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Research Article

The Application of Amino Acid Racemization Geochronology of *Tubipora* sp. in Marine Terraces of Manokwari Region, West Papua, Indonesia

Rahmadi Hidayat[1]*, Sukahar Eka Adi Saputra[2], Salahuddin Husein[1]
1)Department of Geological Engineering, Faculty of Engineering, Universitas Gadjah Mada, Yogyakarta, 55281, Indonesia.
2)Center for Geological Survey, Geological Agency of Indonesia, Bandung, 40122, Indonesia
* Corresponding author, email: rahmadihidayat@ugm.ac.id

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ABSTRACT
The active neotectonics of northern West Papuan coastlines allow the formation of emergent marine terraces associated with Quaternary sea-level high stands. These terraces contain fossils from the coral assemblage, which are useful for geochronological assessments and further estimating uplift rates. Here, we report the applicability of amino acid racemization (AAR) of *Tubipora* sp. to discriminate different ages associated with stages of sea-level high stand, constrained by previous uranium-thorium (U/Th) series dating. The results from amino acid dating of three samples reveal two distinct extents of racemization corresponding to terraces developed during Marine Isotope Stage (MIS) 5 *sensu lato* and 1. However, AAR analysis could not further discriminate interstadial MIS 5a and 5c as determined by published radiometric dating. This indicates the low resolving power of amino acid dating to distinguish sub-sequences beyond the interglacial period. Nevertheless, the cost-effective and rapid analysis of AAR dating of *Tubipora* sp. can be used as preliminary results related to marine terraces formed in different interglacial events.

INTRODUCTION
The emergent coral reef terraces have been widely studied to infer the uplift rate of tectonically active sites such as Barbados (Bard et al. 1990; Schellmann & Radtke 2004), Haiti (Dodge et al. 1983; Dumas et al. 2006), and Huon Peninsula (Bloom et al. 1974). The crucial parameter for estimating the degree of uplift is a robust chronological method to assign palaeosea-level elevation based on calcium carbonate-bearing samples, mostly corals (Tomiak et al. 2016). In most cases, uranium-thorium (U/Th) dating has been applied to evaluate the age of common fossil corals inhabiting near water surfaces as a suitable indicator of the mean sea-level (Hibbert et al. 2016). In principle, U/Th geochronology is a radiometric dating technique focusing on the decay activity of Uranium-234 as parent to Thorium-230 as the daughter isotope contained in the calcium-carbonate fossils. Unlike uranium, thorium is characteristically insoluble in marine water; and thus, in an ideal environment, the abundance of Thorium-230 is considered the main result of radioactive decay from the coral skeleton (Chen et al. 2020). Moreover, the typically closed-system nature of corals, having a limited exchange of both

thorium and uranium uptakes associated with the environment, provides high age resolution and precision determined from the U/Th technique (Chutcharavan & Dutton 2020).

Despite its suitability in coral assemblages, some marine terrace sites present a challenge in establishing U/Th dating due to the introduction of reworked materials from older successions. The occurrence of reworked corals has been identified in several areas within the Pacific coastlines of the US due to the combined effect of vertical displacement and glacial-isostatic adjustment associated with the waxing and waning of the ice sheet (Muhs et al. 2012; Simms et al. 2016). In this case, the age assessment should be conducted carefully, especially when selecting samples that represent the actual age of coral reef terraces. This could be achieved by dating large subsets of coral to determine and remove the reworked fossils, although it may take longer time to complete and be considered costly.

In this case, an alternative dating method of fossils that is able to perform in high-density samples, such as amino acid racemization (AAR), is important to complement the primary geochronological tool. As opposed to U/Th dating, AAR focuses on measuring the degree of protein degradation by determining the ratio between left- and right-handed amino acids. In principle, the concentration of right-handed amino acids began to build up after the death of organisms, so the increasing ratio of left- and right-handed amino acids, namely DL ratios, are proportional to the longer time since death (Demarchi 2020). The current technology of reverse-phase high-performance liquid chromatography (RP-HPLC) and its detector has improved the capability of large amounts of analytical measurement, 2 hours each, with relatively small-sized fossils (Kaufman & Manley 1998; Hidayat et al. 2024). For example, Hidayat et al. (2023) have conducted as many as 472 analyses of individual minuscule fossils of foraminifera (~ 0.5 mm) to understand the nature of reworking in the development of carbonate-rich coastal deposits.

