Munidopsis species (Crustacea: Decapoda: Munidopsidae) from ~~the~~ carcass falls in Weijia Guyot, West Pacific, with recognition of a new species based on integrative taxonomy

Dong Dong[1, 2, *], Peng Xu[3,*], Xinzheng Li[1, 2, 4, 5], Chunsheng Wang[2, 6]

[1] Department of Marine Organism Taxonomy & Phylogeny, Institute of Oceanology, Chinese Academy of Sciences, Qingdao, China
[2] Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao, China
[3] Key Laboratory of Marine Ecosystem and Biogeochemistry, State Oceanic Administration & Second Institute of Oceanography, Ministry of Natural Resources, Hangzhou, China
[4] University of Chinese Academy of Sciences, Beijing, China
[5] Laboratory for Marine Biology and Biotechnology, Pilot National Laboratory for Marine Science and Technology (Qingdao), Qingdao, China
[6] School of Oceanography, Shanghai Jiao Tong University, Shanghai, China
[*] These authors contributed equally to this work.

Corresponding authers:
Dong Dong,
7 Nanhai Road, Qingdao, 266071, China.
dongd@qdio.ac.cn;
Chunsheng Wang,
36 Baochubei Road, Hangzhou, 310012, China
wangsio@sio.org.cn

Abstract

Several squat lobster specimens of the genus *Munidopsis* ~~(squat lobsters)~~ were collected from an artificially placed carcass fall (cow bones) on~~at~~ Weijia Guyot in the western Pacific Ocean. Based on morphological comparisons and molecular analysis, three specimens were confirmed as juveniles of *M. albatrossae* Pequegnat & Pequegnat, 1973, which represents the first record of this species in the western Pacific. The other specimens collected are newly described as *Munidopsis spinifrons* sp. nov., which is distinguished from the closely related species in having a spinose rostrum and basal lateral eyespine on the eyestalk. The *M. albatrossae* from Weijia Guyot exhibited very low genetic distances when compared with a conspecific sample from Monterey Bay, USA, and the ~~sibling~~ closely related species *M. aries* (A. Milne Edwards, 1880) from the northeastern Atlantic. A phylogenetic tree based on the mtCOI gene shows *M. spinifrons* sp. nov. as sister to *M. vrijenhoeki* Jones & Macpherson, 2007 and *M. nitida* (A. Milne Edwards, 1880), although *M. vrijenhoeki* presents a complex relationship with other species in the clade. The systematic status of the new species and the closely related species ~~is~~ are discussed.

Subjects: Taxonomic study of squat lobsters from a deep-sea carcass fall

Keywords: *Munidopsis*, carcass fall, Weijia Guyot, new species, molecular phylogeny

Introduction

The genus *Munidopsis* Whiteaves, 1874 comprises more than 260 species of squat lobsters, distributed worldwide, and is one of the most diverse groups of deep-sea animals (list e.g. Baba et al. 2008 catalog as reference?). *Munidopsis* species are adapted to a variety of abyssal habitats, such as seamounts, ocean plains, hydrothermal vents, cold ~~seepage~~ seeps and whale falls. Whale falls or other types of large organic falls are unique ecosystems creating an island-type habitat that can sustain a specifically adapted benthic community, potentially enduring for decades (Smith et al., 1989; Baco & Smith, 2003; Smith & Baco, 2003; Ristova et al., 2017). The fauna in a carcass fall is often diverse and usually shows a high degree of endemism (Baco & Smith, 2003; Smith & Baco, 2003; Amon et al., 2013; Amon et al., 2017).

Known as opportunists, *Munidopsis* species have often been observed in whale-fall and wood-fall ecosystems (Williams, Smith & Baco, 2000; Jones & Macpherson, 2007; Macpherson, Amon & Clark, 2014; Sumida et al., 2016; Amon et al., 2017). Previous studies found that *Munidopsis* species associated with whale falls or wood falls had a heterogeneous diet and could be scavengers, predators, bacterivorous detritivores and/or even wood-eaters, and were present in the community in every developmental stage (Janßen, Treude & Witte, 2000; Smith & Baco, 2003; Kemp et al., 2006; Macavoy et al., 2008; Hoyoux et al., 2012). Although *Munidopsis* species constitute an important part of organic-fall fauna, there has been little taxonomic study of the group in this ecosystem compared with other habitats (Jones & Macpherson, 2007; Macpherson, Amon & Clark, 2014).

To study the fauna and community characters of a deep-sea carcass-fall ecosystem, artificially placed carcass falls (cow bones) were deployed at Weijia Guyot in the western Pacific, in 2016 and 2018, by the Second Institute of Oceanography, Ministry of Natural Resources of the People's Republic of China. Two cow carcasses mimicking whale falls were placed on the seamount at a depth of 1,427 m and 3,225 m, respectively. ~~Several~~ Eight? specimens of *Munidopsis* were collected among the fauna, together with many amphipod scavengers. After careful examination, three specimens were identified as juveniles of *M.*

Commented [KS1]: In materials section you mention just bones, though, a carcass would imply the entire animal here. I as a reader was curious what exactly you placed on the bottom, large bones, tied together? In a mesh cage?

albatrossae *authority here*, a first record for this species in the West Pacific, and the other five specimens were found to be new to science. In addition to morphological analysis, we performed a barcoding analysis using sequences of the mtCOI gene to help the identification of specimens. Meanwhile, we selected a group of *Munidopsis* species morphologically similar to our specimens for phylogenetic analysis, most of which have mesial eyespines, relatively short P1 and triangular rostrum, and are generally associated with chemosynthetic environments (can you insert the references here where a reader can find their association?). In the present ~~paper~~study, we combined the morphological with molecular methods, in an attempt to clarify the systematic status of the present species from Weijia Guyot and assess their phylogenetic relationships with other congeners.

