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DNA barcoding of crustacean larvae with two new records of *Caridina gracilipes* and *Ptychognathus altimanus* in the western region of Segara Anakan lagoon in Indonesia

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Abstract. *Winarni ET, Simanjuntak SBI, Nuryanto A. 2023. DNA barcoding of crustacean larvae with two new records of* Caridina gracilipes *and* Ptychognathus altimanus *in the western region of Segara Anakan lagoon in Indonesia. Biodiversitas 24: 341-348.* Several studies have been carried out on crustacean larvae diversity, but they focused on the eastern and central regions of the Segara Anakan lagoon/estuary, Cilacap District, Central Java Province, Indonesia. Furthermore, one study has explored crustaceans in the western region but focused on adult individuals and used morphological characteristics. Currently, there is no internet data on the crustacean larvae diversity from the estuary's western region, and there is a high possibility that some species are unidentified. This study aims to describe crustacean larvae diversity in the western region of the Segara Anakan lagoon in Indonesia by using DNA barcoding. Sequence divergence of 3% to databases in GenBank and BOLDsystems was used as a genetic threshold for species border. A total of 14 morphotypes were barcoded, but only nine were successful, of which three were identified as bacteria and six as crustaceans. Based on the predetermined genetic threshold, all crustacean morphotypes can be identified at the species level, and six species were obtained. Meanwhile, a previous study on the adult individual only identified specimens at the genus level, and only one genus was similar to these obtained results. A total of 5 out of 6 species were new records to the western region Cilacap, while 2, namely *Caridina gracilipes* and *Ptychognathus altimanus,* are newly recorded in the Segara Anakan lagoon. These results contribute significantly to the information on crustacea larvae diversity, which is essential for sustainable management.

Keywords: Brackish water, divergence, homology, monophyly, shrimp

INTRODUCTION

The Segara Anakan lagoon/estuary is a unique semi-open estuary in the Southwestern part of Central Java in Indonesia (Herawati et al. 2012; Rimadiyani et al. 2019; Redjeki et al. 2020). It is separated from the open sea by the Nusakambangan Island and connected by two openings in the west and east tips of the island (Rimadiyani et al. 2019). Furthermore, their middle and lagoon areas are highly affected by freshwater logs from the surrounding big rivers. In contrast, the eastern and western areas are significantly affected by high tides from the ocean (Yuniarti et al. 2018). These phenomena make Segara Anakan a complex aquatic ecosystem regarding its water salinity (Winarni et al. 2021). Previous studies revealed that the estuary provides several ecological supports for aquatic faunas (Nuryanto et al. 2017; Pratiwi and Sukardjo 2018; Redjeki et al. 2020).

Crustacea is one of the aquatic faunas living in the Segara Anakan lagoon Cilacap (Kusbiyanto et al. 2020), and several studies have explored this subphylum, such as its biology and production (Akbar et al. 2013; Wagiyo et al. 2018), adult crab (Redjeki et al. 2017; Yuniarti et al. 2018), as well as adult crustacean diversity in its western region (Rimadiyani et al. 2019). Furthermore, two studies were also carried out on larvae diversity, but none collected the larvae in the western region (Kusbiyanto et al. 2020; Winarni et al. 2021), which has led to high spatial, dispersed, and incomplete information regarding the Segara Anakan lagoon.

Determining crustacean larvae diversity in the estuary is challenging due to the limited identification keys, which do not cover all species (Bartilotti et al. 2016; Wakabayashi et al. 2017; Carreton et al. 2019; Muzio et al. 2019; Walczynska et al. 2019). Another constraint is the limited morphological characteristic of the crustacean larvae that can easily be used to diagnose the various crustacean species (Haug et al. 2016). DNA barcoding has successfully solved these constraints using a short fragment of the cytochrome c oxidase 1 (COI) gene (Brando et al. 2016; Rahman et al. 2019; Palecanda et al. 2020; Varela and Bracken-Grissom 2021). Several studies showed that the gene is a reliable barcode for crustacean barcoding (Raupah and Radulovici 2015; Matelatto et al. 2016; Bhagawati et al. 2020), including crustacean larvae (Palecanda et al. 2020; Kusbiyanto et al. 2020). Moreover, other studies proved that the COI gene is a reliable barcode marker to solve cryptic species on crustacea, such as on *Gammarus fossarum* complex (Weiss et al. 2014), *Moina* (Bekker et al. 2016), Ostracoda (Karanovic 2015), and shrimps (Bilgin et al. 2015). It was also successfully used to identify several overlooked species from a particular habitat (Bilgin et al. 2015; Bekker et al. 2016; Muchlisin et al. 2017; Bhagawati et al. 2022).

Previous studies utilized several parameters during species determination using COI barcode, e.g., similarity to reference species deposited in the barcode of life data system (BOLDsystems), homology, coverage, and expected value through BLAST to GenBank database, genetic distance, and monophyly (Xu et al. 2015; Muchlisin et al. 2017; Palecanda et al. 2020; Bhagawati et al. 2022). In addition, various genetic distances were also applied as threshold values for species determination because intraspecific genetic distances highly vary between species (Rahman et al. 2019; Rossel and Arbizu 2019; Rahman et al. 2020). However, the most common genetic threshold for species determination is 3% (Ratnasingham and Hebert 2012).

There is no report on crustacean larvae diversity in the western region of Segara Anakan lagoon, Cilacap District, Central Java, Indonesia; therefore, this study aims to describe crustacean larvae diversity in this region using larvae barcoding.

