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Phylogenetic Position of *Eptatretus chinensis* (Myxinidae: Myxiniformes) Inferred by 16S rRNA Gene Sequence and Morphology

Ya-Wen Chen[1], Hsueh-Wen Chang[2], and Hin-Kiu Mok[1,*]

[1]*Institute of Marine Biology, National Sun Yat-sen University, Kaohsiung, Taiwan 804, R.O.C.*

[2]*Institute of Biological Sciences, National Sun Yat-sen University, Kaohsiung, Taiwan 804, R.O.C.*

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Ya-Wen Chen, Hsueh-Wen Chang, and Hin-Kiu Mok (2005) Phylogenetic position of *Eptatretus chinensis* (Myxinidae: Myxiniformes) inferred by 16S rRNA gene sequence and morphology. *Zoological Studies* **44**(1): 111-118. Analysis of the present dataset indicated variations in external and internal taxonomic morphological characters of *Eptatretus chinensis* occurring in the South China Sea. Molecular evidence from its 16S rRNA gene sequence indicated a close relation of *E. chinensis* with *Paramyxine sheni* and other species of *Paramyxine* with crowded gill apertures. This species is similar to *E. burgeri* and *P. sheni* in the lack of some slime pores in the branchial region. On the other hand, it shares branching of the ventral aorta close to the heart (a derived character state for the Eptatretinae) with some other congeners (but not *E. burgeri*) and *P. sheni*. http://www.sinica.edu.tw/zool/zoolstud/44.1/111.pdf

Key words: Myxinidae, *Eptatretus chinensis*, Morphology, 16S rRNA, Phylogeny.

Eptatretus chinensis (Fig.1) was first described in 1994 based on 5 type specimens collected from the South China Sea (113°14'E, 19°37'N) at a depth of 600 m (Kuo and Mok 1994). It is a 6-gilled hagfish species with a 3-cusp multicuspid in each tooth row and slime pores next to most of the gill apertures. Due to the small number of specimens then available, detailed variations in the morphometric measurements and meristic counts could not be determined in the original description.

A hypothesis of the phylogenetic interrelationships for 11 myxinid species was constructed by Kuo et al. (2003) based on mitochondrial 16S rRNA gene sequence. This study included the following species: 3 *Eptatretus* species (i.e., *E. burgeri*, *E. cirrhatus*, and *E. stoutii*), 2 *Paramyxine* species (*P. cheni* and *P. sheni*), 3 *Quadratus* [*Paramyxine*] species (*Q. nelsoni*, *Q. taiwanae*, and *Q. yangi*), 3 *Myxine* species (*M. circifrons*, *M. formosana*, and *M. glutinosa*), and 3 undescribed *Myxine* species from Taiwanese waters (*Myxine* sp. 1, sp. 2, and sp. 3). The resulting molecular phylogenetic tree (Fig. 2) shows that (1) both the Myxininae and Eptatretinae are monophyletic subfamilies; (2) *P. cheni* is the plesiomorphic sister species for the remaining 7 eptatretine species which form a closely related group; (3) the genus *Paramyxine* is diphyletic (i.e., not monophyletic);

Fig. 1. *Eptatretus chinensis*. Total length, 354 mm.

* To whom all correspondence and reprint requests should be addressed. E-mail: hinkiu@mail.nsysu.edu.tw

(4) the genus *Eptatretus* is paraphyletic; (5) *Q. nelsoni*, *Q. taiwanae*, *Q. yangi*, and *P. sheni* might belong to the same species due to the small differences among their 16S rRNA; and (6) among *Eptatretus* species, *E. burgeri* is the sister species of *Paramyxine* and *Quadratus* species. Similar results for the phylogenetic positions of *E. burgeri*, *P. cheni*, *P. sheni*, *Q. taiwanae*, and *Q. yangi* had been reached in earlier electrophoretic studies (i.e., Kuo 1991, Jansson et al. 1995).

Recently, fishermen at Tungkang (117°46'26''E, 21°44'29''N), southern Taiwan have trapped hagfish from the South China Sea and their catches of *E. chinensis* are sold in the fish market at Tungkang. With more specimens of this species now available to us, our intention was to provide additional data on the morphometric and meristic characters from the new specimens so that variations of these characters can be reported. In addition, with fresh specimens now available, the sequence of the mitochondrial 16S rRNA gene can be determined. The phylogenetic position of *E. chinensis* is then discussed, based on both morphology and the 16S rRNA sequence.

