http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 43(3), 226-232 (2026)

Su Ürünleri Dergisi (2026)
DOI: 10.12714/egejfas.43.3.05

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

New distributional record of *Athanas polymorphus* Kemp, 1915 (Crustacea: Decapoda: Alpheidae) from the Vellar estuary, Southeastern India: An integrated taxonomic approach

Güneydoğu Hindistan'daki Vellar haliçinde *Athanas polymorphus* Kemp, 1915'in (Crustacea: Decapoda: Alpheidae) yeni dağılım kaydı: Bütünleşik bir taksonomik yaklaşım

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Received date: 06.03.2026 Accepted date: 18.05.2026

How to cite this paper:
Arundathy, A., Jana, G., Sanjaikumar, V., Anbuselvan, S., & Thangaraj, M. (2026). New distributional record of *Athanas polymorphus* Kemp, 1915 (Crustacea: Decapoda: Alpheidae) from the Vellar estuary, Southeastern India: An integrated taxonomic approach. *Ege Journal of Fisheries and Aquatic Sciences, 43*(3), 226-232. https://doi.org/10.12714/egejfas.43.3.05

Abstract: This study documents the occurrence of *Athanas polymorphus* in the Vellar estuary on the southeast coast of India, representing a distributional record that further substantiates its presence in estuarine environments. Seven specimens were examined using detailed morphological analysis and molecular confirmation based on mitochondrial 16S rRNA gene sequences. The examined material conforms closely to previously published descriptions of the species, with minor variations observed in the number of meral teeth on the major cheliped and the position of the pterygostomian tooth. Molecular analysis of a 549 bp fragment of the 16S rRNA gene showed 100% similarity with *A. polymorphus* sequences available in GenBank, confirming the species identity. Phylogenetic analysis further supported the placement of the Vellar specimens within the *A. polymorphus* clade. The occurrence of ovigerous females indicates that the species is reproducing within the estuarine environment, suggesting tolerance to fluctuating salinity conditions. This study expands the known habitat range of *A. polymorphus* and highlights the importance of estuarine biodiversity surveys for improving the understanding of caridean shrimp distribution in Indian coastal waters.

Keywords: *Athanas polymorphus*, Alpheidae, new estuarine record, Vellar estuary, molecular identification

Öz: Bu çalışma, *Athanas polymorphus* türünün Hindistan'ın güneydoğu kıyısındaki Vellar haliçinde bulunduğunu belgelemekte ve haliç ortamlarındaki varlığını daha da doğrulayan bir dağılım kaydı sunmaktadır. Yedi örnek, ayrıntılı morfolojik analiz ve mitokondriyal 16S rRNA gen dizilerine dayalı moleküler doğrulama kullanılarak incelenmiştir. İncelenen materyal, türün daha önce yayınlanmış tanımlarıyla büyük ölçüde örtüşmektedir; ancak ana kıskaç üzerindeki meral dişlerin sayısında ve pterygostomian dişinin konumunda küçük farklılıklar gözlemlenmiştir. 16S rRNA geninin 549 bp'lik bir parçasının moleküler analizi, GenBank'ta bulunan *A. polymorphus* sekanslarıyla %100 benzerlik göstererek tür kimliğini doğrulamıştır. Filogenetik analiz ayrıca Vellar örneklerinin *A. polymorphus* clade içindeki yerini desteklemiştir. Yumurtalı dişi bireylerin varlığı, türün haliç ortamında üredigini ve dalgalanan tuzluluk koşullarına tolerans gösterdiğini göstermektedir. Bu çalışma, *A. polymorphus*'un bilinen yaşam alanı aralığını genişletmekte ve Hint kıyı sularındaki caridean karides dağılımının daha iyi anlaşılması için haliç biyoçeşitliliği araştırmalarının önemini vurgulamaktadır.

