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RESEARCH ON THE DIVERSITY OF FRESHWATER SHRIMP SPECIES BELONGING TO THE FAMILY ATYIDAE IN VIETNAM AND PROPOSAL FOR CONSERVATION CLASSIFICATION

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INTRODUCTION

1. Urgency of the topic

The Atyidae family (freshwater shrimp) is an ancient and highly diverse group, comprising approximately 542 species [1, 2]. These shrimp are distributed across all zoogeographical regions, except for the Arctic and Antarctic [3]. Southeast Asia (including southern China) is considered the region with the highest diversity of Atyidae shrimp, with over 210 species belonging to various genera [4]

In order to accurately assess the diversity of species composition to contribute to the conservation of Atyidae shrimp species in Vietnam, an integrated taxonomic study based on a combination of morphological and molecular analysis is necessary. Highly reliable taxonomy together with information on species has provided an important basis for proposing effective conservation measures. Based on the above scientific and practical basis, the researcher has carried out the topic "Research on the diversity of freshwater shrimp species belonging to the family Atyidae in Vietnam and proposal for conservation classification".

2. Research objectives

Identify the species composition of the Atyidae family in Vietnam based on morphological characteristics and 16S gene sequence analysis.

Assess the distribution status and conservation ranking of Atyidae shrimp species in Vietnam according to IUCN classification criteria.

3. Research content

- Research on the species composition of Atyidae shrimp in Vietnam based on morphological and molecular characteristics. Research on the genetic relationship between Atyidae shrimp species in Vietnam

- Study the current distribution status and propose conservation classification of Atyidae shrimp species in Vietnam based on IUCN classification guidelines.

CHAPTER 1. RESEARCH OVERVIEW

1.1. Overview of studies on species composition of the shrimp family Atyidae

1.1.1. General introduction to the shrimp family Atyidae

1.1. 2. Overview of studies on Atyidae shrimp species composition in the world

The Atyidae are the only family in the superfamily Atyoidea, in the order Decapoda, class Malacostraca, phylum Arthropoda. A family of freshwater shrimp, occurring in all tropical and most temperate regions. Most adults of this family are restricted to freshwater [9]. The diversity of freshwater shrimp species in the Asian biogeographic region is three times greater than that in other biogeographic regions [4].

Table 1. 1Number of shrimp species of the family Atyidae and the genus *Caridina* in some countries in the Asian region

Nation	N^0 of species in the family Atyidae	Number of species in the genus *Caridina*
China	147	103
India	31	31
Thailand	15	15
Indonesia	62	
Singapore and Malaysia	14	13
Philippines	36	29

1.1. 3. Overview of Atyidae shrimp classification studies in the world based on molecular biological analysis

Integrative taxonomic research based on the combination of morphological, molecular, biological and ecological data has helped to discover many new species and revise the taxonomy of many species and genera. At the same time, studies on phylogeny, evolution and biogeography are also being vigorously carried out to better understand the Atyidae family.

Thus, most of the genetic studies of Atyidae shrimp species have used 16S rRNA mitochondrial gene sequences. Studies have shown that the genetic distance between species is very low, the distance to separate species can be less than 3%. The taxonomy of this shrimp group requires an integrated approach, including morphological analysis, molecular biology and ecological characteristics, geographical distribution, etc. to increase the level of reliability.

1.1.4. Overview of basic research for conservation and conservation research of Atyidae shrimp in the world

1.1.4.1. Research on conservation classification of Atyidae in the world

Through the above synthesis, it can be seen that there are currently few conservation classification studies for Atyidae shrimp, possibly because this shrimp species has not really received much attention. More research is needed to assess the conservation status of this shrimp family.

1.1.4.2 . Research on conservation of Atyidae shrimp in the world

Currently, conservation studies on Atyidae shrimp species are few, with only a few studies on ecology and reproductive biology that can serve as a basis for future conservation studies.

1.2. Overview of research on the Atyidae shrimp family in Vietnam

1.2.1. Overview of studies on Atyidae shrimp species composition in Vietnam

Synthesis of studies before the PhD student conducted the thesis showed that the Atyidae shrimp fauna of Vietnam has 26 species, including: 2 species in Bouvier's research in 1904 and 1925, *C. weberi sumatrensis* and *C. nilotica typica*; 1 species *C. gracilirostris* De Man, 1892 in the publication of Nguyen Van Xuan; 19 species in the publication of Dang Ngoc Thanh and Ho Thanh Hai in 2012, and 02 new species described in 2020-2021 by Do et al.

Thus, in Vietnam, there are currently only studies on the diversity of Atyidae shrimp species. There have been no in-depth studies on conservation or conservation classification. biological and ecological characteristics,

distribution range, population size, and environmental impacts on this group of organisms. Therefore, conducting in-depth studies related to biological and ecological characteristics and conservation studies of Atyidae shrimp species in Vietnam is necessary.

1.3. Overview of Vietnam's freshwater bodies

1.3.1. Geographical location and terrain

1.3.2. Types of water bodies and biogeographical relationships of inland freshwater aquatic organisms in Vietnam

1.3.3. Geographical zonation of inland freshwater aquatic life in Vietnam

CHAPTER 2. SUBJECTS, LOCATION, TIME AND METHODS OF RESEARCH

2.1. Research subjects, time, and materials

2.2.1. Research time

Research period: from June 2020 to June 2024

2.2.2. Research subjects

The research subjects are species belonging to the family Atyidae, order Decapoda, class Malacostraca, phylum Arthropoda, distributed in Vietnam.