U/Th dating has been utilized to analyze fossil coral embedded in marine terraces to investigate the neotectonics of the Manokwari region, West Papua (Saputra et al. 2022). To determine the uplift rate in this area, Saputra et al. (2022) suggested using soft coral *Tubipora* sp. as specimens for dating. *Tubipora* sp. colonies are widely distributed across the west of Pacific, south of Japan, and the Red Sea, typically inhabiting shallow waters up to 20 meters deep (Ammar 2005). Alongside other coral assemblages such as *Acropora* and *Porites*, *Tubipora* sp. is commonly found in sheltered, low-energy zones rather than fore-reef environments (Manaa et al. 2016). The distinctive organ pipe-like structures and deep red coloration of this species are due to carotenoids in their aragonite skeleton (Ammar 2005). Given its specific habitat requirements, the abundance of *Tubipora* sp. is pertinent for conducting geochronological reconstructions to model vertical ground movements over geological timeframes (Saputra et al. 2022). Despite these valuable attributes, the application of amino acid racemization (AAR) for this species is currently limited, particularly in distinguishing ages related to sea-level highstands.

Here, we investigate the application of amino acid geochronology of fossil soft corals *Tubipora* sp. to determine different ages associated with interglacial sea-levels with references from previous U/Th dating from Saputra et al. (2022). The rapid and cost-effective AAR method for age determination may be valuable to provide large datasets to identify the potential age mixing or reworked fossils and further complement a more

expensive and time-consuming dating method, for example, U/Th series, in selecting the most appropriate sample set to analyze.

REGIONAL SETTING

Manokwari area is geologically situated in the Bird's Head terrane of West Papua (Figure 1), a region considered tectonically active due to oblique convergence of major tectonic plates (Pacific, Caroline and Australia) as evidenced by regional strike-slip faults such as Sorong, Yapen, and Ransiki (Gold et al. 2014). This intense neotectonic setting allows emergent Quaternary coral reef terraces to occur >150 m above the present sea-level (APSL), showing well-developed landforms with northwest-southeast directed escarpments (Saputra & Fergusson 2023). These terraces are characterized by massive and thick reefal limestone comprising calcirudite, calcarenite, conglomerate, and carbonate-rich breccia (Robinson & Ratman 1978). Saputra et al. (2022) documented varying uplift rates of these marine successions of up to 1.28 meter/kilo annum (m/ka) based on U/Th chronology from fossil corals, mainly *Porites* sp., coralline algae, and *Tubipora* sp. The latter specimen is the focus of conducting amino acid dating in this study.