MATERIALS AND METHODS

Thirty specimens of *E. chinensis* (Fig. 1) were purchased from fishermen who captured them at depths of around 500 m. Terminology and the methods of counts and measurement follow McMillan and Wisner (1984).

All counts of external morphological characters were taken from the left side (Fig. 3); all measurements are in percentage of total body length. The position of the bifurcation of the ventral aorta and the distribution of the afferent branchial arteries on the ventral aorta were recorded.

For extraction of mtDNA, 0.2 g of myomere muscle was removed from the back of a specimen of *E. chinensis* and *E. burgeri* and stored at -70 °C.

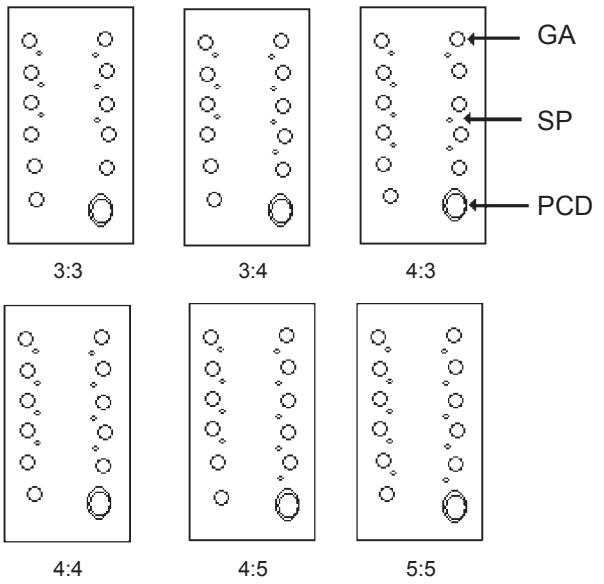

Fig. 3. Patterns of slime pores in the branchial region. GA, gill apertures; SP, slime pores; PCD, aperture of pharyngocutaneous duct.

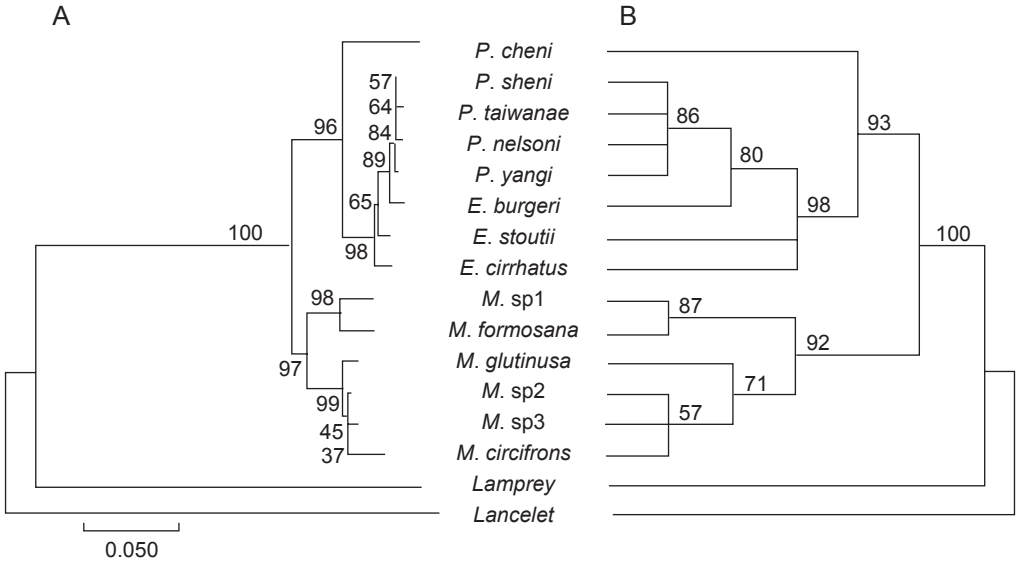

Fig. 2. Phylogenetic trees of hagfish based on mitochondrial 16S rRNA sequence data (566 aligned sites), with trees rooted with the lamprey. (A) Neighbor-joining tree obtained using Kimura 2-parameter distances. (B) The strict consensus of 2 Maximum parsimony trees. Numbers are bootstrap values. (Kuo et al. 2003)