Anahtar kelimeler: *Athanas polymorphus*, Alpheidae, yeni haliç kaydı, Vellar haliçi, moleküler tanımlama

INTRODUCTION

The genus *Athanas* Leach, 1814 is a well-defined taxonomic group of caridean shrimps within the family Alpheidae, comprising numerous species distributed predominantly in tropical and subtropical marine environments. Members of this genus typically inhabit shallow coastal ecosystems such as coral reefs, rocky intertidal zones, and seagrass beds, where they often occur in cryptic microhabitats (De Grave and Fransen, 2011). The genus includes a diverse assemblage of species widely distributed across the Indo-West Pacific region and adjacent marine areas (Anker and Ahyong 2007; Anker and De Grave, 2016). Despite this diversity, regional occurrence records for several *Athanas* species remain incomplete, particularly in estuarine and other marginal marine ecosystems.

Species of *Athanas* are distinguished by morphological characters such as the structure of the rostrum and ocular hoods, arrangement of extra- and infra-corneal teeth, sexually dimorphic chelipeds, and segmentation of the second pereiopod carpus (Anker et al., 2006; Pachelle et al., 2011). However, identification within the genus can be challenging because of subtle interspecific differences and overlapping morphological characters. Consequently, detailed morphological examination is essential and, in some cases, integrative approaches incorporating molecular data are required to resolve species boundaries (Soledade et al., 2019). Estuaries are characterized by strong environmental gradients, particularly fluctuations in salinity, sediment dynamics, and tidal influences, which may limit the distribution of many marine alpheid shrimps (Kennish, 2023).

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The present study documents the first record of *A. polymorphus* from the Vellar estuary on the southeast coast of India. This finding represents an extension of the known ecological range of the species and indicates a potential tolerance to variable salinity conditions. The record contributes to the understanding of the biogeography of the genus *Athanas* and highlights the importance of systematic biodiversity surveys in estuarine ecosystems for improving knowledge of caridean shrimp diversity and distribution.

MATERIALS AND METHODS

Sample collection and measurement

Seven specimens of *A. polymorphus* were collected manually from oyster clusters associated with the edible oyster *Crassostrea madrasensis* at a depth of 0.3–0.5 m in the Vellar estuary (11.4904930° N, 79.7666846° E), southeast coast of India. Sampling was conducted during low tide between November 2024 and January 2025. Specimens were carefully removed from oyster aggregations and immediately transferred to plastic containers containing estuarine water for transport to the laboratory.

Live coloration and external morphological characters were recorded prior to preservation. Subsequently, all specimens were preserved in 95% ethanol. During the sampling period, environmental parameters at the collection site were recorded as follows: water temperature 28–30°C, salinity 10–15 ppt, dissolved oxygen 4.8-5.2mg L^{-1}, and pH 7.7–7.9.

The alpheid shrimp, *Alpheus estuariensis* and a palaemonid shrimp (*Cuapetes* sp.) were also observed in association with *A. polymorphus* at the sampling site during the study period. Morphological observations were carried out using a stereomicroscope. Selected appendages were photographed using a digital camera attached to a compound microscope, and line illustrations were prepared using digital drawing software (Inspiroy-Huion H430P). Morphometric measurements were taken using Mitutoyo CD-6" ASX® digital Vernier calipers with an accuracy of 0.1 mm.

Specimens were identified using previously published taxonomic descriptions by Kemp (1915) and Anker (2003). The examined specimens have been deposited in the reference collection of the Centre of Advanced Study in Marine Biology, Annamalai University with specific voucher numbers for three males (CAS/ATH/TRL01-03); two ovigerous females (CAS/ATH/TRL04-05); two non-ovigerous females (CAS/ATH/TRL06-07).

Molecular identification

Total genomic DNA was extracted from ethanol-preserved tissue following a standard protocol (Sambrook et al., 1989) with the addition of 5 µl proteinase K and 10% SDS in the digestion buffer. Extracted DNA samples were diluted to approximately 20ng/µl using deionized water and used as templates for polymerase chain reaction (PCR).

