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The first report of tropical hermit crab *Calcinus vachoni* (Malacostraca, Decapoda, Calcinidae) in Korea and the preliminary revision of its cryptic diversity

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Abstract

Background

Family Calcinidae is a hermit crab family morphologically diverged from Diogenidae. *Calcinus* is a type genus of Calcinidae. It inhabits tropical coral reefs with a colorful body. Among them, *C. vachoni* was firstly collected in southern Korea. Herein, this study reports Korean *C. vachoni* with its morphological, ecological, and molecular characteristics. Morphological characteristics of 12 *C. vachoni* caught on Jeju Island were examined. Ecological characteristics of *C. vachoni* were reviewed briefly and cox1 DNA barcoding analysis of five Korean *C. vachoni* was conducted with 16 species, 5 genera, and 3 families of Micronesia and GenBank hermit crab data. Korean *C. vachoni* has 13 pairs of gills, a larger left cheliped than the right one, a broad triangular rostrum, a few tufts of setae on ventral margins of dactyl and propodus of the first ambulatory leg, and cream dactyl and bluish-gray propodi of pereopods identical to the original description and previous studies. The cox1 sequences of Korean *C. vachoni* were monophyletic with cox1 sequences of *C. vachoni* in GenBank. *Calcinus vachoni* sequences were divided into three groups regarded as cryptic species. They were correlated with geographical distance as in the previous study. The review of ecological characteristics of *C. vachoni* shows that it inhabits nearby coral which might have a symbiotic relationship. The habitat of *C. vachoni* was extended to the northwest which could be related to recent climate change.

New information

In this study, the tropical hermit crab genus *Calcinus* and Family Calcinidae were first reported in Korea. For molecular identification, the cox1 sequences of Korean *C. vachoni* and other tropical hermit crabs in Kosrae, Micronesia were first obtained and analyzed by

this study. In addition, the cryptic diversity of *C. vachoni* groups noted in the previous study was reviewed and expanded their geographical range. Furthermore, we suggested the association relationship between *C. vachoni* and corals for the first time.

Keywords

Coral, intertidal zone, Jeju Island, symbiosis, climate change, cox1, genetic variation, cryptic species, geographical distance

Introduction

Calcinidae is a hermit crab family diverged from Diogenidae based on the difference in morphological characteristics of the shield (Fraaije et al. 2017). Calcinidae has small or blunt rostrum, a non-spinose massetic region, Y-shape intragastric groove, and degenerated central gastric groove. It consists of 130 species and 7 genera including *Calcinus*, *Allodardanus*, *Aniculus*, *Bathynarius*, *Ciliopagurus*, *Dardanus*, and *Trizopagurus* (Fraaije et al. 2017).

The genus *Calcinus* is the type genus of Calcinidae. It has mostly moderate to large size body, 13 pairs of gills, larger left cheliped than right, moderate to the well-developed triangular rostrum, and colorful carapace (McLaughlin 2003, McLaughlin et al. 2007). The color pattern of the *Calcinus* species is considered an important morphological character to distinguish them (McLaughlin et al. 2007, Malay and Paulay 2010). This genus mostly inhabits the tropical or subtropical coral reef in Indo-west Pacific (Malay and Paulay 2010). A total of 46 species in this genus have been reported worldwide (McLaughlin et al. 2010). However, there are no reports in Korea yet (Jung et al. 2018).

During a continuous systematic study of the Korean hermit crab, 12 specimens of *C. vachoni* were found near soft corals on Jeju Island. Herein, we report Korean *C. vachoni* with a diagnosis. In addition, we conducted cox1 molecular identification of Korean *C. vachoni* by comparison with hermit crab sequences collected from Micronesia and downloaded from GenBank. Furthermore, habitat characteristics of Korean *C. vachoni* revealed by this study were briefly discussed.

