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Cover: Tamil Lacewing *Cethosia nietneri* with colour pencils and watercolours for the background; detailing with fine liners by Elakshi Mahika Molur.



A report on Conidae (Gastropoda) from the Karnataka coast – distribution and shell morphometry

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Abstract: Conidae are a diverse group of predatory marine gastropods known for their highly potent venom, which may hold potential for biomedical applications. This study presents findings from a survey of Conidae species inhabiting the coastal shorelines of Karnataka. Shell measurements and morphometric analyses were conducted on four species: *Conus biliosus*, *C. inscriptus*, *C. milneedwardsi*, and *Conasprella dictator*. Molecular phylogenetic analysis of *C. biliosus* was performed using the partial mitochondrial cytochrome oxidase subunit I (COI) gene sequence.

Keywords: Cone snails, *Conasprella*, *Conus*, Cytochrome oxidase subunit I (COI), marine biodiversity, shell morphometrics, venomous molluscs.

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Author contributions: BSC and MSM planned the work. BSC collected the data. BSC and RSPR analysed the data. BSC drafted the paper and RSPR revised the paper. All authors contributed intellectually and edited/reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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INTRODUCTION

The family Conidae Fleming, 1822 (Dutertre & Lewis 2023) is a widely-distributed species-rich group of marine gastropod molluscs (Rockel et al. 1995). Cone shells are found in all tropical and subtropical oceans, with the Indo-West-Pacific region having the greatest species diversity (Filmer 2001). Studies on the taxonomy and distribution of Conidae in India date back to the latter half of the 19th century (Kohn 1978), and recently 76 of the 93 species known from India were reported from the collections of the Zoological Survey of India (Venkitesan et al. 2019). A total of 77 species of Conidae were documented from Indian waters (Kohn 1978). Regionally, 60 species were documented from Tamil Nadu (Franklin et al. 2009), 84 from the Gulf of Mannar and Lakshadweep Islands (Edward et al. 2022), and 46 from the Kerala coast (Ravinesh et al. 2022).

More than 50 species of cone shells have been identified by various researchers from the Andaman & Nicobar Islands (Rao 2003; Venkataraman et al. 2004; Franklin et al. 2013; Franklin & Apte 2021). A total of 78 cone snail species have been documented from the Lakshadweep archipelago (Smith 1894; Hornell 1921; Nagabhushanam & Rao 1972; Appukuttan et al. 1989; Rao & Rao 1991; Ravinesh & Kumar 2015; Edward et al. 2022). More recently, Ravinesh et al. (2018) recorded 49 species from Lakshadweep, including four newly reported species, three of which had not been previously recorded in India.

Until now, there have been no specific reports of *Conus* from the coast of Karnataka, India. This study presents the findings of a Conidae survey conducted in the year 2022–23 across several coastal regions of Karnataka. Field observations documented the regional distribution of four Conidae species, and shell morphometric analyses were carried out. Only one species was observed alive, and its molecular phylogenetic analysis was performed using the partial mitochondrial cytochrome oxidase subunit I (COI) gene sequence.

MATERIALS AND METHODS

Sample collection

Field surveys were conducted on accessible beaches across three coastal districts of Karnataka: Dakshina Kannada, Udupi, and Uttara Kannada (Image 1A). Transect and trawl net surveys were carried out along the shorelines in various types of coastal marine

habitats, including intertidal and subtidal sandy bottoms, shallow sandy areas, rocky shorelines, and algae-covered rocks. The frequency of each species in these habitats was recorded (Image 1B). The specimens were collected using the handpicking method. Live specimens of *Conus biliosus* ($n = 2$) were observed and collected exclusively from rocky shorelines and algae-covered rocks in Karwar, Uttara Kannada District. The identification of collected specimens was based on the shell morphology descriptions (Rockel et al. 1995). Foot tissue from the live specimen was preserved in 90% ethanol for molecular analysis.

Morphometric analysis

The collected shells were covered with algae, so for easier identification and measurement, they were cleaned using a mixture of commercial liquid bleach ("Rin"), containing nonylphenol ethoxylate, EDTA and sodium xylene sulfonate in water. To preserve the specimens, the surface of the shell was polished with mineral oil. Shell measurements were taken using Vernier Callipers (Kohn & Riggs 1975). The following variables were recorded: weight (W, in grams), shell length (SL), maximum diameter (MD), height of maximum diameter (HMD), aperture height (AH), aperture length (AL), aperture width (AW), height of penultimate whorl (HPW), and spire height (SH) (Fig. S1). All linear measurements were recorded in millimetres (mm).

