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RESEARCH ARTICLE

A review on *Acetes* morphological and molecular identification, and its distribution

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ABSTRACT

This review explores several aspects of *Acetes* spp., covering morphological and molecular identification, and geographical distribution. Identification methods, including morphological and molecular approaches, are employed to improve the understanding of the diversity of *Acetes* spp. The combination of morphological and molecular approaches offers the most accurate and reliable method for species identification and taxonomic classification. This review also examines the global distribution of each species of *Acetes*. The information presented here may serve as a foundational resource for researchers, practitioners and enthusiasts who are interested in the study of the species.

1. INTRODUCTION

Milne-Edwards (1830) employed a morphological approach to classify the newly discovered species from the Ganges estuary, naming them *Acetes indicus*. Krøyer (1859) identified *A. serrulatus* which was discovered in the Norwegian Sea. Ortmann (1893) identified a species, *A. americanus americanus* which was discovered in the estuary of the Amazon River in the Atlantic. Subsequently, Kishinoye (1905) documented and illustrated a new species, *A. japonicus* which was found in multiple locations in the western regions of Japan and Korea. In 1905, Nobili released an initial description which was then followed by a more comprehensive report in 1906, introducing a novel species, *A. erythraeus*, which originated from the Red Sea (Nobili, 1906). Hansen (1919) discovered a few species, *A. sibogae* (Indonesia), *A. chinensis* (Taiwan Strait), *A. dispar* (accepted as *A. japonicus*), *A. spiniger* (accepted as *A. indicus*), *A. brasiliensis* (accepted as *A. americanus*), *A. paraguayensis* (South America) and A. vulgaris (Indonesia, Thailand and Malaysia). Burkenroad (1934) discovered *A. binghami* and *A. americanus limonensis* (accepted as *A. americanus americanus*) from Panama. Colefax (1940) then discovered *A. sibogae australis*, the subspecies of *A. sibogae* in Australia. Nataraj (1949) identified *A. johni* which was discovered in India. Barnard (1955) discovered *A. natalensis* in South African waters. Omori (1975) identified two new species, *A. intermedius* (Taiwan, Philippines and Indonesia) and *A. marinus* (Brazil). In 2024, two new species were discovered, *A. omorii* and *A. maratayama* (Bochini et al., 2024; Hanamura et al., 2024). Each researcher here examined the physical characteristics of the *Acetes* specimens they collected to identify and classify them into different species. Morphological traits can vary among the specimens, influenced by environmental factors (Kennedy et al., 2020). Extensive training and knowledge of the identification keys are important in order to distinguish the variations among the specimens (Wäldchen et al., 2022). Due to the challenges associated with identifying larval stages and damaged specimens based solely on morphological features, there is a high risk of misidentification in the seafood industry (CSIRO, 2013; Hebert et al., 2003; Rajkumar et al., 2015). The existence of cryptic species, which are morphologically similar but genetically distinct, can further complicate the identification process (Bickford et al., 2007).

Researchers have identified and described *Acetes* species from diverse marine environments worldwide, including the Atlantic, Pacific and Indian Oceans. Advances in taxonomy have led to revisions and reclassifications, refining our understanding of their evolutionary relationships. Overall, the field of *Acetes* research has made significant strides in recent

decades, and continued research efforts are essential to fully understand the diversity, ecology, and conservation needs of these ecologically important species.

Molecular identification, which uses DNA to identify species, is commonly used today (Hassan et al., 2024). One popular method is DNA barcoding, which is an excellent tool for exploring and characterizing species richness in an ecosystem (Abbas et al., 2020; Hassan et al., 2024; Mondal & Mandal, 2020). Hebert et al. (2003) introduced the cytochrome c oxidase subunit I (COI) gene as a DNA barcode for species-level identification, delimitation and boundary definition. It has served as a foundation for identifying invertebrate species (Costa, et al., 2007; Mikkelsen et al., 2007). Molecular techniques can be more expensive than morphological identification, especially for large-scale studies (Elbrecht et al., 2017; Ji et al., 2013). Molecular techniques require specialized equipment and expertise. Combining both morphological and molecular approaches can provide the most accurate and reliable method for species identification and taxonomic classification (Odah, 2023).

Morphological identification can be subjective as interpretations of character states may vary among researchers, while molecular techniques provide an objective and quantitative method for species identification (Parins-Fukuchi, 2018). Morphological identification may not be able to differentiate between cryptic species, of which are morphologically similar despite being genetically distinct, but DNA barcoding can accurately identify cryptic species and differentiate between closely related taxa (Bickford et al., 2007; Hebert et al., 2003; Kress & Erickson, 2007).

