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# Morphological and molecular tracing of two ornamental vampire crabs (Decapoda: Sesarmidae) from Central Java

# Морфологическое и молекулярное отслеживание двух декоративных крабов-вампиров (Decapoda: Sesarmidae) Центральной Явы

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KEY WORDS. COI, 16S rRNA, *Geosesarma,* genetic thresholds, haplotype network.
КЛЮЧЕВЫЕ СЛОВА. COI, 16S рРНК, *Geosesarma*, генетические пороги, сеть гаплотипов.

ABSTRACT. *Geosesarma dennerle* and *G. hagen* are the two most popular species of vampire crabs in the ornamental crab trading. These sympatric species typically occupy similar habitats, but variations in carapace coloration often lead to their trade under different trade names, suggesting the same or newly identified species. Therefore, this study aimed to investigate the genetic differences between *G. dennerle* and *G. hagen* using DNA analysis based on COI and 16S rRNA markers from specimens collected in Central Java Province, compared to species obtained from the natural habitat (holotype's locality, Cilacap Regency, Central Java). The results of the phylogenetic analysis showed a monophyletic relationship with well-supported ML and BPP values in some infra clades, while the basal clades were low-supported. The low genetic divergence between closely related species *G. dennerle* and *G. hagen* showed recent separation. However, haplotype network analysis showed a closer connection in the population *of G. hagen* and a larger divergence between trade and wild population in *G. dennerle*. This study suggested the presence of a potential new species in the wild, with promising exploration and description in their natural habitats.

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РЕЗЮМЕ. *Geosesarma dennerle* и *G. hagen* — два самых популярных вида крабов-вампиров в торговле декоративными крабами. Эти симпатрические виды обычно занимают схожие места обитания, но различия в окраске карапакса часто приводят к их торговле под разными торговыми названиями, нередко предполагая один или недавно идентифицированный вид. Целью данного исследования является изучение генетических различий между *G. dennerle* и *G. hagen* с использованием анализа ДНК на основе генных маркеров COI и 16S рРНК из образцов, собранных в провинции Центральная Ява, а также сравнение с видами, собранными из естественной среды обитания (местонахождение голотипа, регентство Силакап, Центральная Ява). Результаты филогенетического анализа показали монофилетическую связь с хорошо поддерживаемыми значениями ML и BPP в некоторых инфракладах, в то время как базальные клады имели низкую поддержку. Низкая генетическая дивергенция между близкородственными видами *G. dennerle* и *G. hagen* показала их недавнее разделение. Однако анализ сети гаплотипов показал более тесную связь в популяции *G. hagen* и большее расхождение между торговой и дикой популяцией у *G. dennerle*. Это исследование предполагает наличие потенциально нового вида в дикой природе, с перспективным исследованием и описанием в их естественной среде обитания.

## Introduction

Sesarmid crabs, particularly *Geosesarma* De Man, 1892, are among the high commodities in the ornamental trades in Europe and Asia under the name "vampire crab" [Rademacher, Mengedoht, 2011]. *Geosesarma* is a large genus, with 73 known species from Southeast and East Asia, the Andaman Islands, Papua New Guinea, and the Solomon Islands [Ng, Davie, 2008; Ng, Wowor, 2019; Shy, Ng, 2019; https://www.marinespecies.org/, accessed 2022]. Currently, 12 species under *Geosesarma* have been identified from Java, which can be divided into four groups [Ng, Wowor, 2019]. The first group consists of *G. noduliferum* (De Man, 1892), *G. bicolor* [Ng, Davie, 1995], *G. dennerle* Ng, Schubart et Lukhaup, 2015, *G. hagen* Ng, Schubart et Lukhaup, 2015, and *G. lebak* Ng et Wowor, 2019. The second group is formed by *G. sukabumi* Ng et Wowor, 2019 and *G. robustum* Ng et Wowor,


Fig. 1. Maps of Java Island: a — red rectangle marks the area of catchment for online shop of ornamental crabs in Jakarta; b — red circle for sampling location in Cipari, Cilacap Regency, Central Java Province; c — habitat of *G. dennerle* and *G. hagen*. Source: https://geoportal.big.go.id/#/viewer.

Рис. 1. Карты острова Ява: a — красный прямоугольник обозначает район сбора для интернет-магазина декоративных крабов в Джакарте; b — красный кружок обозначает место отбора проб в Чипари, округ Чилакап, провинция Центральная Ява; c — местообитание *G. dennerle* и *G. hagen*. Источник: https://geoportal.big.go.id/#/viewer.

2019, the third group comprises *G. confertum* (Ortmann, 1894), *G. sekop* Ng et Wowor, 2019, and *G. cikaniki* Ng et Wowor, 2019, while the last group has only one species *G. rouxi* (Serène, 1968) [Ng, Wowor, 2019]. Additionally, the most recent species is *G. garutense* Ng et Wowor, 2022, which closely resembles *G. rouxi* [Ng, Wowor, 2022].

*Geosesarma* species have very limited local distributions, with some occurring 10 km between sites [Ng *et al*., 2015], presenting a challenge for taxonomists in diversity uncovering. *Geosesarma dennerle* and *G. hagen* are often called vampire crabs due to their bright yellow eyes [Ng *et al*., 2015; Ng, 2017] and are gaining significant attention from traders and ornamental fauna enthusiasts despite the limited scientific description of several crabs in the market. Keeping ornamental fauna in aquarium is a popular hobby with high commercial value globally. Due to commercial wealth and lack of breeding, many indigenous ornamental fauna are caught directly from natural habitats and illegally traded by exporters, posing significant threats to indigenous species.

