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Taxonomy and molecular phylogeny analysis of the genus *Diogenes* "*Edwardsii group*" (Decapoda: Anomora: Diogenidae) based on mitochondrial DNA sequences

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Abstract. *Fitrian T, Madduppa H, Ismet MS, Rahayu DL. 2022. Taxonomy and molecular phylogeny analysis of the genus* Diogenes *"*Edwardsii group*" (Decapoda: Anomora: Diogenidae) based on mitochondrial DNA sequences. Biodiversitas 23: 5302-5313.* Many variations of morphological characters differ from one species to another, showing high interspecific variation within the genus *Diogenes* Dana, 1851. Subtle morphological differences among certain species make it difficult to identify diagnostic characters. Although the genus is very speciose, the study on the molecular is poorly reported. This study aimed to evaluate the taxonomy and phylogeny of the genus *Diogenes* using morphological and molecular features. The molecular phylogeny of 15 species of *Diogenes* included in the "*Edwardsii group*" was constructed based on two mitochondrial genes COI and 16S rRNA. The results of the phylogeny tree construction based on concatenated mitochondrial genes COI and 16S rRNA mtDNA formed 5 clades of monophyletic. Phylogenetic relationships of *Diogenes* species based on concatenated mitochondrial genes COI and 16S rRNA sequenced suggest that the morphological and molecular analyses of the species in this study corroborated one another. Among 15 species studied, three species were challenging to identify due to their resemblance to other species traits. The phylogenetic tree demonstrates that the three taxa denoted by 'sp.' were different from the known species of *Diogenes* so far. *Diogenes* sp.1 was closely related to *Diogenes moosai* Rahayu & Forest, 1995, *Diogenes* sp.2 resembles *Diogenes laevicarpus* Rahayu, 1996, while *Diogenes* sp.3 was closely related to *Diogenes goniochirus* Forest, 1956.

Keywords: 16S rRNA, COI, *Diogenes*, hermit crabs, molecular, morphology

INTRODUCTION

The genus *Diogenes* Dana, 1851 consists of about 80 species and is widely spread in the Indo-Pacific and East Atlantic (Almón et al. 2021; Rahayu 2021; Rahayu and Pratiwi 2022). *Diogenes* is one of the most complex genus of hermit crabs in the family Diogenidae, characterized primarily by the presence of intercalary rostral process between the ocular acicle, the crista dentata of the third maxilliped having only a few teeth, and the endopod of the maxillule lacking lateral processes or having completely developed and curved lateral processes. Four informal groups were defined based on the morphological variances of these features, namely: *Custos*, *Edwardsii*, *Troglopagurus*, and *Pallescens* groups (Forest 1952; Rahayu and Forest 1995; McLaughlin 2005; Asakura and Godwin 2006; Asakura and Tachikawa 2010). *Edwardsii* group is characterized by the simple intercalary rostral process, non-bifurcate antennal acicles, antennal and antennular peduncles being distinctly longer than the ocular peduncles, and a long and setose antennal flagellum (Asakura and Tachikawa 2010). The group comprises 49 species and are found throughout Indo-Pacific and East Atlantic (Rahayu 2015; Trivedi et al. 2016; Igawa and Kato 2017; Landschoff and Rahayu 2018; Almón et al. 2021, 2022). So far, 10 species of *Diogenes* from *the Edwardsii group* have been identified in Indonesian seas.

Species identifications have traditionally been based on morphological characters, but sometimes it is challenging to obtain an accurate identification exclusively based on this approach, especially when cryptic species occurred. To solve these difficulties, the combination of morphological characters with molecular markers has been demonstrated to be a powerful tool in providing accurate identifications in marine organisms in general and in hermit crabs in particular (Raupach et al. 2015; Shih et al. 2016; Landschoff and Gouws 2018; Almón et al. 2021). DNA barcoding is a useful tool for the taxonomic classification and identification of species by sequencing a very short, standardized DNA sequence in a well-defined gene (Purty and Chatterjee 2016; Madduppa et al. 2017; Landschoff and Gouws 2018). The mitochondrial DNA is widely used in identifying a species from Indonesia marine invertebrates (Aprilia et al. 2014; Kurniasari et al. 2014; Sahriyani et al. 2014; Toha et al. 2015; Saleky et al. 2016; Madduppa et al. 2016; Dailami et al. 2018; Pranata et al. 2020; Hikam et al. 2021), discover new species in a wide range of taxa (Wang et al. 2017), and used for phylogenetic

studies (Galan et al. 2018; Sultana et al. 2018; Mantelatto et al. 2021). DNA barcoding has advantages in precision and accuracy in the safe identification of species compared with the morphological observations (Madduppa et al. 2017; Fuentes-López et al. 2020).

The COI and 16S rRNA markers have been extensively used and have proved to be effective tools in the study of decapod crustaceans (Almón et al. 2021; Mantelatto et al. 2022). In the present study, we used partial DNA sequences from the mitochondrial genes cytochrome c oxidase subunit I (COI) and 16S ribosomal RNA (16S rRNA). Additionally, we performed a morphological study based on the traditional taxonomic characters, as well as on a search for new characters, in order to support our molecular findings. Morphological and molecular analysis in this work provides more information about the phylogenetics of *Diogenes* and validates the taxonomic validity of morphological traits using molecular characters. Furthermore, the information of two markers (individually or in combination) can help to support the morphological results, not only for the identification of new species but also to elucidate the taxonomic validity of closely related species (Shih et al. 2016; Almón et al. 2021; Mantelatto et al. 2022).

MATERIALS DAN METHODS

Research materials

The fifteen species of *Diogenes* which are included in the *Edwardsii* group, namely *Diogenes avarus* Heller, 1865, *Diogenes fasciatus* Rahayu & Forest, 1995, *Diogenes foresti* Rahayu & Hortle, 2002, *Diogenes goniochirus* Forest, 1956, *Diogenes holthuisi* Asakura & Tachikawa, 2010, *Diogenes inglei* McLaughlin & Clark, 1997, *Diogenes klaasi* Rahayu & Forest, 1995, *Diogenes laevicarpus* Rahayu, 1996, *Diogenes moosai* Rahayu & Forest, 1995, *Diogenes rectimanus* Miers, 1884, *Diogenes singaporensis* Rahayu, 2015, *Diogenes takedai* Rahayu, 2012, *Diogenes* sp.1, *Diogenes* sp.2 and *Diogenes* sp.3 were used in this study (Figure 1). The specimens used in this study were obtained from several locations in Indonesia, Singapore, Japan, and Thailand and deposited at Research Center Oceanographic, National Research and Innovation Agency (RCO-BRIN) (Table 1).

Procedures

Morphological observation

Morphological characters were observed directly and using Olympus CX23 Binocular Microscope. Animal size, as indicated by shield length (sl) was measured from the midpoint of the rostrum to the midpoint of the posterior margin of the shield.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from pereopod muscle tissue using QIAGEN DNeasy Blood and Tissue Kit (Cat no. 69504). The COI and 16S rRNA molecular marker fragments were amplified by polymerase chain reaction (PCR). The PCR amplification was performed in a 25.5 µL reaction mixture containing 12.5 µL Taq DNA Polymerase (Bioline), 1 µL of each forward and reverse PCR primer, 9 µL of ddH2O, and 2 µL DNA template. The partial COI was amplified using the forward primer LCOI 1490 5'-GGT CAA CAA ATC ATA TTG G-3' and the reverse primer HCOI 2198 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3' (Folmer et al. 1994). The 16S rRNA was amplified using the forward primer 16S-F2 5'-CGR GYT TTA TAT CTG GTT-3' and the reverse primer 16S-R 5'-TTA TGC TAC CTT RGC ACA G-3' (Cabezas et al. 2011). The PCR program on the COI primers was set as follows: initial denaturation at 95°C for 10 s, 30 cycles of denaturation (94°C for 30 s), annealing (50°C for 30 s), and extension (72°C for 30 s) with the final extension step at 72°C for 10 min. The PCR program on the 16S rRNA primers was set as follows: initial denaturation at 94°C for 4 min, 39 cycles of denaturation (94°C for 30 s), annealing (45.5°C for 1 min), and extension (72°C for 1 min) with the final extension step at 72°C for 10 min. The positive PCR products were sent to a sequencing facility in first base Malaysia and loaded into ABI 1377 sequencer machine.