Morphological identification is a quick and cost-effective way to make a preliminary identification, such as in field surveys where immediate identification is necessary, especially for experienced taxonomists (Trail, 2021). On the other hand, molecular identification can provide accurate species identification after the specimens are damaged (Frutos et al., 2022; Mohrbeck et al., 2015). When there is an uncertainty about the taxonomic status of a species, integrating both methods can help to clarify the situation (Odah, 2023).

Despite rich marine biodiversity in Pacific waters, studies on *Acetes* species are still limited (Evans, 2016; Kinch et al., 2010). Indian Ocean areas, on the other hand, could benefit from more comprehensive surveys to understand species diversity and distribution patterns (Saraswat et al., 2022). By prioritizing research in these under-studied regions, we can gain a more comprehensive understanding of the global distribution, diversity and ecological significance of *Acetes* species. Identifying and understanding the distribution and abundance of *Acetes* species in these under-studied regions is crucial for effective conservation and management. Assessing the impact of climate change on *Acetes* populations will aid us in planning future conservation strategies.

Acetes, a small-sized shrimp like Antarctic krill, is an important fishery product in Asian and East African countries due to its large biomass upon accumulation (An et al., 2021; Vereshchaka et al., 2016). There are 13 different gears commonly used to capture *Acetes*, and the most dominant gears are trawl nets, bag nets and push/scoop nets (Stephanie et al., 2021). *Acetes* can be found in tropical and subtropical environments (Amin et al., 2012; Shakawi et al., 2021), creating big aggregations along the beach during certain times of the year, where they are often fished (Omori, 1975). *Acetes* are little shrimps that range in size (10 to 40 mm in length), with females generally bigger than males (Omori, 1975). Their bodies are transparent or semi-transparent on the basipod and endopod of the uropods, with a black cornea and pairs of photogenic red pigment spots (Omori, 1975). Figure 1 depicts a typical male *Acetes* with its body sections labelled.

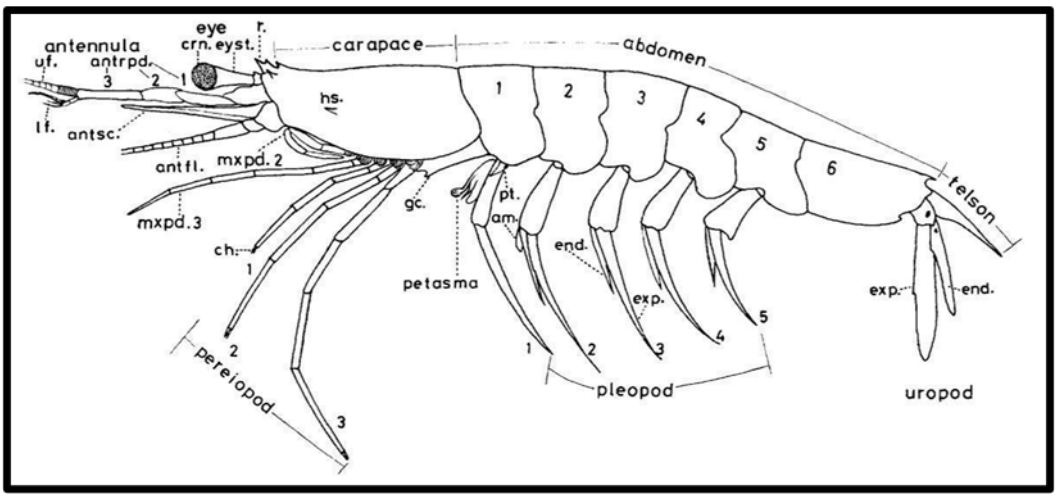

Figure 1: Diagram of a male *Acetes* (Omori, 1975). am: appendix masculine; af: antennal flagellum; ap: antennular peduncle; as: antennal scale; ch: chela; cr: cornea; end: endopod; es: eye stalk; exp: exopod; gc: genital coxa; hs: hepatic spine; lf: lower flagellum; mxpd: maxilliped; pt: procurved tooth; r: rostrum; rps: red pigment spots; uf: upper flagellum.

