

Report of a chiton in the genus *Leptochiton* (Lepidopleurida: Lepidopleurina: Leptochitonidae) from the Yap Trench in the West Pacific Ocean

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Received 1 December 2017; accepted 1 February 2018

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Abstract

Two chiton specimens were collected from sedimentary habitat by China's manned Jiaolong submersible diving to a depth of 6 754 m in the north of the Yap Trench. This is a new locality record for chiton. Both morphological and molecular data support that the two specimens are the same species belonging to the genus *Leptochiton*. Morphologically, this species strongly resembles *L. vanbellei* and *L. deforgesii*. Phylogenetically, it has a close evolutionary relationship with *L. vanbellei*, *L. deforgesii* and *L. boucheti*. This is the third deepest record for deep-sea chitons so far.

Key words: chiton, *Leptochiton*, the West Pacific Ocean, the Yap Trench

Citation: Wang Chunsheng, Zhou Yadong, Jiang Dan, Han Jie. 2018. Report of a chiton in the genus *Leptochiton* (Lepidopleurida: Lepidopleurina: Leptochitonidae) from the Yap Trench in the West Pacific Ocean. Acta Oceanologica Sinica, 37(10): 205–208, doi: 10.1007/s13131-018-1327-9

1 Introduction

The Yap Trench is located on the southeastern boundary of the Philippine Sea Plate in the West Pacific Ocean. The trench forms the part of the Pacific Ring of Fire between the Palau Islands and the Mariana Trench. It is about 700 km long and nearly 9 000 m deep at its deepest point (Fujiwara et al., 2000). Studies on fauna of the Yap Trench are scarce.

Lepidopleuran chitons represent the earliest-diverging living polyplacophorans and are found predominantly in the deep sea, including sunken wood, cold seeps, other abyssal habitats, and occasionally in shallow water at temperate latitudes (Sigwart et al., 2011). Approximately 130 living species are known within the order Lepidopleurida, and all within the extant suborder Lepidopleurina (Sirenko, 2006).

Two chiton specimens were collected from sedimentary habitat by China's manned Jiaolong submersible diving to a depth of 6 754 m in the north of the Yap Trench (9.867 5°N, 138.515 6°E) on May 18, 2016. This is a new locality record for chiton. As all specimens were collected and preserved as part of bulk samples in the field, the two chitons had suffered some post-mortem abrasion from overcrowding in the sample jars and transportation. The larger one was tightly curled (Fig. 1). Thus, characterization of radula and detailed gill arrangement was not applicable. The specimens were fixed in 100% ethanol and then preserved in 70% ethanol. The general morphology was examined focusing on shell and girdle. Their molecular phylogenetic positions in the order Lepidopleurida were reconstructed using mitochondrial cytochrome c oxidase I (COI) gene and nuclear 28S ribosomal

RNA gene (28S rDNA).

The two whole chitons were surface dried, then morphological features were examined using a Zeiss Discovery V12 stereomicroscope and photographed using an AxioCam MRC digital camera (Carl Zeiss MicroImaging Inc., Germany). The preserved lengths were ~9.5 mm and 6 mm respectively. Morphologic description was according to published matrix of Sigwart (2009) and key in Sigwart and Sirenko (2012). Total genomic DNA was isolated from foot tissue using a CTAB (hexadecyltrimethylammonium bromide) protocol (Marko, 2002). Polymerase chain reaction (PCR) amplification of the mitochondrial COI gene was carried out using primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). The 28S rDNA was amplified with primers 28SF1 (5'-ACCCGCTGAATTTAAGCATAT-3') modified from the primer F63mod in Medina et al. (2001) and 28SR1 (5'-CCATTTAAAGTTTGAGAATAGGT-3') which is the reverse complement of primer 28S rd4.8a in Giribet et al. (2006). The 25 µL volume reaction mixture contained 100 ng genomic DNA, 0.15 µmol/L of each primer, 12.5 µL 2×EasyTaq PCR Super-Mix (-dye) (TransGen Biotech, Beijing). The reaction cycling profile involved an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 50°C (for COI)/52°C (for 28S rDNA) for 45 s and extension at 72°C for 2 min, and a final extension at 72°C for 10 min. PCR products were purified using the E.Z.N.A.® Gel Extraction Kit (Omega Bio-tek, Inc., GA, USA), and then were bidirectional sequenced using the PCR primers and the BigDye® Terminator Cycle Sequencing Kit

Foundation item: The National Program on Key Basic Research Project of China under contract No. 2015CB755902.