Materials and methods

We collected 12 individuals of *C. vachoni* from nearby *Palythoa* cf. *mutuki* in the rocky intertidal zone of Gamsan-ri, Andeok-myeon, Seogwipo-si, Jeju-do, the Republic of Korea on 6 Sep 2021. Voucher specimens in this study were deposited in the Honam National Institute of Biological Resources (HNIBR). All specimens used in this study were fixed in 95% ethanol and subjected to morphological examination and molecular analysis. Morphological characters were examined using an MZ8 dissection microscope (Leica, Wetzlar, Germany). Photographs were taken with a D200 digital camera (Nikon, Tokyo, Japan). The shield length of specimens was measured as the length from the tip of the

rostrum to the midpoint of the posterior margin of the carapace using a CD6CSX digital caliper (Mitutoyo, Kawasaki, Japan) to the nearest 0.1 mm.

For molecular analysis, the first or second ambulatory leg of 5 Korean *C. vachoni* was excised for total genomic DNA extraction using the DNeasy Blood & Tissue Kits (QIAGEN, Hilden, Germany). For comparative study, 41 individuals, 16 species, 5 genera, and 3 families of Micronesian hermit crabs in the Marine Arthropod Depository Bank, Marine Biodiversity Institute of Korea (MADBK) were borrowed and their DNA was extracted. To amplify the mitochondrial *cox1* gene, the universal *cox1* primers LCO1490 and HCO2198 were used (Folmer et al. 1994). Polymerase chain reaction (PCR) was performed in a total 50 μ L that included 3 μ L DNA template, 5 μ L 10 x Ex Taq Buffer, 5 μ L dNTP mix (10 mM), 2 μ L of each primer (10 μ M), 0.25 μ L Go Taq DNA polymerase (Promega, Madison City, WI, USA), and 35.75 μ L distilled H₂O. The thermocycling program was as following steps: 10 min denaturation at 94°C followed by 40 cycles of 1 min at 94°C, 1.5 min at 45°C and 2 min at 72°C and a final extension of 10 min at 72°C. PCR products were visualized on 1% agarose gels and sequenced with an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA). Nucleotide sequences of *cox1* were analyzed and edited using Geneious Prime v.2022.0.1 (Biomatters, Auckland, New Zealand) and using Clustal Omega (Sievers et al. 2011) in Geneious. Nucleotide sequences of Korean *C. vachoni* and Micronesian hermit crabs were deposited in GenBank (MZ215675-MZ215720). Thirty GenBank *cox1* sequences of 15 species, 4 genera, and 2 families were downloaded and included in the molecular analyses (Table 1).

Molecular identification of Korean *C. vachoni* was inferred for *cox1* using maximum likelihood (ML) analysis of MEGA10 program (Kumar et al. 2018). Maximum likelihood analyse of *cox1* sequences was performed based on the general time reversible (Tavaré 1986) models with a gamma distribution (+G) and invariable sites (+I) rate categories based on Bayesian Information Criterion (BIC) scores model using JMODELTEST 2.1.7 (Posada 2008). The robustness of individual nodes in the ML trees was assessed by analysis of 1,000 bootstrap replications. Interspecific and intraspecific sequence divergences were estimated based on the K2P distance matrix in MEGA10.

Taxon treatment

Calcinus vachoni Forest, 1958

Nomenclature

Calcinus vachoni Forest, 1958: 285, figs. 2, 3, 9, 10, 15, 19; Morgan 1990: 11, fig. 2; Morgan 1991: 205, figs. 60-62; Poupin 1997: 712, figs. 6E, F, 8A-F; McLaughlin et al. 2007: 170-171, unnumbered figs.; McLaughlin et al. 2010: 19 (list); Arima 2014: 42, unnumbered figs.

Synonym *Calcinus seurati* Miyake 1963: 63; Matsuzawa 1977: pl. 79, fig. 3; Chang and Chen 1992: 108 [not *Calcinus seurati* Forest 1951].

