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Source: Zoological Science, 21(11): 1131-1136

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.21.1131

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## A New Deep-water Lancelet (Cephalochordata) from off Cape Nomamisaki, SW Japan, with a Proposal of the Revised System Recovering the Genus *Asymmetron*

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**ABSTRACT**—*Asymmetron inferum* n. sp. is established for the holotype collected during the Hyper-Dolphin/Natsushima cruise in 2003 of the Japan Marine Science & Technology Center (JAMSTEC) off Cape Nomamisaki, southwestern end of Kagoshima Prefecture, at a depth of 229 m. *A. inferum* is very similar to its congener, *A. lucayanum* Andrews, 1893 (formerly called *Epigonichthys lucayanus*) but easily distinguishable from it by the larger number of total myotomes (83 in the former vs. 55–72 in the latter). The genus *Asymmetron* has been treated as a junior synonym of *Epigonichthys*, but is recovered as a valid genus distinct morphologically from another valid genus *Epigonichthys* (sensu stricto).

Key words: cephalochordates, new species, deep water, morphology, recovery of Asymmetron

## INTRODUCTION

During the Hyper-Dolphin/Natsushima cruise in 2003 of the Japan Marine Science & Technology Center (JAM-STEC), several lancelet specimens were collected from bottom sand near the bones of the sperm whale *Physeter macrocephalus* Linnaeus, 1758 at a depth of 229 m, off Cape Nomamisaki, southwestern end of Kyushu Island, Japan.

The lancelets, or the subphylum Cephalochordata of the phylum Chordata, include ca. 30 known living species, so far collected from tropical to cool-temperate shallow waters all over the world (Poss and Boschung, 1996). The deepest known record for benthic adults of this subphylum as reported by Wickstead (1975), was in collections made by Zietz (1908, p. 288) from "about 100 fathoms [=183 m] of water about 49 miles east of Cape Spencer", South Australia. Thus, it is clear that the present record extends the depth range of lancelets downwards to 229 m, and also represents the first occurrence of the adult lancelets in the deep water, which is usually defined as the region deeper than 200 m.

Further, the material appears to be a new species of the genus *Asymmetron*, as distinct from *Epigonichthys* in the revised system proposed herein, based on morphological considerations (shown in the present study derived from Nishikawa and Nishida's (1997) and molecular studies (Nohara *et al.*, accepted; Kon *et al.*, submitted). The present

\* Corresponding author: Tel. +81-52-789-5761; FAX. +81-52-789-5896. E-mail: nishikawa@num.nagoya-u.ac.jp new species is the first to be established in the lancelets in about 40 years since the proposals of *Branchiostoma bennetti* Boschung and Gunter, 1966 and *B. moretonense* Kelly, 1966.

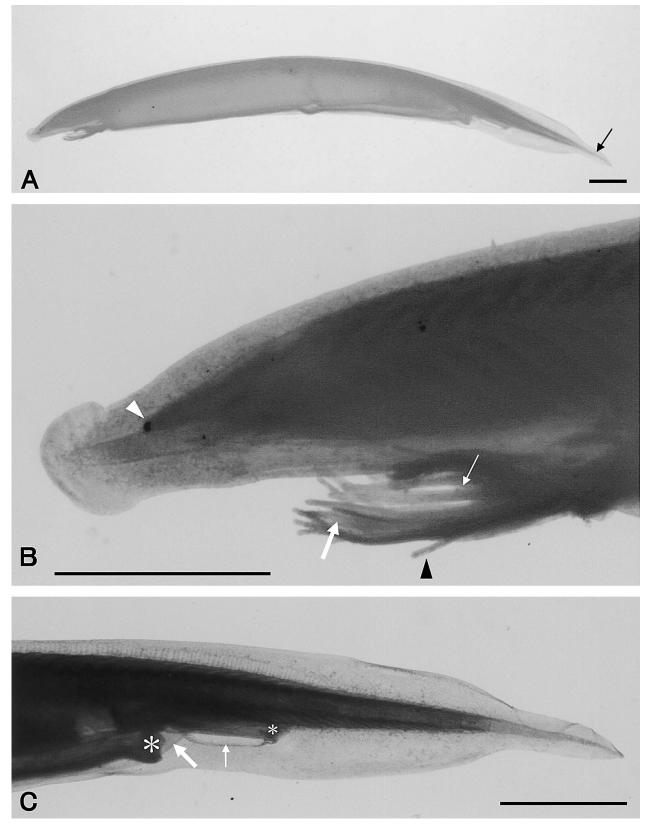
The present finding of the true deep-water lancelet sheds new light on the biodiversity studies of the lancelets, as the closest relative of the vertebrates.

