**Diversity of lanternfish (Myctophidae) larvae along the** **Ninety East Ridge, India Ocean**

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**Abstract**

This study identified 260 lanternfish larvae specimens belonging to 20 species from 12 genera, which were obtained from 544 samples of the Ninety East Ridge waters in the Indian Ocean using the COI DNA barcoding. Among the samples, the intra-specific genetic distances ranged from 0% to 2.99%, while inter-specific genetic distances ranged from 1.88% to 25.71%. With the exception of *Notolychnus valdiviae*, the maximum intra-specific genetic distances were lower than the minimum inter-specific genetic distances for all species. The haplotypes of nine species were analyzed, revealing significant differences in the number, structure, and spatial distribution patterns. Notably, *Ceratoscopelus warmingii* and *Notolychnus valdiviae* displayed a significant north-south divergence pattern. The results indicated that seamount topography may influence the gene flow and horizontal distribution patterns of some lanternfish species, as shown by the distribution patterns of different populations of some species.

Keywords: COI, DNA barcoding, larvae, Myctophidae, Ninety East Ridge

**Introduction**

Seamounts are known to be hotspots of pelagic biodiversity and serve as stepping stones for biological dispersal(Hanel, John, Meyer-Klaeden, & Piatkowski, 2010; Leal & Bouchet, 1991; Wilson Jr & Kaufmann, 1987). They have a significant impact on species composition, productivity, and vertical transfer of nutrients in the surrounding waters(Hubbs, 1959; Lavelle & Mohn, 2010; Morato, Hoyle, Allain, & Nicol, 2010; Rowden, Dower, Schlacher, Consalvey, & Clark, 2010). Previous studies have demonstrated that seamounts tend to increase the abundance and diversity of fish(Hubbs, 1959; K. Krishna, Neprochnov, Rao, & Grinko, 2001; Morato et al., 2010), plankton(Clark et al., 2010; Dai et al., 2020; Dower & Mackas, 1996; Genin, 2004; Mullineau & Mills, 1997), and benthic organisms(Du Preez, Curtis, & Clarke, 2016; Pitcher et al., 2008; Richer de Forges, Koslow, & Poore, 2000) in their vicinity.

Seamounts can impact ecosystems through a combination of factors(Moore et al., 2003; Rowden et al., 2010), such as upwelling(Hubbs, 1959; Pitcher et al., 2008), vertical spatial heterogeneity(Carney, 2005; Du Preez et al., 2016; McClain, Lundsten, Ream, Barry, & DeVogelaere, 2009; White & Mohn, 2004), aggregation of planktonic and planktonic larvae due to blocking currents(Boehlert & Genin, 1987; K. Krishna et al., 2001; Mullineau & Mills, 1997; Rogers, 1994), substrate heterogeneity(Du Preez et al., 2016; McClain, 2007), and the influence of organic matter deposition(Pitcher et al., 2008). Furthermore, seamounts have unique environmental factors, and the distances between them can restrict gene flow(McClain et al., 2009) and result in benthic ecosystems exhibiting distribution patterns similar to those of island organisms(Boehlert & Genin, 1987; Hubbs, 1959; Mullineau & Mills, 1997; Rowden et al., 2010). However, there are significant differences in fauna communities among seamounts in different regions(McClain et al., 2009; Pitcher et al., 2008), and the small coverage of current human investigations on seamounts has led to varying hypotheses about their role in marine ecosystems(McClain, 2007; Rowden et al., 2010; Thoma, Pante, Brugler, & France, 2009).

The Ninety East Ridge is located in the northeastern Indian Ocean and is one of the longest volcanic ridges in the world(K. Krishna et al., 2001; K. S. Krishna et al., 2012). The general north-south trend of the ridge extends from 34°S to 17°N(K. S. Krishna et al., 2012), with the part of the ridge north of 9° to 10°N buried in the Bengal fan sediments(K. S. Krishna et al., 2012; Sclater & Fisher, 1974; Subrahmanyam, Gireesh, Chand, Raju, & Rao, 2008). The average width of the ridge is 200 km, and the average height is 2 km(Bowin, 1973; K. Krishna et al., 2001; Verzhbitsky, 2003). While the east and west sides of the ridge have similar topographic gradients, the seabed depth is deeper on the east side than on the west side between 11° and 21°S(K. Krishna et al., 2001).