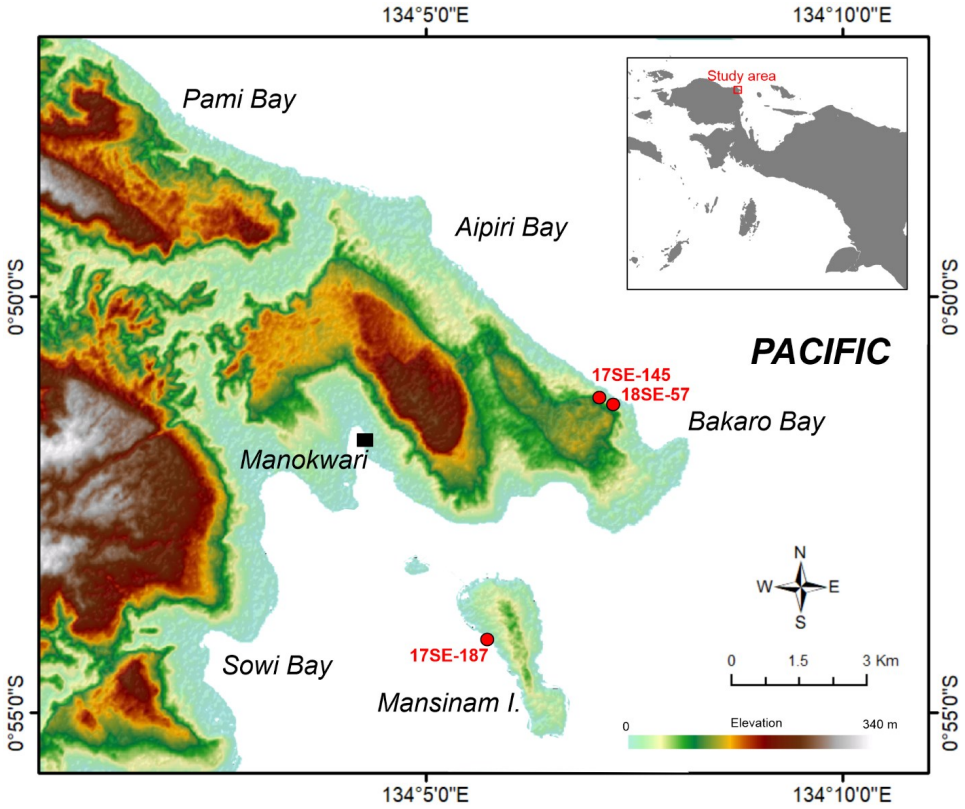

Figure 1. Location of sampling sites within Manokwari region; and an inset of the western part of New Guinea (a territory of Indonesia). The topographic dataset and inset map were retrieved from the Digital Elevation Model Nasional from Badan Informasi Geospasial (2018, https://tanahair.indonesia.go.id/demnas/#/).

MATERIALS AND METHODS

Sampling sites

Three samples previously collected by Saputra et al. (2022) from two different sites within the Manokwari region were used in this study (Figure 1). In Mansinam Island, a sample (coded 17SE-187, Figure 2a) was retrieved from a 150 m wide raised coral reef terrace at an elevation of 3 m APSL. Two remaining *Tubipora* sp. specimens were collected from the terraces of Bakaro Bay (Figure 1), around 6 km north of Mansinam

Island. In more detail, a sample (coded 17SE-145) was obtained from Suweni Terrace, around 77.5 m APSL (Figure 2b), typically comprising a back reef to reef crest facies (Saputra et al. 2022). To the southeast (~120 m), a sedimentary unit consisting of abundant *Tubipora* sp. (18SE-57) was sampled from different coral reef terrace, namely Suweni Dua, with an elevation of 62.5 m APSL (Figure 2c). The characteristics of this faunal assemblage are mostly preserved on the terraces of Bakaro Bay, exhibiting distinctive and unique organ pipe structures filled with sand-sized carbonate particles (Figure 2d).

Amino acid racemization

A suite of *Tubipora* sp. samples, 100 mg each, were initially cleaned with a brush and further immersed in a glass beaker with distilled water and sonicated for 10 min. Subsequently, stoichiometric acid etch (2 mol/L HCl) was applied to remove the outer surface with a potential diagenetic alteration. Following this, samples were treated using a 3% hydrogen peroxide (H_2O_2) solution, rinsed with distilled water five times and air-dried overnight. The dried samples were placed in designated glass tubes, demineralized with 8 mol/L HCl according to the remaining mass, filled with a stream of nitrogen gas and finally sealed. The small amount (50 µL) of samples were transferred to vials for analysis of the free amino acid (FAA) fraction prior to sealing, whereas to recover the free and total bound state of peptides, termed total hydrolyzable amino acids (THAA), the remaining solutions within glass tubes were hydrolyzed using an