A fragment of the mitochondrial 16S rRNA gene was amplified using the primers 16S-SF (5'-GACCGTGCTAAGGT AGCATAATC-3') and 16S-SR (5'-CCGGTCTGAACTCAAATC GTG-3') (Tsang et al., 2009). PCR amplification was performed in a total reaction volume of 25µl containing 1× PCR buffer, 1.5mM $MgCl_2$, 0.4µM of each primer, 0.4mM dNTPs, 1.5U Taq DNA polymerase, 1.25–3.75µl template DNA, and 0.01mg BSA, with the final volume adjusted to 25µl using Milli-Q water.

The PCR cycling conditions consisted of an initial denaturation at 95°C for 3 min, followed by 38 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 40 s, and extension at 72°C for 1 min, with a final extension at 72°C for 5 min. Amplified PCR products were purified using a commercial purification kit and sequenced at the Rajiv Gandhi Centre for Aquaculture (RGCA), Sirkali, India.

The obtained 16S rRNA sequences were edited using the BioEdit sequence alignment editor and compared with available sequences using the NCBI BLASTn tool. The sequences generated in this study have been deposited in the NCBI GenBank database under accession numbers PZ070763 and PZ070764.

To examine phylogenetic relationships, 16S rRNA sequences of six *Athanas* species and one outgroup species (*Alpheus bisincisus*) were retrieved from GenBank. A neighbor-joining phylogenetic tree was constructed using MEGA version 5.0 (Tamura et al., 2011). The robustness of the tree topology was assessed by bootstrap analysis with 1000 pseudoreplicates.

RESULTS

Distribution

Chilka Lake, Orissa, India (Kemp, 1915), Thailand (Banner and Banner, 1966), Australia (Bruce and Coombes, 1997), Singapore (Anker, 2003; Anker and De Grave, 2016) Indonesia (Anker et al., 2015), Vellar estuary, Tamilnadu, India (present study). The *A. polymorphus* distribution localities are illustrated in Figure 1.

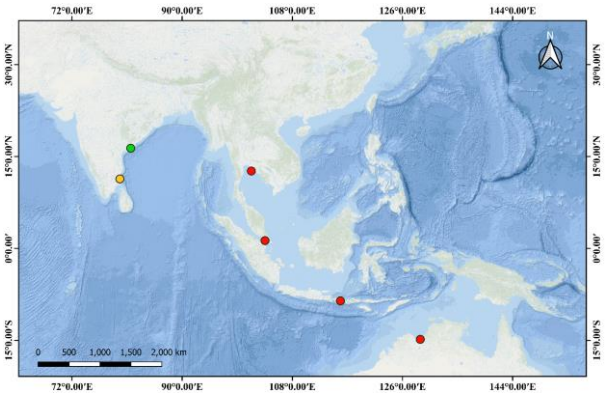

Figure 1. Map showing the distribution of *A. polymorphus* (green dot indicates holotype record, red dots indicate previous records, yellow dot indicates current record)

Colour pattern

Body generally semi-transparent to translucent. Reddish or brownish chromatophores scattered over the body, forming diffuse transverse bands along the posterior margins of abdominal segments. Antennular and antennal peduncles, as well as telson and uropods, often exhibit more intense pinkish or reddish coloration due to higher concentrations of chromatophores. Major cheliped typically hyaline to whitish with scattered reddish chromatophores (Figure 2).

Description

Carapace length 29–32% of total length; rostrum length 11–13% of total length; abdomen length 59–62% of total length. Carapace smooth, without spines. Rostrum laterally compressed, without teeth or spines, reaching the second or third article of the antennular peduncle (Figure 3a); rostral carina weakly developed. Extra-corneal and infra-corneal teeth well developed. Eyes well exposed in both dorsal and ventral view. Anterior pterygostomian angle bearing a distinct projecting tooth.