MATERIALS AND METHODS

Study location, sampling sites, and larvae collection

Crustacean larvae samples were collected from the western region of Segara Anakan lagoon, Cilacap District, Central Java, Indonesia and several sampling tracts, as shown in Figure 1. The sampling sites were located inside the lagoon, river mouth, canals, and opening of the west Segara Anakan lagoon. Site selection was carried out to cover a wide range of environmental conditions. Different from other areas, the lagoon is a part of the Segara Anakan estuary that is much affected by land areas or mangrove forests. Furthermore, 12 sequences (Kusbiyanto et al. 2020) and others additional 17 were retrieved from GenBank.

The field trips for crustacean larvae sampling were carried out on the 20th and 21st of June 2022 with 27 fishing efforts. The samples were collected using larvae nets with a horizontal towing method and boat speed of approximately 3 knots with local people's support. The towing was performed with a time duration of 15 minutes for each fishing effort. These sampling steps were performed in the evening (17:00-21:00) and the morning (06:00-09:00). Crustacean larvae samples from each step were directly preserved in a separated 50 mL bottle filled with technical ethanol 96% and then labeled (Kusbiyanto et al. 2020).

Morphological characterization

Rough and skimmed morphological identification was carried out on all the samples collected; this step was performed to sort them into morphotypes based on differences in their general morphology. In some cases, morphological identification also considered each larva's color and color pattern. These steps were carried out under a magnification lens to differentiate the morphotypes precisely (Kusbiyanto et al. 2020). Morphological identification was carried out following the guide from Chan (1998).

DNA isolation, cytochrome c oxidase 1 gene amplification, and sequencing

The representatives of each morphotype were shipped to PT. Genetika Science Indonesia for molecular barcoding, while the genomic DNA was extracted from each morphotype using DNA Miniprep Kit (Zymo Research, D6016) following the manufacturer's protocol. The fragment of the COI gene was amplified using the MyTaq HS Red Mix (Bioline, BIO-25047) and universal primer pair LCO1490: 5' GGTCAACAAATCATAAA GATATTGG-3' and HC02198: 5'-TAAACTTCAGGGTG ACCAAAAAATCA-3' (Folmer at al. 1994). The thermocycler was set up as follows; double DNA bands were split into single bands using a temperature of 96°C, then the amplification process was carried out for 35 cycles.

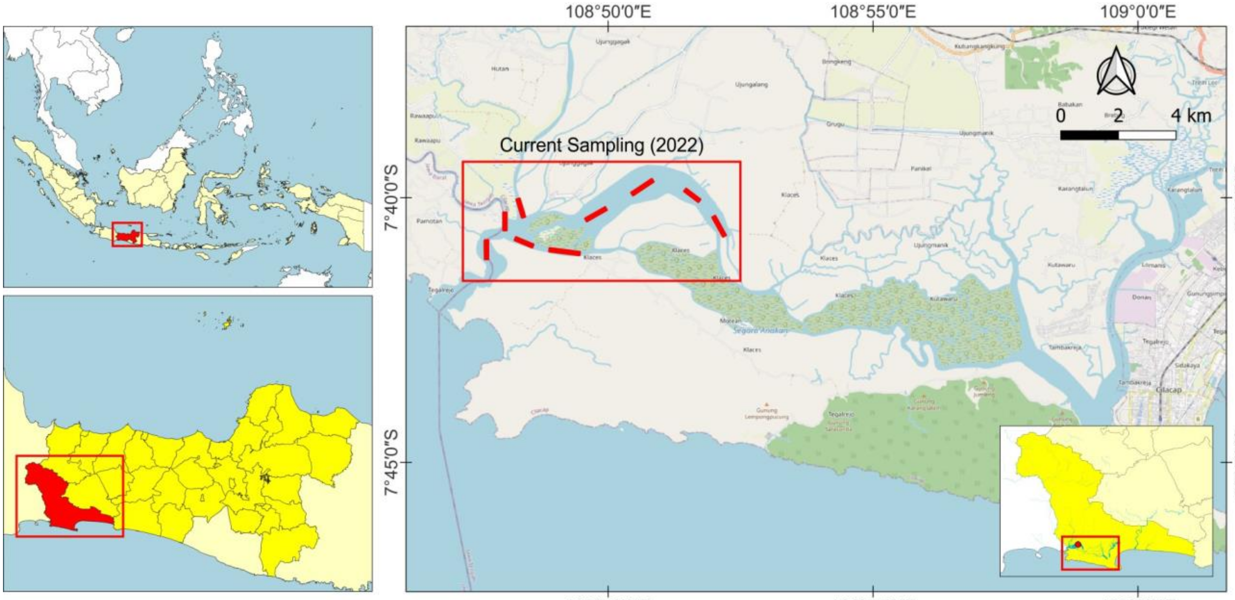

Figure 1. Segara Anakan lagoon, Cilacap District, Central Java, Indonesia sampling sites (map modified from OpenStreet map 2022)

Each step's thermal condition and duration were denaturation at 94° for 10 seconds, annealing at 52° for 30 seconds, and extension at 72° for 45. The final volumes for polymerase chain reaction (PCR) were 25 µL consisting of KOD FX Neo 1 µL, 2XPCR Buffer KOD FX Neo 12.5 µL, 2mM dNTPs 1 µL, 10 pmol/µL of each primer was 1 µL, template DAN 1 µL, and ddH2O 6 µL. The amplification products were sequenced using standard big-dye termination from the Sanger method with a bi-directional sequencing technique.

