

Bifurcating Pattern of the Ventral Aorta and Distribution of the Branchial Arteries of Hagfishes (Myxiniformes), with Notes on the Taxonomic Implications

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Hin-Kiu Mok and Charmion B. McMillan (2004) Bifurcating pattern of the ventral aorta and distribution of the branchial arteries of hagfishes (Myxiniformes), with notes on the taxonomic implication. *Zoological Studies* 43(4): 737-748. Intraspecific and interspecific variations in the position of the bifurcation of the ventral aorta, the distribution of the afferent branchial arteries on the ventral aorta, and connections among the efferent branchial arteries, carotid artery, and medial dorsal aorta of hagfishes are reported. Numbers of afferent branchial arteries on the medial section and side branches of the ventral aorta varied to a certain degree within and among species according to where this aorta is bifurcated. Intraspecific variation is considered low, and the presence of interspecific variation makes the related characters valuable for taxonomy. Whether the ventral aorta bifurcates, the distance of the position of bifurcation from the heart, and the number of afferent branchial arteries arising from the medial section of the ventral aorta were applied to identify species and to interpret hagfish phylogenetic interrelationships. <http://www.sinica.edu.tw/zool/zoolstud/43.4/737.pdf>

Key words: Circulatory system, Hagfishes, Myxiniformes, Systematics.

The ventral aorta (VA) of some myxinids bifurcates into left and right branches at various distances from the heart or at the levels of different gill pouches (GPs) (Fig. 1). The position of bifurcation relative to the GPs has occasionally been described for some hagfishes, e.g., *Eptatretus mendozi* (Hensley 1985), *E. mcconnaugheyi*, *E. fritzi*, *E. sinus*, *E. stoutii*, and *E. deani* (Wisner and McMillan 1990). Relationships among the efferent branchial arteries (EBAs), carotid artery, and medial dorsal aorta are stable among species except for minor variations in the position of the anteriormost anastomoses connecting the carotid artery and the medial dorsal aorta in eptatretines with more than 10 pairs of gill pouches. Such differences can help distinguish between similar species such as *E. deani* and *E. fritzi* or between *E. stoutii* and *E. mcconnaugheyi*. Wisner and McMillan (1990) illustrated the position where the VA bifurcates relative to the dental mus-

cles in the *Eptatretus* species mentioned above. They considered that the length of this muscle might affect the position of bifurcation, but it apparently depends more on the number of GPs along the dental muscle. No comprehensive information about bifurcation of the VAs in most myxinids is available in the literature. As the site of bifurcation varies, so do the lengths of the medial section and of the side branches of the VA. Consequently, the numbers of afferent branchial arteries (ABAs) distributed in these sections vary accordingly.

Besides brief description of the EBAs in *Myxine glutinosa* (Johansen 1963), very little information is available in the literature about these blood vessels in other hagfishes. It remains to be seen whether variations important to the taxonomy of hagfishes exist. As such, the present article provides data on the position of bifurcation in the VA, the distribution of ABAs on the medial and side branches of the VA, and relationships among

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the EBA, carotid artery, and medial dorsal aorta. Taxonomic and systematic implications of these characters of the circulatory system are discussed.

MATERIALS AND METHODS

An incision was made along the ventral midline of a specimen to expose the dental muscle, ventral aorta (VA), heart, gill pouches (GPs), and afferent branchial arteries (ABAs) (Fig. 1). When ABAs are present on the medial section of the VA, the 2 ABAs for a particular pair of GPs are not directly opposite each other. Instead, the one on the right side (i.e., the right side of the body) is usually posterior to the left counterpart. The right ABA of the pair on this section closest to the position of bifurcation of the VA may be on the medial section while its counterpart is on the left side branch (e.g., Fig. 2C, D). Consequently, numbers of ABAs from both sides of the medial section might not be the same. This situation occurs when the bifurcating position is close to the heart and only 1 or 2 pairs of ABAs arise from the medial section. For example, 1 ABA leaves the right side of the medial section of the VA in *E. polytrema*, but none on its left side (Fig. 2B). Similarly, 2 ABAs arise from the right side of the medial section in *E. profundus*, but only 1 from the left side (Fig. 2D).

For other hagfishes, numbers of ABAs are similar on both sides of the medial section of the VA (Fig. 2). To avoid confusion, only the number of ABAs on the right side of the medial section and the right main branch of the VA are reported herein.

After the condition of the ABAs was observed and recorded, the distal ends of these arteries were cut. The gut along the branchial region was then cut anteroposteriorly to expose the medial dorsal aorta, the carotid artery, and the efferent branchial arteries (EBAs) (Fig. 1). The connections between these arteries were observed and recorded. To determine intraspecific variations in the site of bifurcation of the ventral aorta and positions of the ABAs along the VA, these characters were determined in at least 20 specimens each of *Eptatretus chinensis*, *Quadratus yangi*, and *Myxine formosana*.

Due to differences in total GP numbers among hagfishes and for comparative purposes, it might be more appropriate to number the pair of GPs and their corresponding ABAs closest to the heart as the 1st gill-pouch pair and the 1st ABA pair. However, since the site of bifurcation of the hagfish VA is described relative to the anteriormost gill pouch in the existing literature, this reference point is therefore followed in this study to avoid confusion and at the same time to render comparisons among references easier.

