

Geodynamic and molecular characterization of endemic *Cherax* sp. in Wamena Dome, Papua, Indonesia

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Abstract. Hartoko A, Pringgenies D, Melmambessy EHP, Manuel G, Sumantyo JTS, Helmi M, Hariyadi H. 2025. Geodynamic and molecular characterization of endemic *Cherax* sp. in Wamena Dome, Papua, Indonesia. *Biodiversitas* 26: 4090-4102. Foreign aquarium demands threaten the high potential of *Cherax* sp. biodiversity in Papua. The new concept is the integration of geodynamic analysis and molecular characterization of *Cherax* sp. Spectral classification of DEM applied to reconstruct the collision of the Pacific and Australian tectonic plates to form Papua Island. The collision process generates the highland of the Wamena Dome, as well as formations of the northward and southward riverine systems. PCR gene amplification using a mitochondrial DNA-forward JgLCO1490 and reverse JgHCO2198 primers. DNA amplification using a PCR machine and Basic Local Alignment Search. Genetic distance analysis using matrix comparison to GenBank National Center for Biotechnology Information sequences. Phylogenetic tree generated using the Neighbor-Joining method with a bootstrap of 1,000 in MEGA-XII. Phylogenetic analysis of *Cherax monticola* from the Baliem River and *Cherax quadricarinatus*, as representatives of the Australian species, reveals that they belong to a single clade. The nearest genetic distance of *C. monticola* to the Australian origin of *C. quadricarinatus* is 0.17, and is high in *C. monticola* to *C. preissi* (0.234) and to *C. doberai* (1.4570). Most of the 30 *Cherax* sp. are found in the southward rivers of Papua. Haplotype network analysis has confirmed that *C. monticola* is the origin of Papua, and *C. quadricarinatus* is the origin of Australia. Tectonic plate geodynamic and DNA analysis confirming the origin of South Papua and Australia as previously one tectonic plate and induced *Cherax* sp. geographic endemicity.

Keywords: *Cherax*, COI-barcode, endemism, freshwater lobster, geodynamics, Wamena

INTRODUCTION

Most *Cherax* sp. populations in Papua Island were found in the southward riverine system of Papua Island and have their own ecological uniqueness and genetic diversity. Twenty species of Papua's endemic freshwater crayfish, *Cherax* sp., have been studied in terms of their geographical distribution, morphological characteristics, and molecular approach (Lukhaup et al. 2015, 2017; Patoka et al. 2015a, b; Widyasari et al. 2021).

Approximately 10 *Cherax* species and their geographic distributions have been discovered by Abinawanto et al. (2018) and Lekatompessy et al. (2019). These are *Cherax monticola* found in the Baliem River, Central Papua, *C. lorenzi* in Lorentz River, West Papua, and *C. destructor*, *C. quadricarinatus*, *C. longipes*, and *C. murido* in Habemai, Paniai, and Tigi Lakes. Meanwhile, *C. woworae*, *C. pulcher*, *C. boesemani*, and *C. snowden* are found in Ainsok River, Kais River, and Amajaru Lake. A geographical study by Abinawanto et al. (2018) included 13 species of *Cherax* sp.

in the freshwater lakes of Tigi, Habema, Paniai, Tage, and Baliem Rivers. This study also found at Uter Lake at Atinjo Sub-district, Maybrat, Seremuk River at Haha Village, South Sorong, and Baliem River at Pike Village, Hubukiak Sub-district, Jayawijaya, and Baliem Wesaput River at Wesaput Sub-district (Abinawanto et al. 2018). Meanwhile, Lekatompessy et al. (2019) identified freshwater crayfish found in South Sorong, Maybrat, and Jayawijaya Districts as belonging to the family of Parastacidae and the genus of *Cherax* sp..

Further new species described by Lukhaup et al. (2024) are *C. rayko*, endemic at Bian River, Merauke; *C. alyciae*, *C. phing*, *C. wagenknechtae*, *C. boesemani*, and *C. gheradii*. Most recently, Lukhaup et al. (2025) discovered 7 new species: *C. veritas*, *C. arguni*, *C. kaimana*, *C. nigli*, *C. farhadii*, *C. bomberai*, and *C. doberai*. The role of geographic isolation of *Cherax* sp. and an important natural habitat for the endemic freshwater lobster *Cherax* sp. in the Wamena Dome on the mountainous region of central Papua Island at 1,650-1,750 meters above sea level (masl).

IUCN (2015) classified the status of *Cherax* sp. in Papua as "Data Deficient, Vulnerable, and Endangered," and Sari et al. (2011) stated that the existence of *Cherax* sp. population in Papua is under threat. The traditional hand-line fishing of *Cherax* sp. in the southern Papua rivers has recently become a resource threat due to uncontrolled fishing caused by increasing foreign aquarium demand. Widyasari et al. (2021) report a significant increase in catch for export demands, from 32,850 individuals in 2016 to 70,626 individuals in 2020.

The Wamena mountainous area is the collision zone between the Australian Plate in southern Papua and the Pacific Plate in northern Papua, formed over a 55-million-year period (Hall 2001). To date, research on tectonic geodynamic and biological aspects has followed its own distinct path. This study introduces new frameworks for integrating geodynamic concepts, eco-biology, and endemism. An earlier study by Hartoko et al. (2014, 2016) developed a new method for Digital Elevation Model (DEM) spectral classification, aiming to reconstruct paleo-oceanographic zones. This geodynamic process has implications for the origin of all organisms in the northern and southern parts of Papua.

Hall (2001) described that the three tectonic plates of Eurasia-Australia-Pacific had constructed Indonesian islands. The hypothesis of this study posits that the three joining plates will enrich the biodiversity of the organism, more specifically, the paleo-oceanography of the northern and southern parts of Papua Island will differentiate its endemic organisms. Each riverine system is based on the concept that *Cherax* sp. exhibits its own species diversity and shows a familial relationship to Australian endemics. This could provide scientific evidence for geodynamic genetic biodiversity, conservation, the need for local wisdom-based resource management, and in-situ cage culture development for the sustainable income of local communities. Moreover, emerging multidisciplinary research has shed light on the dynamic nature of molecular methods, highlighting its wide

potential for endemism studies. The recognition of distinct geographic origin, such as environmental-induced adaptation, underscores the complexity of the geographic phenotype and its context-dependent conservation and catch regulation.

MATERIALS AND METHODS

Study area

The study area is located in Jayawijaya and Yahukimo Districts, Papua Province, Indonesia. Samples were collected from five sampling sites in the top hill area of the Baliem River, at the border between Jayawijaya and Yahukimo Districts. The sampling sites were located approximately 5 km downstream from each other. Sampling coordinates are (i) 4°8'1.47.9" S/138°58'30.89" E, (ii) 4°11'38.2" S/139°2'0.53" E, (iii) 4°14'26.44" S/139°4'36.83" E, (iv) 4°19'32.47" S/139°3' 14.25" E, (v) 4°23'12.03" S/139°7'47.48" E (Figure 1).

Field sampling

A total of 10 freshwater lobster samples were collected from 5 sampling sites. Samples were collected using hand-line fishing. The field river water temperature at the Baliem River was measured using a HORIBA digital thermometer with an accuracy of 0.01°C, conducted in situ at a depth of 30 cm. Samples of the endemic freshwater lobster *Cherax* sp. were collected during the night from 18.00-21.00 East Indonesian Times.

Animal ethics

Research team members, including Gerson Manuel from STKIP Wamena, Edy H.P. Melmambessy from Universitas Musamus Merauke, and Stasiun Karantina Merauke, strictly use permits for freshwater lobster *Cherax* sp. samples for research purposes.