Antennular peduncle with first article longer than the remaining articles combined; stylocerite exceeding distal margin of first antennular article; ventromesial carina bearing an acute tooth. Outer antennular flagellum biramous, with fused portion comprising five to seven articles (Figure 4a). Antenna with basicerite bearing a distinct acute ventrolateral tooth and a sharp ventromesial carina. Scaphocerite elongate, slightly oval, exceeding antennular peduncle and bearing a distolateral spine (Figure 3f). Carpocerite extending beyond three-quarters of scaphocerite length.

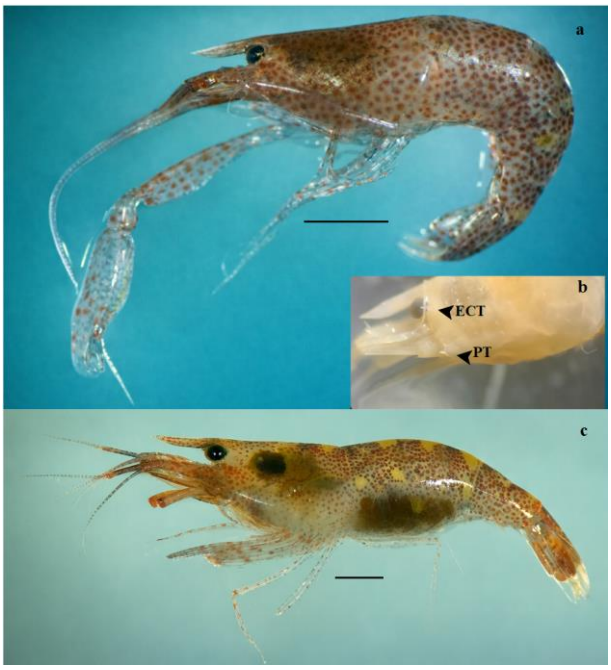

Figure 2. *Athanas polymorphus* (a), adult male CAS/ATH/TRL01 (TL=8.3mm) lateral view; (b). carapace having one pterygostomian tooth (PT) and extra corneal tooth (ECT); (c) adult female CAS/ATH/TRL04 (TL=9.3mm) lateral view, (Scale bar: 1mm)

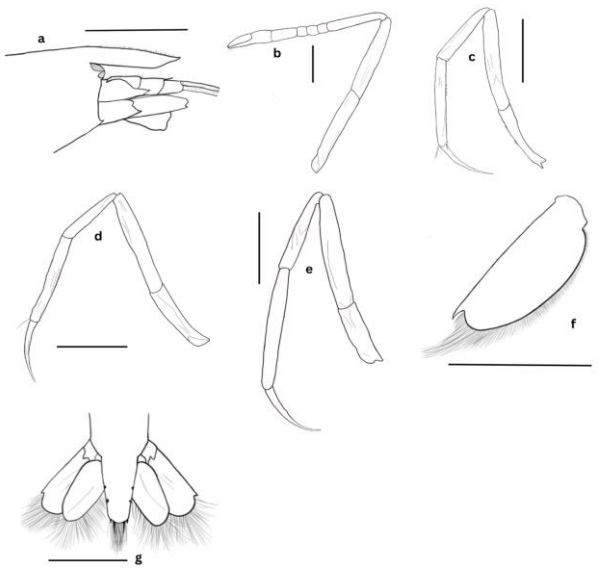

Figure 3. *Athanas polymorphus*, adult female CAS/ATH/TRL04 (TL=9.3mm) a, carapace lateral view; b, right pereiopod 2; c, right pereiopod 3; d, right pereiopod 4; e, right pereiopod 5; f, scaphocerite; g, telson dorsal view (Scale bar=1mm)