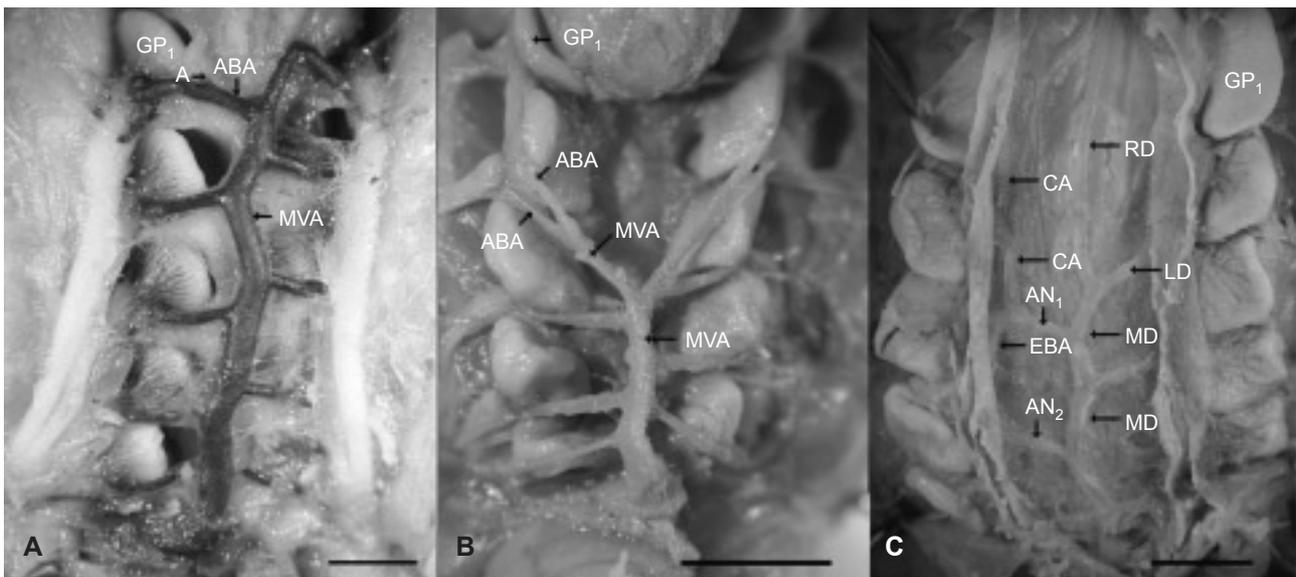


Fig. 1. Ventral view of the branchial region showing the condition of the bifurcation of the ventral aorta, the distribution of the afferent branchial arteries on the ventral aorta (A, B), and the arterial system associated with the efferent branchial arteries, carotid artery, and dorsal aorta (C). A, *Myxine formosana* (312 mm TL); B and C, *Quadratus yangi* (222 mm TL). A, anterior short branch of the 1st ABA; AN, anastomosis connecting the carotid artery and the medial dorsal aorta; ABA, afferent branchial artery; CA, carotid artery; EBA, efferent branchial artery; GP, gill pouch; LD, left dorsal aorta; MD, medial dorsal aorta; MVA, medial section of the ventral aorta; RD, right dorsal aorta; H, systemic heart; SVA, side branch of the ventral aorta. The anterior is to the top. Bar: 4.0 mm.

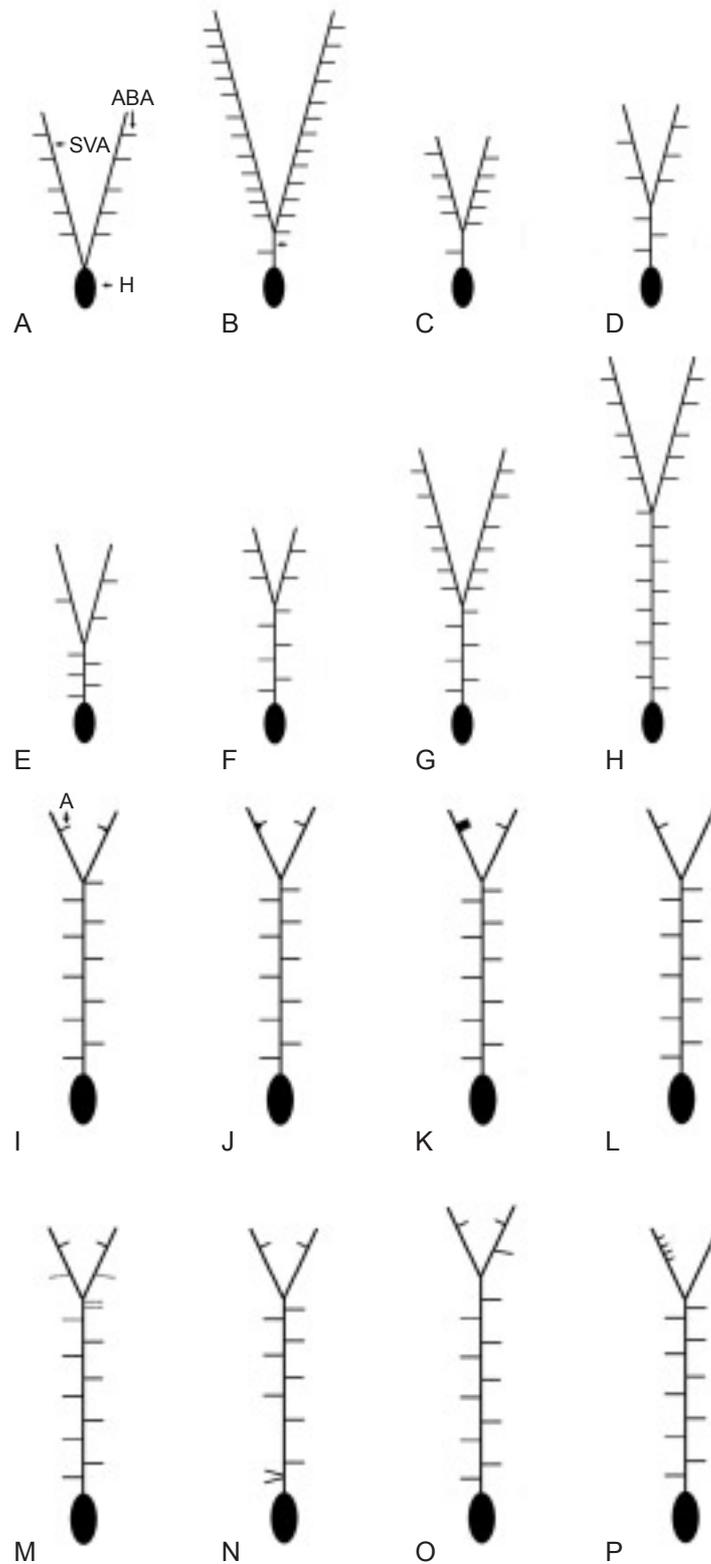


Fig. 2. Simplified diagrams of the ventral view of the ventral aorta (VA), the afferent branchial arteries (ABA) on its medial section (MVA) and side branches (SVA). A small anterior arteriole (A) on the 1st pair of afferent branchial arteries is also shown. A, *Eptatretus mendozi*; B, *E. polytrema*; C, *Paramyxine springeri*; D, *E. profundus*; E, *Quadratus yangi*; F, *E. hexatrema*; G, *E. fritzi*; H, *E. nani*; I, *Myxine affinis*; J, *M. mcmillani*; K, *M. mccoskeri*; L, *M. fernholmi*; M, *M. hubbsi*; N, *M. hubbsoides*; O, *M. ios*; and P, *M. penquenoii*. Dotted lines in (L) represent the variable positions of the second ABA on the left. H, systemic heart.