Editing of the cytochrome c oxidase 1 sequence

The representative of each forward and reverse sequence for each morphotype was edited using Bioedit package 7.2.5 (Hall 2004) to obtain a complete consensus sequence barcoding. This step includes manual editing and trimming the right and left ends of the consensus sequence to avoid ambiguity due to primers. Next, the functional COI coding regions were determined by submitting the product to an online version of the open reading frame (ORF) finder (https://www.ncbi.nlm.nih.gov/orffinder/). The search parameters included a minimal ORF length of 300 nucleotides, genetic code mitochondrial invertebrate, start codon ATG, and alternative initiation codons. Subsequently, the COI coding region was verified through the Basic local alignment search tool (BLAST). At the same time, the aligned query and subject sequences were checked manually using the CDS feature and search parameters to ensure no stop codon along the sequences. Multiple sequence alignments were carried out using ClustalW (Thompson et al. 2003) as included in the Bioedit package version 7.2.5 (Hall 2004).

Taxonomic status of crustacean larvae samples

The current report delineated the taxonomic status of the larvae specimens through sequences comparison to the closely related taxa in GenBank and BOLD data. This study determined the specific category of the samples based on percentage identity (GenBank), similarity (BOLD systems), sequence divergence, and monophyly. Although a genetic threshold of 3% to 5% was used previously (Candek and Kuntner 2015; Karanovic 2015), this study used percentage identity and similarity values of 97% and 3% sequence divergence between samples and reference species available (Ratnasingham and Hebert 2013). A branch length of 0.03 was also utilized as an additional species border parameter. Furthermore, monophyly between the samples and reference species was estimated from the phylogenetic tree, which was reconstructed based on the K2P substitution model using neighbor-joining (NJ) algorithms in MEGAX (Kumar et al. 2018). The topology was polarized through bootstrap replication 1000 times during the reconstruction. *Joryma hilsae* (MT876659) and *Idiosepius biserialis* (MT87664) were selected as the outgroup species.

RESULTS AND DISCUSSION

Taxonomic status of crustacean larvae

A total of 14 larvae morphotypes were collected during the sampling. Unfortunately, the sequencing only yielded nine readable chromatograms for barcoding. Still, three were identified as insects and were not included in the analysis. All the successfully sequenced samples have a high sequence identity of 99.20-100%, with their closest relative taxa deposited in Genbank. They are supported by high query cover and low-expect values (e-values: 0.00). High similarities of 97.52-100% were also obtained when the samples were compared to their closest relatives in the BOLD system database, as shown in Table 1. This study considered high percentage identities and similarities to at least the top three with conspecific references in both databases, namely GenBank and BOLD systems. Additional information about close sister taxa is also shown in Table 1.

Table 1 shows that all specimens have a higher percentage identity than GenBank's predetermined threshold value of 97%. Furthermore, more than 97% genetic similarity was observed when the comparison was carried out with the database available in the BOLD system. These values strongly indicate that all the samples belong to the same species as their conspecific references. The delineation into single species as their references was caused by the 97% homology and similarity threshold value, which is often used widely in animal barcoding (Hubert et al. 2012; Ko et al. 2013; Nuryanto et al. 2018; Amatya 2019).

Crustacean larvae specimens from the western region of Segara Anakan have genetic divergences of 0.00% to 2.25% with their closest relatives in GenBank. A more comprehensive range of 0.00% to 2.43% was observed with the reference species in BOLD systems. The average homology values were between 0.00% to 0.75%, while average similarity values ranged from 0.00% to 0.78%, as shown in Table 2.

Maximum genetic divergences to reference species in GenBank or BOLD system were below the predetermined value of 3%. This result is consistent with previous decisions on the taxonomic status of the samples, where they showed similar traits as the reference conspecific in the databases. Previous studies reported that a 3% genetic divergence is widely used in animal barcoding as a species border (Hubert et al. 2012; Ko et al. 2013; Mohammed et al. 2021; Riani et al. 2021). Several studies have also explored various thresholds as species border during molecular identification from less than 1% to 5% (Cote et al. 2013; Aguilar et al. 2017: Ha et al. 2019; Abdalwahhab et al. 2020). Previous studies stated that genetic divergence in well-identified species ranges from 0.00% to 9.31% (Brando et al. 2016; Landschoff and Gouws 2018; Lipinskaya et al. 2018; Rahman et al. 2019; Rossel and Arbizu 2019; Rahman et al. 2020). Furthermore, higher values were also common among localities (Deli et al. 2018), and these phenomena indicate that no single genetic divergence can be utilized for all crustacean species. The range used is highly dependent on animal phyla (Brando et al. 2016) and the geographic origin of the samples (Deli et al. 2018). That indicates that a predetermined genetic divergence of 3% is a reliable threshold as species border, and it has also been tested in a previous study (Amatya 2019).

The phylogenetic tree was reconstructed using the COI gene with a fragment length of 563 bp, while the Kimura 2-parameter (K2P) neighbor-joining tree topology is presented in Figure 2. The branching pattern revealed that all morphotypes formed monophyletic clades with their conspecific references. Those results also showed branch lengths shorter than the predetermined scale of 0.03. This branch topology indicates that the samples belong to the same species as their references. It has been reported that monophyly with a short branch length can be used to determine the specimen's taxonomic status within a single clade (Xu et al. 2015; Kusbiyanto et al. 2020). Previous studies also used these parameters during species delineation based on molecular barcoding (Bekker et al. 2016; Aguilar et al. 2017; Nuryanto et al. 2017; Lipinskaya et al. 2018; Garibian et al. 2020).