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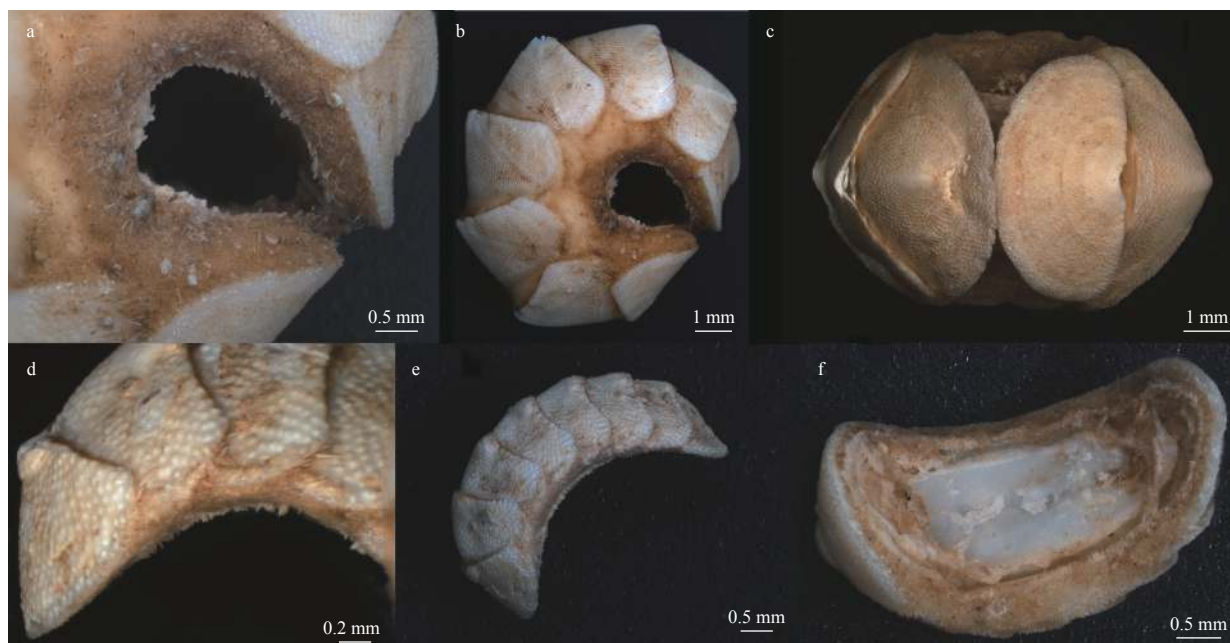


Fig. 1. The two specimens of *Leptochiton* sp. from the Yap Trench. a–c. The larger specimen and d–e. the smaller specimen. In all images, the anterior end is to the left.

(v3.1) on an ABI PRISM[®] 3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The forward and reverse sequences were assembled using SeqMan (Lasergene Version 7; DNASTAR, Inc., Madison, WI, USA), and the assembled files were checked by eye. Then homologous sequences were tested using NCBI BLAST (National Center for Biotechnology Information basic local alignment search tool) to confirm that they corresponded with known polyplacophoran sequences deposited in GenBank. To reconstruct the two chitons phylogenetic positions in the order Lepidopleurida, COI and 28S rDNA sequences used for molecular phylogenetic study (Sigwart et al., 2011) were included. Multiple alignments were performed using ClustalW (Thompson et al., 1994), then the shortest common length was used for phylogenetic reconstruction. Neighbour joining (NJ) trees of the two genes were reconstructed with the Kimura 2-parameter (K2P) model of nucleotide substitution. The nodal reliabilities were assessed using 1 000 non-parametric bootstrap replicates. Sequence alignments, data sets preparations and phylogenetic analyses were carried out using MEGA 6 (Tamura et al., 2013).

