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New Japanese Localities for the Lancelets *Asymmetron lucayanum* complex and *Epigonichthys cultellus* [Cephalochordata], with Notes on their Northward Distribution Extensions

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Abstract The lancelet collection in the National Museum of Nature and Science, Tsukuba, includes a number of Japanese examples of the primarily tropical species *Asymmetron lucayanum* Andrews, 1893 complex and *Epigonichthys cultellus* Peters, 1876 from hitherto unreported localities, including Kushimoto (Wakayama Pref.), Kuchinoerabu-jima Is. (Kagoshima Pref.) and Kyoda, off Nago City, Okinawa Is. (Okinawa Pref.) for the former, and Ôshima Strait between Amami-ôshima Is. and Kakeroma-jima Is. (Kagoshima Pref.) for the latter. Some of these localities represent northward distribution extensions, although these are probably attributable to insufficient sampling efforts in the past, rather than to habitat extensions caused by recent sea warming in northern waters.

Key words: Cephalochordata, NW Pacific, distribution extension, global warming.

Introduction

The lancelet collection of the National Museum of Nature and Science, Tsukuba included Japanese examples of the primarily tropical *Asymmetron lucayanum* Andrews, 1893 species complex and *Epigonichthys cultellus* Peters, 1876. The former complex includes the three species, distinguishable from one another only by molecular analysis. Of the three, *A. lucayanum* (*sensu stricto*) inhabits the Atlantic Ocean, the other two species (both requiring future taxonomic revision) having been so far recorded only from the Indo-Pacific region (Kon *et al.*, 2006). Because the new locality records noted here represent northward distribution extensions of the above species, the possible impact of recent global warming on distribution is examined.

Materials and Methods

The specimens examined in the present study are deposited mainly in the National Museum of Nature and Science, Tsukuba (NSMT), supplemented by those in the Natural History Museum, London (BM), the Museum für Naturkunde der Humboldt-Universität zu Berlin (ZMB), and the Osaka Museum of Natural History (OMNH). A stereo microscope was used for morphological observations, measurements of body length (utilizing calipers), and myomere counts; specimens were not stained. All of the specimens had been fixed with formalin, making them unsuitable for molecular analysis.

Taxonomy and Distribution

Asymmetron lucayanum Andrews, 1893 complex spp. A and B

(Fig. 1)

Asymmetron lucayanum complex spp. A and B: Nishikawa, 2017, pp. 707–708, fig. 27.1b–c.

Asymmetron lucayanum complex clades A and B: Kon *et al.* 2006, pp. 875–883.

Material examined. Fixed with formalin and preserved in 70% ethanol. NSMT-Pc 1175 (immature, deteriorated), Kushimoto, Kii Peninsula, Wakayama Pref., 8 Sept. 1978, Kazushi Okamoto *et al.* coll.; Pc 1148 (immature), Nishinohama beach, Kuchinoerabujima Is., Ohsumi Islands., Kagoshima Pref., 9 m, 10 Sept. 1986, Susumu Ohtsuka coll., SCUBA; Pc 1147 (immature), Kyoda, Nago City, Okinawa Is., Okinawa Pref., 5 m, 14 Dec. 1995, Hirofumi Kubo coll.; Pc 1149 (2 spms., immature), West off Naha City, Okinawa Is., Okinawa Pref., 26°13.60′N, 127°32.63′E, 52 m, 14 May 1991, S. Ohtsuka coll. From Kuroshima Is., Okinawa Pref., all spms. coll. using SCUBA, immature: Pc 1129 (2 spms), Nakamoto, 20 m, 2 July 1986, Hirokuni Noda coll. [described by Noda and Nishikawa, 1989]; Pc 1130 (5 spms.), Nishinohama, depth unknown, 18 Oct. 1997, Hiro'omi Uchida coll.; Pc 1131 (8 spms.), Fuzumari, 5 m, 15 Dec. 1997, H. Uchida coll.; Pc 1132 (2 spms.), Nishino-