## TAXONOMIC DESCRIPTION

Genus *Asymmetron* Andrews, 1893 *Asymmetron* Andrews, 1893, p. 237 (Type species: *A. lucayanum* Andrews, 1893 by monotypy) *Notasymmetron* Whitley, 1932, p. 260 (Type species: *Asymmetron caudatum* Willey, 1896 by original designation)

Diagnosis: Cephalochordates with gonads only on right side, and with a markedly elongated urostyloid process as a posterior extension of notochord without myotomes.

Remarks: The genus *Asymmetron* Andrews, 1893 has recently been regarded as a junior synonym of the genus *Epigonichthys* Peters, 1877 with the type species of *E. cultellus* Peters, 1877. Thus, the lancelets have been generally classified into the two genera *Branchiostoma* Costa, 1834 and *Epigonichthys*, both with many junior synonyms, in a single family Branchiostomatidae (see, e.g., Nishikawa, 1995; Poss and Boschung, 1996). The two valid genera are distinguished from each other by the different position of gonads, i.e., on each side in the genus *Branchistoma*, while only on the right side in *Epigonichthys*; the symmetric (in the former) or asymmetric (in the latter) ending of metepleura



**Fig. 1.** Asymmetron inferum n. sp., the holotype (NUM-Az 0363) from off Cape Nomamisaki, southwestern end of Kagoshima Prefecture, at a depth of 229 m, left side. A, the whole body, showing peculiar urostyloid process (arrow); B, anterior part, showing a black pigment spot in neural tube (white arrowhead), low (thin arrow) and high (thick arrow) intertentacular membrane, and short mid-ventral tentacle (black arrowhead); C, posterior part, showing atriopore (large asterisk), anus (small asterisk), abrupt ending of left metapleuron (thick arrow), and a vacuole between atriopore and anus (thin arrow). Scale bars indicate 1 mm.

has often been mentioned as a difference between the two (see, e.g., Poss and Boschung, 1996). However, this is not always clear (see Nishikawa and Nishida (1997) and the Discussion below). The only recent exception to the prevailing two-genus system is Piyakarnchana's (1962), where the genera *Asymmetron* and *Epigonichthys* are both treated as valid (for the generic distinction see the Discussion below).

It has, however, become clear by molecular analyses of many living species of both genera (Nohara *et al.*, accepted; Kon et al., submitted) that the genus Epigonichthys represents a group of paraphyly with the deepest branch between its species having urostyloid process such as seen in E. lucayanus and allies, and the remaining two monophyletic clusters (one composed of Epigonichthys species lacking the process and the other of Branchiostoma species). Thus, in the lancelets with gonads only on the right side, the presence of urostyloid process may well be regarded an apomorphic character-state to define one taxon at the generic level, markedly distinct from the other taxon composed of Epigonichthys species lacking the process. The former taxon should be called the genus Asymmetron, because its type species is A. lucayanum (=E. lucayanus), while the other the genus Epigonichthys (sensu stricto), which has been confirmed by the mentioned molecular analyses to include its type species, E. cultellus Peters, 1877. All the known species of the genus Branchiostoma lack the distinct urostyloid process, and therefore, its existence is unique to the genus Asymmetron among the lancelets.

