing in topology at the *Dyscolia–Liothyrella* and *Cancellothyris–Terebratulina* nodes. A WP exhaustive search found one tree (length = 42.7 steps, CI = 0.96, RI = 0.97). The topology of this tree was identical to that of the WP bootstrap tree and differed from distance and maximum likelihood trees only in the topology of the *Dyscolia–Liothyrella* node. Whereas in parsimony trees *L. uva* clustered (unexpectedly, from morphology) with *Dyscolia*, in NJ and ML trees the two *Liothyrella* species formed the expected clade, with a low NJ bootstrap value. To determine whether this difference in topology was meaningful, the shortest WP tree that contained the expected *Liothyrella* clade (43.7 steps, CI = 0.938, RI = 0.94) was obtained by a branch-and-bound search for non-minimal trees and compared with the minimal tree using Hasegawa–Kishino, Wilcoxon signed rank and winning-sites tests implemented in Paup*. The trees were not significantly different (Hasegawa–Kishino test, $p=0.33$; Wilcoxon test, $p=0.32$; winning-sites test, $p=1.0$). The cladogram in figure 1*a* is therefore drawn with the *Dyscolia–Liothyrella* clade as an unresolved trichotomy.

(ii) *mtDNA sequences*

Where available, multiple individuals from each taxon in the nDNA alignment were selected for sequencing, together with specimens of *Terebratulina septentrionalis* (Cohen *et al*. 1991, 1993), a species for which no matching nDNA sequence was available. Overall, the 15 articulate brachiopod sequences in the mtDNA alignment represented taxonomic levels from superfamily to species. Two alignments were used, differing in the number of outgroups. These were (i) the 15 brachiopods with a chiton outgroup and (ii) the same ingroup, with outgroups representing seven protostomes (three Lophotrochozoa (Halanych *et al*. 1995) and four Ecdysozoa (Aguinaldo *et al*. 1997)) and four deuterostomes (chordates). After exclusion of ambiguously aligned sites, there were 100 parsimony-informative sites in the smaller alignment and 160 in the larger one. As noted above, these alignments also differed in the number of sites deliberately excluded from analysis.

(iii) *Comparison of nDNA and mtDNA phylogenies*

An MP branch-and-bound search of the ingroup plus chiton mtDNA alignment gave two equally most parsimonious trees (length = 237 steps, CI = 0.675, RI = 0.776), differing only in topology of the *Dyscolia–Liothyrella* clade and consistent with parsimony, distance and ML