An accurate identification of ornamental fauna is often a challenge due to the scarcity of traditional taxonomic practices. For example, several animals' misidentifications have been observed because various trade names are used for different species but are closely related [Dhar, Ghosh, 2015]. *Geosesarma* generally can be diagnosed based on carapace such as size, form, lateral margins, dorsal surface, small round grains on anterior area, front deflection, wide frontal lobe with convex margins, and postfrontal crest prominent, with external orbital tooth including size, form, curved obliquely outward, and tip extends beyond lateral carapace margin, as well as gonopod 1 [Ng *et al*., 2015; Ng, Wowor, 2019]. However, only a few characters can be used to distinguish members of the genus *Geosesarma* that are relatively common among Sesarmid crab [Shahdadi, Schubart, 2015; Shahdadi *et al*., 2018].

DNA barcoding has become a relatively new and universal tool in taxonomic study for assigning specimens to their species, even without all or essential morphological diagnostic features [Hebert *et al*., 2003; Hollingsworth *et al*., 2014]. According to previous studies, DNA barcoding has proven to be an effective and adjunct tool [Hajibabaei *et al*., 2007; Hebert *et al*., 2003], successfully discovering many new species [Mohapatra *et al*., 2013], overcoming taxonomic uncertainty [Laskar *et al*., 2013, 2018], monitoring the ornamental trade [Collins *et al*., 2012; Steinke *et al*., 2009], biodiversity assessments [Laskar *et al*., 2019; Ward *et al*., 2009], and molecular tracing in illegal trading [Zhang *et al*., 2015]. In this study, several vampire crabs were purchased on e-commerce, stated only from Central Java Province, and compared to the natural catchment (Cilacap Regency, Central Java Province, holotype's locality). The sellers gave several trade names for *Geosesarma dennerle* crabs, including "Green Carnaval Crab", "Red Green Carnaval Crab", "Carnaval Violet Green Crab", and "Carnaval Full Violet Crab", and for *G. hagen* as "Red Claw Black Crab" and "Orange Crabs", suggesting either a single species with color va-

rieties or new species. To verify this assumption, species status was evaluated using morphological and molecular analyses for investigating phylogenetic relationships, the genetic threshold, and the haplotype network.

## Material and Methods

FIELD SAMPLING. A total of 15 specimens of *G. dennerle* and 63 *G. hagen* were examined, with sampling location reference being determined by referring to the coordinates in [Ng *et al*., 2015], around Karang Pucung, Cilacap Regency, as shown in Fig. 1. Based on the social media platform information, the two species' samples were collected from Cipari, Cilacap Regency on 20–24 May 2022. After being traced, the sites were not excessively far from the location based on the journal reference at approximately 19.3 km. Specimens examined are deposited in the Museum Zoologicum Bogoriense (MZB), Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN).

MORPHOMETRIC AND MORPHOLOGICAL IDENTIFICATION. The specimens were identified based on an identification key [Ng, Wowor, 2019] and the original description [Ng *et al*., 2015], while the terminology of crab structure was according to technical terms by Ng [2004]. After observing the morphology, all specimens were morphometrically calculated using Leica 125 microscopic stereo and the implemented software, Z6AP0, followed by photographing the whole body of specimens using Sony A600.

MOLECULAR IDENTIFICATION. Samples were extracted using Qiagen Blood Tissue Kit, PCR amplification, and sequencing, followed by DNA isolation from the armpit of crab appendages. DNA was extracted using the Blood and Tissue Kit Qiagen according to the procedures of the extraction handbook. A fragment of 991 bp of mitochondrial gene Cytochrome Oxidase I (COI) was amplified through forward primer GeoCOIF 5'-GGA GCT TGA GCA GGA ATA-3'and reverse primer GeoCOIRmod 5'- CCR AAT ACA GCT CCT ATW G-3' designed in Geneious Software. A fragment of approximately 544 bp of mitochondrial 16s rRNA gene was amplified with the following primer 16Sar-L: 5'–CGCCTGTTTATCAAAAACAT– 3'and 16Sb: 5'– CTCCGGTTTGAACTCAGATCA– 3' [Palumbi, 1996]. DNA template 1 µl was added with Polymerase Chain Reaction (PCR) MyTaq HS Red Mix by Bioline 12.5 µl and $H_2O$ molecular grade 9.5 µl. PCR for COI gene was applied under the condition of initial denaturation of 1 minute at 94 °C, 35 cycles of 30 seconds at 94 °C, 40 seconds at 50 °C, and 1 minute at 72 °C, followed by a final 10-minute extension step at 72 °C. Meanwhile, PCR condition for 16s rRNA was applied under the condition of initial denaturation of 4 minutes at 94 °C, 30 cycles of 30 seconds at 94 °C, 40 seconds at 53 °C, 1 minute at 72 °C, followed by a final 7-minute extension step at 72 °C. After completion, the best PCR product was sent to Macrogen Inc., South Korea, and all sequences were uploaded to BOLD System and in addition to Genebank NCBI. The project of DNA barcode library of *Geosesarma* was built in Barcode of Life Data Systems (BOLD) providing complete molecular, ecological, and taxonomical information under the project "BICC DNA Barcoding of *Geosesarma* spp. from Java". Additionally, sequences were deposited to the National Center for Biotechnology Information (NCBI).

MOLECULAR ANALYSES. Final sequences, 948 bp for COI and 423 bp for 16S RNA, were submitted to BOLD and NCBI, as shown in Table 1. Based on the results, COI gene produced BINs for outgroup BOLD:AFF8991 and *G. hagen* BOLD:AFF5248. *G. dennerle* produced three BINs, namely BOLD:AFF6307 and BOLD:AFF6308 for online purchase samples, BOLD:AFF6306 for naturally caught, and trade specimen BIC-0567 joined BOLD:AFF6306. COI sequences had GenBank NCBI accessions OR147199 to OR147215, while 16S rRNA showed accessions OR257785-OR257801. To validate species, BLAST method compared sequences from GenBank NCBI for COI and 16S genes, there were no COI gene references for the two species. 16S rRNA gene of *G. hagen* was limited in GenBank with accession number ON379433.1 [Tsang *et al*., 2022], but it was excluded from phylogenetic tree due to the absence of COI sequence. Therefore, 16S rRNA gene was only used in genetic distance and haplotype network analysis as a trade-bought specimen. The other genome references of *G. Penangense* accession MZ725941.1 and *G. faustum* accession MZ725940.1 [Lau *et al*., 2021] were used in the phylogenetic analyses of Maximum likelihood and Bayesian analyses by extracting 16S rNA and COI genes.