Lanternfish are small pelagic and benthopelagic fish, with around 250-300 living marine species(Farrell, 2011; Nelson, Grande, & Wilson, 2016; Priede, 2017; van der Land, 2004), making them one of the most widespread and abundant vertebrates in the ocean. They contribute over 50-60% of the biomass of deep-sea fish(Farrell, 2011; Martin, Olson, Girard, Smith, & Davis, 2018; Poulsen et al., 2013). The ventral parts and head of the fish possess well-developed luminescent organs, which harbor symbiotic bacteria(Farrell, 2011; Nelson et al., 2016; Priede, 2017); the number and distribution pattern of these organs are crucial in identifying different species(Helfman, Collette, Facey, & Bowen, 2009; Martin et al., 2018; Moser & Ahlstrom, 1972; Priede, 2017). Lanternfish are known for their diurnal vertical migration behavior, descending to water depths of 300-1200m during the day and ascending to depths of 10-100m at night(Farrell, 2011; Nelson et al., 2016). The family primarily feeds on plankton, while also serving as prey to various marine organisms. Myctophids is a key component of pelagic food webs(Farrell, 2011) (Meincke, 1971)and playing a significant role in the ocean's carbon cycle(Hudson, Steinberg, Sutton, Graves, & Latour, 2014; Priede, 2017).

Most marine bony fishes have a planktonic larval stage(Cochran, Bokuniewicz, & Yager, 2019), and the identification of larval fish is critical for research on species life history, establishing marine protected areas, ecological monitoring, environmental assessment, and formulating fisheries management policies(Maccall, 1979; Moura et al., 2008; Senina et al., 2016; Valdez-Moreno, Vásquez-Yeomans, Elías-Gutiérrez, Ivanova, & Hebert, 2010). In ichthyoplankton samples, lanternfish larvae are usually one of the most common components(Batta-Lona, Galindo-Sánchez, Arteaga, Robles-Flores, & Jiménez-Rosenberg, 2019; Muhling, Lamkin, & Richards, 2012). However, as fish at different developmental stages have different morphological characteristics(Ko et al., 2013), the appearance of individuals at early developmental stages of many taxa is very similar(Valdez-Moreno et al., 2010; Victor, Hanner, Shivji, Hyde, & Caldow, 2009), thus it is difficult to accurately identify fish larvae by morphological characteristics. As a result, many taxa can only be identified to the family level using morphological characters(Ko et al., 2013; Wan & Zhang, 2016). According to Shao, Yang, & Chen (2001), only about one tenth of the known fish can be identified by morphological characters. Although the melanophore pattern of lanternfish larvae can provide a morphological characteristic basis for identification, identifying them still relies heavily on the experience of the identifier, and large genus Diaphus is particularly difficult to morphologically identify(Richards, 2005). In addition, the high morphological variability of lanternfish larvae further complicates their identification(Sabatés & Saiz, 2000).

The application of genetic technology has emerged as a powerful tool for the identification of fish larvae. Hebert et al. (2003) first proposed the concept of DNA barcoding and the use of Cytochrome Oxidase I (COI) sequences for species identification (Hebert, Cywinska, Ball, & DeWaard, 2003; Hebert, Ratnasingham, & De Waard, 2003). The conservative nature of the COI sequence makes it possible to distinguish samples at the species level for most taxa. The absence of introns, high copy number, and matrilineal inheritance make it one of the most widely used barcoded gene fragments(Dasmahapatra & Mallet, 2006; Savolainen, Cowan, Vogler, Roderick, & Lane, 2005; Triverdi, Ansari, Ghosh, & Rehman, 2016). The rapid growth of COI sequence data in the database since 2003(Porter & Hajibabaei, 2018) has provided the necessary resources for accurate identification of fish larvae. According to Ward, Hanner, & Hebert (2009) and Ward, Zemlak, Innes, Last, & Hebert (2005), COI DNA barcoding can identify most fish species, and Ko et al. (2013) has pointed out that the DNA barcoding technique is one of the best methods for identifying fish larvae. DNA barcoding has now been shown to distinguish Lanternfish samples at the species level and even at the geographic population level (Pappalardo et al., 2015; Poulsen et al., 2013).