The morphotypes of the crustacean larvae from the western region of Segara Anakan can be identified as six species based on sequence identity, similarity, and divergence. In addition, their monophyly and branch length in the phylogenetic tree also supported the placement into six species. The taxonomic status of the crustacean larvae from the estuary is shown in Table 3.

Table 1. Sample code, reference species, percentage identity, expect value, and percentage similarity

Sample code	Reference species and accession number	GenBank			BOLD
		Query cover (%)	E-value	Identity (%)	Similarity (%)
WSA_Cr01	*Caridina gracilipes* MN526096	100	0.0	99.36	99.33
	Caridina gracilipes MN526095	100	0.0	99.20	99.16
	Caridina gracilipes MN526091	100	0.0	99.20	99.16
	Paratya australiensis DQ889120	100	0.0	95.99	-
WSA_Cr02	*Macrobrachium esculentum* MN526206	100	0.0	100	100
	Macrobrachium esculentum MN526199	100	0.0	100	100
	Macrobrachium esculentum MN526198	100	0.0	100	100
	Macrobrachium nipponense MN308132	100	0.0	86.60	-
	Macrobrachium scabriculum MN526237	99	0.0	86.56	-
WSA_Cr05	*Cloridopsis scorpio* MT876658	100	0.0	99.84	100
	Cloridopsis scorpio MT876657	100	0.0	99.84	100
	Cloridopsis scorpio MH168247	95	0.0	98.85	-
	Oratosquillina interrupta FJ229791	99	0.0	87.52	-
WSA_Cr08	*Ptychognathus altimanus* MW000766	99	0.0	99.68	99.68
	Ptychognathus altimanus MW000765	99	0.0	99.68	99.68
	Ptychognathus altimanus MW000764	99	0.0	99.68	99.68
	Uca dorotheae LC087961	99	0.0	86.98	-
	Chiromantes haematocheir LC500778	98	0.0	87.00	-
WSA_Cr09	*Macrobrachium* equidens ON753700	100	0.0	99.83	-
	Macrobrachium sp. PK-2018 KX399433	99	0.0	97.75	97.57
	Macrobrachium sp. 1 GFZ-2020	-	-	-	97.68
	Macrobrachium rogersi MW845591	100	0.0	88.26	-
	Macrobrachium sintangense MW845600	100	0.0	85.13	-
WSA_Cr12	*Varuna litterata* MT193719	99	0.0	99.85	-
	Varuna litterata MW125542	99	0.0	99.85	-
	Varuna litterata LC498194	98	0.0	99.84	99.83
	Varuna yui NC_037155	99	0.0	87.42	-
	Ptychognathus altimanus MW000766	97	0.0	86.64	-

Table 2. The average values of sequences divergence between samples and their conspecific references

Sample code	Reference species	Sequence divergence (%)	
		GenBank	BOLDsystems
WSA_Cr01	*Caridina gracilipes*	0.750	0.780
WSA_Cr02	*Macrobrachium esculentum*	0.000	0.000
WSA_Cr05	*Cloridopsis scorpio*	0.490	0.000
WSA_Cr08	*Ptychognathus altimanus*	0.320	0.320
WSA_Cr09	*Macrobrachium equidens*	0.170	0.170
WSA_Cr12	*Varuna litterata*	0.153	0.170

Table 3. Taxonomic status of crustacean larvae in the western region of Segara Anakan lagoon, Cilacap District, Central Java, Indonesia

Sample code	Species	Family	Order	Habitat
WSA Cr01	*Caridina gracilipes*	Atyidae	Decapoda	Brackish water, freshwater
WSA Cr02	*Macrobrachium esculentum*	Palaemonidae	Decapoda	Freshwater
WSA Cr05	*Cloridopsis scorpio*	Squillidae	Stomatopoda	Marine, brackish water, freshwater
WSA Cr08	*Ptychognathus altimanus*	Varunidae	Decapoda	Marine
WSA Cr09	*Macrobrachium equidens*	Palaemonidae	Decapoda	Brackish water, freshwater
WSA Cr12	*Varuna litterata*	Varunidae	Decapoda	Marine, brackish water, freshwater

