Phylogeny of the Apodan Holothurians (Echinodermata) inferred from morphology

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The Apodida is an order of littoral to deep-sea, largely infaunal sea cucumbers with about 270 extant species in 32 genera and three families, Synaptidae, Chiridotidae and Myriotrochidae. In this study, I perform the first phylogenetic test of the taxonomic and palaeontological hypotheses about evolutionary relationships within Apodida by using cladistic analyses of 34 morphological characters. I introduce several previously unconsidered synapomorphic characters, examine the relationships between all recognized suprageneric taxonomic groups and assess the assumptions of monophyly for each family. Maximum-parsimony analyses of type species from 14 genera and use of three rooting methods recovered identical topologies at the subordinal level and subfamily level within Synaptidae. Overall, the current higher-level classification of Apodida was well corroborated. Within Synaptidae, the relationships (Synaptinae, (Leptosynaptinae, Rynkatorpinae)) are well supported. The monophyly of Chiridotidae was not supported and appears paraphyletic at the subfamily level. Calibrating the phylogenetic hypothesis of Apodida against the fossil record indicated that most higher-level divergences occurred within the Palaeozoic, unlike that of extant non-holothuroid echinoderms, which radiated in the early Mesozoic. Synaptidae appears to have radiated during the Lower Cretaceous. Alternatively, and if one discounts the considerable ghost lineage duration that this hypothesis requires, they may have radiated during the Eocene. © 2001 The Linnean Society of London

ADDITIONAL KEY WORDS: holothuroids – Holothuroidea – Synaptidae – Chiridotidae – Myriotrochidae – morphology – sea cucumbers.

INTRODUCTION

Apodida is a group of littoral to deep-sea, largely infaunal holothuroid echinoderms. Apodans are unique among holothuroids in lacking tubefeet, papillae and radial water canals. Apodans are invariably vermiform with a thin, often transparent body wall and range in length from a few millimetres to more than 3 m, making them the largest echinoderms. They are distributed worldwide, with about 270 extant species in 32 genera and three families (Smiley, 1994; Smirnov, 1998). In this study, I make the first phylogenetic test of the taxonomic and palaeontological hypotheses about evolutionary relationships within Apodida by using cladistic analyses of morphological characters. I introduce several previously unconsidered, but important, characters, examine the relationships among

14 terminal taxa representing all currently recognized supergeneric groups and perform a preliminary test of the monophyly of apodan families.

TAXONOMY

The taxonomic history of the apodans has been reviewed most recently by Smirnov (1998). Briefly, Östergren (1898) divided the group into three subfamily-level groups that largely correspond to the present family designations. His classification was modified by Clark (1907) and by Heding (1928, 1929, 1931, 1935), who described numerous new species and introduced important suprageneric distinctions. Several more genera have since been described, notably by Belyaev & Mironov (1980) and Gage & Billett (1986). More recently, Smirnov (1989) revised Synaptidae to include fossil taxa with the stated aim of having the group's taxonomy reflect evolutionary relationships. Smirnov (1998) soon expanded this goal to include the entire

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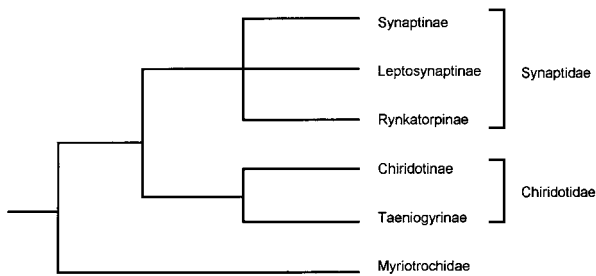
Figure 1. Phylogenetic relationships implied by the taxonomic scheme of Smirnov (1998).

Table 1. Taxa used in this study

Suborder Synaptina Smirnov, 1998
  Family Synaptidae Burmeister, 1837
    Subfamily Synaptinae Burmeister, 1837
      Synapta maculata (Chamisso & Eysenhardt, 1821)
      Opheodesoma spectabilis Fisher, 1907
      Euapta godeffroyi (Semper, 1868)
    Subfamily Leptosynaptinae Smirnov, 1989
      Leptosynapta tenuis (Ayres, 1851)
      Labidoplax buskii (McIntosh, 1866)
    Subfamily Rynkatorpinae Smirnov, 1989
      Oestergrenia digitata Heding, 1931
      Protankyra bidentata (Woodward & Barrett, 1858)
  Family Chiridotidae Östergren, 1898
    Subfamily Chiridotinae Östergren, 1898
      Chiridota laevis (Fabricius, 1780)
      Polycheira rufescens (Brandt, 1835)
    Subfamily Taeniogyrinae Smirnov, 1998
      Taeniogyrus australianus (Stimpson, 1856)
      Trochodota purpurea (Lesson, 1890)
Suborder Myriotrochina Smirnov, 1998
  Family Myriotrochidae Théel, 1877
      Myriotrochus rinkii Steenstrup, 1851
      Acanthotrochus mirabilis Danielssen & Koren, 1879
      Trochoderma elegans Théel, 1877