Materials & Methods

Sample collection and morphological examination

Information on the *Munidopsis* species (~~squat lobsters~~Munidopsidae) collected at Weijia Guyot and the molecular data used in the present study are listed in Table 1. The carcass fall experiments were conducted using deep-sea landers including cow bones deployed on the seabed. The squat lobsters ~~samples~~ were collected when the landers were retrieved one year after the deployment. All specimens collected were found on the bones or within the lander boxes. After being photographed, the specimens were preserved in 80% ethanol. The size of the specimen is given as the postorbital carapace length (PCL), which refers to the carapace length excluding rostrum. All specimens collected in this study were deposited in the Sample Repository of Second Institute of Oceanography (SRSIO), Ministry of Natural Resources, Hangzhou, China.

Munidopsis lauensis Baba & de Saint Laurent, 1992 was used as the comparative material for the phylogenetic analysis. The specimen was collected from a hydrothermal vent in Manus Basin, Bismarck Sea (3°42.25′ S, 151°52.66′ E), at 1,714 m depth, in 19 June 2015. Morphological and molecular data for ~~The~~ specimens of *Munidopsis nitida* (A. Milne Edwards, 1880) used in the study were ~~owned~~ made available by Paula Rodriguez-Flores Museo Nacional de Ciencias Naturales (MNCN-CSIC) and Centre d’Estudis Avançats de Blanes

(CEAB-CSIC), Spain. Th~~ose specimens~~ey were collected off Papua New Guinea (03º31′ S, 148º03′ E), at a depth range of 780–855 m, in 23 April 2014. ~~The morphological and molecular data for *M. nitida* were obtained with the help of Paula Rodriguez-Flores.~~

Molecular data and analysis

Total genomic DNA was extracted from muscle tissue using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), following manufacturer instructions. Extracted DNA was eluted in double-distilled H_2O (ddH_2O). Partial sequences of the COI gene~~s~~ were amplified via polymerase chain reaction (PCR). Reactions were carried out in a 30-µl volume containing: 1.5 µ~~m~~l Premix Taq (TaKaRa Taq^{TM} Version 2.0 plus dye; TaKaRa, Kusatsu, Japan), 1.2 µl each of forward and reverse primers (10 mM), respectively, 1.6 µl DNA template, and 11µl ddH_2O. For *M. albatrossae*, the primer pair gala_COIF and gala_COIR was used to amplify a fragment of 568 base pairs (bp) of the COI gene following the original procedure (Jones & Macpherson, 2007). For *M. spinifrons* sp. nov. specimens, we designed a new reverse primer LCOgala (5'- ATCATAAAGACATTGGAACTTTATA - 3') paired with the universal forward primer HCO2198 (Folmer et al., 1994) to obtain a fragment of ca. 660 bp of the COI gene in the following thermal profile: initial denaturation at 95 °C for 5 min; 40 cycles of 95 °C for 50 s; 49 °C for 50 s; 72 °C for 50 s; and a final extension at 72 °C for 10 min. We also amplified a fragment (672 bp) of the COI gene of *M. lauensis* for phylogenetic analysis using the universal primers HCO2198 and LCO1490 (Folmer et al., 1994) following the thermal profile described above. PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany), and bidirectionally sequenced using the same primers with an ABI 3730xl Analyzer (Applied Biosystems, Foster City, CA, USA).

Commented [KS2]: I assume you didn't add 15 ml

Sequences were checked by the sequence peak height and then assembled based on the contigs using the DNASTAR Lasergene software package (DNASTAR, Inc., Madison, WI, USA). The sequences acquired during this study were uploaded to NCBI~~in~~ GenBank (Table 1). We also downloaded several COI sequences of Munidopsidae species from GenBank (Table 1) for the phylogenetic analysis. Most of the chosen species, which are morphologically similar to either one of the two species presently studied, belong to the *Orophorhynchus* (A. Milne-Edwards, 1880) group and associated with ~~the~~ chemosynthetic environments (can you add references here perhaps?). Two morphologically distinct species, *Munidopsis barbarae* (Boone, 1927) and *Munidopsis corniculata* Rodriguez-Flores, Macpherson & Machordom, 2018, were chosen for comparative analysis, and *Shinkaia crosnieri* Baba & Williams, 1998 was selected as the outgroup in the phylogenetic study.

The sequences were aligned using the software package MEGA 6.06 (Tamura et al., 2013). The average genetic distances within and between species were estimated according to the Kimura 2-parameter (Kimura, 1980) model in MEGA 6.06 (Tamura et al. 2013). The most appropriate nucleotide base substitution model for the alignment data, which is HKY+I+G, was determined by MrModeltest v2 (Nylander, 2004). The maximum likelihoods (ML) for phylogenetic analyses were assembled in PhyML 3.1 (Guindon & Gascuel, 2003) with 1000 replicates. A Bayesian inference tree was constructed using MrBayes 3.2.6 (Huelsenbeck & Ronquist, 2001). Markov chains were run for 10,000,000 generations, sampled every 100 generations; the first 25% trees were discarded as burn-in, after which remaining trees were used to construct the 50% majority-rule consensus tree and to estimate posterior probabilities.

Zoobank registration

The electronic version of this article in portable document format will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new names contained in the electronic version are effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank Life Science Identifiers (LSIDs) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:C1CC52FD-6113-4A53-91C3-8D80E713D255. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.

Results

Taxonomy

Family Munidopsidae Ortmann, 1898

Genus *Munidopsis* Whiteaves, 1874

***Munidopsis albatrossae* Pequegnat & Pequegnat, 1973**

(Figs. 1, 2, 5A)

Munidopsis sp. Wolff, 1961: 148, fig. 16.