DNA extraction and PCR amplification

The foot tissue of *C. biliosus* was used as the source of genetic material and DNA was isolated from 40 mg of tissue using the CTAB method (Doyle & Doyle 1987) yielding approximately 500 ng/ μ l in a total volume of 60 μ l. The mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using universal primers dgLCO: GGTCAACAAATCATAAAGAYATYGG and dgHCO: TAAACTTCAGGGTGACCAAARAAYCA (Folmer et al. 1994). Additionally, 12S1: GGCTTGGCGGTGTTTAGAC and 12S3: GTGCACGTTTCAGAGCCCTA (Simon et al. 1991), and 16Sar: CGCCTGTTACCAAAACAT and 16Sbr: CCGGTCTGAAGTCAGATCACGT (Palumbi 1996) primers were used to amplify 12S rRNA and 16S rRNA genes. PCR reactions were conducted in a total volume of 30 μ l, containing 3 μ l DNA, 1.5 μ l of each primer, 13.5 μ l of Takara master mix, 1.5 μ l MgCl₂, and 9 μ l of PCR gradient water. The protocol began with initial amplification reaction that denatured at 94°C for 4 minutes, followed by 35 cycle denaturation for 40 seconds, annealing at 51°C for 40 seconds and extension at 72°C for 1 minute. A final extension step at 72°C for 5 minutes was

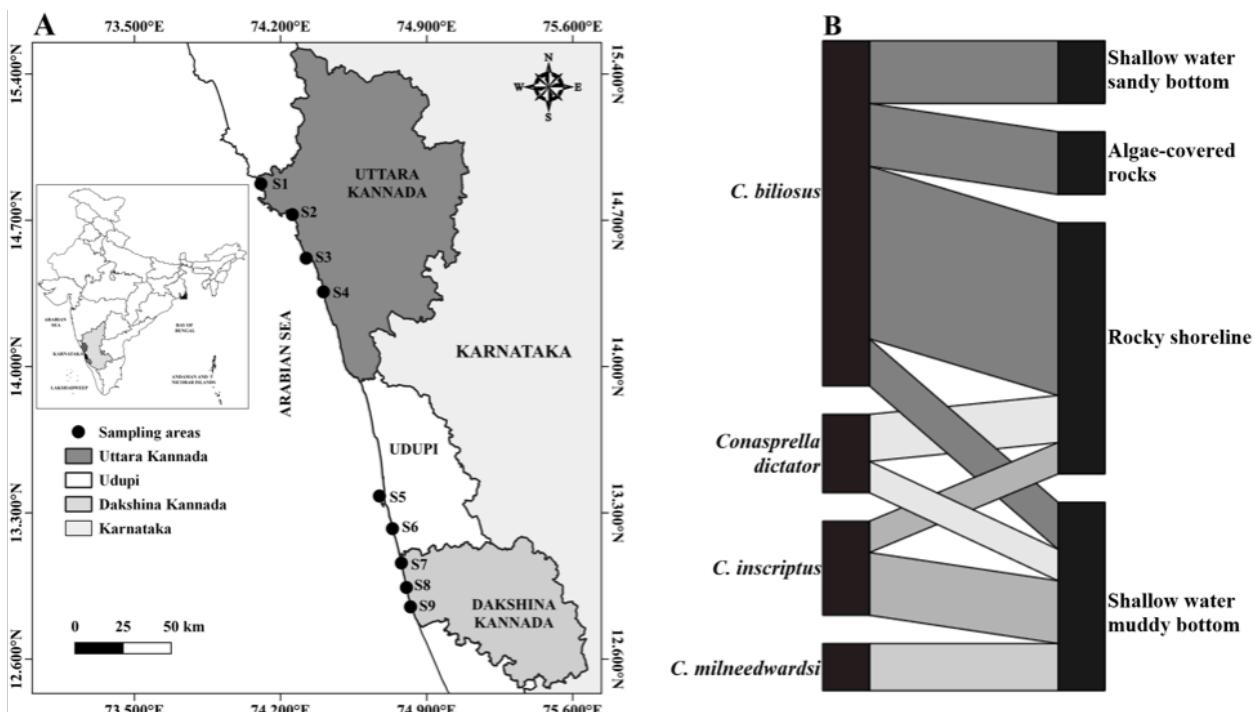


Image 1. Sampling sites (S1–S9) of Conidae in the Karnataka coast. (B) Natural habitat of cone snails collected.

included (Laxmilatha et al. 2021). The genomic DNA concentration and yield of PCR products were quantified using a Nano Drop spectrophotometer (Thermo Fisher Scientific Pvt. Ltd.) and assessed qualitatively using 0.8% agarose gel electrophoresis for DNA and 2% acrylamide gel electrophoresis for PCR products.

Phylogenetic analysis

The amplified PCR products were sequenced using the Sanger sequencing method (Barcode Biosciences). The resulting sequences were compared against the NCBI nr database using BLAST, and the top hits corresponding to *C. biliosus* were downloaded. Additionally, sequences of closely related species (*C. shikamai*) and other COI sequences were retrieved for use as an outgroup. Multiple sequence alignment was performed using MUSCLE, and a phylogenetic tree was constructed using the maximum likelihood (ML) method with the Kimura 2-parameter (K2P) model in MEGA7 software (Kumar et al. 2016). Bootstrap analysis was conducted with 1,000 replicates to assess the tree's robustness.

Data/Statistical analysis:

The morphometric measurements were recorded in a Microsoft Excel spreadsheet and summarized as mean (\pm standard deviation) along with minimum–maximum values. A Spearman's rank correlation coefficient matrix

of the morphometric variables was generated using the R *corrplot* package (Wei & Simko 2021), and scatter plots of morphometric variables (Raup 1961; Kohn & Riggs 1975) were created in Microsoft Excel. Principal component analysis (PCA) was then performed using the R *ggplot2* package (Wickham 2016).

