

***Helice epicure*, A New Species of Varunid Mud Crab (Brachyura, Decapoda, Grapsoidea) from the Ryukyus, Japan**

Ngan Kee Ng¹, Tohru Naruse², and Hsi-Te Shih^{3,*}

¹Department of Biological Sciences, National University of Singapore, 14, Science Drive 4, Singapore 117543.

E-mail: dbsngnk@nus.edu.sg

²Tropical Biosphere Research Center, Iriomote Station, University of the Ryukyus, 870 Uehara, Taketomi, Okinawa 907-1541, Japan

³Department of Life Science and Research Center for Global Change Biology, National Chung Hsing University, 250, Kuo Kuang Road, Taichung 402, Taiwan

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Ngan Kee Ng, Tohru Naruse, and Hsi-Te Shih (2018) *Helice epicure*, a new species of varunid crab, is described from the Ryukyu Islands, Japan. The new species in the genus *Helice* De Haan, 1833 can be distinguished from its congeners by morphological differences in the chelae and suborbital crenulation. The morphological differences between the new species and a sister clade (the *H. latimera* complex, including *H. latimera* Parisi, 1918, *H. tientsinensis* Rathbun, 1931 and *H. formosensis* Rathbun, 1931) are supported by mitochondrial cytochrome oxidase subunit I (*COI*) divergences. However, the phylogenetic relationship within the *H. latimera* complex is unresolved for the time being.

Key words: *Helice epicure*, *H. formosensis*, New species, Varunidae, Mitochondrial cytochrome oxidase subunit I.

BACKGROUND

Crabs of the *Helice* complex (family Varunidae) are common species that burrow in intertidal mudflats, swamps, salt marshes and estuaries (Dai et al. 1986; Dai and Yang 1991; K Sakai et al. 2006; Shih 2007; Shih and Suzuki 2008). There are currently 16 species in six genera distributed mainly in the Indo-West Pacific (K Sakai et al. 2006; Ng et al. 2008). This group's diversity is highest in East Asia (China, Korea, Japan and Taiwan), with 11 species in five genera, namely *Chasmagnathus* De Haan, 1833 (*C. convexus* De Haan, 1835), *Helicana* K. Sakai & Yatsuzuka, 1980 (*H. wuana* Rathbun, 1931; *H. japonica* K. Sakai & Yatsuzuka, 1980; and *H. doerjesi* K. Sakai, Türkay & Yang, 2006), *Helice* De Haan, 1833 (*H.*

tridens De Haan, 1833; *H. formosensis* Rathbun, 1931; *H. latimera* Parisi, 1918; and *H. tientsinensis* Rathbun, 1931), *Parahelice* K. Sakai, Türkay & Yang, 2006 (*P. daviei* (K. Sakai, Türkay & Yang, 2006) and *P. pilimana* (A. Milne-Edwards, 1873)) and *Pseudohelice* K. Sakai, Türkay & Yang, 2006 (*P. subquadrata* (Dana, 1851)) (Ng et al. 2001 2008 2017; K Sakai et al. 2006; Shih 2007; Nishigaki et al. 2011).

The phylogeny of this complex, based on 16S rDNA and *COI* (cytochrome oxidase subunit I) (Shih and Suzuki 2008) recognized the four East Asian genera (*Helice*, *Helicana*, *Chasmagnathus* and *Pseudohelice*) as distinct clades. However, their relationships are not well resolved. K Sakai et al. (2006) and Shih and Suzuki (2008) recovered two subclades within the genus *Helice*, one

*Correspondence: Tel/Fax: 886-4-22856496. E-mail: htshih@dragon.nchu.edu.tw

containing *H. tridens* and the other composed of a mixed clade of *H. latimera*, *H. formosensis* and *H. tientsinensis* - even though the suborbital crenulation among the three species are different. Later studies focusing on the molecular phylogeny of the genus *Helice* supported this finding (Xu et al. 2011 2012).

Helice formosensis (type locality: Yilan, Taiwan) has been reported from Taiwan (including Penghu Islands) and the Ryukyus (K Sakai et al. 2006; Shih 2007; Shih and Suzuki 2008). However, recent collections of the specimens from the Ryukyus have been shown to differ from the Taiwanese population morphologically and molecularly. Based on those differences, the Ryukyu specimens are here described as a new species. This study brings the number of *Helice* species to five.

MATERIALS AND METHODS

Specimens of the new species of *Helice* were collected from the intertidal areas of the Ryukyu Islands (including the islands of Iriomote, Ishigaki, Okinawa, Kume and Amami-Oshima), Japan. Specimens were preserved in 70-95% ethanol after collection. Illustrations were made with the help of a drawing tube attached to a stereomicroscope. Terminology essentially follows that of K Sakai et al. (2006). Measurements provided (in millimetres) are of the carapace length and width. The abbreviations G1 and G2 are used for the male first and second gonopods, respectively. Specimens are deposited in the Zoological Collections of the Department of Life Science, National Chung Hsing University, Taichung, Taiwan (NCHUZOO); Department of Environmental Biology and Fisheries Science, National Museum of Natural Science, Taichung, Taiwan (NMNS); National Taiwan Ocean University, Keelung, Taiwan (NTOU); Naturalis (former Rijksmuseum van Natuurlijke Historie, Leiden, Netherlands (RMNH)); Ryukyus University Museum, Fujukan, University of the Ryukyus, Okinawa, Japan (RUMF); Senckenberg-Forschungsinstitut und Naturmuseum, Frankfurt am Main, Germany (SMF); Smithsonian Institution, National Museum of Natural History, Washington D.C., United States of America (USNM); and the Zoological Reference Collection, Lee Kong Chian Natural History Museum, National University of Singapore, Singapore (ZRC).