The genus *Notasymmetron* Whitley, 1932 was established, with *Asymmetron caudatum* Willey, 1896 being its type species by the original designation; *A. caudatum* is treated a junior synonym of *A. lucayaum* (see, e.g., Poss and Boschung, 1996). Judging from the original description of the genus, it is doubtless that the genus *Notasymmmetron* was defined as the lancelets with gonads only on the right side and with a urostyloid process. Consequently, the genus can be treated as a junior synonym of *Asymmetron*.

For further considerations on morphological peculiarity of the genus *Asymmetron* see Nishikawa and Nishida (1997) and the Discussion below.

## Asymmetron inferum n. sp. (Fig. 1)

Holotype: Deposited in the Nagoya University Museum (NUM-Az 0363), collected from the sand near the bones of the sperm whale, during the Hyper-Dolphin/Natsushima cruise of JAMSTEC (NT03-08 leg1), off Cape Nomamisaki, Kagoshima Prefecture, Japan, 31°20.72'N, 129°59.29'E, 229 m deep, 27 July 2003, JAMSTEC No. 053540; fixed with 10% formalin and preserved in 70% ethanol.

Description: Body, 14.7 mm long and up to 1.5 mm high, elongated longitudinally and much flattened laterally, tapering gradually towards both ends (Fig. 1A); preatrioporal region 10.9 mm, atriopore to anus 1.0 mm, postanal 1.8mm, and urostyloid process 1.0 mm. Rostral fin extensive and semi-circular, clearly separable from other part by dorsal and ventral notches (Fig. 1B). Dorsal fin very low in anterior two-thirds of body, while becoming much higher posterially. Left metapleuron terminated abruptly and markedly just behind atriopore, while the right confluent with well-developed ventral fin (Fig. 1C), followed by thin and high caudal fin, somewhat higher than dorsal fin. Urostyloid process separable from dorsal and caudal fins by its own lower dorsal and ventral fins. Dorsal fin-chambers 270, relative length to width for posterior tall ones up to 3.3; preanal fin-chambers indiscernible, but instead, an elongated vacuole detected along ventral side of rectum between atriopore and anus (Fig. 1C). Myotomes 83 in total, consisting of 58 in preatrioporal region, 9 in region from atriopore to anus, and 16 in postanal, thus giving the myotome formula of 58+9+16=83. A black pigment spot detectable in neural tube just anterior to first myotome (Fig. 1B). Tentacular ring composed of ca. 30 tentacles (including a few dorsal-most minute ones), its anterior margin situated at level of axial (=proximal) part of 5th myotome; intertentacular membrane extremely higher in span of 6 tentacles next to much shorter midventral tentacle than elsewhere. Mid-gut coecum elongated, ranging at level from 20th to 48th myotomes. No gonad.

Remarks: The present specimen resembles A. lucayanum, as the only valid species of the genus and recorded so far from shallow waters of the Indo-West-Pacific and Western Atlantic regions, very much even in detailed features, excepting in the higher number of total myotomes; A. lucayanum is recorded to have 55-72 myotomes, while the present specimen has 83 (see Table 1). This marked morphological difference seems significant for the specific delimitation in the lancelet taxonomy. Moreover, the validity of this delimitation has been confirmed on molecular basis (Kon et al., submitted). Thus, the present specimen may well deserve a new species of the genus Asymmetron with the binomen A. inferum; the specific name derives from the latin adjective "inferus" (=low, underneath) after its deep-sea habitat. It may also be pointed out that A. inferum has far more dorsal fin-chambers than the previous records of A. lucavanum (270 in the former vs. 101-220 in the latter; for the details see Table 1), when Poss and Boschung's (1996) extraordinarily large counts attaining to 484 for the latter species are excluded.