2.2.3. Research materials

Research materials are shrimp specimens currently kept at the Institute of Ecology and Biological Resources, and additional collection will be conducted during 2020-2024.

2.2. Research method

2.2.1. Inheritance method

The PhD candidate received and inherited the specimens collected and stored at the Department of Water Environment Ecology, Institute of Ecology and Biological Resources. In addition, this study also inherited the investigation data, research, and scientific information that have been available up to now related to Atyidae shrimp in Vietnam.

2.2.2. Field survey method, sample collection and sample fixation

2.2.3. Laboratory research methods

2.2.3.1. Method of morphological analysis

All specimens were assigned to species or genera based on current descriptions, according to monographs by Cai (2004) [81], Dang Ngoc Thanh and Ho Thanh Hai (2012), articles describing new species, [74]etc.

2.2.3.2. Molecular biological analysis

Abdominal muscle tissue of Atyidae shrimp samples was preserved in 70% alcohol. Total DNA was extracted from approximately 2 mm^3 of abdominal muscle tissue using the Qiagen BioSprint 96 kit according to the manufacturer's protocol. The 16S gene fragment (590bp) was amplified by polymerase chain reaction (PCR) and sequenced using the primer pairs 16S-F-Car and 16S-R-Car1

2. 2 . 3 . 3. Method of constructing a chemical classification

2.2.3.4. Conservation classification method and proposed conservation measures.

All Atyidae species recorded in Vietnam are assessed according to IUCN 2022 rankings and criteria [79]. Species are assessed into 1 of 8 rankings based on criteria of extinction threat level such as rate of decline, population size, area of geographic distribution, and degree of population and distribution fragmentation.

CHAPTER 3. RESEARCH RESULTS

3.1. Species composition and diagnostic characteristics of Atyidae shrimp in Vietnam

3.1.1. Atyidae species composition in Vietnam

Through analysis of specimens collected at 294 locations (details in Appendix 4) and synthesis of previous research results, a total of 33 species and subspecies of shrimp of 3 genera belonging to the family Atyidae in Vietnam have been recorded. Of these, 4 species have not been scientifically identified and are being kept as species with suspicion that they are new species to science.

There are 5 species and subspecies first recorded in Vietnam, including: *Caridina mertoni* Roux, 1911, *C. peninsularis* Kemp, 1918, *C. serrata* Stimpson, 1860, *C. temasek* Choy & Ng, 1991, *C. typus* Edwards, 1837 .

There are 4 species that have not been recorded, including: *Atyopsis moluccensis* De Haan, 1849; *Caridina gracirostris* De Man, 1892, *C. tonkinensis* Bouvier, 1919 , *C. weberi* De Man, 1892 [74, 86]

There are 15 new species recorded only in Vietnam (accounting for 46.88%): *Caridina cucphuongensis* , *C. pseudoserrata* , *C. rubropunctata* , *C. clinata* , *C. nguyeni* , *C. caobangensis* , *C. haivanensis* , *C. tricincta* , *C. pacbo* , *C. thachlam* , *C. namdat* , *Caridina* sp.1 , *Caridina.* sp.2 , *Caridina* sp.3 , *Caridina* sp.4 are mainly distributed in the Northeast and Northern Delta regions.

3.1.2. Morphological characteristics of Atyidae shrimp species in Vietnam

3.1.2.1. Species belonging to the genus Caridina

Identification characteristics of *Caridina* Edwards, 1837:

Type species: *Caridina typus* H. Milne Edwards, 1837

(1) *Caridina cantonensis* Yu, 1938

Caridina cantonensis Yu, 1938:

Discussion: In previous studies, *C. cantonensis* was only found in streams, and based on egg size, could be considered a land-limited species [89]. However, in this study, *C. cantonensis* was only found on Cu Lao Cham Island, far from the type site, possibly introduced to Vietnam with juveniles from fish farming, or it may also have a natural distribution on the island. Further studies on this species are needed to understand its distribution and ecological characteristics.

(2) *Caridina caobangensis* Li & Liang, 2002

Discussion: The obtained specimens have morphological characteristics consistent with the description of *Caridina caobangensis*.

(3) *Caridina clinata* Cai, Quynh & Ng, 1999

Discussion: The obtained specimens have morphological characteristics consistent with the description *of Caridina clinata*

(4) *Caridina cucphuongensis* Dang, 1980

Caridina serrata cucphuongensis Dang, 1980: 404 - 405;

Discussion: The obtained specimens have morphological characteristics consistent with the description *of Caridina cucphuongensis*.

(5) *Caridina excavatoides* Johnson, 1961

Caridina excavatoides Johnson, 1961:

Discussion: The morphological characteristics of the specimen are consistent with the species description of *Caridina excavatoides* [95].

The specimens of *C. uminensis* collected by Dang Ngoc Thanh and Do Van Tu (2007) from the type site are completely identical to the description of *C. excavatoides* . Therefore, it can be assumed that *C. uminensis* is a synonym of *C. excavatoides* . This species is characterized by a bigger abdominal segment VI, 0.6 times smaller than the length of the carapace, 1.8 times longer than the length of the abdominal segment V; the Endopod of male first pleopod I is slender, 2.7 times longer than the width; the eggs are small, 0.60 - 0.78 × 0.40 - 0.48 mm [71]in size . Genetically, the species identified as *C. excavatoides* in Vietnam and *C. excavatoides* in Thailand has a genetic distance (16S) of 0.28%, consistent with the morphological assessment.

