The Mitochondrial Genome of Ylistrum japonicum (Bivalvia, Pectinidae) from South China Sea and Its Phylogentic Analysis

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Article

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Abstract: The *Ylistrum japonicum* is a commercially valuable scallop known for its long-distance swimming abilities. Despite its economic importance, genetic and genomic research on this species is limited. This study presents the first complete mitochondrial genome of *Y. japonicum*. The mitochondrial genome is 19,475 bp long and encompasses 13 protein-coding genes, 3 ribosomal RNA genes, and 23 transfer RNA genes. By selecting 15 Pectinidae species and additional outgroup taxa, we have constructed phylogenetic trees to elucidate the evolutionary placement of *Ylistrum* within the family Pectinidae. Our analysis reveals that *Ylistrum* is a basal lineage to the Pectininae clade, distinct from its previously assigned tribe, Amusiini. This study offers critical insights into the genetic makeup and evolutionary history of *Y. japonicum*, enhancing our knowledge of this economically vital species.

Keywords: saucer scallop; *Ylistrum japonicum*; mitochondrial genome; taxonomy

1. Introduction

The genus *Ylistrum*, a member of the phylum Mollusca, class Bivalvia, order Pectinoida, family Pectinidae, established by Mynhardt et al. [1] in 2014, derived from the Greek word "ylistro", meaning "glide", reflecting the gliding habits of this group, and dividing the original genus *Amusium* into two genera, *Ylistrum* and *Amusium*. Both *Ylistrum* and *Amusium* primarily found in the Indian-Pacific region and exhibit similar lifestyles and morphological characters [1–3]. Despite this similarities, the evolutionary relationships and placement of *Ylistrum* within the family Pectinidae are not conclusively determined. Research using molecular phylogenetic studies has indicated that the *Ylistrum* is genetically distinct from its close relatives in the genus *Amusium* studies by Alejandrino et al. [4], Mynhardt et al. [1], and Sherratt et al. [5] support this genetic separation. However, the precise phylogenetic placement of *Ylistrum* within the family remains a topic of debate. Serb [6] proposed that the *Ylistrum* might belong to the tribe Decatopectinini, but this suggestion has not been strongly supportted by the abaliable evidence.

Ylistrum species are known for their long-distance swimming or gliding abilities, and their distinctive features include colorful left and right shells and radiating ribs on the inner shell [1,3,7]. There are two extant species within the *Ylistrum* genus that are found across the globe: *Ylistrum japonicum* (Gmelin,1791) was originally discovered in Japan [8], and *Ylistrum balloti* (Bernardi, 1861), which predominantly encountered in Australia's western, eastern, and southern regions [3,9], as well as in New Caledonia [10,11]. There is also a known fossil species from Morgan Limestone [12], *Ylistrum morganense* (Beu and Darragh, 2001). *Y. balloti* is pivotal to the commercial trawl fisheries in Australia, warranting extensive research. In contrast, studies on *Y. japonicum* are scarcer, with most research originating from Japan and South Korea. Okada [13] have delved into the species' ecology and morphology, while Kanmizutaru and Anraku [14] have investigated the effects of $MgCl_2$ injection into the adductor muscle for shell opening, and in 2005, Kanmizutaru et al. [15] have also

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assessed the pallial eyes' light perception through electroretinogram tests. In South Korea, research have illuminated including its reproductive cycle [16], the development of its gonads, the age at first sexual maturity, and the sex ratio [17], as well as the correlation between age and growth [18]. In China, there is limited research available, focusing primarily ecology and the possibilities of artificial breeding [19,20].

The taxonomic classification of *Y. japonicum* is still unclear due to a lack of molecular data. In a recent taxonomic investigation, Dijkstra and Beu [3] have provisionally maintained *Ylistrum* within the Amusiini tribe, awaiting a conclusive molecular phylogenetic analysis of the Pectinidae family.

2. Results

2.1. Mitochondrial Genome Composition

The mitochondrial genome structure of of Y. japonicum is depicted Figure 1 and detailed in Table 1. The complete mitochondrial genome sequence has been submitted to NCBI GenBank database (Accession number: PP571649). The structure is a typical circular closed double-stranded molecule with a total length of 19475 bp, containing 39 genes including 13 protein-coding genes (PCGs), 3 ribosomal RNAs, and 23 transfer RNAs. The base composition is as follows: (A) constitutes 21.9%, thymine (T) 36%, guanine (G) 29%, and cytosine (C) 13.1%. The mitochondrial genome exhibits an A+T content of 57.9% and a G+C content of 42.1%.