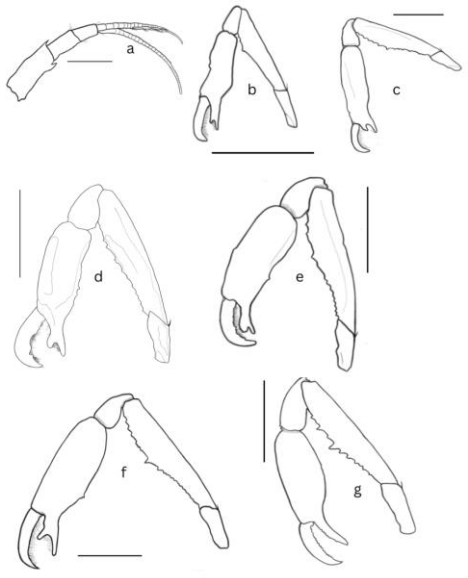

Figure 4. *Athanas polymorphus*, a, antennule; b, right major chela of adult female CAS/ATH/TRL04 (TL=9.3mm); c, right major chela of adult female CAS/ATH/TRL06 (TL=5.9mm); d, right major chela of adult male CAS/ATH/TRL01 (TL=8.3mm); e, right major chela of adult male CAS/ATH/TRL03 (TL=5.7mm); f, right major chela of adult female CAS/ATH/TRL05 (TL=8.7mm); g, right minor chela of adult male CAS/ATH/TRL03 (TL=5.7mm), (Scale bar = 1mm)

First chelipeds highly variable in shape and symmetry. Major chela articles exhibited the following proportional ratio: 1:4.57:1.13:3.02:1.33:0.67 (ischium: merus: carpus: propodus: dactylus: pollex), based on mean values derived from measurements of both male and female specimens (Figure 4b–f). Major chelipeds in both sexes morphologically variable; ischium short, bearing one dorsal spine; merus longest article, armed with 12–19 teeth; carpus cup-shaped and lobed; propodus subcylindrical, bearing 1–5 teeth; dactylus longer than pollex, either serrated or smooth; pollex with a large curved tooth (Figure 4b-f). Both fingers densely setose.

Among the examined specimens, one male possessed a minor cheliped nearly similar to the major cheliped, with long slender fingers bearing small serrated teeth (Figure 4g). Second pereiopod slender; merus slightly longer than ischium. Carpus composed of five articles, with proportional lengths 4.9: 0.9: 0.9: 0.9: 2.4 (Figure 3b). First carpal article slightly shorter than combined length of remaining articles; second, third, and fourth articles subequal; fifth article approximately half the length of the first. Dactylus longer than propodus.

Third pereiopod slender; ischium shorter than merus and bearing two spines; merus and carpus unarmed; propodus with 4-5 spines. Dactylus distally curved with a sharp tip (Figure 3c). The fourth and fifth pereiopods are morphologically similar to the third pereiopod and lack spines (Figure 3d-e).

Uropods bearing a single distal tooth. Telson shorter than uropods, armed dorsally with two pairs of spines. Posterior margin with one pair of median and one pair of lateral spines; median spines approximately four times longer than lateral spines (Figure 3g). Morphological differences between the present specimens and geographically related species of *Athanas* are summarized in Table 1.

Table 1. Morphological comparison between present study specimens and other related *Athanas* species