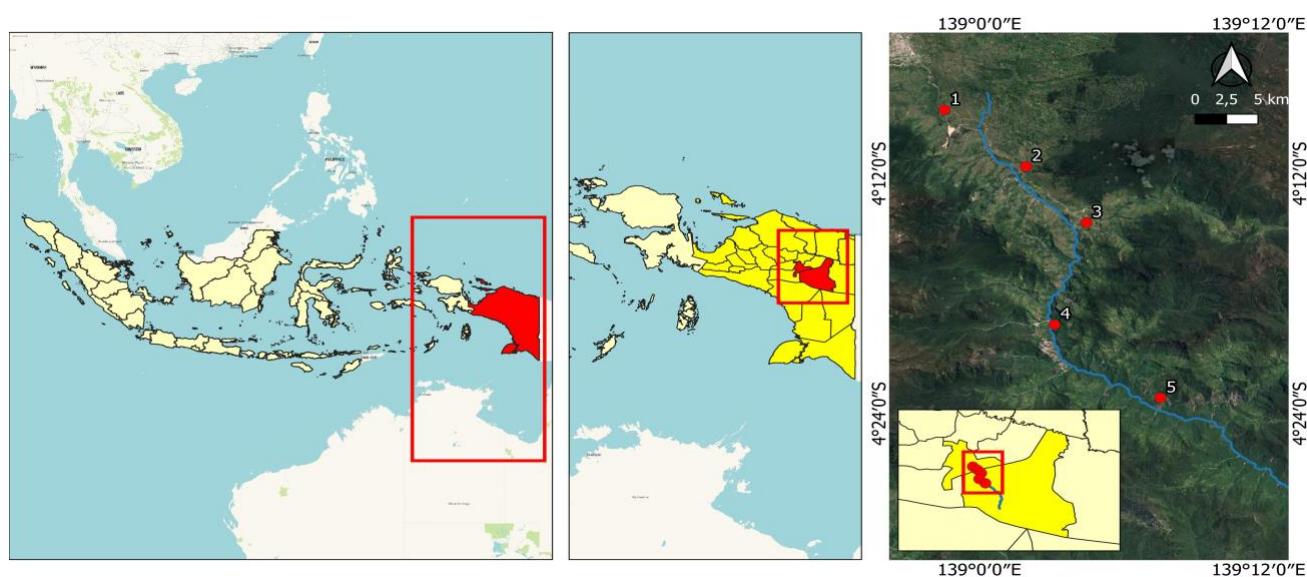


Figure 1. Study area in Jayawijaya and Yahukimo District, Papua, Indonesia. 1-5: Sampling sites at the Baliem River Wamena Dome, Papua

Spatial data processing and analysis

The spatial data used is the Digital Elevation Model Space-Shuttle Radar Terrain Model (DEM-SRTM), with a spatial resolution of 30 m, geodetic datum WGS84, and map projection in geodetic and SUTM. Spatial geodynamic reconstruction using DEM data employs spectral classification method (Hartoko 2014). Numeric data processing was conducted using ERMapper software (Licensed User-Waindo SpecTerra). Spatial data processing was carried out at the Laboratory of the Marine Geomatic Centre, Universitas Diponegoro, Semarang, Indonesia.

Molecular characterization

Ten samples of the freshwater lobster *Cherax* sp. were collected from the Baliem River, Papua, and two samples were used for molecular characterization. A slice of muscle tissue was then euthanized and stored in a cool box. DNA extraction, electrophoresis purification, and PCR were done in the Laboratory of Molecular Biology, Faculty of Fisheries and Marine Science, and Integrated Laboratory, Universitas Diponegoro, Semarang, Indonesia.

DNA extraction, amplification, and sequencing

The genomic DNA size can be found in high molecular weight (Kimura 1980; Pearson 2013; Kumar et al. 2018; Hartoko et al. 2020; Sulardiono et al. 2022). The sample was directly placed into a ZR BashingBead™ lysis tube. The sample was processed for DNA isolation and purification using sterile microtubes and centrifugation at 12,000 rpm for 10 minutes. The DNA from the samples was isolated and purified with Fast-Spin technology. Genomic DNA extraction was carried out using ZR Tissue DNA MiniPrep. The forward primers used were JgLCO1490: 5'-TIT CIA CIA AYC AYA ARG AYA TTG G-3' and reverse primer JgHCO2198: 5'-TAI ACY TCI GGR TGI CCR AAR AAY CA-3' (Geller et al. 2010). The PCR volume for each reaction (total volume 25 µL) consisted of: 1 µL of DNA template, 12.5 µL of MyTaq™ Red Mix (Bioline), 1 µL of each primer, and 9.5 µL of ddH₂O. The thermocycling profile included an initial denaturation at 95°C for 4 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and elongation at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. The PCR products were checked on 1% agarose gels stained with Florosafe. The sequencing process was carried out using the ABI PRISM 3730xl Genetic Analyzer, developed by Applied Biosystems, USA. The kit used for sequencing purposes is BigDye® Terminator v3.1 Cycle Sequencing and Homology Basic Local Alignment Search Tool (BLAST).

Data analysis

Analysis of the sample DNA sequence was compared with the sequence in the DNA database at the National Center for Biotechnology Information (NCBI), National Institute of Health, USA (Pearson 2013) to determine the percentage match of species based on the nucleotide base sequence's homology level. The series of nucleotide bases is compared with a GenBank database. Visual qualitative data, DNA isolation and amplification profiles, and

electropherograms will be presented descriptively. Genetic distances and phylogenetic genetic tree were calculated using MEGA-XII tools using the Neighbor-Joining method, scale 0.20, bootstrap replications of 1,000 (Kimura 1980; Pearson 2013; Kumar et al. 2018; Hartoko et al. 2020; Eprilurahman et al. 2021; Tamura et al. 2021; Lukhaup et al. 2025). Further details on analysis and discussion will refer to Sulardiono et al. (2022) and Abbas et al. (2018). Additional discussion on the freshwater lobster will reference the studies by Abbas et al. (2018, 2021); Abinawanto et al. (2018); Kawai and Patoka (2020); Mazancourt and Audo (2024).

RESULTS AND DISCUSSION

Geodynamic reconstruction of Papua Island

The geodynamic reconstruction of Papua Island using the spectral classification method of DEM-SRTM data, based on the fundamental geological previous knowledge that Papua Island is formed by the collision of the northern part of the Pacific tectonic plate, with the southern part as part of the Australian tectonic plate. This spatial reconstruction process and analysis of Papua Island utilized 5 reconstruction phases, each representing a 10 million-years period (Figure 2). The reconstruction analysis exhibited the formation of the mountainous area of the Wamena Dome (Figures 2, 3, 4, and 5) as a result of the uplift of the ocean floor from the Pacific at the north and the Australian tectonic plate at the south part of Papua and the formation southward of a riverine system, including the Baliem River.

Species diversity and geographic distribution

Morphologically, *Cherax monticola* has a dark brownish color and a length of around 9-12 cm. Dorsal and ventral features of *C. monticola* from the Baliem River are shown in Figure 6. The list of previous geographic locations of *Cherax* sp. in Papua Island is presented in Table 1.

