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Molecular and morphological identification of *Argyrops* and *Acanthopagrus* (Sparidae) in the Java Sea, Indonesia

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Abstract. *Nuryanto A, Fathurahman FR, Bhagawati D, Ardli ER, Wibowo DN, Atang. 2026. Molecular and morphological identification of* Argyrops *and* Acanthopagrus *(Sparidae) in the Java Sea, Indonesia. Biodiversitas 27 (5): d270508. https://doi.org/10.13057/biodiv/d270508.* Accurate species identification is fundamental to effective fisheries management and understanding of marine biodiversity. Previous morphological records have reported *Argyrops spinifer* from Indonesian waters, including the Java Sea, Indonesia, while recent molecular evidence from southern Java identified *Argyrops bleekeri*. These inconsistencies highlight the need for integrative taxonomic approaches. This study aimed to evaluate sparid diversity along the northern coast of Java Island using morphological examination and DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Fish specimens were collected from four landing sites (Pekalongan, Cirebon, Indramayu, Karawang) along the Java Sea. A total of 31 specimens were examined, comprising 15 *Acanthopagrus* and 16 *Argyrops*. High-quality COI sequences were obtained from 30 specimens (96.8% success rate). Molecular identification assigned all *Acanthopagrus* specimens to *Acanthopagrus pacificus* (n:15). Among *Argyrops*, 14 specimens were identified as *A. bleekeri*, while only one specimen was identified as *A. spinifer*, representing just 6.7% of *Argyrops* samples. Genetic similarity values (99.04-100%) and low intraspecific K2P distances (0.0027-0.0036) confirmed species-level assignments, while phylogenetic analysis supported clear separation between taxa. The coexistence of *A. bleekeri* and *A. spinifer* at the Indramayu landing site provides the first molecular confirmation of *A. spinifer* in the Java Sea and demonstrates the limitations of morphology alone, as both species were indistinguishable based on external traits. These findings highlight the risk of species misidentification in fisheries data when relying solely on traditional taxonomy. Integrating molecular tools with morphological examination is therefore critical to avoid compromising stock assessments and biodiversity monitoring in Indonesian marine ecosystems.

Keywords: *Argyrops,* DNA barcoding, genetic diversity, Java Sea, species identification

INTRODUCTION

Sparidae represent a globally important family of marine fishes, playing key economic and ecological roles across tropical and subtropical regions worldwide. Sparids, commonly known as seabreams and porgies, contribute substantially to commercial and artisanal fisheries while helping maintain coastal ecosystem health (Wu et al. 2018). Typical sparid features include laterally flattened bodies, strong jaws, and molar-like teeth, adaptations that allow them to consume diverse benthic prey such as mollusks, crustaceans, and small fish (Pombo-Ayora et al. 2022). Globally, the Sparidae include more than 150 recognized species distributed across the Atlantic, Indian, and Pacific Oceans, reflecting their remarkable ecological plasticity (Iwatsuki et al. 2010).

Despite their broad distribution and economic importance, sparid taxonomy remains incompletely resolved. Species identification based on morphology is often problematic due to overlapping diagnostic characters, broad geographic ranges, and environmentally induced phenotypic plasticity. These factors are particularly pronounced in juveniles or individuals inhabiting variable environments, where morphological variation may mimic interspecific differences or converge among related taxa (D'Iglio et al. 2021). Reliable taxonomic identification is therefore critical, as inaccuracies can propagate through ecological research, biodiversity inventories, fisheries statistics, and management strategies.

Molecular approaches, especially DNA barcoding, have become essential tools over the last twenty years for clarifying taxonomic uncertainties. DNA barcoding employs the mitochondrial COI gene, whose evolutionary rate is sufficiently rapid to differentiate closely related species yet slow enough to remain largely conserved within species boundaries. This technique has proven effective across many taxonomic groups, including sparids, where it shows strong capacity to distinguish species that are morphologically similar (Ceruso et al. 2021). Beyond taxonomic clarification, barcoding has revealed instances where genetically distinct lineages were previously considered single species based on morphology (Wu et al. 2018). Recognition of such hidden diversity has direct implications for fisheries management, as distinct species may differ in growth rates, habitat use, reproductive strategies, population structure, and resilience to exploitation (Martin et al. 2022; Shin and Allmon 2023).

In Indonesia, sparid fishes constitute an important component of coastal fisheries and food security, particularly in areas where small-scale fishing communities depend heavily on reef-associated and demersal fishes. However, molecular data on Indonesian sparids remain limited. To date, seven species have been reported from national waters: *Acanthopagrus pacificus, Argyrops bleekeri, Argyrops spinifer, Dentex spariformis, Pagrus auratus, Rhabdosargus niger,* and *R. sarba* (Iwatsuki et al. 2010; Fricke et al. 2025; Froese and Pauly 2025). Most identifications have relied on morphological features, which are prone to error in regions where multiple congeners co-occur and were preserved or landed specimens may lack complete diagnostic characters. Recent molecular investigations from the Indian Ocean south of Java identified *A. bleekeri*, a species previously unrecorded in that area, suggesting that multiple *Argyrops* species may occur in Indonesian waters (Nuryanto et al. 2023a, b). In contrast, morphological surveys from the Java Sea have consistently reported *A. spinifer* (Mous et al. 2023a; Halim and Wahyu 2023). These contrasting results highlight the need for integrative taxonomic methods that combine morphology and molecular evidence to improve species identification and support more accurate assessments of sparid diversity in Indonesia.

This study aims to resolve taxonomic ambiguity within *Argyrops* and *Acanthopagrus* in the Java Sea by integrating morphological identification with COI-based DNA barcoding. We hypothesize that molecular analysis will reveal species-level distinctions that are not detectable through morphology alone, particularly among closely related *Argyrops* species. Specifically, we predict that DNA barcoding will discriminate *A. bleekeri* from *A. spinifer* despite their morphological similarity, and confirm whether *A. spinifer* occurs in the Java Sea or whether previous records represent misidentifications of *A. bleekeri*. Accordingly, this study addresses whether integrative taxonomy can accurately discriminate sparid species in Indonesian waters and clarify the occurrence and coexistence of *A. bleekeri* and *A. spinifer*.

MATERIALS AND METHODS

Research area and sample collection

Field trips were conducted during two periods: June to August 2024 and July to September 2025. These periods were selected to coincide with peak fishing activity and optimal species availability in the Java Sea, Indonesia, as documented by Napitupulu (2024). Fish samples were purchased from local fishermen at auction centers in four locations along the northern coast of Java: Karawang (6°18'S, 107°18'E), Indramayu (6°20'S, 108°19'E), Cirebon (6°43'S, 108°34'E), and Pekalongan (6°53'S, 109°40'E) (Figure 1). These sites represent major landing areas that receive catches from diverse fishing grounds within the Java Sea.