Character	*Athanas polymorphus*			*Athanas dimorphus*	*Athanas japonicus*
	Present study	(Anker, 2003)	(Kemp, 1915)	(Almeida et al., 2012; Banner and Banner, 1966; Pachelle et al., 2011)	(Anker, 2003)
Carapace setation	Slightly hirsute with erect setae	Slightly hirsute with erect setae	Not described	Not distinctly hirsute	Sparsely setose
Rostrum length	Reaching almost beyond second segment of antennular peduncle	Reaches mid-length of second segment of antennular peduncle	Reaches almost beyond second segment of antennular peduncle	Reaching slightly beyond half-length of second segment of antennular peduncle	Slightly exceeding distal margin of first article of antennular peduncle
Extra corneal teeth	Acute, well developed	Acute, well developed	Well developed	Acute, well developed	Acute, well developed
Intra corneal teeth	Acute, well developed	Acute, well developed	Less spinous than extra corneal teeth	Shorter than extra-corneal with rounded tip	Acute, well developed
Pterygostomial angle	With small anterior tooth projecting in the angle itself	With small anterior tooth projecting in the angle itself	With small anterior tooth projecting in the angle itself or above the angle	Acute in male, rounded in female	Rounded, not projecting, without tooth
Stylocerite length	Exceeds first antennular article but not beyond mid of second article	Exceeds first article but not mid of second article	Exceeds little beyond second article	Reaches half-length of third segment of antennular peduncle	Reaches 1/3 to mid-length of second article
Scaphocerite	Exceeds antennular peduncles, almost oval in shape bearing a disto- lateral spine	Exceeds antennular peduncle, oval and elongated bearing strong disto- lateral spine	Almost reaches antennular peduncle, bearing disto- lateral spine	Reaches the distal end of antennular peduncle	Does not exceed antennular peduncle
Basicerite teeth	Acute single ventrolateral tooth	Acute single ventrolateral tooth	Acute single ventrolateral tooth	Two teeth, one dorsal triangular and one acute inferolateral	Acute single ventrolateral tooth
Meral teeth of the major cheliped	12-19	9-10	15-20	No teeth	7-8
Second pereiopod	Carpal articles ratio 4.9:0.9:0.9:0.9:2.4	Carpal articles ratio 5.5:0.9:0.9:0.9:1.8	Not described	Carpal articles ratio 10:1:1:1:4	Carpal articles ratio 5:1:1:1:2
Telson shape	Elongated, tapering distally	Elongated, tapering distally	As long as uropod	About 2x as long as wide, broadly convex posteriorly	Broadly oval-rectangular
Telson spines	Two pairs, median spines >4x longer than lateral	Two pairs, sometimes three, median spines >4x longer than lateral spines	Two pairs, median spines>4x longer than lateral spines	Two pairs of dorsal and posterolateral spines	Two pairs, median spines<2x longer than lateral spines
Uropod	Well developed, single small lateral spine	Well developed, single small lateral spine	Well developed, single small lateral spine	Well developed, single small lateral spine	Well developed, single small lateral spine

Molecular confirmation

The mitochondrial 16S rRNA gene sequence similarities with related species available in the NCBI GenBank database are presented in Table 2. The specimens were identified as *A. polymorphus* based on both morphological characters and molecular analysis.

Table 2. Mitochondrial 16S rRNA gene sequence similarity with related species

Species	Query coverage (%)	Sequence identity (%)	Accession number	Source
Athanas polymorphus	-	-	PZ070763.1 PZ070764.1	Present study
Athanas polymorphus	99	100	PX631802.1	Unpublished
Athanas japonicus	93	87.02	MZ661669.1	Chow et al., 2021
Athanas ornithorhynchus	90	83.17	MZ661666.1	Chow et al., 2021
Athanas dimorphus	90	82.70	MZ661663.1	Chow et al., 2021
Athanas exilis	94	82.14	LC832869.1	Unpublished
Athanas nitescens	90	81.60	MZ661665.1	Chow et al., 2021

In the present study, two specimens were used for molecular confirmation, generating a 16S rRNA sequence fragment of 549 bp. BLASTn analysis showed 100% sequence similarity with *A. polymorphus* sequences available in GenBank with 99% query coverage, confirming the species identity without ambiguity. The next closest match was *A. japonicus*, which showed only 87.02% sequence similarity and may represent a closely related species.

A neighbor-joining (NJ) phylogenetic tree based on the Kimura two-parameter (K2P) model was constructed using nine sequences to illustrate the divergence pattern among six species of *Athanas* (Figure 5). The NJ tree revealed two distinct clades within the genus with strong bootstrap support. The first clade consisted of *A. polymorphus* and *A. japonicus* with a bootstrap value of 85%. The second clade included *A. ornithorhynchus*, *A. exilis*, *A. dimorphus*, and *A. nitescens* with a bootstrap value of 70%. The outgroup species, *Alpheus bisincisus*, formed a distinct and highly divergent lineage, representing the root of the phylogenetic tree (Figure 5).