According to FAO (2022), *A. japonicus* marine capture production declined sharply from 451,000 tons in 2017 to 251,000 tons in 2020, accounting for 4% of total captured crustaceans. The global decrease is caused by the COVID-19 pandemic which disrupted fishing operations and trade (FAO, 2022; Waldhorn & Autric, 2023). China, who is the leading marine captures producer, showed a 40% decrease in *A. japonicus* captures in 2020 (FAO, 2022). Despite China's top position, there is a voluntary reduction in wild catches, and the specific impact of the new Chinese fishing policies on *A. japonicus* is still unknown (Waldhorn & Autric, 2023).

Although accounting for only 7.4% of the total biomass in 2020, *A. japonicus* constitutes 69.7% to 88.7% of the total abundance, or 3.6 to 50.2 trillion individuals (Waldhorn & Autric, 2023). Akiami paste shrimps, *A. japonicus*, are used to create shrimp paste, a salty and fermented condiment made from crushed and fermented shrimp (Paterson, 2003). Due to the escalating price of brine shrimp cysts, *Acetes* shrimps have gained popularity as a sustainable and affordable feed ingredient for shrimp and fish aquaculture operations (Mahida et al., 2016; Oh et al., 2011; Stephanie et al., 2021).

This paper aims to examine the worldwide distribution of *Acetes* spp. using identification techniques to provide valuable insights and promote a deeper understanding of the species.

2. GEOGRAPHICAL DISTRIBUTION

Acetes aggregations in coastal regions are highly seasonal and influence fishing activities with the swarm timing and intensity fluctuating annually and the catch volumes being unstable from year to year (Omori, 1975). Omari (1975) stated that the general environmental characteristics correlating with the occurrence of this species include shallow water away from shore, water separated from the open sea by peninsulas, underwater hills or numerous islands, noticeable tidal amplitude, and benthic floor covered by mud or sandy silt. *Acetes* are found mostly in estuary and coastal waters of tropical and subtropical parts of the world, which are restricted to Indo-West Pacific, Atlantic and Eastern Tropical Pacific Oceans (Wong, 2013; Omori, 1975). The Indo-West Pacific Ocean and Indo-Malay Archipelago alone accommodate 11 out of 16 species of *Acetes*; *A. erythraeus*, *A. intermedius*, *A. vulgaris*, *A. sibogae*, *A. johni*, *A. natalensis*, *A. serrulatus*, *A. chinensis*, *A. indicus*, *A. japonicus* and *A. omorii* (Barnard, 1955; 1986; George, 1969; Hansen, 1919; Kemp, 1917; Kensley, 1971; Nobili, 1906; Omori, 1975; 1978; Pathansali, 1966; Pérez Farfante & Kensley, 1997; Ravindranath, 1980; Wong, 2013; Hanamura et al., 2024). Global wild shrimp fisheries yield 4.1 to 72 trillion individuals annually, with a single species *A. japonicus* constituting most of the catch (Waldhorn & Autric, 2023). Despite accounting for only 7.4% of the total tonnage in 2020*, A. japonicus* represents 69.7% to 88.7% of all individuals, or 3.6 to 50.2 trillion shrimps (Waldhorn & Autric, 2023).

The distribution of *Acetes* spp. in the America continent is shown in Table 2. Four species were found exclusively in the Atlantic America, which are *A. paraguayensis*, *A. marinus* and *A. americanus*, while *A. binghami* was the only species found at the Pacific coast of America (Allen et al., 2008; D'Incao and Martins, 2000; Melo Júnior, 2006; Omori, 1975; Pérez Farfante & Kensley, 1997; Wong, 2013). A new Atlantic cyptic species were found in Brazil which known as *A. maratayama* (Bochini et al., 2024). However, no *Acetes* species have been reported to be found in the East Atlantic Mediterranean region or the islands of the Central Pacific (Hawaii and New Zealand). *Acetes* have been found in both North and South America, with documented species in the Atlantic and Pacific Oceans. *Acetes* aggregation times vary across the continent depending on the species and locations. *Acetes* are mainly found in warmer waters, thus their aggregation times are linked to seasonal temperature changes.