Mitochondrial DNA was extracted and sequenced. Total DNA was extracted according to Kocher et al. (1989), with 0.2 g of muscle tissue homogenized and incubated in a 1.5 ml microcentrifuge tube to which was then added extraction buffer (1x Tris-EDTA, pH 7.8), 10% SDS, and 10 mg/ml proteinase K. Samples were incubated overnight at 50~55°C with gentle shaking. Samples were PCR-amplified using the universal oligonucleotide primers (16SAR), 5'-CGCCTGTTTAACAAAAACAT-3' and (16SBR) 5'-CCGGTTTTGAACTCAGATCACGT-3' (Palumbi 1996). Long PCR was conducted in a Perkin-Elmer Model 9700 thermal cycler, and reactions were carried out with 35 cycles of a 25 µl reaction volume containing 17.35 µl of sterile distilled H_2O, 2.5 µl 10x PCR buffer, 2.0 µl dNTP (2.5 mM), 1.0 µl of each primer, 0.15 µl of 5 units of TaKaRa Taq, and 1.0 µl of the template. Amplification conditions of 35 cycles were performed in the following order: denaturation at 94°C for 1 min and annealing at 55°C for 1 min, followed by elongation at 72°C for 1 min. Amplified fragments were purified with the TaKaRa purification kit. Sequencing was done at the Department of Biological Sciences, National Sun Yat-sen Univ. Sequences of other ingroup and outgroup species were retrieved from the GenBank database (Table 1), and some were previously reported by Kuo et al. (2003).

Outgroups

Amphioxus (*Branchiostoma belcheri*; AB078191), a lamprey (*Petromyzon marinus*; U11880), and a holocephalan (*Chimaera monstrosa*; AJ310140) were selected as the outgroups for construction of the hagfish phylogenetic trees. Dendrograms constructed using only amphioxus as the outgroup were compared with those dendrograms using the lamprey as the outgroup and the rest of the craniates as ingroups.

Phylogenetic analysis

The 16S rRNA gene sequences of *E. chinensis* and *E. burgeri* we obtained and submitted to GenBank as accession nos. AY619579 and AY619580, respectively, were aligned and compared to those of species studied by Kuo et al. (2003) (Table 1) plus an *E. burgeri* sequence submitted by Delbarbre et al. (accession no., AJ278504). Phylogenetic trees were constructed using unweighted Maximum parsimony (MP), and Neighbor-joining (NJ) with the MEGA2 (Komori et al. 2001) algorithm as implemented in the program packages. One thousand bootstrap replications