2 Systematics

Class Polyplacophora Gray, 1821
 Order Lepidopleurida Thiele, 1910
 Family Leptochitonidae Dall, 1889
 Genus *Leptochiton* Gray, 1847
Leptochiton sp. (Fig. 1).

External morphology. Animal small and elongate. Shell semicarinated, with low elevation (elevation ratio 0.33–0.35 in intermediate valves), valves slightly beaked. Head valve semicircular, of equal width to tail valve. Intermediate valves rectangular, lateral areas not raised, slightly convex anterior margin. Tail valve with median inflated mucro, postmucronal slope straight. Tegumentum densely sculptured with raised, round granules arranged in irregular quincunx. Apophyses subtriangular. Girdle

narrow, dorsally and ventrally covered in elongate, bluntly pointed spicule-scales, distinctive long and sharp chitinous bristles in intersegmental areas. Gills arranged posteriorly.

Genetic sequences. Two COI gene sequences were 684 bp in length after exclusion of the ambiguous region, and there were three polymorphic sites between them (GenBank accession numbers: MG788319 and MG788320), with the genetic *p* distance of 0.004. A BLAST-n of COI resulted 89%, 88% and 87% identical to sequences of *Leptochiton boucheti* (GenBank accession number: JQ950261), *Leptochiton vanbellei* (HQ907871) and *Leptochiton deforgesi* (HQ907856), respectively. The assembled 28S rDNA fragments were 1 132 bp, and there were two variation sites between the two sequences (GenBank accession numbers: MG788321 and MG788322), with the *p* distance of 0.002. A BLAST-n of the two sequences resulted 98%–99% identical to sequences of *L. boucheti* (GenBank accession numbers: HQ907809 and HQ907810), 97%–98% identical to sequences of *Leptochiton* sp. n. 4 (HQ907809 and HQ907810) and 96% to sequence of *L. vanbellei* (HQ907831), respectively.

After alignment analyses, 32 COI and 29 28S rDNA sequences from 36 lepidopleuran taxa (Table 1) were included in NJ tree reconstruction, and the shortest common lengths of which were 554 bp and 993 bp respectively. NJ trees of both COI and 28S rDNA (Fig. 2) showed the two chiton specimens, *Leptochiton* sp._1 (the larger one) and *Leptochiton* sp._s (the smaller one), falling within Clade II which is primarily made up of species found living in sunken wood deposits and from the tropical West Pacific in the phylogenetic study of Sigwart et al. (2011).

Remarks. Full species level description of these two specimens was not possible. Morphologically, this species strongly resembles *L. vanbellei* and *L. deforgesi*. Phylogenetically, it has a close evolutionary relationship with *L. vanbellei*, *L. deforgesi* and *L. boucheti*. This has been the third deepest record for deep-sea chitons so far (Sigwart and Sirenko, 2012).

Table 1. GenBank accession numbers and collection and locality data for specimens used in phylogenetic analyses