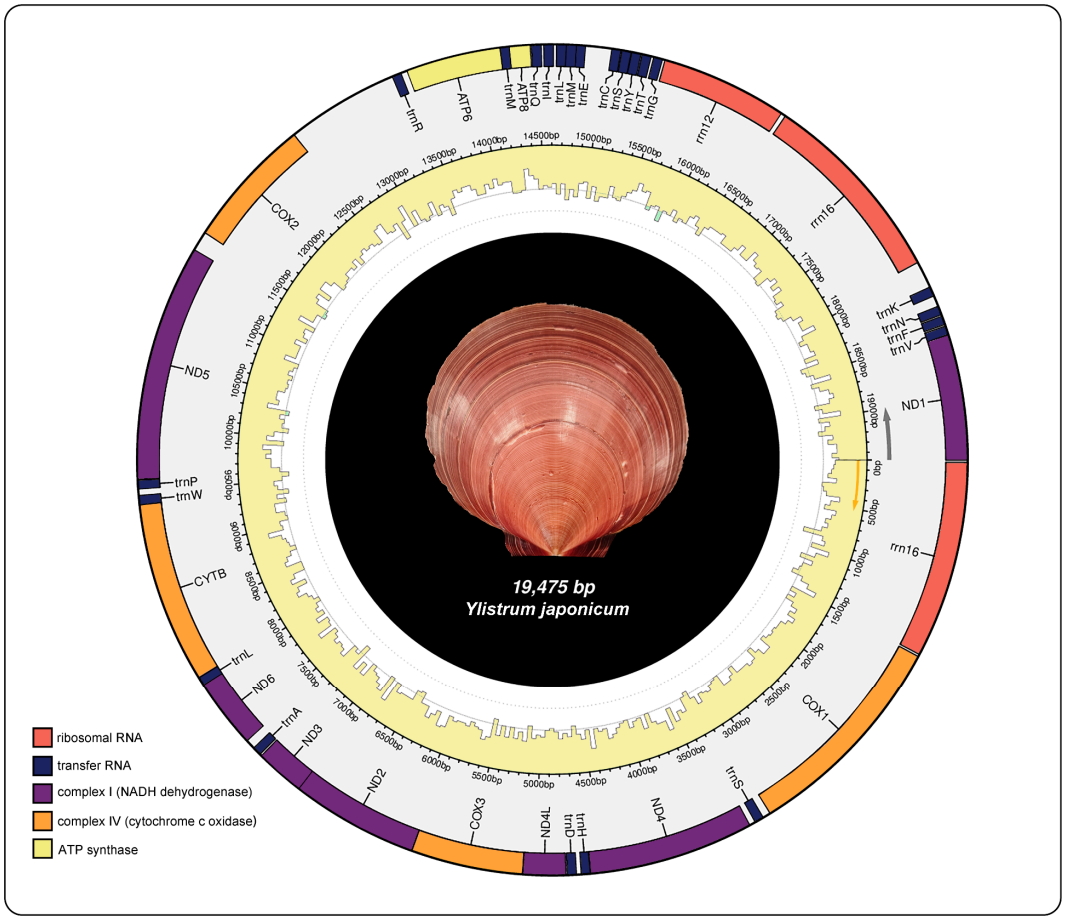

Figure 1. Mitochondrial genome map of *Ylistrum japonicum* in this study.

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Table 2. Structural features of the mitochondrial genome of *Ylistrum japonicum*.

Gene	Sequence location	Size (bp)	Start codon	Stop codon	Intergenic nucleotide (bp)
rrnL	18-1509	1492			17
cox1	1527-3158	1632	ATG	TAA	17
trnS	3219-3286	68			60
nad4	3334-4581	1248	GTG	TAG	47
trnH	4588-4652	65			6
trnD	4690-4757	68			37
nad4L	4769-5089	321	ATG	TAA	11
cox3	5094-5939	846	GTG	TAG	4
nad2	5942-6904	963	GTG	TAA	2
nad3	6904-7275	372	ATC	TAA	-1
trnA	7291-7356	66			15
nad6	7431-7958	528	GTG	TAA	74
trnL	7960-8024	65			1
cytb	8028-9402	1375	TTG	T	3
trnW	9403-9471	69			0
trnP	9522-9586	65			50
nad5	9594-11381	1788	TTG	TAG	7
cox2	11532-12524	993	ATG	TAG	150
trnR	13380-13447	68			855
ato6	13498-14211	714	ATG	TAG	50
trnM	14216-14280	65			4
atp8	14284-14439	156	GTG	TAG	3
trnQ	14451-14521	71			11
trnI	14542-14612	71			20
trnL	14638-14706	69			25
trnM	14713-14784	72			6
trnE	14788-14853	66			3
trnC	15053-15118	66			199
trnS	15129-15195	67			10
trnY	15205-15271	68			9
trnT	15283-15350	68			11
trnG	15372-15437	66			21

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rrnS	15459-16415	957			21
rrnL	16453-17939	1486			37
trnK	18145-18216	72			205
trnN	18301-18367	67			84
trnF	18381-18445	65			13
trnV	18459-18525	67			13
nad1	18528-19475	948	GTG	TAG	2

2.2. Protein-Coding Genes

There are a total of 13 protein-coding genes, 12 out of the 13 Protein-coding genes (PCGs) of Y. japonicum commonly found across most pectinid species [21,22]. Notebly, gene atp8 which is typically abesent in most mitogenomes of most bivalve [23,24] is present in Y. japonicaum. The total length of PCGs was 11884 bp, comprising approximately 61% of the complete genome. Among the genes only four (atp6, cox1, cox2, nad4L) utilize the standard start codon ATG. And the remaining nine had alternative start codon, six genes (atp8, cox3, nad1, nad2, nad4, nad6) had GTG, two TTG (cytb, nad5) and nad3 had ATC. Seven genes had the TAG stop codon, five had TAA, and cytb was terminated by T.