RESULTS

Distribution

Four species were recorded: *Conus biliosus*, *C. inscriptus*, *C. milneedwardsi*, and *Conasprella dictator*; only *C. biliosus* was sampled alive. Altogether, 27 shells, including two live specimens were collected. *Conus milneedwardsi* is listed under Schedule I of the Indian Wildlife (Protection) Act, 1972, Part G: Mollusca (Ravinesh et al. 2019; Samuel et al. 2021).

Based on the sampling data (Image 2A–D), *Conus biliosus*, *C. inscriptus*, and *Conasprella dictator* were observed in the Uttara Kannada and Dakshina Kannada districts, while *C. milneedwardsi* was recorded only in the Udupi District. Sampling of cone shells included several marine habitats. *Conus biliosus* was found in shallow water sandy bottoms and rocky habitats (Image 1B, 2N–Q) with live specimens collected from algae-covered rocks. *Conasprella dictator* and *Conus inscriptus* shells

were found in rocky shoreline areas and shallow muddy bottoms (Image 1B). In contrast, *C. milneedwardsi* shells were collected by trawling in shallow muddy bottoms.

Family: Conidae Fleming, 1822

Genus: *Conus* Linnaeus, 1758

Conus biliosus (Röding, 1798)

Method of collection: Handpicking.

Condition: Live specimens (n = 2) and shells (n = 11).

Habitat: Rocky shore and algae-covered rocks.

Description (Image 2A, 2E–M): Shell length approximately ranges from 20 mm to 40 mm, the body is covered with low, wavy spiral ridges that run from shell base to shoulder and spiral growth ridges are frequently visible, but in some shells they are smooth. We observed this species with different shades (Image 2E–M) of orange, brown, brown-black, and pale brown (juvenile).

Conus inscriptus Reeve, 1843

Method of collection: Trawl bycatch.

Condition: Shells (n = 6).

Habitat: Shallow to subtidal sandy bottom.

Description (Image 2B): Shell length approximately ranges 40–55 mm. Shells are solid with a monotonous finish in a pale brown colour. Anteriorly, spiral grooves present – grooves are wide and contain spiral threads. In the sub-shoulder, bands are usually less noticeable than anterior bands.

Conus milneedwardsi Jousseaume, 1894

Method of collection: Trawl bycatch.

Condition: Shells (n = 3).

Habitat: Subtidal sandy bottoms.

Description (Image 2C): Shell length approximately ranges 86–136 mm. Shells are reddish-white or brownish-white with prominent reticulated patches and spiral bands.

Genus: *Conasprella* Thiele, 1929

Conasprella dictator (Melvill, 1898)

Method of collection: Handpicking and trawl bycatch.

Condition: Shells (n = 5).

Habitat: Shallow sandy bottoms.

Description (Image 2D): Shell length approximately ranges 25–35 mm. Shell is brownish-cream color, surrounded by distinct, dark-brown to reddish-brown bands, which are particularly prominent on the body whorl.

Morphometric analysis

The morphometric data for *C. biliosus* (n = 13), *Conasprella dictator* (n = 5), *C. inscriptus* (n = 6), and *C. milneedwardsi* (n = 3) is given in Table 1.

The Spearman's rank correlation coefficient matrix of the morphometric variables indicated that all variables were positively correlated (Figure 1A), with correlation coefficients ranging from $\rho = 0.38$ to $\rho = 0.99$. The scatter plots of morphometric variables against shell length are shown in Figure 1B–I. The variables such as HMD ($R^2 = 0.994$) and AL ($R^2 = 0.987$) exhibited excellent predictability in relation to shell length. Some variables, such as AW and HPW appeared to be more species-specific as *C. milneedwardsi* samples deviated from the general trend. HPW demonstrated the lowest predictability with respect to shell length among and within species.

A PCA (Figure 2) biplot of morphometric measurements explained 75.6% of the variance in PC1 and 15.5% in PC2. The samples from all four species formed distinct clusters, though the *Conasprella dictator* cluster overlapped with that of *C. biliosus*. As indicated by the lower variability of morphometric measurements (Table 1), *Conasprella dictator* was less spread compared to the other species in the PCA biplot. The variables projected onto the biplot revealed that SL, MD, and

Table 1. The morphometric measurements of the collected cone shells.