higher-level taxonomy of Apodida, creating two suborders, Myriotrochina and Synaptina. The latter suborder unites two families, Chiridotidae and Synaptidae, based, prominently, on similarities in juvenile wheel ossicles. Within Chiridotidae, Smirnov (1998) designated the subfamilies Chiridotinae and Taeniogyrinae and, within the Synaptidae, the subfamilies Synaptinae, Leptosynaptinae and Rynkatorpinae. The second suborder Myriotrochina, in contrast, is monotypic, consisting of the formally unsubdivided family Myriotrochidae.

PHYLOGENY

The phylogenetic relationships implied by the above classification are shown in Figure 1. The family-level arrangement, first illustrated by Östergren (1907) and discussed by Frizzell & Exline (1966), finds support in the extensive palaeontological analysis of wheel ossicles by Gilliland (1993). The concordance of other aspects of the current taxonomy with phylogeny, however, remains untested. In addition, the branching order of subfamilies within Synaptidae is unresolved. Heding (1928) suggests dividing the family into two groups, the Synaptinae and the Leptosynaptinae + Rynkatorpinae, basing his decision on their form of coelomic excretory organs, the ciliary funnels. Alternatively, Semper (1868) and Smirnov (1998) suggest that the division is between the Synaptinae + Leptosynaptinae and the Rynkatorpinae. Here, the distinction is between membership in mutually exclusive sets of ossicle characters and their origination times in the stratigraphic record.

METHODS

INGROUP SELECTION AND ROOTING

Of the 32 currently recognized genera in the three families that constitute the extant Apodida (Smirnov, 1998), 14 genera represented by type species were included in this study (Table 1). The major limitation of this approach is clear: species exemplars must possess the plesiomorphies of the genera they purport to represent. Nevertheless, the explicitness of this approach was reasoned to be superior to the alternative of speculating on the ancestral states of genera. Some genera were excluded because they are little known or, I felt, clearly derived, e.g. the tiny, aspiculate forms *Achiridota*, *Rhabdomolgus* and *Anapta*. Several other genera, although well characterized, were excluded to maintain a high ratio of taxa to characters. Finally, inclusion of genera was based on the desire for multiple representatives from each family and subfamily to perform a preliminary test of the monophyly of these groups. Occasionally other species were used when the character of a genotype was poorly known.

The living proximate outgroup to Apodida is uncertain. Preliminary molecular and morphological analyses (Smith, 1997; Kerr & Kim, 1999) suggest that the closest extant group of holothuroids to apodans is Elasipodida. This and other extant holothuroid outgroups possess several attributes with which to polarize characters within Apodida. Similarly, the oldest unequivocal body fossils assignable to Apodida, Achistridae from as early as the Middle Devonian (Simms *et al.*, 1993), display some plesiomorphies with living apodans. Given these limitations, I explored three

rooting options: First, I rooted trees using a hypothetical ancestor ('ancestor rooting') by combining invariant soft-tissue characters from Elasipodida and the skeletal and gross morphological characters provided by the best-preserved achistrid body fossils, the Francis Creek *Achistrum* from the Middle Pennsylvanian (Sroka, 1988; Sroka & Blake, 1997). Second, to assess the effect of the potentially divergent characters of the living elasipodans, I rooted the tree using only the few skeletal and gross characters available from *Achistrum*. Third, I rooted trees between Myriotrochidae and Chiridotidae *a posteriori* ('mid-point rooting') on the basis of their extended fossil histories compared with that of Synaptidae. The former two clades appeared by at least the Late Permian, while wheel ossicles typical of larval Synaptidae do not occur until the Middle to Upper Triassic (Gilliland, 1993). This indicates that either Myriotrochidae or Chiridotidae is sister to Synaptidae and that the root lies between the two older families. This predicts that the longest branch, the one with the most unambiguous changes, will lie between the two more ancient groups.

PHYLOGENETIC ANALYSES AND CHARACTER SELECTION

Maximum-parsimony analyses of the data were performed using PAUP* 4.0b 2a (Swofford, 1998) with the following options: branch-and-bound search, multistate taxa treated as polymorphisms, zero-length branches collapsed, minimal length trees kept, the initial upper bound computed via stepwise addition and the furthest addition sequence used. I assessed data quality by bootstrapping using 500 replicates, as well as by assessing the skewness of the tree-length frequency distributions generated from 10^5 trees randomly produced from the data. Change of individual characters along branches was examined using MacClade 3.0 (Maddison & Maddison, 1992).