Sampling through auction centers enabled access to specimens from multiple fishing vessels and gear types while ensuring compliance with local fisheries regulations. This approach also minimized additional impact on fish populations, as specimens were obtained from existing commercial catches. A total of 31 specimens were collected (Table 1), comprising 15 individuals morphologically identified as *Acanthopagrus* and 16 as *Argyrops*. The collection procedure followed Indonesian fisheries legislation (Law No. 31/2004 on Fisheries; Regulation No. 19/PERMEN-KP/2021 on Fish Catch Utilization), and no ethical approval was required as specimens originated from legal, non-protected catches.

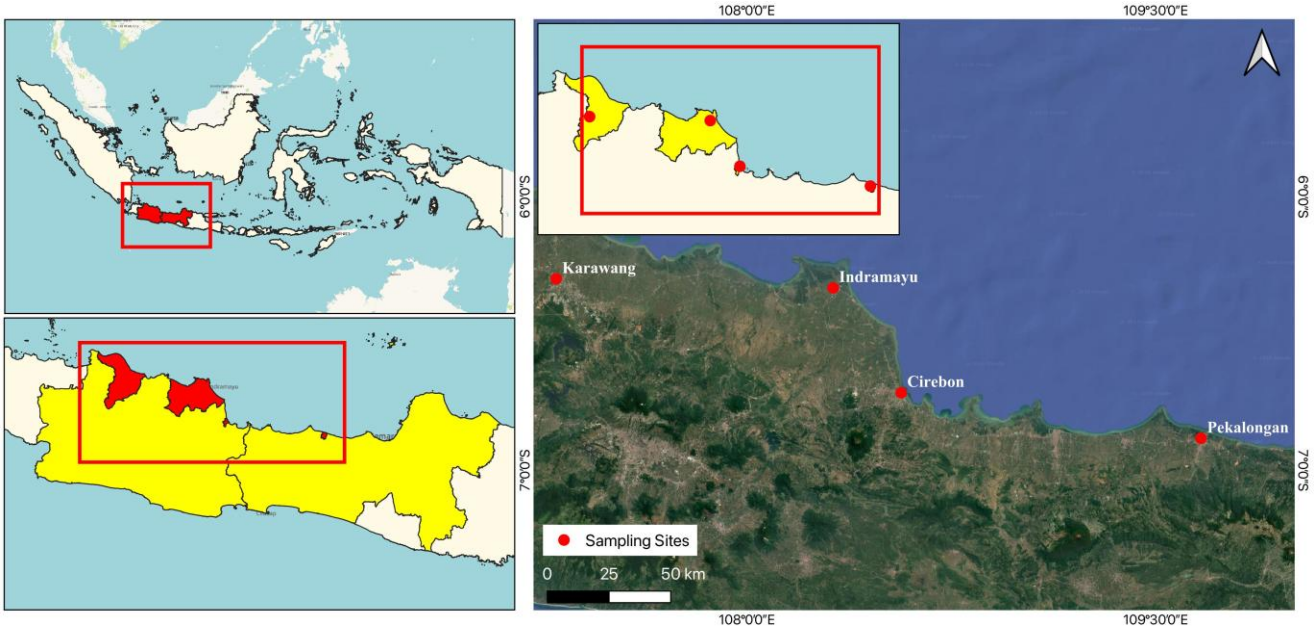


Figure 1. Research area map of Java Island showing sampling locations along the Java Sea Coast, Indonesia

Table 1. Number of fish specimens obtained from each sampling location

Sampling site	Date	Number of specimens
Pekalongan	August 2024	1
Cirebon	July 2025	13
Indramayu	July 2025	16
Karawang	July 2025	1
Total		31

Notably, there are several limitations of the sampling design. All *Argyrops* specimens were obtained from a single landing site (Indramayu), which limits spatial inferences for this genus. Additionally, because specimens were purchased from commercial auction centers, precise capture locations, depths, and habitat characteristics are unknown. Therefore, the findings should be interpreted as applying to the sampled specimens and landing sites rather than to the entire Java Sea Region.

Research procedures

Fish and tissue collection

Complete individual fish were purchased from local fishermen immediately upon landing to ensure freshness for morphological examination. Each specimen was photographed, assigned a specimen voucher code, and processed for preservation. For morphological analysis, whole specimens were preserved in 70% ethanol. For molecular analysis, fin clips (approximately 0.5 cm²) were excised from the right pectoral fin and preserved separately in 96% absolute ethanol. Ethanol was used instead of formalin due to formalin's detrimental effects on DNA integrity, including fragmentation and cross-linking that inhibit PCR amplification (Appleyard et al. 2021).

To ensure proper preservation, the ventral part of each fish was carefully perforated to facilitate ethanol penetration into the abdominal cavity and prevent decomposition of internal organs. Voucher specimens were deposited in the Animal Taxonomy Laboratory, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia. Voucher codes are as follows: *Acanthopagrus* from Pekalongan (JB AP-01), Karawang (SBG AP-01), and Cirebon (CRB AP-01 to CRB AP-13); *Argyrops* from Indramayu (IDR AB-01 to IDR AB-16).

Morphological identification

Morphological identification was performed following established taxonomic references for Sparidae, including Iwatsuki et al. (2010), Iwatsuki and Heemstra (2018), Nuryanto et al. (2023b), and Froese and Pauly (2025). Both qualitative and quantitative characteristics were examined to ensure comprehensive characterization. Qualitative traits assessed included body form (oblong, moderately oblong, or elongated), coloration of dorsal and ventral surfaces (based on fresh specimens and high-resolution photographs), head profile (sloping angle of forehead), and scale type. These features were recorded descriptively and compared with published descriptions. Quantitative characters included meristic counts and morphometric measurements. Meristic characters counted were: dorsal fin spines (D) and soft rays (D′), anal fin spines (A) and soft rays (A′), pectoral fin soft rays (P), total gill rakers on the first arch, and lateral-line scales. Dorsal and anal fin formulas were expressed following standard ichthyological notation, with Roman numerals for spines and Arabic numerals for soft rays.

Morphometric measurements were taken using digital calipers to the nearest 0.1 mm. Measured variables included: standard length (SL, from tip of snout to base of caudal fin), head length (HL, from tip of snout to posterior margin of operculum), body depth (BD, maximum vertical depth), and eye diameter (ED, horizontal orbital diameter). Proportional indices (BD:SL and ED:HL) were calculated to facilitate comparison with literature values. It is important to emphasize that morphological analysis in this study allowed for reliable genus-level identification (separating *Acanthopagrus* and *Argyrops*) but did not provide consistent diagnostic characters to reliably distinguish between the two *Argyrops* species (*A. bleekeri* and *A. spinifer*). This limitation is explicitly acknowledged and underscores the value of molecular analysis for species-level resolution.