Not *Calcinus vachoni* Lewinsohn 1982: 53 [= *Calcinus guamensis* Wooster 1984].

Material

- a. originalNameUsage: *Calcinus vachoni* Forest, 1958; namePublishedIn: Forest, J. 1958. Les Pagures du Viet-Nam. II. Sur quelques espèces du genre *Calcinus* Dana. Bulletin du Muséum national d'Histoire naturelle, ser. 2. 30(2):184–190, 285–290.; acceptedNameUsage: *Calcinus vachoni* Forest, 1958; taxonomicStatus: accepted; scientificNameID: urn:lsid:marinespecies.org:taxname:208673; parentNameUsage: *Calcinus* Dana, 1851; kingdom: Animalia; phylum: Arthropoda; class: Malacostraca; order: Decapoda; family: Calcinidae; taxonRank: species; nomenclaturalCode: ICZN; genus: *Calcinus*; specificEpithet: *vachoni*; scientificNameAuthorship: Forest, 1958; higherGeography: Asia; Korea; Jeju; Seogwipo; Andeok; Gamsan; continent: Asia; waterBody: East China Sea; island: Jeju Island; country: Korea; countryCode: Korea/KR; stateProvince: Jeju; county: Seogwipo; municipality: Andeok; locality: 1008 Gamsan, rocky intertidal zone; verbatimDepth: 0-0.5 m; maximumDepthInMeters: 0.5; minimumDistanceAboveSurfaceInMeters: 0; maximumDistanceAboveSurfaceInMeters: -0.5; locationRemarks: intertidal zone; verbatimCoordinates: 33 14 05N 126 21 30E; verbatimLatitude: 33 14 05N; verbatimLongitude: 126 21 30E; verbatimCoordinateSystem: degrees minutes seconds; samplingProtocol: skin diving; samplingEffort: 1 observer-hour; 0.5 km; eventDate: 2021-09-06T14:22-0900; eventTime: 14:22-0900; startDayOfYear: 249; endDayOfYear: 249; year: 2021; month: 9; day: 6; verbatimEventDate: 20210906; habitat: rocky intertidal zone, nearby colony of *Palythoa* cf. *mutuki*; eventRemarks: a little rainy and windy day; individualCount: 12; lifeStage: adult (sl 2.6-3.9 mm); preparations: whole animal (ETOH); behavior: foraging; establishmentMeans: wild; recordedBy: Jibom Jung; occurrenceStatus: present; identifiedBy: Jibom Jung; dateIdentified: 2021-09-06T21:13-0900; identificationReferences: A catalog of the hermit crabs (Paguroidea) of Taiwan. McLaughlin et al. 2007.; identificationRemarks: The morphological characteristics of Korean samples are identical to the diagnosis of *Calcinus vachoni* by McLaughlin et al. (2007).; modified: 2022-06-14T11:50-0900; language: en & kr; rightsHolder: the Honam National Institute of Biological Resources (HNIBR); institutionID: HNIBRIV911; institutionCode: HNIBRIV; collectionCode: Invertebrates; ownerInstitutionCode: HNIBR; basisOfRecord: PreservedSpecimen

Diagnosis

Thirteen pairs of gills. Shield (Fig. 1) semi-ellipse, length 1.1 time as long as width; rostrum (Fig. 2) and lateral projection broad triangular. Ocular peduncle 0.8 times as long as shield, base inflated; cornea slightly dilated; ocular acicles bi or trifold. Antennular and antennal peduncle shorter than distal corneal margin when fully extended. Antennules with upper rami of flagella terminating in tapered filament. Maxilliped 3 approximate basally. Pereopods with numerous fine granules. Chelipeds unequal, left appreciable larger. Lateral and dorsal surface of palm of left cheliped (Figs 1, 2) almost smooth. Dorsal surface of palm of right cheliped (Figs 1, 2) with 3-6 tuberculates; dorsal margin of carpus with small spines. Ambulatory legs (Figs 1, 2) smooth, 2.7-3.3 times as long as shield. First ambulatory legs with less numerous tufts of setae than second on margins of dactyls and propodi. Tuft of moderate setae on ventral margins of dactyl and propodi of second ambulatory leg, meri and other