	28S rDNA	COI	Species biogeographic region and specimen locality
Lepidopleurida: Ferreiraellidae			
<i>Ferreiraella plana</i>	HQ907795	HQ907844	the southwestern Pacific, Vanuatu; 770–799 m; 2005
<i>Ferreiraella xylophaga karenae</i>	HQ907798	HQ907846	the southwestern Pacific, Vanuatu: Big Bay; 593–630 m; 2005
Lepidopleurida: Hanleyidae			
<i>Hanleya nagelfar</i>	HQ907799		the northern Atlantic/Mediterranean, Iceland: Bioice Sta. 3589; 2002
Lepidopleurida: Leptochitonidae			
<i>Lepidopleurus cajetanus</i>	HQ907802	HQ907847	the northern Atlantic/Mediterranean, Spain: Tossa de mar, Girona; ~10 m; 1997
<i>Leptochiton aequispinus</i>	HQ907803	HQ907848	Japan: Sagami Bay; 240–418 m; 2002
<i>Leptochiton algesirensis</i>	HQ907804	HQ907849	the northern Atlantic/Mediterranean, Italy: Sardinia, S'Archittu; 2003
<i>Leptochiton asellus</i>	HQ907807	HQ907851	the northern Atlantic/Mediterranean, Norway: Aksnestangen, Trondheim; 50–200 m; 2004
<i>Leptochiton boucheti</i>	HQ907809	HQ907853	the southwestern Pacific, Vanuatu: Big Bay; 773–900 m; 2005
<i>Leptochiton cancellatus</i>		HQ907855	the northern Atlantic/Mediterranean, France: Bretagne, off Roscoff; 8 m; 2003
<i>Leptochiton deforgesii</i>	HQ907812	HQ907856	the southwestern Pacific, Vanuatu: Big Bay; 773–900 m; 2005
<i>Leptochiton denhartogi</i>		HQ907858	the eastern Atlantic, Angola: 17°09'S, 11°21'E; 2004
<i>Leptochiton foresti</i>	HQ907815	HQ907860	the southwestern Pacific, Philippines: Bohol Sea, off Pamilacan Island; 273–356 m; 2005
<i>Leptochiton cf. giganteus</i>	HQ907801	HQ907873	the eastern Pacific/northern Pacific, USA: California, Cortes Bank; 367–389 m; 2007
<i>Leptochiton hirasei</i>	HQ907816	HQ907861	Japan: Shibusaki, Miura Peninsula, Japan, intertidal; 2006
<i>Leptochiton intermedius</i>	HQ907817		the northern Atlantic/Mediterranean, Croatia: Istira, Rovinje, Punta Corente; 0–4 m; 2004
<i>Leptochiton japonicus</i>	HQ907818	HQ907862	Japan: Sagami Bay; 94–95 m; 2002
<i>Leptochiton juvenis</i>	HQ907819	HQ907863	the southwestern Pacific, Vanuatu; 618–641 m; 2005
<i>Leptochiton kerguelensis</i>		HQ907864	Antarctica, South Georgia and South Sandwich Islands, 56°42.55'S, 27°57.02'W; 332.3–356.0 m; 2002
<i>Leptochiton laurae</i>	HQ907805	HQ907850	Antarctica, Chile: off Concepcion, 36°21.65'S, 73°44.42'W; 900–904 m; 2004
<i>Leptochiton medinae</i>		HQ907865	Antarctica, South Georgia and Sandwich Islands, 58°44.35'S, 25°10.48'W, 725–815 m; 2002
<i>Leptochiton rugatus</i>	HQ907826	HQ907868	the eastern Pacific/northern Pacific, Russia: Ussuriyskiy Bay, Sea of Japan; 2–4 m; 2004
<i>Leptochiton saitoi</i>	HQ907827	HQ907870	the southwestern Pacific, Philippines: Bohol Sea; 1 764 m; 2005
<i>Leptochiton vanbellei</i>	HQ907831	HQ907871	the southwestern Pacific, Vanuatu: Big Bay; 773–900 m; 2005
<i>Leptochiton vaubani</i>	HQ907825	HQ907867	the southwestern Pacific, Solomon Islands: Sta. Isabel; 664–682 m; 2004
<i>Leptochiton vietnamensis</i>	HQ907832	HQ907872	the southwestern Pacific, Philippines: Bohol/Sulu Seas sill; 982–989 m; 2005
<i>Leptochiton n. sp. 4</i>	HQ907822	HQ907866	the southwestern Pacific, Vanuatu; 350–358 m; 2005
<i>Leptochiton n. sp. 5</i>	HQ907824		the southwestern Pacific, Vanuatu: Big Bay; 593–630 m; 2005
<i>Parachiton acuminatus</i>		HQ907879	the southwestern Pacific, Indonesia: Sulawesi, Mantehage Island; 7.5 m; 2003
<i>Parachiton communis</i>	HQ907840		the southwestern Pacific, Japan: Gahi-jima, Kerama Islands; 9 m; 2006
<i>Parachiton hodgsoni</i>		HQ907880	the eastern Atlantic, South Africa: Cape Aguthas; 2005
<i>Parachiton politus</i>	HQ907841	HQ907881	the southwestern Pacific, Japan: Gahi-jima, Kerama Islands; 9 m; 2006
Lepidopleurida: Nierstraszellidae			
<i>Nierstraszella andamanica</i>	HQ907835	HQ907875	the southwestern Pacific, Philippines: Bohol/Sulu Seas sill; 982–989 m; 2005
<i>Nierstraszella lineata</i>	HQ907836	HQ907876	the southwestern Pacific, Philippines: Bohol/Sulu Seas sill, Dipol Bay; 150–163 m; 2005
Lepidopleurida: Protochitonidae			
<i>Deshayesiella curvata</i>	HQ907794	HQ907843	the eastern Pacific/northern Pacific, Russia: Ussuriyskiy Bay, Sea of Japan; 2–4 m; 2004
<i>Hanleyella oldroydi</i>	HQ907800	HQ907874	the eastern Pacific/northern Pacific, USA: California, Cortes Bank; 367–389 m; 2007
<i>Oldroydia percrassa</i>		HQ907878	the eastern Pacific/northern Pacific, USA: California, off La Jolla; 1972