Variables	<i>C. biliosus</i> (n = 13) Mean (\pm SD) Min–Max	<i>Conasprella</i> <i>dictator</i> (n = 5) Mean (\pm SD) Min–Max	<i>C. inscriptus</i> (n = 6) Mean (\pm SD) Min–Max	<i>C. milneedwardsi</i> (n = 3) Mean (\pm SD) Min–Max
W	4.2 (2.3) 1.3–8.4	2.1 (0.7) 1.4–3.3	10.7 (3.6) 6.8–15.3	41.1 (21.8) 17.7–60.8
SL	28.2 (7.2) 19.1–41.2	29.1 (4.2) 25–35.5	48.8 (5.5) 41.9–54.6	114.7 (25.8) 86–136
MD	17.2 (3.7) 11.2–22.6	13.6 (1.9) 12.1–16.9	24.2 (3.4) 20.3–27.8	38.0 (12.5) 24–48
HMD	19.8 (6.0) 12.7–32.3	20.1 (2.8) 17.8–24.7	36.3 (4.9) 29.2–41.2	72.4 (16.0) 54.1–84
AH	25.5 (6.4) 16.7–35.5	23.1 (2.5) 21–27	39.3 (4.8) 34.8–45.8	76.7 (14.4) 60.1–86
AL	23.0 (6.3) 14.1–32	21.8 (2.6) 19.7–26	39.4 (5.6) 33–45.3	74.7 (16.3) 55.9–85.2
AW	3.2 (1.0) 1.8–4.9	3.0 (1.1) 1.9–4.4	5.0 (0.7) 4.1–5.8	4.2 (0.4) 3.8–4.5
HPW	1.4 (0.4) 0.7–2.3	1.3 (0.4) 0.6–1.9	3.7 (1.4) 2.2–6.0	2.2 (0.2) 2.0–2.3
SH	3.5 (1.1) 2.5–6.1	13.8 (6.3) 6.7–20.0	8.7 (1.0) 7.2–9.9	31.7 (4.1) 27.2–35

Abbreviations: W—Weight | SL—Shell length | MD—Maximum diameter | HMD—Height of maximum diameter | AH—Aperture height | AL—Aperture length | AW—Aperture width | HPW—Height of penultimate whorl | SH—Spire height. The measurements/values for W are in grams, and all others are in millimeter.