The sequence result of the freshwater lobster sample-01 and sample-02 from the Baliem River has been confirmed as *Cherax monticola* with accession No. PQ097958 and PQ097959 had been stored in the NCBI database. Matrix of genetic distance and phylogenetic tree analysis using sequence of ingroup family of Parastacidae sample-01, sample 02, and *C. monticola* NCBI, and seven new species *Cherax* sp., as most recently found by Lukhaup et al. (2025), and outgroup of the freshwater lobster *Macrobrachium rosenbergii* - family of Palaemonidae have resulted in two clades (Table 2). Both *C. monticola* and *C. quadricarinatus* have been found in one group of clade-II. Genetic distance among species is analyzed based on the values in Table 2. The genetic distance between sample-01 and sample-02 to *C. monticola* in the NCBI database is 0.01, and the genetic distance between *C. monticola* and *C. doberai* is 1.4570. The phylogenetic tree is shown in Figure 7.

Secondary supporting matrix of genetic distance had been generated using the ingroup family Parastacidae and outgroup of freshwater lobster *M. rosenbergii*, family of Palaemonidae as in Table 3. Genetic distance at the intraspecies level of sample-01 and sample-02 as *C. monticola*

is 0.019, interspecies genetic distance of *C. monticola* to *C. peknyi* is 0.17 and *C. monticola* to *C. quadricarinatus*, *C. alyciae*, *C. mosessalossa* is 0.18, and the highest genetic distance of *C. monticola* to *C. preissii* is 0.24. The phylogenetic tree resulted in eight clades (Figure 8): Clade

I, *C. crassimanus*; Clade II, *C. preissii*; Clade III, *C. tenuimanus*; Clade IV, *C. quadricarinatus*; Clade V, *C. alyciae*; Clade VI, *C. peknyi*; Clade VII, *C. monticola*; and Clade VIII, *C. mosessalossa*.

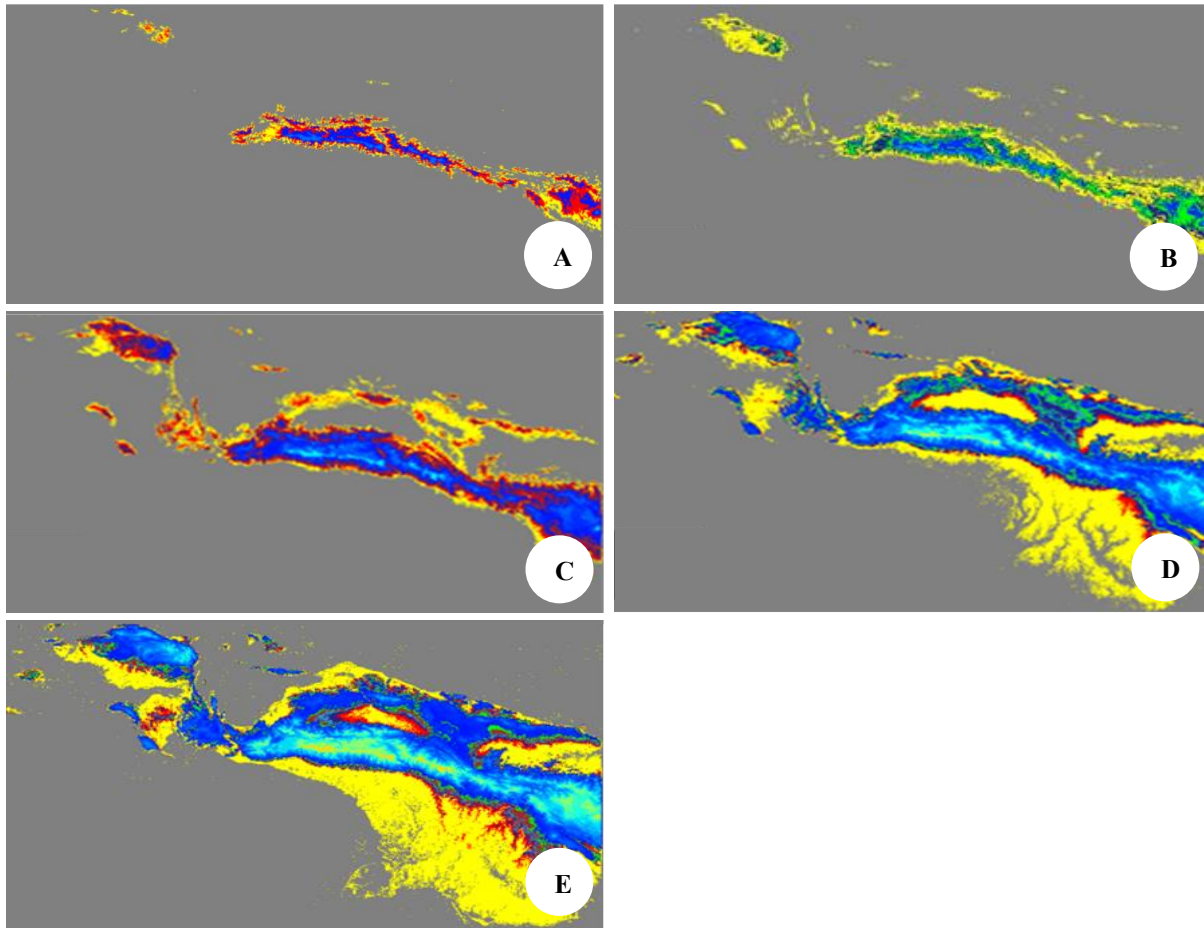


Figure 2. A-E. Five-phase geodynamic reconstruction using spectral classification of Digital Elevation Model (DEM-SRTM, Geodetic) data of the Pacific and Australian tectonic plate of West Papua, Indonesia, each representing of 10 Ma time period

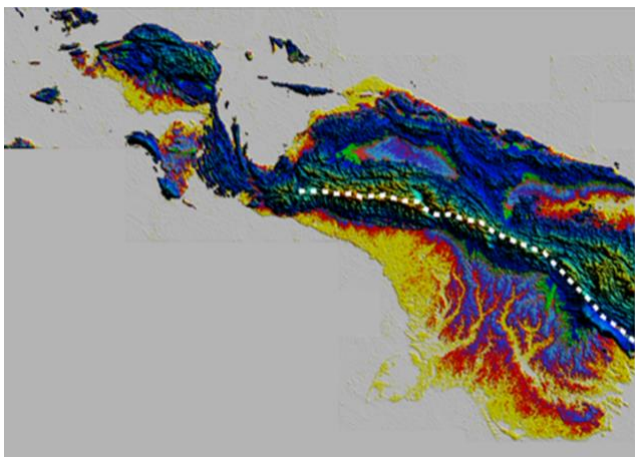


Figure 3. Geological fault, along Wamena Dome at Papua Island, Indonesia (DEM-SRTM, Geodetic)

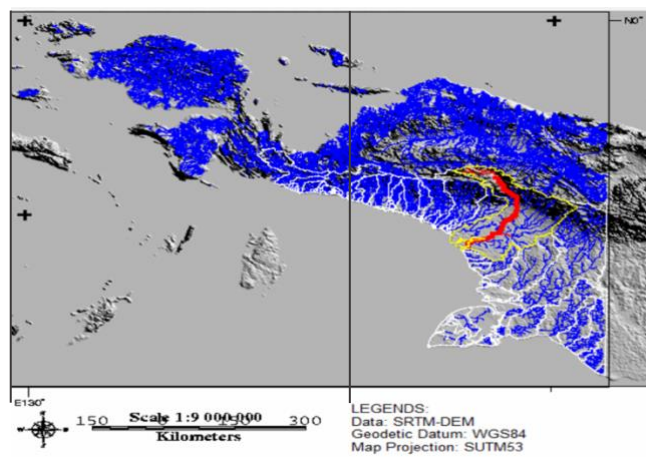


Figure 4. Southward riverine systems and the Baliem River (red line) overlaid on DEM-SRTM of the Wamena Dome, Papua Island, Indonesia