margins with sparse setae or naked; dactyl 0.4-0.5 times longer than propodus, ventral margin with 4-5 corneous spines. Abdomen twisted, membranous. No paired pleopods in either sex, abdominal tergites not well calcified. Uropods asymmetric. Telson (Fig. 2) asymmetric, left posterior lobe larger; terminal and lateral margins of posterior lobes with numerous spines.

Distribution

Jeju Island, Korea; Vietnam (type locality); Indo-Pacific region from South Africa to Easter Island and southern Japan.

Ecology

Nearby living and dead coral in shallow subtidal to 20 m.

Color

Shield cream. Ocular peduncles bluish-gray; acicles cream. Antennular peduncles deep blue. Antennal flagellum red. Chelipeds bluish-gray except cream fingers. Ambulatory legs cream or light bluish-gray; dactyl cream.

Analysis

By molecular analysis, samples of this study were identified as *C. vachoni* because *cox1* sequences of these samples were monophyletic with other *C. vachoni* GenBank sequences with individual variation lower than 3.19% (Fig. 3). Individual variation of *C. vachoni* is lower than the minimum interspecific variation of this study between *C. haigae* and *C. minutus* (12.75 %). Meanwhile, the sequence variation between Korean *C. vachoni* and GenBank *C. aff. vachoni* was 12.16-14.26%, similar to the minimum interspecific variation of this study. In addition, *cox1* sequences of *C. vachoni* and *C. aff. vachoni* were divided into three clades that were closely related to the geographic distance between their collection sites (Fig. 4). The *cox1* sequences of all Micronesian hermit crabs consisted of monophyletic groups with sequences of identical species from GenBank (Fig. 3).

Discussion

There is no distinguished difference between Korean *C. vachoni* and the original description (Forest 1958). *Calcinus vachoni* is distinguished from other hermit crabs in Korea by the following morphological characteristics: 13 pairs of gills, larger left cheliped than right one, no paired pleopods, and a well-developed triangular rostrum. Distinguishing characteristics of *C. vachoni* from other *Calcinus* species are as follows: ventral margins of dactyl and propodus of ambulatory legs with sparse tufts of setae; dorsal margin of right chela with 3-6 tubercles; ventral margins of dactyl and propodi of second ambulatory legs with more numerous tufts of setae than first; telson with numerous spines on the terminal to lateral margins; pereopods with cream dactyl and bluish-gray propodi.

In this study, *C. vachoni* was only found nearby *Palythoa* cf. *mutuki* on Jeju Island. *Calcinus vachoni* is known to inhabit near corals in previous studies (McLaughlin et al. 2007). Hermit crabs have symbiotic relationships with many cnidarians such as hydrozoa, sea anemone, and coral (Williams and McDermott 2004, Jung and Kim 2017). However, a detailed relationship between *C. vachoni* and coral has not been reported. It is necessary to confirm this relationship through further ecological studies.

Calcinus vachoni is mainly a subtropical and tropical species in the Indo-Pacific region. Its range has expanded to the northwest through this report. Recently, inhabitations of many subtropical and tropical organisms have been newly reported in Korea (Jeong et al. 2012, Jung et al. 2013, Jung and Kim 2015, Maran et al. 2015). Because several previous studies have noted that recent climate change shifts the distribution of organisms (Perry et al. 2005, Kelly and Goulden 2008, Thomas 2010), further study is needed on whether the expansion of the range of these tropical marine organisms including *C. vachoni* is related to recent climate change.