An annotated list of the characters used in this study is given below. A total of 34 discrete, equally weighted characters – 23 skeletal, 10 soft-tissue and one behavioural – were scored, including 29 binary and five unordered multistate characters (Table 2). Characters 17–25 were polarized where the states of ossicle characters in *Protankyra* are ancestral on the basis of their identical appearance to the Lower Cretaceous to Oligocene anchor plates of *Rigaudites* and to the Upper Jurassic to Miocene anchors of *Calcancora* (Frizzell & Exline, 1966; Smirnov, 1989). Unknown or logically impossible states were coded as '?' and treated as missing. In the few instances when the character state of the genotype exemplar was unknown, I coded the genus using a congeneric taxon and indicate the substitution in the following list. Characters 2, 8, 10, 17, 18, 19, 26, 31 and 32 were suggested by the taxonomic treatment of Smirnov (1998). Confirmation of these, as well as codings of the remaining characters, was largely achieved using published taxonomic descriptions, primarily Clark (1907), Heding (1928, 1929, 1931, 1935) and Gage & Billett (1986). When doubt still existed about the status of a character, I examined field-caught specimens or collections of extant and fossil material at the following museums: British Museum (Natural History), Museum of Comparative Zoology, National Museum of Natural History and Yale Peabody Museum of Natural History.

CHARACTERS

SOFT-TISSUE AND BEHAVIOURAL FEATURES

1. Maximum body length: 0 = less than 25 cm; 1 = 25 cm or more. Synapta in Synaptinae is the largest recorded echinoderm, reaching lengths of 3 m. Several other synaptines are also quite long, but nearly all other apodans are under 10 cm. The ancestor is scored as 0 because *Achistrum* reaches about 10–13 cm in length.

2. Tentacle morphology: 0 = digitate to peltatodigitate; 1 = pinnate with digits increasing in size proximo-distally; 2 = pinnate with largest digits circa mid-tentacle. Pinnate tentacles possess a conspicuous terminal digit which is lacking in otherwise somewhat similar digitate forms. In peltatodigitate tentacles, occurring in *Polycheira* and a few other genera, digits are arranged distally in a circle. This character is left unscored for the reconstructed outgroup. The tentacle form of the proximate fossil stem member used here, *Achistrum*, cannot be discerned from material examined by me, although may be preserved in other specimens (Sroka, 1988). The only fossil of a possible Palaeozoic stem member to Apodida preserving tentacles is *Palaeocucumaria* (Seilacher, 1961). Their tentacles appear to be without digits (Seilacher, 1961), hence autapomorphic and uninformative in this study. For the most closely related extant member used here, the Elasipodida, tentacles are peltate, and hence also autapomorphic.

3. Number of digits on tentacles: 0 = less than 20; 1 = 20 or more. The number of digits on a tentacle can range from none in *Rynkatorpa* or two in *Dactylapta* to more than 80 in *Synapta*.

4. Ocelli: 0 = absent; 1 = present. Synaptids possess ocelli or optic cups, small patches of pigmented cells at the base of their tentacles, that enclose photosensitive cells (Yamamoto & Yoshida, 1978). Ocelli are found in no other holothuroid, and hence the ancestor is scored as lacking this character.

Table 2. Character matrix for the apodan exemplars, a fossil outgroup *Achistrum* and a reconstructed ancestor. Missing or logically prohibited codings are indicated by '?'

Taxa	Characters						
	5	10	15	20	25	30	34
Synapta	12110	12110	00010	10111	10100	0????	2001
Euapta	12110	12110	10010	10110	01101	0????	2001
Opheodesoma	12110	12110	10010	10100	01101	0????	2001
Leptosynapta	01001	21100	10000	10001	01010	0????	2000
Labidoplax	01001	21100	10000	10001	01010	0????	2000
Oestergrenia	00011	20100	10000	11011	10010	0????	1000
Protankyra	00011	21100	00100	11011	11010	0????	1000
Chiridota	00000	12101	11000	0????	?????	11001	2000
Polycheira	10100	12101	11000	0????	?????	11001	2000
Taeniogyrus	00000	11101	10001	0????	?????	11001	0000
Trochodota	00000	10100	10001	0????	?????	11001	0000
Myriotrochus	00000	00000	00000	0????	?????	10102	2210
Acanthotrochus	00000	00000	00000	0????	?????	10110	2210
Trochoderma	00000	00000	00000	0????	?????	10110	2110
Achistrum	0??0?	????00	??0?1	0????	?????	0????	021?
Ancestor	0??00	00000	00001	0????	?????	0????	0210

5. Sensory cups: 0 = absent; 1 = present. Most non-synaptine synaptids display sensory cups or buds along the proximal base of the tentacles. They are innervated, interiorly ciliated cups and are presumably chemosensory (Clark, 1907). Sensory cups are found in no other holothuroid and the ancestor/outgroup is scored as lacking this character.