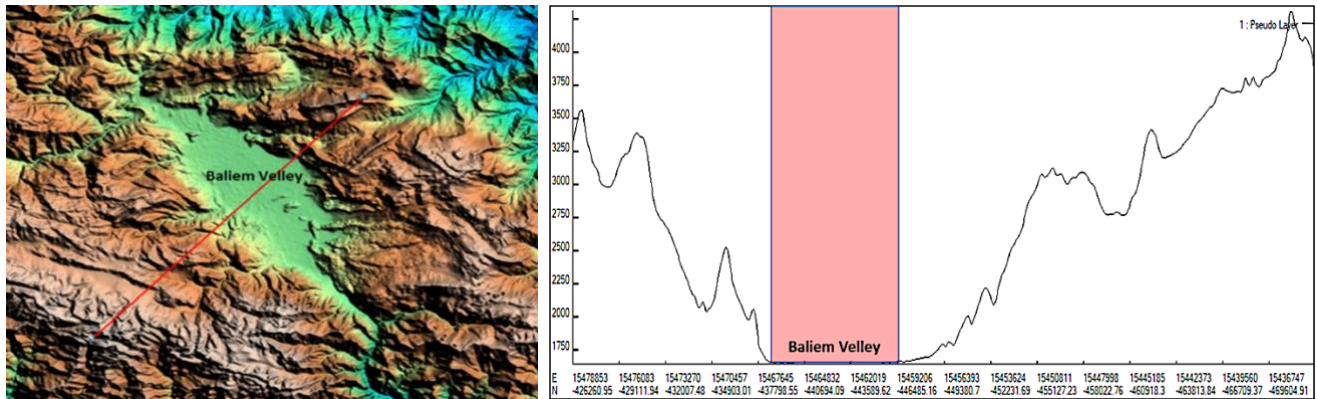


Figure 5. Two-dimensional and traverse spatial processing of DEM-SRTM data of the Baliem valley, as the beginning of the Baliem River at the Wamena Dome, Papua Island, Indonesia

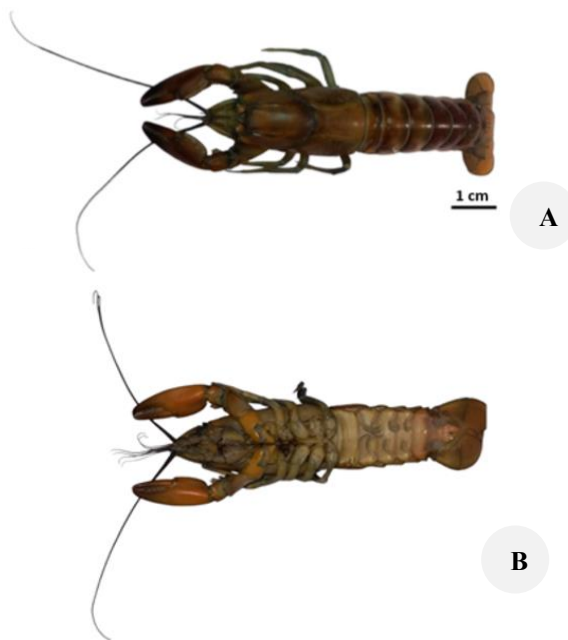


Figure 6. A. Dorsal, and B. Ventral features of *C. monticola* from the Baliem River, Wamena Dome, Papua, Indonesia

Discussion

Geodynamic

The fundamental concept in this reconstruction is that Wamena Dome serves as the junction of Australian Tethyan to Pacific plate tectonic, with its bottom seafloor uplift. This study discovered marine sands and fossils of Nautilidae as part of intra-oceanic subduction processes. The specific freshwater riverine system of the geodynamic processes proves that the southern part of Papua is part of the Australian Tethyan tectonic plates, with its riverine system flowing from the top of the Wamena Dome to the south coast of Papua. North Papua, also called as Melanesian Arc Terrane as part of the Pacific or Caroline Plate, and the southern part called as New Guinea Foreland as part of the Australian Plate (Sapiie et al. 2012; Zahirovic and Garrad 2020). The convergence process of three major

crustal Pacific, Eurasia, and Australian plates has resulted in a complex pattern of subduction zones, volcanic arcs, mountain ranges, and sedimentary basins (Simanjuntak and Barber 1996). Reconstruction begins in the middle Eocene, 50 Ma, late Eocene, 40 Ma, mid-Oligocene, 30 Ma, early Miocene, 20 Ma, late Miocene, 10 Ma, and continues to the present, with arc fragments and trapped ocean basins (McCaffrey et al. 1985; Zahirovic and Garrad 2020). The subducting oceanic slab from the more buoyant continental crust, located near the ocean-continent transition, induces a rebound of the under-thrusted continental margin (McCaffrey et al. 1985; Charlton 1997; Tandon et al. 2000).

The Lengguru wedge is younger than 10 Myr subduction setting in Western Papua of the Bird's Head as a part of the Australian margin beneath the Melanesian Arc. This rapid formation of this wedge is a Fold and Thrust Belt, which may be attributed to a single tectonic event, the core of the wedge in the Wandamen peninsula (François et al. 2016). Papua New Guinea has a complex geodynamic evolution as it is situated at the junction between the Tethyan and proto-Pacific tectonic domains (Zahirovic and Garrad 2020). Plate tectonic reconstruction since the Triassic for this region, evolving network of plate boundary topologies in 1 Myr intervals. This is part of the global plate tectonics dynamics and paleogeography of Southeast Asia (Golonka et al. 2006). The resemblance to the Australian margin forms the basis for the generally accepted idea that many islands of eastern Indonesia are the detached and displaced fragments of that margin. Papua contains an extensive Paleozoic basement that consists of clastic sediments and dolomite with occasional gabbroic intrusions (Dow et al. 1986). Formation of the transitional zone of Papua or Papuan Fold Belt (Dow 1977) was initiated during the collision of Australian Continent and Pacific Oceanic plates in 24 m.y Late Oligocene period. Baliem valley at the top of Wamena Dome belongs to Wamena District, Jayawijaya District of the Mountainous Papua Province, Indonesia, situated at 1,600-1,700 masl (Hall and Morley 2004).

Table 1. Distribution and geographic sites of 30 species of *Cherax* sp. found in Papua Island, Indonesia (Modified from Widyasari et al. 2021; Lukhaup et al. 2025)