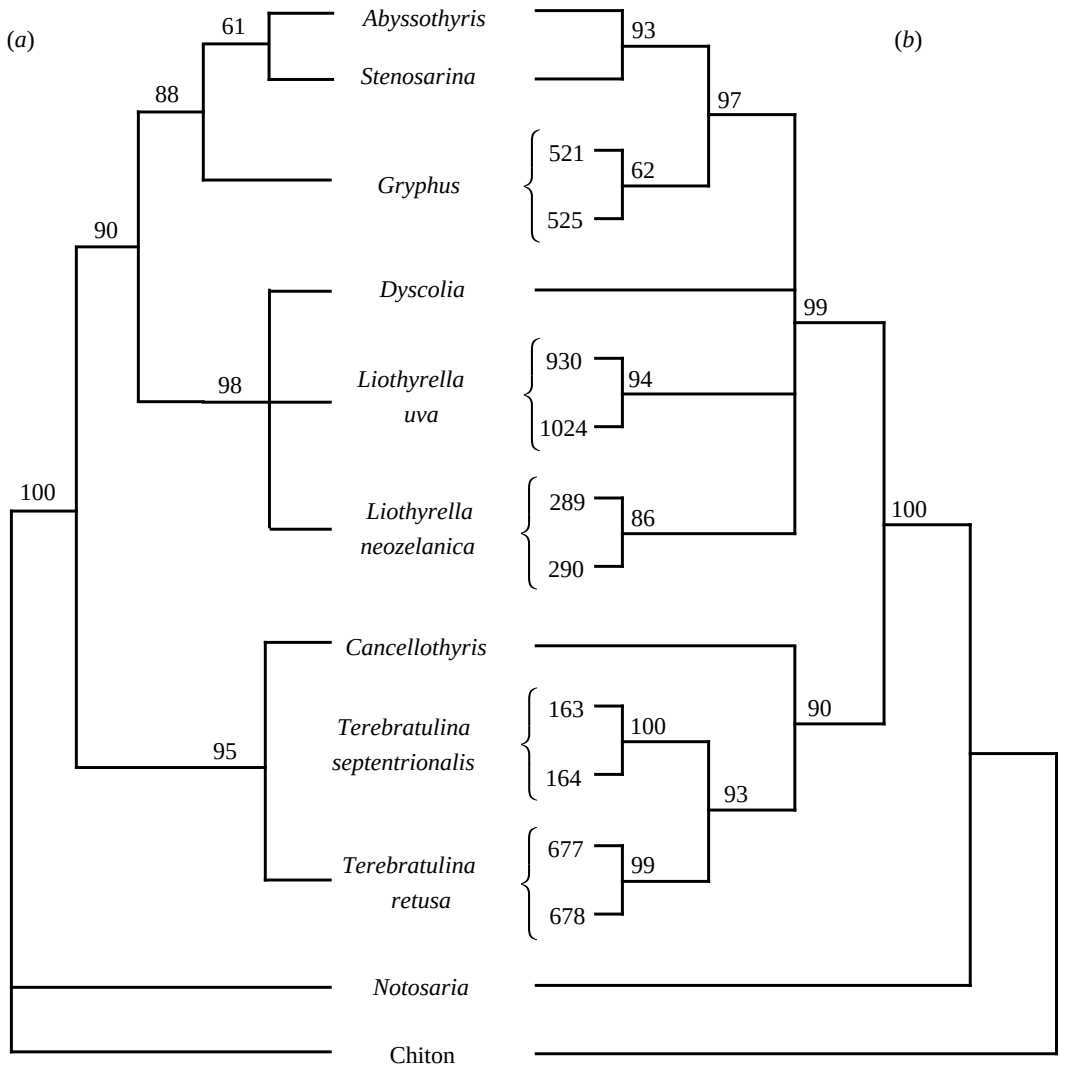

Figure 1. Comparison of nDNA and mtDNA parsimony bootstrap consensus trees. (Numbers adjacent to nodes show the frequency (%) with which the indicated clades occurred in the underlying trees. (*a*) 50% majority-rule consensus of branch and bound searches of 1000 bootstrap replicate nDNA data-sets, after RCI character reweighting. The *Dyscolia–Liothyrella* clade is drawn as a trichotomy for reasons given in the text. (*b*) 50% majority-rule consensus of branch and bound searches of 1000 bootstrap replicate mtDNA data-sets, with equal character weights.)

bootstrap consensus trees, in which this node formed a trichotomy. The parsimony bootstrap tree is compared with the corresponding nDNA tree in figure 1. These trees appear to be fully congruent with one another. Congruence of the underlying data was assessed by a partition homogeneity test, based on comparison of separate and combined tree lengths, with data-randomization (Farris *et al*. 1994). For this test nDNA and mtDNA sequences from the nine individual brachiopods from which both had been obtained (as in figure 1) were concatenated and designated as separate partitions. Using 1000 branch-and-bound replicates, this test found no incongruence in the data ($p=1.00$). Thus, the phylogenetic relationships discovered amongst short-looped brachiopods by analyses of nDNA were fully confirmed by similar analyses of mtDNA.

(iv) *Evidence from mtDNA for protostome affinity of brachiopods*

An MP heuristic search of the larger alignment with protostome and deuterostome outgroups gave 18 equally most parsimonious trees (length=611 steps, CI=0.507, RI=0.657). After reweighting, these reduced to one WP tree (length=180.86 steps, CI=0.614, RI=0.768) which was identical in topology to the WP bootstrap tree. ML and NJ distance and bootstrap consensus trees differed from one another and from the WP tree only by showing less resolution of the deepest nodes. Similar support values for ML tree nodes were also obtained by quartet puzzling (not shown). Since it would be unreasonable to expect fewer than 400 base pairs of mitochondrial sequence to accurately resolve all deep nodes, one of the less-resolved ML cladograms is shown in figure 2. Like all the other trees, this provides strong support for a clade comprising brachiopods, Lophotrochozoa and Ecdysozoa (i.e. undoubted protostomes), which is a sister-clade of the chordates (undoubted deuterostomes, here designated as outgroup). The absence of monophyletic lophotrochozoan and ecdysozoan clades may be due to a combination of the few taxa and small amount of sequence involved, together with nucleotide composition differences and