Table 2: The distribution of *Acetes* spp. in North and South America continent

Species	Country	References
Acetes paraguayensis	Brazil, Venezuela, Argentina, Paraguay, Bolivia and Peru	Hansen, 1919; Aldrich, 1962; Omori, 1975; Rodriguez, 1982; D'Incao & Martins, 2000; Magalhães, 2001; Magalhães, 2002; Collins & Williner, 2003; Collins et al., 2007; Collins & Williner, 2007; Magalhães & Pereira, 2007; Pimentel & Magalhães, 2014; Dos Santos et al., 2018; Tenório et al., 2022; Viera et al., 2022
Acetes marinus	Suriname and Brazil	Omori, 1975; D'Incao & Martins, 2000; Pimentel & Magalhães, 2014; Viera et al., 2022
Acetes americanus	United States of America, Suriname, Panama, French Guiana, Brazil, Costa Rica and Puerto Rico	Pérez-Farfante, 1970; Omori, 1975; Yoshii Oshiro & Omori, 1996; Pérez-Farfante & Kensley, 1997; D'Incao & Martins, 2000; Costa et al., 2003; Vargas & Wehrtmann, 2009; Simões et al., 2013
Acetes binghami	Ecuador and Panama	Omori, 1975; Burkenroad, 1984
Acetes maratayama	Brazil	Bochini et al., 2024

The continent of Asia is home to the greatest number of *Acetes* species. Table 3 shows the distribution of *Acetes* spp. in the Asia continent. *Acetes* shrimp species from *A. japonicus* in Southeast Asia to *A. sibogae* in the Indo-Pacific Ocean exhibit peak abundance during warmer months, with regional variations and potentially peaking year-round depending on the location (Ghani & Zulfahmi, 2012; Yamamoto & Tsurumi, 2005). In 2024, new species were recorded in Malaysia, Thailand and Indonesia which known as *A. omorii* (Hanamura et al., 2024). Even though

the presence of *Acetes* in Asia is well reported, a comprehensive understanding of their exact distribution patterns within the continent can be further investigated. It is important to use both morphological and molecular identification techniques to accurately map *Acetes* distribution in Asia. *A. chinensis* has only been reported in East Asia while *A. johni* only in South Asia. Different *Acetes* species might have different spawning and aggregation periods. Asia is a vast continent with different climates, so their aggregation times differ depending on the location and water temperature changes. Even within the same species, aggregation times might vary across different regions due to water temperature fluctuations.

The distribution of *Acetes* spp. in the Africa continent is shown in Table 4. *A. erythraeus* is present widely along the coast of Africa, as compared to *A. natalensis*. There were only two *Acetes* species distributions reported from Eastern Africa without any recent publications. There is also a lack of extensive studies on *Acetes* diversity in other parts of Africa, which leads to under-reporting of the species. Compared to Asia, research on *Acetes* in Africa seems less extensive, making it hard to find the complete data on their seasonal aggregation patterns there.

The distribution of *Acetes* spp. in the Australia continent is shown in Table 5. *Acetes sibogae australis* is the dominant *Acetes* species in Australia which thrived in the continental shelf and shelf slope waters along the entire Australian coast (Colefax, 1940). There is a possibility that Australia harbours a greater *Acetes* diversity than what is currently documented. Future research may reveal a wider variety of *Acetes* species within Australia's marine ecosystem. Data on previous water temperature variations along each of Australia's coasts provide clues on the peak *Acetes* activity periods as their aggregations depend on the water temperature fluctuations.

Table 4: The distribution of *Acetes* spp. in the Africa continent.

Species	Country	References
Acetes erythraeus	South Africa, Mozambique, Somalia, Tanzania, Kenya and Madagascar	Kensley, 1971; Omori, 1975; Pérez-Farfante & Kensley, 1997; Fischer & Bianchi, 1984
Acetes natalensis	South Africa	Kensley, 1971; Omori, 1975

Table 5: The distribution of *Acetes* spp. in the Australia continent.

Species	States	References
Acetes erythraeus	Queensland	Holthuis, 1980; Pérez-Farfante & Kensley, 1997
Acetes sibogae australis	Western Australia, Northern Territory, Queensland and New South Wales	Hamner & Hamner, 1977; Chan, 1998; Hanamura, 1999; Hanamura & Ohtsuka, 2003

Table 3: The distribution of *Acetes* spp. in the Asia continent.