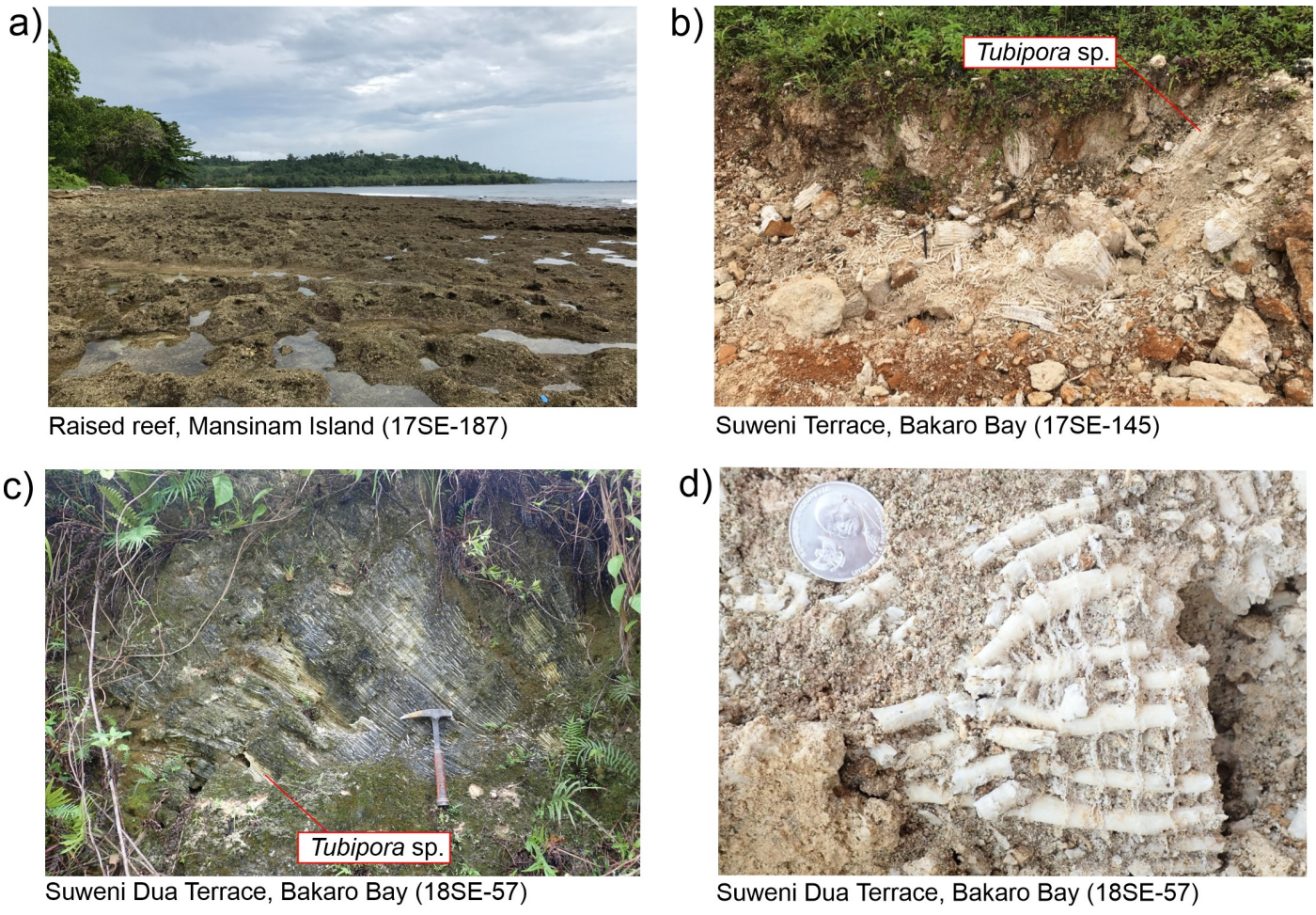

Figure 2. a) Coastal landscape of Mansinam Island (17SE-187) showing raised reef unit close to the present-day sea-level. b) Marine terrace with colonies of *Tubipora* sp. (17SE-145) at Suweni Terrace, Bakaro Bay. c) Organ pipe structures dominate the terrace outcrop of Suweni Dua, Bakaro Bay. d) Close-up of *Tubipora* sp. retrieved from Suweni Dua terrace.

oven for 22 h at 110 °C. After that, the solutions were placed in separate vials and evaporated to dryness via a vacuum desiccator. Finally, all samples were rehydrated using internal standard L-*Homo*-Argenine to estimate amino acid concentration.

The analytical procedure for RP-HPLC generally follows Kaufman and Manley (1998). Pre-column derivatization was performed using ophthaldialdehyde (OPA) and the chiral thiol N-isobutyryl-L-cysteine (IBLC). The instrument used to calculate the extent of racemization (defined by the ratio of D- and L-amino acids or DL ratio) was an Agilent 1100 equipped with a Hypersil BDS C18 column and a fluorescence detector, with mixture of solvents consisting of methanol, aqueous sodium acetate with minor ethylene-diamine-tetraacetic acid (EDTA), sodium azide, and acetonitrile. Inter-laboratory standards (ILC A to C) were used to monitor the instrument's analytical uncertainty. Four amino acids were selected for this study: aspartic acid (ASX), glutamic acid (GLX), valine (VAL), and isoleucine epimerization (A/I), as shown in Table 1. The total abundance of amino acids was calculated based on these three selected amino acids combined with serine (SER), glycine (GLY), alanine (ALA), phenylalanine (PHE), and isoleucine (ILE), as shown in Table 2.

RESULTS

A *Tubipora* sp. from exposed reef outcrop at Mansinam Island (17SE-187) showed the lowest racemization degree compared to other

Table 1. Results of the extent of racemization from *Tubipora* sp. in the Manokwari region's marine terraces with respective U/Th age from *Saputra et al. (2022). Both analyses of total hydrolyzable and free amino acids (in italics) are presented. UWGA: University of Wollongong sample code. ASX: aspartic acid, GLX: glutamic acid, VAL: valine and A/I: isoleucine epimerization.