6. Ciliated cups: 0 = absent; 1 = small with similar shape; 2 = large cups, variously shaped. Ciliated cups and funnels or vibratile urns are small, numerous organs arranged along the insertion of the intestinal mesenteries with the body wall of Chiridotidae and Synaptidae. Their interiors are ciliated and appear to function in removing foreign particulates from the coelomic fluid (Jans & Jangoux, 1989). Ciliated cups are found in no other holothuroid and the ancestor/outgroup is scored as lacking this character.

7. Number of polian vesicles: 0 = always one; 1 = more than one. Polian vesicles are blind, round to elongate sacs extending from the water vascular ring into the coelom. They number from one in most apodans to as many as 50 or more in large synaptids such as *Synapta*. Elasipodans, the extant holothuroids used in the ancestor rooting, possess one polian vesicle.

8. Length of stone canal: 0 = short; 1 = long. The stone canal of apodans, like most holothuroids, terminates distally with an internal madrepore. This character is taken from Smirnov (1998) who reports that the stone canals of Myriotrochidae are relatively much shorter compared with body length than those in the other apodans.

9. Habit: 0 = obligately fossorial; 1 = epibenthic, at least facultatively, or inhabiting crevices. Nearly all apodans are burrowers. The major exceptions are the chiridotid *Polycheira*, which lives in crevices, and several, mostly large-sized, synaptid taxa, which are epibenthic or crevice dwelling. The hypothesized ancestor is considered fossorial on the basis of *Achistrum*'s similarity to non-apodan burrowing holothuroids with elongate, fusiform bodies and small tentacular rings.

OSSICLES

10. Body wall ossicles restricted to 'papillae': 0 = absent; 1 = present. In *Chiridota*, *Polycheira* and *Paradota* body-wall ossicles occur only in small clusters, usually adjacent to the radii. The ossicles of the fossil *Achistrum* are distributed throughout the integument.

11. Straight rods: 0 = absent; 1 = present. Short, straight to slightly curved rods – with their ends simply branched – are found in the tentacles of most apodans and, in a few taxa, the body wall.

12. C-shaped rods: 0 = absent; 1 = present. C-shaped rods are smaller than straight rods, occur in the body wall and differ from C-shaped rods found in non-apodan holothuroids. They have expanded, rounded,

knobbed, often flattened ends and occur only in some chiridotid genera.

13. Irregularly branched rods: 0 = absent; 1 = present. Irregular and multiply branched rods are found in the synaptid Apodida in *Protankyra* and two genera not used in this study, *Pendekaplectana* and *Polyplectana*. While phylogenetically uninformative in this study, this character is still a synapomorphy of groups within Apodida and is included to assist future work.

14. Miliary rosettes: 0 = absent; 1 = present. Tiny irregularly branched rosettes occur in the body wall and tentacles of four genera of Synaptinae.

15. Hooks: 0 = absent; 1 = present. Hooks occur in three living genera of chiridotids, *Taeniogyrus*, *Scoliorhapis* and *Trochodota*. An open loop or eye is formed by pronounced curvature of the ossicle at one end. The hooks of the fossil *Achistrum* differ from those of extant forms by the straighter shaft and a closed loop, sometimes with internal crossbars. Despite the differences between these two types, they are homologized here on the basis of similarly pointed ends, curvature of the shank, the way the eye is formed (Reich, 1999, pl. 1) and life positions in which the hooks can lie scattered in the body wall (Sroka, 1988).

16. Anchors and anchor plates: 0 = absent; 1 = present. Anchors and anchor plates occur in all spiculate genera of Synaptidae. The two types of ossicles are functionally related and invariably co-occur, so are considered as a single character. Anchors and their associated plates do not occur in *Achistrum*, which originated in the Devonian, and do not appear in the fossil record until the Middle Jurassic. The following nine characters (17–25) are derived from features of anchors or anchor plates and are scored as missing in taxa lacking these ossicles.

17. Development of anchor plate: 0 = rod primordium parallel to anchor shaft; 1 = perpendicular to anchor shaft. Smirnov (1998) divides Synaptidae into two groups on the basis of the development of the anchor plates. In Rynkatorpinae, the anchor plate arises from a rod-like element resting parallel to the shaft of the anchor. In Synaptinae and Leptosynaptinae, the plate primordium is positioned orthogonally to the anchor shaft.

18. Base of anchor plate: 0 = with a ledge; 1 = with a bridge. The base of the anchor plate articulates with the anchor keel via a flattened end ('ledge') or a raised crossbeam ('bridge').

19. Width of base of anchor plate: 0 = narrow; 1 = wide. In most synaptids, the base of the anchor plate is considerably narrowed. Only *Synapta* and *Protankyra* in this study possess plates with wide bases.