(6) *Caridina gracilipes* De Man, 1892

Discussion: The morphological characteristics of the collected specimens are consistent with De Man's description of the species *Caridina wyckii* var *gracilipes*. [25].

Dang Ngoc Thanh (1975) described the species *C. acuticaudata* Dang, 1975 mainly based on the absence of appendix interna of the male first pleopod. The species *C. acuticaudata was also mentioned in his and* his colleagues' later [68, 72, 97] publications . Dang Ngoc Thanh & Ho Thanh Hai (2012) commented that this species is endemic to northern Vietnam, in the Red River basin [74]. However, based on the descriptions of these authors and analysis of specimens collected at many locations in Vietnam, it can be

determined that *C. acuticaudata* is *C. gracilipes* De Man, 1892. Genetic analysis based on 16S gene fragment, *C. gracilipes* samples obtained in Vietnam with samples obtained in Sulawesi Indonesia (published in Genbank) forms a separate clade.

(7) *Caridina gracillima* Lanchester, 1901

Discussion: The morphological characteristics of the specimen are consistent with the description of the species *Caridina gracillima* Lanchester, 1901 [98].

(8) *Caridina gracirostris* De Man, 1892

Research specimens: in the NCS specimens obtained and inherited, there was no *C. gracilirostris species* .

(9) *Caridina haivanensis* Do & Dang, 2010

Discussion: The morphological characteristics of the specimen are consistent with the description of the species *Caridina haivanensis* by Do Van Tu and Dang Ngoc Thanh (2010).

(10) *Caridina johnsoni* Cai, Ng & Choy, 2007

Discussion: The morphological characteristics of the studied specimens are consistent with the description of the species *C. johnsoni* by Cai, Ng & Choy (2007) [96].

Dang Ngoc Thanh and Ho Thanh Hai (2012) described the species *C. propinqua* , which has many characteristics of the rostrum, rostral formula, stylocerite, pereiopods and proportions relatively similar to *C. propinqua*. in the description of De Man (1908) and *C. johnsoni* Cai, Ng & Choy, 2007 [96][100]. From the collected specimens and from the description of Dang Ngoc Thanh and Ho Thanh Hai (2012) it can be determined that *C. propinqua* in the description of Dang Ngoc Thanh and Ho Thanh Hai is *C. johnsoni* Cai, Ng & Choy, 2007. Compared with the original description, the specimens identified as *C. johnsoni* from Vietnam sometimes also have pre-anal spines. Genetically, it also shows that *C. johnsoni specimens* not *C. propinqua* with a 16S gene genetic distance of

15.8% with the *C. propinqua sample* obtained in Sri Lanka (sample published in Genbank). The phylogenetic diagram shows that *C. propinqua* is on a branch independent of *C. johnsoni* .

(11) *Caridina lanceifrons* Yu, 1936

Synonym *: Caridina flavilineata* Dang, *Caridina vietriensis* Dang & Do, 2007 , *Caridina pseudoflavilineata* Do & Dang

Discussion: The morphological characteristics of the studied specimens are consistent with the morphological description of *Caradina lanceifrons* Yu, 1936, and the re-description of Liang (2004) and Cai (2014) [12, 82, 96].

Thus, the populations in Vietnam do not show significant differences when compared with the original description of *Caridina lanceifrons* Yu, 1936 or the re-descriptions by Liang (2004) and Cai (2014) [14, 88, 102]. The three species include *C. flavilineata* Dang, 1975, *C. vietriensis* and *C. pseudoflavilineata* Do & Dang, 2010 [70]is considered a synonym of *Caridina lanceifrons* .

(12) *Caridina macrophora* Kemp, 1918

Synonym: *Caridina subnilotica* Dang, 1975

Discussion: The morphological characteristics of the studied specimens are consistent with the description of the species *C. macrophora* [99].

(13) *Caridina mertoni* Roux, 1911

Discussion: The morphological characteristics of the studied specimens are consistent with the descriptions of Roux, 1911 [106]and de Mazancourt et al [51].

(14) *Caridina namdat* Do, Dang & von Rintelen, 2021

Discussion: The morphology of the studied specimen is consistent with the description of the species *Caridina namdat* by Do et al. (2021) [76].

(15) *Caridina nguyeni* Li & Liang, 2002

Discussion: Morphological characteristics of the studied specimen are consistent with the description of the species *Caridina nguyeni* [70].

(16) *Caridina pacbo* Do, von Rintelen & Dang, 2020

Discussion: The morphological characteristics of the studied specimen are consistent with the description of the species *C. pacbo* Do, von Rintelen & Dang, 2020 by Do Van Tu et al (2020) [72].

(17) *Caridina peninsularis* Kemp, 1918

Caridina brachydactyla peninsularis Kemp, 1918: 279, fig 10. [Type locality: Botanical Garden, Penang, Malaysia; lectotype assigned by Cai, Ng & Choy, 2007] [100].

Discussion: The morphological characteristics of the specimen are consistent with the description of the species *Caridina peninsularis* [95].

(18) *Caridina pseudoserrata* Dang & Do, 2007

Discussion: Morphological characteristics of the studied specimens are similar to the species description. *Caridina pseudoserrata* Dang & Do, 2007 by Dang Ngoc Thanh and Do Van Tu (2008) [68].