Location	Code	UWGA (replicates)	U/Th age* (ka)	ASX	GLX	VAL	A/I
Mansinam I.	17SE-187	11407 (4)	2.7 ± 0.3	0.552 ± 0.005 *0.715 ± 0.005*	0.278 ± 0.013 *0.487 ± 0.033*	0.256 ± 0.023 *0.455 ± 0.023*	0.555 ± 0.033 *0.755 ± 0.083*
Suweni, Bakaro Bay	17SE-145	11404 (4)	90 ± 5.0	0.779 ± 0.008 *0.910 ± 0.008*	0.551 ± 0.010 *0.680 ± 0.008*	0.522 ± 0.021 *0.858 ± 0.059*	0.738 ± 0.029 *1.087 ± 0.076*
Suweni Dua, Bakaro Bay	18SE-57	11403 (4)	102 ± 1.5	0.765 ± 0.008 *0.910 ± 0.004*	0.544 ± 0.005 *0.665 ± 0.020*	0.503 ± 0.011 *0.834 ± 0.066*	0.705 ± 0.030 *0.947 ± 0.049*

Table 2. Results of the amino acid concentrations of *Tubipora* sp. in marine terraces of Manokwari region with respective U/Th age from *Saputra et al. (2022). Both analyses of total hydrolyzable and free amino acids (in italics) are presented. UWGA: University of Wollongong sample code. ASX: aspartic acid, GLX: glutamic acid, VAL: valine and ILE: isoleucine. Total amino acid concentrations are measured from aspartic acid, glutamic acid, valine, serine, glycine, alanine, phenylalanine and isoleucine.

Location	Code	UWGA (replicates)	U/Th age* (ka)	ASX (pmol/mg)	GLX (pmol/mg)	VAL (pmol/mg)	ILE (pmol/mg)	Total (pmol/mg)
Mansinam I.	17SE-187	11407 (4)	2.7 ± 0.3	972.2 ± 4.3 *50.6 ± 14.9*	608.6 ± 2.7 *23.2 ± 1.6*	470.7 ± 0.4 *12.8 ± 1.3*	328.6 ± 1.2 *6.0 ± 0.9*	4429.0 ± 1.2 *216.5 ± 22.7*
Suweni, Bakaro Bay	17SE-145	11404 (4)	90 ± 5.0	392.2 ± 7.8 *178.9 ± 0.6*	151.6 ± 7.0 *25.9 ± 0.4*	93.0 ± 8.6 *35.9 ± 1.0*	50.6 ± 1.2 *13.1 ± 0.4*	1152.4 ± 1.2 *473.3 ± 8.3*
Suweni Dua, Bakaro Bay	18SE-57	11403 (4)	102 ± 1.5	430.8 ± 6.1 *183.2 ± 1.4*	104.7 ± 1.8 *25.6 ± 0.2*	44.2 ± 0.7 *40.9 ± 2.1*	23.7 ± 0.8 *21.7 ± 0.8*	894.6 ± 0.8 *501.9 ± 8.8*

specimens with ASX and GLX DL ratios 0.552 ± 0.005 and 0.278 ± 0.013 respectively for THAA (Figure 3a). The AAR results based on THAA show a lower extent of racemization than FAA, which is expected as a result of the introduction of low amino acid racemization within bound state peptides that were broken down via hydrolysis (Wehmiller 1993) by exposing them to a temperature of 110 °C for 22 h. In this case, the results from FAA of *Tubipora* sp. at Mansinam Island (17SE-187) exhibit ASX and GLX DL ratios of 0.715 ± 0.005 and 0.478 ± 0.033 (Figure 3b). Similarly, these values are lower compared with two specimens obtained from Bakaro Bay.

The remaining samples collected from Bakaro Bay yielded a distinctly greater extent of ASX racemization at 0.779 ± 0.008 (17SE-145) and 0.765 ± 0.005 (18SE-57) for THAA (Figure 3a). Regarding GLX, the AAR results from Bakaro Bay samples consistently showed higher DL ratios at roughly twice that of the sample from Mansinam Island at 0.551 ± 0.010 (17SE-145) and 0.544 ± 0.005 (18SE-57) for THAA. The racemization extent of VAL from these specimens is also roughly twice greater than *Tubipora* sp. from Mansinam Island. The FAA analyses for two specimens of Bakaro Bay yielded almost racemic for ASX (~0.910), whereas for GLX, the DL ratios are within the range of 0.665 – 0.680 (Figure 3b).