2.3. rRNA and tRNA Genes

The rrnS (rrn12) gene spans 957 bp (from position 15459 to 16415) while rrnL (rrn16) gene has two copies with length 1492 bp and 1486 bp (18-1509, 16453-17939 respectively). The mitochondrial genome of Y. japonicum contains 23 tRNA genes ranging in length from 65 to 72 nucleotides. Three tRNA genes are present in two copies, all of three tRNA genes was found with distict anticodons. Two trnS (rRNA-ser) have UCU or UGA, two trnL (tRNA-Leu) had UAA or UAG, and two trnM (rRNA-Met) had UAU or CAU. The occurrence multiple trnM genes in the mitochondiral genomes of bivalves is common [21], and the presence of two copies of trnS also frequently observed in most mitochondiral genomes of most animals [25]. None of the tRNA genes overlapp with any protein-coding genes (PCGs).

2.4. Gene Order

The occurrence of mitogenomes rearrangement is prevalent among mollusks [26], arrangement observed in mitogenome of Y. japonicum is also a novel configuration for the family Pectinidae, with no matching gene junctions found in other Pectinidae species (Figure 2). Species with higher gene order similarity were selected for comparison and newly annotated atp8 genes according to Malkócs et al. [25]. Due to the lacks of annotation of rRNA sequence, Mizuhopecten yessoensis (FJ595959) was excluded from the gene order analysis, and based on the high similarity of gene order between three Argopecten species [27], only one species was selected as a representative.

Comparing gene arrangements of four selected species, there is one gene cluster “nad6-trnL-cytb” were shared. When excluding the tRNA genes, four scallop species shared another gene clusters “nad1-rrnL-cox1”; Gene cluster “nad4L-cox3” present in Y. japonicum, Argopecten irradians irradians, Amusium pleuronctes and gene cluster “cox3-nad2-nad3” were shared by Y. japonicum, A. pleuronctes and Chlamys farreri. The “nad5-atp6” cluster in Y. japonicum is split by the insertion of cox2, make it different from the other two Pectininae species. The gene cluster “nad5-atp6-rrnS” was also shared between A. irradians irradians and A. pleuronctes when those variable tRNA were excluded, this indicates the close phylogenetic affinity between two species as well.

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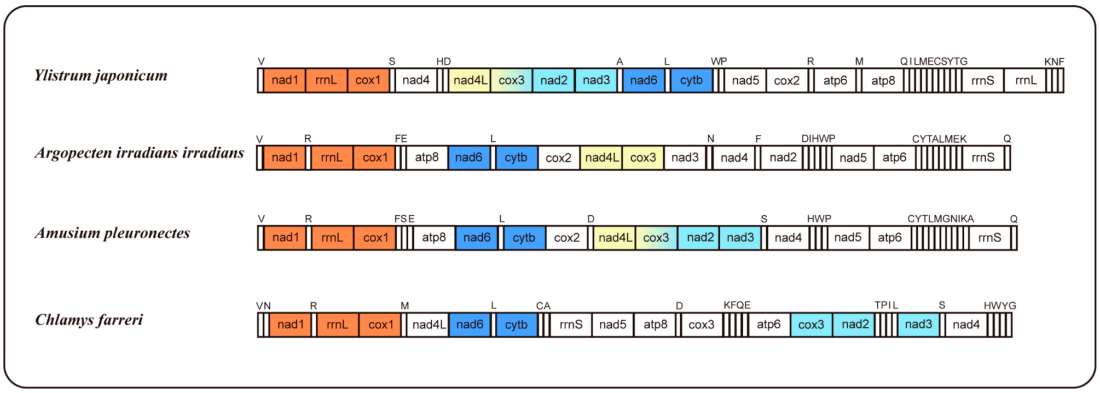

Figure 2. Gene orders of *Ylistrum japonicum*, *Argopecten irradians irradians*, *Amusium pleuronctes* and *Chlamys farreri*, with newly annotated atp8 genes. The same color indicate identical gene junctions (excluding the tRNA genes).

2.5. Gene Collinearity

Gene collinearity analysis using the progressiveMauve algorithm in Mauve, delineated 7 locally collinear blocks (LCBs) across complete mitochondrial genome of 5 Pectininae species (Figure 3). These LCBs are conserved across all mitogenomes analyzed, although variations in the sequence order are evident among the different species. The order of LCBs demonstrated a high degree of similarity among the three Argopecten species, indicating their close evolutionary relationship. In contrast, Y. japonicum exhibited a significantly distict order of LBCs arrangement when compared to both three Argopecten species and A. pleuronectes.