(19) *Caridina rubropunctata* Dang & Do, 2007

Discussion: The morphology of the tested specimens is consistent with the description of the species *Caridina rubropunctata* Dang & Do, 2007 by Dang Ngoc Thanh and Do Van Tu (2007) [68].

(20) *Caridina serrata* Stimpson, 1860

Discussion: Morphological characteristics of the specimen are similar to the species description. *Caridina serrata* Stimpson, 1860 [107]. This is the first time this species is recorded in Vietnam. The genetic results also confirm the morphological results, when the genetic distance (16S gene) between the *C. serrata samples* obtained in Vietnam with *C. serrata samples* collected in Hong Kong (gene bank) is 0.2%. The genetic relationship diagram also shows that *C. serrata samples* all belong to an independent branch, proving that they all belong to the same species. .

(21) *Caridina temasek* Choy & Ng, 1991

Discussion: The morphological characteristics of the studied specimen are consistent with the description of the species *Caridina temasek.* [108].

(22) *Caridina thachlam* Do, Cao, von Rintelen, 2021

Discussion: The morphological characteristics of the specimen are consistent with the description of Do Van Tu et al. (2021) [109].

(23) *Caridina tonkinensis, Bouvier, 1919*

Research specimens: in the NCS specimens obtained and inherited, there was no *C. tonkinensis species* .

(24) *Caridina tricincta* Do, von Rintelen & Dang, 2020

Discussion: The morphology of the studied specimen is consistent with the species description of Do Van Tu et al. (2020) [75].

(25) *Caridina typus* H. Milne Edwards, 1837

Synonym: *Caridina exilirostris* Stimpson, 1860: 98 (Type location: Okinawa (Loo Choo) Island, Ryukyu Islands, Japan).

Discussion: The morphological characteristics of the specimen are consistent with the description of the species *Caridina typus* [112].

(26) *Caridina weberi* De Man, 1892

Research specimens: specimens obtained and inherited, there was no *C. weberi species* .

(27) *Caridina weberi sumatrensis* De Man, 1892

Caridina weberi var. *sumatrensis* De Man, 1892

Discussion: Morphological characteristics of the studied specimen are consistent with the morphological description of the species *Caridina weberi. sumatrensis* of De Man, 1892 [25].

(28) *Caridina* sp.1

Discussion: *Caridina* sp.1 differs from *C. serrata* in: the rostrum is longer (reaching to the beginning to the middle of the second segment of antennular peduncle to vs. nearly reaching to or slightly exceeding the end of the basal segment of antennular peduncle); number of ventral teeth of the rostrum is smaller (0–2 vs. 0–6); scaphocerite is stouter (2.6 vs. 3.0 times as long as wide); endopod of male first pleopod is more slender (2.9 vs. 2.5

times as long as proximal width); egg size is bigger (1.33 × 0.84 mm vs. 0.9–1.0 × 0.7–0.6 mm)

Genetically, *Caridina* sp.1 and *Caridina cucphuongensis* have a genetic distance of 2.9% in the 16S gene segment. However, morphologically, the two species have differences in the length of the rostrum (reach to the second segment of the antennal compared to the reach to the end of the base segment of the antennal) and the rostral formula (5 - 11 + 4 - 6/0 - 2 vs 0 - 3 + 2 - 7/0 - 2) and Stylocerite (approaching the middle of the 2nd antennal segment compared to reaching the tip of the 2nd antennal segment).

(29) *Caridina* sp.2

Discussion: The obtained specimens of *Caridina* sp.2 have some morphological characteristics similar to *C. caobangensis* described by Li and Liang (2002) with straight, long rostrum reaching the middle of the 3-segmented antennae, fomular rostral teeth [70]. However, the male appendages of *Caridina* sp.2 collected at Xuan Son National Park are completely different from the male appendages described in the publication of Li and Liang (2002) [70], as well as from the morphology of specimens collected at the type site. The species found at Tan Son has the appendix interna of the the first pleopod male exceeding terminal margin of endopod by 0.52 times its length, compared to 0.66 times longer and folded.

Appendix interna of male second of *Caridina* sp.2 obtained in Tan Son reaching to proximal 0.5 times appendix masculina, appendix interna of male second of *C. caobangensis* as long as the appendix masculina. Molecular biological analyses also showed that the genetic difference between *Caridina* sp.2 and *C. caobangensis* is 2 different species with a genetic distance (16S) of 9.6%.

(30) *Caridina* sp.3

Discussion: *Caridina* sp.3 looks similar to *C. pacbo* in the shape of the rostrum and the male first pleopod [75]. However, it can be distinguished by a suite of characteristics: the lower number of teeth in the rostrum (0–8+0–4/0–2

vs. 5–12+2–6/0–3); stylocerite extending (mostly reaching to end of basal segment, sometimes to the beginning of second segment vs. reaching to the middle of the second segment of the antennular peduncle); slender merus of the first pereiopod (2.39–2.67 times as long as wide vs. 2.75–3.8 times as long as wide); slender chela and merus of second pereiopod (3.16–3.5 times as long as wide vs. 2.67–3.09 times as long as wide, 5.92–6.38 times as long as wide vs. 5.0–5.86 times as long as wide, respectively); the distal margins of the endopod of the male first pleopod (slightly extended on both left and right sides vs. not extended). The genetic distance between *Caridina* sp.3 and *C. pacbo* is 4.2%. The genetic relationship diagram also shows that *Caridina* sp.3 is separated into a separate branch, separate from the species *C. pacbo*.