The high number of myotomes in *A. inferum* is reminiscent of *B. elongatum* Sundevall, 1852 from the Peruvian and Chilean coasts and the Galapagos Islands with 78 myotomes in an average (ranging from 75 to 86) and *E. hectori* (Benham 1901) endemic to New Zealand with 84 (84 to 85) myotomes (the data from Poss and Boschung, 1996). However, these species lack urostyloid process completely.

The three specimens of this new species were examined before used for molecular analysis (see above), but rather insufficiently because they were somewhat deformed due to fixation with pure ethanol, collected during the Hyper-Dolphin/Natsushima cruise of JAMSTEC, from the same locality as the holotype, 28 July 2003 (JAMSTEC Nos.

Table 1. Comparison in the number of dorsal fin-chambers and myotome formula of A. inferum n. sp. with all the previous records with	
enough information of Asymmetron lucayanum and its junior synonyms (A. caudatum, A. macricaudatum, and A. orientale)	

		Average <sup>2)</sup> and range <sup>3)</sup> in number of						
Species name <sup>1)</sup>	Locality	myotomes					- References	
	opecies name	Locality	Locality	dorsal fin- chambers	preatrioporal	atriopore to anus	postanal	total
A. inferum n. sp. (n=1)	Off Kyushu, Japan 229 m deep	270	58	9	16	83	in the present study	
<i>A. lucayanum</i> (n=22)	Bahamas	N.D.	48.8 (42–46)	8.7 (8–9)	12.7 (11–14)	65.3 (62–68)	Andrews (1893)	
A. lucayanum (n=12)	Bahamas	N.D	(43–46)	(8–10)	(12–13)	(63–69)	Kirkaldy (1895)	
<i>A. lucayanum</i> (n=24) <sup>4)</sup>	NW Atlantic & Maldives	(170–180)	(42–46)	(8–9)	(11–14)	(62–68)	Bigelow & Farfante (1948)	
A. lucayanum	Red Sea ca. 11 m deep	N.D.	42.7 (41–44) (n=3)	6.9 (6–8) (n=7)	8.4 (7–10) (n=9)	57.7 (56–59) (n=3)	Steiniz (1962)	
A. luayanum (n=3)	Persian Gulf	(114–142)	(38–39)	(6–7)	(12–16)	(57–61)	Dawson (1964)	
<i>A. lucayanum</i> (n=28)	Maldives	N.D.	44.36 (43–46)	9.79 (9–13)	12.61 (11–13)	66.71(64–71)	Punnett (1903)	
A. lucayanum	New Caledonia	174.3 (130–220) (n=44)	44.8 (40–48) (n=43)	8.4 (6–10) (n=44)	13.3 (11–16) (n=44)	66.3 (63–72) (n=77)	Wickstead (1970)	
A. lucayanum (A) (n=9)	Solomon Isls. down to 35 m	161.4 (142–176)	39.2 (38–41)	8.1 (8–9)	11.9 (11–13)	59.2 (58–61)	Gibbs & Wickstead (1969)	
A. lucayanum (B) (n=9)	Solomon Isls. down to 35 m	189.5 (166–214)	44.3 (41–46)	8.7 (8–10)	13.1 (12–14)	66.1 (64–68)	Gibbs & Wickstead (1969)	
<i>E. lucayanus</i> (A) (n=27)	Great Barrier Reef, 22 m	N.D.	39-40 (39–41)	8–10 (7–12)	11–13 (10–14)	60-61 (59-64)	Gibbs & Wickstead (1996)	
<i>E. lucayanus</i> (B) (n=48)	Great Barrier Reef, 22 m	N.D.	43-45 (42–47)	9–11 (8–12)	12–15 (12–16)	66–69 (64–70)	Gibbs & Wickstead (1996)	
A. caudatum (n=2)	Papua New Guinea	N.D.	40, 44	9	11	60, 64	Willey (1897)	
E. lucayanus	Australia	133 (101–170) (n=13)	42.8 (39–46) (n=13)	9.2 (8–12) (n=13)	12.0 (9–15) (n=13)	63.8 (58–71) (n=14)	Richardson & McKenzie (1994)	
A. lucayanum (n=1)	Palau Isls.	170	41	9	11	61	Nishikawa (1980)	
<i>A. lucayanum</i> (n=14)	Mindro Is.	N.D.	43.1 (40–45)	9.4 (9–10)	11.6 (10–14)	64.3 (62–67)	Franz (1922)	
<i>A. lucayanum</i> (n=2)	Okinawa Is. ca. 15 m deep	140, 170	42, 44	9, 10	12	63, 66	Nishikawa (1980)	
<i>E. lucayanus</i> (n=151)	Various areas	307 (167–484)	36.5 (35–52[sic])	15.9 (13–18)	7.7 (6–9)	60 (55–62)	Poss & Boschung (1996)	