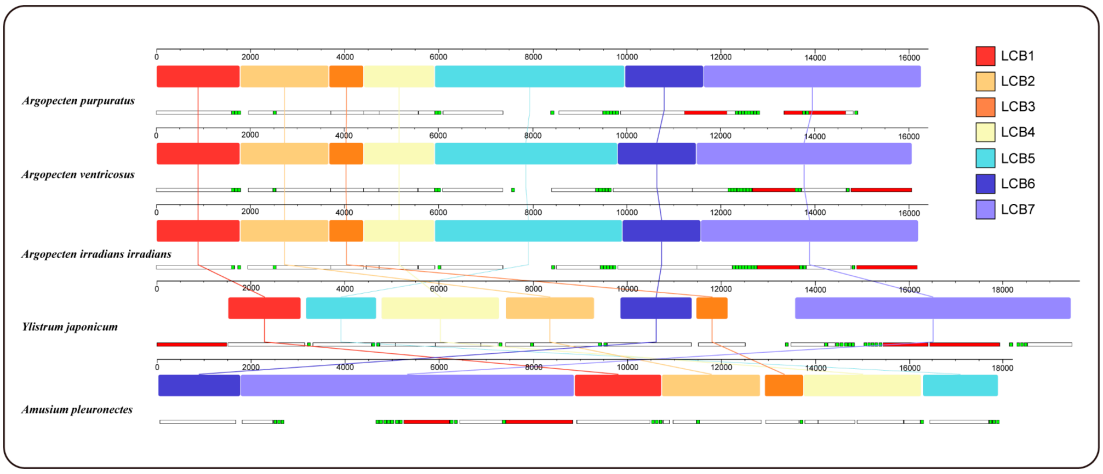

Figure 3. Gene collinearity analysis of 5 Pectininae species. The level of similarity at each position is shown in the blocks. The white, red and green boxes represent protein coding, rRNA, tRNA genes.

2.6. Phylogenetic Analysis

To delve deeper into the phylogenetic position of Y. japonicum and the taxonomic status of family Pectinidae, a phylogenetic tree (Figure 4) was constructed based on complete or nearly complete mitochondrial genome data of various Pectinidae species and outgroup taxa. The results of the phylogenetic analysis were found to be comparable with previous studies by Smedley et al. [28], Yao et al. [29], and Malkócs et al. [25], and were mostly consistent with Waller's classification [30,31]. Phylogenies based on two methods (Maximum Likelihood and Bayesian inference) of the concatenated protein sequences showed almost complete agreement, with high bootstrap values or posterior probabilities supporting all nodes. The systematic arrangement, as proposed by Waller [31],

 doi:10.20944/preprints202407.2376.v1

subdivides the family Pectindae into four subfamilies: Pectininae, Chlamydinae, Pallioline, and Camptonectinae. The outgroup Mytilinae and Crassotreinae were found to be consistent with the phylogenetic position proposed by Xu et al. [21], where the clade Mytilinae forms a sister group with the clade Osteridae+Pectinidae.

Our study has confirtmed the previously hypothesized monophylicity of Pectinidae, as concluded by Waller [32]. However, due to the absence of complete mitochondrial genome sequences of the Camptonectinae, no representatives from this subfamily were included in the phylogenetic analysis. The species within Pectinidae were effectively categorized into three subfamilies Palliolinae, Chlamydinae, and Pectininae. Placopecten magellanicus, serving as the representative of the Palliolinae, was positioned at the basal position of the branch Palliolinae+Chlamydinae. The clade Palliolinae+Chlamydinae was well supported as the sister group to the Pectininae clade [33,34]. Within the subfamily Chlamydinae, M. yessonesis and C. farreri were found to have the closest relationship, forming a sister taxon with Mimachlamys, consistent with the results obtained by Xu et al. [21]. Ylistrum was identified as a lineage basal to the clade Pectininae, separated from its considered tribe Amusiini, a conclusion supported by Alejandrino et al. [4], Sherratt et al. [5], and Serb [6]. Argopecten were clustered on the same branch, forming a sister group with the clade Amusium+Pecten. The close relationship between A. pleuronectes and Pecten maximus + Pecten albicans was also noted, consistent with studies of Barucca et al. [35], Alejandrino et al. [4], and Feng et al. [34].

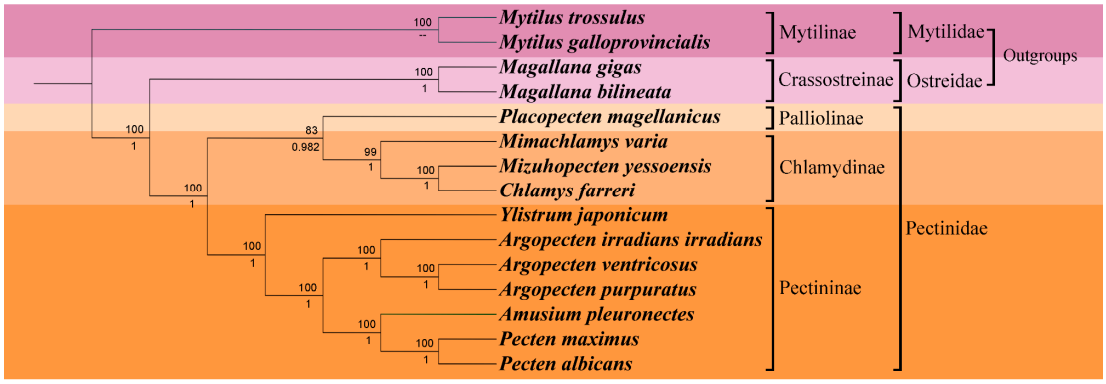

Figure 4. Phylogenetic tree derived from Maximum likelihood (ML) and Bayesian inference (BI) based on the sequences of mitochondrial protein-coding genes (PCGs). Numbers above the branches indicate bootstrap support; numbers below branches are Bayesian posterior probability. A dash indicates no support for that node.