(31) *Caridina* sp.4

Discussion: *Caridina* sp.4 quite similar to C. nguyeni from Cao Bang province, northeast Viet Nam, in the shape of the rostrum and endopod of male first pleopod [70]. However, it can be separated by the number of dorsal teeth on the rostrum anterior to the orbital margin (3–9 vs. 9–12), the number of ventral teeth of the rostrum (0–2 vs. 1–4); the length of stylocerite (reaching to the beginning of the second segment of antennular peduncle vs. reaching to beyond the middle to the end of second segment of the antennular peduncle); the appendix interna of the second male pleopod (about 0.6 times the length of the appendix masculina vs. about 0.4 times length of appendix masculina); the uropodal diaeresis (with 18–21 movable spiniform setae vs. with 12–18 movable spiniform setae). Genetically, it also shows that these are two distinct species with a genetic distance based on the 16S gene segment between the two species of 8.8%.

Genetic evaluation showed that *Caridina* sp. 4 compared with *C. clinata* and *C. haivanensis* are quite closely related to each other with genetic distances (16S) of 1.3% and 2.5%, respectively. However, in terms of morphology, the three species also have many differences that require

further analysis of other genes to more accurately assess the relationship between these species.

3.1.2.2. The genus Neocaridina Kubo, 1938

Identification characteristics of the *Neocaridina* Kubo, 1938 variety:

Distribution: Southeast Asia.

Standard species: *Neocaridina denticulate* (De Haan, 1844)

***Neocaridina palmata palmata* Shen, 1948**

Discussion: The morphological characteristics of the studied specimen are consistent with the description of the species *Neocaridina palmata palmata* by Shen [99].

Dang Ngoc Thanh (1967) described the species *Caridina denticulata vietnamensis* in Northern Vietnam. In later works, this author raised this subspecies to the species level *C. vietnamensis.* [68]. Cai (1996) considered this species as a synonym of *Neocaridina palmata palmata* (Shen, 1948) [104]. Later, Dang Ngoc Thanh and colleagues transferred the species *C. vietnamensis* to the genus *Neocaridina* (*N. vietnamensis*) [72, 74, 97]. Li & Liang (2002) based on the classification of Cai (1996) considered *C. denticulata vietnamensis* as a synonym of *N. palmata palmata* (Shen, 1948) [70]. However, Dang Ngoc Thanh and Do Van Tu (2008) and Dang Ngoc Thanh and Ho Thanh Hai (2012) commented that the species *N. vietnamensis* can be distinguished from the species *N. Palmata palmata* or a subspecies of this species by the endopod of the male first pleopod (round shape and stable vs. variable); appendix masculina of male second pleopod (perpendicular vs. rounded) and appendix interna (reaching nearly to the tip of appendix masculina vs. not reaching to the tip of appendix masculina) and larger egg size (1 - 1.25 × 0.65 - 0.85 mm). However, Liang (2004) stated that the shape of the endopod of the first pleopod is very variable in *N. palmata*, even in the specimens collected from the same localities. Genetic analysis also confirmed that *N. palmata palmata samples* collected from different locations in Northeast Vietnam are all subspecies of *N. palmata palmata*, with a genetic

distance of 0 - 0.2% (16S) vs. Sample published in gene bank (obtained in Hong Kong, China).

3.1.2.3 The genus Atyopsis

Identification characteristics of *Atyopsis genus* Chace, 1983 :

Distribution: Southeast Asia [82].

Type species: *Atya spinipes* Newport, 1847

***Atyopsis moluccensis* De Haan, 1849**

Atya moluccensis De Haan, 1849

Research specimens: specimens obtained and inherited, there was no *Atyopsis moluccensis* species .

3.1.3. Genetic characteristics of Atyidae shrimp species distributed in Vietnam based on 16S gene sequence analysis

The 590bp long 16S ribosomal subunit gene fragment, after correction, the remaining 535bp of 75 samples from 30 species of the Atyidae family in Vietnam (30 species analyzed for morphology) was used to analyze the genetic relationship. The researcher used 09 more sequences of some *Caridina species* published in GenBank for comparison. *The Neocaridina palmata palmata species* was used as an outgroup in the analysis due to its taxonomic closeness to the genus *Caridina*. [13]

3.1.3.1. K2P genetic distance among Atyidae shrimp species in Vietnam

The results of K2P genetic distance analysis based on the 535bp segment of the 16S rRNA gene of shrimp species belonging to the family Atyidae in Vietnam showed the average genetic difference between shrimp species belonging to the family Atyidae in Vietnam showed the average genetic difference between shrimp species belonging to the genus *Caridina* is 12.1%, the smallest genetic distance is between the two species *Caridina* sp.4 and *C. clinata* was 1.3% and the largest was 20.3% between *C. temasek* and *Caridina* sp.2. The genetic distance between species of the genus *Caridina* and *Neocaridina palmata palmata* was 11.9% on average, the lowest distance between *N. palmata palmata* and *C. thachlam* was 7.7%.

3.1.3.2. Genetic relationship of Atyidae shrimp in Vietnam

The results of genetic relationship analysis based on 16S gene sequence analysis showed that all Atyidae shrimp species in Vietnam (including species of the genera *Caridina* and *Neocaridina*) are separated into independent groups. This shows that Atyidae shrimp species in Vietnam are all monophyletic.