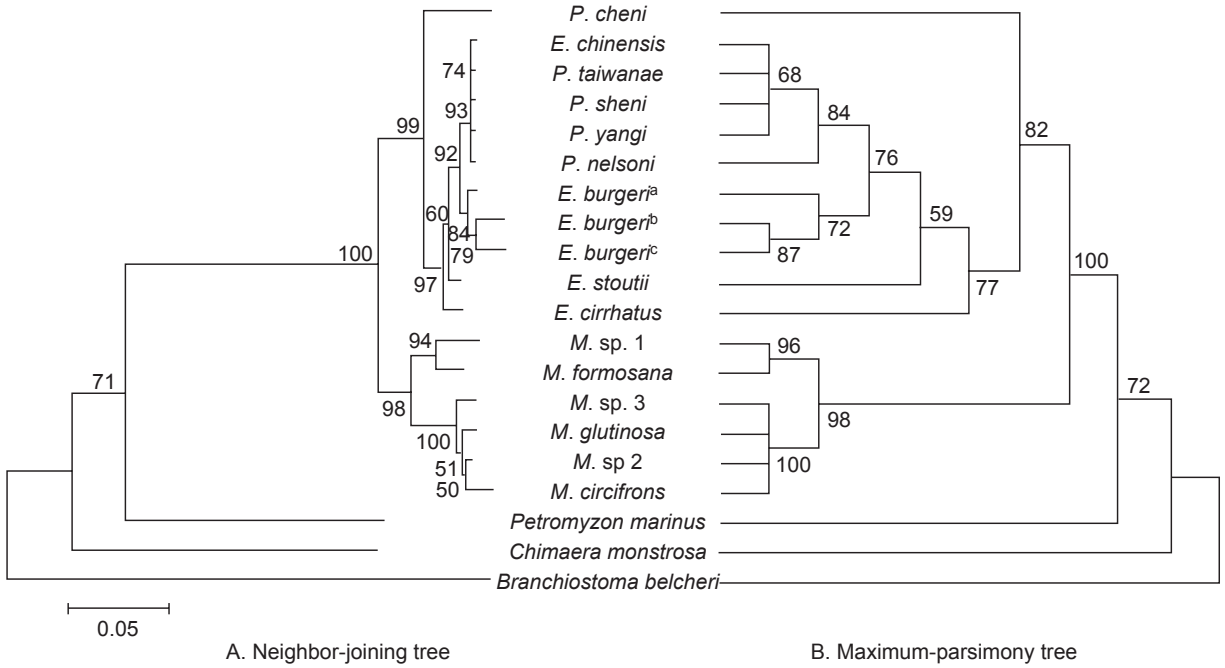

Fig. 4. Phylogenetic trees of hagfish based on mitochondrial 16S rRNA sequence data, with trees rooted using *Branchiostoma belcheri*. (A) Obtained Neighbor-joining tree. (B) Obtained Maximum-parsimony tree. Values at the nodes represent those among the 1000 bootstrap replicates which support that node. [a]Kuo et al. 2003. [b]Delabre et al. 2002. [c]The present paper.

were performed for the MP and NJ analyses.

Table 1. Species used with hagfish (Kuo et al. 2003) and their GenBank accession numbers

GenBank accession nos. *Taxon*	Accession no.
Paramyxine cheni	AF364620
P. nelsoni	AF364608
P. sheni	AF364610
P. taiwanae	AF364611
P. yangi	AF364612
Eptatretus burgeri[a]	AF364616
E. burgeri[b]	AJ278504
E. burgeri[c]	AY619580
E. cirrhatus	AF364619
E. stoutii	AF364618
Myxine circifrons	AF364629
M. formosana	AF364625
M. glutinosa	AJ404477
Myxine sp. 1	AF364622
Myxine sp. 2	AF364626
Myxine sp. 3	AF364627
Petromyzon marinus	U11880
Chimaera monstrosa	AJ310140
Branchiostoma belcheri	AB078191

[a]Kuo et al. 2003. [b]Delabre et al. 2002. [c]The present paper.

RESULTS

Morphological variation

Body proportions and meristic counts are given in Table 2. In *E. chinensis*, as shown in Fig. 3, six gill apertures were arranged in a straight line on each side of the body; the last gill aperture on the left side was confluent with the pharyngocutaneous duct aperture. A slime pore often occurred posteromedially to most gill apertures. Numbers of slime pores in the 4 sections of the body (prebranchial, branchial, trunk, and tail) were (15~17)+(3~5)+(45~47)+(11~14), respectively. There were 3 (2~4) cloacal slime pores on average. Not all gill apertures had an associated slime pore. (i.e., some slime pores were absent from the branchial region). When a slime pores did not occur, it was more often in the posterior gill apertures, and slime pores were never found next to the last pair of gill apertures. Numbers of slime pores on both sides of the branchial region were not necessarily equal. The total number of branchial slime pores varied (Fig. 5); a majority of specimens had slime pores associated with all 4 anterior pairs of gill apertures (30, or 70% of all specimens), while other categories included 3: 3 (right: left anterior pairs of gill apertures) in 3.3% of specimens, 3: 4 in 6.6%; 4: 3 in 3.3%, 4: 5 in 10%, and 5: 5 in 6.6%. On the dental plates, the cusp