Sequences were edited and arranged by using Geneious [Kearse *et al*., 2012], while species were delineated using DNA sequences referred to as Operational Taxonomy Units (OTUs) with Refined Single Linkage (RESL) algorithms as implemented in BOLD to produce Barcode Index Numbers (BIN) [Ratnasingham, Hebert, 2013]. The best substitution model TIM2+G4 was calculated using JmodelTest [Darriba *et al*., 2012]. Phylogenetic trees based on Bayesian inference (BI) and Maximum Likelihood (ML) analyses were performed by combining two gene partitions of COI and 16sRNA using MrBayes 3.2.6 [Ronquist *et al*., 2012] and Raxml GUI software [Silvestro, Michalak, 2012] with *Terrathelphusa chilensis* as the outgroup. ML tree reconstruction showed bootstrap support (> 70%), consistent with established phylogenetic analysis standards, while BI using Mrbayes [Ronquist *et al*., 2012] produced a prior value above 0.85 (Bayesian Posterior Probability/BPP), indicating strong initial belief in species relationships. Genetic distance based on Kimura 2 Parameter was analyzed in MEGA 5.0 with 1000 bootstrap replications [Tamura *et al*., 2011], followed by the determination of Haplotype number of the concatenated sequences using dnsAP. Haplotype network was constructed through PopArt [Leigh, Bryant, 2015] based on the median joining network method, while the phylogenetic tree and haplotype network were edited using Adobe Illustrator.

## Results

PHYLOGENETIC ANALYSIS. 16S rRNA gene in *G. hagen* (BIC-0552–0556) from the natural catchment and BIC-0565 from trading showed consistent pairwise identity (about 99.6%) to *G. hagen* on GenBank [Tsang *et al*., 2022]. Genetic variation threshold based on COI and 16S rRNA genes between *G. dennerle* and *G. hagen* was low, reaching approximately 1.6–2.7% and 1–1.5% (Suppl. Tables 1 and 2). *Geosesarma* sp.1 (BIC-0563 and BIC-0564), *Geosesarma* sp.2 (BIC-0569), *Geosesarma* sp.3 (BIC-0570 and BIC-0571), and *Geosesarma* sp.4 (BIC-0566) had COI genetic differences of approximately 1.8–2.4% and 1.8–2.6%, 1.8–2.6% and 2.1–2.8%, both 8–8.7%, 1.9–2.2%, and 2.1–2.6%, for *G. dennerle* and *G. hagen,* respectively. *Geosesarma* sp. 1 (BIC-0563 and BIC-0564), *Geosesarma* sp.2 (BIC-0569), *Geosesarma* sp.3 (BIC-0570 and BIC-0571), and *Geosesarma* sp.4 (BIC-0566) showed 16S rRNA genetic differences of approximately 8–2.4% and 1.8–2.6%, 1.8–2.6%, and 2.1–2.8%, both 8–8.7%, 1.9–2.2%, and 2.1–2.6%, for *G. dennerle* and *G. hagen*, respectively.

Table 1. Access code of sequences of *Geosesarma dennerle* and *G. hagen* (BOLD and NCBI databases).
Таблица 1. Коды сиквенсов *Geosesarma dennerle* и *G. hagen* (базы данных BOLD и NCBI).

| Species | Museum Code | Access Code (BOLD) | Access Code for COI Gene (NCBI) | Access Code for 16S Gene (NCBI) | References |
|---|---|---|---|---|---|
| *Geosesarma dennerle* | BIC-0559 | BOLD:AFF6307 | OR147204 | OR257790 | This study |
| *Geosesarma dennerle* | BIC-0560 | BOLD:AFF6307 | OR147205 | OR257791 | This study |
| *Geosesarma dennerle* | BIC-0561 | BOLD:AFF6307 | OR147206 | OR257792 | This study |
| *Geosesarma* sp.1 | BIC-0563 | BOLD:AFF6308 | OR147207 | OR257793 | This study |
| *Geosesarma* sp.1 | BIC-0564 | BOLD:AFF6308 | OR147208 | OR257794 | This study |
| *Geosesarma dennerle* | BIC-0567 | BOLD:AFF6308 | OR147211 | OR257797 | This study |
| *Geosesarma* sp.2 | BIC-0569 | BOLD:AFF6308 | OR147213 | OR257799 | This study |
| *Geosesarma* sp.3 | BIC-0570 | BOLD:AFF6308 | OR147214 | OR257800 | This study |
| *Geosesarma* sp.3 | BIC-0571 | BOLD:AFF6308 | OR147215 | OR257801 | This study |
| *Geosesarma hagen* | BIC-0552 | BOLD:AFF5248 | OR147199 | OR257785 | This study |
| *Geosesarma hagen* | BIC-0553 | BOLD:AFF5248 | OR147200 | OR257786 | This study |
| *Geosesarma hagen* | BIC-0554 | BOLD:AFF5248 | OR147201 | OR257787 | This study |
| *Geosesarma hagen* | BIC-0555 | BOLD:AFF5248 | OR147202 | OR257788 | This study |
| *Geosesarma hagen* | BIC-0556 | BOLD:AFF6307 | OR147203 | OR257789 | This study |
| *Geosesarma hagen* | BIC-0565 | BOLD:AFF5248 | OR147209 | OR257795 | This study |
| *Geosesarma* sp.4 | BIC-0566 | BOLD:AFF6308 | OR147210 | OR257796 | This study |
| *Geosesarma hagen* | BRA1017 | – | – | ON379433.1 | Tsang et al., 2022 |
| *Geosesarma penangense* | MZ725941.1 | – | MZ725941.1 | MZ725941.1 | Lau *et al*. 2021 |
| *Geosesarma faustum* | MZ725940.1 | – | MZ725940.1 | MZ725940.1 | Lau *et al*. 2021 |
| *Terrathelphusa chilensis* | BIC-0568 | BOLD:AFF8991 | OR147212 | OR257798 | Outgroup in this study |