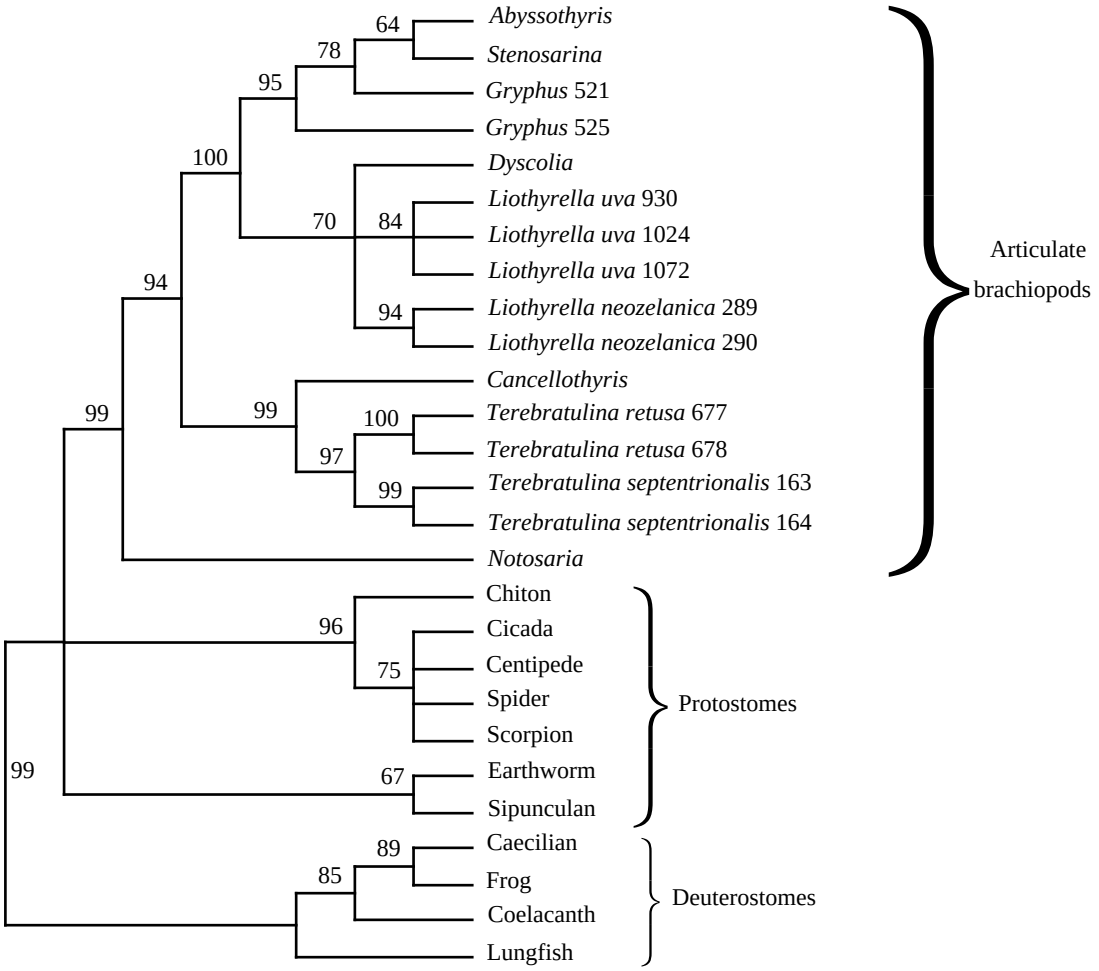

Figure 2. Maximum likelihood distance bootstrap consensus tree of mtDNA sequences with undoubted protostome and deuterostome outgroups. (50% majority-rule consensus of NJ trees built from 100 bootstrap replicate mtDNA data-sets using maximum likelihood distances calculated under the 2ST (HKY) model, with all available parameters estimated from the data. In exploratory analyses (not shown) the topology of ingroup-outgroup relationships was unaffected by omission of sites 1–77, which were present in the ingroup but absent from the outgroup sequences.)

residual ambiguity in alignment of the more phylogenetically distant outgroup sequences. Despite these potential difficulties, an ecdysozoan clade is clearly present.