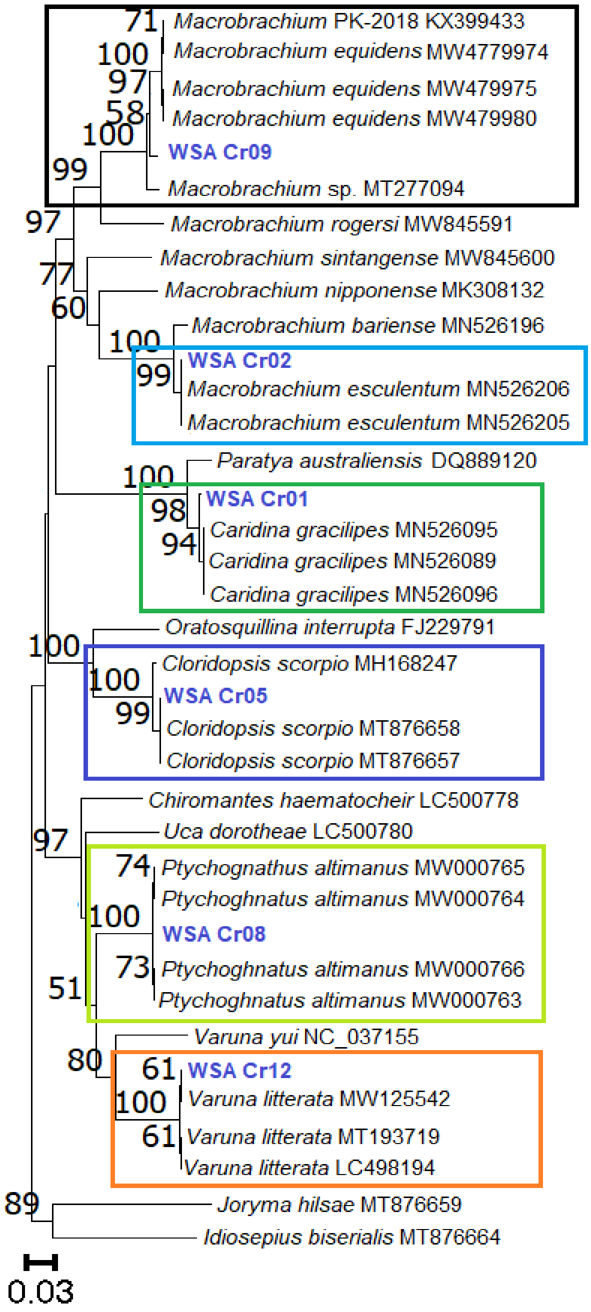

Figure 2. The K2P neighbor-joining tree showing monophyly between samples (blue words) from the western region of Segara Anakan lagoon, Cilacap District, Central Java, Indonesia with their conspecific references retrieved from GenBank

The diversity of crustacean larvae in the selected region is far below the expected number because previous studies showed higher diversity from the other areas, such as the central region, which was reported to have 11 species (Winarni et al. 2021), while eight were found in the east (Kusbiyanto et al. 2020). However, this study recorded two species not reported by Winarni et al. (2021) and Kusbiyanto et al. (2020), namely *Caridina gracilipes* and *Ptychognathus altimanus*, as shown in Table 4. This finding proved that this study provides new contributions, namely two new records about crustacean larvae diversity in the Segara Anakan lagoon Cilacap, Central Java. This finding is essential for fisheries management in the estuary, especially in the western region.

Another comparison proved that this study obtained fewer species than previous studies, which were also carried out in the western region of Segara Anakan, as shown in Table 5. This study found six species of Crustacea, while based on adult individual identification, Rimadiyani et al. (2019) reported 17 species. The difference is presumably because different sampling gears were utilized between this study and Rimadiyani et al. (2019). This study used a larvae net for collecting only planktonic larvae, while Rimadiyani et al. (2019) utilized grab-targeting macro zoobenthos. In addition, it has been reported that not all crustacean species nurses their larvae in the estuary. Therefore, obtaining a lower number of species is reasonable than those previously reported.

The comparison between the present study and Rimadiyani et al. (2019) showed only one similar genus was obtained, namely *Caridina*. However, the specimen was identified at the species level, namely *C. gracilipes*, while Rimadiyani et al. (2019) only carried out identification at the genus level, as shown in Table 5. The results show that DNA barcoding with the COI gene can precisely identify specimens at the species level compared to previously used morphological identification. Therefore, this study has improved the previous research by providing new taxonomic status of the crustacean specimen in the western region of Segara Anakan, especially in the genus *Caridina*. The five crustacean species found were not reported by Rimadiyani et al. (2019). Although this study obtained a far smaller number, it still essentially contributed to the diversity of data in the location, especially by adding five new records. Furthermore, compared to previous studies on crustacea across Segara Anakan (Redjeki et al. 2017; Yuniarti et al. 2018) revealed that the finding of *P. altimanus* was a new record.

Table 4. Comparison barcoding result of crustacean larvae from a different region of the Segara Anakan lagoon, Cilacap District, Central Java, Indonesia

Current study (West)	Winarni et al. 2021 (Central)	Kusbiyanto et al. 2020 (East)
Caridina gracilipes*	*Portunus pelagicus*	*Acetes* sp.
Macrobrachium esculentum	*Stomatopoda* sp	*Penaeus merguiensis*
Cloridopsis scorpio	*Cloridopsis scorpio*	*Acetes sibogae*
Ptychognathus altimanus*	*Macrobrachium rosenbergii*	*Neocallichirus* sp.
Macrobrachium equidens	*Macrobrachium esculentum*	*Neodorippe* sp.
Varuna litterata	*Neodorippe* sp.	*Cloridopsis scorpio*
	Grandidierella sp.	*Joryma hilsae*
	Macrobrachium equidens	*Rhopalophthalmus indicus*
	Palaemon sp.	
	Macrobrachium sp.1	

Note: * new records from Segara Anakan based on larvae barcoding

Table 5. Comparison between crustacean larvae barcoding and morphological identification of adult individuals of crustacea from the western region of Segara Anakan lagoon, Cilacap District, Central Java, Indonesia

Current study (Larvae barcoding)	Rimadiyani et al. 2019* (Morphological identification of the adult individual)	Finding status for the western region of the Segara Anakan lagoon
Caridina gracilipes	*Caridina* sp.	New record
Macrobrachium esculentum	No record	New record
Cloridopsis scorpio	No record	New record
Ptychognathus altimanus	No record	New record
Macrobrachium equidens	No record	New record
Varuna litterata	No record	New record

This study found new species records from the Segara Anakan lagoon on a limited number of crustacean larvae morphotypes. Unfortunately, the barcoding was also only successful on fewer morphotypes. Therefore, further studies are still needed to evaluate crustacean larvae diversity in the Segara Anakan lagoon Cilacap, Central Java, Indonesia. The possible efforts are applying a new molecular method, named environmental DNA (e-DNA) technique, and extending sampling periods. In the case of *P. altimanus*, further studies on adult individuals are also essential.