As presented in Fig. 2, the phylogenetic tree of *Geosesarma* spp. showed a monophyletic relationship with highly supported (> 70) ML and (> 0.90) BPP values in infraclades of *G. hagen* and *Geosesarma* sp.3, with highly supported (> 0.90) BPP values *G. dennerle,* while the basal clades were poorly supported. Haplotype network analysis of yellow eye *Geosesarma* based on COI gene produced four haplotypes in *G. dennerle*, six in *G. hagen*, and one in *Geosesarma* sp.4., while 16S gene showed four haplotypes in *G. dennerle*, one in *G. hagen*, and one in *Geosesarma* sp.4, as presented in Fig. 3.

## TAXONOMY

### Family Sesarmidae Dana, 1851
### *Geosesarma* De Man, 1892
### *Geosesarma dennerle* Ng, Schubart et Lukhaup, 2015
### Fig. 4a–d.

MATERIAL EXAMINED. 4 males 9.16–12.04 × 8.51–12.20 mm, 5 females 10.00–11.74 × 9.18–13.46 mm (MZB Cru. 5698), (7°25′S, 108°55′E), Cipari, Cilacap, Central Java, coll. Dhany H. Mahendra & Saeful, 23 May 2022, DNA: BIC-0559-BIC-0561, 1 male 10.06 × 9.7, (MZB Cru. 5236), online trading from Jakarta, 09 Dec. 2020, coll. Rena T. Hernawati, DNA: BIC-0567.

OTHER MATERIAL. *Geosesarma* sp.1: 2 males 10.26–11.37 × 9.77–10.42 mm, 1 female 11.29 × 10.21 mm (MZB Cru. 5236), online trading from Jakarta, 09 Dec. 2020, coll. Rena T. Hernawati, DNA: BIC-0563, BIC-0564; *Geosesarma* sp.2: 1 male 10.17 × 9.81mm (MZB Cru. 5700), online trading from Jakarta, 01 Jun. 2022, coll. Dhany H. Mahendra, DNA: BIC-0569; *Geosesarma* sp.3: 2 males 10.19–12.198 × 11.25–13.05 mm (MZB Cru. 5777), online trading from Jakarta, 01 Jun. 2022, coll. Dhany H. Mahendra, DNA: BIC-0570 and BIC-0571; *Geosesarma* sp.4: 1 male 10.51 × 10.00 mm (MZB Cru. 5195) online trading from Jakarta, 09 Dec. 2020, coll. Rena T. Hernawati, DNA: BIC-0566; *Terrathelphusa chilensis* as outgroup. 1 male 39.68 × 28.80 mm (MZB Cru. 5198:) (7°18′S, 108°44′E), DNA: BIC-0568.

DIAGNOSIS. Carapace squarish, dorsal surface with obvious divided area, and the anterior regions covered with small round grains; outer orbital teeth large, triangular, obliquely outward. External surface of the palm of adult male chelae granulated, the dorsal edge of dactylus with 7–9 tubercles, pectinated tip. Male abdomen broad, with semicircular telson, somit 6 with convex lateral margin. Gonopod 1 (G1) slender, with straight proximal part, distal region pectinated, bent, elongate, spatuliform.

COLOR. Coloration in wild (Fig. 4a–c) and trading (Fig. 4d) samples show significant variation. The posterior half or third of carapace is mostly cream to yellow and the anterior carapace is purple. Moreover, the anterior half of carapace and ambulatory legs are violet-purple to purplish brown and the abdomen has small spots, with dark gray to purplish gray. The chelae of adult male is bright purple, while juveniles show grayish purple to pale purple, and white, with bright yellow eyes.

HABITAT. The habitat of *Geosesarma* is in shrubs characterized by dense vegetation and flowing with small water current. These species also make holes in riverbank of small creeks and eat plant litter. The adults are occasionally found in the water to hydrate the carapace during the day, while juveniles stay close to rivers.

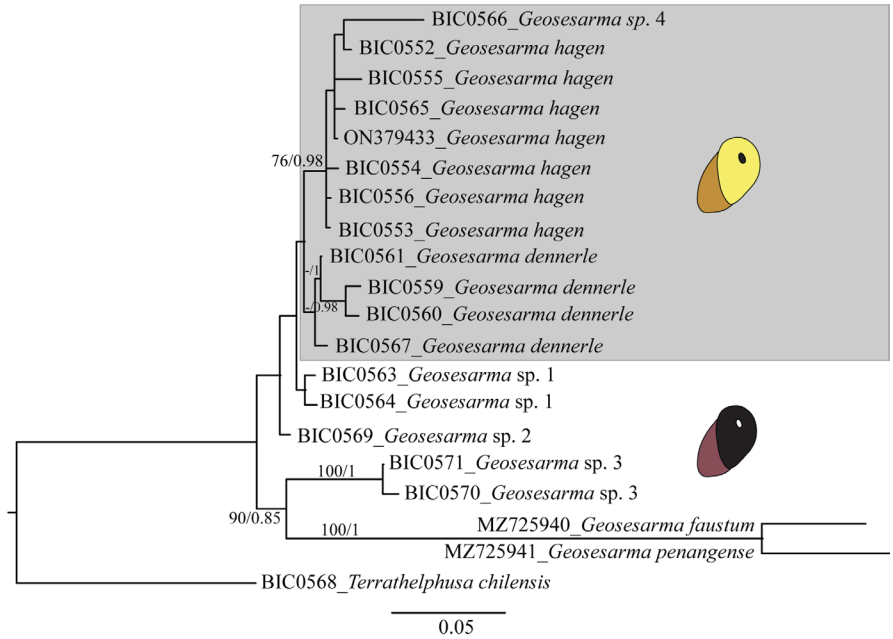


Fig. 2. Phylogenetic tree of the concatenated COI and 16S RNA genes based on Maximum Likelihood and Bayesian Inference methods (-/-), respectively.