Munidopsis albatrossae Pequegnat & Pequegnat, 1973: 163, figs. 1, 2 (type locality: Eastern Pacific South of Madalena Bay, Baja California).—Baba, 2005: 284.—Jones & Macpherson, 2007: 480, fig. 2A.—García Raso et al., 2008: 1282, fig. 2.

Munidopsis aries Ambler, 1980: 17.—Wicksten, 1989: 315 (not *M. aries* (A. Milne Edwards, 1880)).

Materials examined. SRSIO1709000X, 1 male (PCL 6.29 mm), 1 female (PCL 6.51 mm), 1 sex indet. (PCL 4.02 mm). Carcass fall experimental field site, Weijia Guyot, West Pacific. R/V *Haiyang 6*, stn. MCMX1605, 12°43.0149’N, 156°27.2057’E, 3225 m, 30 September 2017.

Diagnosis. Carapace (Fig. 1A) (excluding rostrum) as long as broad. Frontal margins oblique, with blunt outer orbital angle above antennal peduncle. Anterolateral corners blunt, followed by notch at end of anterior cervical groove; anterior branchial margins rugose, slightly convex, each with blunt anterior tooth; posterior branchial margins converging posteriorly. Dorsal surface with numerous rugae; gastric region elevated, with pair of low epigastric processes. Rostrum broadly triangular, 1.3 times longer than broad, 0.6 length of remaining carapace; lateral margins straight, weakly serrated distally; dorsal surface and ventral surface (Fig. 1B) each with median, longitudinal ridge. Pterygostomial flaps (Fig. 1C) with oblique rugae on lateral surface; anteriorly acute. Sternal plastron (Fig. 1D) as long as broad; sternite 4 moderately elongated anteriorly, ventral surface depressed. Abdominal tergites unarmed;

Commented [KS3]: Looks like 2–3 low processes (blunt anterolateral, larger than antennal, followed by subequal low spine), I think these should be mentioned tergites 2 and 3 each with two transverse ridges on dorsal surface, anterior ridges more elevated. Telson (Fig. 1E) composed of 8 plates. Eyestalks short, mesial eyespines present but reduced; cornea small. Basal article of antennular peduncle (Fig. 1B) with 2 ?lateral (mesially unarmed?) anterior spines. Antennal peduncle (Fig. 1B) overreaching eyestalks, unarmed? Looks like A2–3 have minute mesial spines.~~.~~? Merus of third maxilliped (Fig. 1F) armed with 3 or 4 small spines on flexor margin. Pereopod 1 (P1, chelipeds) (Fig. 2A) subequal, approximately 1.1 ×~~of~~ ~~posterior carapace length~~PCL; palms with rows of spines on lateral and mesial margins, dorsal surfaces with scattered spines; fingers 0.8 × palm length, opposable margins distally spooned; fixed fingers with denticulate carina on distolateral margin. Pereopods 2–4 slender (Fig. 2B–E); P2 slightly overreaching distal end of P1, approximately 1.6 × ~~posterior carapace length~~PCL; meri spinulose on extensor and flexor margins; carpi each with 2 longitudinal, spinulose carinae, *(plural)* on extensor surface; propodi each with 2 carinae on extensor surface and 1 pair of distal spines on flexor margin; dactyli (Fig. 2E) approximately 0.7 propodi length; flexor margin straight, with 7 elevated teeth, each bearing small corneous spines. P1 with epipods.

Coloration. Grey white (5A).

Distribution. East Pacific: south of Madalena Bay, Baja California, Costa Rica, East Pacific Rise, and Monterey Bay (California). Antarctic waters: Bellingshausen Sea. West Pacific: Weijia Guyot. Depth 1,920–3,680 m.

Habitats. The specimens described here were collected from cow bones in an artificial carcass fall, deployed at 3,225 m at Weijia Guyot. This species was previously found on a whale fall in Monterey Bay (Jone & Macpherson, 2007), and on soft sea bottom in the Bellingshausen Sea (García Raso, García Muñoz & Manjón-Cabeza, 2008).

Remarks. The specimens from Weijia Guyot show a few differences from the holotype and the specimen from Monterey Bay. In the present specimens, the mesial eyespines are blunt and the anterior branchial margins are faintly serrated, whereas in the holotype, the mesial eyespines are prominent and the anterior branchial margins have numerous small spines (Pequegnat & Pequegnat, 1973). The present specimens are all juveniles, with the PCL not longer than 7 mm; in contrast, the specimens described from the East Pacific exceed 70 mm PCL. Therefore, the slight morphological differences can be considered intraspecific

Commented [KS4]: Thise whole section just describes P2? What about P3-4?

Formatted: Font: Italic

variations due to size. Genetic distances are discussed in molecular section below?

***Munidopsis spinifrons* sp. nov.**

urn:lsid:zoobank.org:act:B1A381D7-7BF1-4C37-A15F-33ACF009C833

(Figs. 3, 4, 5B)

Munidopsis vrijenhoeki Jones & Macpherson, 2007: 496 (part, small leg fragments?).

Materials examined. SRSIO18100001, holotype, 1 male (PCL 24.89 mm); SRSIO18100002, paratypes, 4 females (PCL 15.79–20.45 mm). Carcass fall experimental field site, Weijia Guyot, West Pacific. R/V *Dayang 1*, stn. DY48-II-MX1802, 12°56.96'N, 156°57.25'E, 1427.5 m, 25 October 2018.