4. DISCUSSION

(a) *Terebratuloid brachiopod phylogeny*

The systematics of fossil and Recent rhynchonellate brachiopods is based principally on the ontogeny and morphology of the brachidia (lophophore supports or 'loops'), and articulation-related shell characters (Williams 1965, 1997; Carlson 1995; Williams *et al*. 1996). Owing to a general lack of well-defined morphological characters, short-looped, terebratuloid forms ('this difficult group'; Cooper 1983, p. 35) offer the taxonomist particular problems, and recent developments in molecular systematics create the first prospect of resolving genealogical relationships within such groups. This prospect provided the main impetus for the present investigation for it was clear that the nDNA sequences then available from different, well-established terebratuloid genera differed by very few substitutions and predicted some relationships that were not morphologically expected (e.g. the sister-group relationships between, on the one hand *Gryphus* and *Abyssothyris–Stenosarina* and, on the other hand, *Dyscolia* and *Liothyrella*). Moreover, a morphologically well-defined subgroup of terebratuloids (cancellothyrids) whose origin dates at least from the Jurassic (Williams 1997), received only modest support in the nDNA tree. (The complete lack of support for this clade apparent in some analyses (Cohen *et al*. 1998) is now known to be an artefact, resulting from inclusion of one related but distant, and one related but highly imperfect, sequence.) Thus, sequence data from a more rapidly evolving genome region were needed to test these important, but relatively weak predictions of the nDNA gene tree. The nuclear and mitochondrial SSU gene trees compared in figure 1 and the underlying sequence data are clearly congruent. Thus, despite short branches and weak to moderate bootstrap support, all the terebratuloid clades predicted from analysis of nDNA sequences, including both a clade expected from morphology and unexpected clades (Cohen & Gawthrop 1996, 1997; Cohen *et al*. 1998), have been verified by the mtDNA results. Therefore, both nDNA and mtDNA gene trees appear to be reliable guides to the articulate brachiopod species tree.

Furthermore, these mtDNA data contain enough phylogenetic signal to resolve relationships between orders (Rhynchonellida: e.g. *Notosaria*; and Terebratulida: all other genera), super-families (Terebratuloidea, e.g. *Liothyrella* and Cancellothyridoidea, e.g. *Cancellothyris*), families (e.g. Tichosininae: *Stenosarina* and Gryphinae: *Gryphus*), confamilial genera and congeneric species. Thus, these data strongly suggest that 1000 or more nucleotides of mtDNA per taxon from a comprehensive selection of terebratuloids would provide a realistic and useful phylogeny of this difficult group. Such data would make sense of a rather featureless morphological landscape and would provide a sound basis for inferences about the biogeographic and plate tectonic correlates of articulate brachiopod evolution. For example, the superficially unexpected finding that *Gryphus*, a genus found today principally in the Mediterranean Sea, clusters with *Abyssothyris* and *Stenosarina* from the South Pacific but that the latter do not cluster with *Dyscolia* and *Liothyrella* from the same region may be consistent with the geologic record if the relatively short-lived planktonic larvae of terebratuloid, articulate brachiopods dispersed widely in Tethys, the circum-global tropical sea that opened in the Mid-Jurassic. Continental drift has now closed most of Tethys (except the Mediterranean) and north–south ocean basins have opened instead (Stanley 1993; Smith *et al*. 1994), creating dispersal barriers that could have triggered the phylogenetic radiation seen today. An ancestral Tethyan distribution would explain why *Stenosarina*, a genus originally described from the Caribbean but present also in the South Pacific (Cooper 1977; Laurin 1997), clusters with *Abyssothyris* from the South Pacific and with *Gryphus* from the Mediterranean and Atlantic. Perhaps dispersal in the ancient Tethys explains what would otherwise be a conundrum of modern biogeography. Clearly, a future, comprehensive molecular phylogeny of articulate brachiopods should reveal much about their historical biogeography.

(b) *Protostome versus deuterostome affinities of brachiopods, ectoprocts and phoronids*