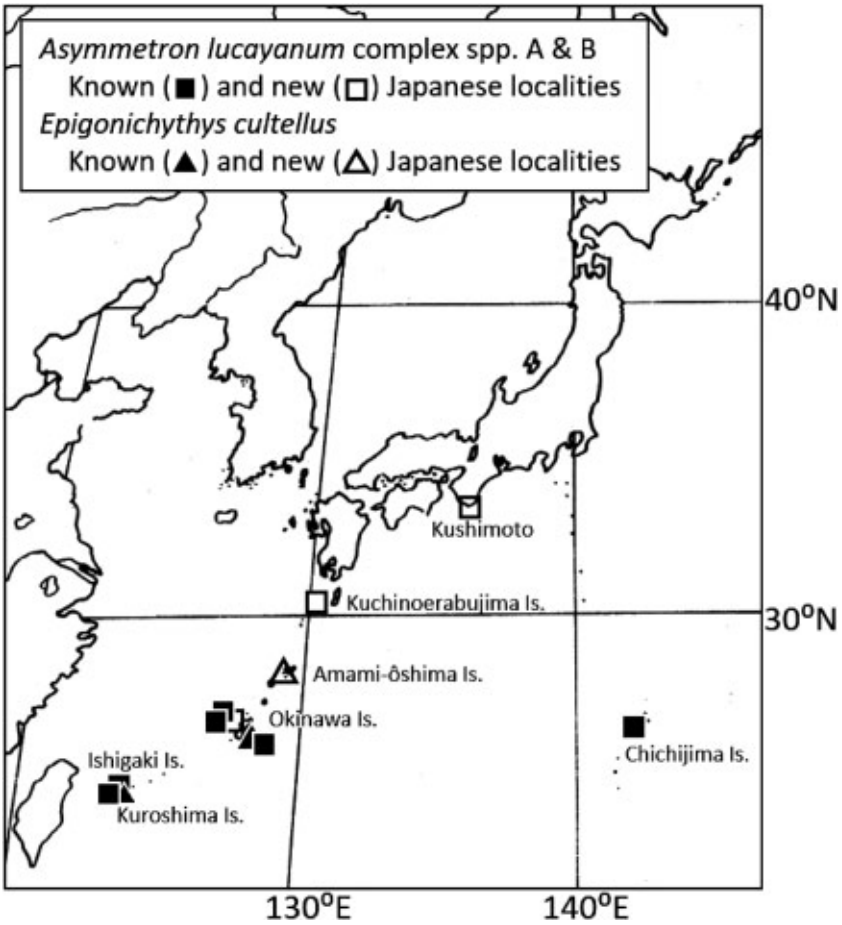

Fig. 1. Map showing known and new Japanese localities of *Asymmetron lucayanum* complex and *Epigonichthys cultellus*.

hama, 8 m, 17 Dec. 1997, H. Uchida coll.; Pc 1133, off Kuroshima Hbr, 30 m, 18 Apr. 1998, H. Uchida coll.; Pc 1134, Fuzumari lagoon, depth unknown, 20 Apr. 1998, H. Uchida coll. From Miyasato lagoon, Kuroshima Is., Okinawa Pref., 2.2–4.5 m, all spms. coll. using SCUBA, coll. Kenji Kuroyanagi, immature unless otherwise noted [described by Kuroyanagi and Nomura, 2000]: Pc 1135 (8 spms., mature), 8 May 1998; Pc 1136 (12 spms., mature), 21 June 1998; Pc 1137 (11 spms, mature),11 July 1998; Pc 1138 (2 spms., mature), 9 Aug. 1998; Pc 1139 (7 spms., mature), 3 Sept. 1998; Pc 1140 (3 spms., mature), 8 Oct. 1998; Pc 1141, 5 Nov. 1998; Pc 1142, 21 Jan. 1999; Pc 1143 (4 spms.), 22 Feb. 1999; Pc 1144, 20 Mar. 1999; Pc 1145 (5 spms.), 17 Apr. 1999; Pc 1146 (7 spms., mature), 25 May 1999. OMNH-Iv 6238 (immature), Amitori Bay, Iriomote Is., Okinawa Pref., Apr. 1995 [listed by Yamanishi *et al.*, 1998].