Materials examined. *Eptatretus bischoffii* USNM 260037, 208 mm; *E. burgeri* NSYSU uncat., 9 (225~580 mm); *E. caribbeanus* UF 27892, 378 mm; USNM 218405, 356 mm; *E. carlhubbsi* BPBM 33933, 845 and 930 mm; *E. cirrhatus* AM 35438001, 185 and 188 mm; NMV A6935, 340 mm; *E. chinensis* NSYSU uncat., 30 (246~496 mm); *E. deani* CAS 56104, 370 mm, CAS 56105, 342 mm; *E. fritzi* SIO66-23, 385 and 412 mm; *E. grouseri* SIO97-77, 142 mm; *E. hexatrema* SII 29421, 492 mm, SAM 13040, 467 mm; *E. laurahubbsae* SIO 65-643, 85 mm, USNM 227441, 192 mm; *E. mcconnaugheyi* USNM 296320, 5 (236~400 mm); *E. mccoskeri* USNM 344905, 280 mm; *E. menodozi* USNM 268924, 364 mm; *E.*

minor UF27893, 276 mm, USNM 218398, 340 mm; *E. multidens* USNM 218402, 406 mm; *E. nani* USNM 272583, 432 and 550 mm; *E. okinoseanus* NSYSU uncat., 308 and 470 mm; *E. polytrema* USNM 272582, 388 mm; *E. profundus* SII 47212, 575 mm, SAM 33338, 630 mm; *E. sinus* USNM 296319, 300 315 and 360 mm; *E. stoutii* NSYSU uncat., 234 and 360 mm, USNM 59834, 325 mm; *E. strahani* USNM 227442, 450 mm; *E. wisneri* SIO 97-76, 300 mm; *E. wayuu* INVEMAR-PEC 2410, 216 mm; INVEMAR-PEC 2411, 194 mm; *Paramyxine atami* USNM 16442, 495 mm (3/3 multicuspid); *Paramyxine atami* (3/2 multicuspid) IZAS uncat., 499 mm; *P. cheni* NSYSU uncat., 9 (207~450 mm); *P. fernholmi* NSYSU uncat., 12

Table 1. Number of afferent branchial arteries on the medial section of the ventral aorta of selected hagfish species. GP, number of gill pouches. Area of occurrence: CP, central Pacific Ocean; CS, Caribbean Sea; GM, Gulf of Mexico; JP, Japan; PH, Philippine; SA, South Africa; SAU, South Australia; SCS, South China Sea; TW, Taiwan; WCAM, western coast of Central America; WNAM, western coast of North America; WSAM, western coast of South America

Species	GP	Area of occurrence	Number of ABA on the medial ventral aorta						
			0	1	2	3	4	5	6
<i>Paramyxine cheni</i>	5	TW					V		
<i>Quadratus nelsoni</i>	5	TW			V				
<i>Q. yangi</i>	5	TW			V	V			
<i>Eptatretus profundus</i>	5	SA			V				
<i>P. atami</i>	6	JP-TW			V				
<i>E. burgeri</i>	6	JP-TW				V			
<i>P. fernholmi</i>	6	TW				V			
<i>P. wisneri</i>	6	TW				V			
<i>P. sheni</i>	6	TW	V	V					
<i>E. chinensis</i>	6	SCS	V	V					
<i>E. mendozi</i>	6	CS	V						
<i>E. multidens</i>	6	CS	V						
<i>E. minor</i>	6	GM	V	V					
<i>P. springeri</i>	6	GM		V					
<i>E. hexatrema</i>	6	SA				V			
<i>Q. ancon</i>	6	CS				V			
<i>Q. taiwanae</i>	6	TW				V			
<i>E. cirrhatus</i>	7	NZ-SAU	V						
<i>E. caribbeaus</i>	7	CS	V						
<i>E. strahani</i>	7	SCS-PH	V						
<i>E. carlhubbsi</i>	7	CP			V				
<i>E. okinoseanus</i>	8	JP			V				
<i>E. bischoffii</i>	10	WSAM	V						
<i>E. sinus</i>	10	WNAM							V
<i>E. deani</i>	11	WNAM						V	
<i>E. fritzi</i>	11	WNAM				V			
<i>E. stoutii</i>	12	WNAM					V		
<i>E. mcconnaugheyi</i>	13	WNAM		V	V	V			
<i>E. nani</i>	13	WSAM							V
<i>E. polytrema</i>	14	WSAM		V					

(226~370 mm); *P. wisneri* NSYSU uncat., 192 and 265 mm; *P. sheni* NSYSU uncat., 230, 345, 374, and 410 mm; *P. springeri* UF 34267, 460 mm, USNM 161513, 340 mm; *Quadratus nelsoni* NSYSU uncat., 5 (190~242 mm); *Q. taiwanae* NSYSU uncat., 16 (170~302 mm); *Q. yangi* NSYSU uncat., 20 (125~249 mm), *Q. ancon* INVE-MAR-PEC 2412, 220 mm; *Myxine affinis* USNM 39039, 386 and 558 mm; *M. australis* USNM 153395, 320 mm, USNM 103769, 316 mm, USNM 117329, 204 mm; *M. capensis* SII 34846, 395 and 416 mm; *M. circifrons* SIO 068-118, 4 (206~270 mm); *M. dubueni* SIO 90-140, 523 mm; *M. dorsum* SIO 92-21, 482 mm; *M. fernholmi* SIO 90-139, 570 mm; *M. formosana*, NSYSU uncat., 28 (115~760 mm); *M. garmani* NSYSU uncat., 268 mm; *M. glutinosa* AMNH 56015, 480 mm, NSYSU uncat., 302 mm; *M. hubbsi* SIO 60, 415 and 440 mm, USNM 325214, 11 (242~435 mm); *M. hubbsoides* SIO 90-142, 657 mm; *M. ios* SIO 80-52, 412 mm; *M. knappi* SIO 90-144, 504 mm; *M. limosa* USNM 197132, 335 mm, ROM 18057, 392 mm; *M. mccoskeri* SIO 92-117, 226 mm; *M. mcmillanae* USNM 308407, 273 mm; *M. pequenoii* SIO 90-146, 168 mm; *M. robinsi* SIO 90-149, 505 mm; and *Neomyxine biniplacata* NMNZ P.24388, 318 and 354 mm.

Institutional abbreviations follow Leviton et al. (1985), with the addition of INVEMAR-PEC, Instituto De Investigaciones Marinas y Costeras; NSYSU, National Sun Yat-sen University, and IZAS, Institute of Zoology, Academia Sinica.