20. Holes in anchor plate: 0 = few, in a '6 + 1' pattern; 1 = many irregularly arranged. Anchor plates with a '6 + 1' pattern show six, occasionally seven, holes surrounding a central hole. Conversely, *Synapta* and Rynkatorpinae have plates with numerous holes.

21. Margin of anchor plate: 0 = smooth; 1 = spiny or irregular.

22. Margin of holes in anchor plate: 0 = smooth; 1 = dentate.

23. Vertex of anchor: 0 = smooth; 1 = with minute knobs.

24. Anchor arms: 0 = smooth; 1 = serrated. Synaptine synaptids possess smooth anchor arms; the remaining synaptids, serrated arms. However, *Epitomapta*, although not included in this study, is interesting in that it is polymorphic: according to the type description of Heding (1928), it has serrated anchors in the posterior of the body and smooth anchor arms anteriorly.

25. Keel of anchor: 0 = not branched; 1 = irregularly branched.

26. Wheel ossicles in adult: 0 = absent; 1 = present. Synaptids possess wheel ossicles only as larvae and juveniles (Mortensen, 1938; Pawson, 1971), while all other apodan holothuroids retain wheels in the body wall as adults. The similarities between synaptid larval wheels and those of adult chiridotids have been used to suggest a close relationship between the two families (Smirnov, 1998). However, too little comparative data exist on synaptid larvae for their wheel characters to be incorporated into this study. Currently, larvae and their ossicles are known from only three species in three genera of the 17 genera in Synaptidae (Semon, 1888; Mortensen, 1937, 1938). Examples of wheels from potentially other, but unidentified, synaptids are reported by Pawson (1971) and Inaba (1934). A potential coding, but not one adopted here, for the ancestor outgroup is presence of wheels based on their occurrence in Paleochiridotidae and *Theelia*, likely stem-member apodans from the Lower Mississippian that precede the occurrence of myriotrochid- and chiridotid-like wheels. The older wheels, however, cannot be entirely aligned with those of Recent taxa, but most

resemble those of taeniogyrine chiridotids, in having a sub-hexagonal outline, interior dentate rim and few spokes (Gilliland, 1993). The following four characters (27–30) are based on characteristics of wheels and are scored as missing in taxa lacking this type of ossicle.

27. Hub of wheel: 0=flat; 1=concave with a stellate superstructure. The wheel hub of myriotrochids is flat and either solid or multiperforate, while chiridotids have a star-shaped structure raised over an imperforate concave hub.

28. Number of spokes on wheel: 0=six; 1=more than six. Chiridotids possessing wheels invariably have the form with six spokes. Myriotrochids by contrast have wheels with from at least 8 to 16 spokes.

29. Outward-pointing teeth on wheel rim: 0=absent; 1=present. Outward-pointing teeth are absent in chiridotid and larval synaptid wheels and are variable among myriotrochid genera.

30. Inward-pointing teeth on wheel rim: 0=absent; 1= small denticles; 2=large teeth. The inner rims of wheel ossicles have numerous tiny teeth in chiridotids, but, among myriotrochid genera, teeth on the inner margin are either large or absent.

CALCAREOUS RING

31. Passage of radial nerve through radial plate of calcareous ring: 0=via an anterior groove; 1=via a perforation. All living apodans, with the exception of Taeniogyrinae, the myriotrochid *Achiridota* and synaptid *Epitomapta*, have perforate radial plates. The ancestral outgroup, represented by *Achistrum*, is coded as imperforate based on examination of museum specimens. These same rings appear similar to the 'Type 2' putative *Achistrum* calcareous rings pictured by Gilliland (1992).

32. Anterior projections on radial plates of calcareous ring: 0=absent or very low and blunt; 1=one projection on all radial plates; 2=two projections on dorsolateral radial plates. The central anterior projection of the radial plate serves as the insertion point for the radial muscles and separates adjacent tentacle ampullae. Long projections are found only in Myriotrochidae, several genera of which also possess two projections on their dorsolateral radials. This character in the reconstructed ancestor is coded as having one projection per plate. This is based on observations of *in situ* calcareous rings in *Achistrum* from the Middle Pennsylvanian. Note, however, that Gilliland (1992) found isolated radials from Lower Jurassic apodans,

evidently *Achistrum*, that occasionally carried two projections.

33. Position of fossae for tentacle ampullae: 0=anterior; 1=exterior. The calcareous ring is grooved to receive the tentacle ampullae, either on the anterior surface, as in Myriotrochidae, or on the exterior, as in the remaining apodans. Calcareous ring plates from *Achistrum* have anteriorly positioned tentacular fossae (Gilliland, 1992).

34. Connective tissue ring: 0=absent; 1=present. A well-developed ring of clear connective tissue extends posteriorly from the calcareous rings of synaptine synaptids. This feature is found in no other Recent holothuroids and is coded as absent in the ancestor outgroup.