Description. Body 6.6 to 24.2 mm in length [20 mm in northern-most (Kushimoto) specimen (Pc 1175)]. Urostyloid process distinct, 0.3 mm in length in 6.6 mm specimen (Pc 1148). Number of myomeres *ca.* 64 to 68. No preanal fin-chambers. Anterior margin of buccal tentacular ring situated at level of axial (= proximal) part of 6th or 7th myotome. Intertentacular membrane abruptly higher between 4 or 5 longer tentacles than between adjacent shorter tentacles. Gonads discernible on right side only. Metapleurae markedly asymmetrical. About 12 U-shaped gill slits on each side in a 6.6 mm long specimen (Pc 1148) probably represent an early post-metamorphosis stage.

Remarks. Of the Japanese lancelets belonging to the *A. lucayanum* complex, a molecular analysis by Kon *et al.* (2006) of Kuroshima Island specimens showed both species to be represented (see above). Because the specimens examined here had been formalin-fixed, they can no longer be subjected to molecular analysis and their specific identity remains unknown; hence their present identification as "*A. lucayanum* complex spp. A and B."

In Japanese waters, the complex has so far been reported (in chronological order) from Sesokojima Is., off Nago City of Okinawa Is. in 1978 (Nishikawa, 1979, 1980); from Kuroshima Is., Yaeyama Islands in 1986 (Noda and Nishikawa, 1989) and in 1998–1999 (Kuroyanagi and Nomura, 2000); from Chichijima Is., Ogasawara Islands in 1993–1994 (Tachikawa and Nishikawa, 1997); from Amitori Bay, Iriomote Is. in 1995 (Yamanishi *et al.*, 1998; present study); from Kabira Bay, Ishigaki Is. in 2007 (Nishikawa and Kubo, unpublished); from west of Naha City, Okinawa Is. in 2010 (Urata and Ohtsuka, 2014); and from Kin Bay, Okinawa Is. in 2012 (Nishikawa and Kon, unpublished). The new Japanese localities [Kushimoto (NSMT-Pc 1175), Kuchinoerabujima Is. (Pc 1148), and Kyoda (Pc 1147)] are indicated in Fig. 1.

Northward extensions to the reported distribution of the complex are represented here by two specimens, collected from Kushimoto, Kii Peninsula in 1978 and Kuchinoerabujima Is., Ohsumi Islands in 1986, respectively. The dates of these records, particularly that from Kii Peninsula, indicate insufficient sampling efforts in the past, rather than a northward expansion of habitat caused by recent warming of northern waters.

Epigonichthys cultellus Peters, 1876

(Figs. 1–2 and Table 1)

Epigonichthys cultellus Peters, 1876, pp. 325–327, figs. 1–5; Whitley, 1932, p. 260; Richardson and McKenzie, 1994, pp. 1453–1454, text-fig. 1B and tab. 5; Poss and Boschung, 1996, pp. S62–S63; Nishikawa and Nishida, 1997, p. 243; Li *et al.* 2015, pp. 780–790; Nishikawa, 2017, pp. 710–711.

Amphioxus (*Epigonichythys*) *cultellus*: Semon, 1893, p. 7.

Asymmetron cultellus: Franz, 1922, pp. 418–424, text-figs. 26–28; Gibbs and Wickstead, 1969, p. 139 and tab. 4; Wickstead, 1970, p. 239.

Asymmetron cultellus ?: Franz, 1926, pp. 221–222, text-figs. 1–2.

Asymmetron cultellum: Tattersall, 1903, pp. 219–220, pl. 1, figs. 8–10; Tchang, 1962, pp. 525–528, text-figs. 1–2.

Branchiostoma cultellum: Günther, 1884, pp. 32–33; Willey, 1894, pp. 361–370, 1 unnumbered text-fig.

Heteropleuron cultellum: Kirkaldy, 1895, pp. 316–317, pl.