In summary, the molecular phylogenetic results demonstrate that Vietnam lies on the biogeographic boundary between East Asia and Southeast Asia, as evidenced by the fact that the distribution ranges of the two species groups meet in Vietnam. The high species density and genetic diversity in northern Vietnam are remarkable and warrant further investigation.

3.1.3. Identification key for species of the Atyidae family in Vietnam

I	The carpus of pereiopod I and II are the same. The chela has no palm.	*Atypia* (*A. moluccensis*
	The carpus of pereiopod I and II are different. The chela has palm.	II
II	Coxa of pereiopod II with a curved spine. Endopod of male first pleopod expanded into pear shape, with tiny spiniform setae on distal margin of dorsal surface; appendix interna is at the base of inner border	*Neocaridina (N. palmata palmata)*
	Coxa of pereiopod II with a curved spine. Endopod of male first pleopod is usually bean-shaped or conical, with no spiniform setae on distal margin of dorsal surface, appendix interna is at the tip of the inner edge.	*Caridina*

Identification key for species of the genus Caridina recorded in Vietnam

1	Normal eye	3
	Reduced or absent eyes	2

2	Eyes slightly reduced; eye stalks still present	*C. namdat*
	Eyes greatly reduced or absent; eyestalk absent	*C. thachlam*
3	Long stylocerite, exceeding the basal segment of antennal peduncle I	4
	Short stylocerite peduncle, not exceeding the basal of antennal peduncle I	15
4	Very short rostrum, not exceeding the basal segment of antennal peduncle I.	5
	Rostrum exceeds the basal segment of antennal peduncle I.	7
5	Appendix interna of male second pleopod reaches 0.3-0.4 times the length of appendix masculina	*Caridina serrata*
	Appendix interna of male second pleopod reaches 0.6 times the length of appendix masculina	6
6	Rostral formula 5 - 12 + 2 - 6/0 - 3, stylocerite reaching to the middle of the second segment of antennal peduncle I; merus of the first pereiopod 2.75 - 3.8 times as long as wide	*Caridina pacbo*
	Rostral formula 0 - 8 + 0 - 4/0 - 2, stylocerite reaching to the end of basal segment segment of antennal peduncle I, merus of the first pereiopod 2.39–2.67 times as long as wide	*Caridina sp.3*
7	Rostrum does not reach the middle of the second segment of antennal peduncle I	8
	Rostrum reaching beyon the middle of the second segment of antennal peduncle I	9
8	Rostrum slightly curved downward, Rostral formula 5 - 11 + 4 - 6/0 - 2, stylocerite reaching to nearly the middle of the second segment of antennal peduncle I.	*Caridina sp. 1*

	Rostrum straight, Rostral formula 5 - 11 + 4 - 6/0 - 2, stylocerite reaching to nearly the beginning of the second segment of antennal peduncle I.	*Caridina cucphuongensis*
9	First pereiopod dactylus shorter than palm	10
	First pereiopod dactylus the same or longer than palm	11
10	Rostrum reaching the end of antennal peduncle I, Rostral formula 11 - 14 + 9 - 12/5, stylocerite reaching to middle of the second segment of antennal peduncle I.	*Caridina nguyeni*
	Rostrum reaching beginning of the third segment of antennal peduncle I, Rostral formula 2 - 7 + 3 - 9/0 - 2, stylocerite reaching to middle of the second segment of antennal peduncle I.	*Caridina sp. 4*
11	Stylocerite reaching beyond the middle to near the tip of second segment of antennal peduncle I.	*C. rubropunctata*
	Stylocerite rarely reaching beyond the middle of second segment of antennal peduncle I	12
12	3 - 5 ventral rostral teeth, Endopod of male first pleopod anterior part folded backwards, Uropodal diaeresis with 15 - 18 movable spiniform setae	*C. cantonensis*
	0 - 2 ventral rostral teeth, Endopod of male first pleopod anterior traight, Uropodal diaeresis with 19 - 22 movable spiniform setae	13
13	Rostrum reaching the middle of the second segment of antennal peduncle I	*C. pseudoserrata*
	Rostrum reaching beyon of the third segment of antennal peduncle I	14
14	Appendix interna of male second pleopod almost as long as the tip of appendix masculina	*C. caobangensis*
	Appendix interna of male second pleopod 0.6 times the length of appendix masculina	*Caridina sp. 2*

15	Rostrum not exceeding the tip of the second segment of antennal peduncle I	16
	Rostrum beyon the tip of the second segment of antennal peduncle I	19
16	Stylocerite reaching tip of the basal segment of antennal peduncle I.	17
	Stylocerite just over half the first segment of the antennal peduncle I	18
17	Appendix interna of male second pleopod 0.9 times the length of appendix masculina	*Caridina tricincta*
	Appendix interna of male second pleopod 0.5 times the length of appendix masculina	*Caridina haivanensis*
18	Endopod of male first pleopod roughly rectangular in shape.	*C. clinata*
	Endopod of male first pleopod roughly conical in shape.	*C. mertoni*
19	No dorsal rostral teeth and dorsal rostral teeth posterior to orbital margin	*C. typus*
	Has dorsal rostral teeth and dorsal rostral teeth posterior to orbital margin	20
20	Rostrum curves upward, extending far beyond the edge of the scaphocerite.	21
	Rostrum curves nearly straight or curved downwards, may reach the edge of the scaphocerite.	24
21	Rostrum very long, extending far beyond the the edge of the scaphocerite, about 1.5 times the length of the carapace; dorsal rostral teeth fewer than 12 widely spaced teeth, including 1 dorsal rostral teeth posterior to orbital margin	22