RESULTS

The parsimony analyses under all rooting schemes give the same 50% majority rule consensus tree. Ancestor rooting produced six most parsimonious trees, a consensus of which is presented in Figure 2. This tree has a length 50 steps, a consistency index (CI) of 0.76, rescaled CI (RC) of 0.65, retention index (RI) of 0.86. Rooting the tree on just the fossil characters of *Achistrum* recovered nine most parsimonious trees. The shortest trees are 52 steps long and each has a CI of 0.75, an RC of 0.63 and an RI of 0.85. Rooting on the longest branch (with eight unambiguous changes) yielded a single most parsimonious tree identical in topology to that of the ingroup from the other rooting procedures. This tree has a length of 47, a CI of 0.81, an RC of 0.72 and an RI of 0.88. The frequency distributions of tree lengths in all analyses were highly left skewed, with g_1 scores (i.e. the distributions' third moments) of -0.82, -0.84 and -0.84 respectively, suggesting considerable 'hierarchical' signal in the data sets (Hillis & Huelsenbeck, 1992). The ancestral rooting gave a topology identical to, and descriptive statistics nearly identical to, the topologies recovered using *Achistrum* or long-branch rooting. Hence, the following discussion is restricted to the tree founded on a reconstructed ancestor, except where notable differences between the analyses occur.

There is strong support via bootstrapping and the number of unambiguous changes in character states for Myriotrochidae as sister to Chiridotidae+Synaptidae. The latter two clades are joined by seven synapomorphies: gain of ciliated cups, increased length of the stone canal, presence of rod ossicles, gain of a complex wheel hub, inward-pointing teeth on the wheel rim, loss of anterior projections on the calcareous ring and the switch to exterior tentacular ampullae. The
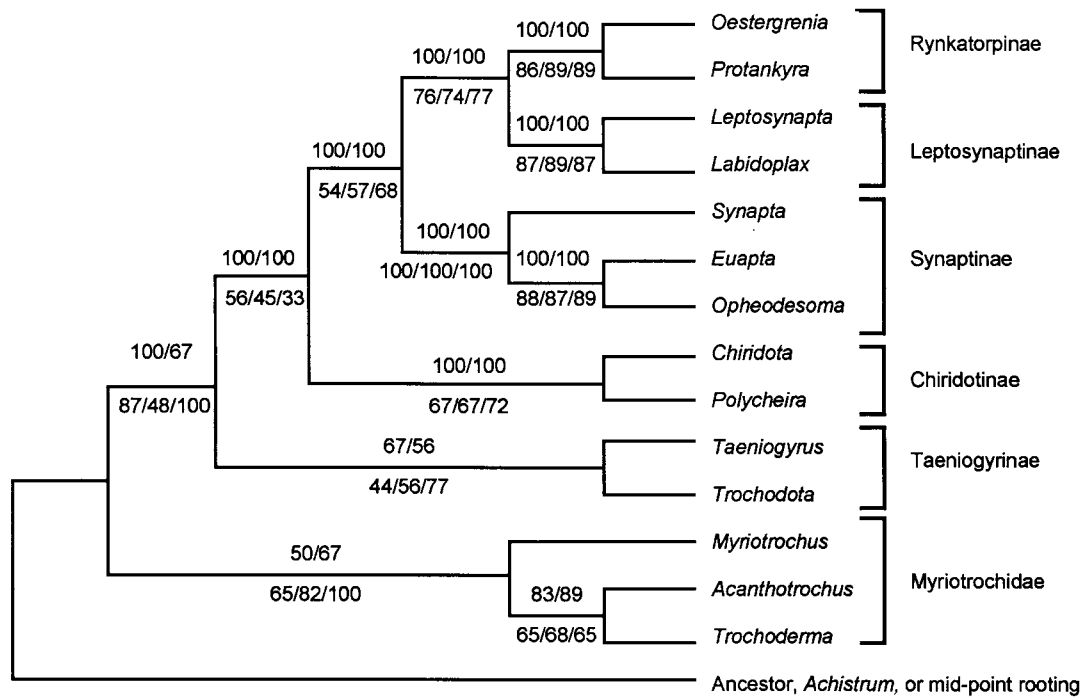
Figure 2. Topology of majority rule consensus trees, for ancestral and *Achistrum* rootings, and topology of single most parsimonious tree, using mid-point rooting. Numbers above branches indicate percentage of trees in which branch appears for ancestral and for *Achistrum* rootings. Numbers below branches indicate supporting percentages of 500 bootstrap samples for ancestral, *Achistrum*, and mid-point rootings.
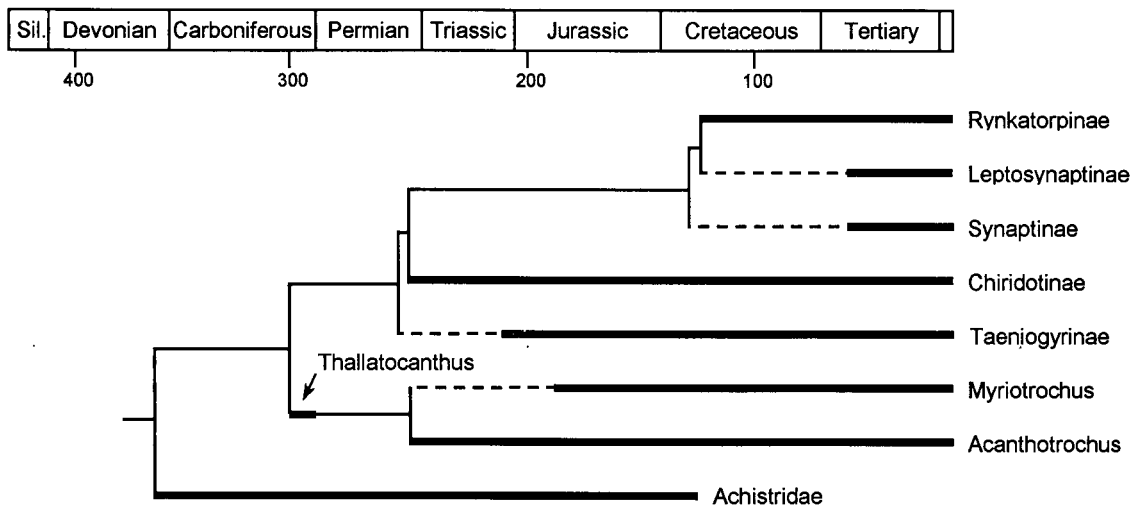