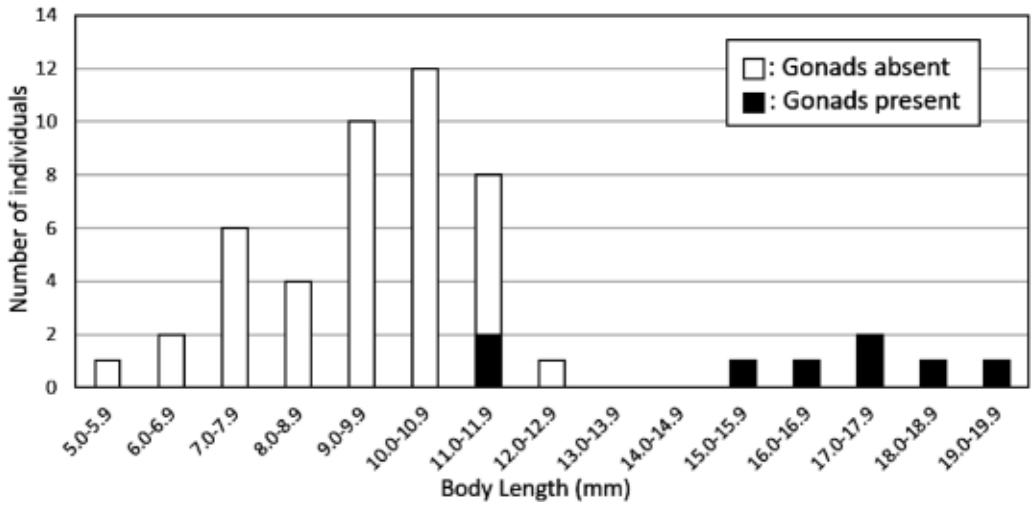

Fig. 2. Body length compositions of 50 specimens of *Epigonichthys cultellus* from Ôshima Strait (NSMT-Pc 1224).

34, fig. 2; Römer, 1896, pp. 113–115.

Bathyamphioxus franzi Whitley, 1932, p. 260 (Established by a bibliographical reference to Franz's (1926) "*Asymmetron. cultellus* ?" from Shark Bay; later synonymized with *Epigonichthys cultellus* by Richardson and McKenzie (1994, see above)).

Heteropleuron hedleyi Haswell, 1908, pp. 33–35, text-fig. 1

"Branchiostomatidae, genus and species *incertae sedis*": Kon *et al.*, 2004, pp. 48–50, 1 text-fig. and 1 tab.

Material examined. Ôshima Strait between Amami-ôshima Is. and Kakeroma-jima Is. Fixed with formalin, preserved in 70% ethanol. NSMT-Pc 1223, 50 m, 1 July 1989, Minoru Imajima coll., dredge (Stn. 5); Pc 1224 (50 spms.), 65 m, 1 July 1989, M. Imajima coll., dredge (Stn. 4). ZMB 3729 (3 out of 5 spms.; those having 60 or 61 myomeres excluded) [described by Franz, 1922], Bagamoyo, Exp. "Prinz Adalbert" [described by Franz, 1922]. BM 1972.1.21.18-46 (18 out of 22 spms.; remaining 4 identifiable with *A. lucayanum* complex), Maravo Lagoon, Solomon Islands, 2–18 m, Royal Society Expedition to the Solomon Islands, 29 Oct to 10. Nov. 1965 [described by Gibbs and Wickstead, 1969].

Description. Body 5.3 mm to 19.0 mm in length. No urostyloid process. Up to 17 or more gonads on right side only in 11.5 mm or longer specimens. Metapleural ending symmetrical. Dorsal fin-chambers ca. 200 to 220 in number, length/width ratio ca.4.5. Preanal fin-chambers 20–25, length/width ratio *ca.* 1.5. Number of myomeres 51 to 56 (ave. 53.8, n = 16), myomere formula 34 + 12 + 9 = 55 in a very well preserved 18.3 mm specimen (Pc 1224).

Remarks. Figure 2 shows the body length compositions of 50 specimens (NSMT-Pc 1224), probably representing two different cohorts (smaller mostly immature and larger mature) in a well-established local population.