	Rostrum very long, extending far beyond the the edge of the scaphocerite, about 1.5 times the length of the carapace; dorsal rostral teeth more than 12 closely spaced teeth, including at least 1 dorsal rostral teeth posterior to orbital margin	23
22	Preanal carina lacking a spine, average egg size 0.55 - 0.66 × 0.35 - 0.40 mm	*C. gracillima*
	Preanal carina with spine, average egg size small 0.4 × 0.25 mm	*C. gracilirostris*
23	Telson triangular, with a short central point; Endopod of male first pleopod lacking appendix interna or only in the form of notches; egg size small 0.3 - 0.32 × 0.48 - 0.52 mm	*C. gracilipes*
	Telson edge slightly rounded at tip, without central point; appendix interna of male first pleopod horn-shaped appendage, growing at apical edge; average egg size 0.53 - 0.59 × 0.84 - 0.93 mm	*Caridina macrophora*
24	Rostrum almost straight, with teeth covering dorsal rostral	25
	Rostrum slightly curved downwards at the front or sigmoid shaped, with no teeth on the front of the dorsal rostral.	28
25	Rostrum long, beyon of the antennal peduncle I	26
	Rostrum short, usually reaching only the middle of the second of the antennal peduncle I	27
26	Less than 3 dorsal rostral teeth posterior to orbital margin; the egg size is small 0.40 - 0.42 × 0.25 - 0.30 mm	*Caridina peninsularis*
	Less than 4 - 6 dorsal rostral teeth posterior to orbital margin; large egg size 0.80 - 0.90 × 0.50 - 0.60 mm	*Caridina lanceifrons*
27	No dorsal rostral teeth posterior to orbital margin	*C. weberi*
	4 - 6 dorsal rostral teeth posterior to orbital margin	*C. weberi sumatrensis*

28	Endopod of male first pleopod lacking appendix interna	*C. temasek*
	Endopod of male first pleopod has appendix interna	29
29	Abdominal segment VI is larger, the ratio of abdominal segment length VI/cephalothorax length is less than 0.60 times	*C. excavatoides*
	The abdominal segment VI is slender, the ratio of the length of the abdominal segment VI/the length of the cephalothorax is more than 0.65 times.	30
30	Preanal carina lacking a spine; length/width ratio carpus of pereiopod I and II are 2.9 and 4.0, respectively.	*C. johnsoni*
	Preanal carina with spine; length/width ratio carpus of pereiopod I and II are 3.5 and 4.4, respectively.	*C. tonkinensis*

3.2. Distribution characteristics and proposed conservation classification of Atyidae shrimp in Vietnam

3.2.1. Evaluation of distribution characteristics of Atyidae shrimp species in Vietnam

3.2.1.1. Distribution by region

Synthesizing data from 294 points where Atyidae shrimp samples were collected, it shows that the North has a rich number of shrimp species belonging to the Atyidae family with 21 species accounting for 64% of the total number of recorded species (Figure 3.51). Of the 21 species distributed only in the North, 6 species, *Neocaridina palmata palmata, C. serrata, C. tonkinensis, C. weberi sumatrensis, C. weberi* and *C. cantonensis*, have a wide distribution recorded in some neighboring countries and Vietnam. The remaining species are currently only found in Vietnam and most are only recorded in one or two provinces.

The results in Table 3.14 show that Atyidae shrimp are less distributed in the Northwest region, with up to 78% of the survey points not collecting Atyidae shrimp samples. The Central Highlands region is also the region with less Atyidae shrimp samples, with the number of points not

collecting samples accounting for 88%. The North Central region, the Northern Delta region, the Northeast region, and the South Central region are the regions with a large distribution of shrimp species belonging to the Atyidae family, with the percentage of points collecting samples accounting for over 90% of the survey points.

3.3.1.2. Distribution by altitude and landscape topography

a. Distribution according to landscape terrain

- Distribution of Atyidae shrimp in mountainous water bodies:
- Distribution of Atyidae shrimp in delta water bodies:
- Distribution of Atyidae shrimp in coastal estuary waters:

b. Altitude distribution

Thus, it can be seen that most shrimp species of the Atyidae family are distributed at low altitudes below 300 m. m above sea level. However, altitudes below 300 This area is heavily impacted by human activities such as agricultural cultivation as well as industrial production activities, which is a major threat to shrimp species of the Atyidae family.

3 .2.2. Assessment of conservation classification and factors affecting Atyidae species in Vietnam

3.2.2.1. Conservation classification assessment of Atyidae species in Vietnam

Of the total 33 species of Atyidae shrimp recorded in Vietnam, 23 species have been recognized globally by the International Union for Conservation of Nature and Natural Resources (IUCN). Accordingly, 8 species at Least Concern (LC) and 1 species (*C. serrata*) at Near Threatened (NT).