Рис. 2. Филогенетическое дерево конкатенированных генов COI и 16S рРНК, основанное на методах максимального правдоподобия и байесовского анализа (-/-), соответственно.

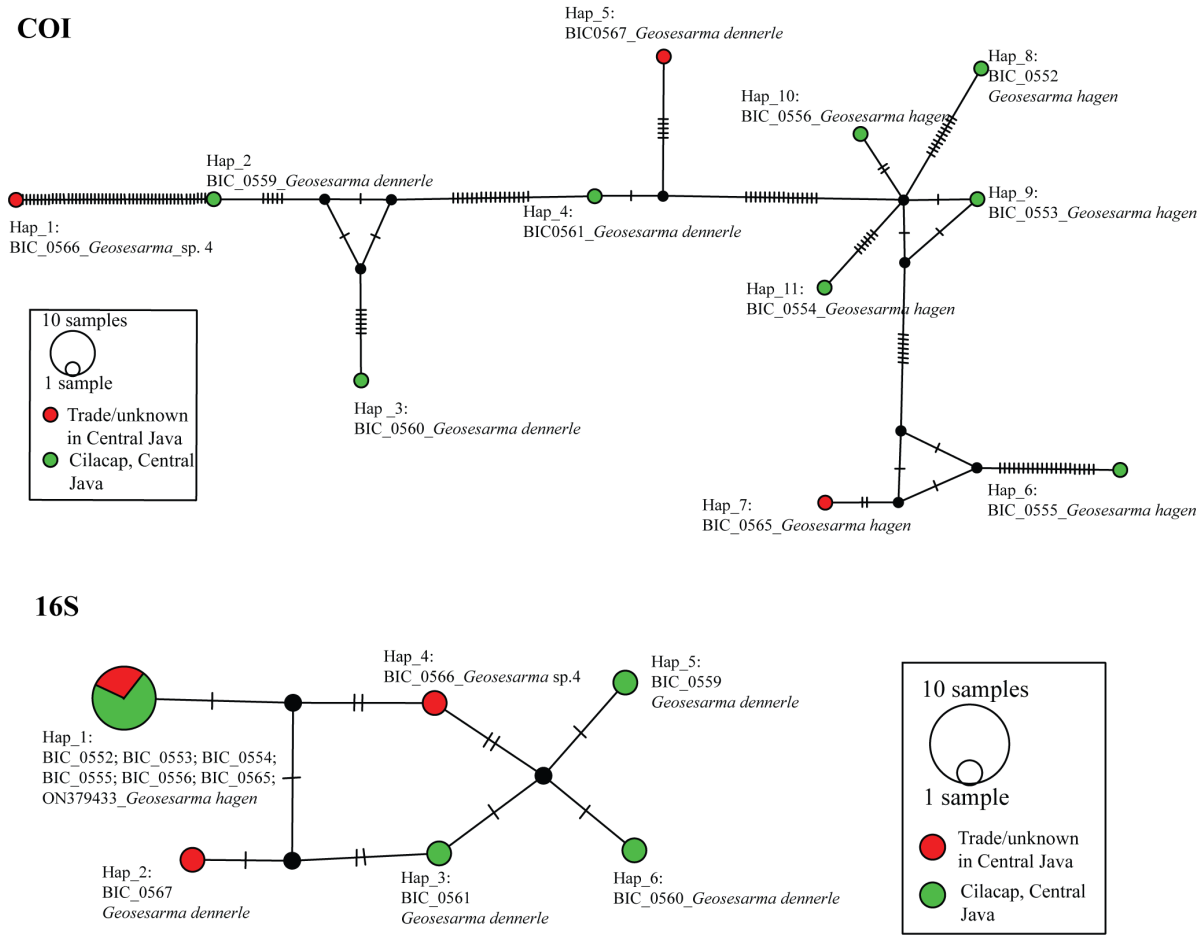


Fig. 3. Haplotype network COI (upper) and 16S rRNA (lower) genes of "yellow eye" group. *Geosesarma dennerle* (a,c) and *G. hagen* (b, d).

Рис. 3. Сеть гаплотипов COI (вверху) и 16S рРНК (внизу) группы "желтые глаза". *Geosesarma dennerle* (a, c) и *G. hagen* (b, d).


Fig. 4. *Geosesarma dennerle*: (a, b, c) BIC-0559–0561 from natural catchment and (d) BIC-0567 from online trading; *Geosesarma* spp. from online trading*: Geosesarma* sp. 1 (e, f,) BIC-0563, BIC-0564; *Geosesarma* sp. 2 (g) BIC-0569; *Geosesarma* sp. 3 (h, i) BIC-0570 and BIC-0571.

Рис. 4. *Geosesarma dennerle*: (a, b, c) BIC-0559–0561 из естественной среды обитания и (d) BIC-0567 из интернет-магазина; *Geosesarma* spp. из интернет-магазина: *Geosesarma* sp.1 (e, f,) BIC-0563, BIC-0564; *Geosesarma* sp.2 (g) BIC-0569; *Geosesarma* sp.3 (h, i) BIC-0570 и BIC-0571.

REMARKS. Samples BIC-0559–0561 collected from Cipari, Cilacap, Central Java, resemble *G. dennerle*, showing square-shaped carapaces, small solid grains, and 8–9 tubercles, although some overlap with *G. hagen*'s 7–9 tubercles. *G. dennerle* has a black anterior and yellowish posterior carapace, purple chelae, wide triangular orbital teeth, and a semicircular telson. G1 basal segment correlated with [Ng, Wowor, 2019], and the female gonophore has a narrowed valve. Trade specimen BIC-0567 fits morphologically and genetically to *G. dennerle*, but several fresh specimens have gradations in carapace color distribution.