Table 1. The specimens sequenced for this study with collection locality and NCBI GenBank accession numbers for partial sequences of COI and 16S rRNA

Species name	Collected	Locality	GenBank Acc. no.	
			COI	16S rRNA
Diogenes avarus Heller, 1865	Reference Collection-RCO	Timika-Papua, Indonesia	OM956218	ON064098
Diogenes fasciatus Rahayu & Forest, 1995	Reference Collection-RCO	Ubin Island, Singapore	OM992268	ON064088
Diogenes foresti Rahayu & Hortel, 2002	Reference Collection-RCO	Timika-Papua, Indonesia	OM992271	ON064092
Diogenes goniochirus Forest, 1956	Reference Collection-RCO	Phuket, Thailand	0M992266	ON064086
Diogenes holothuisi Asakura & Tachikawa, 2010	Reference Collection-RCO	Kumejima, Japan	OM992270	ON064091
Diogenes inglei McLaughlin & Clark, 1997	Reference Collection-RCO	Singapore	OM992265	ON064085
Diogenes klaasi Rahayu & Forest, 1995	Reference Collection-RCO	Teluk Banten, Indonesia	OM992264	ON064084
Diogenes laevicarpus Rahayu, 1996	Reference Collection-RCO	Ubin Island, Singapore	OM992275	ON064096
Diogenes moosai Rahayu & Forest, 1995	Reference Collection-RCO	Serangoon Island, Singapore	OM992272	ON064093
Diogenes rectimanus Miers, 1884	Reference Collection-RCO	Singapore	OM992273	ON064094
Diogenes singaporensis Rahayu, 2015	Reference Collection-RCO	Ubin Island, Singapore	OM992274	ON064095
Diogenes tekedai Rahayu, 2012	Reference Collection-RCO	Lombok, Indonesia	OM992269	ON064090
Diogenes sp.1	Reference Collection-RCO	East Lombok, Indonesia	OM964805	ON064089
Diogenes sp.2	Reference Collection-RCO	Timika-Papua, Indonesia	OM992267	ON064087
Diogenes sp.3	Reference Collection-RCO	North Sulawesi, Indonesia	OM992276	ON064097

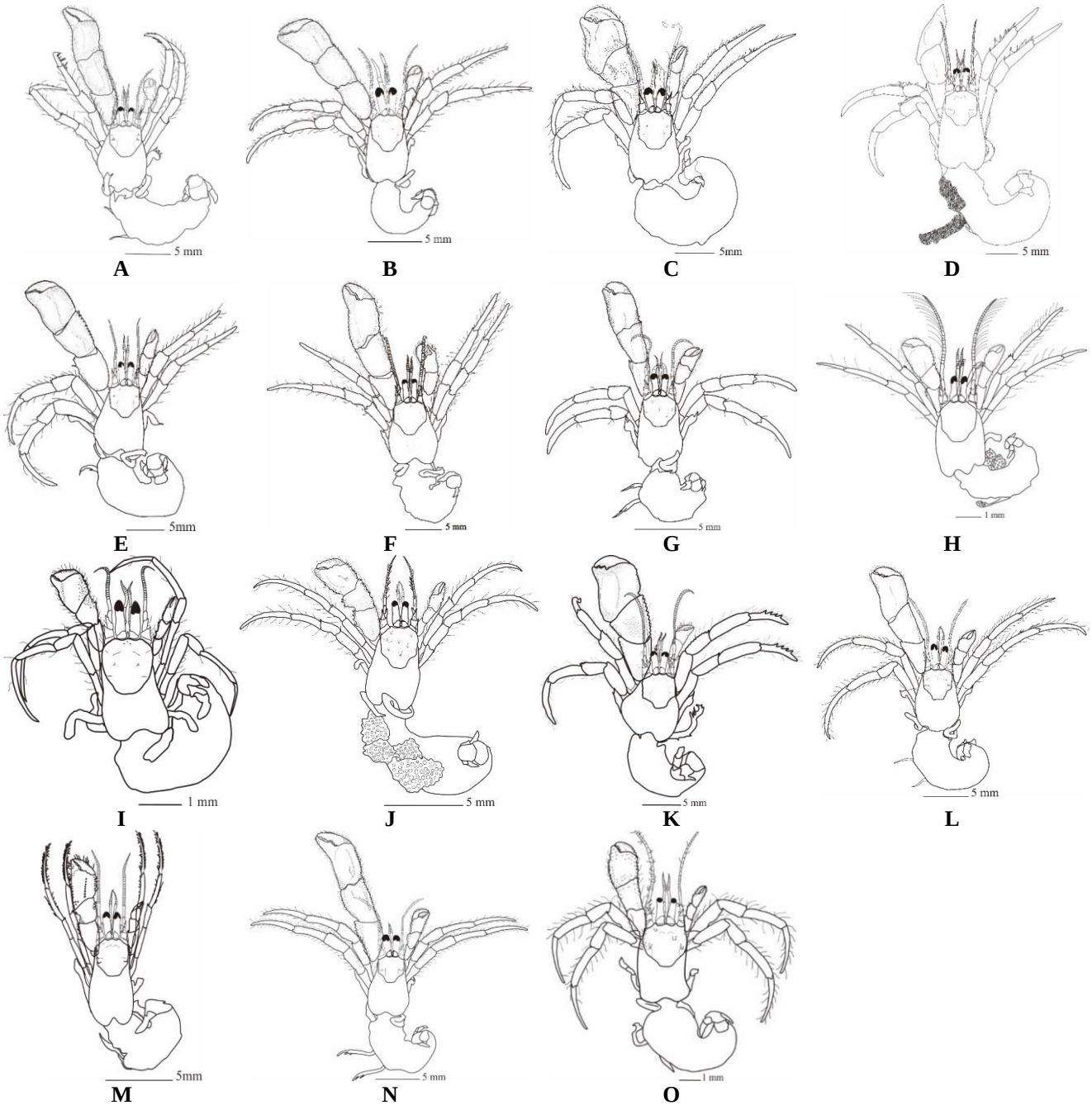

Figure 1. The fifteen species of the genus *Diogenes* used in the study. A. *Diogenes avarus* Heller, 1865, B. *Diogenes* sp.1, C. *Diogenes* sp.2, D. *Diogenes fasciatus* Rahayu & Forest, 1995, E. *Diogenes foresti* Rahayu & Hortel, 2002, F. *Diogenes* sp.3, G. *Diogenes goniochirus* Forest, 1956, H. *Diogenes holthuisi* Asakura & Tachikawa, 2010, *Diogenes inglei* McLaughlin & Clark, 1997, J. *Diogenes klaasi* Rahayu & Forest, 1995, K. *Diogenes laevicarpus* Rahayu, 1996, L. *Diogenes moosai* Rahayu & Forest, 1995, M. *Diogenes rectimanus* Miers, 1884, N. *Diogenes singaporensis* Rahayu, 2015, O. *Diogenes takedai* Rahayu, 2012

Data analysis

Species of the specimens are identified morphologically according to Rahayu and Forest (1995), Rahayu and Hortle (2002), Siddiqui et al. (2004), McLaughlin et al. (2007), Asakura and Tachikawa (2010), and Rahayu (1996, 2012, 2015). In addition, detailed morphological characterization was conducted in *Diogenes* sp.1, sp.2, and sp.3.

Alignment and sequence visualization was carried out using ClustalW on MEGA X (Kumar et al. 2018) and manually checked in the MEGA X. The final alignment consisting of COI gene and 16S rRNA was then verified into the BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to ensure the identity of the samples and to test homology with the available sequences in GenBank. The 15 sequence

data for the *Diogenes* obtained in this study have been deposited in GenBank (Table 2). The total number analyzed in the COI gene sequences was 53 sequences consisting of 15 specimen sequences from this study and 38 sequences from the GenBank database (Tabel 2) as a reference used to test the homology between the sequences studied and the sequences in the GenBank database. The phylogenetic tree analysis using 17 sample sequences from each marker (COI and 16S rRNA), consisting of 15 sample sequences used in this study and 2 sequences of *Clibanarius infraspinatus* Hilgendorf, 1869 (accession number MK076144) and *Dardanus arrosor* Herbst, 1796 (accession number MH482004) sequences from GenBank as outgroups. Phylogenetic analysis was performed by constructing a phylogenetic tree using the Neighbor-Joining method (Saitou and Nei 1987) with Kimura-2-Parameter substitution models (Kimura 1980) was reconstructed with 1000 replicates of bootstrap value (Felsenstein 1985) by using the MEGA X. The genetic distance between species was investigated using the MEGA X (Kumar et al. 2018).