Species	Country	References
Acetes japonicus	Malaysia, Singapore, Indonesia, India, Pakistan, Bangladesh, Japan, China, Taiwan, Korea, Philippines, Vietnam, Thailand, Cambodia, Myanmar, Iran and Kuwait	Pathansali, 1966; Harada, 1968; Omori, 1975; Omori, 1978; Ravindranath, 1980; Tirmizi & Ghani, 1982; Fischer & Bianchi, 1984; Miquel, 1984; Aravindakshan et al., 1985; Grabe & Lees, 1992; Xiao & Greenwood, 1993; Jo & Omori, 1996; Zafar & Alam, 1997; Chan, 1998; Pan, 2000; Chiou et al., 2003; Seok et al., 2004; Amin, 2008; Aziz et al., 2008b; Amin et al., 2009b; Amin et al., 2009c; Amin et al., 2010a: 2010b; Aziz et al., 2010; Oh et al., 2010; Amani, 2011; Amani et al., 2011a; Amani et al., 2011b; Amani et al., 2011c; Dore, 2012; Arshad et al., 2013; Khairina et al., 2013; Wong, 2013; Kharina et al., 2016; Singh et al., 2017; Yang & Chang, 2018; Ruethers et al., 2019; Tangkrock-olan et al., 2019; Shakawi et al., 2021; Helmi et al., 2022a
Acetes serrulatus	Malaysia, Singapore, Indonesia and China	Omori, 1975; Chan, 1998; Oh et al., 2010; Oh et al., 2011; Rahouma et al., 2013; Wong, 2013; Wong et al., 2015; Wong et al., 2017
Acetes chinensis	Korea, Japan, China and Taiwan	Omori, 1975; Oh & Jeong, 2003; An et al., 2021
Acetes intermedius	Malaysia, Philippines, Indonesia, Taiwan and India	Omori, 1975; Motoh, 1980; Chan, 1998; Chen & Chen, 2002; Chiou et al., 2003; Arshad et al., 2007; Aziz et al., 2008b; Amin et al., 2009c; Saini et al., 2011; Saini, 2013; Musel et al., 2019
Acetes erythraeus	Malaysia, India, China, Taiwan, Philippines, Thailand, Indonesia, Singapore and Yemen	Omori, 1975; Fischer & Bianchi, 1984; Chan, 1998; Sajeevan & Kurup, 2016; Tangkrock-olan et al., 2019; An et al., 2021; Hassan & Othman, 2021; Shakawi et al., 2021; Ann et al., 2022
Acetes vulgaris	Malaysia, Thailand, Vietnam, Singapore, China, Indonesia and Timor-Leste	Omori, 1975; Chan, 1998; Arshad et al., 2008; Amani et al., 2011c; Rahouma et al., 2013; Pongsetkul et al., 2017a; Pongsetkul et al., 2017b; Tangkrock-olan et al., 2019
Acetes indicus	Malaysia, India, Pakistan, Vietnam, Bangladesh, Cambodia, Indonesia, Myanmar and Singapore	Omori, 1975; Omori, 1978; Ravindranath, 1980; Tirmizi & Ghani, 1982; Chan, 1998; Deshmukh, 2003; Amin, 2008; Aziz et al., 2008b; Amin et al., 2009c; Amin et al., 2009d; Amani et al., 2011c; Rahouma et al., 2013; Tanimura et al., 2013; Zodape, 2014; Khan et al., 2015; Sajeevan & Kurup, 2016; Yap et al., 2019; Kaur et al., 2021
Acetes sibogae sibogae	Malaysia, Philippines, Japan, Indonesia, Australia, India, Thailand and Singapore	Pathansali, 1966; Omori, 1975; Hamner & Hamner, 1977; Motoh, 1980; Ravindranath, 1980; Chan, 1998; Hanamura, 1999; Hanamura & Ohtsuka, 2003; Tanimura et al., 2013; Wong, 2013; Sajeevan & Kurup, 2016; Fukuchi et al., 2017; Ann et al., 2022; Hardianto et al., 2022
Acetes johni	Pakistan, India and Sri Lanka	Nair, 1977; Tirmizi & Ghani, 1982; Fischer & Bianchi, 1984; Goswami & Shrivastava, 1996; Aravindakshan et al., 1998; Deshmukh, 2003; Sajeevan & Kurup, 2016
Acetes omorii	Malaysia, Thailand and Indonesia	Hanamura et al., 2024

3. MORPHOLOGICAL APPROACH

Omori's (1975) global keys are considered the most suitable for identifying *Acetes* species, as it provides detailed description for each species. However, it should also be noted that there are numerous other regional keys available for identifying *Acetes* species, as evidenced by the works of Barnard (1955), Chan (1998), D'Incao & Martins (2000), George (1969), Hansen (1919), Kemp (1917), Miquel (1984), Pathansali (1966), and Ravindranath (1980).