The diversity of larval fishes in the Ninety East Ridge has received little attention in previous studies(L. Zhang et al., 2021). To better comprehend seamount ecosystems in the Arabian Ocean and gain further insights into the role of seamounts in biological evolution, it is crucial to investigate the diversity of larval fishes in this region.

**Materials and Methods**

**Study area and Sample collection**

Larvae samples used in this study were based on zooplankton samples collected in the summer of 2021 in the Ninety East Ridge region of the India Ocean, as depicted in Figure 1. The sampling area, situated to the north of the equator and adjacent to Sumatra Island in the northeast, covered approximately 20 latitudes (~1170 nautical miles) from north to south and 5 longitudes (~285 nautical miles) from east to west.

In this study, "larval fish" refers to individuals from the preflexion stage to the juvenile stage. The three collection methods used were: (1) WP2 plankton net, towed vertically to collect samples over 200 m, with a mouth diameter of 57cm and mesh size of 0.198-0.202mm, equipped with a mechanical flow meter (Hydro-Bios); (2) Large plankton net, towed vertically to collect samples over 200 m, with a mouth diameter of 80cm and mesh size of 0.505-0.507mm, equipped with a mechanical flow meter (Hydro-Bios); (3) Horizontal trawling using large plankton net, with a trawling time of 0.5-1 hours and speed of 0-1 knots. However, due to the slow speed of flowmeter, it was unable to accurately count, hence, the samples captured in this way were only used for qualitative analysis. As per the sampling plan limitations, not every station in the figure was sampled. A total of 14 stations were sampled using the WP2 plankton net, 17 stations were sampled using the large plankton net, and 19 stations were sampled using horizontal trawling with a large plankton net. Details of the sampling methods and stations are presented in Table 1.

Samples collected using the above-mentioned methods were placed in a collection bottle, and transferred to a sample tube. They were fixed by adding 95% alcohol or RNAhold® (Transgen Biotech) and stored in a refrigerator at 4℃. Photographs of the samples were taken using a Leica S9D microscope and initially classified based on morphological identification.

**DNA Extraction and Amplification**

For DNA extraction, the DNeasy Blood & Tissue Kit (QIAGEN, China) was used following the manufacturer's protocol for animal tissues. Amplification of the COI mitochondrial sequence was performed using two pairs of primers: 5’-LCO1490: GGTCAACAAATCATAAAGATATTGG-3', HCO2198: 5’-TAAACTTCAGGGTGACCAAAAAATCA-3'(Vrijenhoek, 1994); dgLCO1490: 5’-GGTCAACAAATCATAAAGAYATYGG-3', dgHCO2198: 5’-TAAACTTCAGGGTGACCAAARAAYCA-3'(Leray et al., 2013). A 25 µL PCR reaction system was prepared containing 12.5 µL PCR mixture [Taq polymerase plus Master Mix II (Dye Plus)], 1 µL of each primer (10 µM), 2.5 µL DNA template, and 8 µL of pure water. The PCR reaction conditions were annealing at 45 ℃, increasing by 0.5 ℃ per cycle for 15 cycles, 49 ℃ for 20 cycles, resulting in a target product of approximately 690 bp. Samples that met the sequencing concentration were subjected to bidirectional sequencing.

Some samples failed to amplify via PCR, and those that were successfully amplified were sent to Sangon Biotech Co., Ltd (Shanghai, China) for PCR cleaning and sequencing.