According to a global molecular phylogenetic analysis of the genus *Epigonichythys* (in prep., see also Kon *et al.*, 2011), cryptic speciation within the genus is highly likely, implicating *E. cultellus*. Therefore, the synonyms and records of this species listed above may need to be revised in the future. In the analyses, many Indo-West Pacific samples, including those from Okinawa, which conformed morphologically to *E. cultellus*, constituted a single cluster markedly distinct from other clusters. The former cluster included samples from the Great Barrier Reef and New Caledonia, and despite a lack of comparable material from the type locality of *E. cultellus* (Moreton Bay) is likely referable to that species. It further holds that Kon *et al.*'s (2004) immature specimen, at that time referred to as "Branchios-

Table 1. A morphological comparison of lancelets referred to *Epigonichthys cultellus*

Locality and depth	Body length	Number of myomeres	Number of dorsal fin-chambers	Number of preanal fin-chambers	Asymmetrical ending of metapleurae	Remarks & references
Ôshima Strait, 50–65 m	5.3–19.0 mm (n = 51)	51–56 (n = 16)	200–220	20–25	No	Present study
Nakagusuku Bay, Okinawa Is., 9 m	25.8 mm (n = 1)	54	227	21	No	Kon *et al.* (2004)
Kin Bay, Okinawa Is.	16.5 mm (n = 1)	51	*ca.* 185	*ca.* 20	Uncertain	Nishikawa and Kon (unpublished)
Off Shantou, South China Sea and Gulf of Tonkin, 0–49 m	8.5–21.5 mm (n = ?)	48–55	*ca.* 220	*ca.* 20	No	Tchang (1962); information on metapleuran asymmetry from figure; also from Hong Kong (Chen *et al.*, 2007)
Maravo lagoon, New Georgia Group, Solomon Islands, 2–18 m	8.3–25.3 mm (n = 29)	49–52	182–228	13–19	Yes, but obscure	Gibbs and Wickstead (1969); also re-examination of specimens registered as BM 1972.1.21.18–46
Bay of St. Vincent, New Caledonia	ND	*ca.* 202–219 (n = 3)	51–52	ND	Yes, but obscure	Wickstead (1970); Nishikawa and Nishida (1997)
Ternate, Indonesia	33 mm (n = 1)	52	239	ND	ND	Römer (1896); number of dorsal fin-ray chambers from Franz (1922)
Thursday Is., Torres Strait	ND	52 (n = 2?)	ND	ND	ND	Günther (1884)
Thursday Is., Torres Strait	40 mm (n = 1)	53	240	ND	ND	Franz (1922); possibly the same material collected by Semon (1893) between Friday Is. and Prince of Wales Is., close to Thursday Is.
Murray Is., Torres Strait, 5–8 ftms	28 mm (n = 3)	54	ND	ND	ND	Haswell (1908)
Torres Strait, 6–30 ftms	Up to 35 mm (n = ?)	51–55	ND	24–27	ND	Willey (1894)
Torres Strait	ND	50–56	170–200	ND	Yes, markedly	Willey (1894); Kirkaldy (1895) (same material); information on metapleuran asymmetry from figure
Moreton Bay, E Australia, 8 ftms (Type locality)	12.0–22.0 mm (n = 4)	49–50	*ca.* 260 (n = 1)	0? (n = 2)	Yes, but obscure	Name-bearing type specimens; Nishikawa (2017)
Shark Bay, W Australia, 6–9 m	17.5 mm (n = 1)	48	214	ND	ND	Franz (1926)
New South Wales, Queensland, and Western Australia	Up to 34 mm (n = 61)	46–54 (n = 66)	180–254 (n = 66)	14–31 (n = 54)	ND	Richardson and McKenzie (1994)
Tuticorin, India, 8–10 ftms	22.6–24 mm (n = 2)	55–57	ND	ND	ND	Franz (1922)
W coast of Ceylon Is., 3–6 ftms	23–25 mm (n = 2)	54–55	ND	ND	ND	Tattersall (1903)
Bagamoyo, Tanzania	11.5–16.5 mm (n = 3)	50–52	205–220	18–22	Yes, but obscure	Three of 5 specimens registered as Nr. 1573 (now ZMB 3729). Described as "Nr. 1–3" of "*Asymmetron cultellus*" by Franz (1922); Nishikawa and Nishida (1997); present study

tomatidae, genus and species *incertae sedis*", may also prove to be *E. cultellus* since it shares 54 myomeres with the latter (Nishikawa, 2017).