Molecular identification

Total genomic DNA was extracted from fin clips using the Invitrogen PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol for animal tissue. Briefly, fin clips were air-dried to remove excess ethanol, then incubated in digestion buffer with proteinase K at 55°C until complete lysis. DNA was bound to spin column membranes, washed with buffer solutions, and eluted in 50 µL of elution buffer. DNA quality and concentration were assessed by electrophoresis on a 1% agarose gel stained with SyBr Safe DNA gel stain (Invitrogen, USA) and visualized under a UV transilluminator. Samples showing clear, high-molecular-weight bands without smearing were considered suitable for PCR amplification.

A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal fish primer pair FishF1 (5'-TCAACCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGCCAAAGAATCA-3') developed by Ward et al. (2005). PCR reactions were performed in a total volume of 50 µL containing 25 µL of 2× MyTaq HS Red Mix (Bioline, UK), 1 µL each of forward and reverse primers (10 µM), 1 µL of 0.5% Bovine Serum Albumin (BSA, Promega, USA) to improve amplification efficiency by binding PCR inhibitors, 2 µL of DNA template (approximately 50-100 ng), and nuclease-free water to volume.

Thermal cycling was conducted in a Primus 25 Thermal Cycler (Peqlab hain, Germany) under the following conditions: initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 15 s, annealing at 50°C for 15 s, and extension at 72°C for 10 s; and a final extension at 72°C for 5 min. Successful amplification was verified by electrophoresis of 5 µL PCR product on a 1.5% agarose gel stained with SyBr Safe, visualized under UV light, and

compared to a 1kb DNA ladder (Smobio, China) to confirm fragment size (approximately 650-700 bp).

Strong, single-band PCR products were purified and sequenced bidirectionally by 1st BASE Asia (Singapore) using Sanger sequencing technology with the same primers used for amplification. Raw chromatograms (ABI files) were received and processed using BioEdit version 7.0 (Hall 2005). For each specimen, forward and reverse sequences were assembled into a consensus sequence. Low-quality bases at the 5' and 3' ends were trimmed by visual examination of chromatogram peaks to ensure unambiguous base calls, following standard practices in DNA barcoding research (Wulandari and Rais 2021; Jarulis et al. 2022). The trimmed COI sequences ranged from 570 to 652 base pairs in length, depending on read quality at the fragment ends. To verify that sequences represented functional mitochondrial COI gene fragments, Open Reading Frames (ORFs) were checked using the ORF Finder tool available through the National Center for Biotechnology Information (NCBI), applying the vertebrate mitochondrial genetic code. Sequences showing internal stop codons or frameshifts would have been excluded as possible pseudogenes.

Data analysis

Morphological and genetic species concepts were applied in an integrated framework to ensure robust specimen identification. Morphological characteristics were analyzed descriptively through comparison with reference data (Nuryanto et al. 2023a) to delineate fish specimens at the genus level. The genetic species concept was subsequently employed to determine the taxonomic status of samples collected from the northern coast of Java (Figure 3).

Consensus sequences were compared against reference sequences in BOLD Systems v5 and GenBank's nr/nt database using BLAST and the BOLD identification engine. A 99% genetic similarity threshold (or 1% divergence) was applied for species-level assignment in Sparidae, based on thresholds established by Ahmed et al. (2021) and Nuryanto et al. (2023b). Sensitivity analysis using alternative similarity thresholds of 98.5% and 99.5% confirmed consistent species assignments across all samples. Reference sequences for target species were obtained from GenBank, including: *A. pacificus* (GU673695, OR512858, GU674104, PQ319711), *A. bleekeri* (GU673360, KU682551, OQ387142), and *A. spinifer* (DQ107836, DQ107838, MT943701, MT943702; Hasan et al. 2021). Two members of the family Lethrinidae, *Lethrinus harak* and *Lethrinus ornatus*, were included as outgroups based on their phylogenetic position as a closely related but distinct family within Perciformes (Nuryanto et al. 2023b).

Pairwise genetic distances and phylogenetic analyses were performed to assess species delineation. The Kimura 2-parameter (K2P) model was selected to provide a reliable estimation of genetic divergence for mitochondrial COI sequences by accounting for unequal rates of transition and transversion, while maintaining model simplicity suitable for short barcode fragments (Kimura 1980; Hebert et al. 2003). This model has been extensively validated and standardized in DNA barcoding research to obtain consistent and comparable distance estimates across taxa (Collins et al. 2012). A genetic distance threshold of 0.03 was applied for species delimitation (Ratnasingham and Hebert 2013; Miralles et al. 2024), consistent with previous research on marine fishes (Amatya 2019; Kusbiyanto et al. 2020; Chen et al. 2021).

Phylogenetic trees were reconstructed using the Maximum Likelihood (ML) method based on the K2P model with codon positions 1, 2, and 3 partitioned. Sequences were aligned using MUSCLE with default parameters, and alignments were trimmed to a common length to ensure all sequences covered the same gene region. Bootstrap resampling (1000 replicates) was performed to assess node support, with values ≥70% considered strong support, 50-69% moderate support, and <50% weak support. Initial trees for heuristic searches were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances, with the topology having the highest log-likelihood value selected as the final tree. Tree polarity was inferred by comparison with outgroup sequences.

Species delineation was confirmed when specimens met three criteria: (i) genetic similarity ≥99% to reference sequences of known species; (ii) clustering with reference sequences in a monophyletic clade with bootstrap support >70%; (iii) exhibiting short branch lengths within clades consistent with intraspecific variation (Xu et al. 2015; Bhagawati et al. 2020; Kusbiyanto et al. 2020; Palecanda et al. 2020). All genetic distance calculations and phylogenetic reconstructions were conducted in MEGA version 12 (Kumar et al. 2024).

RESULTS AND DISCUSSION

The integration of morphological examination and COI DNA barcoding provided clarification of Sparidae species composition at the sampled landing sites along the Java Sea. The results verified the identities of the collected specimens and revealed the coexistence of *A. bleekeri* and *A. spinifer* at the Indramayu landing site. The combined evidence demonstrates the limitations of relying solely on morphology in groups with overlapping diagnostic traits and highlights the value of molecular tools for accurate species identification. The following sections present the morphological features, genetic distances, and phylogenetic relationships of Sparidae specimens from the Java Sea, with interpretation carefully scaled to the evidence provided by the dataset.