Based on the parameters used during barcoding, six crustacean larvae were successfully identified at the species level: *Caridina gracilipes, Macrobrachium esculentum, Cloridopsis scorpio, Ptychognathus altimanus, Macrobrachium equidens,* and *Varuna litterata.* A total of 5 new records were obtained from western areas, while *C. gracilipes* and *P. altimanus* constitute two new records across the Segara Anakan lagoon.

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REFERENCES

Abdalwahhab O, Galal-Khallaf A, Saber SAEl-L, Osman AGM, Mohammed-Geba K. 2020. A case study for application of DNA barcoding in identifying species and genetic diversity of fish from the Suez city market, Egypt. Aquat Living Resour 33: 11. DOI: 10.1051/alr/2020012.

Aguilar A, Maeda-Martinez AM, Murugan G, Obregon-Barboza H, Rogers DC, McClintock K, Krumm JL. 2017. High intraspecific genetic divergence in the versatile fairy shrimp *Branchinecta lindahli* with a comment on cryptic species in the genus *Branchinecta* (Crustacea: Anostraca). Hydrobiologia 801: 59-69. DOI: 10.1007/s10750017-3283-3.

Akbar PP, Solichin A, Saputra SW. 2013. Analysis of length-weight relationship and condition factor of rebon shrimp (*Acetes japonicas*) in Cilacap of Central Java. Manag Aquat Resour J 2 (3): 161-169. DOI: 10.14710/marj.v2i3.4211. [Indonesian]

Amatya B. 2019. DNA barcoding of cyprinid fish *Chagunius chagunio* Hamilton,1822 from Phewa Lake, Nepal. Intl J Biol 11 (4): 88-100. DOI: 10.5539/ijb.v11n4p88.

Bartilotti C, Salabert J, Dos Santos A. 2016. Complete larval development of *Thor amboinensis* (De Man, 1888) (Decapoda: Thoridae) described from laboratory-reared material and identified by DNA barcoding. Zootaxa 4066 (4): 399-420. DOI: 10.11646/zootaxa.4066.4.3.

Bekker EI, Karabanov DP, Galimov YR, Kotov AA. 2016. DNA barcoding reveals high cryptic diversity in the North Eurasian *Moina* species (Crustacea: Cladocera). Plos One 11 (8): e0161737. DOI: 10.1371/journal.pone.0161737.

Bhagawati D, Nuryanto A, Winarni ET, Pulungsari AE. 2022. Morphological and molecular characterization of mole crab (Genus: *Emerita*) in the Cilacap coastlines of Indonesia, with particular focus on genetic diversity of *Emerita* sp. nov. Biodiversitas 23 (5): 2395-2404. DOI: 10.13057/biodiv/d230517.

Bhagawati D, Winarni ET, Nuryanto A. 2020. Molecular barcoding reveals the existence of mole crabs *Emerita emeritus* in North Coast of Central Java. Biosaintifika 12 (1): 104-110. DOI: 10.15294/biosaintifika.v12i1.20497.

Bilgin R, Utkan MA, Kalkan E, Karhan SU, Bekbolet M. 2015. DNA barcoding of twelve shrimp (Crustacea: Decapoda) from Turkish sea reveals cryptic diversity. Mediterr Mar Sci 16 (1): 36-45. DOI: 10.12681/mms.548.

Brando MC, Freire AS, Burton RS. 2016. Estimating diversity of crabs (Decapoda: Brachyura) in a no-take protected areas in the SW Atlantic coast through DNA barcoding of larvae. Syst Biodivers 14: 288-302. DOI: 10.1080/14772000.2016.1140245.

Candek K, Kuntner M. 2015. DNA barcoding gap: Reliable species identification over morphological and geographical scales. Mol Ecol Resour 15 (2): 268-277. DOI: 10.1111/1755-0998.12304.

Carreton M, Company JB, Planella L, Heras S, García-Marín J-L, Agulló M, Clavel-Henry M, Rotllant G, dos Santos A, Roldán MI. 2019. Morphological identification and molecular confirmation of the deep-sea blue and red shrimp *Aristeus antennatus* larvae. PeerJ 7: e6063. DOI: 10.7717/peerj.6063.

Chan TY. 1998. Shrimps and prawns. FAO species identification guide for fishery purposes. In: Carpenter KE, Niem VH (eds). The living Marine Resources of the Western Central Pacific. FAO, Rome.

Cote IM, Green SJ, Morris Jr JA, Akins JL, Steinke D. 2013. Diet richness of invasive Indo-Pacific lionfish revealed by DNA barcoding. Mar Ecol Prog Ser 472: 249-256. DOI: 10.3354/meps09992.

Deli T, Kalkan E, Karha SU, Uzunova S, Keikhosravi A, Bilgin R, Schubart CD. 2018. Parapatric divergence among deep evolutionary lineages in the Mediterranean green crab, *Carcinus aestuarii* (Brachyura, Portunoidea, Carcinidae), accounts for a sharp phylogeographic break in the Eastern Mediterranean. BMC Evol Biol 18: 53. DOI: 10.1186/s12862-018-1167-4.

Garibian PG, Neretina AN, Taylor DJ, Kotov AA. 2020. Partial revision of the neustonic genus *Scapholeberis* Schoedler, 1858 (Crustacea: Cladocera): Decoding of the barcoding results PeerJ 8: e10410. DOI 10.7717/peerj.10410.

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

Ha TTT, Huong NT, Hung NP, Guiguen Y. 2019. Species identification using DNA barcoding on processed panga catfish products in Viet Nam revealed important mislabelling. Turkish J Fish Aquat Sci 18: 457-462. DOI: 10.4194/1303-2712-v18_3_11.