Barnard (1955) established early identification keys and described the basic morphological features used for differentiating *A. erythraeus* and *A. natalensis*, followed by George (1969), Ravindranath (1980) and Chan (1998) who then updated and refined the identification keys for *A. erythraeus*. Kemp (1917) and Pathansali (1966) used the identification keys provided by Nobili (1906) without making any updates. D'Incao and Martin (2000), then, employed those laid out by Omori (1975) and Hansen (1919) to identify *Acetes* and examine its distribution in Brazil. Some of the authors classified some *Acetes* species as separate due to the variations within the same species. Hansen (1919) was the one to identify *A. carolinae* (later known as *A. americanus carolinae*), while George (1969) and Kemp (1917) were the ones who discovered *A. cochinensis* (later known as *A. japonicus*) and *A. insularis* (later known as *A. serrulatus*) respectively. These shrimps are some of the variations of existing species, and the descriptions provided by these researchers were useful in having these natural variations documented.

Omori in 1975 reviewed the classification of the genus *Acetes* and decided that *A. cochinensis* and *A. insularis* were the same as an existing species. *A. brasiliensis* previously documented by Hansen (1919) and the males of *A. americanus limonensis* by Burkenroad (1934) were placed into the synonymy of *A. americanus americanus*, and two new species, *A. intermedius* and *A. marinus*, were identified (Omori, 1975). Previously, Pathansali (1966) was unable to identify *A. intermedius* which shows a limitation in the regional keys' ability in encompassing all the morphological variations found within certain *Acetes* species in each region. Miquel (1984) collected *A. japonicus* from Kuwait and found that the female specimens were slightly different from the diagnosis by Omori (1975). Their genital area is more similar with *A. chinensis* examined by Hansen (1919). Despite these variations among the females, the structure of the male petasma proved that they are indeed *A. japonicus*. This shows that the identification keys proposed by Omori (1975) are still the best choice despite the variations present in certain species.

Kemp (1917) provided the distinction between adult males of *A. indicus*, *A. serrulatus* (also known as *A. insularis*), *A. erythraeus* and *A. japonicus*, based on their petasma and lower antennular flagellum structure, while the characteristic differences in the third thoracic sternite were observed in the females of these species. This observation aligns with Omori's (1975) study on detailed description of all 14 currently known *Acetes* species, where distinct differences in petasma and lower antennular flagellum structure were observed in males, and variations in the structure of the third thoracic sternite were noticed in females. The combination of these three characteristics, along with the use of Omori's (1975) global identification keys, proves to be highly practical. He also stated that the number of denticles on the rostrum behind the terminal point, the size of the eye, the proportional lengths of the 3 segments of the antennular peduncle, the detailed structure of the basis (trochanter) and the coxa of the third pereiopod, the presence of a procurved tooth between the bases of the first pair of pleopods, the shape of the telson, and the proportional length of the non-ciliated part of the outer margin of the exopod of the uropod to the entire margin can be used to differentiate the species.

Over time, taxonomic classifications can change due to new discoveries, genetic analyses and evolving understanding of species relationships (Feher, 2017). Outdated keys may not reflect these changes which can lead to incorrect identifications (Piemontese, 2020). While older keys can be useful, it is important to supplement them with recent literature and consider using molecular techniques for confirmation. Omori's (1975) global keys remain a valuable resource, but regional keys and molecular approaches can also contribute to accurate identification of *Acetes* species.

Table 6 shows the general characteristics of *Acetes* spp. groups. The *Acetes* spp. are divided into two groups: the *erythraeus* and the *japonicus*. The unique morphology of a female's third thoracic sternite and a male's lower antennular flagellum serves as clear diagnostic markers for readily identifying *A. marinus* and *A. paraguayensis* within the *erythraeus* group (Omori, 1975).

4. MOLECULAR APPROACH

Molecular markers identify the genetic distinctiveness of a specific individual, species, or population (Maralit & Santos, 2015). The wide range of markers utilized serves various purposes including observing the variations that can occur due to mutation in the genomic loci. The effectiveness of a study relies on the efficiency of the chosen marker (Al-Samarai & Al-Kazaz, 2015; Maralit & Santos, 2015). There are many markers frequently used in genetic studies of shrimp, which include

allozymes markers, mitochondrial DNA (mtDNA) and nuclear markers such as restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), microsatellite, single nucleotide polymorphisms (SNP), and expression sequence tag (EST) markers (Vaseeharan et al., 2013). Allozymes are the different versions of enzymes caused by variations in genes which can be seen using protein electrophoresis (Al-Samarai & Al-Kazaz, 2015). mtDNA is a circular molecule found within mitochondria which is solely inherited from the mother and does not undergo any recombination (Vaseeharan et al., 2013; Al-Samarai & Al-Kazaz, 2015). mtDNA is proven to be effective in defining relationships among closely related species (Askari et al., 2013). Most nuclear genetic markers come from parts of the genome that do not code for proteins, as these areas face weaker selection pressures and tend to evolve faster than the protein-coding parts (Magoulas, 1998).