Description. Carapace (Fig. 3A) (excluding rostrum) distinctly longer than broad. Frontal margins slightly concave, antennal spines well developed. Lateral margins approximately parallel, bearing short, sparse setae. Anterolateral spines relatively short. Anterior branchial margin with 3 or 4 spines; anteriormost spine strongest; posterior two spines usually rudimental. Posterior branchial margin rugose, with distinct spine at base of posterior cervical groove. Posterior margin unarmed, slightly concave. Dorsal surface covered with transverse, ~~and~~ interrupted ridges, bearing long setae. Gastric region elevated, with 2 strong epigastric spines (followed with 2 tiny spines in holotype). Cervical groove distinct. Cardiac region with distinct transverse uninterrupted ridge. Rostrum spiniform (Fig. 3B), 0.4 times as long as remaining carapace length, 1.2 times broader than long (base at level of antennal spine base); dorsal surface evenly and longitudinally carinate; distal 0.3 length of lateral margins strongly upturned, ~~each~~ bearing 1 or 2 small but distinct spines. Pterygostomial (Fig. 3C) flap~~s~~ with oblique rugae on lateral surface.

Commented [KS5]: They look more oblique (convex instead of concave for sure)

Sternal plastron (Fig. 3D) slightly longer than broad, widening posteriorly. Sternite 3 broader than anterior margin of sternite 4, divided into two parts by median longitudinal groove; anterior margins laterally biconcave, with median notch. Sternite 4 narrowly elongated with longitudinal groove in anterior part; posterior part broad, surface with short scales, ~~ventral~~ posterior surface depressed. Sternites 5–7 each with elevated, transverse ridges~~ on ventral surface~~, bearing simple setae.

Abdominal tergites smooth and unarmed; tergites 2–4 each with 2 transverse ridges bearing stiff setae anteriorly, posterior ridge relatively short.

Telson (Fig. 3E) composed of 10 distinct plates.

Eyestalk (Fig. 3F) hardly movable. Cornea oval, globular, broader than long. Ocular peduncle short, nearly invisible in dorsal view, broader than cornea; mesial eyespine prominent, anterolaterally directed, reaching to distal 0.6 of rostrum; lateral eyespine relatively short, closely adjacent to cornea and followed with distinct spine on base of peduncle (usually covered by carapace in dorsal view).

Antennular peduncle (Fig. 3G, H) with basal article longer than broad; distal margin bearing strong ventrolateral spine and dorsolateral spine (rarely bearing another minute intermedian spine); lateral face slightly inflated, covered with short rugae; mesial margin straight.

Antennal peduncle (Fig. 3G) reaching to half length of rostrum, bearing setae on lateral and mesial margins. Article 1 immovable, with strong distomesial and distolateral spines. Article 2 armed with strong distolateral spine, small mesial spine at midlength?. Article 3 subrectangular, with strong distomesial and distolateral spines, and minute dorsodistal spine. Article 4 short and unarmed.

Third maxilliped (Fig. 3I) slender. Ischium and basis approximately as long as merus length, disto-extensor corner acute; crista dentata well-developed, extending onto basis. Merus subrectangular, extensor margin with distinct distal spine followed ~~with~~ by small tubercle; flexor margin irregularly denticulate. Carpus unnamed. Propodus with distoflexor margin convex. Dactylus short. Dactylus flexor margin, propodus distoflexor margin, and carpus dorsal distoflexor margin densely covered in long setae.

Pereopod 1 (P1, chelipeds) (Fig. 4A, B) subequal, 1.4 ×~~times as long as~~ PCL, densely covered in long and stiff setae on rugae and base of spines on surface and margins. Ischium short, approximately 0.7 merus length (measured at midline?), distal margin with distinct dorsolateral spine and small ventrolateral spine; ventrodistal margin anteriorly produced, with strong subterminal spine. Merus approximately 0.4 PCL, subtriangular in cross-section, with short rugea on surfaces; dorsal surface armed with longitudinal row of spines (strongest on distal margin, successively decreasing in size); dorsodistal margin with another strong spine on mesial side; ventrodistal margin with strong mesial and lateral spines. Carpus less than half merus length, dorsomesial margin with strong subdistal spine and small median spine (~~disappearing~~ small or indistinct? on right P1 of holotype); dorsolateral margin with strong distal spine; ventrodistal margin produced into triangular lobe. Chela relatively compressed, approximately 1.3 merus length (including fixed finger), twice as longer as broad; palm unarmed. Fingers 0.8 palm length, opposable margins distally spooned and crenulated; occlusal margins sinuous, with low, triangular tooth proximally on fixed finger, and broad, low tooth medially on movable finger; distolateral margin of fixed finger with distinct denticulate carina *(very indistinct in figure)*.

Pereopods 2–4 (P2–4, ambulatory legs 1–3) (Fig. 4C–F) setose, bearing long, stiff setae ~~densely~~ on margins and surfaces of each segment. P2 approximately 1.8 times PCL, overreaching distal end of cheliped. Meri somewhat compressed; P2 merus approximately 0.7 PCL (P3 merus 0.9 P2 merus length; P4 merus 0.8 P2 merus length), 4.3 times as long as broad (P3 3.8 times, P4 3.3 times); extensor margin armed with row of spines, distal-most spine prominent; flexor margin rugose, with strong distal spine. Carpi each with 2 longitudinal ridges on dorsal surface; lateral carina rugose, armed with small distal spine (P2, sometimes absent) or unarmed (P3 and P4); mesial carina armed with row of 3–6 spines, distal spine laterally situated and subequal (or smaller) in size to penultimate spine; flexor margin armed with small but acute distal spine. Propodi subcylindrical, P2 propodus 0.8 merus length (P2–4 propodi subequal in length); extensor surface nearly flat, with 2 longitudinal carinae; flexor margin rugose, with pair of distal corneous spines. Dactyli (Fig. 4F) 0.4–0.5 propodi length; extensor margin rugose; flexor margin straight, with 11 movable corneous spines (increasing in size distally) each based on triangular tooth.