RESULTS AND DISCUSSION

Morphological characters

Based on morphological characters, three species in this study (*Diogenes* sp.1, sp.2, and sp.3) cannot be attributed to all known species of the genus. *Diogenes* sp.1 resembles *D. avarus* in the following characters: short and moderately stout of ocular peduncle; palm of left cheliped with medially elevated outer surface armed with moderately closely spaced tubercles; second and third pereopods slender and carpi with rows of small spines. However, the differences are detected in the proportion of ocular peduncle length and shield, which is 0.5, with no dilated cornea in *D. avarus* versus 0.8 and dilated cornea in *Diogenes* sp.1; the absence of small spines on the fourth segment of antennal peduncle in *D. avarus*, versus presence of moderately strong spine in *Diogenes* sp. 1; propodi of second pereopods with rows of small spines on dorsal margin in *D. avarus*, versus without rows of small spines on dorsal margin in *Diogenes* sp.1.

Table 2. The 38 reference sequences of the genus *Diogenes* and 2 outgroups used in this study

Species name	Locality	No. accession	References
Diogenes armatus Almón, Cuesta, Schubart & García Raso in Almón, Cuesta, Schubart, Armenia & García Raso, 2021	Spain	MW776699	Almón et al. (2021)
Diogenes pugilator P. Roux, 1829	Spain	MW776677	Almón et al. (2021)
Diogenes sp.	Spain	MW776712	Almón et al. (2021)
Diogenes curvimanus Clément, 1874	France	MW776661	Almón et al. (2021)
Diogenes ovatus Miers, 1881	Guinea-Bissau	MW776720	Almón et al. (2021)
Diogenes sp.	Spain	MW776710	Almón et al. (2021)
Diogenes sp.	Spain	MW776713	Almón et al. (2021)
Diogenes curvimanus Clément, 1874	Belgium	MW776660	Almón et al. (2021)
Diogenes sp.	Singapore	MN690174	Ip et al. (2019)
Diogenes edwardsii De Haan, 1849	South Korea	MN114253	Jung and Kim (2019)
Diogenes sp	South Africa	MH482041	Landschoff and Gouws (2018)
Diogenes brevirostris Stimpson, 1858	South Africa	MH482027	Landschoff and Gouws (2018)
Diogenes sp.	South Africa	MH482016	Landschoff and Gouws (2018)
Diogenes sp.	South Africa	MH481965	Landschoff and Gouws (2018)
Diogenes sp.	South Africa	MH482061	Landschoff and Gouws (2018)
Diogenes albimanus Landschoff & Rahayu, 2018	South Africa	MH482073	Landschoff and Gouws (2018)
Diogenes costatus Henderson, 1893	South Africa	MH482075	Landschoff and Gouws (2018)
Diogenes aff. Pallescens	South Africa	MH482068	Landschoff and Gouws (2018)
Dardanus arrosor Herbst, 1796	South Africa	MH482004	Landschoff and Gouws (2018)
Diogenes goniochirus Forest, 1956	China	MW411962	Li et al. (2020)
Diogenes deflectomanus Wang & Tung, 1980	China	MK747781	Li et al. (2020)
Diogenes rectimanus Miers, 1884	China	MK747782	Li et al. (2020)
Diogenes edwardsii De Haan, 1849	China	MK747784	Li et al. (2020)
Diogenes nitidimanus Terao, 1913	China	MK747783	Li et al. (2020)
Diogenes avarus Heller, 1865	China	MK747780	Li et al. (2020)
Clibanarius infraspinatus Hilgendorf, 1869	China	MK076144	Li et al. (2019)
Diogenes sp.	India	MT590694	Mazumder et al. (2020)
Diogenes costatus Henderson, 1893	Pakistan	MW697229	Narejo et al. (2021)
Diogenes pugilator P. Roux, 1829	North Sea: German Bight	KT208810	Raupach et al. (2015)
Diogenes canaliculatus Komai, Reshmi & Kumar, 2013	India	KJ150701	Reshmi and Bijukumar (2014)
Diogenes merguiensis De Man, 1888	India	KM043474	Reshmi and Bijukumar (2014)
Diogenes alias McLaughlin & Holthuis, 2001	India	KM043472	Reshmi and Bijukumar (2014)
Diogenes violaceus Henderson, 1893	India	KJ150705	Reshmi and Bijukumar (2014)
Diogenes dubius Herbst, 1804	India	KM043473	Reshmi and Bijukumar (2014)
Diogenes miles Fabricius, 1787	India	KJ150703	Reshmi and Bijukumar (2014)
Diogenes manaarensis Henderson, 1893	India	KM043475	Reshmi and Bijukumar (2014)
Diogenes planimanus Henderson, 1893	India	KJ150704	Reshmi and Bijukumar (2014)
Diogenes custos Fabricius, 1798	India	KJ150702	Reshmi and Bijukumar (2014)

The morphological characters of *Diogenes* sp.2 are similar to *D. edwardsii* and *D. laevicarpus*. These three species have ocular peduncles slightly shorter than length of shield; left cheliped stout, with longitudinal sulcus on the outer surface of dactyl, palm shorter than dactyl, outer surface with longitudinal ridge medially; strong ridge on a fixed finger. The differences are on the following characters: *D. edwardsii* has a small cornea (0.5 times as long as ocular peduncles); sulcus on the left cheliped dactyl flanked by pointed spines; ridge on the fixed finger consists of large, pointed spines; palm always carries sea anemone on its surface; second and third pereopod with a row of spines on the dorsal margin of propodi and carpi. The *D. laevicarpus* has characters as follows: cornea 0.3 times as long as ocular peduncle; sulcus on left cheliped dactyl broad, flanked by sparse tubercles; ridge on fixed finger low, consists of small tubercles; a row of spines only on the dorsal margin of carpi of the second pereopod. *Diogenes* sp.2 has cornea same as *D. laevicarpus*, sulcus of left cheliped dactylus deep, broad, flanked by a row of large tubercles on either side; prominent ridge on fixed finger consists of smalls tubercles; anemones have never affixed palm; second and third pereopod with a row of spines on the dorsal margin of propodi and carpi.

Diogenes sp.3 resembles *D. foresti* in having ocular peduncles slightly shorter than length of shield, stout, slightly inflated basally; the outer surface of the palm of the left cheliped with numerous small acute tubercles, longitudinal ridge medially; second and third pereopods long and slender, small distal spine on the carpus of third pereopod. The differences can be seen in the shape of ocular peduncles, which are dilated distally and proximally in *D. foresti* (only dilated on the cornea in *Diogenes* sp.3); the fourth segment of antennal peduncle with small spine latero-distally in *D. foresti* (unarmed in *Diogenes* sp.3); second and third pereopods long with dactyl curved, with a strong distal spine on each dorsal margin of carpi in *D. foresti* (second and third pereopod long but the dactyls quite straight, carpi of the second pereopod with row of spines on dorsal margin in *Diogenes* sp.3).

Molecular identification

BLASTn examination by comparing the sample sequence with the reference sequence in the NCBI GenBank database found homologous sequences on COI markers with several species of *Diogenes* sequences from the NCBI GenBank. However, the 16S rRNA markers identified by BLAST, on average, were not homologous to several species of *Diogenes* in the GenBank database. The BLASTn results of COI marker sequence data through GenBank NCBI showed a sequence homologous, however, the average of the query coverage showed less close to 100%, E(Expect)-Value > 0, and PerIdent was not close to 100% in each database and the type of difference morphologically observed species of *Diogenes* by classical taxonomy with species of *Diogenes* based on BLAST in the GenBank database (Table 3).

This shows that the availability of *Diogenes* sequence data in the GenBank database is still very limited. The collection of DNA sequence codes along with accurate taxonomic, systematic references, will allow for an effective identification system and will be very useful in determining subtle/cryptic species. The construction of a phylogeny tree for 15 sample sequences with 38 reference references (Neighbor Joining, Kimura 2 parameter 1000x bootstrap) using COI markers is shown in Figure 2. The COI gene shown the number of data sets consisting of 617 sites with 334 conserved sites and 283 variable sites, the nucleotide frequency was a T/U (thymine/uracil base)= 37.0%, C (cytosine base)= 16.8%, A (adenine base)= 25.2%, G (guanine base)= 20.9%. Overall phylogenetic analysis for 53 COI gene sequences (using the neighbor-joining method and bootstrapping with 1000 replicates) indicated that all sequences formed a monophyletic clade or derived from the same ancestor (Figure 2).

Phylogenetic analysis

The phylogenetic tree construction used 17 sequences, consisting of 15 sequences used in this study and 2 samples from *C. infraspinatus* and *D. arrosor* sequences downloaded from the NCBI GenBank (Figure 3). The COI gene markers shown the number of data sets consisting of 617 sites with 365 conserved sites and 252 variable sites, the nucleotide frequency was a T/U (thymine/uracil)= 36.5%, C (cytosine)= 16.8%, A (adenine)= 25.2%, and G (guanine)= 21.5%. The 16S rRNA gene marker shown the number of data sets consisting of 583 sites with 103 conserved sites and 438 variable sites, the nucleotide frequency was a T (thymine/uracil)= 38.0%, C (cytosine)= 12.7%, A (adenine)= 36.2%, and (guanine)= 13.2%). The combined dataset of the two gene markers (COI marker and 16S rRNA) reconstructs the phylogeny tree are shown in Figure 4. The total data set of the combining COI gene markers and 16S rRNA consists of 1146 sites with 450 conserved sites and 656 variable sites, the nucleotide frequency was a T/U (thymine/uracil)= 37.3%, C (cytosine)= 15.0%, A (adenine)= 30.1%, and G (guanine base)= 17.5%.