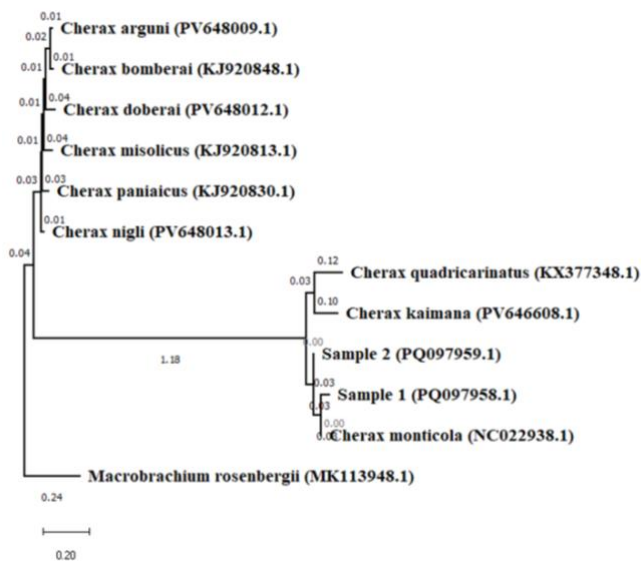
Species	Authors	Distribution	Coordinates
<i>C. albertisii</i>	(Blaha et al. 2016; Sugianti and Satria 2017)	Merauke	8°26'35.66" S; 140°28'2.10" E
<i>C. alyciae</i>	(Lukhaup et al. 2018; 2025)	Boven Digoel District	6°5'1.78"S; 140°17'38.56"E
<i>C. monticola</i>	(Holthuis 1956; Blaha et al. 2016; Gan et al. 2016; Hartoko et al. 2025)	Baliem River, Habbema Lake, Wamena	4° 8'47.9"S; 138° 58'30.89"E
<i>C. arguni</i>	(Lukhaup et al. 2025)	Kaimana District	3 35'14.88"S; 133 47'22.22"E
<i>C. kaimana</i>	(Lukhaup et al. 2025)	Kaimana District	3 35'14.88"S; 133 47'22.22"E
<i>C. minor</i>	(Holthuis 1996; Blaha et al. 2016)	Eragayam (ex. Wurigelebur) Jaya Wijaya District	3 40'3.15"S; 138 50'18.90"E
<i>C. boschmai</i>	(Holthuis 1949; Blaha et al. 2016; Crandall and De Grave 2017)	Paniai Lake	3°53'53.33"S; 136°19'4.50"E
<i>C. buitendijkae</i>	(Holthuis 1949; Crandall and De Grave 2017)	Paniai Lake	3°53'53.33"S; 136°19'4.50"E
<i>C. communis</i>	(Yogi et al. 2007; Blaha et al. 2016; Crandall and De Grave 2017)	Paniai Lake, Nabire District	3°53'53.33"S; 136°19'4.50"E
<i>C. paniaicus</i>	(Holthuis 1949; Franssen et al. 1997; Blaha et al. 2016)	Paniai Lake, Tage Tigi	3°53'53.33"S; 136°19'4.50"E
<i>C. lorentzi</i>	(Holthuis 1949; Holthuis 1956; Blaha et al. 2016; Crandall and De Grave 2017)	Painai Lake	3°53'53.33"S; 136°19'4.50"E
<i>C. destructor</i>	(Lekatompessy and da Costa 2019)	Tigi Lake, Deiyai District	4° 3'58.00"S; 136° 14'37.00"E
<i>C. longipes</i>	(Holthuis 1949; Blaha et al. 2016; Lekatompessy and da Costa, 2019)	Tigi Lake, Deiyai District,	4° 3'58.00"S; 136° 14'37.00"E
<i>C. murido</i>	(Holthuis 1949; Blaha et al. 2016; Lekatompessy and da Costa, 2019)	Tigi Lake, Deiyai District	4° 3'58.00"S; 136° 14'37.00"E
<i>C. quadricarinatus</i>	(Blaha et al. 2016; Lekatompessy and da Costa 2019)	Tigi Lake, Deiyai District	4° 3'58.00"S; 136° 14'37.00"E
<i>C. pallidus</i>	(Holthuis 1949; Blaha et al. 2016)	Tigi, Paniai, Wissel Lakes	3° 54'57.76"S; 136° 15'4.91"E
<i>C. solus</i>	(Lukhaup and Herbert 2008; Blaha et al. 2016)	Tigi Lake	4° 3'58.00"S; 136° 14'37.00"E
<i>C. gherardii</i>	(Patoka et al. 2015a; Blaha et al. 2016; Sedik et al. 2018)	Maybrat	1° 17'30.12"S; 132° 19'33.46"E
<i>C. holthuisi</i>	(Lukhaup and Pekny 2006; Blaha et al. 2016)	Aitinyo Lake, Uter	1° 25'58.43"S; 132° 23'15.73"E
<i>C. misolicus</i>	(Holthuis 1949; Blaha et al. 2016; Lukhaup et al. 2017)	Misool Island, Raja Ampat,	1° 54'37.86"S; 130° 10'42.07"E
<i>C. mosessalossa</i>	(Lukhaup et al. 2018)	Klademak Creek, Sorong	0°52'45.96"S; 131°16'22.44"E
<i>C. pulcher</i>	(Lukhaup 2015; Blaha et al. 2016)	Hoa Creek (Teminabuan), Sorong	0° 52'23.06"S; 131° 15'57.27"E
<i>C. snowden</i>	(Lukhaup et al. 2015; Lukhaup et al. 2018)	Oinsok (Ainsok River Drainage) and Sawiat Sub-district,	1° 8'58.34"S; 131° 56'39.37"E
<i>C. subterigneus</i>	(Patoka et al. 2015b; Blaha et al. 2016)	Aitinyo Lake	1° 25'58.43"S 132° 23'15.73"E
<i>C. warsamsonicus</i>	(Lukhaup et al. 2017)	Warsamson River, Sorong	1° 13'45.16"S; 131° 33'28.26"E
<i>C. bomberai</i>	(Lukhaup et al. 2025)	Fakfak District	2° 55'3.48"S; 132° 18'56.08"E
<i>C. doberai</i>	(Lukhaup et al. 2025)	Teluk Bintuni District	2°8'29.60"S; 133°52'8.99"E
<i>C. farhadii</i>	(Lukhaup et al. 2024)	Teluk Bintuni District	2°8'29.60"S; 133°52'8.99"E
<i>C. misolicus</i>	Eprilurahman et al. UnPublished	Misool Island	1°54'37.86"S; 130°10'42.07"E
<i>C. boesemani</i>	(Lukhaup and Pekny 2008; Blaha et al. 2016)	Amajaru Lakes, Kais River Drainage	1°16'29.31"S; 132°12'28.21"E

Table 2. Matrix of genetic distances using sequence sample-01, sample-02, *C. monticola* NCBI, and seven new *Cherax* sp. species found by Lukhaup et al. (2025), and the outgroup of *Macrobrachium rosenbergii*

Species	1	2	3	5	6	7	8	9	10	11	12
Sample 1											
Sample 2	0.04										
<i>Cherax monticola</i>	0.01	0.03									
<i>Cherax quadricarinatus</i>	0.19	0.16	0.18								
<i>Cherax kaimana</i>	0.21	0.21	0.20	0.22							
<i>Cherax nigli</i>	1.28	1.22	1.22	1.33	1.37						
<i>Cherax arguni</i>	1.28	1.22	1.22	1.35	1.33	0.07					
<i>Cherax paniaicus</i>	1.33	1.27	1.27	1.37	1.39	0.04	0.08				
<i>Cherax misolicus</i>	1.34	1.28	1.28	1.42	1.36	0.06	0.07	0.07			
<i>Cherax bomberai</i>	1.38	1.31	1.31	1.45	1.46	0.06	0.01	0.07	0.07		
<i>Cherax doberai</i>	1.45	1.37	1.37	1.53	1.45	0.05	0.07	0.06	0.07	0.06	
<i>Macrobrachium rosenbergii</i>	1.65	1.47	1.54	1.65	1.50	0.34	0.35	0.34	0.35	0.35	0.36

Table 3. Matrix result of genetic distances of *Cherax monticola* sample-01 and sample-02 with the other seven *Cherax* sp. and outgroup of *Macrobrachium rosenbergii*, with comparison to *Cherax* sp. sequence from the GenBank NCBI database