Another ML tree (Figure 5) was constructed based on 16S rRNA sequences, three specimens of Y. japonicum from China (PP571649) and Japan (HM622702, KF982785) [4,36] were selected. The result was comparable with Mynhardt et al. [1]. Genus Amusium and Pecten forming a sister group again. Antillipecten antillarum as a lineage basal, forming a sister group with the clade Anguipecten+Ylistrum. Two Ylistrum taxa formed a sister clade and well separated with Amusium. Specimens HM622702 and KF982785 were in same branch, sharing an ancestor with the Chinese individual. All Y. japonicum finally converged into the same branch, this provided evidence that it was a monophyletic clade, and also shown the closely genetic distance between its individual.

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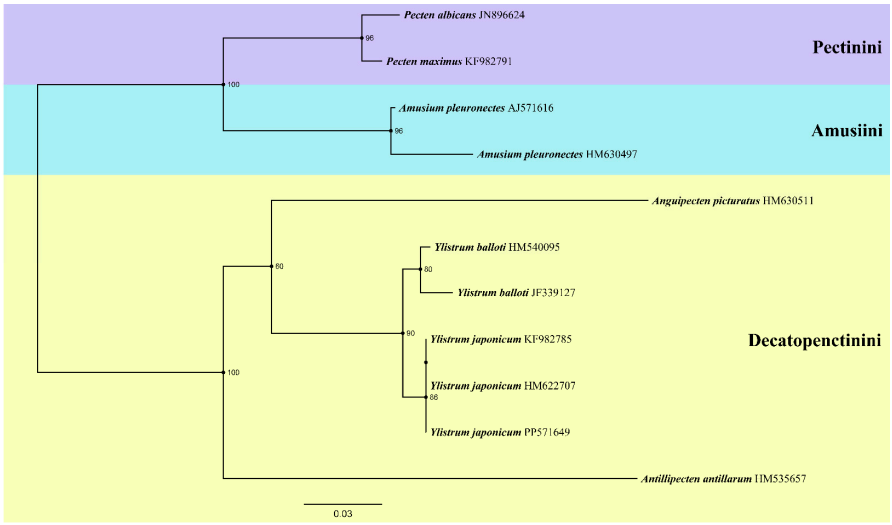

Figure 5. Phylogenetic tree of genus *Ylistrum* and some species from three tribe of Pectininae inferred by Maximum likelihood (ML) of *16S rRNA* sequences. Numbers indicate bootstrap support.

2.7. Systematic Descriptions

The systematic arrangement has been adopted from Waller [31] and Serb [6].

Order Pectinida Gray, 1854
Superfamily Pectinoidea Rafnesque, 1815
Family Pectinidae Rafnesque, 1815
Subfamily Pectininae Rafnesque, 1815
Tribe Decatopectinini Waller, 1986
Genus *Ylistrum* Mynhardt and Alejandrino, 2014
Ylistrum japonicum (Gmelin, 1791)

Type locality: Japan.
Distribution: Japan (South of central Honshu Island), Korean (Jeju Island), China (Taiwan Province, Guangdong Province, Guangxi Province and Hainan Province.).
Morphological description: Shell large, round, smooth, and glossy. The left valve is dark red to reddish-brown, covered with concentrically arranged dark brown fine lines and spots, The color is slightly lighter at the umbo, with small light-colored spots. The right valve is slightly flat, pale yellow to light tan, white near the umbo, also concentrically arranged brown spots on surface. Two small auricles and slightly different in size, The color of auricles on left valve is darker The inner surface of the shell is white, with the left valve having a yellow to light brown edge, sometimes the inner edge of left valve is pale brown. Interior radial ribbing on both valves, the specimen collected from the Hailing Island with 33-43 ribs on the left valve and 42-49 ribs on the right valve .
Remarks: In original description, the species group from China were record as a subspecies *Amusium japonicum taiwanicum* Habe, 1992 [37], and now its a synonymised name of *Y. japonicum*. Unlike individuals from Japan, the color of specimens from China are not bright, and the concentrically arranged brown spots are presence on the right valve, these are the morphological difference between the individuals from above two producing areas. Despite there are morphological differences, their molecular biological evidence indicates that they are the same species. The counting of internal rib by different author is not always the same (e.g. Zhang et al. [38], Wang [7], Mynhardt et al. [1]). In addition, as counts had completely overlapping rangesand it also could not be used to differentiate between the two *Ylistrum* species [1]. Overall, the most significant difference between *Y. japonicum* and *Y. balloti* are the color of their auricles on right valve and the spots in a concentric pattern on both two valves, to the former species, the auricles on right valve are generally darker, and spots always appear along with theirs valves repair marks.

 doi:10.20944/preprints202407.2376.v1

When mixed with the long-ribbed scallop in trawl nets, 15 out of 52 scallops were *Y. japonicum* in one trawl, with sandy bottom sediment and others are *A. pleuronectes*. The average shell length of *Y. japonicum*, in the population was 84.27±10.63mm (n=15), the average shell height was 82.97±9.48mm, and the average shell width was 16.70±2.23mm.