Table 6: The general characteristics of *Acetes* spp. groups (summarized from Omori, 1975).

erythraeus group		
Species	Female	Male
A. erythraeus *A. intermedius* *A. marinus* *A. paraguayensis* *A. sibogae* *A. vulgaris*	a pair of conspicuous protuberances present on anterior part of third thoracic sternite	• pointed anterior margin of genital coxa • petasma with pars astringens • third segment of antennular peduncle shorter than first segment except for *A. sibogae*
japonicus group		
A. americanus *A. binghami* *A. chinensis* *A. indicus* *A. japonicus* *A. johni* *A. natalensis* *A. serrulatus*	conspicuous protuberances absent on the thoracic stemite	• petasma without pars astringens • rounded anterior margin of genital coxa • third segment of antennular peduncle longer than first segment

Researchers have delved into the genetic makeup of *Acetes* shrimp using various molecular markers. Cytochrome oxidase I (COI) has been instrumental in studies across the globe (Simões et al., 2023; Hassan & Othman, 2021; Kaur et al., 2021; Park et al., 2021; Deng et al., 2020; Wong et al., 2017; Maktar, 2013). Simões et al. (2023) and Kang et al. (2015), as a case in point, turned to 16S ribosomal RNA (rRNA) for broader phylogenetic insights, while Hardianto et al. (2022) and Aziz et al. (2010) explored the potential of 12S rRNA and RAPD respectively.

Simões et al. (2023) unravelled the diversity of A. *americanus* in both the US and Brazil. Similarly, Wong et al. (2017) identified four species, *A. indicus*, *A. serrulatus*, *A. japonicus* and *A. sibogae*, along the Peninsular Malaysia's west coast, while Hassan and Othman (2021) identified two species, *A. erythraeus* and *A. serrulatus*, in Sarawak. From the coast of Mumbai (Kaur et al., 2021) to Korea (Park et al., 2021) to East China Sea (Deng et al., 2020) and lastly to Indonesia (Kusbiyanto et al., 2020), researchers have mapped the genetic landscape of these tiny shrimps. While Jamaluddin et al. (2019) could only identify the Malaysian samples on the genus level, Maktar (2013) successfully distinguished five *Acetes* species in Kedah, Malaysia, which were *A. indicus*, *A. japonicus*, *A. serrulatus*, *A. intermedius* and *A. vulgaris*. Kang et al. (2015) added to the knowledge of Korean *Acetes* with his discovery, and Hardianto et al. (2022) pinpointed *A. sibogae* in the Western Pacific coast. Aziz et al. (2010) used RAPD in his research to identify *A. japonicus* in Malaysia, while Kim et al. (2012) sequenced the complete mitochondrial genome of *A. chinensis* from a beach in South Korea.

Various DNA extraction methods of *Acetes* spp. have been reported in the literature. Simões et al. (2023) and Kaur et al. (2021) utilized a modified cetyltrimethylammonium bromide (CTAB) protocol, whereas Wong et al. (2017), Maktar (2013) and Aziz et al. (2010) opted for a commercial extraction kit. Kang et al. (2015), for one, employed an automated DNA extraction system. It must also be noted that there are several studies that did not specifically mention the extraction method used.

According to Abbas et al., (2020), in crustaceans, the most effective approach is the cytochrome oxidase subunit I (COI) mitochondrial gene region using primer pairs outlined by Folmer et al., (1994). Utilizing DNA barcoding proves to be an excellent method for identification of crustaceans' species and the exploration of species diversity within an ecosystem (Abbas et al., 2020).