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
PQ0979581.1 Sample 1															
PQ097959.1. Sample 2	0.04														
NC022938.1 <i>C. monticola</i>	0.01	0.03													
AF493624.1 <i>C. crassimanus</i>	0.24	0.24	0.24												
AF493625.1 <i>C. crassimanus</i>	0.23	0.23	0.23	0.01											
KM039099.1 <i>C. crassimanus</i>	0.24	0.24	0.24	0.01	0.01										
AF493626.1 <i>C. tenuimanus</i>	0.22	0.23	0.22	0.13	0.12	0.13									
AF493627.1 <i>C. tenuimanus</i>	0.22	0.23	0.22	0.13	0.12	0.13	0.00								
AF493630.1 <i>C. tenuimanus</i>	0.22	0.22	0.22	0.12	0.12	0.13	0.00	0.00							
DQ006294.1 <i>C. quadricarinatus</i>	0.18	0.16	0.17	0.22	0.22	0.22	0.21	0.21	0.21						
MW591032.1 <i>C. quadricarinatus</i>	0.18	0.16	0.17	0.22	0.22	0.22	0.21	0.21	0.21	0.00	0.00				
KX377345.1 <i>C. quadricarinatus</i>	0.18	0.15	0.17	0.21	0.21	0.21	0.21	0.21	0.20	0.01	0.01	0.01			
EF118818.1 <i>C. preissii</i>	0.24	0.24	0.24	0.14	0.14	0.14	0.16	0.16	0.16	0.23	0.23	0.23	0.24		
EF118819.1 <i>C. preissii</i>	0.24	0.24	0.24	0.14	0.14	0.14	0.16	0.16	0.16	0.23	0.23	0.23	0.24	0.00	
EF11821.1 <i>C. preissii</i>	0.24	0.24	0.24	0.14	0.14	0.13	0.16	0.16	0.16	0.23	0.23	0.23	0.24	0.01	0.01
MH457597.1 <i>C. alyciae</i>	0.18	0.17	0.17	0.22	0.22	0.22	0.21	0.21	0.21	0.17	0.17	0.17	0.17	0.21	0.21
MH457598.1 <i>C. alyciae</i>	0.19	0.17	0.18	0.22	0.22	0.22	0.21	0.21	0.21	0.18	0.18	0.18	0.18	0.21	0.21
MH457599.1 <i>C. alyciae</i>	0.18	0.17	0.17	0.22	0.22	0.22	0.21	0.21	0.21	0.17	0.17	0.17	0.17	0.21	0.21
MH457600.1 <i>C. peknyi</i>	0.17	0.15	0.17	0.22	0.22	0.22	0.21	0.21	0.21	0.18	0.18	0.18	0.18	0.22	0.22
MH457604.1 <i>C. peknyi</i>	0.17	0.15	0.17	0.22	0.22	0.22	0.21	0.21	0.21	0.18	0.18	0.18	0.18	0.22	0.22
MH457601.1 <i>C. peknyi</i>	0.17	0.15	0.17	0.22	0.22	0.23	0.22	0.22	0.21	0.18	0.18	0.18	0.18	0.23	0.23
MH457602.1 <i>C. mosessalossa</i>	0.18	0.17	0.17	0.22	0.22	0.22	0.22	0.22	0.22	0.18	0.18	0.18	0.17	0.25	0.25
MH457603.1 <i>C. mosessalossa</i>	0.18	0.17	0.17	0.22	0.22	0.22	0.22	0.22	0.22	0.18	0.18	0.18	0.17	0.25	0.25
MK113948.1 <i>M. rosenbergii</i>	0.65	0.62	0.63	0.72	0.71	0.72	0.66	0.66	0.66	0.71	0.71	0.71	0.69	0.75	0.75

**Figure 7.** Phylogenetic tree of *Cherax monticola* (Parastacidae) from the Baliem River, Wamena, to the 7 new species found by Lukhaup et al. (2025) from Papua, with outgroup of *M. rosenbergii* (Palaemonidae)

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The molecular characterization confirmed that the two freshwater lobster samples from the Baliem River are *C. monticola*; the first phylogenetic analysis using the sequence of *Cherax* sp. Family Parastacidae from this study was compared to other sequences from the GeneBank, and the outgroup of other freshwater lobster *M. rosenbergii* - family Palaemonidae. The second phylogenetic analysis using *Cherax* sp. - family Parastacidae to outgroup of *Liopenaeus vannamei* - family Penaeidae have revealed III clades. In which *C. monticola* from Baliem River is present

in clade-III along with the Australian *C. quadricarinatus*. This suggests a conceptual origin from the same tectonic plate as Australia. This study confirmed the close genetic distance analysis of *C. monticola* to *C. quadricarinatus*, *C. mosessalossa*, *C. peknyi*, and *C. alyciae* with 0.177 (Table 2 and Figure 7).

Four nucleotides of *C. monticola* from the sample-01 and 02 from this study, GeneBank of PQ097958.1, and *C. quadricarinatus* MW591032.1 were compared to analyze the DNA-sequence length to obtain percentage nucleotide composition (Table 5). Alignment based on the mitochondrial COI gene sequence, and calculate the number of C, A, G, and T in the Timin RNA represented by Urasil with T(U) code (Reunov et al. 2021; Raupach et al. 2022). Fragment lengths of the analyzed nucleotides of *C. monticola* in this study ranged from 647 to 665 bp (Table 4). Intraspecific genetic distance of sample *C. monticola* and GeneBank is 589 to 656 bp, interspecific genetic distance of *C. monticola* and *C. quadricarinatus* is 656 to 658 bp (Table 4).

The example genome of *C. monticola* from Papua New Guinea mitogenome consists of 15,917 base pairs containing 13 protein-coding genes, 2 ribosomal subunit genes, 22 transfer RNAs, and a non-coding AT-rich region. The base composition of *C. monticola* is 33.46% for T, 21.48% for C, 33.71% for A, and 11.35% for G, with an AT bias of 67.17% (Gan et al. 2014). The molecular phylogeny based on a mitochondrial gene fragment, 16S, supports the morphology-based description of the seven new species, which can also be clearly distinguished by sequence differences (Lukhaup et al. 2025). Nucleotide composition analysis found that *C. monticola* from this study consists of T(U) 32.1%, C 21.7%, A 27.2% and G 19%. Meanwhile, *C. quadricarinatus* has T(U) 30.6%, C 24.6%, A 26.7% and G 18.2% (Table 4).