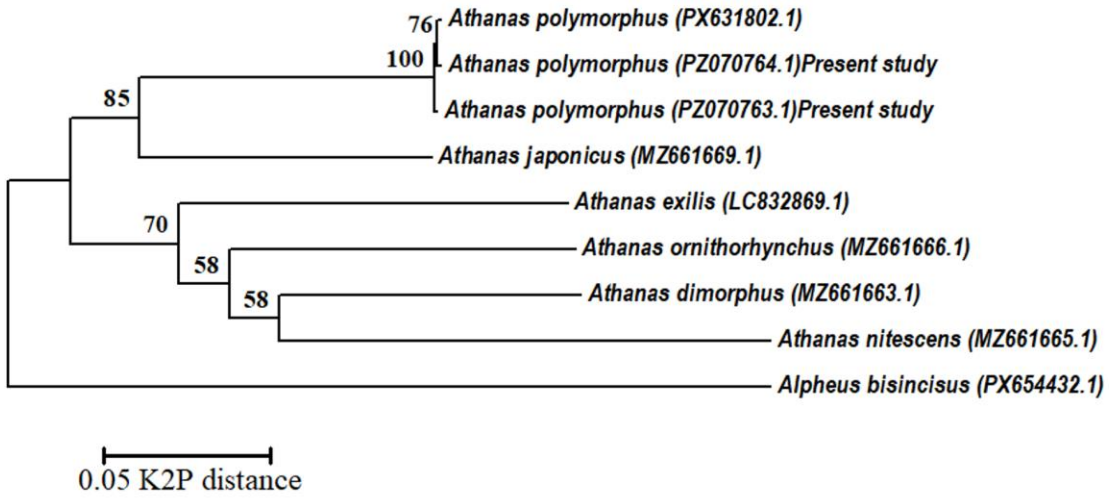

Figure 5. Neighbor-joining phylogenetic tree based on mitochondrial 16S rRNA sequences showing the relationship of *A. polymorphus* with related species

DISCUSSION

Previous records of *A. polymorphus*, including those from Chilka Lake (India) and other Indo–West Pacific localities, indicate that the species commonly inhabits brackish, or fully marine environments (Kemp, 1915; Banner and Banner, 1966; Bruce and Coombes, 1997; Anker, 2003; Anker et al., 2015; Anker and De Grave, 2016). After eleven decades, the present record from the second Indian locality (Vellar estuary) after Chilka further confirms the species' ability to persist under estuarine conditions characterized by fluctuating salinity, tidal influence, and dynamic sediment regimes. This observation suggests that *A. polymorphus* possesses a broader physiological tolerance than previously recognized and may be capable of adapting to transitional coastal environments.

Morphologically, the specimens from the Vellar estuary closely conform to the diagnostic characters described by Kemp (1915) and refined by Anker (2003), including a smooth carapace, unarmed laterally compressed rostrum, well-developed extra- and infra-corneal teeth, and a five-articulated carpus of the second pereiopod. Nevertheless, several minor variations observed in the present material warrant further consideration.

The number of teeth on the merus of the major cheliped (12–19) agrees with the range reported by Kemp (1915) for Indian specimens (up to 20), but exceeds the lower counts reported by Anker (2003) (up to 10). This discrepancy may reflect environmental heterogeneity and phenotypic plasticity rather than taxonomic divergence, with similar patterns reported in caridean shrimps (Konan et al., 2010; Duarte et al., 2016).

All examined specimens possessed a five-articulated carpus in the second pereiopod, consistent with reports for *A. dimorphus* (Almeida et al., 2012) and *A. ahyongi* (Anker and Komai, 2010), supporting the view that the five-segmented carpus is a stable morphological trait within *Athanas*. In contrast, Kemp (1915)'s report of both four- and five-articulated forms from Chilka Lake likely reflects observational limitations rather than true intraspecific variation.