The polymerase chain reaction (PCR) thermal regimes are shown in Table 7. Hassan & Othman (2021) and Wong et al. (2017) adopted different approaches with touch down PCR. It is

a way to make PCR more precise by preventing primers from attaching to incorrect locations on the DNA (Green & Sambrook, 2018). Denaturation separates the two strands of DNA by breaking the weak bonds that hold them together (Rahman et al., 2013). Majority of the studies opted for 95 ºC for initial denaturation. Kaur et al. (2012) and Hassan & Othman (2021), for instance, opted for 96 ºC and 94 ºC respectively with time ranging from 1 minutes to 10 minutes. Prolonged initial denaturation beyond three minutes will inactivate the DNA polymerase, which can compromise its enzymatic function (Lorenz, 2012). However, in the case of extended initial denaturation period of 3 to 9 minutes, hot-start PCR can be employed to overturn this, where DNA polymerase is added to the reaction mixture to prevent the enzyme from inactivation (Lorenz, 2012). The denaturation temperature is generally either 94 ºC or 95 ºC in the researches studied opting for 30 seconds, except for Kang et al. (2015) who opted for 1 minute.

Annealing is where primers are attached to the single strand DNA. The choice of the temperature at this stage depends on the primers used and is usually below their melting temperature (Rahman et al., 2013). The annealing temperature ranges from 32 to 52 ºC. The annealing duration used by Kaur et al. (2012) and Aziz et al. (2010) is the shortest (30 seconds), while that of Hassan & Othman (2021) and Wong et al. (2017) the longest (1 minute 30 seconds). DNA polymerase builds new complementary strands that match the target sequence, which starts from the annealed primer then progresses along the DNA template during extension (Rahman et al., 2013). The extension and final extension temperatures were at 72 ºC in all studies, but varying time ranging from 30 seconds to 1 minutes and 5 to 10 minutes. Among the studies examined, the number of PCR cycles needed for amplification ranges from 35 to 40. Simões et al. (2023) required the highest number of cycles in his study, as compared to Hassan & Othman (2021), Wong et al. (2017), Kang et al. (2015) and Kaur et al. (2012).

Overall, molecular markers provide a versatile approach for *Acetes* species identification that offers significant advantages over traditional morphological methods in validating and potentially enhancing the accuracy of morphological identification in *Acetes* shrimp. COI is the preferred marker that is widely used for identifying various *Acetes* shrimp species across different geographical locations. Both traditional and conventional methods for DNA extraction have proved to be successful for *Acetes* sp. The PCR conditions, however, differ among studies, including the annealing and extension times. An optimized and standardized DNA extraction and PCR protocols for *Acetes* sp. should be developed to ensure an efficient and reliable molecular analysis.

The absence of sequence data for certain *Acetes* species, including *A. intermedius*, *A. johni* and *A. natalensis*, in the GenBank database represents a significant gap in our understanding of their genetic diversity, phylogenetic relationships and ecological adaptations, which is actually crucial for planning the effective conservation and management strategies. By addressing the challenges and leveraging the latest technological advancements, researchers can gain a deeper understanding of the diversity and evolution of these important species. Investigating other choices of markers also might show further insights into the *Acetes* spp. population genetic.

Table 7: PCR thermal regime of *Acetes* spp.

References	Initial denaturation		Denaturation		Annealing		Extension		Final extension		Cycles
	Temp. (ºC)	Time	Temp. (ºC)	Time	Temp. (ºC)	Time	Temp. (ºC)	Time	Temp. (ºC)	Time	
Kaur et al., 2012	96	5 min	95	30 s	50	30 s	72	30 s	72	10 min	35
Simões et al., 2023 (COI)	95	3 min	95	30 s	44-50	45 s	72	1 min	72	10 min	40
Hassan & Othman, 2021; Wong et al., 2017	94	1 min	94	30 s	45	1 min 30 s	72	1 min	-	-	5
	-	-	94	30s	51	1 min 30 s	72	1 min	72	5 min	30
Kang et al., 2015	95	10 min	94	1 min	52	1 min	72	1 min	72	7 min	35
Simões et al., 2023 (16s)	95	3 min	95	30 s	42-44	45 s	72	1 min	72	10 min	40
Aziz et al., 2010	95	3 min	94	30 s	32	30 s	72	35 s	72	5 min	39

5. CONCLUSION

In conclusion, this review has touched on various topics regarding *Acetes* spp., enlightening on its morphological and molecular identification methods. The geographical distribution discloses the key ecological drivers that shape the habitat preferences of *Acetes* spp. This review offers a comprehensive perspective which covers from

identification to distribution by combining various data on the species. The number of reference sequences for *Acetes* species in public databases must be increased. Comprehensive DNA barcoding techniques which focus on the COI gene are needed to generate comprehensive dataset for the species identification. Next-generation sequencing (NGS) technologies can also be utilized to obtain a more detailed genetic profile of *Acetes* spp. Researches on these crustaceans have contributed significantly to science, conservation and product development, of which benefit both human health and industry.

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