P1 with epipod.

Distribution. Known only from the type locality, Weijia Guyot, West Pacific.

Coloration. White.

Habitats. The species is recorded only from the artificially placed deep-sea carcass fall (cow bones) at Weijia Guyot, western Pacific, but is probably associated with hydrothermal vents

Commented [KS6]: This is a hypothesis not further discussed, should probably be deleted

(Jones & Macpherson, 2007).

Etymology. Latin words "*spini*-" means spinose or spiny, and "*frons*" means the rostrum. The new specific name refers to the special character that discriminates it from the closely related species.

Remarks. The new species is morphologically similar to *M. nitida* in having a narrow rostrum, parallel carapace lateral margins, pair of strong epigastric spines, mesial and lateral eyespines, broad cornea, unarmed abdominal segments, anterior branchial margin with 2–4 spines, P1 shorter than P2, and epipod present on P1. *Munidopsis spinifrons* sp. nov. can be readily distinguished from *M. nitida* in having small distal spines on the lateral margins of the rostrum, and a basal lateral eyespine; in *M. nitida*, the lateral margins of the rostrum are entire (Baba, 2005) and the eyestalk bears only a distal lateral eyespine. The latter character has not been mentioned in previous literature, but according to the illustration in Baba (2005: Fig. 72e), *M. nitida* lacks such a basal lateral eyespine. This was also supported by examination of specimens from New Guinea (Paula Rodríguez Flores, pers. comm.). The character of the spinose rostrum can be observed on all five of the present specimens, regardless of the size and sex, although the spines are weak on the smallest specimen; therefore we accept it as a consistent and reliable interspecific character. The new species resembles *Munidopsis exuta* Macpherson & Segonzac, 2005 in having a narrow rostrum with small lateral marginal spines and a pair of strong epigastric spines; *M. spinifrons* sp. nov. differs from *M. exuta* in having antennal spines, lateral eyespines, and denticulate carina on the P1 fixed finger.

COI sequence data (see below) show that the new species is closely related to *M. vrijenhoeki*. However, those two species differ morphologically. Besides the spinose rostrum, the new species has a narrow rostrum, pair of strong epigastric spines, large cornea, anterior branchial margins with 2 or 3 spines, 3rd maxillipeds meri with irregular denticles on the flexor margins, P1 with epipods, and P2–4 meri each with a row of spines only on the extensor margin. In contrast, *M. vrijenhoeki* has a broad rostrum, pair of small epigastric spines, small cornea, branchial margins nearly unarmed, 3rd maxillipeds meri with well-developed spines on the flexor margin, P1 without epipod, and P2–4 meri with a row of spines on the flexor margin. The genetic relationships are discussed in the Discussion section.

Molecular data analysis

Kimura’s two-parameter pairwise genetic distances between *M. albatrossae* from the Weijia Guyot and a specimen from the Monterey Bay (Jones & Macpherson, 2007) was 0.4%, suggesting that the specimens are the same species. The genetic distances between *M. aries* from the northeastern Atlantic (Jones & Macpherson, 2007) and *M. albatrossae* from both the Weijia Guyot and Monterey Bay were 1.8% and 1.4%, respectively, indicating a close relationship between these two species.

Kimura’s two-parameter pairwise genetic distance between *M. spinifrons* sp. nov. and *M. nitida* was 4%, indicating clear genetic divergence. However, *M. spinifrons* sp. nov., including all five sequences, showed no significant genetic distance compared with *M. vrijenhoeki* Mvri2 (DQ677675), yet displayed high genetic distance compared with *M. vrijenhoeki* Mvri3 (DQ677676), at 1.7%.

The combined phylogenetic trees reconstructed from both the maximum-likelihood (ML) and Bayesian inference (BI) analyses are generally congruent. In the combined trees, *M. albatrossae* from both the west and east Pacific cluster together (BP [maximum likelihood bootstrap percentage] = 91), although the Bayesian posterior probability (PP) are modest. Meanwhile, *M. albatrossae* and *M. aries* form a highly supported monophyletic clade A (BP = 100, PP = 1.00) suggesting the close relationship of these two species.

In the phylogenetic tree, *M. spinifrons* sp. nov., *M. vrijenhoeki* (both Mvri2 and Mvri3), and *M. nitida* form highly supported clade B (BP = 94, PP = 1.00), indicating their close relationship. The five specimens of the new species cluster together into a subclade, although the bootstrap value is modest (BP = 65); nevertheless, *M. vrijenhoeki* Mvri3 and *M. nitida* form a strongly supported subclade (BP = 98, PP = 1.00), illustrating that they are more genetically related than the rest of the species (individuals) within clade B. *Munidopsis lauensis* and *Munidopsis myojinensis* Cubelio, Tsuchida, Hendrickx, Kado & Watanabe, 2007, together with clade B, compose a large clade C with high Bayesian support (PP = 0.99).

Discussion

The COI gene is considered much conserved in the genus *Munidopsis*, following studies between populations and among sibling species (Jones & Macpherson, 2007; Thaler et al., 2014; Coykendall, Nizinski & Morrison, 2017). The smallest nucleotide divergences for mtCOI ~~observed in the~~between species of *Munidopsis* from the EP Rise are 1.6%–1.9% (Jones & Macpherson, 2007). The genetic distance (Kimura's two-parameter pairwise), however, observed between *M. albatrossae* (from Monterey Bay) and *M. aries* (northeastern Atlantic) was 1.4%, which is smaller than that ~~calculated~~ presented/reported by Jones and Machpherson (2007) (2.8%), probably because data from different specimens were used. Similarly, *M. albatrossae* from the Weijia Guyot displayed very low genetic distance compared with specimens from the East Pacific, although their geographic distance is vast. Harino et al. (2005) mentioned the capture of *M. albatrossae* at the Nankai Through, off the south coast of Japan, but that record was not confirmed by taxonomic examination. Therefore, the current ~~finding can be seen as~~report is considered the first definite record of *M. albatrossae* distributed in the West Pacific. García Raso, García Muñoz & Manjón-Cabeza (2008) also reported the occurrence of this species in Antarctic waters (west of Peter I Island). Together, these findings suggest that *M. albatrossae* has a wide distribution range in the Pacific Ocean. Among the sampling localities reported in the present and previous studies, the Weijia Guyot and Monterey Bay are the only two places where the specimens were observed in a whale-fall or carcass-fall environment.