### *Geosesarma hagen* Ng, Schubart et Lukhaup, 2015
Fig. 5a–f.

MATERIAL EXAMINED. 1 female ovigerous 11.93 × 10.57mm (MZB Cru. 5235) online market in Jakarta, 09 Dec. 2020, coll. Rena T. Hernawati, DNA: BIC-0565; 29 males 11.42–12.31 × 10.01–11.36 mm, 33 females 10.11–10.5 × 9.00–9.32 mm (MZB Cru. 5697) (7°25′S, 108°45′E) Cipari, Cilacap, Central Java, 23 May 2022, coll. Dhany H. Mahendra & Saeful, DNA: BIC-0552–0556.

DIAGNOSIS. Carapace squarish, dorsal surface with obvious divided area, postfrontal crest protruding, large triangular outer orbital teeth curving obliquely outward, anterior surface of adult male chelae granules, dorsal margin of dactylus with 7–9 tubercles, with pectinated ends. Male abdomen broad, telson semicircular, somite 6 with strongly convex lateral edge. Gonopod 1 (G1) slender, proximal part straight, distal region pectinated, bent, elongated, spatuliform.

COLOR. Coloration in wild (Fig. 5a, c–f) and trading (Fig. 5b) samples show significant variations. The anterior half or third of carapace and ambulatory legs are dark brown, chelae of male adults are bright orange to reddish orange, while juveniles are generally more reddish. The eyes are bright yellow, abdomen has small spots, with dark gray to brown orangish, and chelae of adult male are bright purple, while juveniles are grayish purple to pale purple, and white.

HABITAT. The habitat of *G. hagen* is similar to *G. dennerle*.

REMARKS. BIC-0552–0556 resemble *G. hagen*, having square carapaces, solid grains, and 9 tubercles. *G. hagen* has an orange carapace, red chelae, narrow triangular orbital teeth, a semicircular telson, and a wider gonophore valve compared to *G. dennerle*. Trade specimen BIC-0565 correlates morphologically and genetically to wild specimens, but several fresh specimens have gradations on carapace color distribution.

## Discussion

PHYLOGENETIC RELATIONSHIPS. Vampire crabs have a similar life strategy to freshwater shrimps, colonizing the environment by tolerating the low salinity, having abbreviation of larval development, producing fewer and bigger egg size becoming a strong phylogenetic signal [Wowor *et al*., 2009; Pileggi, Mantelatto, 2010; Vogt, 2013]. Phylogenetic reconstruction among species in genus *Geosesarma* based on concatenated mitochondrial genes leads to monophyletic clades with *G. faustum* and *G. penangense,* as the ancestral species. Meanwhile, the genus *Geosesarma* shows a consistency signal of the