3. Discussion

The family Pectinidae, as a clade within Bivalvia, exhibits a significant range of morphological and behavioral variations, rendering it of great importance in ecology, evolution, and commercial activities. Nevertheless the taxonomy of Pectinidae has long been a source of debate within the scientific community.

Waller [30] hypothesis for the classification and evolution of Pectinidae based on morphology, particularly focusing on pre-radial stage shell microsculpture, and incorporating fossil data and geological evidence. In 2006, Waller revised his scallopidae phylogenetic hypothesis in conjunction with previous molecular genetic studies, leading to the successful establishment of a stable classification method. Much of the contemporary taxonomic research on Pectinidae is grounded in the taxonomy framework developed by Waller, as evidenced by the work of Serb [6] and Smedley et al. [28]. Despite the enhancements to Waller's hypothesis, which have been fueled by advances in molecular techniques and the expansion of fossil evidence, controversies over the correct classification of Pectinidae remain In molecular phylogeny, discrepancies in findings can stem from a variety of factors, such as the choice of single genetic sequences [39] or the concatenation of multiple sequences [40,41]. Additionally, the accuracy and limitations of phylogenetic tree construction methods [6,42,43], the number and distribution of sampled species, the selection of outgroups, and the processing of sequences can all influence the results obtained. A case in point is the phylogenetic placement of the Palliolinae, a monophyletic subgroup within the Pectinidae. Different studies have placed the Palliolinae within various clades, either with the Pectininae or the Chlamyinae, highlighting the ongoing inconsistencies in phylogenetic resolution within this family (e.g. Alejandrino et al. [4], Sherratt et al. [5], Xu et al. [21], Lin et al. [22], Malkócs et al. [25], Li et al. [27], Smedley et al. [28], Saavedra and Peña [33], Feng et al. [34] and Malkowsky and Klussmann-Kolb [40]). In contrast, Waller's hypothesis placed Palliolinae with Pectininae as a sister group [31]. On the mito-phylogenomics level, differences in sequence selection and methodologies can lead to varying results, as evidenced by studies by Lin et al. [22], Malkócs et al. [25] and Li et al. [27]. The divergence time estimation analysis conducted by Lin et al. [22] based on concatenated mitochondrial PCG gene sequences yielded a similar topology to our studies, suggesting that similarities and differences in results may be related to sample selection. In summary, differences in these results can be attributed to variations in sequence selection, analytical approaches, and species sampled.

Although the robustness of a phylogenetic tree can be affected by a variety of factors, it is noteworthy that Ylistrum and Amusium have consistently been distinguished in past molecular phylogenetic investigations. The placement of Ylistrum within the subfamily Pectininae is well-supported, as evidenced by the study of Matsumoto and Hayami [39] and subsequent research. Nevertheless, due to the significant morphological and distributional similarities between Y. japonicum and A. pleuronectes, Ylistrum has historically been grouped with the Amusiini, even though molecular genetic studies have consistently pointed to its distinctiveness from Amusium. Our phylogenetic study based on complete mitochondrial genome indicate that Ylistrum has ancient origins, but its preciseplacement within the subfamily Pectininae remains ambiguous due to insufficient sample data. Alejandrino et al. [4] analyzed the phylogeny of 81 extant taxa from the Pectinidae, based on the nuclear Histone H3, 12S rRNA, 16S rRNA data and 28S rRNA data, the result shows that Ylistrum was placed among the species of the tribe Decatopectinini, and Aumsium were nest in different clade (Pectinini). Subsequently, Smedley et al. [28] expanded the dataset to 62 Pectinidae species on the basis of Alejandrino et al. [4] into a new phylogenetic analysis, clade Ylistrum was once again placed in the Decatopectinini tribe. Interestingly, it forms a sister clade with two Annachlamys species belongs to the tribe Pectinini, but the phylogenetic location of Annachlamys was still debated [3]. Mynhardt et al. [1] focused on the phylogenetic analysis of

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Aumsium and Ylistrum, restored their respective monophyletic clades and described Ylistrum as a new genus. In this study, Ylistrum also form a sister groups with a Decatopectinini species (A. antillarum). Our phylogenetic analysis based on 16S rRNA indicates that Y. japonicum from Japan and China are monophyletic, and same as former studies, two Ylistrum taxa were still nest in the tribe Decatopectinini. In light of these findings and previous studies, we adhere to Serb's classification [6], positioning Ylistrum within the Decatopectinini tribe.

The complete mitochondrial genome sequence data for Ylistrum remains inadequate. The available data for Y. balloti (accession number ON041136) is in fact, based on an erroneous identification. When analyzed using NCBI-BLAST (with an alignment length greater than 1200), this sequence exhibits a high degree of similarity (greater than 98.99%) to Y. japonicum, but less than 94.10% similarity to Y. balloti. This strongly suggests that ON041136 is more closely related to Y. japonicum. Additionally, the collection location of ON041136 is Beihai, Guangxi, China, which is problematic since there are no documented distributions of Y. balloti within China. The mention of Y. balloti in the Chinese Zoology book [7] is considered a misidentification, highlighting the need for a critical revision of this information.