Genomic DNA was isolated from leg muscle tissue using the GeneMark tissue and cell genomic

DNA purification kit (Taichung, Taiwan). A portion of the *COI* gene was amplified with polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al. 1994). The PCR conditions for the above primers were denaturation for 50 s at 94°C, annealing for 70 s at 45-47°C, and extension for 60 s at 72°C (40 cycles), followed by extension for 10 min at 72°C. Sequences were obtained by automated sequencing (Applied Biosystems 3730), after verification with the complimentary strand. Sequences of different haplotypes were deposited in the DNA Data Bank of Japan (DDBJ); others can be found in Shih and Suzuki (2008).

The best-fitting model for sequence evolution of the *COI* dataset was determined to be TrN+I+G by jModelTest (v. 2.1.4, Posada 2008), selected by the AIC (Akaike information criterion), and was subsequently applied to the Bayesian inference (BI) analyses. The BI analysis was performed using MrBayes (v. 3.1.1, Ronquist and Huelsenbeck 2003). The search was run with 4 chains for 10 million generations and 4 independent runs, with trees sampled every 1000 generations. The convergence of chains was determined by the average standard deviation of split frequency values below the recommended 0.01 (Ronquist et al. 2005) and the first 1050 trees were discarded as the burnin. A consensus MP tree was constructed using PAUP* (v. 4.0b10, Swofford 2003), with 2000 bootstrap iterations of a simple heuristic search, TBR branch-swapping (tree bisection-reconnection) (100 random-addition sequence replications; max no. of trees to retain = 10000). All characters were equally weighted. Basepair (bp) difference, as well as pairwise estimates of Kimura 2-parameter distance (Kimura 1980) and *p*-distance, for inter- and intraspecific genetic diversities were also calculated by PAUP*.

RESULTS

TAXONOMY

Family Varunidae H. Milne Edwards, 1853
Subfamily Varuninae H. Milne Edwards, 1853
Genus *Helice* De Haan, 1833 (sensu K Sakai et al. 2006)

***Helice epicure* sp. nov.**

(Figs. 1, 2A, 3)

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Material examined: Holotype: male (25.3 × 30.7 mm) (RUMF-ZC-5009), Miyara River (= R.), Ishigaki Island (= I.), coll. T. Naruse, 23 June 2011. Paratypes: 1 male (19.4 × 23.5 mm) (ZRC 2014.0244), Funaura jetty and beach, Iriomote I., coll. N.K. Ng, Y. Cai & T. Naruse, 15 June 2000; 1 male (26.4 × 30.1 mm) (RUMF-ZC-3069), Fukari R., Iriomote I., coll. T. Naruse, 30 October 2010; 1 male (21.7 × 26.1 mm), 2 females (16.1-19.1 × 20.1-23.3 mm) (RUMF-ZC-5010), Fukari R., Iriomote I., coll. R. Yoshida & Y. Endo, 19 August 2016; 1 female (23.8 × 29.5 mm) (RUMF-ZC-3070), Fukari R., Iriomote I., coll. T. Naruse, 30 October 2010; 1 male (28.9 × 34.4 mm), 1 female (22.3 × 27.5 mm) (ZRC 2014.0204), Fukari R., Iriomote I., coll. T. Naruse, 30 October 2010; 1 male (18.8 × 23.3 mm) (NCHUZOOL 12898), Shira R., Iriomote I., coll. T. Naruse, 30 August 2015.

Others: 1 female (18.4 × 23.5 mm) (RUMF-ZC-5011), Sashiki, Okinawa I., coll. T. Naruse, 30 July 2007; 2 males (15.7-27.4 × 18.9-32.7 mm) (SMF-29177), Oyama, Ginowan City, Okinawa I., coll. W. Ishigaki, N. Isa & M. Miyagi, 20 August 1983; 1 female (18.7 × 23.7 mm) (ZRC 2014.0243), Shirase R., Kumejima, coll. KUMEJIMA 2009 Expedition, 19 November 2009; 2 males (17.1-17.5 × 21.1-21.6 mm), 2 females (13.0-14.2 × 16.1-18.3 mm) (NCHUZOOL 12899), Konase, Setouchi, Amami-Oshima I., coll. T. Maenosono, 26 April 2012; 2 females (12.3-17.0 × 15.0-20.0 mm), 1 male (16.4 × 18.5 mm) (RUMF-ZC-3071), Tekebu, Kasari, Amami-Oshima I., coll. T. Maenosono, 25 April 2012; 3 males, 14.3-18.0 × 17.2-21.0 mm), 5 females (14.4-20.1 × 17.7-24.1 mm) (RUMF-ZC-1576), Sashiki tidal flat, Okinawa Island, coll. T. Naruse and K. Kobayashi, 11 August 2011; 11 males (9.9-17.2 × 12.6-21.2 mm), 5 females (9.3-14.5 × 12.0-18.2 mm) (RUMF-ZC-3072), Konase, Setouchi, Amami-Oshima I., coll. T. Maenosono, 26 April 2012.