Similarly, the ASX and GLX amino acid concentrations in the Mansinam Island sample (17SE-187) indicate limited protein degradation, with the highest values at 972.2 ± 4.3 pmol/mg and 608.6 ± 1.5 pmol/mg for THAA, respectively (Figure 4a). Overall, the sample from Mansinam Island yielded about 4429 ± 17.7 pmol/mg of measured THAA abundance. In contrast, two *Tubipora* sp. specimens from Bakaro Bay displayed significantly lower total amino acid abundance, approximately one-fourth of the sample from Mansinam Island. The ASX concentration in the Bakaro Bay samples ranged from 430 to 392 pmol/mg, while GLX contents were around 104 to 151 pmol/mg. For total amino acid abundance detected from the FAA pool, *Tubipora* sp. from Bakaro Bay yielded higher values (Figure 4b), particularly for ASX,

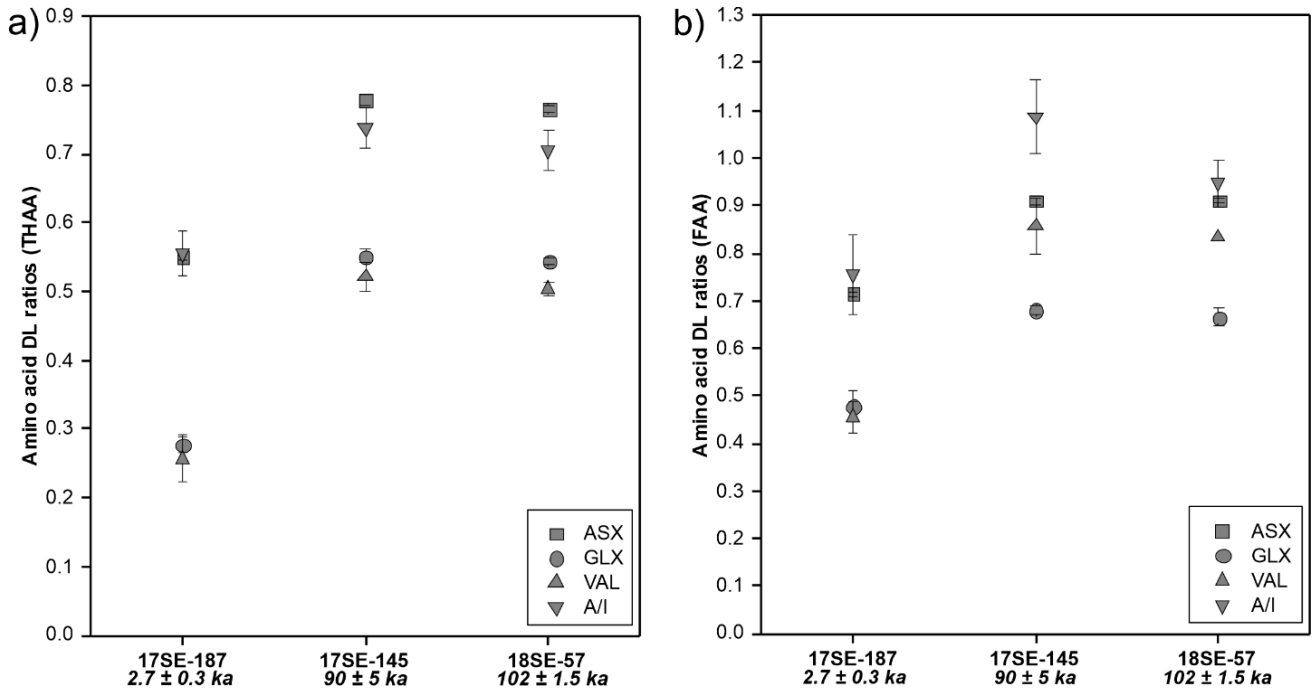

Figure 3. Amino acid DL ratios of three *Tubipora* sp. samples for a) THAA and b) FAA. Note that the MIS 1 sample from Mansinam Island (17SE-187) showed a substantially lower extent of amino acid racemization. Independent ages were reported by Saputra et al. (2022) from the U/Th series.