4. Materials and Methods

4.1. Sample Collection

A total of 50 specimens were collected during 2022 to 2023 from Hailing Island, Yangjiang City, Guangdong Province, China (21.61N, 111.93E) (Figure 6). Morphometric measurements were performed using an electronic vernier caliper (0.1mm), body measurement traits (shell length, shell height, shell width, shell weighet, etc.) were recorded for further investigation. All collected samples are intended for commercial purposes, and there are no concerns regarding animal ethics. The morphological characteristics of these specimens were categorized and compared in accordance with Zhang et al. [38] and Zhang [44]. From this collection, one specimen had its adductor muscles extracted (5g), which were then preserved in a -80°C freezer for subsequent analytical procedures.

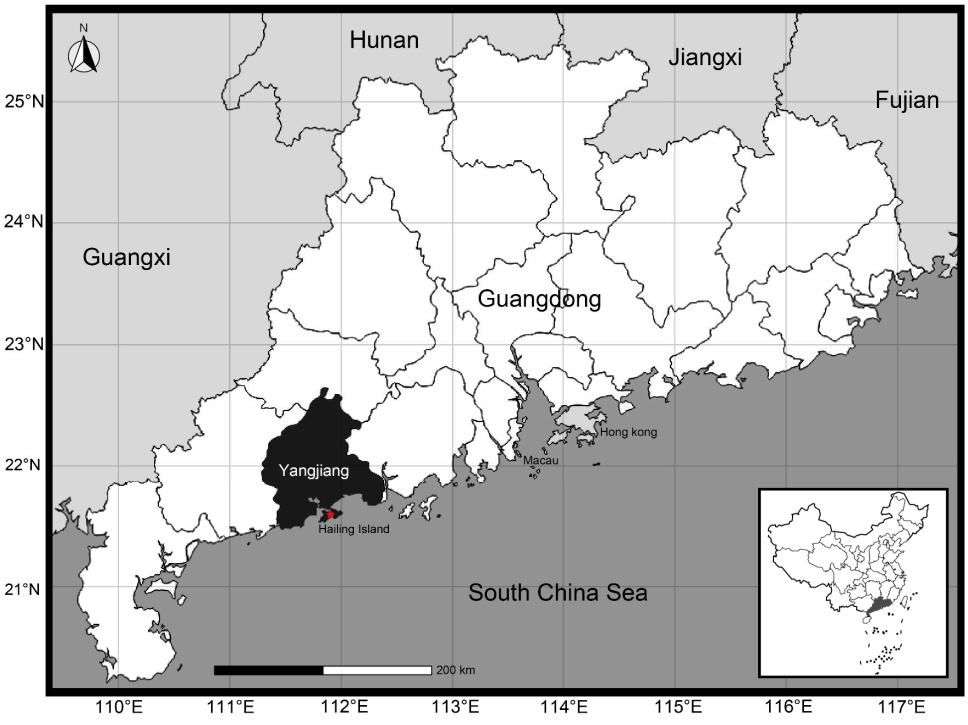

Figure 6. Sampling location of *Ylistrum japonicum* (modified from d-maps: https://d-maps.com).

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4.2. DNA Extraction, Library Preparation and Next Generation Sequence

gDNA was extracted by MagPure Bacterial DNA Kit (Magen, Guangzhou, China) following pre-grind in liquid nitrogen. Qubit dsDNA HS assay kit (Sangon, Shanghai, China) was used to test concentration and 1% Agarose gel electrophoresis to confirm intergrity. The library preparation and next generation sequence was finished by Sangon Biotech (Shanghai) Co., Ltd.. First, 500ng quantified DNA was randomly fragmented by Covaris (Woburn, USA). Next, Hieff NGS®MaxUp II DNA Library Prep Kit for Illumina® (YEASEN, Shanghai, China) was used for next steps. Briefly, Endprep enzyme was added to repair end and 3′.end A tail ligation. Then adaptor was ligated by enhancer and Fast T4 DNA ligase. Index primer was added by PCR and the amplified product about 400bp was selected by DNA selection beads. The library concertration and size was confirmed by Qubit 4.0 (Thermo, Waltham, USA) and 2% agarose gel electrophoresis respectively.

Then the libraries were pooled and loaded on Novaseq 6000 (Illumina. San Diego, USA)/DNBseq-T7 (BGI, Shenzhen, China) sequencer by 2×150 bp paired end sequence kit according to the manufacture´s instructions.

4.3. Sequence Assembly and Annotation

Rawbases yielded at least 6 GB was used for downstream analysis. First, all of the raw reads were trimmed by Fastqc v0.11.2 [45]. The software SPAdes v3.15 [46] was used to assemble the raw sequence reads into contigs. tBLASTn and GeneWise were used to obtain the CDS gene boundary by reverse alignment with the near-source reference database, the tRNA sequence annotation was obtained by MiTFi, Rfam used cmsearch alignment identifies non-coding rRNA and the final summary into complete annotation results. The circular gene maps of the specie Y. japonicum was drawn by Circos.