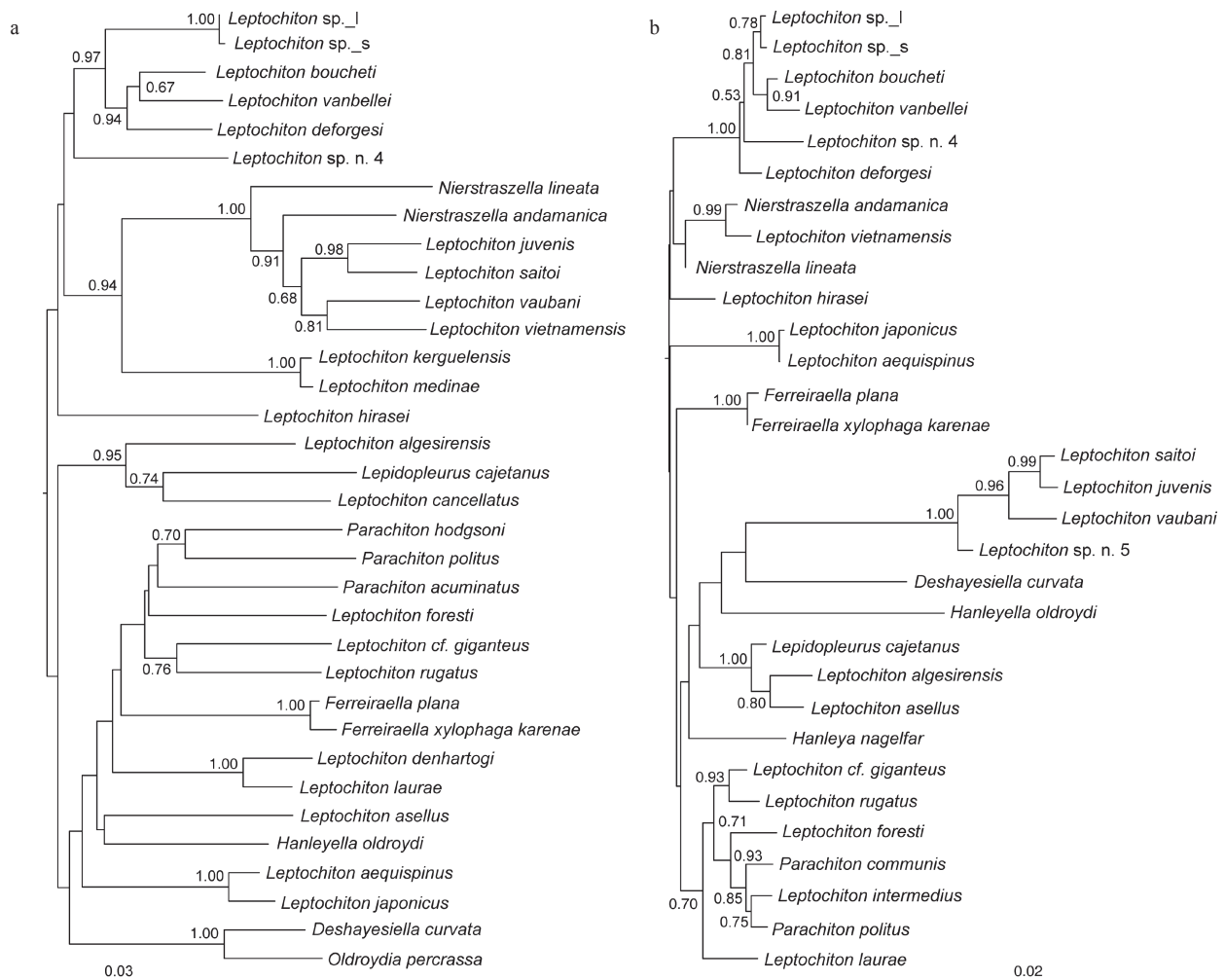


Fig. 2. Neighbour-joining phylogram for the COI (a) and 28S rDNA (b) Bootstrap values above 50% are indicated at nodes. *Leptochiton* sp._l and *Leptochiton* sp._s are the larger and smaller chiton specimens from the Yap Trench, respectively.

References

- Folmer O, Black M, Hoeh W, et al. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3(5): 294–299
- Fujiwara T, Tamura C, Nishizawa A, et al. 2000. Morphology and tectonics of the Yap Trench. *Marine Geophysical Researches*, 21(1–2): 69–86
- Giribet G, Okusu A, Lindgren A R, et al. 2006. Evidence for a clade composed of molluscs with serially repeated structures: monoplacophorans are related to chitons. *Proceedings of the National Academy of Sciences of the United States of America*, 103(20): 7723–7728, doi: [10.1073/pnas.0602578103](https://doi.org/10.1073/pnas.0602578103)
- Marko P B. 2002. Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution*, 19(11): 2005–2021, doi: [10.1093/oxfordjournals.molbev.a004024](https://doi.org/10.1093/oxfordjournals.molbev.a004024)
- Medina M, Collins A G, Silberman J D, et al. 2001. Evaluating hypotheses of basal animal phylogeny using complete sequences of large and small subunit rRNA. *Proceedings of the National Academy of Sciences of the United States of America*, 98(17): 9707–9712, doi: [10.1073/pnas.171316998](https://doi.org/10.1073/pnas.171316998)