**Molecular data processing and analysis**

The molecular data processing and analysis were performed as follows. First, the sequences were joined, aligned, and trimmed using SeqMan v. 7.1.0 (DNAStar, U.S.A.) (Burland, 1999), with low signal strength ends removed, resulting in an edited length of approximately 650bp. Next, PhyloSuite was used for molecular phylogeny analyses(D. Zhang et al., 2020). Multiple sequences were aligned using the 'auto' strategy in MAFFT(Katoh & Standley, 2013). One sequence obtained from GenBank was considered as an outgroup (*Scopelengys tristis*). The best-fit evolutionary models were determined by selecting for Bayesian Information Criterion (BIC) using ModelFinder(Kalyaanamoorthy, Minh, Wong, Von Haeseler, & Jermiin, 2017). Maximum likelihood (ML) phylogenies were inferred using IQ-TREE(Nguyen, Schmidt, Von Haeseler, & Minh, 2015) for 2000 standard bootstraps, as well as the Shimodaira–Hasegawa-like approximate likelihood-ratio test(Guindon et al., 2010). Finally, the phylogenetic trees were viewed and edited using iTOL (available at https://itol.embl.de/), following Letunic and Bork (2021).

Pairwise distance analyses were conducted by K2P model(Kimura, 1980) in MEGA11(Tamura, Stecher, & Kumar, 2021), and all ambiguous positions were removed. In order to minimize experimental error resulting from different sampling ranges, only samples captured during this cruise were selected for genetic distance analysis.

Samples were identified using the Basic Local Alignment Search Tool (BLAST) against the GenBank database to confirm their species. Samples with a sequence similarity greater than or equal to 98% (percentage identity) to a known species were considered to belong to the same species as the reference sequence in GenBank(Leray et al., 2013; Machida, Hashiguchi, Nishida, & Nishida, 2009). To ensure the reliability of the results, reference sequences were selected based on the following principles: preference was given to sequences from published articles, and sequences of the same species uploaded by different authors (except for species with only one sequence in the GenBank database) were used with priority. For samples with a sequence similarity below 98%, the best-matched sequences in the GenBank were downloaded for further analysis, and their taxonomic status was confirmed based on their phylogenetic relationships with known species sequences. Larval fish samples that did not belong to the family Myctophidae were excluded from this study based on both morphological and molecular identification methods.

Haplotype network graphs were constructed for all species with sample sizes larger than 10. The sequences from each species were aligned using the MAFFT(Katoh & Standley, 2013) plugin available in the Phylosuite software(D. Zhang et al., 2020). Haplotype diversity statistics were calculated using the DnaSP software(Librado & Rozas, 2009). A haplotype TCS network graph was created using PopART (Population Analysis with Reticulate Trees)(Leigh & Bryant, 2015). Since PopART can only accommodate up to 10 colors (collection locations), some font and color adjustments were made using Adobe Illustrator.

**Results**

**Specimen morphology**

The photographs of the specimens are presented in Supplementary Figure 1-36. The specimens of *Diaphus lucidus* and *Symbolophorus evermanni* are not shown due to their poor state of preservation and the resulting low reference value of the photographs.

**Species abundance and sample composition**

In this experiment, a total of 544 larval fishes were collected and COI sequences were successfully amplified for 519 of them. Of these, 260 were myctophids, comprising 50% of the total number of larval fish samples. The sequences of them were submitted to GenBank (http://www.ncbi.nlm.nih.gov), and the details are provided in Table 2. Of all myctophid samples, 53 samples were collected at 14 stations using wp2 plankton net (vertical) with individual density of 0.0666ind/m^3 on average. 77 samples were collected at 17 stations using large plankton (vertical) with individual density of 0.0381ind/m^3 on average. The rest of the samples were collected by horizontal trawl.