Morphological identification

Morphological examination of the 31 specimens revealed two clearly differentiated groups corresponding to the genera *Acanthopagrus* and *Argyrops*. Fifteen individuals were identified as *Acanthopagrus*, exhibiting a moderately oblong body, greyish-silver dorsal surfaces, and dark silvery ventral regions (Figure 2.A). These traits are consistent with the genus' characteristic morphology

described by Iwatsuki et al. (2010) and Ebner (2021). The head profile was gently sloping, and the body was moderately compressed laterally.

Sixteen specimens were classified as *Argyrops*, distinguished by a more elongated body shape, pinkish-silver dorsal areas, and silvery-rose to pinkish-silver ventral surfaces (Figure 2.B). These features align with descriptions by Iwatsuki and Heemstra (2018). The forehead exhibited a steeper slope compared to *Acanthopagrus*, and the body was slightly more elongated. The clear visual separation between these two groups reflects the morphological distinctiveness of these genera within the Sparidae family.

Diagnostic comparisons between *Acanthopagrus* and *Argyrops* supported the visual separation (Table 2). Both genera exhibited similar dorsal and anal fin formulas (D XI, 11-13; A III, 8-10) and overlapping gill-raker counts (9-11), indicating that these meristic characters alone are insufficient for genus-level discrimination. However, *Acanthopagrus* had higher lateral-line scale counts (42-48) than *Argyrops* (36-40), consistent with previous taxonomic descriptions (Iwatsuki et al. 2010; Nuryanto et al. 2023b). This character provided a reliable diagnostic feature for genus-level identification.

Morphometric ratios also differed between the genera. *Argyrops* showed a deeper body relative to standard length (BD:SL: 0.38±0.01) compared to *Acanthopagrus* (BD:SL: 0.37±0.01). Eye diameter relative to head length was also slightly larger in *Argyrops* (ED:HL: 0.22±0.01) than in *Acanthopagrus* (ED:HL: 0.21±0.01). These proportional differences provided quantitative evidence distinguishing the two genera at the morphological level.

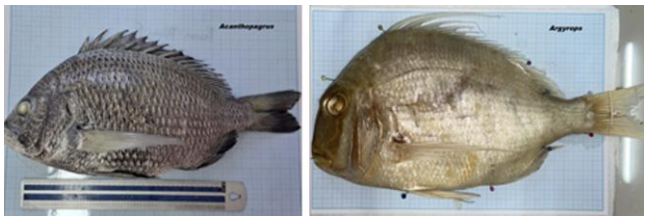
Notably, however, the morphological analysis did not allow a reliable distinction between *A. bleekeri* and *A. spinifer* within the *Argyrops* genus. Specimens of both species shared overlapping external features, including body shape, coloration patterns, and meristic counts. This limitation underscores the necessity of molecular analysis for species-level identification in this group and is consistent with the known morphological conservatism within *Argyrops* (Iwatsuki and Heemstra 2018).

Molecular identification

Sequence recovery and characteristics

High-quality COI sequences were successfully obtained from 30 of the 31 specimens, representing a 96.8% amplification and sequencing success rate. The single unsuccessful sequenced sample (IDR AB-16) was excluded from all molecular analyses. The 30 successfully sequenced specimens were deposited in GenBank under accession numbers PX210350-PX210378 and PQ394093 (Table 3). Sequence lengths ranged from 570 to 652 base pairs after trimming, with the majority (24 of 30) exceeding 600 bp. All sequences were verified as functional mitochondrial COI fragments through ORF analysis, which confirmed the absence of internal stop codons and frameshifts when translated using the vertebrate mitochondrial genetic code.

BLAST searches against BOLD and GenBank demonstrated that every successfully sequenced specimen exhibited genetic identities exceeding the 99% species-level threshold, providing strong molecular support for taxonomic assignments. All 15 *Acanthopagrus* specimens showed high similarity to *A. pacificus* reference sequences (BOLD: AAF1278). Thirteen specimens exhibited 100% identity, while two specimens (SBG AP-01 and JB AP-01) showed 99.04% identity. The slightly lower similarity in these two specimens resulted from 3-4 nucleotide differences across the sequenced fragment, which is well within the range of intraspecific variation reported for this species (Iwatsuki et al. 2010; Nuryanto et al. 2023b).



A B

Figure 2. Fish morphotypes obtained during the study. A. *Acanthopagrus*, B. *Argyrops*

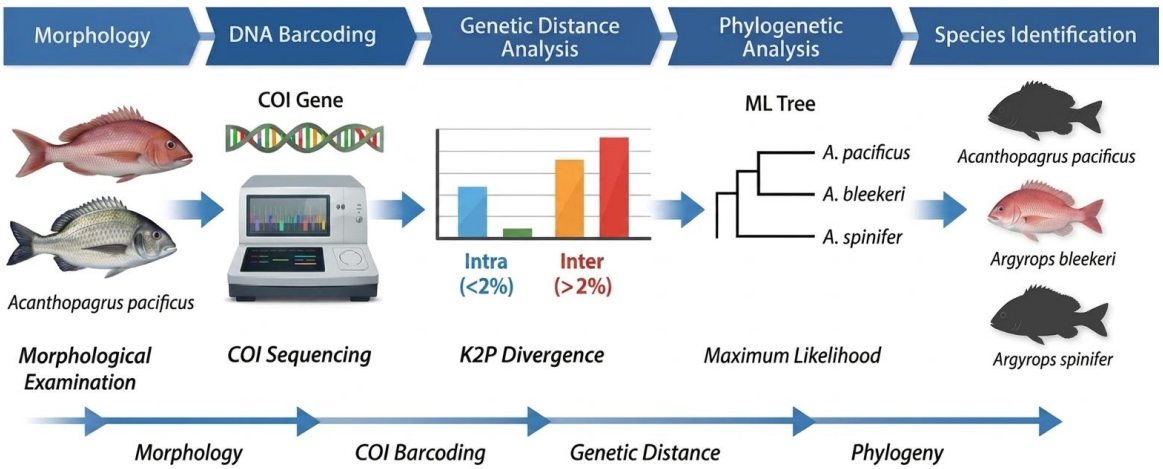


Figure 3. Pipeline for morphological assessment, COI barcoding, and ML phylogeny of *Acanthopagrus* and *Argyrops* species

Among the 15 successfully sequenced *Argyrops* specimens, 14 were assigned to *A. bleekeri* (BOLD: AAB3719). Eight of these showed 100% identity to reference sequences, while seven exhibited 99.82-99.84% identity. The minor sequence variation among *A. bleekeri* specimens (1-2 nucleotide differences) is consistent with intraspecific polymorphism and does not suggest the presence of additional cryptic lineages. One *Argyrops* specimen (IDR AB-04) matched *A. spinifer* (BOLD: ACA9486) with 100% identity across all three reference sequences compared. This specimen showed only 97.69% similarity to *A. bleekeri* references, clearly distinguishing it from the congeneric species. In total, molecular analysis identified 15 *A. pacificus*, 14 *A. bleekeri*, and one *A. spinifer* from the 30 successfully sequenced specimens. The single *A. spinifer* specimen originated from the Indramayu landing site, the same location where all 14 *A. bleekeri* specimens were collected, indicating sympatric occurrence of these two species in catches landed at this site.