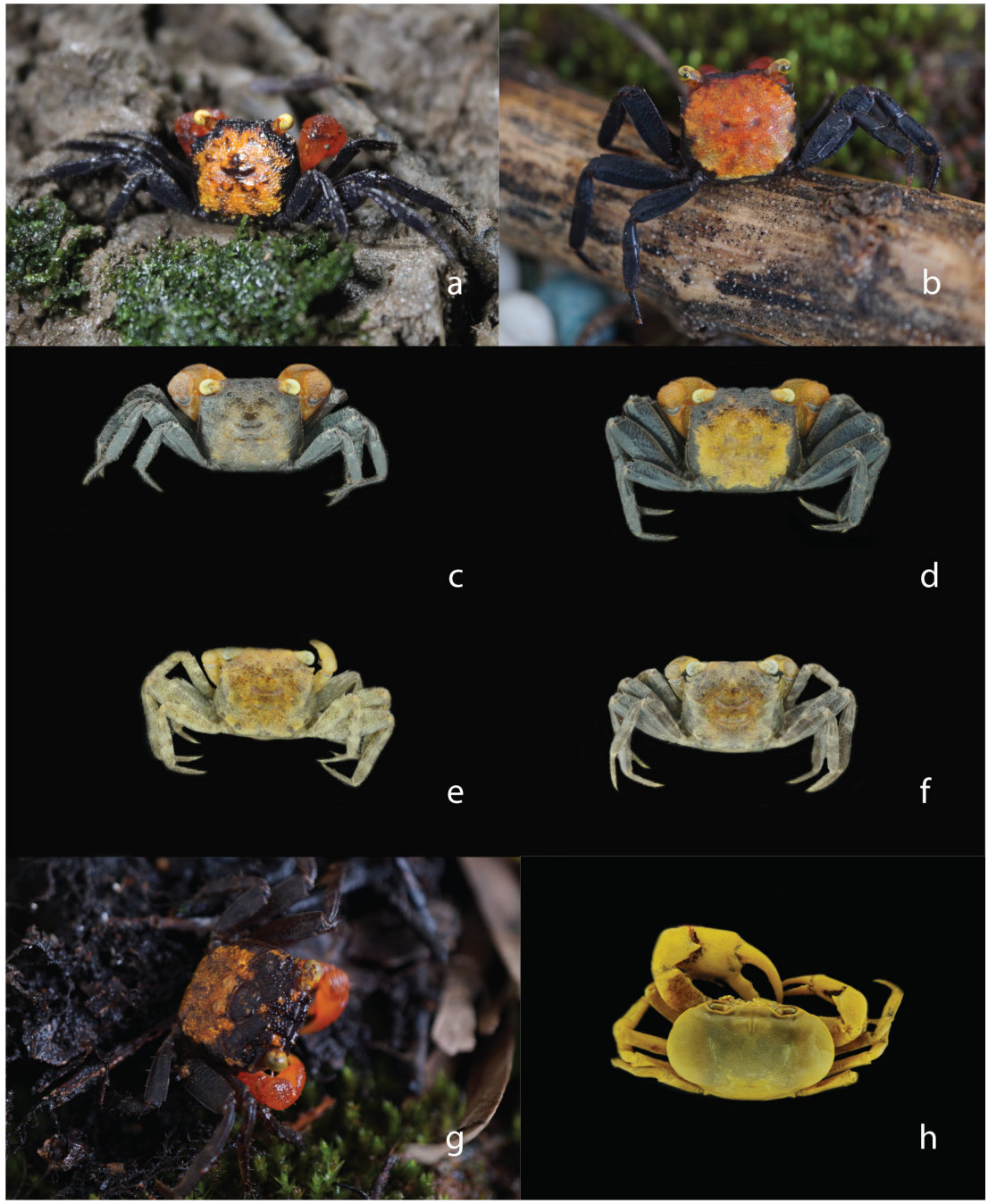


Fig. 5. *Geosesarma hagen*: (a, c–f) BIC-0552–0556 from natural catchment; (b) BIC-0565 from online trading. *Geosesarma* sp.4: (g) BIC-0566 from online trading. *Terrathelphusa chilensis* (h) as an outgroup.

Рис. 5. *Geosesarma hagen*: (a, c–f) BIC-0552–0556 из естественной среды обитания; (b) BIC-0565 из интернет-магазина. *Geosesarma* sp.4: (g) BIC-0566 из интернет-магазина. *Terrathelphusa chilensis* (h) как внешняя группа (outgroup).

eye colors on the phylogenetic tree, as ancestral species, namely *G. penangense* and *G. faustum* have black eyes to the recent species in *G. dennerle* and *G. hagen* clades having yellow eyes (Fig. 2).

Previous studies on morphological phylogenetic relationships suggested that ancestral birds had intermediate OMT (transmittance of lenses and corneas). Other characteristics included bigger and unpigmented eyes, as well as UV sensitivity occurring several times, some birds had developed in the opposite direction [Olsson *et al*., 2021]. Transmittance of lenses showed a high phylogenetic signal, in nocturnal frogs having unpigmented lenses and UV sensitivity. Meanwhile, diurnal frogs had more pigmented lenses that absorbed maximally on the violet and blue range of the spectrum [Yovanovich *et al*., 2020]. In addition, domestic pigeons had a greyish iris color due to a mutation of the wild ancestor with an orange iris color approximately 5,400 years ago, based on a genomic study

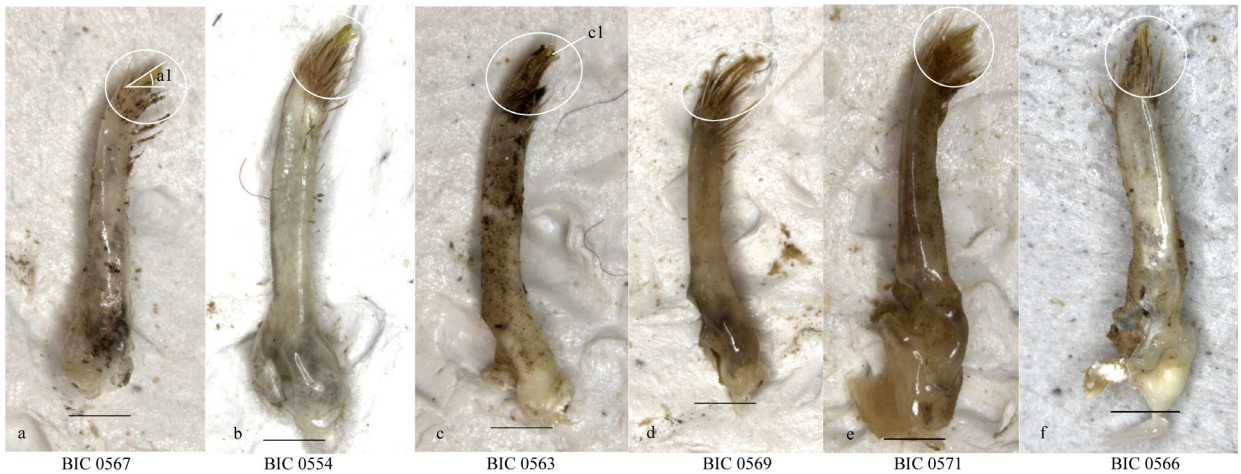


Fig. 6. Gonopod 1 (G1): *Geosesarma dennerle* (a); *G. hagen* (b), *Geosesarma* sp.1 (c), *Geosesarma* sp.2 (d), *Geosesarma* sp.3 (e), *Geosesarma* sp.4 (f). Abbreviations: a1 — angle inclination; cl — cleft. Scale: 1 mm.

Рис. 6. Гонопод 1 (G1): *Geosesarma dennerle* (a); *G. hagen* (b), *Geosesarma* sp.1 (c), *Geosesarma* sp.2 (d), *Geosesarma* sp.3 (e), *Geosesarma* sp.4 (f). Сокращения: a1 — угол наклона; cl — углубление (расщелина). Масштаб: 1 мм.

[Xi *et al*., 2021]. Therefore, the eye colors or pigment can be another phylogenetic signal in the freshwater crab lifestyle, showing the need for further study.

An obvious color pattern is found on the carapace of the genus *Geosesarma,* as shown in Figs 4–5. The chelae and carapace color of *G. faustum* is orange and *G. penangense* is red, while *G. dennerle* has a bicolor carapace with purple chelae, *G. hagen* has an orange carapace with red or range chelae. However, several specimens of *G. dennerle* and *G. hagen* have slight differences in pigmentation coverage on the carapace. This difference is attributed to the effect of substrate on infraspecific variation in body color [Tong *et al*., 2019], limiting the reliability of color patterns on the carapace for species identification. Freshwater shrimps in Sulawesi's Lakes depict conspicuous body patterns, particularly for *Caridina spongicola* due to living in specific sponge host, while *C. spinata* has two patterns, with and without stripe color [Zitzler, Cai, 2006; von Rintelen *et al*., 2007; von Rintelen, Cai, 2009].