RESULTS

Bifurcation of the ventral aorta (VA) and distribution of its afferent branchial arteries (ABEs)

Intraspecific variation. The VA of all 9 examined *E. burgeri* (a 6-gilled species) specimens bifurcated at the level of the 3rd gill pouch, and 3 ABAs were on the medial section of the VA. In all 30 examined specimens of another 6-gilled hagfish, *E. chinensis*, the VA bifurcated at the level of the 5th gill pouch. In 80% of those specimens, the last ABA on the right was situated at the junction of the bifurcation, whereas the ABA of the remaining specimens was either on the medial section of the VA (in 16.7% of specimens) or on the right side branch (in 3.3% of specimens). As such, the sites of bifurcation were consistently close to the heart (i.e., without intraspecific variation in this aspect), and at the same time, the last ABA on the right was located on the medial section of the VA in 96.7% of specimens. Nine (75%) of the 12 specimens of *P. fernholmi* with 6 pairs of gill apertures (which might not have been arranged in a straight line, particularly for those on the left side) had 3 ABAs on the medial section of the VA, whereas the remaining 3 specimens had only 2 ABAs on this section. In all 8 specimens of *Q. taiwanae* (with 6 gill apertures crowded together), the site of bifurcation was at the level of either the 2nd or 3rd GP, and 3 ABAs were on the medial section of the VA

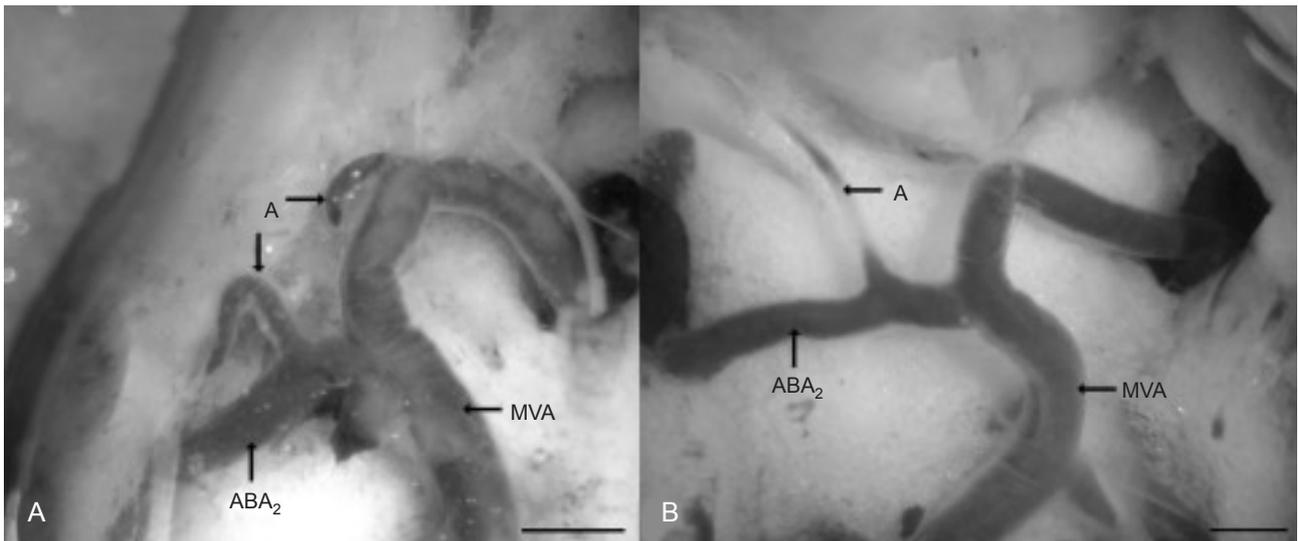


Fig. 3. Ventral view showing variation of the short anterior branch on the 1st pair of afferent branchial arteries (ABA) of *Myxine formosana*. A, anterior short branch of the 1st ABA; DM, dental muscle; MVA, medial section of the ventral aorta. Bar: 1.0 mm.