4.4. Gene Collinearity

Gene collinearity among complete mitochondrial sequences of five Pectininae species was explored to assess their phylogenetic relationship with Y. japonicum, using the progressiveMauve algorithm and default parameters (including default seed weight, determine locally collinear blocks, and full alignment) in the Mauve v2.4.0 [47].

4.5. Phylogenetic Analysis with Mitochondrial Genome

Two phylogenetic analyses were conducted based on the complete mitochondrial sequences of Y. japonicum in this study. Following the meyhodologies established in previous studies by Malkowsky and Klussmann-Kolb [39], Xu et al. [21] and Malkócs et al. [25], a total of 15 mitochondrial sequences of Pectinidae species and outgroup taxa were selected to construct phylogenetic trees. This selection includes 11 Pectinidae species across three subfamilies: Pectininae, Palliolinae, and Chlamydinae. The available mitochondrial genome sequences were obtained from GeneBank, incorporating 13 complete mitochondrial genomes and 2 incomplete sequences (KP900974, KP900975), each over 16,000 base pairs in length. Two Ostreidae species Magallana bilineata, Magallana gigas and two Mytilidae species Mytilus galloprovincialis, Mytilus trossulus were used as outgroups.

PhyloSuite v1.2.3 [48] was utilized to extract the protein-coding genes (PCGs) from each sequence. All Sequences were aligned in batches with MAFFT v7.505 [49]. The alignments were refined using the codon-aware program MACSE v2.06 [50] which preserves reading frame and allows incorporation of sequencing errors or sequences with frameshifts. Ambiguously aligned fragments of the alignments were removed in batches using Gblocks 0.91b [51]. ModelFinder v2.2.0 [52] was used to select the best-fit partition model. The phylogenetic tree was subsequently constructed using both the Maximum-likelihood (ML) method in IQ-TREE v2.2.0 [53,54] and Bayesian inference (BI) in MrBayes v3.2.7a [55]. Branch support was determined with 5000 bootstrap iterations for best-scoring ML tree. Markov Chain Monte Carlo (MCMC) analyses were run for 1,000,000 generations (sampling

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every 1000 generations), in which initial 50% of sampled data were discarded as burn-in. The result was beautified with FigTree v1.4.4.

To explore in more detail the monophyletic development of Y. japonicum and its taxonomic position in Pectininae, another ML tree was constructed using 16S rRNA by PhyloSuite v1.2.3 [48], 3 Y. japonicum specimens from different regions (China and Japan) and the other 8 specimens of subfamliy Pectininae were selected. All Sequences were aligned in batches with MAFFT v7.505 [49] and pruned by Gblocks 0.91b [51]. Branch support was determined with 5000 bootstrap iterations for best-scoring ML tree. List of specimens included in molecular studies shown in Table 2.

Table 2. List of specimens included in the molecular studies.

Subfamily	Tribe in this research	Previous Tribe	Species	Genbank accession numbers	Genetic compartments
Ingroup					
	Aequipectinini	Aequipectinini	*Argopecten irradians irradians*	DQ665851	mitogenome
	Aequipectinini	Aequipectinini	*Argopecten purpuratus*	KT161260	mitogenome
	Aequipectinini	Aequipectinini	*Argopecten ventricosus*	KT161261	mitogenome
Pectininae	Amusiini	Amusiini	*Amusium pleuronectes*	MT419374	mitogenome
	Amusiini	Amusiini	*Amusium pleuronectes*	AJ571616	16S rRNA
	Amusiini	Amusiini	*Amusium pleuronectes*	HM630497	16S rRNA
	Decatopectinini	Amusiini	*Ylistrum japonicum*	PP571649	mitogenome
	Decatopectinini	Amusiini	*Ylistrum japonicum*	KF982785	16S rRNA
	Decatopectinini	Amusiini	*Ylistrum japonicum*	HM622707	16S rRNA
	Decatopectinini	Amusiini	*Ylistrum balloti*	HM540095	16S rRNA
	Decatopectinini	Amusiini	*Ylistrum balloti*	JF339127	16S rRNA
	Decatopectinini	Decatopectinini	*Anguipecten picturatus*	HM630511	16S rRNA
	Decatopectinini	Decatopectinini	*Antillipecten antillarum*	HMS35657	16S rRNA
	Pectinini	Pectinini	*Pecten maximus*	KP900975	mitogenome
	Pectinini	Pectinini	*Pecten maximus*	KF982791	16S rRNA
	Pectinini	Pectinini	*Pecten albicans*	KP900974	mitogenome
	Pectinini	Pectinini	*Pecten maximus*	JN896624	16S rRNA
Palliolinae	Palliolini	Palliolini	*Placopecten magellanicus*	DQ088274	mitogenome
	Chlamydini	Chlamydini	*Chlamy farreri*	EF473269	mitogenome
Chlamydinae	Fortipectinini	Fortipectinini	*Mizuhopecten yessoensis*	FJ595959	mitogenome
	Mimachlamydini	Mimachlamydini	*Mimachlamys varia*	MZ520326	mitogenome
Outgroup					
Crassostreinae			*Magallana bilineata*	MT985154	mitogenome
			Magallana gigas	MZ497416	mitogenome
Mytilinae			*Mytilus galloprovincialis*	DQ399833	mitogenome

Mytilus trossulus	AY823625	mitogenome	

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