The high genetic similarity values (99.04-100%) observed between Java Sea specimens and reference sequences in the BOLD system provide strong molecular support for assigning these specimens to *A. pacificus, A. bleekeri,* and *A. spinifer*. These values substantially exceed the widely used 97% threshold for species delimitation in many animal groups (Ratnasingham and Hebert 2013) and are consistent with the more stringent 99% threshold recommended for closely related Sparidae species (Ahmed et al. 2021; Nuryanto et al. 2023a, b). The adoption of this conservative threshold is particularly important for Sparidae, which contains morphologically similar and genetically close species with limited sequence divergence (Ha et al. 2018; Alam et al. 2020; Al-Zaidan et al. 2020; Salem et al. 2021).

Genetic distance analysis

Pairwise genetic distances calculated using the Kimura 2-parameter (K2P) model provided quantitative support for the species assignments (Table 4). Intraspecific variation was low across all three species, consistent with expectations for conspecific populations. Within A. *pacificus*, the mean K2P distance was 0.0027±0.0011 (range: 0.000-0.0048), indicating minimal sequence divergence among specimens from different landing sites (Cirebon, Karawang, and Pekalongan). This low level of variation is typical for conspecific populations of marine fishes with high dispersal potential and suggests genetic cohesion across the sampled range. Within *A. bleekeri*, the mean intraspecific distance was 0.0036±0.0013 (range: 0.000-0.0052), based on the 14 specimens from Indramayu. This value is slightly higher than that observed in *A. pacificus* but remains well within the range expected for intraspecific variation in sparid fishes (Wu et al. 2018).

The single *A. spinifer* specimen (IDR AB-04) showed minimal divergence from reference sequences (0.0008±0.0008), confirming its identity. The distance between IDR AB-04 and the *A. spinifer* references (0.0008) was an order of magnitude lower than its distance to *A. bleekeri* specimens (0.0144±0.0036), clearly distinguishing it from the congeneric species.

Interspecific distances between *A. bleekeri* and *A. spinifer* averaged 0.0113±0.0024 (range: 0.0085-0.0145). This value is relatively low compared to typical interspecific distances in many fish genera (often 2-5% or higher), which is consistent with patterns reported for closely related sparid congeners (Ha et al. 2018; Alam et al. 2020; Salem et al. 2021). The low divergence suggests either recent speciation or slow evolutionary rates in this lineage, highlighting the need for careful interpretation of COI data in this group. The clear clustering of specimens with respective reference sequences in phylogenetic analysis supports the interpretation that these represent distinct species despite modest sequence divergence. Intergeneric distances between *Argyrops* and *Acanthopagrus* averaged 0.0986±0.0104, an order of magnitude higher than congeneric comparisons. This substantial divergence confirms the distinctness of these genera and provides a useful benchmark for evaluating species-level separations.

Phylogenetic analysis

Maximum Likelihood phylogenetic reconstruction based on the K2P model (Figure 4) strongly supported the genetic distance results and provided independent confirmation of species assignments. The tree topology was well-resolved, with most major clades receiving strong bootstrap support. All Sparidae specimens from the Java Sea formed a monophyletic group (bootstrap support 100%), clearly separated from the outgroup species *L. harak* and *L. ornatus*. This confirms the family-level assignment of all specimens and demonstrates the utility of COI for distinguishing Sparidae from related families.

Table 2. Morphometric and meristic characteristics of *Acanthopagrus* (n: 15) and *Argyrops* (n: 16).

Character observed	*Acanthopagrus* (n: 15)	*Argyrops* (n: 16)
Body form	Moderately oblong	Oblong to slightly elongated
Dorsal coloration	Greyish-silver	Pinkish-silver
Ventral coloration	Silvery dark	Silvery rose to pinkish silver
Upper profile (forehead)	Gently sloping	Steeply sloping
Scale type	Ctenoid	Ctenoid
Lateral-line scales	42-48	36-40
Dorsal spines (D)	XI	XI
Dorsal soft rays (D′)	11-13	11-13
Anal spines (A)	III	III
Anal soft rays (A′)	8-9	8-10
Pectoral soft rays (P)	14-16	15-17
Gill rakers (total)	9-11	9-11
Body depth (BD, mm)	67.3±9.0	84.0±6.7
Standard length (SL, mm)	181.1±24.2	221.9±16.1
Head length (HL, mm)	58.5±7.8	67.3±5.6
Eye diameter (ED, mm)	12.2±1.2	14.6±1.0
BD:SL	0.37±0.01	0.38±0.01
ED:HL	0.21±0.01	0.22±0.01

Note: Values for BD:SL and ED:HL are presented as descriptive summaries (mean±standard deviation) and were not statistically tested for species discrimination; they are provided for comparative purposes with literature values

Table 3. Genetic identity of Sparidae samples from the north coast of Java Island, Indonesia

Species (BOLD ID)	Sample Code	GenBank accession No.	Genetic identity (%)	Sequence length (bp)
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-01	PX210350	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-02	PX210351	100.00	632
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-03	PX210352	100.00	632
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-04	PX210353	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-05	PX210354	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-06	PX210355	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-07	PX210356	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-08	PX210357	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-09	PX210358	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-10	PX210359	100.00	647
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-11	PX210360	100.00	570
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-12	PX210361	100.00	570
Acanthopagrus pacificus (BOLD: AAF1278)	CRB AP-13	PX210362	100.00	652
Acanthopagrus pacificus (BOLD: AAF1278)	SBG AP-01	PX210363	99.04	621
Acanthopagrus pacificus (BOLD: AAF1278)	JB AP-01	PQ394093	99.04	616
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-01	PX210364	100.00	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-02	PX210365	100.00	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-03	PX210366	100.00	612
Argyrops spinifer (BOLD: ACA9486)	IDR AB-04	PX210367	100.00	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-05	PX210368	100.00	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-06	PX210369	100.00	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-07	PX210370	100.00	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-08	PX210371	100.00	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-09	PX210372	99.82	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-10	PX210373	99.82	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-11	PX210374	99.82	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-12	PX210375	99.84	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-13	PX210376	99.84	611
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-14	PX210377	99.84	612
Argyrops bleekeri (BOLD: AAB3719)	IDR AB-15	PX210378	99.84	612