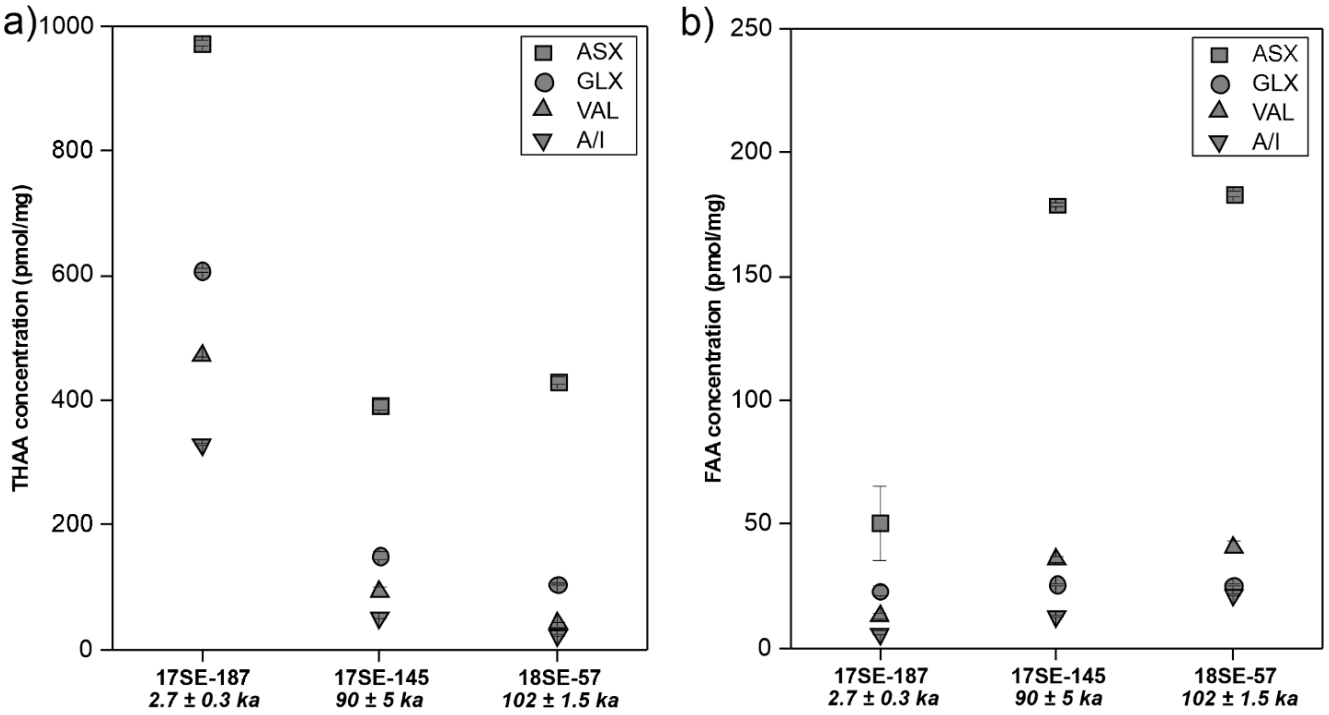

Figure 4. Selected amino acid concentration of three *Tubipora* sp. samples for a) THAA and b) FAA. Note that the MIS 1 specimen from Mansinam Island (17SE-187) yielded noticeably high amino acid contents for THAA. On the contrary, smaller amino acid concentrations are observed in the same sample for FAA, particularly ASX. Independent ages were reported by Saputra et al. (2022) from the U/Th series.

which ranged from 178 to 183 pmol/mg, compared to the sample from Mansinam Island (50.6 ± 14.9 pmol/mg). A slight decrease in amino acid abundance was also observed for both GLX and VAL in *Tubipora* sp. from Mansinam Island.

DISCUSSION

Three *Tubipora* sp. specimens analyzed in this paper show two distinct groups determined from AAR dating. A sample derived from Mansinam Island showed a typically low extent of racemization, suggesting a younger age. This supports the previous U/Th dating from *Porites* sp. within the same depositional unit, resulting in 2.7 ± 0.3 ka of age corresponding to the interval of Marine Isotope Stage (MIS) 1 sea-level highstand (Saputra et al. 2022). The extent of ASX racemization (DL ratio 0.552 ± 0.005), however, is relatively high for Holocene calcareous skeletons, most likely due to warm temperature within the region West Papua. AAR dating from the coral specimen of *Montastraea annularis*, dated 6.64 ka based on radiocarbon geochronology, also yielded a DL ratio of >0.5 at Glover Reef, Caribbean carbonate atoll with a mean annual temperature of 26 °C (Van Ee et al. 2012). Fossil coral *Favia* sp. from the Holocene marine terrace of New Guinea has also been dated using AAR with an extent of epimerisation (A/I) of 0.4 (Wehmiller et al. 1976). Here, the epimerisation extent of *Tubipora* sp. from Mansinam Island was recorded at 0.555 ± 0.033 for THAA (Figure 3a). Thus, despite the fact that different taxa affect the rate of racemization (Miller & Brigham-Grette 1989), the high racemization degree is generally found within coral assemblages from tropical climate regimes.