GENETIC THRESHOLD AND HAPLOTYPE NETWORK. 16S rRNA sequences in *G. hagen* from wild (BIC-0552–0556) and trade specimen (BIC-0565) show the consistent pairwise identity of approximately 99.6% with *G. hagen* the GenBank [Tsang *et al*., 2022]. *G. hagen* had approximately 0.2–1% intraspecific variation (Suppl. Table 2) between trade and wild samples, while *G. dennerle* had 0.3–1.4%. Although interspecific variation between *G. dennerle* and *G. hagen* was not more than 2.7%, there is still genetic separation. A previous study on insects found that species divergence often exceeded 3% [Hebert *et al*., 2003], showing significant genetic divergence due to mutations, isolation, or selective pressures, such as geological, chemical, and environmental factors [Baksir *et al*., 2022].

*G. dennerle* and *G. hagen* are sympatric species in this study. They also co-occur in habitat approximately 10.5 km apart [Ng *et al*., 2015]. *Geosesarma* spp. has two shapes and colors of chelae similar to *G. dennerle* and *G. hagen*. These include big purple chelae and relatively smaller reddish-orange chelae, which can also co-occur sympatrically on Mount Slamet [Hernawati, 2019]. Thereby the genetic difference probably has a consequence of their similar morphology due to close distance. Moreover, low genetic differentiation of closely related species *G. dennerle*, *G. hagen*, and *Geosesarma* sp.4 morphologically having yellow eyes show their recent separation due to the presence of hybridization among ancestors in the genus *Geosesarma*. Hybrid species have less obvious and stable color patterns, which provokes matting errors [von Rintelen *et al*., 2007; von Rintelen, Cai, 2009; Richards *et al*., 2018]. The divergence in morphology and low genetic distance in *Geosesarma* sp. suggest subspecies, such as Apidae [Martinet *et al*., 2019].

Haplotype network analysis of yellow eye species group based on origin location and 16S rRNA, as presented in Fig. 3, showed a similar pattern to the phylogenetic tree in Fig. 2. This is consistent with the morphological characters, while different haplotypes for each wild and trade specimen were found based on COI gene. The higher number of haplotypes in COI gene and the low genetic distance of *G. dennerle* and *G. hagen* populations showed the recent fragmentation in Java Island, such as between two mangrove crabs *Parasesarma semperi* (Bürger, 1893) and *P. longicristatum* (Campbell, 1967) [Shahdadi *et al*., 2018] and two shore crab species *Hemigrapsus penicillatus* (De Haan, 1835) and *H. sanguineus* (De Haan, 1835) [Shin *et al*., 2019]. Based on 16S rRNA, there was local connectivity in *G. hagen* population, compared to *G. dennerle,* which produced different lineages or haplotypes between natural and trade populations. Semiterrestrial crabs, such as members of genus *Geosesarma* do not need brackish water in life stages, suggesting their classification as locally limited distributed. Moreover, several volcanoes are spread across Java Island that can be barriers for vampire crab populations to disperse or connect, leading to the development of subspecies in gastropods [Poitrimol *et al*., 2022].

SYSTEMATICS. Morphological observations on yellow eye specimens are consistent with the phyloge-

netic tree, which shows a large clade of *G. hagen*, and *G dennerle*. Trade specimen BIC-0565, namely "Red Claw Black Crab" is *G. hagen*, while BIC-0566 "Orange Crabs" is a distinct species. The difference is based on variation in the coloration of carapace, which is orange in the half posterior, as well as form and angle inclination of distal pectinated part of the first gonopod (Fig. 6b, f), suggesting potentially varying species.

*G. dennerle* and *Geosesarma* sp.1–3 have yellow and black eyes, respectively, and can be distinguished by the first gonopod form, the angle inclination of the distal pectinated part (Fig. 6), and coloration patterns on carapace (Fig. 4). BIC-0567 trade specimen namely "Green Carnaval Crab" resemble the real *G. dennerle. Geosesarma* sp.1 (BIC-0563 and BIC-0564) and *Geosesarma* sp.3 (BIC-0570 and BIC-0571) have different coloration carapace and seller gave namely "Red Green Carnaval Crab" and "Violet Green Carnaval Crab", respectively. Specifically, *Geosesarma* sp.1 specimens have a posterior carapace that divides into three color regions, with the left and right sides being cream (Fig. 4h–i). Trade specimen, BIC-669, *Geosesarma* sp.2 namely "Carnaval Full Violet Crab" has ambulatory legs and carapace (Fig. 4g) that are completely purple to greyish on the middle grove region.

Trade specimens of *Geosesarma* spp. are not related in the phylogenetic tree (Fig. 2), except for *Geosesarma* sp.4, which is separated into several clades from *G. dennerle* and *G. hagen* clades. The number of haplotype networks in Fig. 3 on the yellow eye group shows similar results. Therefore, several candidates of new species in genus *Geosesarma* are considered for formal descriptions, showing the need for tracing in the wild to obtain the real distribution of *Geosesarma* sp.1–4. Morphological and molecular species validation of genus *Geosesarma* is essential due to global exploitation for aquarium trade. Small genetic differences lead to a high level of extinction risk [Kleinhans, Willows-Munro, 2019], showing the benefits of valid names in trade and DNA library to identify and monitor the wild populations.

**Supplementary data**. The following materials are available online.

Supplement Table 1. Genetic distance based on COI gene of *Geosesarma* spp.

Supplement Table 2. Genetic Distance based on 16S rRNA gene of *Geosesarma* spp.

**Compliance with ethical standards**

**Conflict of interests:** The authors declare that they have no conflict of interest.

**Ethical approval:** No ethical issues were raised during our research.

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