Comparative material: *Helice formosensis* Rathbun, 1931: Holotype: male (25.6 × 31.1 mm) (USNM-55371), Giran (= Yilan), Taiwan, coll. Taihoku Normal School, August 1918. Others: 1 female (12.5 × 15.8 mm) (NCHUZOOL), estuary of Gaoping R., Kaohsiung, Taiwan, coll. 29 November 2004; 1 female (21.7 × 27.5 mm) (NCHUZOOL), Yuanli, Miaoli, Taiwan, coll. 5 March 2006; 1 male (27.0 × 31.4 mm) (NCHUZOOL), Anping, Tainan, Taiwan, coll. 26 March 1998; 1 male (18.1 × 21.5 mm) (NCHUZOOL), Haisangu, Hsinchu, Taiwan, coll. 16 March 2006; 1 female (19.6 × 25.5 mm) (NCHUZOOL), Cingluo,

Penghu, Taiwan, coll. 23 Nov 2006; 1 female (21.5 × 26.6 mm) (RUMF-ZC-5013), Dongshih, Chiayi, Taiwan, coll. 9 April 1996; 1 male (17.1 × 20.3 mm) (NCHUZOOL), Jhuwei, New Taipei City, coll. 25 October 2009; 1 male (17.2 × 20.5 mm) (NCHUZOOL), estuary of Houlong R., Miaoli, Taiwan, coll. 14 November 1992; 2 males (17.9 × 22.0-22.3 mm) (NCHUZOOL), Cingluo, Penghu, Taiwan, coll. 27 June 2006; 2 males (16.8-23.9 × 20.8-27.7 mm) (NCHUZOOL), 4 females (13.9-19.4 × 17.2-24.5 mm) (NCHUZOOL), Wenliao, Taichung, Taiwan, coll. 20 May 2002; 1 male (20.7 × 25.8 mm) (RUMF-ZC-5012), Gaomei, Taichung, coll. 12 July 2006; 4 males (16.4-19.0 × 19.7-23.4 mm), 5 females (18.4-20.0 × 23.6-25.5 mm) (NCHUZOOL), Wenliao, Taichung, Taiwan, coll. 20 August 2002; 1 male (14.7 × 16.9 mm), 1 female (19.9 × 24.5 mm) (NCHUZOOL), Cingluo, Penghu, coll. 23 November 2006; 1 male (23.3 × 27.3 mm) (NCHUZOOL), Dadu R., Shengang, Changhua, Taiwan, coll. 24 October 1995; 1 male (24.4 × 29.4 mm) (NMNS-040701), Taiwan, coll. 4 July 2001; 1 male (19.2 × 23.3 mm) (NMNS-04129); 2 males (22.0-24.3 × 28.6-29.4 mm) (NTOU), Luodong, Yilan, Taiwan, coll. H.-S. Lai, 2 November 1985; 2 males (1.7-14.3 × 14.8-17.5 mm) (NTOU), estuary of Fengshan R., Hsinchu, Taiwan, coll. J.-F. Huang, 27 September 1987; 1 male (21.5 × 26.2 mm) (NTOU), Taichung, Taiwan, coll. J.-F. Huang, 29 March 1988; 3 males (7.8-12.9 × 9.7-16.2), 2 females (17.8-19.9 × 22.2-24.3 mm) (ZRC 1999.1420), mangroves at Sinfeng, Hsinchu, Taiwan, coll. H.-C. Liu & C.D. Schubart, 17 September 1999; 2 males (30.0-30.5 × 25.4-25.8 mm) (ZRC.2001.0071), north Taiwan, coll. K.-X. Lee, no collection date; 1 male (19.5 × 23.4 mm), river mouth, Taiwan, coll. 29 November 2004. *H. tridens* De Haan, 1835: Lectotype, 1 male (24.9 × 30.4 mm) (RMNH-D188), Japan, coll. von Siebold. Paralectotypes, 1 female, (23.1 × 28.3 mm) (RMNH-D189), Japan, coll. von Siebold; 1 male (25.0 × 29.8 mm) (RMHN-D190), Japan, coll. von Siebold. Others: 1 male (23.7 × 28.9 mm) (MNHN-B3476), Japan, no collector information, no collection date, from Leiden Museum. *H. latimera* Parisi, 1918 (= *H. tridens pingi* Rathbun, 1931, see K Sakai et al. 2006): Holotype of *H. tridens pingi*, 1 male (25.7 × 31.1 mm) (USNM-59163), Hainan I., China, coll. C. Ping, November 1924. Others: 1 male (23.3 × 27.2 mm), 1 female (25.1 × 31.1 mm) (NCHUZOOL), Kinmen, Taiwan, coll. L.-R. Tung, 8 May 1989. *H. tientsinensis* Rathbun, 1931: Holotype, 1 male (24.9 × 28.8 mm) (USNM-25431), Tientsin (= Tianjin), China, coll.