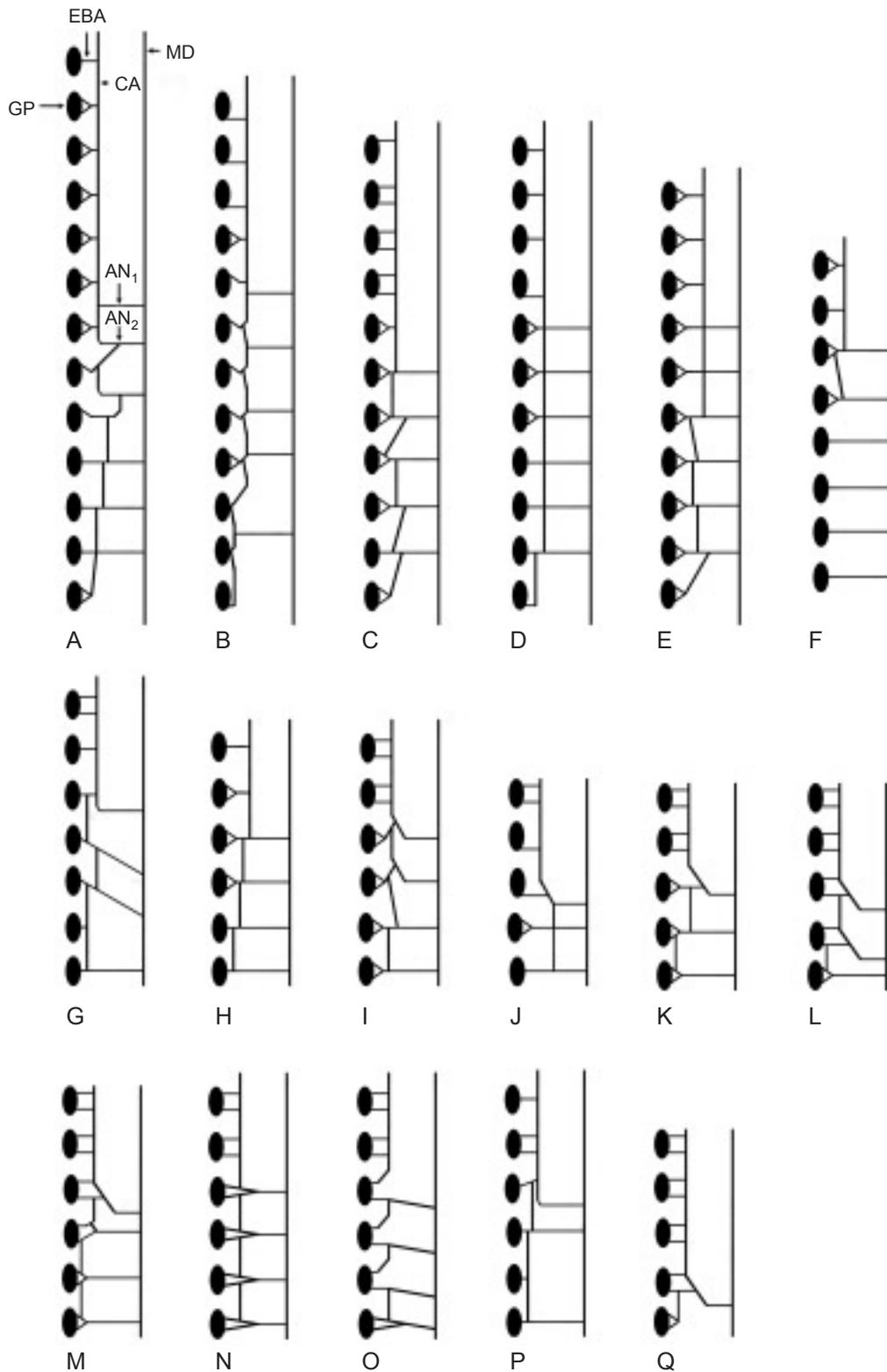


Fig. 4. Simplified diagrams on ventral view showing the right side of the arterial system related to the efferent branchial arteries, carotid artery, and dorsal aorta. A, *Eptatretus mcconnaugheyi*; B, *E. stoutii*; C, *E. deani*; D, *E. fritzi*; E, *E. sinus*; F, *E. okinoseanus*; G, *E. cirrhatu*s; H, *E. burgeri*; I, *Paramyxine sheni*; J, *P. cheni*; K, *Quadratus yangi*; L, *Myxine circifrons*; M, *M. hubbsi*; N, *M. affinis*; O, *M. garmani*; P, *M. glutinosa*; and Q, *M. formosana*. AN, anastomosis; CA, carotid artery; EBA, efferent branchial artery; GP, gill pouch; MD, medial dorsal aorta.

(Table 1). Fourteen (70%) of the 20 specimens of *Q. yangi* examined, which has 5 gill apertures crowded together, had 3 ABAs on the medial section of the VA (in four of these 14 specimens, one of these ABAs was located at the junction of the side branches of the VA), whereas the other 6 of 20 specimens had only 2 ABAs on the medial section of the VA (Table 1).

For all 28 examined specimens of the 5-gilled *M. formosana*, the VA did not bifurcate, and all ABAs were connected to the medial VA. Intraspecific variation in the number of ABAs on the medial section of VA also existed in *E. minor* (0 or 1 ABA), *E. mcconnanaugheyi* (1~3 ABAs), and *P. sheni* (a 6-gilled species; 0 or 1 ABA; Table 1), and *E. wayuu*. *Eptatretus wayuu* is a 5-gilled species of the Caribbean Sea in which the configuration of the bifurcation differs between the holotype and paratype; one does not bifurcate while the other does, leaving 3 ABAs on the medial section of the VA (Mok et al., 2001).

In most hagfishes, the last ABA on the right on the medial section of the VA next to the heart is posterior to the last ABA on the left side (Fig. 1). The relative position of these 2 arteries in one of the 2 examined *E. nani* specimens was reversed (Fig. 2H).

The position of the origin of the 2nd ABA on the left varied in the 6-gilled *M. hubbsi*, *M. affinis*, and *M. limosa*. It either connected to (1) the medial section of the VA posterior to the point where

the 1st pair of ABAs diverged, (2) the point where the 1st pair of ABAs diverged, or (3) the 1st ABA on the left (Fig. 2M). For the 13 *M. hubbsi* specimens examined, 46% exhibited condition 1, 23% condition 2, and 31% condition 3. *Myxine affinis* and *M. limosa* (both with 2 specimens examined) showed both conditions 1 and 2.

Interspecific variation. Thirteen of the 32 eptatretine species examined had 6 pairs of gill pouches. Comparisons among them may provide insights into the degree of interspecific variation in the branching position among species with the same number of gill pouches. In the 6-gilled *E. burgeri*, *E. hexatrema*, *Q. ancon*, and *Q. taiwanae*, and 3 six-gilled *Paramyxine* species, "*P. atami*" specimens with a 3/2 cuspid formula, *P. fernholmi*, and *P. wisneri* (except for *P. atami* specimens with a 3/3 cuspid formula, *P. sheni*, and *P. springeri*; also see below), the VA bifurcates at the level between the 3rd and 4th GPs (i.e., at about halfway between the GPs). The 4th to 6th pairs of GPs are drained by the ABAs from the medial section of the VA (i.e., 3 ABAs on this section), while the 1st to the 3rd pairs of GPs are drained by the ABAs from the branches of the VA (Fig. 2F). In most *P. sheni* specimens, the Pacific 6-gilled *Eptatretus* spp. (*E. grouseri* and *E. chinensis*, but not *E. burgeri*), the Caribbean *P. springeri*, and 1 *E. minor* specimen, the VA bifurcates close to the heart (at the level of the 6th or 5th GP). ABAs of the last pair of gill pouches are from the medial