The tree topologies on COI and 16S rRNA markers using 17 sequences obtained a slightly different form of the branching position of the species *Diogenes* sp.1 and *Diogenes* sp.3. The tree topologies on COI markers obtained *Diogenes* sp.3 form clade together with *D. goniochirus* and *D. fasciatus* (bootstrap=100), with *Diogenes* sp.2 (bootstrap=45) and with *D. laevicarpus* (bootstrap=57), *Diogenes* sp.1 form clade together with *D. avarus* and *D. foresti* (bootstrap=92). The tree topologies on 16S rRNA markers obtained *Diogenes* sp.1 cluster together and form clade *D. goniochirus* and *D. singaporensis* (bootstrap=47), *Diogenes* sp.2 occurs in a branch group with *D. laevicarpus* (bootstrap=70), *Diogenes* sp.3 cluster together and form clade with *D. avarus* and *D. foresti* (bootstrap=99).

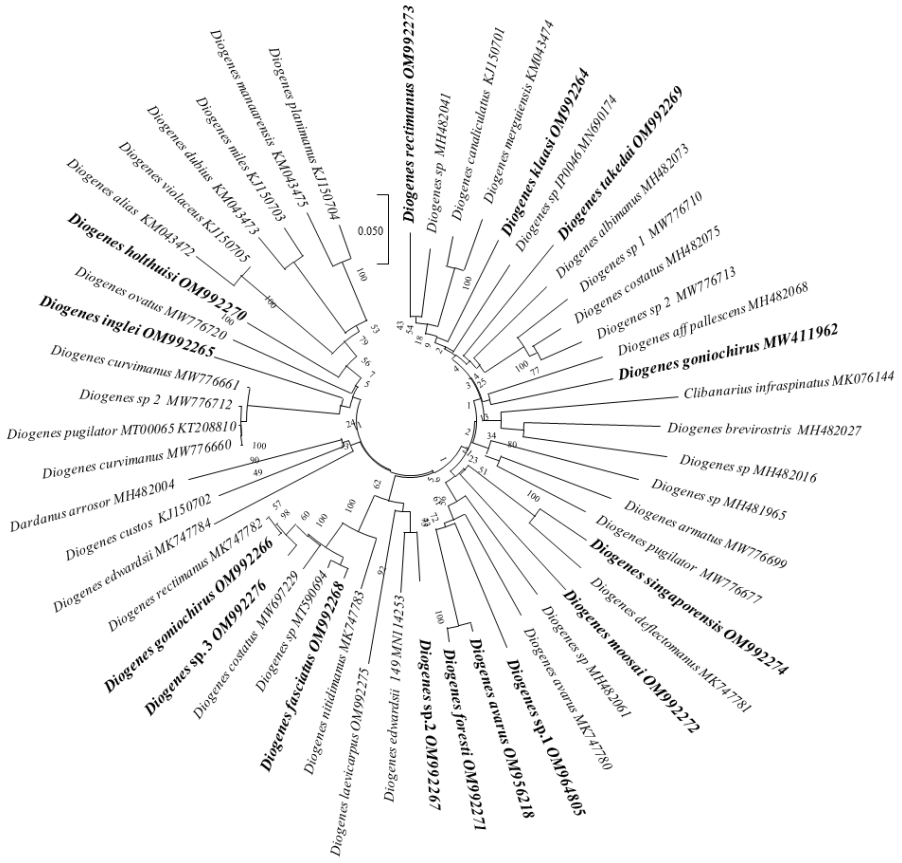

Figure 2. Neighbor-joining phylogenetic tree of 15 sample sequences and 38 sequences downloaded from GenBank generated by COI gene with 1000x bootstrap

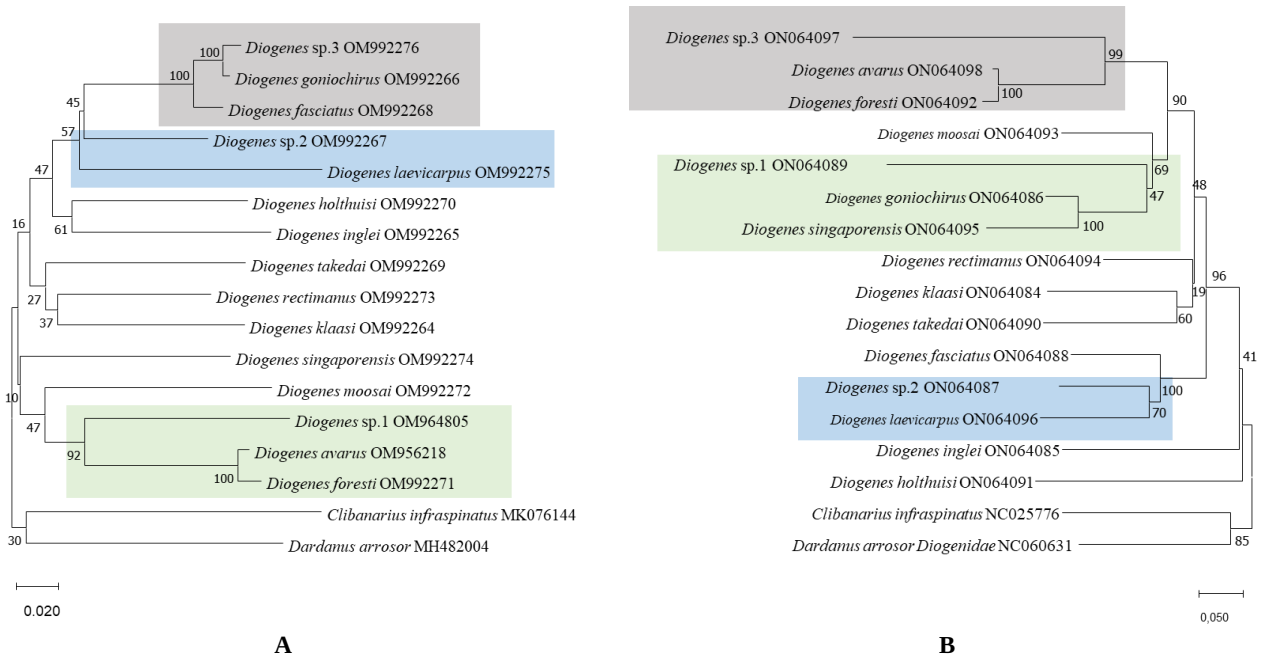

Figure 3. Phylogenetic tree of *Diogenes* generated by (A) COI genes (617 bp) and (B) 16S rRNA genes (583 bp)

Table 3. BLAST identification results based on the COI genes

Species based on morphological character	Query cover	E-value	Per.Ident	Species based on BLAST NCBI	Genbank accesion number
Diogenes avarus Heller, 1865	100%	3,00E-178	85.65%	*Diogenes avarus* Heller, 1865	MK747780.1
Diogenes sp.1	99%	3,00E-143	80.49%	*Diogenes costatus* Henderson, 1893	MH481985.1
Diogenes sp.2	86%	00.00	92.61%	*Diogenes edwardsii* De Haan, 1849	MN114253.1
Diogenes fasciatus Rahayu & Forest, 1995	92%	00.00	97.83%	*Diogens rectimanus* Miers, 1884	MK747782.1
Diogenes foresti Rahayu & Hortel, 2002	99%	00.00	85.52%	*Diogenes avarus* Heller, 1865	MK747780.1
Diogenes sp.3	95%	00.00	98.41%	*Diogenes rectimanus* Miers, 1884	MK747782.1
Diogenes goniochirus Forest, 1956	98%	00.00	99.54%	*Diogenes rectimanus* Miers, 1884	MK747782.1
Diogenes holthuisi Asakura & Tachikawa, 2010	100%	00.00	84.82%	*Diogenes nitidimanus* Terao, 1913	MK747783.1
Diogenes inglei McLaughlin & Clark, 1997	95%	1,00E-177	85.62%	*Eumunida picta* Smith, 1883	EU243556.1
Diogenes klaasi Rahayu & Forest, 1995	95%	00.00	87.54%	*Diogenes costatus* Henderson, 1893	MH481993.1
Diogenes laevicarpus Rahayu, 1996	94%	4,00E-152	83.62%	*Diogenes nitidimanus* Henderson, 1893	MK747783.1
Diogenes moosai Rahayu & Forest, 1995	97%	7,00E-169	83.93%	*Diogenes costatus* Terao, 1913	MH481993.1
Diogenes rectimanus Miers, 1884	96%	00.00	89.93%	*Diogenes* sp.	MH482041.1
Diogenes singaporensis Rahayu, 2015	97%	00.00	91.48%	*Diogenes deflectomanus* Wang & Tung, 1980	MK747781.1
Diogenes takedai Rahayu, 2012	95%	00.00	86.36%	*Diogenes costatus* Terao, 1913	MH481985.1