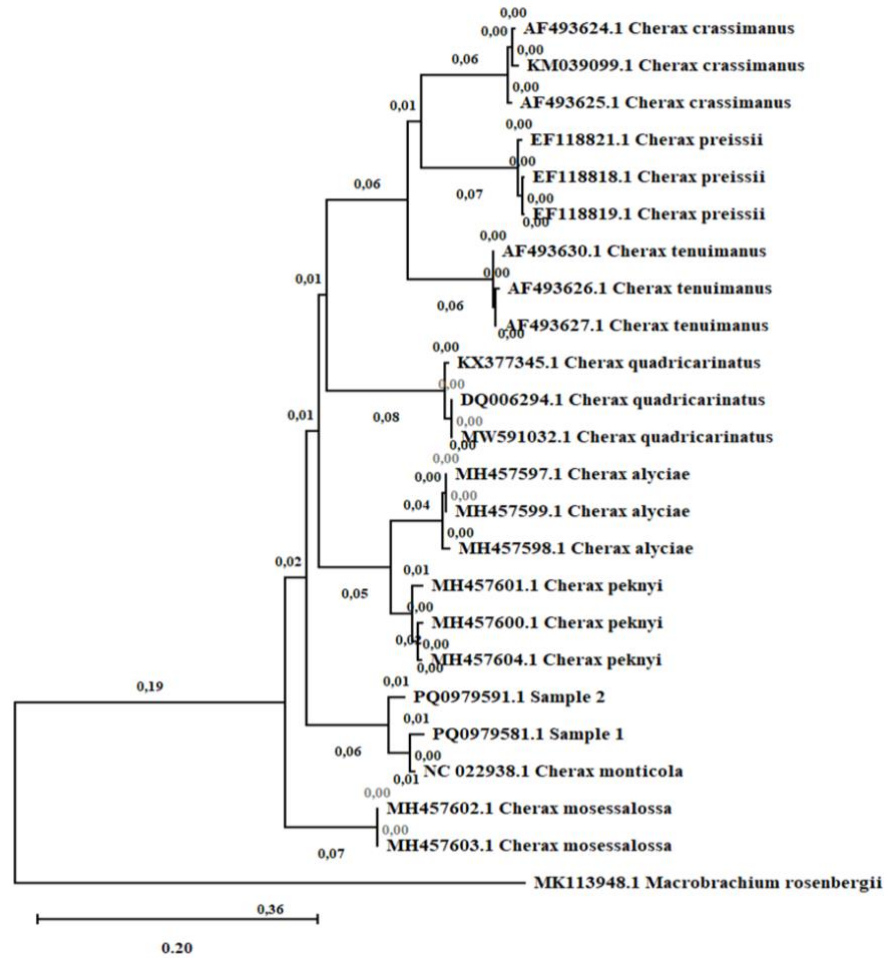


Figure 8. Phylogenetic tree of *Cherax monticola* sample-01 and sample-02 (family Parastacidae) from Baliem River Wamena Dome to other *Cherax* sp. found in Papua Island and outgroup of *Macrobrachium rosenbergii* (family Palaemonidae)

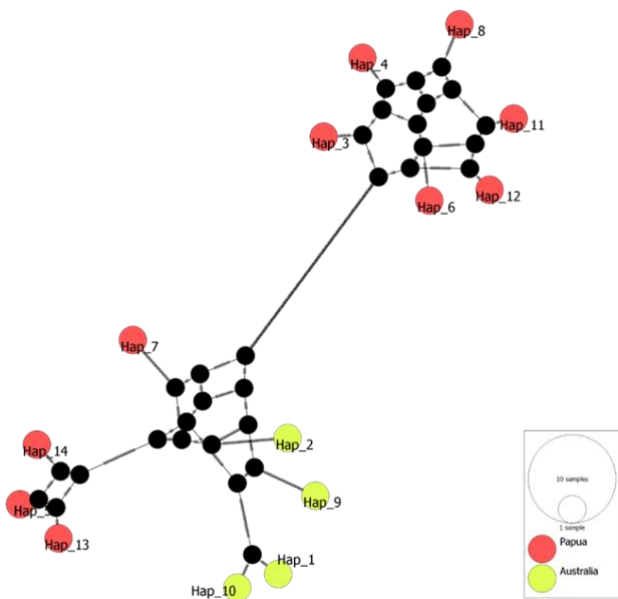


Figure 9. Haplotype network analysis of *Cherax* sp., origin of Papua and Australia

The molecular characterization used in the study, using a similar method used by the latest study by Lukhaup et al. (2025) had found seven new species of *Cherax* sp. from Papua New Guinea, with the approach of molecular phylogeny based on a mitochondrial gene fragment 16S supports the morphology-based description of the seven new species, whose sequence differences can be clearly distinguished (He et al. 2011; Eprilurahman et al. 2021; Tamura et al. 2021; Lukhaup et al. 2025). Molecular genetic advancement of *Cherax* sp. with a newly developed nucleotide microsatellite marker of AC, GT, CT (Dali et al. 2023; Wei et al. 2024).

This study confirmed the genetic uniformity based on comparison of sequence 656 to 658 bp of the south Papua *C. monticola* and the Australian-originated *C. quadricarinatus* (Table 4). This finding is supported by the verification of Haubrock et al. (2021) that *C. quadricarinatus* is a freshwater lobster found in the freshwater rivers of the tropical Australian Northern Territory and Papua Island. Earlier study of Lukhaup et al. (2017), which discovered the species of *C. warsamsonicus* at Warsamson River and is closely related to *C. misolicus*, *C. mosessalossa* at Klademak River, Sorong, *C. peknyi*, *C. alyciae* at Papua New Guinea, *C. tenuimanus*, and *C. preissii* originated from Australia.

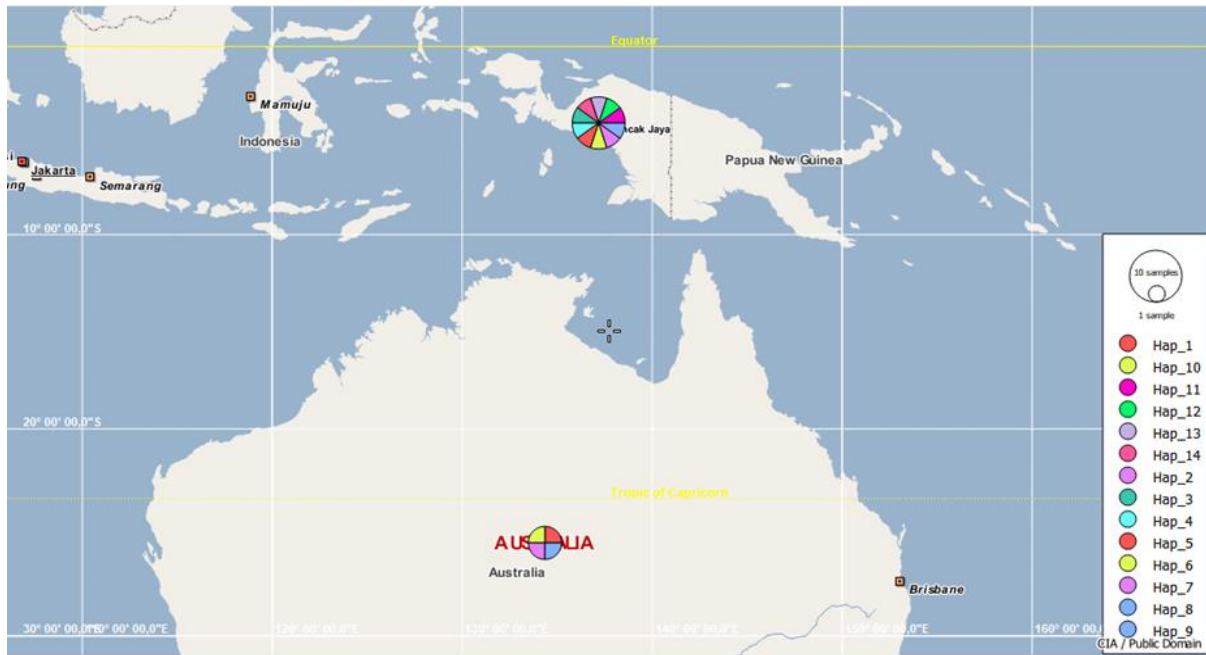


Figure 10. Map of *Cherax* sp. geographical origin based on the sequence haplotype network

Table 4. Comparison of sequence 589 bp *Cherax monticola* to *Cherax quadricarinatus* (uniformity-%)