M. Robb. Paratype, 1 female (26.2 × 33.0 mm) (USNM-171410), Tientsin, China, coll. May 1901.

Description: Carapace rectangular (Fig. 1A), distinctly broader than long, width to length ratio 1.17-1.29 (mean = 1.24, $n = 43$), dorsal surface convex, regions defined, punctuated, with single short seta on each punctum; cervical grooves indistinct, shallow, broad. Front sloping ventrally, frontal margin (Fig. 1A) bilobed, medially divided

by deep, wide sinus; median sinus distinctly separating epi-, protogastric regions. Supraorbital margins gently sinuous, divergent posteriorly. Exorbital tooth distinct, tip pointing anteriorly; anterolateral, posterolateral margins confluent as lateral margin, with 3 branchial teeth behind exorbital tooth, first, second lateral teeth large, separated from anterior tooth by sharp V-shaped notch, third tooth small. Two low, oblique ridges on



Fig. 1. *Helice epicure* sp. nov. Paratype, male (26.4 × 30.1 mm) (RUMF-ZC-3069). (A) dorsal entire view; (B) cephalothorax, ventral view; (C) right chela, outer view.

mesobranchial region, terminating laterally at notch between first, second and second, third teeth, respectively. Another low ridge present above base of P5. Infraorbital margins (Fig. 2A) granulate, setose, incomplete, not congruent laterally with exorbital tooth. Male with distinct suborbital ridge just below infraorbital margin, extending laterally to sub-branchial region; suborbital ridge with 19-26 (mean = 21.9, $n = 22$) well-spaced heteromorphic tubercles; mesial tubercles small, rounded, usually fused, non-mesial tubercles medially (especially below constricted portion to distal end of eye when retracted) large, vertically-elongated, inclined mesially (Fig. 2A). Female suborbital ridge weaker than male, with 23-29 (mean = 26.2, $n = 15$) small,

isomorphic round granules.

Epistome broad, short longitudinally, sloping anteriorly, posterior margin with 2 submedian shallow concavities, median part produced roundly, margin granulated.

Eyes (Figs. 1A, 2A) well-developed, eye stalk weakly constricted near base of cornea. Third maxillipeds (Fig. 1B) leaving rhomboidal space when closed; ischium long, narrow; with longitudinal shallow groove, merus almost as long as ischium, widened distally, with diagonal groove running from distomesial corner of merus to proximolateral corner of ischium, mesial margin of diagonal groove setose; carpus articulated at distolateral corner of merus, auriculiform. Exopod