The amino acid DL ratios from two samples from Bakaro Bay are also consistent with older age representing MIS 5, especially sub-stages 5a and 5c, provided by U/Th dating studies by Saputra et al. (2022) with age 90 ± 5 ka (17SE-145) and 102 ± 1.5 ka (17SE-145). AAR results from both terraces showed a cluster of ASX and GLX DL ratios at >0.75

and >0.54, respectively. These are comparable to the Pleistocene-age corals (*Acropora* and *Montastraea*) dated in Glover Reef, showing >0.7 and >0.45 for ASX and GLX DL ratios, respectively (Van Ee et al. 2012).

AAR dating based on FAA shows two different clusters, as seen in the THAA analysis. The older cluster is represented by two samples from Bakaro Bay with ASX DL ratios close to the racemic point. However, AAR results based on the free amino acid pool are lacking from previous research, particularly in tandem with other independent ages. Despite different taxa, the specimens from Bakaro Bay also yielded A/I values around 1 for FAA, which is in good agreement with MIS 5a dated *Porites* sp. from New Guinea, yielding an extent of epimerization at 1.03 (Wehmiller et al. 1976).

The AAR dating conducted in this study could not separate sub-stages of MIS 5 as demonstrated by U/Th dating. Both samples show a relatively similar extent of ASX and GLX racemization, including the FAA analysis. Moreover, the ASX DL ratio for MIS 5a (0.779 ± 0.008) was recorded higher than MIS 5c (0.765 ± 0.005). This is also shown in GLX and VAL DL ratios where MIS 5c dated *Tubipora* sp. has lower values. These values are likely due to the lower resolution of AAR dating with an increasing extent of racemization. The inability of AAR dating of corals to discriminate sub-stages of MIS 5 in tropical climates has been shown in some sites. In Barbados, Wehmiller et al. (1976) noted the challenge of determining the age of MIS 5a (80 ka) and 5e (120 ka) due to the statistically similar GLX DL ratios.

In addition to the low precision of AAR dating, the warm temperature in tropical climate sites, such as the Middle Pleistocene and beyond, makes it difficult to assess older age. This is mainly due to the high sensitivity of racemization rates and diagenetic temperature history. The warmer temperature increases the racemization rate exponentially (Miller & Brigham-Grette 1989; Kaufman & Miller 1992). The exponential trend of racemization or epimerization rate has been shown from the collection of fossil mollusks of MIS 5e succession from temperate settings (e.g., southern Australia) towards tropical sites (e.g., Huon Peninsula, New Guinea) (Murray-Wallace 2000). For example, the extent of epimerization of the large mollusk *Tridacna maxima* was reported close to a racemic point beyond 125 ka (Hearty & Aharon 1988). In contrast, several 125 ka mollusks within a colder, temperate region of southern Australia yielded A/I around 0.4 (Murray-Wallace 2000).

CONCLUSION

The fossils of soft coral *Tubipora* sp. deposited in the uplifted marine terraces of the Manokwari area, West Papua, are considered a potential specimen for conducting amino acid geochronology. In this site, the aminozone or cluster associated with MIS 5 from a sample collected in Bakaro Bay can be confidently defined based on a greater extent of amino acid racemization than MIS 1-aged specimen from Mansinam Island. Significantly reduced total amino acid (THAA) concentrations are evident from those older *Tubipora* sp. samples, signifying the longer term of protein degradation. Moreover, the increasing abundance of total amino acid of FAA, ASX in particular, was observed within the MIS 5-aged specimens due to prolonged peptide breakdown that leads to enrichment of free amino acid pool. However, despite the warmer temperature in the West Papuan region, the resolution of AAR dating is deemed insufficient to discriminate sub-stages of MIS 5. In addition, the warm temperature of the West Papuan area substantially increases the racemization rates, particularly ASX and A/I, which likely reduce the

capability to date beyond the late Pleistocene age (>125 ka). Nonetheless, AAR dating may be complementary to establish along with an independent dating method (e.g., U/Th series or radiocarbon) to discriminate the Holocene from the late Pleistocene interglacial period due to rapid and cost-effective analysis.

AUTHORS CONTRIBUTION

R.H. made the research design, data analysis and original manuscript writing; S.E.A.S. did field data collection and manuscript writing; SH did the supervision and manuscript editing.

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CONFLICT OF INTEREST

The authors declare that there are no competing interests concerning the research or research funding.

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