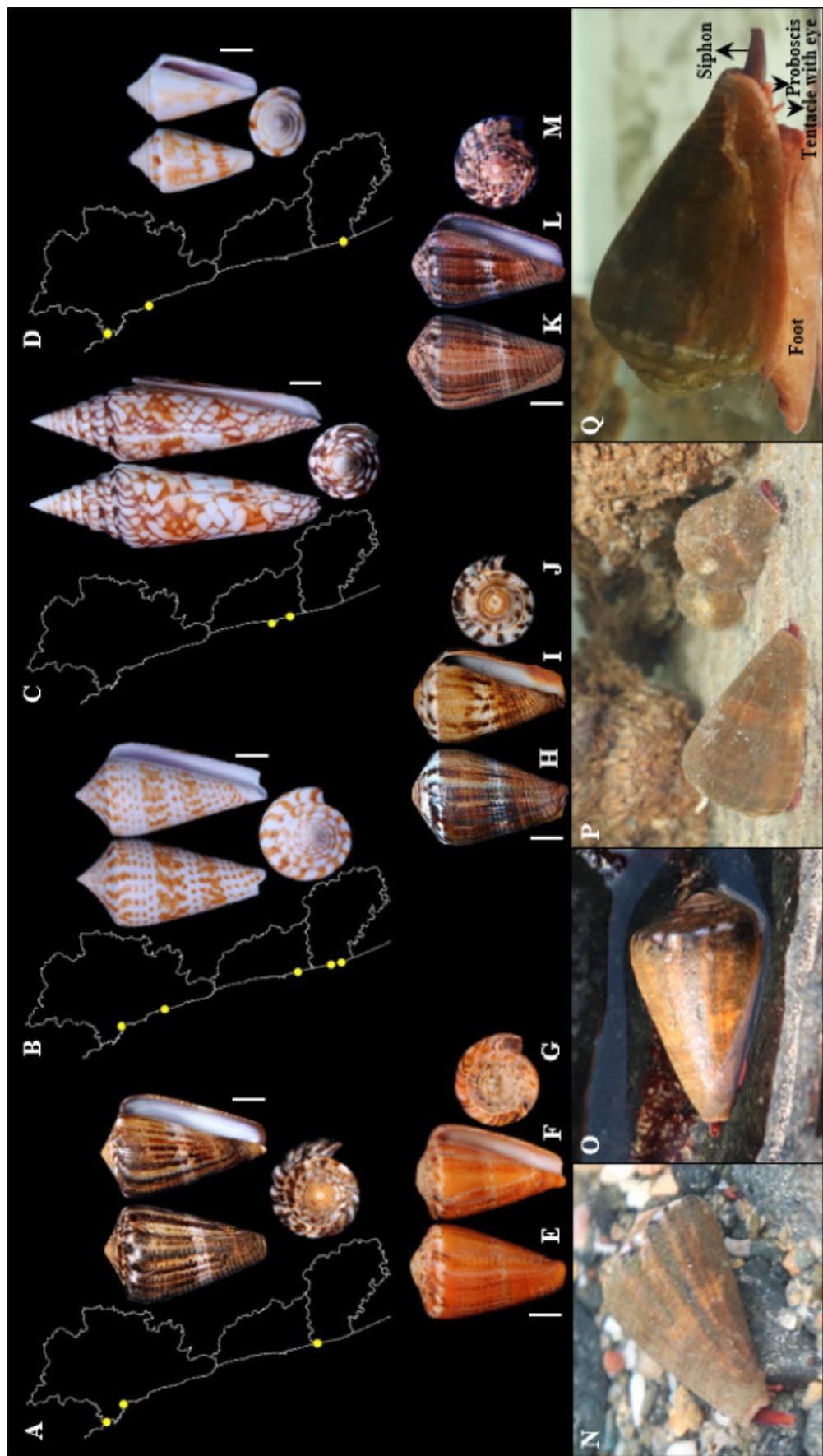


Image 2. Distribution (locality data) of Conidae in the Karnataka coast. (A) *C. biliosus*, (B) *C. inscriptus*, (C) *C. milneedwardsi*, and (D) *Conasprella dictator*. (E-M) Colour morphs in *C. biliosus*. (A-M) Dorsal, ventral, and top views of cone snail species. Scale bar is 1 cm. (N-Q) *C. biliosus* in the natural habitat. (P-Q) *C. biliosus* in aquarium. © B S Chandan.

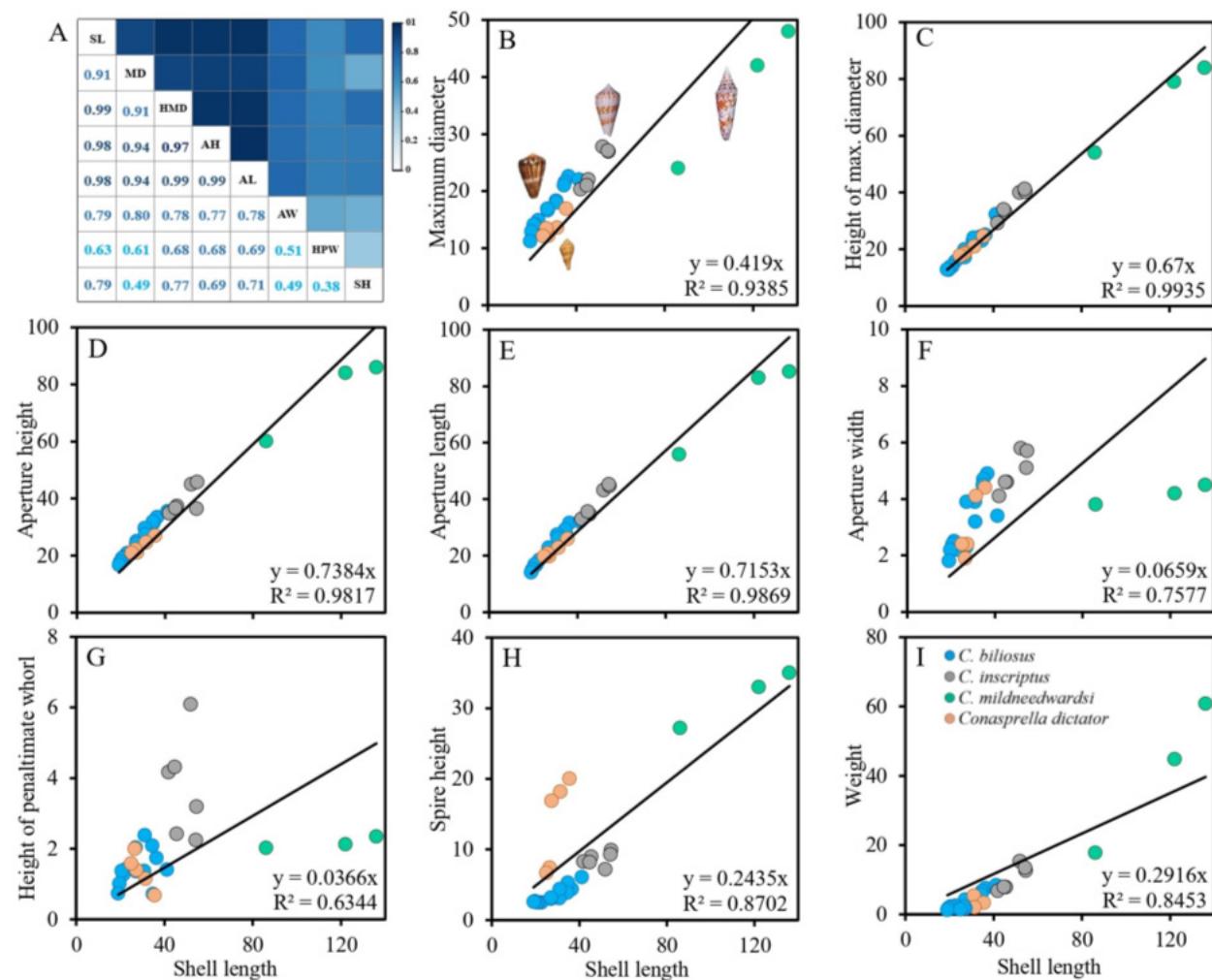


Figure 1. (A) The Spearman's rank correlation coefficient matrix of morphometric variables. (B-I) Scatter plots of various shell measurements (all in mm, except weight which is in g) against the shell length (in mm). Abbreviations: AH - aperture height, SL - shell length, MD - maximum diameter, HMD - height of maximum diameter, AL - aperture length, AW - aperture width, APW - height of penultimate whorl, and SH - spire height.

other factors primarily contributed to PC1, as evident by the spread of long-shelled *C. milneedwardsi* along PC1. The variables HPW, AW and SH primarily contributed to PC2.