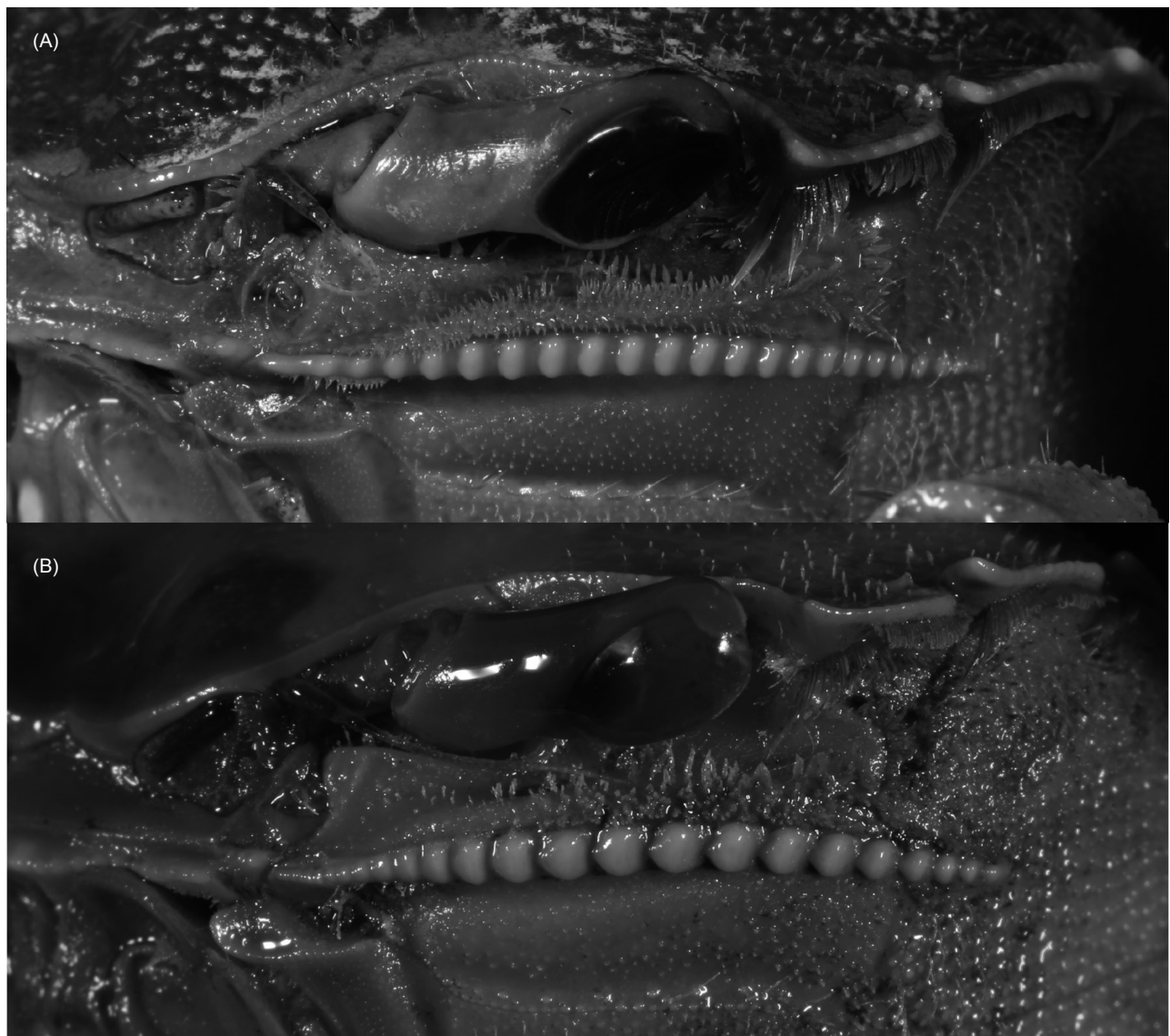


Fig. 2. Left suborbital ridge of *Helice epicure* sp. nov. and *H. formosensis* Rathbun, 1931. (A) *H. epicure* sp. nov., holotype, male (25.3 × 30.7 mm) (RUMF-ZC-5009); (B) *H. formosensis*, male (27.0 × 31.4 mm) (NCHUZ00L).

narrow, long, reaching to distal third of lateral margin of merus, flagellum longer than width of merus.

Chelipeds (Fig. 1A) relatively short, stout, symmetrical in both male, female, much larger in male. Merus with triangular cross-section, lower margins lined with granules. Carpus rhomboidal in upper view, inner angle acute. Large male chela (Fig. 1C) longer than exorbital width; palm almost as long as high, outer surface weakly convex, smooth, upper part thin, upper margin keel-like, inner surface concave around articulation with carpus and base of immovable finger; movable finger longer than immovable finger, almost as long as lower margin of palm; both fingers (including occlusal margins) straight, directed slightly downwards, leaving narrow gap when closed, occlusal margin lined with small teeth.

Ambulatory legs (Fig. 1A) moderately long, slender; second ambulatory leg longest. Anterior-upper margin of meri with subdistal tooth. Mat of short setae on lower surfaces of propodus and distal portion of carpus of first ambulatory legs and propodus of second ambulatory leg. Dactyli long, slender, tapering to slender, acute, corneous tip.

Male pleon narrowly triangular (Fig. 3A), all somites and telson free; lateral margins with short setae; first pleonal somite arched, with transverse ridge; second somite narrow, short; third somite short, widest, lateral margin acutely convex; fourth somite broader but shorter than fifth somite; fifth somite with distal margin straight, lateral margins slightly concave; sixth pleonal somite subquadrate, lateral margins slightly convex on distal third, proximal margin straight; telson lateral margins subparallel, distal margin rounded, with transverse line of long setae on subdistally. Female pleon very broadly rounded.

Male thoracic sternites 3 and 4 (Fig. 1B) demarcated by straight suture; sternite 4 with lateral margins strongly concave, subdistal portions of lateral portion and anterior portion of sternopleonal cavity with setose area. Sternopleonal cavity narrow, reaching imaginally line joining almost distal end of bases of chelipeds, sutures between sternites 4-7 continuous mesially to near base of slope of cavity, ending in narrow depressions; median grooves present on sternites 5-7 each, but not connected to each other. Penis emerged from near anterior margin of thoracic sternite 8.

G1 (Fig. 3B, C) long, slender, gently curving dorsally along thorax, distally reaching to almost anterior margin of sternite 5 *in situ*; distal portion

of G1 with terminal and subterminal (= palp) lobes, terminal lobe elongated, with thick corneous endpiece, genital opening lateral to base of terminal lobe; palp rounded, semitriangular, densely setose (Fig. 3B, C). G2 (Fig. 3D) short, broad, tip bilobed.

Female thoracic sternum similar in form but relatively broader. Female gonopore (= vulva; Fig. 3E) on anterior two-fifth of sternite 6, conically-shaped sternal cover developed from lateral margin, almost entirely covering vulva except for crescentic slit along anterior, mesial, posterior margins.

Etymology: The species name 'epicure' is used here as a Latin noun, derived from 'epikouros', a noun in Greek for 'food lover' or a person who takes particular pleasure in fine food and drink; this alludes to the late Professor Michael Türkay, a food connoisseur, who strongly believed in eating well and working hard. 'Epikouros' in Latin is 'Epicurus', also the name of a famous ancient Greek philosopher, whose insistence that nothing should be believed except that which was tested through direct observation and logical deduction made him a key figure in the development of science and scientific methodology.

Distribution: The new species is found in central to southern Ryukyus, including the islands of Amami-Oshima, Okinawa, Kumejima, Ishigaki and Iriomote. Authors who recorded *Helice* from the Ryukyu Islands are almost certainly referring to the new species instead (Table 1), although we were not able to examine their material. Our sampling covered most of the Ryukyu Islands (from Amami to Iriomote), and all the specimens belong to the new species. Paratypes are designated to the specimens collected from Yaeyama Islands (including the islands of Ishigaki and Iriomote), as the other main localities, including the islands of Okinawa and Amami, are located relatively far from the type locality (> 300 km).