Table 1 compares some morphological characters of specimens so far referred to *E. cultellus* and provides a rough outline of the mainly tropical distribution of the species, supplemented in the NW Pacific by Lin *et al.*'s (2015) records from *ca.* 30–45 m deep on the Taiwan Banks (Taiwan Strait), surveyed from 1999 to 2003 and the closest locality to the Japanese records for the species.

In Japanese waters, *E. cultellus* has so far been reported (in chronological order) from Kuroshima Is., Yaeyama Islands in 2001 (used for molecular analyses by Kon *et al.*, 2011), Nakagusuku Bay, Okinawa Is. in 2003 (Kon *et al.*, 2004; Tab. 1) and from Kin Bay, Okinawa Is. in 2012 (Nishikawa and Kon, unpublished; Tab. 1). Although Ôshima Strait is newly-reported here as a northward extension of the distribution range (Fig. 1), the specimens were collected in 1989 (NSMT-Pc 1223, 1224), before those collected from Taiwan. As in the previous species account, insufficient sampling efforts is a likely reason for the distribution extension, rather than a northward habitat expansion as suggested by Nishikawa (2017).

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References

Chen, Y., S. G. Cheung, R. Y. C. Kong and P. K. S. Shin 2007. Morphological and molecular comparisons of dominant amphioxus populations in the China seas. Marine Biology, 153: 189–198.

Franz, V. 1922. Systematische Revision der Akanier. Jenaische Zeitschrift für Naturwissenschaft, 58: 369–452.

Franz, V. 1926. Acrania. In: Michaelsen, W. and R. Hartmeyer (eds.): Die Fauna Südwest-Australiens, vol. 5, pp. 219–222, Gustav Fischer, Jena.

Gibbs, P. E. and J. H. Wickstead 1969. On a collection of Acrania (Phylum Chordata) from the Solomon Islands. Journal of Zoology, London, 158: 133–141.

Günther, A. 1884. Reptilia, Batrachia, and Pisces. In: Anonymous (ed.): Report on the zoological collections made in the Indo-Pacific Ocean during the voyage of H.M.S. 'Alert' 1881-2, pp. 29–33, Trustees of the British Museum (Natural History), London.

Haswell, W. A. 1908. Note on the Cephalochorda in the Australian Museum. Records of Australian Museum, 7: 33–35.

Kirkaldy, J. W. 1895. A revision of the genera and species of the Branchiostomidae. Quarterly Journal of Microscopical Science, 37: 303–323.

Kon, T., M. Nishida and T. Nishikawa 2011. A molecular phylogenetic analysis of the genus *Epigonichthys* (Cephalochordata) and the discovery of a species new to Japan. In: Abstracts of the 48th annual meeting of the Biological Society of Okinawa, University of the Ryukyus, Nishihara, 4 June 2011.

Kon, T., M. Nohara, M. Nishida, W. Sterrer and T. Nishikawa 2006. Hidden ancient diversification in the circumtropical lancelet *Asymmetron lucayanum* complex. Marine Biolology, 149: 875–883.

Kon, T., Y. Sakurai and T. Yoshino 2004. A lancelet collected from Nakagusuku Bay, Okinawa Island. Biological Magazine Okinawa, 42: 47–52.

Kuroyanagi, K. and K. Nomura 2000. Population survey of a lancelet species, *Epigonichthys lucayanus* around Kuroshima Island, Yaeyama Group, the Southern Ryukyus. Nankiseibutu, 42: 85–88.

Lin, H. C., J. P. Chen, B. K. K. Chan and K. T. Shao 2015. The interplay of sediment characteristics, depth, water temperature, and ocean currents shaping the biogeography of lancelets (Subphylum Cephalochordata) in the NW Pacific waters. Marine Ecology, 36: 780–793.

Nishikawa, T. 1979. A short report on the lancelet *Asymmetron lucayanum* Andrews, 1893 from Sesokojima Island, Okinawa. Biological Magazine Okinawa, 17: 15–18.