The pterygostomian tooth in the Vellar specimens projects directly from the pterygostomian angle rather than from a position slightly above it. This condition corresponds closely with the description provided by Anker (2003), suggesting that the discrepancy noted by Kemp (1915) may reflect observational differences or geographic variation among populations. Such minor inconsistencies highlight the importance of re-examining historical descriptions using modern imaging methods and comparative material.

The occurrence of *A. polymorphus* in association with the edible oyster *Crassostrea madrasensis* suggests that oyster reefs provide suitable microhabitats for the species. Its co-occurrence with *Alpheus estuariensis* and *Cuapetes* sp. indicates that oyster clusters may support diverse shrimp assemblages by providing refuge and feeding opportunities, thereby functioning as biodiversity hotspots (Beck et al., 2011). The presence of ovigerous females further suggests that *A. polymorphus* is not merely a transient inhabitant but may reproduce within the estuarine environment, suggesting successful ecological establishment in the Vellar estuary.

Morphologically, *A. polymorphus* closely resembles *A. japonicus*, particularly in general body form and coloration, which necessitates careful examination of diagnostic characters. However, differences in the configuration of the pterygostomian region and cheliped morphology provide reliable distinguishing features between the two species.

Molecular analysis further supports the morphological identification. The mitochondrial 16S rRNA gene sequence obtained in the present study showed 100% similarity and 99% sequence coverage with *A. polymorphus* sequences available in GenBank, confirming the species identity. In contrast, the sequence similarity with *A. japonicus* was only 87.02%, indicating clear interspecific divergence and suggesting that *A. japonicus* represents a closely related sister species. The phylogenetic tree shows, *A. polymorphus* of the present study is clustered with *A. polymorphus* of Goa specimen with a bootstrap value of 76% and shows moderate closeness. This might be due to the genotypical variation or the primer attachment site on the target gene during PCR.

DNA-based identification requires recoverable DNA, analyzable sequences, and sufficiently variable markers for species-level resolution (Peris et al., 2009), all of which were met in this study. Mitochondrial 16S rRNA sequences were successfully amplified and analysed, and the resulting phylogeny was congruent with morphological taxonomy, supporting its reliability for species identification in alpheid shrimps.

CONCLUSION

The present study reports the first confirmed record of *A. polymorphus* from the Vellar estuary on the southeast coast of India, extending the known ecological distribution of the species to estuarine environments. The examined specimens generally conform to previously published descriptions, with only minor variations observed in meral dentition and the position of the pterygostomian tooth, which likely represent intraspecific or geographical variation. Key diagnostic characters, particularly the five-articulated carpus of the second pereiopod and the configuration of the chelipeds, support the identification of the Vellar specimens as *A. polymorphus*. The presence of ovigerous females indicates active reproduction within the estuary, suggesting that the species is capable of adapting to fluctuating salinity conditions. This record highlights the ecological plasticity of *A. polymorphus* and emphasizes the importance of continued taxonomic surveys in estuarine and marginal marine ecosystems. Further integrative studies incorporating additional molecular markers and broader geographic sampling will be valuable for clarifying population structure, assessing potential cryptic diversity, and improving understanding of the biogeography of the genus *Athanas* in Indian coastal waters.

ACKNOWLEDGEMENTS AND FUNDING

The authors are highly grateful to the Authorities of Annamalai University for providing facilities and encouragement. The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

AUTHOR CONTRIBUTIONS

Anandanatesan Arundathy: Sample collection, measurement, drawing, manuscript draft writing, Gothandapani Jana: Sample collection, measurement, DNA isolation, Vengatesan Sanjaikumar: Sample collection and DNA isolation, Subramaniyan Anbuselvan: Sample collection and DNA isolation, Muthusamy Thangaraj: Concept of manuscript, data analysis, revision of manuscript.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

ETHICAL APPROVAL

Ethical approval is not required for this study.

DECLARATION OF AI USE

We have not used AI-assisted technologies in creating this article. All content was originally produced by the authors.

DATA AVAILABILITY

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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