Table 4. Kimura 2-parameter (K2P) genetic distances among Sparidae samples. Values are mean±standard deviation. Values in bold indicate within-species comparisons

Sample code	K2P genetic distance		
	Argyrops bleekeri	***Argyrops spinifer***	***Acanthopagrus pacificus***
IDR AB-01 to 03 and IDR AB-05 to 15	**0.0036±0.0013**	0.0113±0.0024	0.0815±0.0089
IDR AB-04	0.0144±0.0036	**0.0008±0.0008**	0.0531±0.0058
CRB AP-01 to 13, SBG AP-01, and JB AP-01	0.0569±0.0062	0.0531±0.0060	**0.0027±0.0011**
Intergenera (*Argyrops* vs *Acanthopagrus*)		**0.0986**±0.0104	

Within the Sparidae clade, two major lineages corresponding to the genera *Acanthopagrus* and *Argyrops* were recovered with high bootstrap support (99%). While such values exceed the conventional 70% threshold (Lemoine and Gascuel 2024), for strong support, they are interpreted here as robust indicators of genuine phylogenetic structure, especially given their consistency with morphological and distance-based evidence. The observed support levels may reflect a limited number of variable sites within the COI gene for resolving deeper divergences or the effects of incomplete lineage sorting.

The *Acanthopagrus* clade was highly resolved, with all AP-coded specimens (CRB AP-01 to AP-13, SBG AP-01, and JB AP-01) clustering tightly with *A. pacificus* reference sequences (GU673695, OR512858, GU674104, PQ319711). Bootstrap support for this clade was 99%, and branch lengths within the clade were extremely short, consistent with the low intraspecific genetic distances observed. The inclusion of specimens from three different landing sites (Cirebon, Karawang, and Pekalongan) within a single, well-supported clade suggests genetic connectivity among *A. pacificus* populations along the north coast of Java, although population-level sampling would be needed to confirm this pattern.

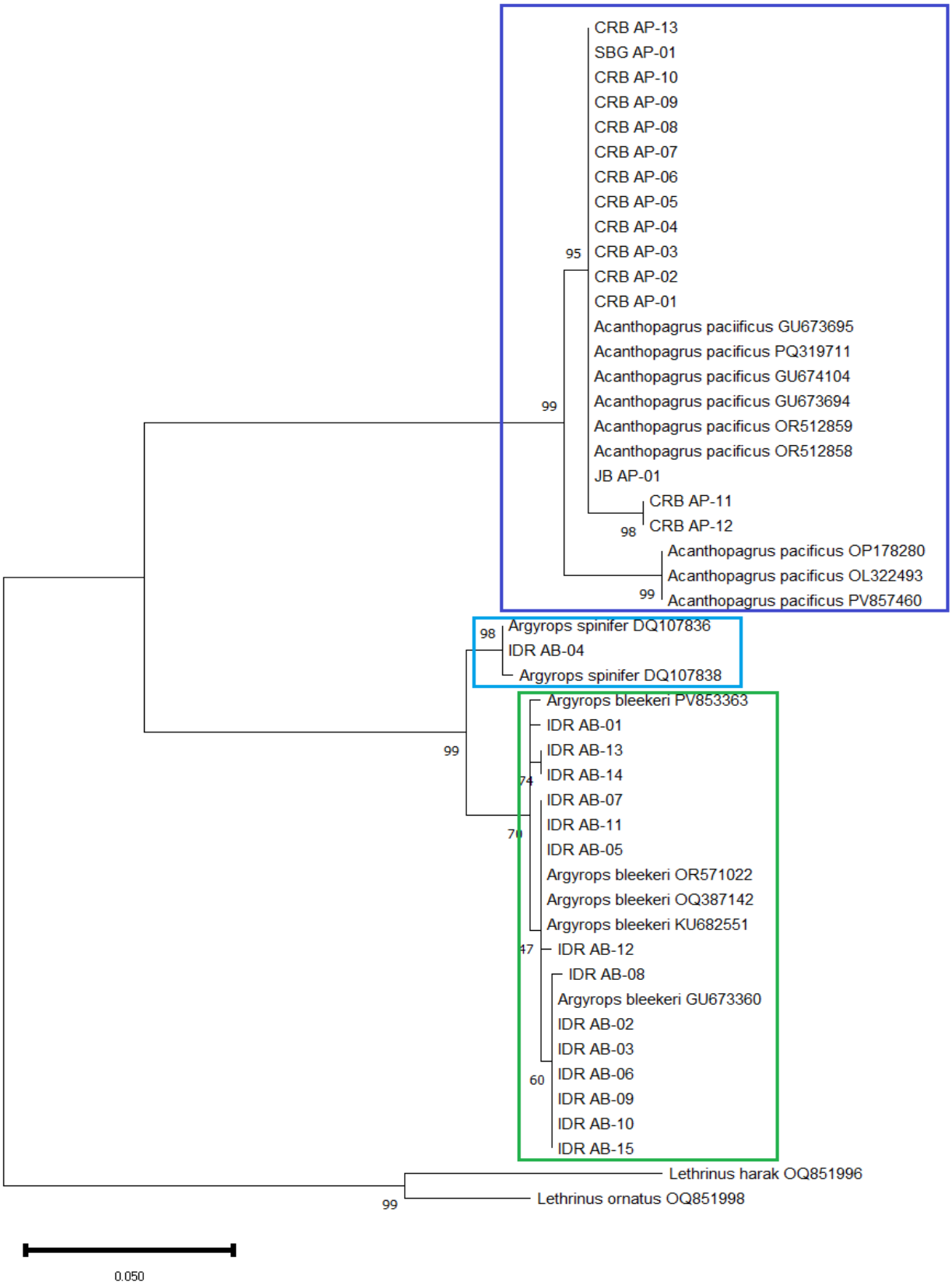


Figure 4. Maximum Likelihood phylogenetic tree based on COI sequences (Kimura 2-parameter model) showing relationships among Sparidae specimens from the Java Sea, Indonesia. Bootstrap support values (1000 replicates) are shown at nodes; values ≥70% are considered strong support, 50-69% moderate support. Sequences generated in this study are indicated by specimen codes (CRB AP-, JB AP-, SBG AP-, IDR AB-). Reference sequences from GenBank include: *Acanthopagrus pacificus* (GU673695, OR512858, GU674104, PQ319711), *Argyrops bleekeri* (GU673360, KU682551, OQ387142), and *Argyrops spinifer* (DQ107836, DQ107838). *Lethrinus harak* and *L. ornatus* were used as outgroups. Scale bar indicates substitutions per site

The *Argyrops* clade showed clear bifurcation into two well-supported subclades corresponding to the two species. The first subclade (bootstrap support 98%) comprised the single specimen IDR AB-04, which clustered with *A. spinifer* reference sequences (DQ107836, DQ107838). The second subclade (bootstrap support 99%) included the remaining 14 AB-coded specimens, which grouped with *A. bleekeri* references (GU673360, KU682551, OQ387142). This clear separation confirms that these represent distinct evolutionary lineages despite their morphological similarity and modest genetic divergence (2%). The high bootstrap support for species-level clades provides confidence in the taxonomic assignments.