The type material of *M. vrijenhoeki* ~~involves~~ comprised of three specimens: the holotype and the small leg fragments of two other specimens (Jones & Macpherson, 2007); ~~accordingly,~~ three COI sequences were published in GenBank based on these materials (DQ677674–DQ677676, representing Mvri1–Mvri3). The sequence of DQ677674 (Mvri1) was assigned to the holotype, but annotated as including two individuals. However, as compared with other molecular data, the Mvri1 sequence was too short (211 bp, identical with part of Mvri2) to be used for phylogenetic analysis in the present study. The genetic distance observed between *M. spinifrons* sp. nov. and Mvri2 specimen was 0, but was significantly higher (1.7%) ~~between~~ compared to~~the new species and~~ Mvri3. Moreover, the combined phylogenetic tree confirms that Mvri3 is more closely related to *M. nitida* than to Mvri2 and *M. spinifrons* sp. nov., with high support. These results suggest that the type material of *M. vrijenhoeki* represents at least 2 valid species. Consequently, we think that one or two of the leg fragments attributed to *M. vrijenhoeki* might instead belong to the present new species, and that the Mvri3 sequence might be derived from the holotype. Still, the morphological differences were sufficiently distinct ~~significant enough~~ to distinguish our specimens as representing a valid species separate from *M. vrijenhoeki*~~, even if part of their COI sequences were alike~~. Nonetheless, additional samples accompanied by molecular examination involving multi-~~sequences~~ genes are needed to verify their actual systematic relationship.

The phylogenetic relationships among species within *Munidopsis* genus has been fully discussed (Jones & Macpherson, 2009; Ahyong, Andreakis & Taylor, 2011; Coykendall, Nizinski & Morrison, 2017). According to the phylogenetic tree in the present study, the species from the West Pacific (except *M. albatrossae* in Weijia Guyot) are all clustered together in clade C, while species from the East Pacific and Atlantic are scattered in other clades, suggesting a level of genetic divergence between fauna from different geographic regions. However, some West-Pacific species are morphologically more similar to congeners from the East Pacific and Atlantic Ocean. For example, *M. spinifrons* and *M. nitida* from the West Pacific resemble the East-Pacific species, ~~*Munidopsis*~~ *M. bracteosa* Jones & Macpherson, 2009 and ~~*Munidopsis*~~ *M. scotti* Jones & Macpherson, 2009, in having strong spines on the anterior branchial margins; on the contrary, *M. lauensis* and *M. vrijenhoeki* from the West Pacific lack such spines. The broad rostrum and small cornea also link *M. vrijenhoeki* to *M. aries* and ~~*Munidopsis*~~ *M.s* *bermudezi* Chace, 1939 in Atlantic Ocean. The current result supports the idea that there is no correlation between morphological and genetic divergences (at least based on COI) for squat lobsters (Jones & Macpherson, 2009). Since the COI gene of *Munidopsis* species is much conserved and indirectly correlated to the morphological differentiations, more barcoding genes are needed to be explored for the species identity, and multi-genes conjoint analysis is necessary to reveal the phylogenetic relationship among species from different geographic areas.

Juveniles of squat lobsters have seldom been described, either from a natural or artificial carcass fall or wood fall. The *M. albatrossae* collected from Weijia Guyot were all juveniles, with the longest being only 6.51 mm PCL; in contrast, specimens of this species from other localities reach 87 mm PCL (Pequegnat & Pequegnat, 1973). Jones and Macpherson (2007) also examined a juvenile collected from a whale fall. Hoyoux et al. (2012) found adults of a

Munidopsis species within mesh boxes (containing woody baits), with body sizes larger than the mesh size, meaning that the species would have entered the boxes as larvae. The available evidence supports the view that large organic falls may attract *Munidopsis* larvae by acting as a nursery area, but where their growth into other life stages may be sustained. Carcass falls are considered as "stepping stones" for species endemic in chemosynthetic environment to disperse over large distances (Smith et al., 1989; Distel et al., 2000; Smith & Baco, 2003; Amon et al., 2013). Accordingly, this unique habitat would function likewise for squat lobsters, which are widespread and opportunistic in this ecosystem.

Acknowledgement

We are extremely grateful to Dr Paula Carolina Rodriguez-Flores and Prof. Enrique Macpherson for providing us with valuable morphological and molecular data on *M. nitida*. We extend sincere thanks to associate professor Dongsheng Zhang for help with the sample collection.

References

Ambler JW. 1980. Species of *Munidopsis* (Crustacea, Galatheidae) occurring off Oregon and in adjacent waters. *Fishery Bulletin* 78:13–34.

Amon DJ, Glover AG, Wiklund H, Marsh L, Linse K, Rogers AD, Copley JT. 2013. The discovery of a natural whale fall in the Antarctic deep sea. *Deep-Sea Research II* 92:87–96.

Amona DJ, Copley JT, Dahlgren TG, Horton T, Kemp KM, Rogers AD, Glover AG. 2017. Observations of fauna attending wood and bone deployments from two seamounts on the Southwest Indian Ridge. *Deep-Sea Research II* 136:122–132

Ahyong ST, Andreakis N, Taylor J. 2011. Mitochondrial phylogeny of the deep-sea squat lobsters, Munidopsidae (Galatheoidea). *Zoologischer Anzeiger* 250:367–377.