The myctophid larvae belong to the 12 genus 38 species, which contains 16 undefined species. *Ceratoscopelus warmingii* had the highest number of samples among species with 31, while *Diaphus* had the highest number of samples among genera with 109. Out of the 26 sampling stations, samples of myctophids were not collected at four stations. The species composition of each sampling station is presented in Figure 2-4.

**Phylogenetic trees and genetic distances**

After editing, the length of the successfully sequenced sample sequence was approximately 650-690 bp. The best-fit model was selected using the Bayesian Information Criterion (BIC) with ModelFinder. According to the BIC, the best-fit model was GTR+F+R5. The resulting phylogenetic tree is presented in Figure 5.

In this study, the intraspecific genetic distances among sample sequences ranged from 0-2.99%, with an average of 0.32%. Meanwhile, interspecific genetic distances ranged from 1.88%-25.71% (Figure 6), with an average of 18.28%. Except for *Notolychnus valdiviae*, the maximum intraspecific genetic distances were smaller than the minimum interspecific genetic distances for all species. The genetic distance increases significantly as the taxonomic rank increases.

**Haplotype statistics**

The TCS haplotype network graph for nine species with sample sizes greater than 10 was constructed based on COI sequences. The analysis revealed significant variations in the number and distribution patterns of haplotypes across different species (Fig. 7-Fig. 15). The number and distribution patterns of haplotypes varied significantly among species. Notably, *Ceratoscopelus warmingii* and *Notolychnus valdiviae* exhibited significant geographic distribution patterns, while the remaining species did not show any distinct geographic distribution patterns. The haplotype diversity (Hd) of the 9 species ranged from 0.3778 to 0.9869. *Diaphus mollis* had the lowest Hd, while *Diaphus richardsoni* had the highest.

**Discussion**

**Species abundance and sample composition**

Zooplankton abundance in the Indian Ocean region is closely associated with monsoons and displays substantial seasonal variation(Koné, Aumont, Lévy, & Resplandy, 2009; Veldhuis, Kraay, Van Bleijswijk, & Baars, 1997). Following the onset of the southeast monsoon, algal blooms appear and zooplankton abundance peaks in the following one to two months. Previous studies have indicated that decapods, amphipods, ichthyoplankton, and cnidarians are most abundant in the eastern Indian Ocean region during winter (June-September) and lowest in early summer, with intermittent sub-peaks in early autumn (March). Horizontally, the abundance of plankton in this area tends to decrease with increasing latitude between 9°S and 32°S, while the ichthyoplankton abundance reaches its peak between 24°S and 25°S(Tranter & Kerr, 1977). The productivity of the Ninety East Ridge region surpasses that of surrounding areas, mainly because of the impact of monsoons and seamounts(Brewer et al., 2015).

Lanternfish are the most common fish in marine environments(Richards, 2005), and collecting data on its larvae is a prerequisite for understanding the ecosystem of Nine East Ridge region. We utilized the COI DNA barcoding technique to identify the larvae of lantern fish in the Nine East Ridge waters during the summer season.We identified 38 species of larvae in 11 genera, accounting for 15% of the total number of known living lanternfish. Genus *Diaphus* had the largest number of samples and is also the largest genus in the family Myctophidae(Elloran, 2012). Stations SEI-24, SEI-25, and SEI-A2 had the highest abundance of larval fish, with lower abundances observed at southern stations. The distribution pattern of lanternfish larvae is similar to the zooplankton described by Tranter & Kerr (1977), but it differs from the ichthyoplankton.

The density of individuals captured by the large plankton net was lower than the WP2 net, probably due to the larger mesh size, which missed some smaller-sized larvae. Conversely, the total number of individuals and species captured by the large net was higher than the WP2 net, likely due to the smaller mouth diameter of the WP2 net, which resulted in inadequate sampling.

At the four stations where no samples of lanternfish were collected, horizontal trawling was the sampling method used. This may be related to the shallow water depth (~1m) of horizontal trawling sampling.The samples contained 16 unknown species, and the taxonomic status of these samples require further studies of adult myctophids in the Nine East Ridge region to be determined.