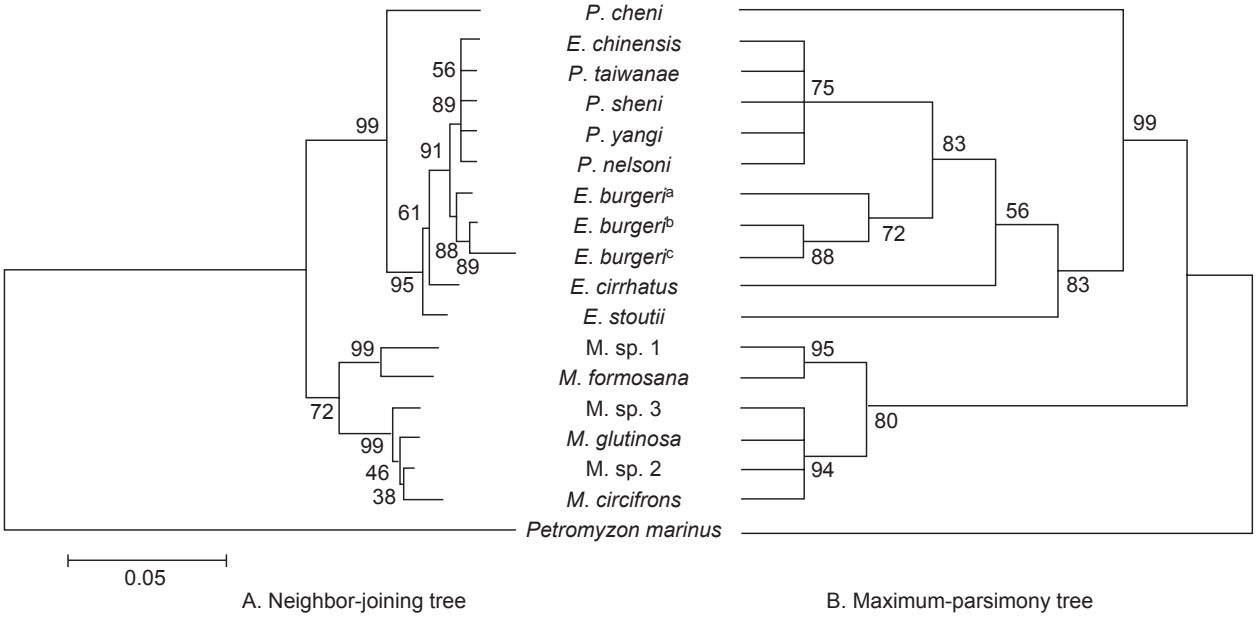

Fig. 5. Phylogenetic trees of hagfish based on mitochondrial 16S rRNA sequence data, with trees rooted using *Petromyzon marinus*. (A) Obtained Neighbor-joining tree. (B) Obtained Maximum-parsimony tree. Values at the nodes represent those among the 1000 bootstrap replicates which support that node. [a]Kuo et al. 2003. [b]Delabre et al. 2002. [c]The present paper.

formula was 8~10+3/3+8~10, with a mode of 9+3/3+9. The ventral aorta bifurcated near the heart (i.e., at gill pouch 6); the last or 6th afferent branchial artery connected to the point of bifurcation in 80% of examined specimens, whereas it connected to the medial section of the ventral aorta in 17% of specimens and to the right-side branch of the ventral aorta in only 3% of specimens. The dental muscle length averaged 15.1% (13.3%~17.7%) of TL; its width was 32.9% (27.5%~40.7%) of its length; and the distance between the tip of the dental muscle and the branching point of the ventral aorta measured 1.7%~26.8% of the dental muscle length, obviously showing great variation.

Phylogenetic position of *E. chinensis*

The 16S rRNA sequence obtained for *E. chinensis* (accession no. AY619579) was 544 base pairs long, and is shown in table 5. The *E. chinensis* sequence contains an insert of GGTA at position 72 of the 16S rRNA gene (Table 5). This insert was not found in any of the hagfish sequences analyzed by Kuo et al. (2003). It is, however, found in *E. burgeri* sequences (accession no. AJ278504 submitted by DeLabre et al. and accession no. AY619580 submitted by us) and *Myxine glutinosa* (an insert of GATA at position 72; accession no. AJ40447). In addition to this unusual insert, position 233 of the 16S rRNA gene of *E. chinensis* has just 3 T's (Table 5) instead of 5 T's as occurs in many other hagfishes.

The composition of the 16S rRNA gene varied among species and genera. Data of the 18 hagfish taxa and *Petromyzon marinus*, *Branchiostoma belcheri*, and *Chimaera monstrosa* were analyzed.

Transition/transversion ratios and the distance matrix obtained from the analysis of the alignment of all sequences of the species compared are shown in Tables 3 and 4, respectively. Values of the pairwise Kimura 2-parameter distance among *Myxine* spp. ranged between 0.010 and 0.076, while that between the species of the *Myxine* group and *P. cheni* ranged between 0.127 and 0.148. The Neighbor-joining trees constructed from the Kimura 2-parameter distances are shown in Figs. 4A and 5A. The Maximum-parsimony trees (strict consensus trees) are shown in Figs. 4B and 5B.

Dendrograms constructed using the 2 algorithms and different outgroups reached similar conclusions that *E. chinensis* is more closely related to the *Paramyxine* and *Quadratus* species than to its traditional (i.e., *Eptatretus*) congeners. The *E. chinensis* sequence was especially close to that of *P. sheni*, showing a distance of only 0.006 (Table 4).