"*Helice leachi* Hess, 1865" was reported from the Ryukyus (e.g. Sakai 1976; Shokita et al. 1998; Nakasone and Irei 2003), but those records are now regarded as *Pseudohelice subquadrate* (Dana, 1851) instead (Sakai et al. 2006).

Remarks: The taxonomy of the genus *Helice* De Haan, 1833 has been treated at length by K Sakai et al. (2006), and there is no need to discuss it further here. K Sakai et al. (2006) provided a key to identifying the extant four species. The main identification characters are the forms of the suborbital ridge (K Sakai et al. 2006: 16) and the structure of the G1 tip (K Sakai et al. 2006: 17).

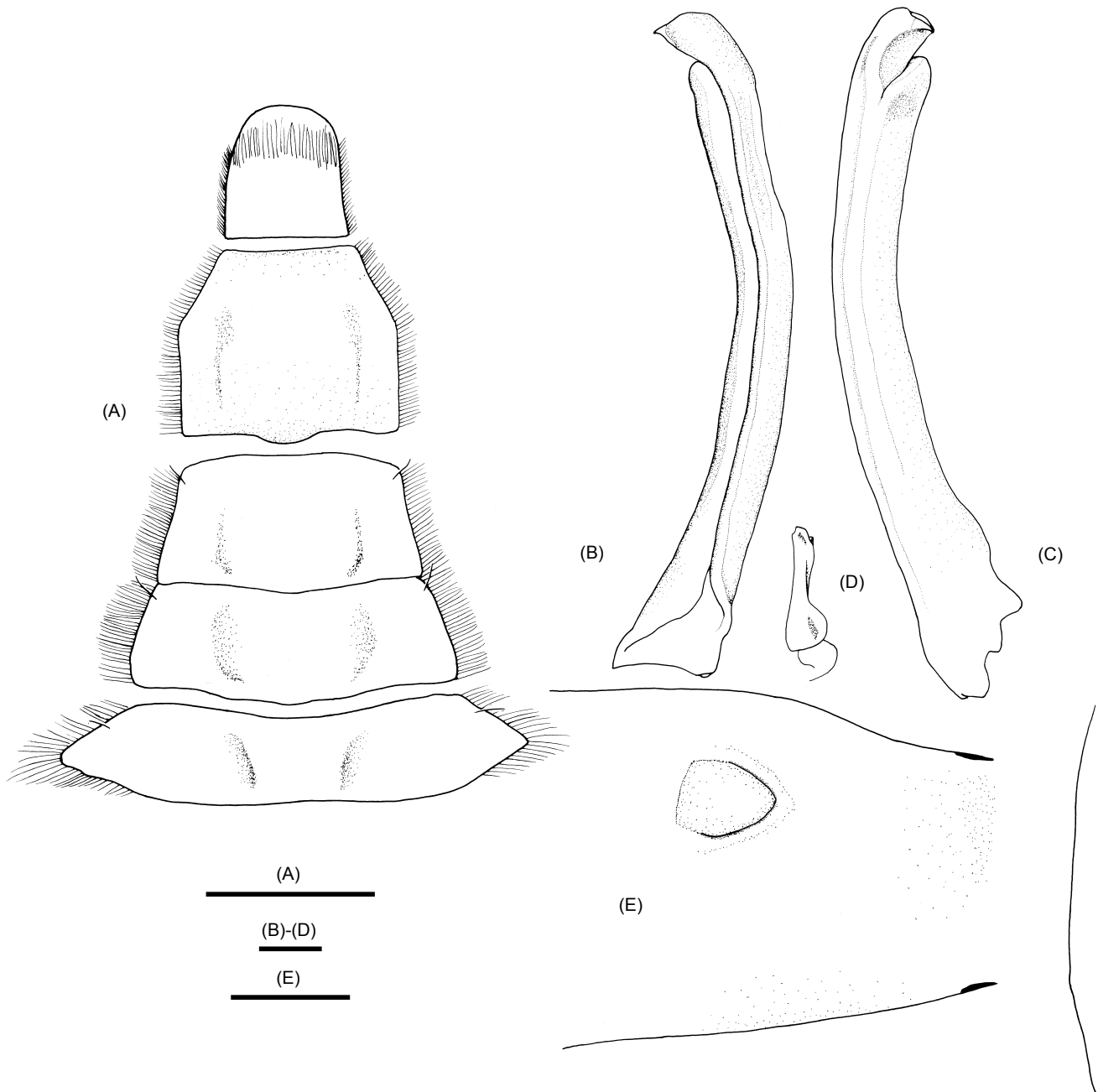


Fig. 3. *Helice epicure* sp. nov. Holotype, male (25.3 × 30.7 mm) (RUMF-ZC-5009) (A-D), female (18.4 × 23.5 mm) (RUMF-ZC-5011) (E). (A) pleonal somites 3-6 and telson; (B) left G1, mesial view; (C) left G1, lateral view; (D) left G2, ventral view; (E) gonopore (= vulva). Scale bars: A = 5.0 mm, B-E = 1.0 mm.