Some internal nodes within the *A. bleekeri* clade exhibited lower bootstrap support (47-74%), reflecting the limited phylogenetic signal at the population level and the close relationships among individuals. However, these lower values do not affect the species-level conclusions, as all *A. bleekeri* specimens formed a monophyletic group relative to *A. spinifer* and the outgroup.

The congruence between tree-based and distance-based methods strengthens confidence in the species identifications. All three analytical approaches (BLAST similarity, genetic distance, and phylogenetic placement) yielded consistent assignments, with no conflicting signals. This methodological concordance is particularly important for closely related species like *A. bleekeri* and *A. spinifer*, where reliance on any single criterion could be misleading. ML phylogenetic reconstruction based on the Kimura-2 Parameter (K2P) model (Figure 4) supported the genetic distance results. All Sparidae specimens from the Java Sea formed a single, well-defined monophyletic group clearly separated from the outgroup species *L. harak* and *L. ornatus*. In the main Sparidae cluster, two major clades were identified corresponding to the genera *Acanthopagrus* and *Argyrops*, supported by high bootstrap values (99%). The topology of the tree reflected genus-level structuring since the examined specimens were correctly assigned to the respective taxonomic groups.

Co-occurrence of *Argyrops bleekeri* and *Argyrops spinifer* in the Java Sea

Previous studies have reported the occurrence of *A. spinifer* in Indonesian waters based on morphological identification. Mous et al. (2023a, b) documented this species in deepwater demersal fisheries surveys, while Halim and Wahyu (2023) reported it from artisanal catches in East Java. However, these records relied solely on morphology and lacked molecular confirmation.

In contrast, recent DNA barcoding studies from the Indian Ocean south of Java identified *A. bleekeri* rather than *A. spinifer* in that region (Nuryanto et al. 2023a, b). These studies suggested that previous morphological records of *A. spinifer* from southern Java might represent misidentifications of *A. bleekeri*, highlighting the need for molecular verification.

This study provides the first molecular confirmation of *A. spinifer* from the Java Sea, based on a single specimen (IDR AB-04) collected from the Indramayu landing site. This specimen showed 100% sequence similarity with *A. spinifer* references from BOLD (FOA675-04.COI-5P, FOA676-04.COI-5P, FOA677-04.COI-5P) and substantially lower similarity (97.69%) to *A. bleekeri* references (Table 5). The specimen was morphologically indistinguishable from the co-occurring *A. bleekeri* specimens, with overlapping external features that rendered visual identification uncertain. This morphological convergence underscores the value of molecular methods for accurate species identification in this genus.

It should be noted that the identification of *A. spinifer* is based on a single successfully sequenced specimen. While the COI sequence provides clear evidence for this species' presence in the Java Sea, broader sampling is needed to determine its distribution, abundance, and ecological role in the region. The single specimen may represent a rare species, a sporadic visitor to the area, or a species that is under-sampled due to fishing practices or market preferences. Without additional specimens, these possibilities cannot be distinguished.

The coexistence of *A. bleekeri* (14 specimens) and *A. spinifer* (1 specimen) in the same sampling location (Indramayu) demonstrates that two genetically distinct *Argyrops* species occur in sympatry in catches landed at this site. This finding highlights the value of molecular methods for detecting species that may be overlooked when identification relies solely on morphology. It also raises questions about ecological partitioning between these congeners, which could be addressed through future studies with an ecological sampling design.

The detection of two genetically distinct *Argyrops* species in the Java Sea is consistent with patterns reported in other studies where DNA barcoding has revealed overlooked species diversity in marine fishes. Similar findings have been documented in deep-sea Antarctic polychaetes (Brasier et al. 2016), freshwater halfbeaks in Sundaland (Lim et al. 2016), Nigerian freshwater fishes (Iyiola et al. 2018), Chinese sparids (Wu et al. 2018), and Malaysian marine fishes (Abidin et al. 2024). These studies collectively demonstrate that molecular methods frequently uncover diversity that is not apparent from morphology alone, particularly in groups with conservative body plans or recent evolutionary radiations.

The discovery of three Sparidae species (*A. pacificus, A. bleekeri,* and *A. spinifer*) from the Java Sea sampling sites is broadly consistent with the known diversity of the family in Indonesian waters (Nuryanto et al. 2023a, b). The molecular confirmation of *A. spinifer* adds to the growing body of DNA barcode data for Indonesian marine fishes and provides a foundation for future studies on the distribution, ecology, and conservation of these species.

Integrative synthesis across analytical layers

All analytical approaches employed in this study, morphological characterization, genetic similarity thresholds (99.04-100% identity), K2P distance analysis (intraspecific: 0.0027-0.0036; interspecific: 0.0113), and Maximum Likelihood phylogeny (bootstrap support 98-99% for species clades), convergently support the delimitation of *A. pacificus*, *A. bleekeri*, and *A. spinifer* as distinct species in the Java Sea (Table 6).

Critically, the integration of these layers resolves the central taxonomic ambiguity that motivated this study. Morphological analysis alone failed to distinguish *A. bleekeri* from *A. spinifer* because these two species exhibit overlapping external traits. The molecular data explain why: the interspecific genetic divergence between them is only 1.13% (K2P distance 0.0113). This level of divergence is among the lowest reported for congeneric sparids and falls within a range where morphological differentiation often lags behind molecular lineage separation. In many fish groups, species with less than 2% COI divergence frequently lack clear diagnostic morphological characters, a phenomenon attributed to recent speciation, incomplete lineage sorting, or stabilizing selection on body form (Pereira et al. 2013).