- Sigwart J D. 2009. Morphological cladistic analysis as a model for character evaluation in primitive living chitons (Polyplacophora, Lepidopleurina). *American Malacological Bulletin*, 27(1–2): 95–104, doi: [10.4003/006.027.0208](https://doi.org/10.4003/006.027.0208)
- Sigwart J D, Schwabe E, Saito H, et al. 2011. Evolution in the deep sea: a combined analysis of the earliest diverging living chitons (Mollusca: Polyplacophora: Lepidopleurida). *Invertebrate Systematics*, 24(6): 560–572
- Sigwart J D, Sirenko B I. 2012. Deep-sea chitons from sunken wood in the West Pacific (Mollusca: Polyplacophora: Lepidopleurida): taxonomy, distribution, and seven new species. *Zootaxa*, 3195: 1–38
- Sirenko B. 2006. New outlook on the system of chitons (Mollusca: Polyplacophora). *Venus*, 65(1–2): 27–49
- Tamura K, Stecher G, Peterson D, et al. 2013. MEGA6: molecular evolutionary genetics analysis version 6. 0. *Molecular Biology and Evolution*, 30(12): 2725–2729, doi: [10.1093/molbev/mst197](https://doi.org/10.1093/molbev/mst197)
- Thompson J D, Higgins D G, Gibson T J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22(22): 4673–4680, doi: [10.1093/nar/22.22.4673](https://doi.org/10.1093/nar/22.22.4673)