**Phylogenetic trees and genetic distances**

The phylogenetic tree demonstrates that COI DNA barcodes can effectively differentiate most species at the species level. Notably, for species such as *Notolychnus valdiviae*, *Ceratoscopelus warmingii*, *Diaphus parri*, *Diaphus splendidus* and *Lampanyctus nobilis*, COI barcodes can distinguish between different samples at the population level. However, the monophyly of the genus *Benthosema* appears problematic as *Benthosema glaciale* and genus *Diogenichthys* form the same crown group. To eliminate the possibility of species misidentification in the GenBank database, we meticulously examined all COI sequences of *Benthosema glaciale* in the database and found that at least 50 sequences (not shown) and three published articles(Baillon, Hamel, Wareham, & Mercier, 2012; Poulsen et al., 2013; Sparks et al., 2014) support the sequence belonging to *Benthosema glaciale*.

The COI sequences obtained in this experiment differed somewhat from the sequences of *Diaphus effulgens* obtained from GenBank. However, the similarity was greater than 98%, leading to the conclusion that they belong to the same species(Machida et al., 2009). Further study may be necessary to confirm the validity of this identification, particularly after capturing adult fish with sequences more similar to the samples.

Although most samples in this study had maximum intraspecific genetic distances smaller than the minimum interspecific genetic distances, there were significant differences in interspecific genetic distances among different genera. For instance, the average interspecific genetic distance of genus *Benthosema* was 16.94%, genus *Diaphus* was 11.20%, andgenus *Lampadena* was only 5.03%. It is possible that there may not be a universal threshold for interspecific genetic variation that can be applied across all genera in the family Myctophidae to successfully distinguish species.

**Haplotype statistics**

This study identified a distinct geographic population distribution pattern for *Ceratoscopelus warmingii* and *Notolychnus valdiviae*. *Notolychnus valdiviae* is divided into two well-defined assemblages, separated by the SEI-10 station. The lineage S, marked with blue on the haplotype network map, was located southeast of the station, while the lineage N, marked with red, was distributed northwest of the site. The haplotype network map (Fig. 15&16) reveals limited genetic exchange between the two assemblages, with no shared haplotypes between the southern and northern lineages. Within the lineage N, all samples of the interior haplotype (Hap\_2) were located on the western side of the seamount.

It was observed that *Ceratoscopelus warmingii* had multiple short evolutionary branches, with the longest branch located in the southern part of the seamount, which was categorized as lineage S and marked in blue (Fig. 7&17). However, no clear geographical distribution pattern was observed for the remaining branches. Based on haplotypes, the samples could be classified into lineage S, lineage 1, and lineage 2, where lineage 1 and lineage 2 together formed lineage N. It was noted that lineage S and lineage N had distinct distribution ranges with no shared haplotypes between different stations. lineage 1 and lineage 2 underwent at least three mutations, but their distribution ranges significantly overlapped, indicating a possible population expansion following a short period of isolation. Out of the 26 haplotypes, only 2 were distributed in multiple populations, both of which were interior haplotypes, likely due to ancestral polymorphism. Interestingly, the distribution range of these haplotypes, except for one sample collected at station SEI-38, was predominantly located in the north-central or northwestern side of the seamount, possibly spreading from the western to the eastern side.

In conclusion, the geographic population distributions of *Ceratoscopelus warmingii* and *Notolychnus valdiviae* are correlated with seamounts, suggesting that the presence of seamounts could limit genetic exchange between different populations of these species. The northern lineage of both species displays a tendency to expand towards the eastern side of the seamount, which may be related to the existence of a mountain pass located near 10°S on the seamount. Furthermore, populations located in the western side of the seamount may have spread towards the southern side of the seamount via this pass or another northern mountain pass. However, more samples and intensive sampling are required to confirm this hypothesis.