The combination of low interspecific genetic divergence (1-2%) and overlapping morphological traits therefore suggests recent divergence or ongoing cryptic speciation within *Argyrops*, where lineage separation is evident at the molecular level but not yet reflected in clear morphological differentiation. This pattern is consistent with incomplete lineage sorting or evolutionary radiation, where COI mutations have accumulated faster than discernible morphological changes (van Velzen et al. 2012). The reciprocal monophyly observed in the ML tree (bootstrap 98%), despite the low divergence, confirms their status as independent evolutionary lineages. This finding demonstrates that even low levels of genetic divergence, when coupled with concordant phylogenetic evidence, can reliably delimit species in recently diverged groups, but only when molecular tools are employed, as morphology alone is insufficient.

Implications for fisheries management and future research

The accurate species identification provided by this study carries significant implications for fisheries management in the Java Sea. The three species confirmed, *A. pacificus, A. bleekeri,* and *A. spinifer*, are all targets of commercial and artisanal fisheries in the region. Currently, management measures treat them collectively as "sparids" or "seabreams" without species-specific considerations. However, if these species differ in growth rates, maturation sizes, habitat preferences, or vulnerability to fishing pressure, as has been documented for other sparids (Lin et al. 2021), then this mixed-species management approach could lead to the unsustainable exploitation of the most vulnerable species.

The DNA barcode data generated in this study establish a critical baseline for future monitoring. These sequences can be used to develop rapid identification tools, such as species-specific PCR assays or metabarcoding approaches for assessing catch composition in mixed fisheries. Furthermore, they contribute to the global reference database for Sparidae, an essential resource for the continued advancement of DNA-based biodiversity assessment methods. Despite these contributions, several important limitations of this study should be acknowledged and addressed in future research. The sampling was geographically restricted, with all *Argyrops* specimens originating from a single landing site (Indramayu). Broader sampling across the northern and southern coasts of Java, as well as adjacent regions like Madura, Kalimantan, and Sumatra, is needed to determine the full distributional ranges of these species and to assess whether the patterns observed at Indramayu are representative of the wider Java Sea. This geographical limitation is compounded by the fact that only a single *A. spinifer* specimen was collected, which severely limits conclusions about this species' abundance and ecology. Targeted sampling efforts, possibly involving different gear types or fishing grounds, would help determine whether this species is consistently present but rare, or whether its occurrence in this study represents an unusual event.

Methodologically, the study used only a single mitochondrial marker (COI). Future research incorporating additional markers, particularly nuclear genes or genome-wide approaches, could provide deeper insights into species boundaries, population structure, and evolutionary relationships within *Argyrops*. The reliance on specimens from commercial landings also precluded the collection of crucial ecological data, such as precise capture locations, depths, and habitat types. Studies designed with ecological sampling frameworks could investigate habitat partitioning, dietary differences, and other ecological factors that may facilitate the coexistence of these congeners.

Finally, the morphological similarity between *A. bleekeri* and *A. spinifer* warrants further investigation. Detailed morphometric analysis with larger sample sizes, geometric morphometrics, or examination of additional characters, such as otolith morphology or scale microstructure, might reveal previously overlooked diagnostic features. Such studies would enhance the utility of morphological identification in field settings where molecular analysis is not feasible.

Table 5. Genetic similarity (GM) between a Java Sea, Indonesia, *Argyrops spinifer* specimen and two *Argyrops spinifer* from BOLD System

Sample code	Accession number	*Argyrops spinifer* (BOLD: ACA9486)		*Argyrops bleekeri* (BOLD: AAB3719)	
		GM (%)	Sequence ID	GM (%)	Sequence ID
IDR AB-04	PX210367	100	FOA675-04.COI-5P	97.69	FOAH923-08.COI-5P
		100	FOA676-04.COI-5P	97.69	FOAH923-08.COI-5P
		99.82	FOA677-04.COI-5P	97.69	FOAO1207-18.COI-5P

Table 6. Integrative synthesis of morphological, genetic distance, and phylogenetic evidence for species delimitation of Sparidae from the Java Sea, Indonesia

Species (n)	Morphological distinguishability	Genetic identity to reference (%)	Intraspecific K2P distance (mean±SD)	Interspecific K2P distance to congener	Phylogenetic placement (ML bootstrap)
A. pacificus (15)	Clearly distinguishable from *Argyrops*	99.04-100%	0.0027±0.0011	0.0986±0.0104 (vs. *Argyrops* genus)	Monophyletic clade, 99%
A. bleekeri (14)	Not distinguishable from *A. spinifer*	99.82-100%	0.0036±0.0013	0.0113±0.0024 (vs. *A. spinifer*)	Monophyletic clade, 99%
A. spinifer (1)	Not distinguishable from *A. bleekeri*	100%	0.0008±0.0008*	0.0113±0.0024 (vs. *A. bleekeri*)	Monophyletic clade with references, 98%

Note: Based on single specimen; intraspecific distance calculated against reference sequences only

In conclusion, this study provides molecular evidence for three Sparidae species at sampled landing sites along the Java Sea: *A. pacificus* (n: 15), *A. bleekeri* (n: 14), and *A. spinifer* (n: 1). COI sequencing achieved a high success rate (96.8%), with genetic similarity values ranging from 99.04-100% and low intraspecific K2P distances (0.0027-0.0036), supporting robust species-level identification. The results represent the first molecular confirmation of *A. spinifer* even only representing at 6.7% of *Argyrops* samples in the Java Sea and document the coexistence of two *Argyrops* species at the Indramayu landing site. This finding highlights the importance of integrating molecular tools with traditional taxonomy for accurate species identification, as the two *Argyrops* species were morphologically alone was insufficient to distinguish between the two *Argyrops* species.

The present dataset has several limitations that should be considered when interpreting these findings. All *Argyrops* specimens were obtained from a single landing site (Indramayu), which limits spatial inferences for this genus, and by the identification of *A. spinifer* based on a single specimen, which limits inference on its distribution and ecological role. Additionally, reliance on a single mitochondrial marker (COI) constrains deeper evolutionary and population-level insights. Additionally, because specimens were purchased from commercial auction centers, precise capture locations and habitats are unknown.

Despite these limitations, the DNA barcode data generated in this study provide a valuable baseline for future monitoring of sparid fishes in the Java Sea. They contribute to the growing reference database for Indonesian marine fishes and demonstrate the value of integrative taxonomic approaches for documenting marine biodiversity. Future research with broader geographic sampling, larger sample sizes, and additional genetic markers (e.g., nuclear genes or genomic approaches) would strengthen understanding of the distribution, population structure, and evolutionary relationships of these species. Such efforts could ultimately contribute to more informed on the implications for species-specific fisheries management, and conservation of coastal fish resources in Indonesia.

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