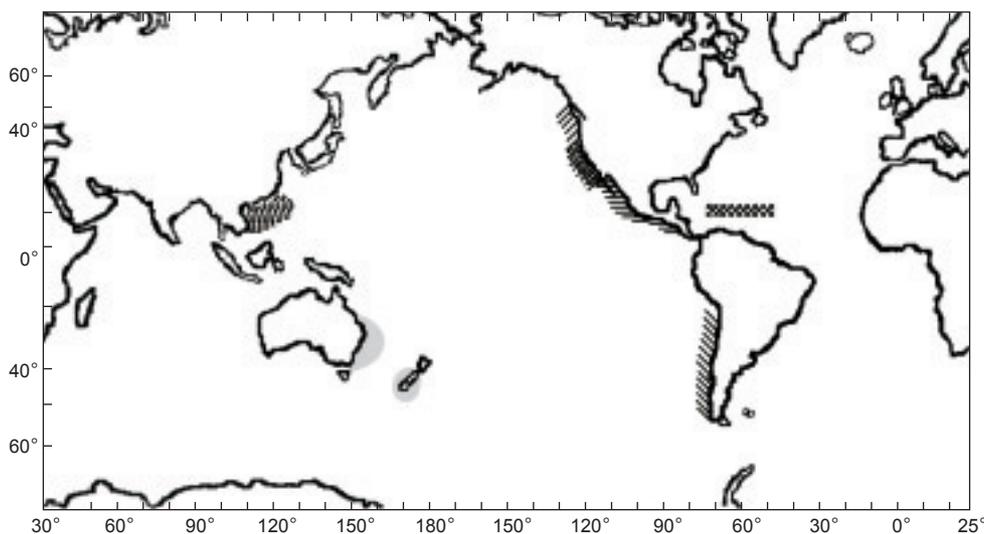


Fig. 5. Map showing distributions of the eptatretine species in which the ventral aorta bifurcates very close to the systemic heart leaving no ABA on the medial section of this artery. Numbers in parentheses are numbers of gill pouches. ▨: *E. deani* (11), *E. stoutii* (12); ▩: *E. mcconnaugheyi* (13); ▧: *E. mendozai* (6), *E. multiden* (6), *E. caribbeus* (7); ▨: *E. sinus* (10), *E. fritzi* (11); ▩: *E. bischoffii* (10), *E. nani* (13), *E. polytrema* (14); ▧: *E. strahani* (7); ▨: *E. cirrhatus* (7)

section of the VA, while the other 5 pairs of GP are drained by the ABAs from the side branches (Fig. 2C; Table 1). In other 6-gilled species (i.e., *E. mendozi*, *E. multidentis*, a 346 mm specimen of *P. sheni*, and 1 *E. minor* specimen) the VA splits right after leaving the heart, and all ABAs are from the side branches (Fig. 2A).

Of the following *Eptatretus* species examined, all are 7-gilled except for *E. okinoseanus* with 8 GPs. *Eptatretus caribbeanus*, *E. cirrhatus*, and *E. strahani* had no ABAs on the medial section of the VA; *E. laurahubbsae* had 3 ABAs; and *E. okinoseanus*, *E. mccoskeri*, and *E. carlhubbsi* had 2 ABAs on the medial section (Table 1). The VA of the 7-gilled *E. menodozi* from Brazilian waters branches at GP 5~6 (Mincarone 2000); it is expected to have 2 ABAs on the medial section of the VA.

Only 6 eptatretine species (*E. eos*, *P. cheni*, *Q. nelsoni*, *Q. yangi*, *E. profundus*, and *E. wayuu*) are 5-gilled. Two ABAs were from the medial segment of the VA in *E. profundus*, *P. nelsoni*, and about 30% of the *Q. yangi* specimens (Fig. 2D, Table 1). Three ABAs were on the medial section of the VA of *P. cheni*, most *Q. yangi* specimens (70%), and the paratype of *E. wayuu* – a condition similar to the majority of 6-gilled eptatretines (see above). Exceptionally, the VA of the holotype of *E. wayuu* does not branch (Mok et al., 2001).

Eight of the *Eptatretus* species examined 10 or more pairs of GPs (Table 1). In *E. bischoffii*, all ABAs were on the side branches. Only the last gill pair in *E. polytrema* (Fig. 2B) and the last 3 pairs in *E. mcconnaugheyi* and *E. fritzi* (Fig. 2G) are fed by the ABAs from the medial section of the VA (Table 1). In the other 4 *Eptatretus* spp. with more than 10 pairs of GPs (i.e., *E. deani*, *E. nani*, *E. sinus*, *E. stoutii*), the VAs split more anteriorly. Consequently, more than 3 pairs of GPs close to the heart are supplied by the ABAs from the medial section of the VA (Table 1, Fig. 2H). The VAs of almost all examined myxinines (5- or 6-gilled, with most being 6-gilled) do not bifurcate into 2 main side-branches. The VA sends out a pair of ABAs at its front end to the 1st pair of GPs. In the majority of these species, this pair of ABAs does not bifurcate into major side branches, and all ABAs originate from the VA with a certain distance between adjacent ones on the same side of the aorta. When intraspecific variation occurs in 6-gilled species, it is most likely associated with the site of origin of the 2nd pair of ABAs, particularly the left one. Of all the myxinine species examined, in only one 435 mm *M. hubbsi* specimen did both

the left and right 1st ABAs split into 2 arteries for the 1st and 2nd GPs (Fig. 1M). In the only *M. ios* examined, the 5th and 6th ABAs on the left were connected (but not the right ones; Fig. 2O). The 1st and 2nd ABAs in no other myxinine specimens examined were connected. Unlike the general condition in which the ABAs on the same side of the medial section are equally spaced, the only *M. hubbsoides* specimen examined displayed an unusual difference from other hagfish species by having the 5th and 6th ABAs on the right side of the medial section connecting at the same point on the VA (Fig. 2N).

For the majority of myxinines, including *Neomyxine biniplicatus*, some *M. formosana*, *M. garmani*, *M. glutinosa* specimens, *M. hubbsi*, *M. hubbsoides*, *M. ios*, *M. knappi*, *M. mccoskeri*, and *M. mcmillanae*, there is a very short anterior branch (or arteriole) on the 1st ABAs on both the left and right (Figs. 2I, M-O, 3). This anterior branch runs into the posterolateral side of the dental muscle or its adjacent soft tissue. In most cases, its end did not taper (e.g., *M. fernholmi*, Fig. 2L). However, an abrupt reduction in diameter was noticed in the distal section of these anterior branches in *M. ios* and the right anterior branch in *M. mcmillanae* (Fig. 2J). In some species, one of these anterior branches was either smaller or absent; in *M. hubbsi* the left anterior branch was either smaller or missing, and was missing on the left side branch in *M. fernholmi*, some *M. limosa* specimens, and *M. mccoskeri*. Of the 20 specimens of *M. formosana* examined, this anterior branch was absent from the 1st left ABA in 5 specimens, and was absent from the 1st ABAs on both the left and right sides in only 1 specimen. It was also noted that the anterior branch was less developed in large specimens (e.g., in the 490 and 540 mm specimens of *M. formosana*) and was missing on the 1st ABA on the right in some specimens of *M. glutinosa*. Unlike most other myxinines mentioned above, the short anterior branch in *M. mccoskeri*, found only on the 1st ABA on the right, was thick instead of tapered (Fig. 2K). This branch on the 1st ABA was absent in *M. australis*, some *M. glutinosa* specimens, some *M. limosa* specimens, *M. robinsi*, and all eptatretinines (Fig. 2A-H). Interestingly, in *M. penquenoii*, 4 short side branches were seen on the 1st ABA on the right (Fig. 2P).