Baba K. 2005. Deep-sea chirostylid and galatheid crustaceans (Decapoda: Anomura) from the

Indo-West Pacific, with a list of species. *Galathea Reports* 20:1–317.

Baba K, de Saint Laurent M. 1992. Chirostylid and galatheid crustaceans (Decapoda: Anomura) from active thermal vent areas in the southwest Pacific. *Scientia Marina* 56:321–332.

Baba K, Williams AB. 1998. New Galatheoidea (Crustacea, Decapoda, Anomura) from hydrothermal systems in the West Pacific Ocean: Bismarck Archipelago and Okinawa Trough. *Zoosystema* 20:143–156.

Baco AR, Smith CR. 2003. High species richness in deep-sea chemoautotrophic whale skeleton communities. *Marine Ecology Progress Series* 260:109–114.

Boone L. 1927. Scientific results of the first oceanographic expedition of the "Pawnee", 1925. Crustacea from the tropical east American Seas. *Bulletin of the Bingham Oceanographic Collection* 1:1–147.

Chace FA. 1939. Reports on the scientific results of the first Atlantis Expedition to the West Indies, etc. Preliminary descriptions of one new genus and seventeen new species of decapod and stomatopod Crustacea. *Memorias de la Sociedad Cubana de Historia Natural* 13:31–54.

Coykendall DK, Nizinski MS, Morrison CL. 2017. A phylogenetic perspective on diversity of Galatheoidea (*Munida, Munidopsis*) from cold-water coral and cold seep communities in the western North Atlantic Ocean. *Deep-Sea Research II* 137:258–272.

Cubelio SS, Tsuchida S, Hendrickx ME, Kado R, Watanabe S. 2007. A new species of vent associated *Munidopsis* (Crustacea: Decapoda: Anomura: Galatheidae) from the Western Pacific, with notes on its genetic identification. *Zootaxa* 1435:25–36.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3:294–299.

García Raso JE, García Muñoz JE, Manjón-Cabeza ME. 2008. First record of *Munidopsis albatrossae* (Crustacea: Decapoda: Galatheidae) from Antarctic waters. *Polar Biology* 31:1281–1285.

Goffredi SK, Johnson S, Tunnicliffe V, Caress D, Clague D, Escobar E, Lundsten L, Paduan JB, Rouse G, Salcedo DL, Soto LA, Spelz-Madero R, Zierenberg R, Vrijenhoek R. 2017.

Hydrothermal vent fields discovered in the southern Gulf of California clarify role of habitat in augmenting regional diversity. *Proceedings of the Royal Society B* 284:20170817.

Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52:696–704.

Harino H, Iwasaki N, Arai T, Ohji M, Miyazaki N. 2005. Accumulation of organotin compounds in the deep-sea environment of Nankai Trough, Japan. *Archives of Environmental Contamination and Toxicology* 49(4):497–503

Hoyoux C, Zbinden M, Samadi S, Gaill F, Compère P. 2012. Diet and gut microorganisms of *Munidopsis* squat lobsters associated with natural woods and mesh-enclosed substrates in the deep South Pacific. *Marine Biology Research* 8:28–47.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogeny. *Biometrics* 17:754–755.

Janßen F, Treude T, Witte U. 2000. Scavenger assemblages under differing trophic conditions: A case study in the deep Arabian Sea. *Deep Sea Research II* 47:2999–3026.

Jones WJ, Macpherson E. 2007. Molecular phylogeny of the East Pacific squat lobsters of the genus *Munidopsis* (Decapoda: Galatheidae) with the descriptions of seven new species. *Journal of Crustacean Biology* 27:477–501.

Kemp KM, Jamieson AJ, Bagley PM, McGrath H, Bailey DM, Collins MA, Priede IG. 2006. Consumption of large bathyal food fall, a six month study in the NE Atlantic. *Marine Ecology Progress Series* 310:65–76.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.

Macavoy SE, Carney RS, Morgan E, Macko SA. 2008. Stable isotope variation among the mussels *Bathymodiolus childressi* and associated heterotrophic fauna at four cold-seeps communities in the Gulf of Mexico. *Journal of Shellfish Research* 27:147–51.

Macpherson E, Segonzac M. 2005. Species of the genus *Munidopsis* (Crustacea, Decapoda, Galatheidae) from the deep Atlantic Ocean, including cold-seep and hydrothermal vent areas. *Zootaxa* 1095:1–60.

Macpherson E, Amon D, Clark PF. 2014. A new species of *Munidopsis* from a seamount of the Southwest Indian Ocean Ridge (Decapoda: Munidopsidae). *Zootaxa* 3753: 291-296

Milne Edwards A. 1880. Reports on the results of dredging under the supervision of Alexander Agassiz, in the Gulf of Mexico and in the Caribbean Sea, etc. VIII. Etudes preliminaires surles Crustacés. *Bulletin of the Museum of Comparative Zoology at Harvard College* 8:1–68.

Nylander JAA. 2004. MrModeltest v 2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Ortmann AE. 1898. Crustacea, Malacostraca. In: Gerstäcker A, Ortmann AE eds. *Die Klassen und Ordnungen der Arthropoden wissenschaftlich dargestellt in Wort und Bild, in H.G. Bronn's Die Klassen und Ordnungen der Thier-Reichs wissenschaftlich dargestellt in Wort und Bild.* Leipzig: Winter'sche Verlagshandlung, 5 (2):1057–1168.