Figure 3. Phylogeny of Apodida inferred from the morphological analyses (Fig. 2) and the stratigraphic record of oldest fossil representatives for the included groups. Thin solid lines indicate phylogeny based on the present study; thick solid lines indicate range from the earliest reliable occurrences of fossil members to the latest ones or present; thin dashed lines indicate potential range extensions. *Thallatocanthus* is used to anchor the deepest apodan divergence *sensu* Gilliland (1993).

Table 3. Earliest stratigraphic occurrences of apodan taxa

Taxon	Fossil representative	Earliest record	Age (Mya)	Reference
Rynkatorpinae	*Rigaudites*	Lower Cretaceous	144.2	Smirnov (1989)
Leptosynaptinae	*Synaptites*	Tertiary (Eocene)	56.5	Smirnov (1989)
Synaptinae	*Croneisites*	Tertiary (Eocene)	56.5	Smirnov (1989)
Chiridotinae	*Theelia multiplex*	Middle Triassic (Ladinian)	234.3	Gilliland (1993)
Taeniogyrinae	*Theelia koeveskelensis*	Upper Triassic (Carnian)	227.4	Gilliland (1993)
Myriotrochus	*Hemisphaeranthos*	Lower Jurassic (Hettangian)	205.7	Gilliland (1993)
Acanthotrochus	*Acanthotheelia*	Upper Permian (Dzhulfian)	256.0	Gilliland (1993)
Achistridae	*Porachistrum*	Middle Devonian (Givetian)	380.8	Gilliland (1993)
Stem Myriotrochidae	*Thallatocanthus*	Middle Pennsylvanian	310.0	Gilliland (1993)

Myriotrochidae is supported by one unambiguous character, an increase in the number of wheel spokes. Less, but still moderate, bootstrap support is found for a clade of Chiridotinae + Synaptidae. This node is subtended by a single character, increase from one to several polian vesicles. The Taeniogyrinae possesses two unambiguous synapomorphies, gain of sigmoid hooks and the loss of perforate radial plates. The Synaptidae received only moderate bootstrap support and is identified by three unambiguous characters, the gain of optic cups, anchors plus anchor plates and the loss of wheel ossicles in the adult. The Chiridotinae are diagnosed by having C-shaped rods. Within Synaptidae, the Synaptinae is the best supported group. This clade is identified by eight unambiguous synapomorphies; they are increased body size, pinnate tentacles with large central digits, rosette ossicles, a cartilagenous ring, epibenthic habit and three features found on the anchor and anchor plate. The Leptosynaptidae + Rynkatorpinae are also well delimited by four features, gain of sensory cups, large ciliated cups, one polian vesicle and serrated arms on anchor ossicles. Both Leptosynaptidae and Rynkatorpinae are well supported by bootstrapping and are identified by three and two unambiguous character changes respectively.