The idea that there exist animal forms with distinct body plans (*Baupläne*) is a somewhat controversial corollary of the fact that metazoa are classified into separate phyla. Protostomes and deuterostomes (sometimes elevated to taxonomic ranks as Protostomia and Deuterostomia) are supra-phylum aggregates (Grobben 1908) that have been widely recognized amongst the metazoan phyla on the basis of mutually exclusive suites of ontogenetic characters. Whether brachiopods (together with ectoprocts, phoronids and pterobranchs) belong amongst protostomes or deuterostomes (or neither group) has long been a matter of debate, arising because they variously display mixtures of the supposedly diagnostic characters. Whereas many recent morphology-based reviews have favoured deuterostome affinities of these lophophorates (Brusca & Brusca 1990; Schram 1991; Eernisse *et al*. 1992; Nielsen *et al*. 1996), current molecular data unequivocally associate pterobranchs with other deuterostomes but place brachiopods, ectoprocts and phoronids with other protostomes (Field *et al*. 1988; Patterson 1989; Lake 1990; Halanych 1995; Halanych *et al*. 1995; Cohen & Gawthrop 1996, 1997; Conway Morris *et al*. 1996; Cohen *et al*. 1998). However, these molecular studies have so far been based exclusively on data from a single gene (strictly, a gene family), which specifies the nuclear-encoded small subunit of ribosomal RNA (nDNA). Although results from this gene are widely accepted as reliable, and to our knowledge no definitely misleading phylogeny based upon it has yet been recognized, the implications for the interpretation of ontogeny of the hypothesis that lophophorates 'are' protostomes are so far-reaching that it should receive independent confirmation before it is widely accepted. The mtDNA results illustrated in figure 2 appear to provide such confirmation. They show strong support for a clade uniting lophotrochozoan and ecdysozoan protostomes with articulate brachiopods, distinct from a clade containing undoubted deuterostomes. Thus, these mtDNA data appear to confirm the conclusion drawn from all previous nDNA studies, that brachiopods and other lophophorates (excluding pterobranchs) are genealogically, protostomes. The clarity of this result depends upon selection amongst available mtDNA (but not nDNA) sequences. Homologous nDNA sequences from the two echinoids *Psammechinus* and *Strongylocentrotus* cluster amongst deuterostomes as expected, but the small segment of mtDNA analysed here unexpectedly clusters amongst or adjacent to protostomes. A similar, surprising effect has been reported for sea urchin mitochondrial protein-coding sequences in a different context (Nei 1996; Russo *et al*. 1996). Whatever the significance of these anomalies, they do not affect the argument below, except possibly to raise a question over echinoderm genealogy.

Acceptance that brachiopods, ectoprocts and phoronids are genealogically allied to undoubted protostomes implies that, at least amongst lophophorates, the ontological features on which these assemblages have been defined (mode of coelom formation, embryological location of mouth and anus, etc.) have been misinterpreted or are misleading in at least some cases, perhaps due to convergence (Moore & Willmer 1997). Thus, operational definitions of the terms 'protostome' and 'deuterostome' based upon the usual ontogenetic characters cannot be relied upon: protostomes and deuterostomes as traditionally defined must be abandoned. If these categories are to have any future utility it must be on the basis of a clade-based taxonomy (de Querioz & Gauthier 1990, 1994) and clearly, at present, only operational definitions based on molecular phylogenetic analyses are available. Using such a definition, protostomes are animals that cluster in gene trees with other (undoubted) protostomes and are excluded from clusters comprising deuterostomes, whilst deuterostomes are defined *mutatis mutandis*. It is therefore opportune to replace the terms 'protostome' and 'deuterostome' by clade-based names (de Querioz & Gauthier 1990, 1994). Although 'Protostomia' and 'Deuterostomia' have been used in a related context (Valentine 1997), these terms predate the concept of clade-based names and we therefore suggest that the (admittedly ugly) neologisms 'Protostomozoa' and 'Deuterostomozoa' adequately combine historical continuity with phylogenetic principle and should henceforth be adopted. Retention of the reference to mouth position is unfortunate, but in the absence of other uniting characters this historical connection is justified as a useful mnemonic.

5. TAXONOMY

Protostomozoa

Etymology: from the Greek, *protos*, combining form, first; *stoma*, a mouth; *zoa*, combining form, plural of *zoion*, an animal.

Diagnosis: the last common ancestor of Lophotrochozoa, Ecdysozoa and all their included phyla, and all its descendants.

Deuterostomozoa

Etymology: from the Greek, *deuteros*, combining form, second; *stoma*, a mouth; *zoa*, combining form, plural of *zoion*, an animal.

Diagnosis: the last common ancestor of Echinodermata, Hemichordata, Chordata, and all its descendants.

The mtDNA amplification and sequencing were initiated and largely completed during the summer of 1993 by S.S., working in partial fulfilment of an MLA degree in the University of Pennsylvania under the supervision of C.W.T. S.S.'s laboratory work was planned and supervised by B.L.C. M.E.B. subsequently finished the mtDNA sequencing. The nDNA sequencing was done by A.B.G. and B.L.C. Phylogenetic analyses and manuscript preparation were undertaken (eventually!) by B.L.C., with geological input from C.W.T. We are grateful to Dr J. A. Sheps, Dr A. Williams, both of the University of Glasgow, and Dr P. Willmer, University of St Andrews, for critical reading of a draft manuscript, and to Dr D. L. Swofford, Smithsonian Institution, for permission to report results obtained with Paup* test versions d57 and d59. Work in Glasgow was supported by the UK Natural Environment Research Council (GR3/8708 and GST/02/832).

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