N.D.= No data <sup>1)</sup> A. lucayanum (A) and (B) mean two forms found sympatrically and distinguishable from each other by different number of myotomes (for further details see Discussion)

<sup>2)</sup> Bigelow and Farfante (1948), Dawson (1964), and Kirkaldy (1895) gave no data ; the figures of the New Caledonian and Mindro populations were calculated here based on Wickstead's (1970) and Franz's (1922) data, respectively; the figures of the Great Barrier Reef population indicate for each column the range "containing 85% or more of the samples", instead of the average, following Gibbs and Wickstead (1996).

<sup>3)</sup> In parentheses.

<sup>4)</sup> Includes the syntypes of *A. macricaudatum* Parker, 1904 from Florida and *A. orientale* Parker, 1904 from the Maldive Isls.

053515 to 053517) ; they were 10.7, 12.5, and 13.0 mm long, furnished with ca. 80 myotomes, with the left metapleuron ending abruptly and markedly just behind the atriopore, while the right confluent with well-developed ventral fin, with an urostyloid process, 0.58, 0.46, and 0.58 mm long, respectively, but without any preanal fin-chambers; gonads uncertain. The occurrence of urustyloid process, the marked asymmetry of metapleura, and ca. 80 myotomes are the reasons for the present identification, although the gonads were uncertain in the specimens.

Abandoned was my examination of Zietz's (1908, pp.

288-289) South Australian specimen of the "Genus, Asym[sic]etron, Andrews", the previous holder of the deepest record as stated above, because during the cruise it "was lost sight of for several days. When examining it later it was found to be decomposed".

## DISCUSSION

## Asymmetry of metapleura in generic definition

Piyakarnchana (1962) rightly distinguished Asymmetron from Epigonichthys (sense stricto), both having gonads only on the right, on the basis of the difference that the former genus was furnished with "right metapleuron continuous with ventral fin, left metapleuron terminating behind atriopore", while Epigonichthys with "both metapleura terminating a short distance behind atriopore" (p. 96). However, the metapleuran asymmetry, as claimed in Asymmetron by Piyakarunchana above, is often detectable (though unclearly) also in Epigonichthys, as discussed in detail by Nishikawa and Nishida (1997) on the basis of the examination of specimens and the literature survey. So far as I am aware, the mentioned asymmetry is markedly observable only in A. lucayanum and A. inferum because their ventral fin in between atriopore and anus is always high enough to allow for easy recognition of its continuity with the right metapleuron and the abrupt disappearance of the left one just behind the atriopore (see Fig. 1C). Thus, Piyakarynchana's definition of the genus Asymmetron is not adopted here.