Table 2. Characteristics of *Eptatretus chinensis*. Thirty *E. chinensis* specimens were examined (including the holotype, paratypes, and the specimens used in the molecular studies)

Character	*E. chinensis* *N* = 30	*E. chinensis*[a] *N* = 5
Total length (TL, mm)	261~540	350~375
Weight (g)	40.3~244.6	74~106
Measurements in thousandths of TL:		
Prebranchial L	246.9~353.2	250~257
Branchial L	43.7~63.6	74~80
Trunk L	456.4~531.3	517~520
Tail L	138.7~186.4	52~148
Tail depth	71.4~101.9	77~85
Counts:		
Cusps on multicusp	3/3	3/3
Outer	8~10 + 8~10	10 + 10
Inner	8~10 + 8~10	10 + 10
Slime pores (left side):		
Prebranchial	15~17	15~19
Branchial	3~5	4 (5)
Trunk	40~47	42~45
Tail	11~14	11~14

[a]Kuo and Mok 1994.

DISCUSSION

Molecular evidence from the 16S rRNA gene sequence suggests a close phylogenetic affinity of *E. chinensis* to *P. sheni*, *Q. nelsoni*, *Q. taiwanae*, and *Q. yangi* instead of *E. chinensis* to its *Eptatretus* congeners (Figs. 4, 5, and its sequence is most like that of *P. sheni* (981 similarities with 6 gaps). In the absence of molecular data from *E. chinensis*, Kuo et al. noted tiny differences in 16S rRNA gene sequences among *Q. nelsoni*, *Q. taiwanae*, *Q. yangi*, and *P. sheni*. However, they retained the specific validity of these 4 species on the basis of unpublished isozyme data (Kuo et al. 2003).

Mok and McMillan (2004) concluded that the Eptatretinae is a monophyletic group in having a bifurcated ventral aorta (a synapomorphic character state; the plesiomorphic character state of the ventral aorta lacking bifurcation as in myxinines). Within this subfamily, transformation series of the bifurcation site have evolved from a site midway between the heart and the 1st gill pouch (e.g., as occurs in *E. burgeri*, *E. hexatrema*, *P. taiwanae*, *P. fernholmi*, *P. wisneri*, and *Quadratus* spp.) toward sites closer to the heart leaving fewer or no afferent branchial arteries on the medial section of the ventral aorta (e.g., *E. chinensis*, *E. caribbeanus*, *E. cirrhatus*, *E. strahani*, and *P. sheni*; Mok and

Table 3. Nucleotide percentage composition and length of the 16S rRNA sequence

	T(U)	C	A	G	Total
Paramyxine nelsoni	39.3	13.7	30.0	17.0	540
P. sheni	39.5	13.5	30.1	17.0	542
Q. taiwanae	39.7	13.5	29.5	17.3	542
Q. yangi	39.6	13.5	29.4	17.6	541
P. cheni	38.5	13.4	29.4	18.7	545
Eptatretus burgeri[a]	39.4	13.4	30.4	16.8	543
E. burgeri[b]	39.7	13.2	30.2	17.0	547
E. burgeri[c]	38.9	13.5	30.7	16.8	547
E. chinensis	39.7	13.4	30.0	16.9	544
E. stoutii	38.8	13.9	29.6	17.7	541
E. cirrhatus	39.3	13.7	29.9	17.2	542
Myxine sp. 1	37.1	15.3	29.8	17.8	544
M. formosana	37.4	14.7	29.9	18.0	545
Myxine sp. 3	37.2	15.5	29.4	17.9	541
Myxine sp. 2	36.8	15.7	29.4	18.1	541
M. circifrons	36.1	15.2	29.6	19.1	540
M. glutinosa	37.2	15.2	29.3	18.3	546
Petromyzon marinus	33.3	20.5	23.6	22.5	550
Chimaera monstrosa	31.4	21.2	26.4	20.9	579
Branchiostoma belcheri	30.7	22.7	32.5	14.1	560
Average	37.4	15.3	29.4	17.9	546

[a]kuo et al. 2003. [b]Delabre et al. 2002. [c]The present paper.