Table 4. Genetic distance among species of the genus *Diogenes* used in the study based on combined (COI and16S rRNA)

No.	Species name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	*Diogenes avarus*																
2	*Diogenes fasciatus*	0.282															
3	*Diogenes foresti*	0.021	0.291														
4	*Diogenes goniochirus*	0.268	0.148	0.278													
5	*Diogenes holthuisi*	0.342	0.279	0.337	0.290												
6	*Diogenes inglei*	0.336	0.280	0.342	0.291	0.289											
7	*Diogenes klaasi*	0.330	0.244	0.338	0.276	0.313	0.305										
8	*Diogenes laevicarpus*	0.337	0.216	0.345	0.276	0.310	0.319	0.307									
9	*Diogenes moosai*	0.271	0.257	0.273	0.223	0.337	0.312	0.278	0.337								
10	*Diogenes rectimanus*	0.277	0.229	0.283	0.230	0.276	0.273	0.217	0.265	0.241							
11	*Diogenes singaporensis*	0.302	0.292	0.309	0.180	0.331	0.335	0.312	0.326	0.246	0.255						
12	*Diogenes takedai*	0.307	0.257	0.322	0.259	0.323	0.329	0.246	0.296	0.271	0.219	0.262					
13	*Diogenes* sp.1	0.315	0.346	0.314	0.317	0.387	0.395	0.345	0.383	0.295	0.324	0.313	0.340				
14	*Diogenes* sp.2	0.283	0.164	0.303	0.224	0.297	0.287	0.244	0.208	0.290	0.216	0.285	0.260	0.332			
15	*Diogenes* sp.3	0.284	0.206	0.289	0.168	0.351	0.351	0.330	0.334	0.300	0.315	0.344	0.323	0.388	0.288		
16	*Clibanarius infraspinatus*	0.349	0.339	0.352	0.349	0.341	0.327	0.328	0.374	0.345	0.295	0.366	0.352	0.382	0.318	0.405	
17	*Dardanus arrosor*	0.334	0.285	0.343	0.321	0.325	0.309	0.333	0.361	0.349	0.300	0.350	0.335	0.384	0.318	0.378	0.313

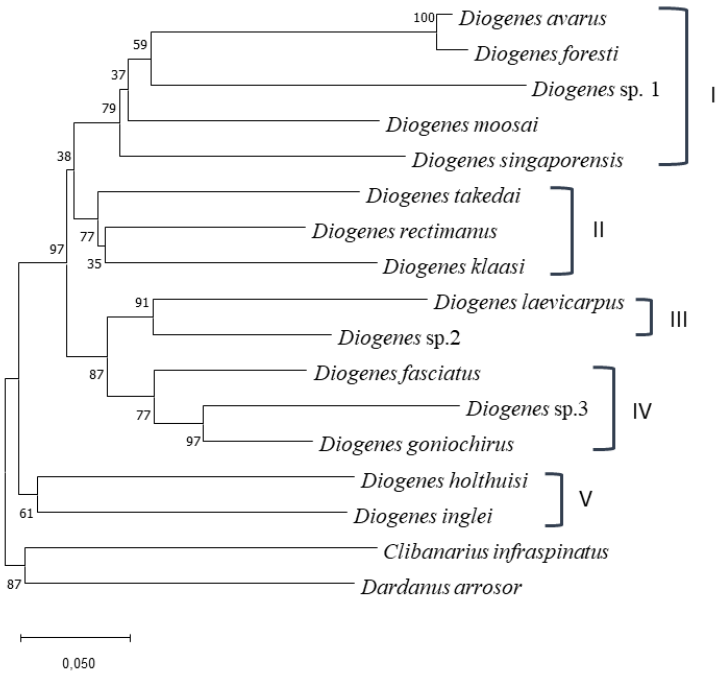

Figure 4. Phylogenetic tree of *Diogenes* species based on concatenated mitochondrial genes (COI and 16S rRNA) (Neighbor Joining, Kimura 2 parameter 1000x *bootstrap*, 1146 bp)

The tree topologies of the genus *Diogenes* based on two combined data on the COI and 16S rRNA formed 5 clades consisting of I, II, III, IV, and V with a significant bootstrap mean value. The clustering of form clade I was composed of *D. singaporensis* (bootstrap=79), *D. moosai* (bootstrap=37), *Diogenes* sp.1 (bootstrap=59) with the subclades *D. avarus*, and *D. foresti* (bootstrap=100). Based on genetic distance, *Diogenes* sp.1 is closer to *D. moosai* (0.925). The clustering of form clade II was composed of *D. takedai* (bootstrap=77) with the subclades *D. rectimanus* and *D. klaasi* (bootstrap=35). The clustering of form clade III was composed of *D. laevicarpus* and *Diogenes* sp.2 (bootstrap=91). Based on genetic distance, *Diogenes* sp.2 is more closer to *D. fasciatus* (0.164). The clustering of form clade IV was composed of *D. facsiatus* (bootstrap=77) with the subclade *Diogenes* sp.3 and *D. goniochirus* (bootstrap=97). Based on genetic distance, *Diogenes* sp.3 is more closer to *D. goniochirus* (0.168). The clustering of form clade V was composed of *D. inglei* and *D. holthuisi* (bootstrap=61). Based on data analysis, the genetic distance of the investigated specimens between species is shown in Table 4. The genetic distance of the investigated between 17 species shows a range of 0.021- 0.405. Genetic distance shows the relationship between each sequence, the smaller the value means the more closely related to the sequence/individual (Madduppa et al. 2017).

Discussion

Species of *Diogenes* are generally defined as having a spinose (intercalary rostriform) process developed from the ocular plate between the ocular acicles, *Diogenes* was established by Dana (1851) for a relatively few species in which the intercalary rostral process forms a large, somewhat flattened, marginally pinose structure (McLaughlin and Holthuis 2001); Thirteen pairs of biserial phyllobranchiate gills; Antennal acicle broadened basally and often developed as pair of bifurcate spinose processes; flagella often with long ventral setae. Crista dentata of third maxillipeds frequently with few teeth, without an accessory tooth. Endopod of maxillule with external lobe obsolete or well developed and recurved. First, maxilliped usually with flagellate exopod. Left cheliped much larger than right; dactyls and fixed fingers opening obliquely. Males with paired gonopores, no sexual tubes; females with paired, or less frequently, single gonopore; no paired pleopods in either sex; with unpaired pleopods on second to fifth segments in both sexes; male pleopods, with few exceptions, uniramous. Uropods asymmetrical or rarely symmetrical. Telson without transverse indentations; terminal margin entire or divided into 2 lobes by small median cleft; terminal and lateral margins armed or unarmed (Siddiqui et al. 2004).

Morphological characteristics of hermit crabs in the species belonging to the genus *Diogenes* vary greatly. McLaughlin (2002) proved this, who made *D. gardineri* Alcock, 1905 and *D. serenei* Forest, 1956, synonymous with *D. pallescens* Whitelegge, 1897. Morphological analysis of the three species showed variations in several characters previously considered key characters to distinguish the type so that the character can no longer be used to distinguish the three types. These characters include the ratio of the length and width of the shield, the

ratio of the length of the ocular peduncle to the antennular peduncles, the presence or absence of ventral spines under the intercalary rostral process, the size of the spines on the cheliped, and the number of spines on the pereopod. McLaughlin (2005) showed morphological variations in the species belonging to the *Troglopagurus* Group, which made *D. stenops* Morgan & Forest, 1991 and *D. setrocristatus* Morgan & Forest, 1991 synonyms of *D. jousseaumei* Bouvier, 1897 and *D. platyops* Rahayu & Forest, 1995 synonymous with *D. jubatus* Nobili, 1903. Variations in this *Troglopagurus* Group include: the number of spines on the ocular acicle, the ratio of the length of the ocular peduncle to that of the cornea, the dactylus and propodus of pereopods 2 and 3, and the shape of the telson. In addition, variations in intraspecific characters differ from one species to another, making the key characters for species also different. For example, variations in shield form can be used as key characters in one species but may not apply to other species; the size and density of spines/setae depend on the size or sex of the organism for one species but not for another. Based on the definition of the morphological variations, the 3 species of *Diogenes* in this study, namely *Diogenes* sp.1, sp.2, and sp.3 cannot be identified yet.