Molecular analysis results (Fig. 3) showed that *C. vachoni* was divided into three groups that were correlated with geographical distance as in the study of Malay and Paulay (2010). Malay and Paulay (2010) have found that *C. vachoni* is divided into three geographic groups (i.e., *C. vachoni*, *C. aff. vachoni* Mascarenes, and *C. aff. vachoni* Cooks) through molecular analysis of three genes (cox1, 16S rDNA, and H3). In this study, the *C. vachoni* group was newly identified in Korea and China and the *C. aff. vachoni* Mascarenes group was newly identified in South Africa (Fig. 4). Since the habitat of *C. vachoni* is a wide area in the Indo-Pacific region ranging from South Africa to Easter Island and southern Japan, it is necessary to examine distributions of the three groups through extensive sampling in these areas.

All three groups of *C. vachoni* are considered cryptic species due to their large genetic variations and low morphological differences. The minimum interspecies distance in this study was 12.75% between *C. haigae* and *C. minutus*. This value is similar to the genetic variation (12.16-14.26%) among the three groups of *C. vachoni*. On the other hand, Malay and Paulay (2010) did not find any morphological differences between these three groups of *C. vachoni*. Therefore, each of these *C. vachoni* groups is considered to be different cryptic species. Additional sampling and detailed morphological observation are needed to determine whether these groups are new species.

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Figure 1.

Dorsal view of *Calcinus vachoni* Forest, 1958 (male, shield length 3.5 mm, HNIBRIV911, abdomen lost).

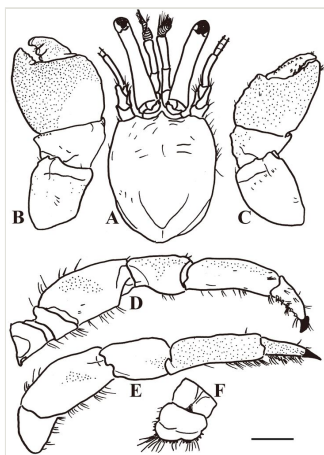


Figure 2.

Calcinus vachoni Forest, 1958 (male, shield length 2.9 mm, HNIBRIV911) **A** Shield and cephalic appendages, dorsal view **B** Left cheliped, dorsal view **C** Right cheliped, dorsal view **D** Right first ambulatory leg, mesial view **E** Left second ambulatory leg 2, lateral view **F** telson, dorsal view. Scale bar: A-F=2 mm.

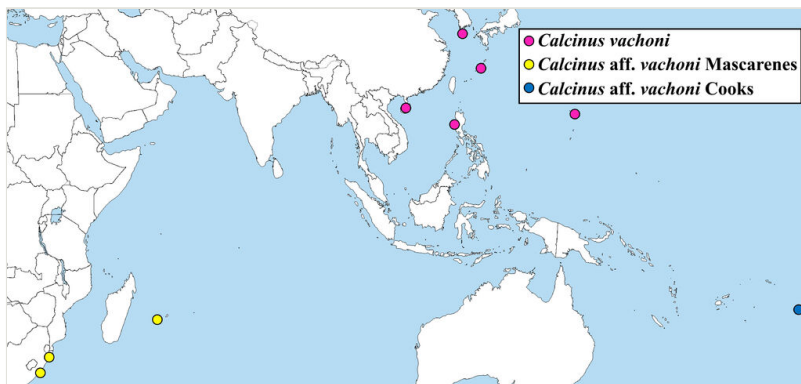


Figure 4.

Map showing the collection sites of *Calcinus vachoni* samples that *cox1* sequences were analyzed in this study. The name of *C. vachoni* groups were those of Malay and Paulay (2010)

Table 1.

GenBank accession numbers and geographic information of Paguroidea species used for phylogenetic analysis in this study. *: sequences downloaded from GenBank.