DISCUSSION

TAXONOMIC IMPLICATIONS

The results of this study constitute the first phylogenetic test of the classification of Apodida. Smirnov (1998) recently modified subordinal- and subfamily-level designations, introducing several new groups with the intent of delimiting evolutionary units within Apodida. He recommends subdividing the apodans into two suborders, Synaptina and Myriotrochina. Synaptina consists of two families, the Chiridotidae, with subfamilies Chiridotinae and Taeniogyrinae, and the Synaptidae, comprising subfamilies Synaptinae, Leptosynaptinae and Rynkatorpinae. Smirnov's suborder Myriotrochina is monotypic. My analyses largely corroborate the reorganization: each taxon, with the exception of Taeniogyrinae, is supported by a high bootstrap percentage and up to seven unambiguous synapomorphies (Fig. 2).

The analysis also indicates strong support for a group not delimited in the latest classification of Apodida. Smirnov (1998) speculated that within Synaptidae there are two main lineages, the Synaptinae + Leptosynaptinae and the Rynkatorpinae. He based this split on the distinctive development and form of each group's anchor plate ossicles (*sensu* Semper, 1868). In contrast, Heding (1928) had suggested that the division runs through Synaptidae and Leptosynaptinae + Rynkatorpinae. Heding (1928) founded the split on the size and shape of their ciliary urns, naming the two groups Micrournae and Heterounae respectively. In this study, Heding's grouping receives high bootstrap support and is subtended by four synapomorphies.

The investigation also uncovered a second possible relationship not seen in Smirnov (1998): the most striking difference between the phylogeny and apodan classification schemes is the possible paraphyly of Chiridotidae (Fig. 2). The family's two subfamilies appear as a grade with Chiridotinae sister to Synaptidae. Phylogenetic studies often uncover evidence that the Linnean designations encompass paraphyletic rather than monophyletic units (e.g. Mooi, 1990; Smith & Paterson, 1995). In this study, however, the paraphyly of Chiridotidae was not strongly supported, and hence it is premature to suggest a taxonomic revision. If further support for the chiridotid grade is found, then Taeniogyrinae should be removed from Chiridotidae, provided that its monophyly is also firmly established. Regardless of the outcome, an important point remains. The characters used in this study were largely derived from taxonomic descriptions. Thus the lack of concordance between the phylogenetic estimate and the Linnaean classification indicates that some groups have not been described cladistically, i.e. not through

the most parsimonious ascription of diagnostic characters. This exposes the fact that there are no unambiguous characters uniting Chiridotidae as currently conceived. The family is diagnosed via a suite of traits, only overlapping subsets of which are possessed by any one genus and are often exhibited by non-chiridotid apodans.

FOSSILS AND THE CALIBRATION OF DIVERGENCE TIMES

Fossil exemplars in phylogenetic analyses may lower the resolution of branching order and increase the number of most parsimonious solutions. This is because some characters with poor preservation potential must be coded as missing, which in turn increases the number of possible resolutions of trees now bearing polytomies defined by missing data. My analyses appeared minimally affected by the potential problems of missing data from fossils, in part because I constructed a hypothetical ancestor by combining the skeletal and gross morphological characters of the fossil taxon with the soft-tissue characters invariant in Recent holothuroids. Using this outgroup, six most parsimonious trees were recovered, a strict consensus of which was still resolved at the nodes of interest discussed in the previous section. Nevertheless, as a further test of the robustness of the rooting, I ran the parsimony analysis using only the nine characters available for *Achistrum* in the hypothetical ancestor. The analysis recovered a majority-rule consensus of nine most parsimonious trees was identical to the estimate based on the ancestral and long-branch rootings, while bootstrap values differed by 0–10% (Fig. 2).

We used the *Achistrum*-rooted tree and the fossil record to estimate lineage divergence times in Apodida (Fig. 3). The oldest reliable stratigraphic records of the studied taxa are shown in Table 3. Despite a fossil record for Holothuroidea that is on the whole "really appalling" (Smith, 1988), the record of the Apodida is relatively good compared with that of other holothuroid orders (Frizzell & Exline, 1966). This permits us at least to make preliminary estimates on the times of some divergence events at the family and subfamily level. The Upper Permian through Upper Triassic *Acanthotheelia* is here considered *sensu* Gilliland (1993) ancestral to Recent *Acanthotrochus* on the basis of the uniquely shared presence of wheel ossicles with a toothed inner rim and a few, but enlarged, spines on the outer margin opposite the interspoke spaces. The calibrated tree suggests that there was little radiation of extant groups at the subfamily level during the Triassic like that reported for other echinoderms. Instead, most deep apodan splits appear to have happened in the late Palaeozoic. Synaptidae, however,

underwent a much later radiation during the Lower Cretaceous. Alternatively and taking the earliest recorded occurrences of Leptosynaptinae and Synaptinae at face value, Synaptidae radiated in the early Tertiary, perhaps in concert with the Eocene expansion of scleractinian coral reefs that is the habitat of most synaptids today.

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