Table 1. References that cited species referable to *Helice epicure* sp. nov. from the Ryukyu Islands

Species name in references	References
<i>Helice tridens</i>	Stimpson 1858: 105 (part); 1907: 133 (part); Hirata et al. 1988: 22, 1 unnumbered fig.
<i>Helice tridens tridens</i>	Miyake 1963: 68; Sakai 1976: 669 (part).
<i>Helice formosensis</i>	Miyake 1983: 185, pl. 62(2); Nakasone and Irei 2003: 272.
<i>Helice tridens latimera</i>	Nagai and Nomura 1988: 49, 1 unnumbered fig.

The new species does not bear any resemblance to *Cyclograpsus latreilli* H Milne Edwards, 1837, which has been synonymized under *Helice tridens* (MNHN-B4647S) and *Pseudohelice subquadrata* (Dana, 1851), a senior synonym of *Helice latreilli* H. Milne Edwards, 1853 (MNHN-B3468S) by K Sakai et al. (2006), respectively.

Helice epicure sp. nov. can be clearly distinguished from *H. tridens* (De Haan, 1835), *H. latimera* Parisi, 1918, and *H. tientsinensis* Rathbun, 1931 by the shape of suborbital crista and/or number of granules on the suborbital ridge (Table 2).

In the general morphology, the new species is most similar to *H. formosensis*. However, it can be distinguished from *H. formosensis* by the following characters: viz. the shape of the middle large tubercles of suborbital ridge (relatively smaller, each tubercle mesially high, well-spaced from each other (Fig. 2A) vs. relatively large, each tubercle medially high, densely lined in *H. formosensis* (Fig. 2B)) and the base of the occlusal margin of the immovable finger of large male (almost straight (Fig. 1C) vs. deeply concave in *H. formosensis*). The new species also resembles *H. latimera* and *H. tientsinensis* in the general form of the carapace, but it can be easily distinguished from *H. latimera* by the following characters viz. a) forms, including the numbers and types of tubercles on the suborbital ridge (see Table 2); b) the gap between the terminal and subterminal lobes on the tip of the G1 is broad (vs. narrow gap of G1 in *H. latimera*) (see K Sakai et al. 2006: 17, fig. 22a-b), and c) the female gonopore is raised, and rounded (vs. is slightly raised and slightly elongated in *H. latimera*) (see K Sakai et al. 2006: 17, fig. 26). The new species can be easily distinguished from the *H. tientsinensis* in the following characters viz. a) forms, including the numbers and types of tubercles on the suborbital ridge (see Table 2);

b) the gap between the terminal and subterminal lobes on the tip of the G1 is broad (vs. slightly narrower gap of G1 in *H. tientsinensis*) (see K Sakai et al. 2006: 17, fig. 21a-b), and c) the female gonopore is raised, and rounded (vs. is slightly raised and slightly elongated in *H. tridens*) (see K Sakai et al. 2006: 17, fig. 25).

Molecular analyses and discussion

The phylogenetic tree of *COI* based on 658-bp segments (Fig. 4) showed *H. epicure* sp. nov. and the *H. latimera* complex (including *H. latimera*, *H. formosensis* and *H. tientsinensis*) to be sister. There are three haplotypes, named “Hep-C1”, “Hep-C2” and “Hep-C3” (accession numbers LC375187, LC375188, and LC375189, respectively), differing by ≤ 3 bp or 6.92% K2P divergence, among 17 individuals of the new species. The haplotype HRY-C1 is the most common one, found in 14 individuals from Iriomote, Ishigaki, Okinawa and Amami; HRY-C2 is found in one individual from Okinawa; and HRY-C3 in two individuals from Kumejima and Amami.

With regard to *Helice*, the mean divergence within and between subclades (species or complex) are shown in table 3. The minimum divergence between *H. epicure* and the *H. latimera* complex is 2.97%, which is much larger (4.9 times) than the maximum divergence (0.61%) within members of the *H. latimera* complex. Therefore, this new species is supported by both its phylogenetic relationship (Fig. 4) and its nucleotide divergence (Table 3).

Although the minimum interspecific divergence 2.97% is smaller, compared with other taxa of the superfamily Grapsoidea, viz. 4.7% (between *Helice tridens* and the *Helice latimera* complex; Shih and Suzuki 2008); 4.66% (between *Eriocheir japonica* (De Haan, 1835) and *E. hepuensis* Dai,

Table 2. Number and types of tubercles present on the suborbital ridge of different *Helice* species (Fig. 4)

	Male	Female
<i>H. formosensis</i>	15-33 heteromorphic roundish tubercles, including 2-8 striated, mesial ones	24-33 small, rounded, isomorphic tubercles
<i>H. latimera</i>	64-67 vertically expanded, striated ridges	37-45 small, rounded isomorphic tubercles
<i>H. tientsinensis</i>	33-37 heteromorphic tubercles; clearly higher medially, laterally confluent with one another	26-37 small, rounded isomorphic tubercles
<i>H. tridens</i>	9-17 heteromorphic rounded tubercles, including 2-3 striated mesial tubercles	15-28 small, rounded isomorphic tubercles
<i>H. epicure</i> sp. nov.	19-26 heteromorphic roundish tubercles with several striated, mesial ones	23-29 small, rounded isomorphic tubercles

1991) (GTR + G model; Chu et al. 2003); and 4.1% (for the Jamaican *Sesarma reticulatum* group; Schubart et al. 1998), this value is still greater than the 1.6% (between *Neosarmatium africanum* Ragionieri, Fratini & Schubart, 2012 and *N. meinerti* (De Man, 1887)) (*p*-distance; Ragionieri et al. 2009 2012).