Table 4. Pairwise distance (lower diagonal) and transition/transversion ratio (upper diagonal) matrix for 16S rRNA gene fragments (Kimura 2-parameter distance)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Paramyxine nelsoni	—	—	2.007	—	0.917	6.071	—	2.708	0.333	4.063	2.030	1.320	1.179	0.799	0.730	0.863	0.839
P. sheni	0.002	—	1.001	—	0.917	5.049	—	2.364	—	4.063	2.030	1.320	1.179	0.799	0.730	0.863	0.839
P. taiwanae	0.006	0.004	—	3.017	0.924	3.035	7.097	2.03	0.249	3.053	1.830	1.266	1.131	0.769	0.704	0.834	0.808
P. yangi	0.006	0.004	0.008	—	0.917	7.097	—	3.053	0.668	5.099	2.548	1.415	1.273	0.799	0.808	0.938	0.921
P. cheni	0.074	0.074	0.078	0.074	—	0.969	0.917	0.978	0.789	0.804	0.693	0.713	0.784	0.586	0.542	0.616	0.586
Eptatretus burgeri[a]	0.014	0.012	0.016	0.016	0.071	—	3.017	1.003	1.26	3.053	1.417	1.176	1.176	0.830	0.758	0.894	0.873
E. burgeri[b]	0.014	0.012	0.016	0.016	0.074	0.008	—	1.682	2.022	5.099	2.030	1.320	1.320	0.758	0.691	0.826	0.718
E. burgeri[c]	0.022	0.020	0.024	0.024	0.080	0.008	0.016	—	1.772	2.246	1.306	1.171	1.171	0.848	0.779	0.906	0.887
E. chinensis	0.008	0.006	0.010	0.010	0.080	0.018	0.018	0.022	—	1.623	1.158	1.171	1.045	0.716	0.656	0.781	0.752
E. stoutii	0.020	0.020	0.024	0.024	0.069	0.024	0.024	0.032	0.026	—	2.030	1.390	1.390	0.865	0.789	0.927	0.909
E. cirrhatus	0.024	0.024	0.028	0.028	0.065	0.024	0.024	0.032	0.030	0.024	—	1.043	1.043	0.629	0.571	0.703	0.629
Myxine sp. 1	0.114	0.114	0.116	0.118	0.141	0.111	0.114	0.120	0.120	0.107	0.109	—	3.598	1.889	1.755	2.096	2.431
M. formosana	0.107	0.107	0.109	0.111	0.139	0.111	0.114	0.120	0.113	0.107	0.109	0.036	—	2.675	2.600	2.188	3.284
Myxine sp. 3	0.100	0.100	0.102	0.100	0.127	0.098	0.098	0.107	0.106	0.095	0.098	0.063	0.065	—	5.049	1.306	3.548
Myxine sp. 2	0.100	0.100	0.102	0.104	0.127	0.098	0.098	0.107	0.106	0.095	0.098	0.055	0.057	0.012	—	1.009	4.031
M. circifrons	0.116	0.116	0.118	0.120	0.148	0.113	0.113	0.123	0.122	0.111	0.113	0.074	0.076	0.032	0.024	—	1.158
M. glutinosa	0.102	0.102	0.104	0.107	0.127	0.100	0.095	0.109	0.109	0.098	0.098	0.061	0.059	0.018	0.010	0.030	—

[a]kuo et al. 2003. [b]Delabre et al. 2002. [c]The present paper.

McMillan, 2004). The phylogenetic affinity of *E. chinensis* and *P. sheni* as suggested by the molecular evidence, therefore, does not conflict with that of the site of bifurcation of the ventral aorta being close to the heart leaving only 1 or no afferent branchial artery on the medial section of the ventral aorta. However, these 2 species differ greatly in the distribution of slime pores in the gill-aperture region. For *P. sheni*, the vast majority of specimens have no slime pores in this region, with only a very few specimens having just 2 pairs of slime pores in this region (Mok et al. 2001). *Eptatretus chinensis*, by contrast, has many more. We reexamined the holotype and 2 paratypes available to this study and recorded the slime pore counts in this region as 4: 4 (in the holotype; right and left sides, counting from the anteriormost gill aperture) and 5: 5 and 5: 4 (in the 2 paratypes). No slime pores were found next to the last pair of gill apertures-a condition which also applied to some other *Eptatretus* spp. (i.e., *E. burgeri*, *E. cirrhatus*, and *E. stoutii*, but not *E. deani*). The absence of a few slime pores in the branchial region was also noted in the 6-gilled *E. burgeri*; 2 examined specimens showed a 4: 5 pattern (i.e., an absence of 2 slime pores on the right side). The present dataset on the slime pore count shows that the majority of *E. chinensis* specimens have a slime pore next to most gill apertures (i.e., the 1st 4 pairs), and this character places it closer to the *Eptatretus* species assemblage and clearly distinguishes it from *P. sheni*. The variability of slime pore numbers in this region in *E. chinensis* indicates that it is a species at a transitional stage of a transformation series between other *Eptatretus* species in which a slime pore is always placed next to every gill aperture (e.g., *E. deani* with 12: 11 and *E. caribbeanus* with 6: 5) and *P. sheni* and other *Paramyxine* (e.g., *P. springeri* with 2: 3, specifically 1,0,1,0,0,0: 1,0,1,1,0,0) and *Quadratus* spp. in which no slime pores are found in the branchial region. Therefore, despite the close, derived morphological similarity between *E. chinensis* and *P. sheni*, differences in slime pores suggest that they represent 2 separate species.