Sample code	Sequence length (bp)	T(U)	C	A	G
Sample-01	665	32.1	21.7	27.2	19.0
Sample-02	647	32.4	21.9	27.7	18.0
PQ097958.1 <i>C. monticola</i> voucher-01	589	32.1	21.7	27.2	19.0
MW591032.1. <i>C. quadricarinatus</i>	658	30.6	24.6	26.7	18.2

Table 5. Sequence of *Cherax* sp. used for haplotype network analysis (Green-Australian; Red-Papua)

Code	Species
Hap-1	<i>Cherax tenuimanus</i> (AF493630.1)
Hap-2	<i>Cherax quadricarinatus</i> (KX377348.1)
Hap-3	<i>Cherax paniaicus</i> (KJ920830.1)
Hap-4	<i>Cherax nigli</i> (PV648013.1)
Hap-5	<i>Cherax monticola</i> (NC022938.1)
Hap-6	<i>Cherax misolicus</i> (KJ920813.1)
Hap-7	<i>Cherax kaimana</i> (PV646608.1)
Hap-8	<i>Cherax doberai</i> (PV648012.1)
Hap-9	<i>Cherax destructor</i> (MW220065.1)
Hap-10	<i>Cherax cainii</i> (KM039098.1)
Hap-11	<i>Cherax bomberai</i> (KJ920848.1)
Hap-12	<i>Cherax arguni</i> (PV648009.1)
Hap-13	Sample 1 (PQ079758.1)
Hap-14	Sample 2 (PQ079759.1)

The sample of *C. monticola* of this study was collected from the Baliem River at the top hill of the Wamena Dome, which flows southward, confirming the reconstruction

during the formation of Papua Island (Figures 1, 2, and 3). In general, *C. monticola* inhabits high mountainous river areas, sheltering in sand and volcanic boulders. Geographic analysis by Abinawanto et al. (2018) found that the most distant genetic relatedness was observed between the Uter Lake population and the Baliem River, Pike Village at Jayawijaya District population. Conversely, the closest genetic distance is found between the Baliem River, Wesaput Village of Jayawijaya District population and Baliem River-Pike Village, Jayawijaya District (Abinawanto et al. 2018). Geographic factors caused the populations to become isolated and may form different characters, the tight relatedness between groups is indicated by an inter-sample allusion or overlapping. Also, the distribution of Uter Lake and Seremuk River populations was closely related. Still, the population of the Baliem River-Pike Village, Jayawijaya District, and Baliem River, Wesaput Village, Jayawijaya District were distantly related (Abinawanto et al. 2018). Freshwater lobster *C. monticola* is a species that belongs to the family of Parasticidae found in the Baliem River and Wisel Lake at the southern part, 1,600-2,000 masl, mountainous area of Papua Island (Abinawanto et al. 2018).

Baliem River is the main river at the Baliem Dome, with winding, meandering flows, to the south, with several waterfalls and a steep canyon with natural embankment. To the south of the Baliem River meet to Uwe and the Wamena River. Bottom sediments consist of calcareous and volcanic sands, fine sands. Baliem River belongs to Jayawijaya District, South Papua Island. *C. monticola* in the Baliem river can reach a maximum weight of 800g, much larger than those in Australia (Kawai and Patoka 2021). Ecological parameters measurement at freshwater Baliem River from this study found the river water temperature in the range of 14-18°C, pH 6.8-7.5, and dissolved oxygen 4.1-4.3 mg L⁻¹, down-hill river current

0.09-0.92 m.sec⁻¹, water transparency 17-160 cm, and depth of 0.7-3.7m. Water temperature range at Tigi Lake is 23-25°C, and pH range of 6-7 (Abinawanto et al. 2018). The optimum growth of *Cherax* sp., in a temperature range of 21-29°C, and a variety of protein feed of 14-39% and the optimum water parameters for suitable growth of *Cherax* sp. with dissolved oxygen above 1 mg L⁻¹, alkalinity range 20-300 mg L⁻¹, and pH 6.5-9.0. (Taufiq et al. 2017; Timumun et al. 2022; Asnawi et al. 2023; Safir et al. 2023; Tarina et al. 2023; Miptah et al. 2024). Low or high river water temperature will disrupt the growth, and they will tend to be submerged in the sediment and less active.

Haplotype network analysis based on four *Cherax* sp., the sequence of Australian *C. tenuimanus* (AF493630.1), *C. quadricarinatus* (KX377348.1), *C. destructor* (MW220065.1), *C. cainii* (KM039098.1), and ten species from Papua origin was constructed using PopArt software with Integer Neighbor-Joining Net method (Table 5, Figure 9, Figure 10). Four species of Australian *Cherax* sp. are known to originate mainly from the Northern Territory and Western Australia (Prastowo et al. 2020).

Morphologically, *C. monticola* from Baliem River in this study is small, with a length of 10 cm, black color on the dorsal part, and light brown color on the ventral part. Meanwhile, *C. quadricarinatus* is relatively large, exhibiting a bluish green color and a reddish tinge. Adult males display a bright reddish color on their outer main chelae. Gill morphology and formulae of this family had been described by Kawai and Patoka (2021). This species has four longer carinae at the dorsal cephalon and can reach a maximum length of 25 cm and a weight of 600 g; the length-weight relationship of *C. quadricarinatus* from Paniai Lake exhibits a negative growth pattern (Tampubolon and Maitindom 2023). At present, *C. quadricarinatus* has been found spread all over the aquaculture sites of Malaysia (Naquiddin et al. 2016). *C. quadricarinatus* mainly originates and is well distributed in the northern part of Australia (Haubrock et al. 2021).

Other organisms, such as fish, also live in the Baliem River; the river is locally-wisdom considered an important biome, has a high economic value, and is used for daily bathing and washing. Freshwater lobster *C. monticola* is locally called 'huna', has a high local wisdom value, as also sweet-potato locally called "hipiri", and pig locally known as "wam". The local community hopes to develop the use of the Baliem River and *Cherax* sp. for sustainable natural resources and eco-tourism. The database from this study was used as an applied scientific base for the need of the nucleus conservation zone development, a step-wise local wisdom-based community socialization for mindset shifting from traditional catch to develop freshwater culture of each 30 species existence in each river site.

In conclusion, this study has presented a new conceptual framework for understanding the species endemism, based on the tectonic geodynamic reconstruction of Papua Island and geographical habitat characters. Spatial geodynamics revealed the reconstruction of the collision process using the DEM data of the two tectonic plates of the Pacific in the north and the Australian in the south, leading to the formation of Papua Island. The uplift of the ocean tectonic

edge follows the collision process to form the highland mountainous zones along the Wamena Dome. This highland along the Wamena Dome with the southward slopes at the south of Papua Island then generates the southward riverine system. The database of the earlier study revealed the genetic biodiversity, in which most of the 30 species of the freshwater lobster *Cherax* sp. were found in 25 southward river flows and lakes on South Papua Island. This fact has confirmed the endemism of *Cherax* sp. due to the geographical riverine system. COI-molecular analysis on the sample collected from the southward Baliem River found *C. monticola* as the representative of South Papua. Following the genetic distance, phylogenetic tree, and haplotype network analysis, it is confirmed that *C. monticola*, originating from Papua, and *C. quadricarinatus*, of Australian origin, both belong to the same clade. This evidence concludes that *Cherax* sp., in the southern part of Papua Island, is found in one group with the Australian representative and originated from one tectonic plate, and provides a new paradigm to illustrate the geodynamic process that underpins organismal endemism. The genetic biodiversity richness of *Cherax* sp. in the southern part of Papua Island provides a scientific basis for conservation, resource management, and cultural development, leading to sustainable income for local communities.

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