Hall TA. 2004. Bioedit: A User-friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Ibis Therapeutics, A Division of Isis Pharmaceuticals 1891 Rutherford Road Carlsbad, North Carolina.

Haug C, Ahyong ST, Wiethase JH, Olesen J, Haug JT. 2016. Extreme morphologies of mantis shrimp larvae. Nauplius 24: e2016020. DOI: 10.1590/2358-2936e2016020.

Herawati VE, Hartoko A, Suminto. 2012. The suitability of Segara Anakan waters, Cilacap, Central Java as cultivation area of Polymesoda erosabased on primary productivity using satellite image. Bonorowo Wetl 2 (2): 41-51. DOI: 10.13057/bonorowo/w020201.

Hubert N, Meyer CP, Bruggemann HJ, Guérin F, Komeno RJL, Espiau B, Causse R, Williams JT, Planes S. 2012. Cryptic diversity in Indo-Pacific coral-reef fishes revealed by DNA-barcoding provides new support to the centre-of-overlap hypothesis. Plos One 7 (3): e0028987. DOI: 10.1371/journal.pone.0028987.

Karanovic I. 2015. Barcoding of ancient lake Ostracods (Crustacea) reveals cryptic speciation with extremely low distances. Plos One 10 (3): e0121133. DOI: 10.1371/journal.pone.0121133.

Ko HL, Wang YT, Chiu TS, Lee MA, Leu MY, Chang KZ, Chen WY, Shao KT. 2013. Evaluating the accuracy of morphological identification of larval fishes by applying DNA barcoding. Plos One 8 (1): e0053451. DOI: 10.1371/journal.pone.0053451.

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.

Kusbiyanto, Bhagawati D, Nuryanto A. 2020. DNA barcoding of crustacean larvae in Segara Anakan, Cilacap, Central Java, Indonesia using cytochrome c oxidase gene. Biodiversitas 21 (10): 4878-4887. DOI: 10.13057/biodiv/d211054.

Landschoff J, Gouws G. 2018. DNA barcoding as a tool to facilitate the taxonomy of hermit crabs (Decapoda: Anomura: Paguroidea). J Crustacean Biol 38 (6): 780-793. DOI: 10.1093/jcbiol/ruy084.

Lin X, Stur E, Ekrem T. 2015. Exploring genetic divergence in a species rich insect genus using 2790 DNA barcodes. Plos One 10 (9): e0138993. DOI: 10.1371/journal.pone.0138993.

Lipinskaya T, Radulovici A, Makaranka A. 2018. First DNA barcoding-based record of *Echinogammarus trichiatus* (Martynov, 1932) (Crustacea, Gammaridae) in Belarus. BioInvasions Rec 7 (1): 55-60. DOI: 10.3391/bir.2018.7.1.08.

Matelatto FL, Carvalho FL, Simoes SM, Negri M, Souza-Carvalho EA, Terossi M. 2016. New primers for amplification of cytochrome c oxidase subunit I barcode region designed for species of Decapoda (Crustacea). Nauplius 24: e2016030. DOI 10.1590/2358-2936e2016030.

Mohammed MA, Nuryanto A, Kusmintarsih ES. 2021. Genetic differentiation of dengue vector *Aedes aegypti* in the small geographical scale of Banyumas District, Indonesia based on cytochrome oxidase I. Biodiversitas 22 (2): 675-683. DOI: 10.13057/biodiv/d220219.

Muchlisin ZA, Batubara AS, Fadli N, Muhammadar AA, Utami AI, Farhana N, Siti-Azizah MN. 2017. Assessing the species composition of tropical eels (Anguillidae) in Aceh Waters, Indonesia, with DNA barcoding gene Cox1. F1000Res 6: 1-10. DOI: 10.12688%2Ff1000research.10715.1.

Muzio GD, Sartor RM, Nurra N, Battuello M, Pessani D, Cervella P, Cuesta JA. 2019. Morphology of planktonic zoeal stages of *Palicus caronii* (Decapoda, Brachyura), identified by DNA barcoding, provides novelties to Palicoidea larval systematics. Sci Rep 9: 19132. DOI: 10.1038/s41598-019-55412-3.

Nuryanto A, Amalia G, Khairani D, Pramono H, Bhagawati D. 2018. Molecular characterization of four giant gourami strains from Java and Sumatra. Biodiversitas 19 (2): 528-539. DOI: 10.13057/biodiv/d190228.

Nuryanto A, Pramono H, Sastranegara MH. 2017. Molecular identification of fish larvae from East Plawangan of Segara Anakan, Cilacap, Central Java, Indonesia. Biosaintifika 9 (1): 33-40. DOI: 10.15294/biosaintifika.v9i1.9191.

Palecanda S, Feller KD, Porter ML. 2020. Using larval barcoding to estimate stomatopod species richness at Lizard Island, Australia for conservation monitoring. Sci Rep 10: 10990. DOI: 10.1038/s41598020-67696-x.

Pereira LHG, Hanner R, Foresti F, Olivera C. 2013. Can DNA barcoding accurately discriminate megadiverse Neotropical freshwater fish fauna? BMC Genet 14: 20. DOI: 10.1186/1471-2156-14-20.

Pratiwi R, Sukardjo S. 2018. Effect of rainfall on the population of shrimps *Penaeus monodon* Fabricius in Segara Anakan Lagoon, Central Java, Indonesia. Biotropia 25 (3): 156-169. DOI: 10.11598/btb.2018.25.3.830.