Molecular analysis can be performed to identify cryptic/sibling species (Pante et al. 2015), mainly the mitochondrial genes, which have demonstrated the existence of many cryptic lineages (Hultgren and Brandt 2015; Cunha et al. 2022). The incorporation of molecular tools into systematic studies has confirmed the taxonomic status of many taxa described based on morphological data (Landschoff and Gouws 2018), for example, in the genus *Cryphiops* (Mantelatto et al. 2021) and the genus *Diogenes* (Almón et al. 2021). The BLASTn results of COI marker sequence data through GenBank NCBI showed a sequence homologous, however, the average of the query coverage showed less close to 100%, E(Expect)-Value > 0 and PerIdent were not close to 100% in each database and the type of difference morphologically observed species of *Diogenes* by classical taxonomy with species of *Diogenes* based on BLAST in the GenBank database. The collection of DNA sequence codes along with accurate taxonomic, systematic references, will allow for an effective identification system and will be very useful in determining subtle/cryptic species. The results of molecular identification of 15 sequences using the cytochrome oxidase subunit I (COI) gene by comparing sample sequences with reference sequences in the NCBI GenBank database supported by topologies tree construction with the Neighbor-Joining method showed that all sequences formed a monophyletic clade or derived from the same ancestor supported by the Bootstrap Method variation estimation model (1000x replication).

The phylogenetic relationship among the genus *Diogenes* species was mainly delineated based on the COI, 16S rRNA, and combined COI and 16S rRNA data sets. We analyzed the phylogenetic relationships among *Diogenes* to test whether the three taxa denoted by 'sp' are distinct species with morphologically closest species. Results of the reconstruction tree topologies on the COI and 16S rRNA markers showed there were different positions of *Diogenes* sp.1 and *Diogenes* sp.3 and the difference in bootstrap support values for each marker. The bootstrap value is not considered a reliable measure of clade support (Craig and Felder 2021). However, based on the effective assessment, it shows that the tree is better with a higher bootstrap proportion and to get an accurate bootstrap value with the number of taxa analyzed since tree space itself increases exponentially with the number of taxa (Regier et al. 2013). The discrepancy positions of *Diogenes* sp.1 and *Diogenes* sp.3 are thought to be due to the different base pair lengths and evolutionary rates resulting from the two markers. The COI marker resulted in a longer base pair length and a faster evolutionary rate than the 16S rRNA marker. Previous research recommended that the long DNA sequences can yield a sufficient number of characters for robust phylogenetic inference and provide high phylogenetic resolution of relationships among closely related congeneric species of the genus *Clibanarius* (Li et al. 2019), while the relatively short DNA sequences used for phylogeny construction may explain the inconsistent phylogenetic patterns observed in previous reconstructions of *Clibanarius* phylogeny (Negri et al. 2014). Research about assessing the effectiveness of mitochondrial COI and 16S rRNA genes also showed 16S has a lower species identification success than COI on DNA barcoding of farmland spiders (Wang et al. 2017).

Concerning the phylogenetic validity of *Diogenes* based on two genes concatenated datasets, clades I, II, III, IV, and V were formed. The clustering based on molecular analysis or phylogeny tree topologies supports the clustering based on morphological analysis. The clade I comprised *Diogenes* sp.1, *D. moosai*, *D. singaporensis* with subclade *D. avarus*, and *D. foresti*. These five species' common morphological characters were found shields longer than broad, ocular peduncles stout, left cheliped with irregular rows of small tubercles or subacute spines, second and third pereopods slender. Based on the morphological characters, *Diogenes* sp.1 is closer to *D. avarus*, but there are several variations of different morphological characters. However, based on molecular analysis and genetic distance, *Diogenes* sp.1 is closer to *D. moosai*. Based on the morphological and molecular characters, *Diogenes* sp.1 is a different species from *D. avarus* and it is suspected that *Diogenes* sp.1 is a new species. The clade II was composed of *D. takedai, D. rectimanus* and *D. klaasi*. The common morphological characters of these three species are as follows: a shield that slightly longer than broad, left cheliped dactyl longer than palm; row of strong spines on upper and lower margin of palm; right cheliped with dactyl longer than palm; upper surface with a row of small spines, slight hiatus between dactyl and fixed finger. The clade III was composed of *D. laevicarpus* and *Diogenes* sp.2, their common morphological characteristics were that found shield was longer than broad, left cheliped dactyl with an elongated sulcus, and had a fixed finger with a longitudinal ridge. Based on morphological characters, *Diogenes* sp.2 is closer to *D. edwardsii* but there are several variations of different morphological characters, while based on molecular analysis, *Diogenes* sp.2 is closer to *D.*

laevicarpus which is suspected that *Diogenes* sp.2 is a new species and very close to *D. laevicarpus.* The clade IV was composed of *D. facsiatus, Diogenes* sp.3 and *D. goniochirus.* The common morphological characteristics of these three species were found in the form of a shield, which is approximately as long as broad, ocular peduncles stout, left cheliped covered by small, pointed spines. Based on morphological characters *Diogenes* sp.3 is closer to *D. foresti*, but there are several variations of different morphological characters. However, based on molecular analysis *Diogenes* sp.3 is closer to *D. goniochirus*. It is suspected that *Diogenes* sp.3 is a new species. The clade V was composed of *D. inglei* and *D. holthuisi.* Their common morphological characteristics were in having an ocular peduncle, which is shorter than a shield, tapering in the cornea, an antennal acicle short not reaching the base of the fifth segment of the antennal peduncle, left cheliped with a row of prominent spines on the upper margins of dactyl, palm and carpus, and lower margins of palm and fixed finger; the outer surface of dactyl with a short row of spines near upper margin.

In the combined-markers (COI+16S rRNA) genetic approaches, the tree topologies showed substantially similar phylogenetic relationships among the terminal taxa with the previously assigned morphological trait-based species groups. The molecular and morphology analysis results suggested that *Diogenes* sp.1 is different species with *D. avarus*, *Diogenes* sp.2 and *D. foresti* are also distinct species, and Diogenes sp.3 and *Diogenes edwardsii* as well as different species. The application of molecular tools, combined with traditional morphological analyses, has confirmed the taxonomic relationships of the species in the genus *Diogenes*. These results strengthen the analysis of the observed morphological differences. This also proves that an error in identification can occur in the species with subtle morphological differences if a molecular analysis is not carried out. Previous research has shown that the application of molecular tools, combined with traditional morphological analyses and the study of live color patterns, has revealed the existence of three different species of hermit crab along the coasts of the Iberian Peninsula (Almón et al. 2021).

In conclusion, subtle differences in morphological data indicate phylogenetic importance for the genus *Diogenes*. Most sample sequences of the genus *Diogenes* from GenBank formed a cluster together with specimens from this study. The concatenation of COI and 16S rRNA partial sequences led to higher confidence in the phylogenetic relationships. The analysis of the phylogeny tree construction based on the combined COI gene data and 16S rRNA mtDNA formed 5 monophyletic groups/clades. An overall phylogenetic analysis indicated that all species form monophyletic clades or are derived from the same ancestor by previous classic taxonomic work. The morphology and molecular analysis inference shown here demonstrate that *Diogenes* sp.1, *Diogenes* sp.2, and *Diogenes* sp.3 are different from any other species known in the genus *Diogenes*, a description of these three species will be done in a future study.

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REFERENCES

Almón B, Cuesta JA, García-Raso JE. 2022. Two new hermit crab species of *Diogenes* Crustacea: Decapoda: Diogenidae) from Atlanto-Mediterranean coasts of Iberian Peninsula: Poleward migrants or merely overlooked indigenous species? Ecol Evol 12 (5): e8844. DOI: DOI: 10.1002/ece3.8844.

Almón B, Cuesta JA, Schubart CD, Armenia L, Raso JEG. 2021. Redescription of the hermit crab *Diogenes pugilator* (Decapoda: Anomura) reveals the existence of a species complex in the Atlanto-Mediterranean transition zone, resulting in the resurrection of *D. curvimanus* and the description of a new species. Zool J Linn Soc 20: 1-31. DOI: 10.1093/zoolinnean/zlab093.

Aprilia FE, Soewondo A, Widodo, Toha AHA. 2014. Amplification of COI and 16s rRNA genes from the marine invertebrate *Plakobranchus ocellatus*. Biotropika 2 (5): 276-278. [Indonesian]

Asakura A, Godwin S. 2006. *Diogenes patae* n. sp. a new species of hermit crab (Crustacea, Decapoda, Anomura, Diogenidae) from American Samoa. Zoosystema 28: 457-463.