In other species with number of samples greater than 10, such as *Diaphus brachycephalus*, *Diaphus richardsoni*, *Diaphus fragilis*, *Diaphus perspicillatus*, and *Lampanyctus* sp.4, no discernible north-south population divergence distribution pattern was observed. For instance, the haplotype network map indicates that *Diaphus brachycephalus* is centered around Hap\_3, with the rest of the haplotypes distributed radially, indicating that the species may have undergone a rapid population expansion, and the distribution range of Hap\_3 haplotypes includes multiple stations from south to north. It is speculated that the differences in the geographical distribution patterns of haplotypes in different species may be linked to variations in the length of the larval planktonic period and different vertical migration patterns. Although almost all adult myctophids exhibit diurnal vertical migration, some species have different vertical migration patterns for larvae and adult fish(Richards, 2005), and various subfamilies have different vertical distribution patterns for larvae(Sassa, Kawaguchi, Hirota, & Ishida, 2004). The upper flank and Taylor cap of the Seamount may intercept the vertical migration of larvae at certain depths(Pitcher et al., 2008). These findings suggest that seamounts may play a complex role in shaping the evolutionary history and biogeography of lanternfish

SEI-29 appears to have a high specificity for several species, as it contains the most haplotypes for *Lampanyctus nobilis*, *Diaphus richardsoni*, *Diaphus brachycephalus*, and *Ceratoscopelus warmingii*. Additionally, for *Notolychnus valdiviae*, *Lampanyctus nobilis*, *Diaphus richardsoni*, *Diaphus perspicillatus*, *Diaphus mollis*, *Diaphus fragilis*, *Diaphus brachycephalus*, and *Ceratoscopelus warmingii*, internal haplotype distribution was observed at SEI-29.

Meincke’s (1971) study on the Great Meteor Seamount demonstrated that the anticyclonic vortex of isolated seamounts can trap plankton. SEI-29 is also situated above a relatively isolated seamount. It is possible that some haplotype-specific larvae drifted in from the southwest or further out and were subsequently captured and concentrated at this location. Another explanation for the high haplotype diversity at SEI-29 is that it is simply the result of dispersal. To determine which hypothesis is correct, data from the area surrounding the station, particularly from the southwest, are required.

In summary, this study utilized COI barcodes to identify 38 species of lantern fish larvae in the Ninety East Ridge, India Ocean. The effects of different net types on sampling results were compared. A phylogenetic tree was constructed and the haplotype network revealed that the species *Ceratoscopelus warmingii* and *Notolychnus valdiviae* had similar geographic population distribution patterns, which were related to the topography of seamounts. Among the species analyzed in this paper, the SEI-29 station typically aggregated the most haplotypes, potentially due to its location on a relatively isolated seamount in the area. The topography of the seamount may significantly impact gene exchange and population distribution of larval fish.

This paper is part of the survey of larval fish in the Ninety East Ridge area, and we plan to continue expanding their DNA barcoding data to cover other fish families.

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**Declaration of Generative AI and AI-assisted technologies in the writing process**

*Statement: During the preparation of this work the authors used ChatGPT in order to polish the writing, check grammar and improve readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.*

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**Figure Captions**

Fig. 1. Map of sample collection sites. Background plot from The General Bathymetric Chart of the Oceans (GEBCO) (available at https://www.gebco.net/)

Fig. 2. The species composition and abundance of lanternfish larvae at each station (wp2 plankton net, vertical).

Fig. 3. The species composition and abundance of lanternfish larvae at each station (large plankton net, vertical).

Fig. 4. The species composition of lanternfish larvae at each station (large plankton net, horizontal).

Fig. 5. Phylogenetic tree based on mitochondrial sequences (COI). The analysis was carried out using Bayesian Inference under the GTR+F+R5 substitution model.

The black numbers near nodes are bootstrap support values; only those higher than 85% are indicated. The samples obtained from this experiment all start with "F" and are not labeled with the species name, while the reference sequences obtained from GenBank are labeled with the species name and GenBank accession number.

Fig. 6. The heatmap displays pairwise K2P distances values based on COI gene sequences for both intra- and interspecific comparisons.