Rahman MAU, Kumar CP, Mohanchander P, Manikantan G, Khan AS, Lyla PS. 2020. All of a piece: Identification of the different life stages of *Scylla serrata* (Forsskal, 1775) using DNA barcodes. J Aquat Biol Fish 8: 12-17.

Rahman MAU, Kumar CP, Mohanchander P, Manikantan G, Khan AS, Lyla PS. 2019. Identification of eggs, larvae and adults of *Scylla serrata* (Forsskal, 1775) using DNA barcodes. J Aquat Biol Fish 7: 24-30.

Ratnasingham R, Hebert PDN. 2013. A DNA-based registry for all animal species: The barcode index number (BIN) system. Plos One 8 (8): e66213. DOI: 10.1371/journal.pone.0066213.

Raupach MJ, Radulovici AE. 2015. Looking back on a decade barcoding crustaceans. Zookeys 539: 53-81. DOI: 10.3897/zookeys.539.6530.

Redjeki S, Arif M, Hartati R, Pinandita LK. 2017. Density and distribution of crab (Brachiura) in mangrove forest ecosystem of Segara Anakan Segara Anakan Cilacap. Jurnal Kelautan Tropis 20 (2): 131-139. DOI: 10.14710/jkt.v20i2.1739. [Indonesian]

Redjeki S, Hartati R, Endrawati H, Widianingsih, Nuraini RAT, Riniatsih I, Agus EL, Mahendrajaya RT. 2020. Growth pattern and condition factor of mangrove crab (*Scylla tranquebarica*) in Segara Anakan Cilacap District. E3S Web Conf 147: 02005. DOI: 10.1051/e3sconf/202014702005.

Riani S, Prabowo RE, Nuryanto A. 2021. Molecular characteristics and taxonomic status of morphologically similar barnacles (*Amphibalanus*) assessed using the cytochrome coxidase 1 gene. Biodiversitas 22 (3): 1456-1466. DOI: 10.13057/biodiv/d220349.

Rimadiyani W, Krisanti M, Sulistiono. 2019. Macrozoobenthos community structure in the Western Segara Anakan Lagoon, Central Java, Indonesia. Biodiversitas 20 (6): 1588-1596. DOI: 10.13057/biodiv/d200615.

Rossel S, Arbizu PM. 2019. Revealing higher than expected diversity of *Harpacticoida* (Crustacea: Copepoda) in the North Sea using

MALDI-TOF MS and molecular barcoding. Sci Rep 9: 9182. DOI: 10.1038/s41598-019-45718-7.

Setyaningrum N, Lestari W, Krismono, Nuryanto A. 2022. Genetically continuous populations of Striped Snakehead *(Channa striata*) in the Cingcingguling River fragmented by Sempor Reservoir, Central Java, Indonesia. Biodiversitas 23 (1): 222-230. DOI: 10.13057/biodiv/d230128.

Thompson JG, Gibson TJ, Higgins DG. 2003. Multiple sequence alignment using ClustalW and ClustalX. Curr Protoc Bioinform 2 (1): 2.3.1-2.3.22. DOI: 10.1002/0471250953.bi0203s00.

Varela C, Bracken-Grissom H. 2021. A mysterious world revealed: Larval-adult matching of deep-sea shrimps from the Gulf of Mexico. Diversity 13: 457. DOI: 10.3390/d13100457.

Wagiyo K, Damora A, Pane ARP. 2018. Biological aspects, population dynamics and stock density of banana prawns *(Penaeus merguiensis* de man, 1888*)* in the nursery habitat of Segara Anakan estuaries, Cilacap. Jurnal Penelitian Perikanan Indonesia 24 (2): 127-136. [Indonesian]

Wakabayashi K, Yang C-H, Shy J-Y, He C-H, Chan T-Y. 2017. Correct identification and redescription of the larval stages and early juveniles of the slipper losbter *Eduarctus martensii* (Pfeffer, 1881) (Decapoda: Scyllaridae). J Crustacean Biol 37 (2): 204-2019. DOI: 10.1093/jcbiol/rux009.

Walczynska KS, Soreide JE, Weydmann-Zwolicka A, Ronowicz Gabrielsen TM. 2019. DNA barcoding of Cirripedira larvae reveals new knowledge on their biology in Artic coastal ecosystems. Hidrobiologia 837: 149-159. DOI: 10.10007/s10750-019-3967-y.

Weiss M, Macher JN, Seefeldt MA, Leese F. 2014. Molecular evidence for further overlooked species within the *Gammarus fossarium* complex (Crustacea: Amphipoda). Hydrobiologia 721: 165-184. DOI: 10.1007/s10750-013-1658-7.

Winarni ET, Kusbiyanto, Nuryanto A. 2021. Estimating crustacean species utilize Segara Anakan lagoon Cilacap, Indonesia, as nursery ground through DNA barcoding. J Hunan Univ Nat Sci 48 (10): 275282.

Xu X, Liu F, Chen J, Li D, Kuntner M. 2015. Integrative taxonomy of the primitively segmented spider genus *Ganthela* (Araneae: Mesothelae: Liphistiidae): DNA barcoding gap agrees with morphology. Zool J Linn Soc 175 (2): 288-306. DOI: 10.1111/zoj.12280.

Yuniarti, Ihsan YN, Asdak C, Dahiyat Y, Kamarudin MKA, Gasim MB, Yusra AFI, Juahir H. 2018. Impact sedimentation to community structure macrozoobenthos in Segara Anakan lagoon. J Fundam Appl Sci 10 (1S): 565-579. DOI: 10/4314.jfas.v10i1s.40.