Efferent branchial arteries, the carotid artery, and the dorsal aorta

For all 5- to 8-gilled hagfishes, EBAs from the

3 anteriormost GPs drain to the carotid artery (Figs. 1, 4F-P). The carotid artery extends posteriorly and tapers towards the posterior GPs. For the anterior GP, there might be 2 EBAs from 1 GP entering separately into the carotid artery (Fig. 4). For the posterior pouches, these 2 arteries might merge into 1 artery before entering the carotid artery. The EBAs for the posteriormost 2 gill pouches of *E. deani*, *E. stoutii*, *E. sinus*, *E. fritzi*, *E. mcconnaugheyi*, and *M. formosana* join before draining into the carotid artery (Fig. 4A, C-E, Q).

For a majority of hagfishes, the 1st anastomosis connecting the carotid artery and the medial dorsal aorta is present at the level of the 3rd GP. For hagfishes with 10~13 pairs of GPs, the 1st anastomosis between the carotid artery and medial dorsal aorta emerges more posteriorly, i.e., at the level of the 5th or 6th GP (Fig. 4A-E): in *Eptatretus sinus* (a 10-gilled species) at the 6th gill pouch of the 10 pouches (6/10), *E. fritzi* 5/11, *E. deani* 6/11, *E. stoutii* 5/12, and *E. mcconnaugheyi* 6/13. These data suggest that the site of the 1st anastomosis is consistent in species with fewer than 10 pairs of GPs. However, for those with more pouches, this site is located more posteriorly, but its position did not clearly correlate with the number of gill pouches present. As the number of GPs also varies in some of these species (e.g., *E. sinus* has 9 or 10 GPs, *E. deani* has 10~12 GPs, *E. stoutii* 11~13 GPs, and *E. mcconnaugheyi* has 13 or 14 GP), intraspecific variation in the position of the 1st anastomosis can be expected in these species.

DISCUSSION

Intraspecific variation in the number of ABAs on the medial section of the VA occurs in *E. chinensis*, *E. minor*, *E. mcconnaugheyi*, *P. sheni*, *Q. taiwanae*, *Q. yangi*, and *E. wayuu* among the species examined. The present dataset on intraspecific variation in eptatretines suggests that (1) numbers of ABAs on the medial section of the VA vary between 0 and 1 for 6-gilled eptatretine species in which the gill apertures are in a straight line and the VA bifurcates close to the heart (e.g., *E. chinensis*), (2) 3 ABAs are on the medial section of the VA of 6-gilled eptatretine species in which the gill apertures are in a straight line but the VA does not bifurcate close to the heart (i.e., *E. burgeri*), and (3) numbers of ABAs on the medial section of the VA of 5 or 6-gilled eptatretines, in which the gill apertures are not in an obviously straight

line and the VA does not bifurcate close to the heart (i.e., *Q. taiwanae*, *P. fernholmi*, and *Q. yangi*), vary from 2 to 3 with a mode of 3 (i.e., in many as 70%~100% of the specimens examined).

Variations in the diverging position of the 5th ABA on the left were only observed in *M. affinis*, *M. hubbsi*, and *M. limosa*. These characters are considered consistent and reliable for taxonomic implications.

The number of ABAs on the medial section of the VA is informative only to a certain extent for resolving some taxonomic problems of hagfishes. Most hagfishes are recognized based only on several meristic characters (e.g., slime pore and tooth counts) and the arrangement of the gill apertures. Overlaps of ranges of these characters between or among species often exist making validity of a species' status doubtful in some cases. The branching position of the VA and the distribution of ABAs on the VA provide additional characters for species identification or justification of taxonomic statuses. The taxonomic status of *P. atami*, with 6-gill apertures in a straight line, has been problematic. The holotype (Dean 1904), the specimen at the USNM (USNM 16442), and a specimen the SIO from Tokyo, for instance, have a 3/3 multicuspoid formula, while many of the specimens described by other ichthyologists under the same scientific name may have a 3/2 multicuspoid formula. Fernholm (1986 1998) stated the probability that specimens formerly identified as *P. atami* may represent at least 2 species. These *Paramyxine* specimens with a 3/2 fused cusps that had been collected in the Sagami Bay area, Japan were probably the new species, *Paramyxine moki*, recently described by McMillan and Wisner (2004). For the examined specimen with a 3/3 condition, only 2 ABAs are present in the medial section of the VA, whereas the specimen with a 3/2 condition examined has 3 ABAs. Despite such differences, variations presented in the number of ABAs on the medial section of the VA of 6-gilled eptatretines in which the gill apertures are not in an obviously straight line (including *P. atami* and *P. fernholmi*) result in no useful evidence in resolving this taxonomic problem (see above).

The unique relative position of the 5th and 6th ABAs on the right in *M. hubbsoides* supports its species status. Whether this unusual pattern is an individual variation remains to be seen.

In the phylum Chordata, the subphylum Cephalochordata (including the amphioxus, Amphioxiformes) is the plesiomorphic sister group of the subphylum Craniata (or the Vertebrata)

(Nelson 1994). There are 2 alternative hypotheses about the phylogenetic interrelationships among hagfishes, lampreys, and gnathostomes. Similarity in nucleotide sequences of the 18S ribosomal RNA molecule supports the close relationship between hagfishes and lampreys (Stock and Whitt 1992). Conversely, the presence of a cerebellum, an optic tectum in the brain, neural arches along the notochord, nervous control of the heart, etc. supports the alternative hypothesis that lampreys and gnathostomes are close relatives (Forey and Janvier 1994). Morphologically speaking, the latter hypothesis seems to prevail: the Myxiniformes is the most primitive group in the subphylum Craniata in which the chordates Petromyzontiformes and Gnathostomata are included. To interpret character polarity for hagfishes, amphioxus is a legitimate out-group; the condition of the circulatory system in amphioxus can be taken as a reference for hypothesizing the polarity of related character states in hagfishes. The circulatory system of amphioxus is vertebrate-like, with a ventral and a dorsal aorta, and aortic arches about the pharynx; no bifurcation in the ventral aorta occurs (Smith 1960) – a condition similar to that in myxinines. For chordates, the VA of lampreys bifurcates at the level of the 4th and 5th gills; 4 pair of ABAs are on the medial section of the VA (Bond 1979). In elasmobranchs, however, the ventral aorta does not bifurcate (Bond 1979). According to the principle of parsimony of the cladistic methodology, it is more parsimonious to hypothesize herein that the plesiomorphic character state for the Myxinidae should resemble that of the amphioxus (i.e., the VA without bifurcation). Consequently, the Eptatretinae is a monophyletic group in having a bifurcated VA – a synapomorphic character state. Within this subfamily, the transformation series have evolved toward the bifurcation site being closer to the heart, leaving fewer or no ABAs on the medial section of the VA.