Phylogeny of *C. biliosus*

The mitochondrial COI sequences from two *C. biliosus* samples were obtained and submitted to NCBI (supplemental information). The phylogenetic tree based on these COI sequences revealed that *C. biliosus* samples (PQ390234 and PQ392002) from Karnataka were distinct, but clustered within the same clade of other *C. biliosus* (KJ549870.1 and KJ550138.1) from Indo-West-Pacific region (Puillandre et al. 2014) (Figure 3). The *C. shikamai* and other species formed a clear outgroup. Since COI sequences were found and used

widely for cone snail databases, 12S rRNA and 16S rRNA sequences were not included in the analysis.

DISCUSSION

This study documents four species of cone snails: *C. biliosus*, *C. inscriptus*, *C. milneedwardsi*, and *Conasprella dictator* from the Karnataka coast. While Conidae species generally share a similar shell shape (Rockel et al. 1995), morphometric measurements are widely used as distinguishing features between species. The ranges of values observed in this study are consistent with previous records of morphometric measurements for cone shells of the corresponding species from the Indo-Pacific region (Rockel et al. 1995), Lakshadweep

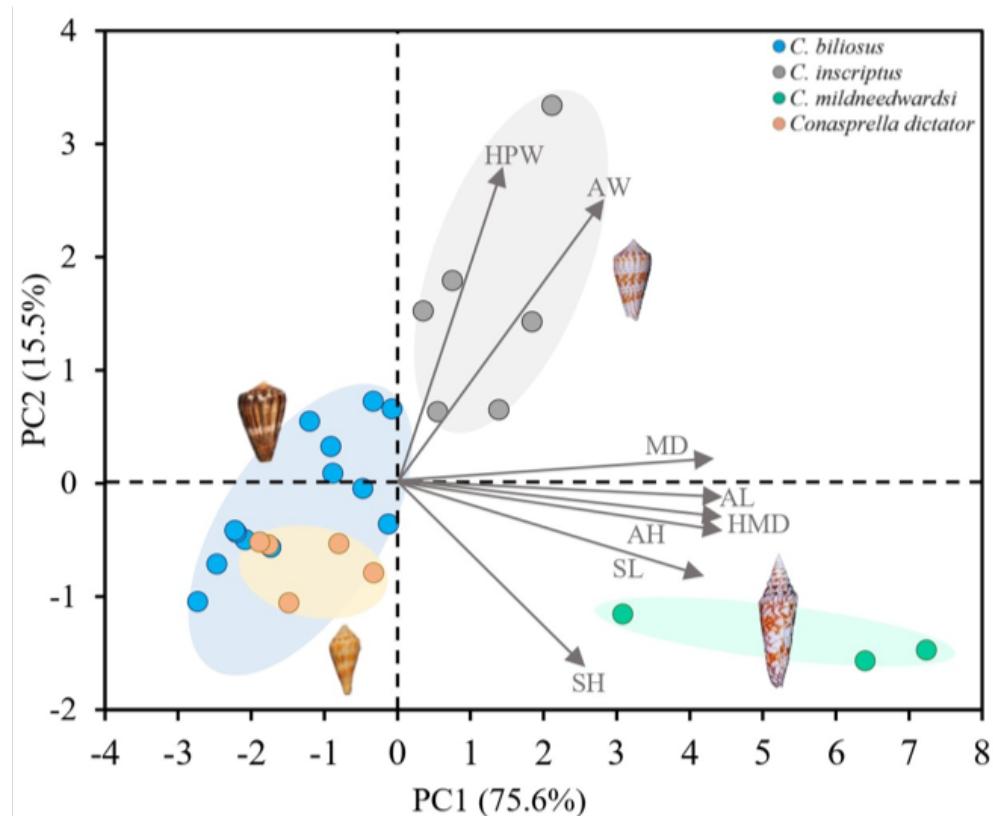


Figure 2. PCA biplot of Conidae species based on morphometric variables. Groups based on species. The arrows indicate the relationships of the variables with species group.