Family	Species	Location	Specimen number	cox1 GenBank accession no		
Annuntiogenidae	<i>Paguristes jalur</i>	Kosrae, Micronesia	MADBK	ON763589		
			160550_001	ON763590		
				ON763591		
Calcinidae	<i>Aniculus erythraeus</i>	Kosrae, Micronesia	MADBK	ON763551		
			160538_001			
		MADBK	ON763552			
		160538_002				
	<i>Aniculus retipes</i>	Kosrae, Micronesia	MADBK	ON763553		
			160539_001			
			MADBK	ON763554		
		160539_002				
		China		MK610038*		
	<i>Calcinus vachoni</i>	Jeju, Korea	HNIBRIV911		ON763592	
					ON763593	
					ON763594	
					ON763595	
					ON763596	
				Balingasay, Philippines	UF 6748	FJ620291*
				Maug Island, Mariana Islands	UF 5742	FJ620339*
				Okinawa, Japan	UF 6992	FJ620395*
				China		MK747767*
				<i>Calcinus aff. vachoni</i> Mascarenes	Reunion Island, Mascarene Islands	
	UF 13011	FJ620294*				
	FJ620295*					
Sodwana Bay, South Africa		MB-A066068	MH482034*			
		MB-A065989	MH482078*			
Port Shepstone, South Africa		MB-A066419	MH481935*			
		MB-A066420	MH482017*			
Pumula, South Africa		MB-A066399	MH481962*			
		MB-A066397	MH482022*			
		MB-A066398	MH482045*			
<i>Calcinus aff. vachoni</i> Cooks	Rarotonga Island, Cook Islands		UF 1377	FJ620296*		
			UF 11702	FJ620292*		

<i>Calcinus elegans</i>	Kosrae, Micronesia	MADBK 160518_004	ON763555
		MADBK 160518_006	ON763556 ON763557
	Rangiroa Atoll, Tuamotu Archipelago	UF 1351	FJ620284*
<i>Calcinus guamensis</i>	Kosrae, Micronesia	MADBK 160535_002	ON763558 ON763559
	Hiva Oa Island, Marquesas Islands	UF 5171	FJ620288*
<i>Calcinus haigae</i>	Kosrae, Micronesia	MADBK 160534_004	ON763560 ON763561 ON763562
			American Samoa
	<i>Calcinus laevimanus</i>	Kosrae, Micronesia	MADBK 160519_016
Reunion Island, Mascarene Islands		UF 5426	FJ620270*
<i>Calcinus lineapropodus</i>	Kosrae, Micronesia	MADBK 160524_007	ON763565 ON763566 ON763567
			Guam Island, Mariana Islands
	<i>Calcinus minutus</i>	Kosrae, Micronesia	MADBK 160536_005
American Samoa		UF 3263	FJ620303*
<i>Calcinus morgani</i>	Kosrae, Micronesia	MADBK 160530_001	ON763570
	American Samoa	UF 3236	FJ620277*
<i>Calcinus pulcher</i>	Kosrae, Micronesia	MADBK 160537_003	ON763571 ON763572 ON763573
			Pohnpei Island, Micronesia
	<i>Dardanus deformis</i>	Kosrae, Micronesia	MADBK 160523_005
MADBK 160523_007			ON763584
MADBK 160523_008			ON763585
China			MK747778*

	<i>Dardanus guttatus</i>	Kosrae, Micronesia	MADBK 160526_003	ON763586	
		China		MK747774*	
	<i>Dardanus lagopodes</i>	Kosrae, Micronesia	MADBK 160528_008	ON763587 ON763588	
		China		MK747771*	
	Diogenidae	<i>Clibanarius corallinus</i>	Kosrae, Micronesia	MADBK 160525_004	ON763574
					ON763575
					ON763576
			China	MK076135*	
		<i>Clibanarius eury sternus</i>	Kosrae, Micronesia	MADBK 160531_001	ON763577
					ON763578
ON763579					
China		MK076141*			
<i>Clibanarius striolatus</i>		Kosrae, Micronesia	MADBK 160532_003	ON763580	
				MADBK 160532_004	ON763581
	MADBK 160532_005			ON763582	