Kuo et al. (2003), supported by mtDNA evidence, suggested that (1) the Myxininae and Eptatretinae are both monophyletic groups, and (2) *P. cheni* is a plesiomorphic sister group for the other eptatretine species they studied (including *Paramyxine* and *Eptatretus* spp.). Their data also indicated a close phylogenetic affinity among the 4 species they studied (i.e., *P. sheni*, *Q. nelsoni*, *Q. taiwanae*, and *Q. yangi*). Those authors also pointed out that small differences in their molecular analysis of 16S rRNA suggest that these 4 species belong to the same species. Despite this notion, they retained the prevailing specific statuses on

the basis of unpublished isozyme data of Kuo, who found that each of them possessed certain fixed alleles. Regarding the last inference, bifurcation of the VA at a posterior site in *P. sheni* and the different arrangement of GAs in a straight line in *P. sheni* versus being crowded together in an irregular pattern in the other 3 species clearly distinguishes *P. sheni*, which is more likely to be a separate species.

The absence of ABAs on the medial section of the VA, a condition associated with a close affinity of the bifurcation site to the heart, is hypothesized to be a full apomorphic character state for hagfishes (see above). Accordingly, it can be suggested that *E. mendozai*, *E. multiden*, *E. caribbeanus*, *E. cirrhatus*, *E. strahani* (and possibly *E. minor*; see above), and even *E. bischoffi* may be closely related. These species are as widely separated geographically as the Caribbean Sea, Australia, New Zealand, and the Philippines. No species on the western coast of North America has this character state. Despite the geographic closeness, *P. springeri* (Bigelow and Schroeder 1952), with 1 ABA on the medial section of the VA, does not share this derived character state. Fernholm and Hubbs (1980) reviewed *P. springeri* and re-assigned it as *E. springeri*. Later Fernholm (1998) considered *E. minor* to be its closest relative. The ABA count indicates that *P. springeri* does not coincide with the majority of *Paramyxine* species (except for *P. sheni* and *P. atami* with a 3/3 multicupid formula) that carry 3 ABAs on the medial section. Conversely, *P. springeri* looks more similar to *P. sheni*, *P. atami* (also see below), and *E. minor* in this aspect (i.e., *E. minor* has 0 or 1 ABA on the section of the VA). The relationships between *E. minor* and *P. springeri*, on the one hand, and *E. minor* and the other *Eptatretus* species from the Caribbean Sea with the same character state on the other, cannot be determined on the basis of this character alone.

Wisner (1999) recognized a new genus and subfamily (*Quadratus* and the Quadatinae) on the grounds of the character of the gill apertures being closely crowded in a square, rectangular, or rhomboidal pattern. Accordingly, he assigned 3 *Paramyxine* species (*P. nelsoni*, *P. yangi*, and *P. taiwanae*) to this new genus. Among them, the 1st 2 species have 5 pairs of gill pouches and have 2 ABAs on the medial section of the VA. The 6-gilled *Q. taiwanae* (with 3 to 5 ABAs on the medial section of the VA) shares a state of having 3 ABAs on the medial VA with *P. fernholmi* and *P. wisneri* among other *Paramyxine* species. The Caribbean

Sea *Q. ancon* with 6 crowded gill apertures (Mok et al., 2001) is the only representative of this genus not in Taiwanese waters. Unlike *Q. taiwanae* which shares similar branchial characters, 2 ABAs are in the median section of the VA in *Q. ancon* – the same condition as that in *P. atami* and *P. sheni*. The present dataset, therefore, offers no supporting evidence for the monophyly of these 2 supraspecific taxa as proposed by Wisner. The present dataset, on the other hand, points to a possibility that *P. atami* and *P. sheni* may be more remotely related to their congeners for having fewer ABAs on the medial VA. Interestingly, *E. burgeri* and *E. hexatrema* are two of the 6-gilled *Eptatretus* species examined that share the pleiomorphic character state with *P. fernholmi*, *P. wisneri*, and *Q. taiwanae*, i.e., species having 1/2 of the ABAs on the medial section of the VA. This similarity does not conflict with the possibility suggested by some previous workers on hagfish systematics (e.g., Huang et al. 1994, Jansson et al. 1995) that *E. burgeri* might have a closer relationship to the *Paramyxine* species in Asia than to other congeners.

The position of the bifurcating site of the VA may be related to the length of the dental muscle (see above). If this is true, the posterior tip of the dental muscle is expected to be very close to the bifurcation site, and the space between them should be limited. In most cases, this expected condition holds (e.g., in *E. mendozi*, *E. mcconnaugheyi*, *Q. yangi*, and *Myxine* spp., to name a few). On the other hand, a rather long distance between these 2 sites was also seen in some hagfishes, e.g., *E. fritzi*, *E. sinus*, *E. strahani*, and *P. springeri*. Therefore, a clear correlation between the dental muscle and the bifurcation site of the VA in hagfishes as a whole does not hold. The anterior extension of the medial section of the myxiniine VA, however, does possibly correlate with a shorter dental muscle in comparison with the eptatretines; the medial VA reaches the posterior tip of the dental muscle.

All species with 10 or more pairs of gill pouches are restricted to the western coasts of North and South America. All 4 eptatretine species off coastal South America (Chile) have 10~14 pairs of gill pouches (Fernholm 1998, no specimen of *E. decatrema* was available for this study). Two of the remaining 3 species from Chile, namely, *E. bischoffii* and *E. polytrema*, lack any afferent branchial arteries on the medial section of the ventral aorta. *Eptatretus nani* from Chile is similar to the other species with 10 or more pairs of gill

pouches on the western coast of North America in having more than 2 afferent branchial arteries on the section of the ventral aorta. It seems possible that the 4 *Eptatretus* species from Chile do not form a clade. If possession of 10 or more pairs of gill pouches have been derived more than once during hagfish evolution, *E. bischoffii* and *E. polytrema* may be more closely related to species in the Caribbean and other parts such as the Philippines (e.g., *E. multidentis*, *E. mendozi*, *E. caribbeanus*, *E. strahani*, and possibly *E. minor*) in which the ventral aorta bifurcates very close to the heart, leaving no afferent branchial arteries on the medial section of this artery.

The appearance of the anteriormost anastomosis between the carotid artery and the medial dorsal aorta may vary among eptatretines with more than 10 pairs of gill pouches, and this variation may be of taxonomic value.

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