Asakura A, Tachikawa H. 2010. *Diogenes holthuisi*, a new species of hermit crab (Decapoda: Anomura: Diogenidae) from shallow water of the Ogasawara (Bonin) Islands, Japan. In: Fransen C, De Grave S, Ng PKL (eds). Studies on Malacostraca: Lipke Bijdeley Holthuis Memorial Volume. Crustaceana Monographs 14: 133-144. DOI: 10.1163/9789047427759_006.

Cabezas P, Macpherson E, Machordom A. 2011. *Allogalathea* (Decapoda: Galatheidae): A monospecific genus of squat lobster. Zool J Linn Soc 162: 245-270. DOI: 10.1111/j.1096-3642. 2010. 00681.x.

Craig CW, Felder DL. 2021. Molecular phylogenetic analysis of the *Paguristes tortugae* Schmitt, 1933 complex and selected other Paguroidea (Crustacea: Decapoda: Anomura). Zootaxa 4999 (4): 301-324. DOI: 10.11646/zootaxa.4999.4.1.

Cunha AM, Terossi M, Mantelatto FL, Almeida AO. 2022. Genetic variation and cryptic diversity of the *Alpheus lobidens* complex (Decapoda: Alpheidae) associated with marine ecoregions. Mar Freshw Res 73: 319-327. DOI: 10.1071/MF21043.

Dailami M, Santi D, Murtihapsari, Abubakar H, Toha AHA. 2018. Genetic analysis of cytochrome oxidase subunit 1 gene fragment from *Cirrhilabrus* cf. *ryukyuensis* Ishikawa 1904 (Labridae) from Cenderawasih Bay and Raja Ampat. Ikhtiologi Indonesia 18 (3): 209-222. DOI: 10.32491/jii.v18i3.347. [Indonesian]

Dana JD. 1851. Conspectus crustaceorumquae in orbis terrarium circumnavigatione, Carlos Wilkes e classe reipublicae foederatae duce, lexit et descripcit. Proc Acad Nat Sci Philadelphia 5: 267-272.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39 (4): 783-791. DOI: 10.1111/j.1558- 5646. 1985.tb00420.x.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I diverse metazoan invertebrates. Mol Mar Biol Biotechnol 3 (5): 294-299.

Forest J. 1952. Rémarques sur les genres *Diogenes* Dana et *Troglopagurus* Henderson (à propos de la description d'un Paguridae nouveau de la côte occidentale d'Afrique, *Diogenes mercatoris* sp. nov. Bull Inst Royal Sci Nat Belgique 28 (11): 1-15.

Forest J. 1956. Les Pagures du Viet-Nam. I. Le genre *Diogenes* Dana. Bull Mus Natl Hist Nat 228 (6): 524-532.

Fuentes-López A, Ruiz C, Galián J, Romera E. 2020. Molecular identification of forensically important fly species in Spain using COI

barcodes. Sci Justice 60 (3): 293-302. DOI: 10.1016/j.scijus.2019.12.003.
Galan GL, Mendez NP, Cruz RYD. 2018. DNA barcoding of three selected gastropod species using cytochrome oxidase (COI) gene. Ann West Univ Timis Ser Biol 21 (1): 93-102.
Heller C. 1865. Crustaceen. In: Reise der Osterreichischen Fregatte Novara um die erde in den Jahren 1857, 1858, 1859 unter den Befehlen des Commodors B. von Wüllerstorf-Urbair. Zoologischer Theil. 2(3). Kaiserlich-königlichen Hof-und Staatsdruckerei, Wien.
Hikam AM, Mubarakati NJ, Dailami M, Toha AHA. 2021. DNA barcode on marine invertebrates. Biologi Udayana 25 (1): 46-56. DOI: 10.24843/JBIOUNUD.2021.v25.i01. p06. [Indonesian]
Hultgren KM, Brandt A. 2015. Taxonomy and phylogenetics of the *Synalpheus paraneptunus*-species-complex (Decapoda: Alpheidae), with a description of two new species. J Crust Biol 35: 547-558. DOI:10.1163/1937240X-00002354.
Igawa M, Kato M. 2017. A new species of hermit crab, *Diogenes heteropsammicola* (Crustacea, Decapoda, Anomura, Diogenidae), replaces a mutualistic sipunculan in a walking coral symbiosis. PLoS ONE 12 (9): e0184311. DOI: 10.1371/journal.pone.0184311.
Ip YCA, Tay YC, Gan SX, Ang HP, Tun K, Chou LM, Huang D, Meier R. 2019. From marine park to future genomic observatory? Enhancing marine biodiversity assessments using a biocode approach. Biodiversity Data Journal 7: e46833. DOI: 10.3897/BDJ.7.e46833
Jung J, Kim W. 2019. DNA Barcoding of Six Diogenid Species (Crustacea: Decapoda: Paguroidea) in Korea. Anim. Syst. Evol. Divers 35 (4): 182-185. DOI: 10.5635/ASED.2019.35.4.021
Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16: 111-120. DOI: 10.1007/BF01731581.
Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35 (6): 1547-1549. DOI: 10.1093/molbev/msy096.
Kurniasari RD, Soewondo A, Toha AHA. 2014. Identification of *Synaptula* (Echinoderms: Holothuroidea) Raja Ampat Based on COI Gene. Biotropika 2 (5): 265-268. [Indonesian]
Landschoff J, Gouws G. 2018. DNA barcoding as a tool to facilitate the taxonomy of hermit crabs (Decapoda: Anomura: Paguroidea). J Crust Biol 38: 780-793. DOI: 10.1093/jcbiol/ruy084.
Landschoff J, Rahayu DL. 2018. A new species of the hermit crab genus *Diogenes* (Crustacea: Decapoda: Diogenidae) from the coast of KwaZulu-Natal, South Africa. Zootaxa 4379 (2): 268-278. DOI: 10.11646/zootaxa.4379.2.7.
Li W, Cheng J, Hui M, Sha Z. 2019. Molecular phylogeny of the genus *Clibanarius* (Decapoda: Anomura: Diogenidae) based on mitochondrial and nuclear DNA sequences. J Oceanol Limnol 37 (5): 1686-1697. DOI: 10.1007/s00343-019-8329-0.
Li W. 2020. Molecular phylogeny and evolutionary radiation of the diogenid hermit crabs (Decapoda: Anomura: Diogenidae) inferred from mitochondrial and nuclear genes. Instititute of Oceanology, Chinese Academy of Science, NanHai Road, Qingdao, Shandong, China. https://www.ncbi.nlm.nih.gov/nuccore/MW411962.1/
Madduppa H, Ayuningtyas RU, Subhan B, Arafat D. 2016. Exploited but unevaluated: DNA barcoding reveals skates and stingrays (Chordata, Chondrichthyes) species landed in the Indonesian fish market. Ilmu Kelautan: Indonesian J Mar Sci 21 (2): 77-84. DOI: 10.14710/ik.ijms.21.2.77-84. [Indonesian]
Madduppa H, Taurusman AMA, Subhan B, Anggraini NP, Fadillah R, Tarman K. 2017. Short Communication: DNA barcoding reveals vulnerable and not evaluated species of sea cucumbers (Holothuroidea and Stichopodidae) from Kepulauan Seribu reefs, Indonesia. Biodiversitas 18 (3): 893-898. DOI:10.13057/biodiv/d180305.
Mantelatto FL, Pileggi LG, Pantaleão JAF, Magalhães C, Villalobos JL, Álvarez F. 2021. Multigene phylogeny and taxonomic revision of American shrimps of the genus *Cryphiops* Dana, 1852 (Decapoda, Palaemonidae) implies a proposal for reversal of precedence with *Macrobrachium* Spence Bate, 1868. ZooKeys 1047: 155-198. DOI: 10.3897/zookeys.1047.66933.
Mantelatto FL, Souza-Carvalho EA, Araujo SR, Magalhães C. 2022 Combined multigene and morphological analysis reveals lineage-specific diversification of the neotropical freshwater crabs of the genus *Fredius* Pretzmann, 1967 (Brachyura, Pseudothelphusidae). Syst Biodivers 20 (1): 1-15. DOI: 10.1080/14772000.2021.2008042.
Mazumder A, Dhar B, Choudhury A, Ghosh SK. 2020. DNA barcoding of Crabs from Sunderban of Esatern India. N Bose Innovation Centre, University of Kalyani, West Bengal, India. https://www.ncbi.nlm.nih.gov/nuccore/MT590694
McLaughlin PA, Clark PF. 1997. A review of the *Diogenes* (Crustacea, Paguridea) hermit crabs collected by Bedford and Lanchester from Singapore, and from the 'Skeat' Expedition to the Malay Peninsula, with a description of a new species and notes on Diogenes intermedius De Man, 1902. Bull Nat Hist Mus Zool 63: 33-49.
McLaughlin PA, Holthuis LB. 2001. In pursuit of J.F.W. Herbst's Species of *Diogenes* (Anomura: Paguridea: Diogenidae). J Crust Biol 21 (1): 249-265. DOI: 10.1163/20021975-99990121.
McLaughlin PA, Rahayu DL, Komai T, Chan TY. 2007. A Catalog of the Hermit Crabs (Paguroidea) of Taiwan. National Taiwan Ocean University, Keelung.
McLaughlin PA. 2002. *Diogenes pallescens* Whitelegge, *D. gardineri* Alcock and *D. serenei* Forest (Decapoda: Anomura: Paguroidea: Diogenidae): Distinct species or morphological variants? Raffles Bull Zool 50 (1): 81-94.
McLaughlin PA. 2005. The "Troglopagurus Group" of *Diogenes* (Decapoda: Anomura: Paguroidea: Diogenidae) revisited. J Crust Biol 25: 598-619. DOI: 10.1651/C-2598.1.
Miers EJ. 1884. Crustacea. In: Report on the Zoological Collections Made in the Indo-Pacific Ocean during the Voyage of H.M.S. 'Alert' 1881-2. British Museum, London. DOI: 10.1080/00222938409459811.
Narejo AH, Saher NU, Odhano S, Kamal M. 2021. Genetic analysis of hermit crab species along the coast of Pakistan. Centre of Excellence in Marine Biology, University of Karachi, University Road, Karachi, Sindh, Pakistan. https://www.ncbi.nlm.nih.gov/nuccore/MW697229.1/
Negri M, Lemaitre R, Mantelatto FL. 2014. Molecular and morphological resurrection of *Clibanarius symmetricus* (Randall, 1840), a cryptic species hiding under the name for the "Thinstripe" hermit crab *C. vittatus* (Bosc, 1802) (Decapoda: Anomura: Diogenidae). J Crust Biol 34 (6): 848-861. DOI: 10.1163/1937240X-00002277.
Pante E, Schoelinck C, Puillandre N. 2015. From integrative taxonomy to species description: One step beyond. Syst Biol 64 (1): 152-160. DOI:10.1093/sysbio/syu083.
Pranata B, Toha AHA, Kolibongso D. 2020. Genetic of *Panulirus versicolor* Lobster in Cendrawasih Bay Papua and Lombok Waters West Nusa Tenggara. J Enggano 5 (2): 2527-5186. DOI: 10.31186/jenggano.5.2.249-257. [Indonesian]
Purty RS, Chatterjee S. 2016. DNA barcoding: An effective technique in molecular taxonomy. Austin J Biotechnol Bioeng 3 (1): 1059.
Rahayu DL, Forest J. 1995. Le genre *Diogenes* (Decapoda, Anomura, Diogenidae) en Indonésie, avec la description de six espèces nouvelles. Bull Mus Nat Hist Nat Paris Ser 16: 383-415.
Rahayu DL, Hortle KG. 2002. The genus *Diogenes* (Decapoda, Anomura, Diogenidae) from Irian Jaya, Indonesia with description of a new species. Crustaceana 75 (3-4): 609-619. DOI: 10.1163/156854002760095633.
Rahayu DL, Pratiwi R. 2022. Diogenes matabiru, a new species of hermit crab from Lombok Island, Indonesia (Crustacea, Decapoda, Anomura, Diogenidae). Zootaxa 5093 (4): 493-500. DOI: 10.11646/zootaxa.5093.4.7.
Rahayu DL. 1996. Notes on littoral hermit crabs (excluding Coenobitidae) (Crustacea: Decapoda: Anomura) mainly from Singapore and Peninsular Malaysia. Raffles Bull Zool 44 (2): 335-355.
Rahayu DL. 2012. A new species of the hermit crab genus *Diogenes* Dana, 1851 (Decapoda, Anomura, Diogenidae) from Lombok, Indonesia. In: Komatsu H, Okuno J, Fukuoka K (eds). Studies on Eumalacostraca: A Homage to Masatsune Takeda. Crustaceana Monographs 17: 263-274. DOI: 10.1163/9789004202894_022.
Rahayu DL. 2015. New record and new species of the hermit crab genus *Diogenes* Dana, 1851 (Decapoda: Anomura: Diogenidae) from Singapore. Raffles Bull Zool Suppl 31: 182-192.
Rahayu DL. 2021. Hermit crabs of the family Diogenidae (Crustacea: Decapoda: Anomura) collected during the South Java Deep-Sea 2018 biodiversity cruise in Indonesia, with description of two new species. Raffles Bull Zool 36: 162-180.
Raupach MJ, Barco A, Steinke D, Beermann J, Laakmann S, Mohrbeck I, Neuman H, Kihara TC, Pointner K, Radulovici A, Segelken VA, Wesse C, Knebelsberger T. 2015. The application of DNA barcodes for the identification of marine crustaceans from the North Sea and adjacent regions. PLoS ONE 10: e0139421. DOI: 10.1371/journal.pone.0139421.