Fig. 7. Haplotype network from *Ceratoscopelus warmingii* COI sequences, obtained from the TCS analysis. The diameter of the circles represent the frequency of each haplotype. Mutational steps are symbolized by short line, and black dots mark missing steps.

Fig. 8. Haplotype network from *Diaphus brachycephalus* COI sequences, obtained from the TCS analysis.

Fig. 9. Haplotype network from Diaphus fragilis COI sequences, obtained from the TCS analysis.

Fig. 10. Haplotype network from *Diaphus mollis* COI sequences, obtained from the TCS analysis.

Fig. 11. Haplotype network from *Diaphus perspicillatus* COI sequences, obtained from the TCS analysis.

Fig. 12. Haplotype network from Diaphus richardsoni COI sequences, obtained from the TCS analysis.

Fig. 13. Haplotype network from *Lampanyctus nobilis* COI sequences, obtained from the TCS analysis.

Fig. 14. Haplotype network from *Lampanyctus* sp.4 COI sequences, obtained from the TCS analysis.

Fig. 15. Haplotype network from *Notolychnus valdiviae* COI sequences, obtained from the TCS analysis.

Fig. 16. Distribution map of the two main *Notolychnus valdiviae* lineages in the sampling area. The northern and southern clades are marked in blue and red respectively.

Fig. 17. Distribution map of the main *Ceratoscopelus warmingii* lineages in the sampling area. The northern and southern clades are marked in blue and red respectively.

**Table Captions**

Table. 1. Sampling stations characteristics and sampling method.

Table. 2. NCBI GenBank accession numbers obtained in this study.

**Supplementary Figures Legends**

Supplementary Figure. 1. *Benthosema suborbitale*

Supplementary Figure. 2. *Bolinichthys distofax*

Supplementary Figure. 3. *Bolinichthys photothorax*

Supplementary Figure. 4. *Bolinichthys* sp.1

Supplementary Figure. 5. *Ceratoscopelus warmingii*

Supplementary Figure. 6. *Dasyscopelus* sp.

Supplementary Figure. 7. *Diaphus brachycephalus*

Supplementary Figure. 8. *Diaphus effulgens*

Supplementary Figure. 9. *Diaphus fragilis*

Supplementary Figure. 10. *Diaphus mollis*

Supplementary Figure. 11. *Diaphus parri*

Supplementary Figure. 12. *Diaphus perspicillatus*

Supplementary Figure. 13. *Diaphus phillipsi*

Supplementary Figure. 14. *Diaphus richardsoni*

Supplementary Figure. 15. *Diaphus* sp.1

Supplementary Figure. 16. *Diaphus* sp.2

Supplementary Figure. 17. *Diaphus* sp.3

Supplementary Figure. 18. *Diaphus* sp.4

Supplementary Figure. 19. *Diaphus splendidus*

Supplementary Figure. 20. *Diaphus termophilus*

Supplementary Figure. 21. *Hygophum* sp.

Supplementary Figure. 22. *Lampadena luminosa*

Supplementary Figure. 23. *Lampadena* sp.1

Supplementary Figure. 24. *Lampadena* sp.2

Supplementary Figure. 25. *Lampanyctus* nobilis

Supplementary Figure. 26. *Lampanyctus* sp.1

Supplementary Figure. 27. *Lampanyctus* sp.2

Supplementary Figure. 28. *Lampanyctus* sp.3

Supplementary Figure. 29. *Lampanyctus* sp.4

Supplementary Figure. 30. *Lampanyctus tenuiformis*

Supplementary Figure. 31. Myctophidae incertae sedis

Supplementary Figure. 32. *Notolychnus valdiviae*

Supplementary Figure. 33. *Symbolophorus rufinus*

Supplementary Figure. 34. *Symbolophorus* sp.1

Supplementary Figure. 35. *Symbolophorus* sp.2

Supplementary Figure. 36. *Triphoturus nigrescens*