Nishikawa, T. 1980. Records of two lancelet species, *Asymmetron maldiense* and *A. lucayanum*, from the western Pacific. Publications of the Seto Marine Biological Laboratory, 25: 167–173.

Nishikawa, T. 2017. Chapter 27 Taxonomic review of lancelets (Cephalochordata) in Japanese waters. In:

Motokawa, M. and H. Kajihara (eds.): Species Diversity of Animals in Japan, Diversity and Commonality in Animals, pp. 703–714, Springer Japan, Tokyo.

Nishikawa, T. and M. Nishida 1997. Problems in lancelet systematics. In: Kawashima, S. and S. Kikuyama (eds.): Advances in Comparative Endocrinology. 13th International Conference of Comparative Endocrinology, Yokohama, 17–21 Nov 1997, vol. 1, pp. 241–246, Monduzzi Editore, Bologna.

Noda, H. and T. Nishikawa 1989. New finds of the two lancelet species, *Asymmetron lucayanum* and *A. maldivensis*, on the coast of Kuroshima Island, Yaeyama Islands. Nankiseibutu, 31: 37–38.

Peters, W. 1876. Über *Epigonichythys cultellus*, eine neue Gattung und Art der Leptocardii. Monatsberichte der Königlichen Preussische Akademie des Wissenschaften zu Berlin, 1876: 322–327.

Poss, S. G. and H. T. Boschung 1996. Lancelets (Cephalochordata: Branchiostomatidae): how many species are valid? Israel Journal of Zoology, 42, Supplement, S13–S66.

Richardson, B. J. and A. M. McKenzie 1994. Taxonomy and distribution of Australian cephalochordates (Chordata: Cephalochordata). Invertebrate Taxonomy, 8: 1443–1459.

Römer, F. 1896. Über des Vorkommen von *Heteropleuron cultellum* J. W. Kirkaldy bei Ternate. Zoologischer Anzeiger, 19: 113–115.

Semon, R. 1893. Reisebericht und Plan der Werkers. Denkschriften der Medicinisch-Naturwissenschaftlichen Gesellschaft zu Jena, 4 (Zoologische Forschungsreisen in Australien und dem Malayischen Archipel von Dr. Richard Semon, 1): 1–10.

Tachikawa, H. and T. Nishikawa 1997. First finds of the circum-tropical lancelet *Epigonichthys lucayanus* (Andrews) from Chichijima Island, Ogasawara Islands, Japan (Cephalochordata). Nankiseibutu, 39: 145.

Tattersall, W. T. 1903. Report on the Cephalochorda collected by Professor Herdman, at Ceylon, in 1902. In: Herdman, W. A. (ed.): Report to the Government of Ceylon on the pearl oyster fisheries of the Gulf of Manaar, pp. 209–226, The Royal Society, London.

Tchang, S. 1962. Sur la presence du genre *Asymmetron* dans la mer de Chine et la distribution géographique de *Branchiostoma belcheri* (Gray). Acta Zoologica Sinica, 14: 525–528.

Urata, M. and S. Ohtsuka 2014. Occurrence of *Asymmetron lucayanum* Andrews, 1893 complex off Naha, Okinawa. Bulletin of the Setouchi Field Science Center, Graduate School of Biosphere Science, Hiroshima University, 12: 21–25.

Whitley, G. P. 1932. The lancelets and lampreys of Australia. Australian Zoology, 7: 256–264.

Wickstead, J. H. 1970. On a small collection of Acrania (Phylum Chordata) from New Caledonia. Cahiers du Pacifique, 14: 237–243.

Willey, A. 1894. Report on a collection of amphioxus made by Professor A. C. Haddon in Torres Straits, 1888-9. Quarterly Journal of Microscopical Science, 35: 361–371.

Yamanishi, R., Y. Ide, M. Hanaoka and K. Hatooka 1998. Macrobenthic fauna on the sandy bottom of Amitori Bay, Iriomote Island, the Ryukyus, Southern Japan: Results of surveys in April and October of 1995. Bulletin of Institute of Oceanic Research and Development, Tokai University, 19: 79–86.