Pequegnat WE, Pequegnat LH. 1973. *Munidopsis albatrossae*, a new species of deep-sea Galatheidae (Decapoda, Anomura) from the Eastern Pacific Ocean. *Crustaceana* 24:163–168.

Pop Ristova P, Bienhold C, Wenzhöfer F, Rossel PE, Boetius A. 2017. Temporal and Spatial Variations of Bacterial and Faunal Communities Associated with Deep-Sea Wood Falls. *PLoS ONE* 12(1): e0169906. DOI:10.1371/journal. pone.0169906

Rodríguez-Flores PC, Macpherson E, Machordom A. 2018. Three new species of squat lobsters of the genus *Munidopsis* Whiteaves, 1874, from Guadeloupe Island, Caribbean Sea (Crustacea, Decapoda, Munidopsidae). *Zootaxa* 4422(4):569–580.

Shen Y, Kou Q, Chen W, He S, Yang M, Li X, Gan X. 2016. Comparative population structure of two dominant species, *Shinkaia crosnieri* (Munidopsidae: *Shinkaia*) and *Bathymodiolus platifrons* (Mytilidae: *Bathymodiolus*), inhabiting both deep-sea vent and cold seep inferred from mitochondrial multi-genes. *Ecology and Evolution* 6 (11):3571–3582.

Smith CR, Baco AR. 2003. Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology* 41:311–354.

Smith CR, Kukert H, Wheatcroft RA, Jumars PA, Deming JW. 1989. Vent fauna on whale remains. *Nature* 34:27–128.

Sumida PYG, Alfaro-Lucas JM, Shimabukuro M, Kitazato H, Perez JAA, Soares-Gomes A, Toyofuku T, Lima AOS, Ara K, Fujiwara Y. 2016. Deep-sea whale fall fauna from the Atlantic resembles that of the Pacific Ocean. *Scientific Reports* 6: 22139. DOI: 10.1038/srep22139.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729.

Thaler AD, Plouviez S, Saleu W, Alei F, Jacobson A, Boyle EA, Schultz TF, Carlsson J, Van Dover CL. 2014. Comparative population structure of two deep-sea hydrothermal-vent-associated Decapods (*Chorocaris* sp. 2 and *Munidopsis lauensis*) from Southwestern Pacific Back-Arc Basins. *PLoS ONE* 9(7):e101345. DOI:10.1371/journal.pone.0101345.

Whiteaves JF. 1874. On recent deep-sea dredging operations in the Gulf of St. Lawrence. *American Journal of Science (ser. 3)* 7:210–219.

Wicksten MK. 1989. Ranges of offshore decapod crustaceans in the eastern Pacific Ocean. *Transactions of the San Diego Society of Natural History* 21:291–316.

Williams AB, Smith CR, Baco AR. 2000. New species of *Paralomis* (Decapoda, Anomura, Lithodidae) from a sunken whale carcass in the San Clemente basin off southern California. *Journal of Crustacean Biology* 20 (special number 2):281–85.

Wolff T. 1961. Animal life from a single abyssal trawling. *Galathea Reports* 5:129–162.

Figure 1 *Munidopsis albatrossae* Pequegnat & Pequegnat, 1973. SRSIO1709000X, male. (A) carapace and abdominal tergites 1–3, dorsal view; (B) rostrum, eye, left antennule and antenna, ventral view; (C) carapace and right pterygostomian, lateral view; (D) sternal plastron, ventral view; (E) telson, dorsal view; (F) left third maxilliped and ischium crista dentata, ventral view. Scales equal 1.0 mm.

Figure 2 *Munidopsis albatrossae* Pequegnat & Pequegnat, 1973. SRSIO1709000X, male. (A) left cheliped (P1), dorsal view (setae omitted); (B) left pereopod 2 (P2), lateral view; (C) left pereopod 3 (P3), lateral view (setae omitted); (D) left pereopod 4 (P4), lateral view (setae omitted); (E) dactylus of left P2, lateral view (setae omitted). Scales equal 1.0 mm.

Commented [KS7]: You didn't state (setae omitted) in Fig 1, I don't think you need to repeat every time.

Figure 3 *Munidopsis spinifrons* sp. nov., SRSIO18100001, holotype. (A) carapace and abdominal tergites 1–3, dorsal view; (B) distal part of rostrum, dorsal view; (C) carapace and left pterygostomian, lateral view; (D) sternal plastron, ventral view; (E) telson, dorsal view; (F) left eyestalk, dorsal view; (G) left antennule and antenna, ventral view; (H) right antennule, ventral view; (I) left third maxilliped and ischium crista dentata, ventral view. Scales equal 1.0 mm.

Figure 4 *Munidopsis spinifrons* sp. nov. SRSIO18100001, holotype. (A) right cheliped (P1), dorsal view (setae only shown on lateral margin of merus); (B) right cheliped (P1), ventral view (setae omitted); (C) right pereopod 2 (P2), lateral view; (D) right pereopod 3 (P3), lateral view (setae omitted); (E) right pereopod 4 (P4), lateral view (setae omitted); (F) dactylus of right P2, lateral view (setae omitted). Scales equal 1.0 mm.

Figure 5 ~~Photos~~ Live coloration? Or preserved specimens? of specimens examined. (A) *Munidopsis albatrossae* Pequegnat & Pequegnat, 1973, SRSIO1709000X, male; (B) *Munidopsis spinifrons* sp. nov., SRSIO18100001, holotype.

Figure 6 Phylogenetic tree obtained by the Maximum likelihood analysis based on the COI gene sequences. Maximum likelihood bootstrap scores (BP, above) and Bayesian posterior probabilities (PP, below or right) are indicated adjacent to each node. Values of BP ≥ 75% and PP ≥ 0.95 are marked red. Only values of BP ≥ 50% and PP ≥ 0.5 are shown.