The small interspecific divergence distance suggests that the insular *H. epicure* and the continental *H. latimera* complex diverged relatively recently in geological time. If the substitution

rate of *COI* - 1.66%/106 yr for marine sesarmids (Schubart et al. 1998) - is applied, then the two species separated about 1.8 million years ago (with the *p*-distance 2.97%). This suggests that this separation was the result of isolation by early Pleistocene glaciation events (Haq et al. 1987; Woodruff 2003) around the Ryukyu Islands, when it separated from continental Asia, probably by the deep Okinawa Trough, while the Taiwan Island was still connected to China.

While *H. epicure* forms a distinct clade, its

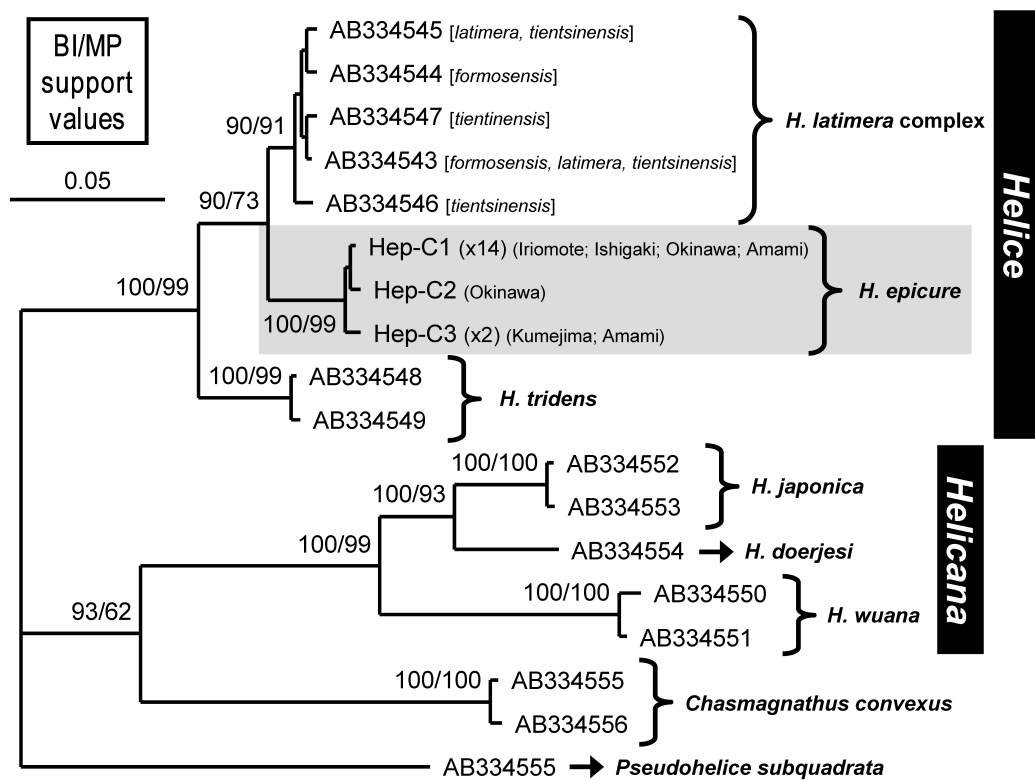


Fig. 4. A Bayesian inference (BI) of species of the *Helice/Chasmagnathus* complex from Japan (including the Ryukyus), Korea, China, and Taiwan (including Penghu) based on cytochrome oxidase subunit I (*COI*) genes. Support values at the nodes represent the posterior probability for BI and the bootstrap values for maximum parsimony (MP).

Table 3. Matrix of percentage pairwise nucleotide divergences with K2P distance (and value range) based on 658 bp of *COI* within and between three subclades (species or complex) of *Helice*

	within species/complex	between species/complex		
		<i>H. latimera</i> complex	<i>H. epicure</i>	<i>H. tridens</i>
<i>H. latimera</i> complex	0.17 (0-0.61)	-		
<i>H. epicure</i> sp. nov.	0.09 (0-0.46)	3.07 (2.97-3.46)	-	
<i>H. tridens</i>	0.1 (0-0.15)	4.89 (4.74-5.4)	6.51 (6.41-6.92)	-

sister clade - the members of the *H. latimera* complex - remains unresolved (Fig. 4) (see Shih and Suzuki 2008; Xu et al. 2011 2012). Shih and Suzuki (2008) have already suggested *H. latimera*, *H. formosensis* and *H. tientsinensis* may be a single species based on the unresolved clade. Recently, Xu et al. (2010 2011) analysed the morphometrics of *H. latimera* and *H. tientsinensis*. Based on morphological characters, the two species cannot be separated by cluster, discriminant or principal component analyses. In addition, intermediate forms of suborbital crenulation were found in Ningbo, Zhejiang, China, around the delta of Yangtze River; the northern population of which was closer to the “*tientsinensis*-form” and the southern population the “*latimera*-form”. These studies suggest that *H. latimera* and *H. tientsinensis* are probably conspecific. Similar morphometric studies including a morphologically distinct *H. formosensis* will be necessary to solve taxonomic issues among these three species.

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