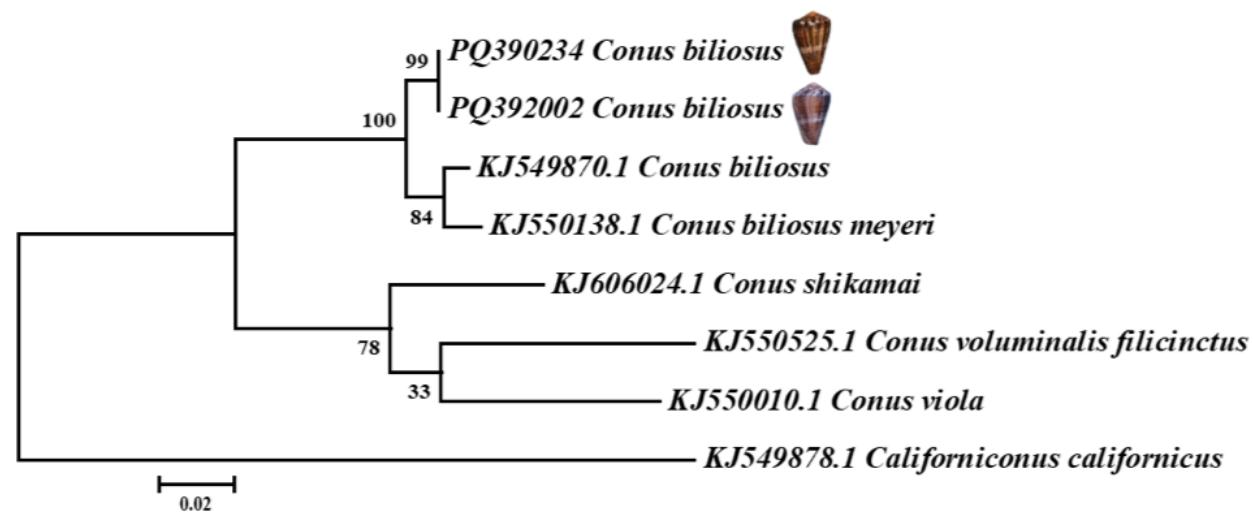


Figure 3. Maximum likelihood (ML) based phylogenetic tree of *C. biliosus* using partial mitochondrial COI gene sequences. The *C. shikamai* and others were used as the out group. Numbers next to nodes indicate percentage bootstrap values based on 1000 iterations. Scale bar indicates the number of substitutions per site.

(Ravinesh et al. 2018), Tamil Nadu (Venkitesan et al. 2019), and Kerala (Ravinesh et al. 2022).

The phylogenetic analysis based on mitochondrial COI sequences of live *C. biliosus* specimens collected in

this study placed them in a monophyletic group with *C. biliosus meyeri*, a southern subspecies found in Indian marine habitats (Puillandre et al. 2014).

This pilot study on the previously unexplored

Karnataka coast may inspire researchers to conduct more intensive surveys and acquire accurate data on habitat and distribution of cone snails of the Karnataka coast.

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Supplemental information

Notes:

Partial gene sequences of 12S rRNA, 16S rRNA, and COI from two specimens of *C. biliosus*. The sequences have been submitted to NCBI and the accession numbers are given.

>PQ393374| 12S rRNA (partial) from *C. biliosus* 1

```
ATTCGACATTCACGTTTCCCGACCTCCTTATTCCGAGTTTACCGCTTGCTCAGGTAACATCTTAAACATAGTAGTTAGCTACGAAAATTTAATTAAACACGTCA
AATCAAGGTGAGCTAATAAGAGGGAGGAGATGGGTACAATTATATAATCACGAAAAACGTCTAAATAAGGTGTTGAAGGAGGACTGAAAGTAATTAAATTATAAAAGAGA
ATGAATAGGGCTCTAAACACGTGACA
```

>PQ394594| 16S rRNA (partial) from *C. biliosus* 1

```
CGGACCTTGCAGTGAGTTTCAACGGCCGGTACTACTCACCGTCAAAGGTAGCATAATCATCCTGCCTTATAATTGAAGGCTGGAATGAATGGTTGACAAGAATACAACGTCTC
TATATGATTCTCTAGAATTATTATTTGGATGAAAAGTCAGATATTAAAGACAAGAAGACCCATCGAGCTTAGAGAAAATTAGTAGACTAATAATAATCAAGAAATAAAAGAAA
AACTACTAAACACTTGGTGGGCAACCGAGGAGAAAAAGCCTCTTAAGTTAACTCTGATGTGCTGATCAAAGGAAATTGATCAAAGGAAATTGACCTGAGTCTTAAGGGTGGTGTGAC
CATTATCTTTCAAGGCCATATCGAAAAAAAGGTTGTGACCTGATGTTGACCAGAATACCTAAAGATGCGAGTCTTAAGGGTGGTGTGACCTAAATTCTACGTGAT
CTGT
```

>PQ390234| COI (partial) from *C. biliosus* 1

```
GGGTTGGTGGTACTGCCTTAAGTTATTGATCGCAGAATTAGGTGAGCTGCTTACCTGGAGACGATCAGTTGATAATGTAATTGAGCACATGCTTTGTTATAATTGTTT
CTTAGTGTGCAATAATAATTGGGGGATTGGGATTGATTAGTAGCTCTTATATTGGGGGCTCCAGATATGGTTTCTCGACTAAATAATAAGTTTGGCTTCCGCTGCGTT
GCTCTCTATCGCAGCGGTAGAAAGGGGTGTTGAGCAGTATATCCTTTAGCAGGAAATCTAGCTCATGCTGGAGGTTCTGAGATCTGGGATTTCTCTCCAT
CTGCTGGGTTCTTATTTGGGTGAGTAAATTACACAAATTATAATACGATGGCAGGGATAAAATTGAAACGCCCTCGTTGTTGTTGTCGTAAGAAATTACTGCTT
TTATTGCTTTATCTTACCTGTTAGCAGGAGCAATTACGATACTCTAACCGATCGAAATTAAACTGCTTCTTGACCAGCAGGAGGTGGGATCCTATTATACCAGCATTGTT
```