Mok et al. (2001) discussed delimitation of the genera *Eptatretus* and *Paramyxine* in length and concluded that the diagnostic importance of the absence of at least 1 slime pore in the gill-aperture region (between the 1st and the penultimate gill aperture) is questionable. *Eptatretus* has also been diagnosed by the character of having all efferent branchial ducts about equal in length (Norman 1957, Wisner 1999). For *E. chinensis*, the 1st branchial duct is about twice the length of the last one.

Fernholm (1998: 39-40) stated, "It is obvious that major revisions are needed of *Eptatretus* and

Table 5. Total 16S rRNA nucleotide sequence from 544 bases of *Eptatretus chinensis*

10	20	30	40	50
GTTTTTTACT	CAGATCACGT	AAGATATTAT	TCGTTGAACA	AACGAACCAT
60	70	80	90	100
TAGTAGCTTT	TGCACCACTT	GGGTATCTTA	ATCCAACATC	GAGGTCGTAA
110	120	130	140	150
GCTTCTTTGT	CGATGTGAAC	TTTAAAAAGA	AATAGCGCTG	TTATCCCTAA
160	170	180	190	200
AGTAACTTGT	TCATTGATCA	GAAGATTCTG	GGTCATTTTA	ATAAGTTTCT
210	220	230	240	250
TATTTAACTA	AGTTGTTACT	TTGTTTTAAG	AAGATTTCGT	TTAATTATTG
260	270	280	290	300
ATGATAGTTT	TATTCAATGG	TTGCCCCAAC	CAAATTTTTA	AAATTAGTAT
310	320	330	340	350
TTTTCTTTAT	TGTTTTTATA	GATAGGTTAA	TTTAATGTTT	GAAGCTTTTA
360	370	380	390	400
GGGTCTTTTC	GTCTTATAAG	AGAATTTCTG	TCTTTGAACA	GAAAGGATAA
410	420	430	440	450
TTTCATTGAT	TAAAATTAGG	AGACAGTTTG	GCTTTCGTTA	ATCCATTCAT
460	470	480	490	500
TCTAGTCTAT	AATTAATAGA	CAATTGATTA	TGCTACGTTA	TCATCAGAGA
510	520	530	540	
TGGCCGTTGA	AAAAATCACT	GGGCAGGTAG	GACTTATTAT	ATTA

Paramyxine to determine their generic delimitation. In anticipation of this, we find it most helpful at present not to distinguish *Paramyxine* but include it in *Eptatretus*." In view of (1) the absence of a convincing diagnosis for these 2 genera, (2) the broadly accepted inference that these 2 genera are paraphyletic groups (e.g., Kuo et al. 2003), and (3) the intermingled similarities shown among some species in these 2 genera (i.e., *E. burgeri*, *E. chinensis*, and *P. sheni*), it seems appropriate to agree with Fernholm's synonymy of these 2 genera and to abandon the genus *Paramyxine*. *Paramyxine atami*, the type species of the genus *Paramyxine* described by Dean in 1904, has an apomorphic character state of the ventral-aortal bifurcation site of the subfamily Eptatretinae. If one judges only by this character, *Paramyxine* should include an eptatretine species group with a similar derived character state. However, this is not the case (see above). By contrast, *E. hexatrema* which was originally named *Bdellostoma hexatrema* in 1836 has the plesiomorphic character state of the ventral aorta. Occurrence of the plesiomorphic state in this and many other more species of *Eptatretus* (see above) makes it more reasonable to retain this genus name.

It is noteworthy that (1) South China specimens of *E. chinensis* are more closely related to the western Pacific *Paramyxine* and *Quadratus* spp. than these western Pacific species are to the northwest Pacific *E. burgeri* (Figs. 4, 5) which is sympatric with some *Quadratus* species (Mok and Chen 2001), and (2) all of these species just mentioned are more closely related to the eastern Pacific *E. stoutii* than they are to the southern ocean *E. cirrhatus* (Figs. 4, 5).

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