# Some features peculiar to known species of Asymmetron

Nishikawa and Nishida (1997) claimed that Epigonichthys lucayanus (=A. lucayanum in the present study) has two more features unique among all the known species of lancelets, other than the existence of elongated urostyloid process and the marked metapleuran asymmetry stated above. One is the peculiar morphology of the intertentacular membrane which is "much higher around the ventral side of oral hood than laterally, where the tentacles on either side are interconnected only near their bases" (Bigelow and Farfante, 1948, p. 21), thus the membrane changes its height abruptly between lateral and ventral ones; further, the membrane of the ventral tentacles is also much higher than its ventral neighbor interconnecting the most ventral one of the tentacles with the midventral tentacle (see e.g., Fig. 6 of Pl. 13 of Andrews (1893); Fig. 2 of Nishikawa (1980)); in the other lancelets, the membrane's change in height is guite gradual, with the height gradually increasing ventrally, slightly (in many species of Branchiostoma) or remarkably (e.g., in Epigonichthys maldivensis; unlike Poss and Boschung's (1996) misinterpretation of its descriptions given by Nishikawa (1980) etc.). The other is the posterior shift of the tentacular ring, with its anterodorsal extremity located at (or sometimes behind) the 4th myotome; in other lancelets, the extremity is discerned around the 1st myotome. These two features are shared with A. inferum (see Fig. 1B).

### Preanal vacuole in A. inferum

In the holotype of *A. inferum*, a longitudinally elongated vacuole is detectable along the ventral side of the rectum between atriopore and anus (Fig. 1C). This structure is not detected in the pure ethanol-preserved specimens identified with this species, and therefore, may be unstable. Its true nature remains open until histological studies on additional material are performed. The structure might possibly be explained as the preanal fin-chamber. If this is true, *A. inferum* can also be distinguished from *A. lucayanum* by the

existence of the chamber in the former.

The lack of distinct preanal fin-chambers is recognized as a diagnostic feature of *A. lucayanum* by Poss and Boschung (1996), and followed in the present study. Although it is true that a few old records of this species show the existence of preanal fin-chambers (for the details see Bigelow and Farfante (1948)), there is agreement among all of the recent literature which includes any reference to this character that it is not present. Thus, the old information was put aside here, like Nishikawa (1986).

### New explanation on pelagic larvae with many myotomes

Gibbs and Wickstead (1996) proposed a beautiful hypothesis to explain the variation in the myotome formula of A. lucayanum, based on their discoveries that its certain adult populations were composed each of the two forms (A and B), distinguished from each other by the difference in myotome formula (see Table 1); typically, the total and preatrioporal numbers were 58-61 and 38-40, respectively in the form (A), while 65-69 and 43-45 in the form (B). The hypothesis suggests "that the full complement of myotomes was formed prior to the completion of metamorphosis" (for original references see Gibbs and Wickstead), and holds that the two forms are "derived from different larval stages", the form (A) "from an amphioxus larva-like stage that has metamorphosed after a short planktonic stage" and the form (B) "from the amphioxides stage which has undergone a long, more widely dispersed, planktonic existence" (p. 615). According to this hypothesis, amphioxides larvae with 83-88 total myotomes, which have been rarely collected offshore, are regarded to be "held in oceanic currents until death" (p. 524).

Another hypothesis is also possible, however, that the present new species *A. inferum*, so far known to have 83 total myotomes, may represent adults of the mentioned amphioxides larvae with 83–88. It is true that in the sea area not far from its type locality, two such larvae were recorded: one, having 80 myotomes, from the Tsushima Strait, less than 200 m deep, and the other with 83 myotomes from the southern end of East China Sea, 2,080 m (Nishikawa, 1981). Then, the forms (A) and (B) might possibly be two good species, instead of representing two phenotypic forms of a single species as predicted by Gibbs and Wickstead's hypothesis. Molecular analyses will be indispensable to examine the validity of these hypotheses.

#### ACKNOWLEDGEMENTS

Sincere thanks are due to Dr. Y. Fujiwara of JAMSTEC for the material, to Prof. M. Nishida and Dr. T. Kon of the Ocean Research Institute of University of Tokyo and Dr. M. Nohara of HITEC Co. for citation of the unpublished data of molecular analyses, to Prof. E. B. Cutler of Harvard University for critical reading of the manuscript. Financially supported in part by the Grants-in-Aid from JSPS (Nos. 13440253 and 16370044).

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(Received June 29, 2004 / Accepted September 29, 2004)