>PQ393375| 12S rRNA (partial) from *C. biliosus* 2

```
TACAAAAAAAGAACATACAGTGGTAAGTCTATCACGCTTACCGACCCCTATAGCAGTTGAGCTGTTACCGTTGTCGTCAGGTAACCTTAAACATAGTAGTTAGCTGAAAAT
TATTAATAACACGTCAATCAAGGTGAGCTAATAAGAGGGAGGAGGGTACAATTATATAATCACGAAAAACGTCTAAATAAGGTGTTGAAGGAGGACTGAAAGTAATT
TTAATTATAAAAGAGAATGAATAGGGCTCTGAAACACGTGACAAA
```

>PQ380238| 16S rRNA (partial) from *C. biliosus* 2

```
TTTTAACGGCCGGTACTGACCGTCAAAGGTAGCATAATCATTGCTTATAATTGAAGGCTGGAATGAATGGTTGACAAGAATACAACGTCTATATGATTCTAGAATTGTT
TTTGGATGAAAAGTCAGATATTAAAAGACAAGAACCCATCGAGCTTAGAGAAAATTAGTAGACTAATAATAATCAATAGAAAATAAGAAAACTAAACACACTTGGT
GGGGCAACCGAGGAGCAAATAAGCCTCTTAAGTTAACTCTGATGTGCTGATCAAAGGAAATTGATCAAAGGAAATTGACCTGATGGATAACAACGTATC
```

>PQ392002| COI (partial) from *C. biliosus* 2

```
GGGTTGGTGGTACTGCCTTAAGTTATTGATCGCAGAATTAGGTGAGCTGCTTACCTGGAGACGATCAGTTGATAATGTAATTGAGCACATGCTTTGTTATAATTGTTT
CTTAGTGTGCAATAATAATTGGGGGATTGGGATTGATTAGTAGCTCTTATATTGGGGGCTCCAGATATGGTTTCTCGACTAAATAATAAGTTTGGCTTCCGCTGCGTT
GCTCTCTATCGCAGCGGTAGAAAGGGGTGTTGAGCAGTATATCCTTTAGCAGGAAATCTAGCTCATGCTGGAGGTTCTGAGATCTGGGATTTCTCTCCAT
CTGCTGGGTTCTTATTTGGGTGAGTAAATTACACAAATTATAATACGATGGCAGGGATAAAATTGAAACGCCCTCGTTGTTGTTGTCGTAAGAAATTACTGCTT
TTATTGCTTTATCTTACCTGTTAGCAGGAGCAATTACGATACTCTAACCGATCGAAATTAAACTGCTTCTTGACCAGCAGGAGGTGGGATCCTATTATACCAGCATTGTT
```

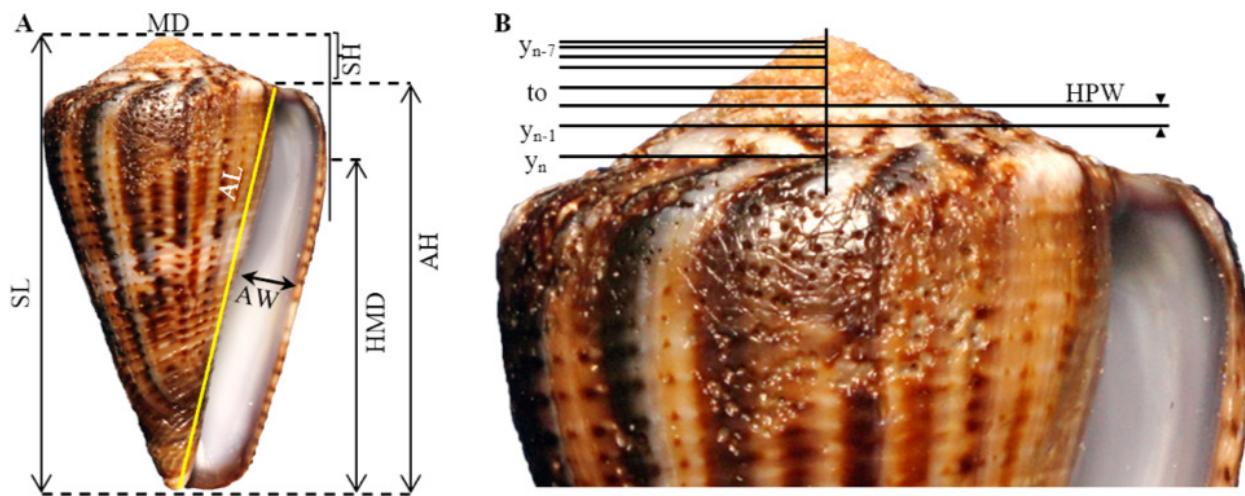


Image S1. Morphometric measurements of the Conidae shell (*C. biliosus*): A—Ventral view | B—Posterior or spire view. Abbreviations: AH—aperture height | AL—aperture length | AW—aperture width | HPW—height of penultimate whorl | HMD—height of maximum diameter | MD—maximum diameter | SH—spire height | SL—shell length. In the spire, the successive whorls are labelled as y_n to y_{n-7} . © B S Chandan.

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