Regier JC, Mitter C, Zwick A, Bazinet AL, Cummings MP, Kawahara AY, Sohn JC, Zwickl DJ, Cho S, Davis DR, Baixeras J. 2013. A large-scale, higher-level, molecular phylogenetic study of the insect order Lepidoptera (moths and butterflies). PLoS ONE 8 (3): e58568 DOI: 10.1371/journal.pone.0058568.

Reshmi R, Bijukumar A. 2014. Taxonomy and diversity of anomuran crabs (Decapoda: Anomura) off Kerala Coast. Aquatic Biology and Fisheries, University of Kerala, India. https://www.ncbi.nlm.nih.gov/nuccore/KJ150701.1/

Sahriyani N, Widodo, Toha, AHA. 2014. Amplification of the COI (Cytochrome C Oxidase Subunit I) gene in *Holothuroidea* collected from the Raja Ampat islands. Biotropika 2 (4): 232-234. [Indonesian]

Saitou N, Nei M. 1987. The neighbor-joining: A new method for reconstructing phylogentic tree. Mol Biol Evol 4: 406-425.

Saleky D, Setyobudiandi I, Toha HA, Takdir M, Madduppa HH. 2016. Length-weight relationship and population genetic of two marine gastropods species (Turbinidae: *Turbo sparverius* and *Turbo bruneus*) in the Bird Seascape Papua, Indonesia. Biodiversitas 17: 208-217. DOI: 10.13057/biodiv/d170130.

Shih HT, Ng PKL, Davie PJF, Schubart CD, Türkay M, Naderloo R, Jones D, Liu MY. 2016. Systematics of the family Ocypodidae Rafinesque, 1815 (Crustacea: Brachyura), based on phylogenetic relationships, with a reorganization of subfamily rankings and a review of the taxonomic status of *Uca* Leach, 1814, sensu lato and its subgenera. Raffles Bull Zool 64: 139-175.

Siddiqui FA, Kazmi OB, McLaughlin PA. 2004. Review of the Pakistani species of *Diogenes* Dana, 1951 (Decapoda Anomura Paguroidea Diogenidae). Trop Zool 17: 155-200. DOI: 10.1080/03946975.2004.10531205.

Sultana Z, Asakura A, Kinjo S, Nozawa M, Nakano T, Ikeo K. 2018. Molecular phylogeny of ten intertidal hermit crabs of the genus *Pagurus* inferred from multiple mitochondrial genes, with special emphasis on the evolutionary relationship of *Pagurus lanuginosus* and *Pagurus maculosus*. Genetica 146 (4-5): 369-381. DOI: 10.1007/s10709-018-0029-8.

Toha AHA, Sumitro SB, Widodo, Hakim L. 2015. Color diversity and distribution of sea urchin *Tripneustes gratilla* in Cenderawasih Bay Ecoregion of Papua, Indonesia. Egypt J Aquat Res 41: 273-278. DOI: 10.1016/j.ejar.2015.05.001.

Trivedi JN, Osawa M, Vachhrajani KD. 2016. A new species of the genus *Diogenes* Dana, 1851 (Crustacea: Decapoda: Anomura: Diogenidae) from Gujarat, northwestern India. Zootaxa 4208 (2): 189-197. DOI :10.11646/zootaxa.4208.2.6.

Wang ZL, Yang XQ, Wang TZ, Yu X. 2017. Assessing the effectiveness of mitochondrial COI and 16S rRNA genes for DNA barcoding of farmland spiders in China. Mitochondrial DNA Part A: 1-8. DOI: 10.1080/24701394.2017.1350949.