The results of the assessment of the conservation status of Atyidae shrimp species in Vietnam according to IUCN rankings and standards show that the number of species assessed at Vulnerable (VU) is 7 (accounting for 21%), Near Threatened (NT) is 13 (accounting for 40%), Least Concern (LC) is 11 (accounting for 33%) and Data Deficient (DD) is 2 (accounting for 6%) (Table 3.9). Thus, the threat level of Atyidae shrimp is higher than that of other freshwater invertebrate groups. Notably, the group of species assessed

at Near Threatened accounts for a fairly high proportion. If the habitat continues to be destroyed at the current rate, the proportion of species threatened with extinction in this group could reach nearly 50%.

3.3.3. Proposed conservation measures

With species groups having wide distribution

With some endemic species having narrow distribution areas

CONCLUSION AND RECOMMENDATIONS

CONCLUSIONS

1. The Atyidae shrimp fauna in Vietnam comprises 33 species belonging to three genera: *Atyopsis*, *Caridina*, and *Neocaridina*. Among them, four species (*Caridina sp.1*, *Caridina sp.2*, *Caridina sp.3*, *Caridina sp.4*) are suspected to be new to science. Additionally, five species (*C. mertoni*, *C. peninsularis*, *C. serrata*, *C. temasek*, and *C. typus*) have been newly recorded for Vietnam. Six species have undergone taxonomic revisions: *C. flavilineata*, *C. vietriensis*, and *C. pseudoflavilineata* are now considered synonyms of *C. lanceifrons*, while *C. acuticaudata*, *C. uminensis*, and *C. subnilotica* are synonyms of *C. gracilipes*, *C. excavatoides*, and *C. nilotica macrophora*, respectively. A classification key has been developed for the 33 Atyidae species currently identified in Vietnam.

The genetic distance based on the 16S rRNA gene among *Caridina* species averages 12.1%, with the smallest genetic distance (1.3%) found between *Caridina sp.4* and *C. clinata*, and the largest (20.3%) observed between other species. Species with a narrow distribution in Vietnam have a genetic distance of 7.7%, which is lower than that of widely distributed species (14.8%).

2. Among the Atyidae species found in Vietnam, 64% are distributed in the northern region, 12% in the southern region, and 24% are found nationwide. The Northeast region has the highest diversity of Atyidae shrimp, with 17 species recorded, while the Northwest region has only one

species. In terms of elevation, 26 species are found at altitudes of 0–300 m, 15 species between 300–800 m, and three species above 800 m. Most Atyidae species inhabit areas where human activities and production occur, making them vulnerable to pollution, overexploitation, and habitat loss.

3. Regarding the conservation status of Atyidae shrimp in Vietnam:

Vulnerable (VU): 7 species (*Atyopsis moluccensis*, *Caridina cantonensis*, *C. serrata*, *C. haivanensis*, *C. namdat*, *C. thachlam*, and *C. weberi sumatrensis*). Near Threatened (NT): 13 species, Least Concern (LC): 11 species, Data Deficient (DD): 2 species.

RECOMMENDATIONS

Further Research

Conduct additional field surveys across various regions to rediscover lost specimens of species such as *Atyopsis moluccensis*, *Caridina tokinensis*, and *C. weberi*, and to identify new species of Atyidae shrimp in Vietnam.

Perform further mitochondrial and genomic analyses to clarify the taxonomic status and genetic diversity of Vietnamese Atyidae shrimp.

Study the biological and ecological characteristics, as well as artificial breeding techniques, of Atyidae species—particularly endemic species—to support conservation efforts.

Conservation Recommendations

For species classified as Vulnerable, protective measures should be implemented, including habitat conservation, restrictions on harvesting, public awareness campaigns, and artificial breeding programs to facilitate ex situ conservation of Atyidae shrimp.

Conduct stock assessments of *Caridina* shrimp to establish sustainable exploitation and conservation strategies.

Apply molecular markers to evaluate population diversity and guide conservation planning for *Caridina* shrimp.

LIST OF PUBLISHED WORKS RELATED TO THE THESIS

A. INTERNATIONAL ARTICLES

1. Do Van Tu, Kristina von Rintelen, Werner Klotz, Le Hung Anh, Tran Anh Tuan, Dang Van Dong, **Phan Thi Yen** , Nguyen Tong Cuong, Hoang Ngoc Khac, Phan Doan Dang, Thomas von Rintelen (2021). Taxonomy notes and new occurrence data of four species of atyid shrimp (Crustacea: Decapoda: Atyidae) in Vietnam, all described from China. Biodiversity Data Journal 9: e70289. doi: 10.3897/BDJ.9.e70289

B. DOMESTIC NEWSPAPERS

1. **Phan Thi Yen** , Werner Klotz, Thomas von Rintelen, Dang Van Dong, Do Van Tu (2021). Redescription of *Caridina cucphuongensis* Dang, 1980 (Crustacea: decapoda: Atyidae) from cuc phuong national park, northern Vietnam. Academia journal of biology 2021, 43(4): 45–54

2. Phan Doãn Đăng, Lê Hùng Anh, **Phan Thị Yến**, Nguyễn Văn Tú, Nguyễn Xuân Đồng, Đỗ Văn Tứ*, 2021. Ghi nhận mới về loài tôm nước ngọt *Caridina typus* H. Milne Edwards, 1837 (Crustacea: Decapoda: Atyidae) ở Việt Nam. Tạp chí Nông nghiệp và Phát triển nông thôn. Số chuyên đề "Nguồn lợi Thủy sản Việt Nam: Đa dạng sinh học, nuôi trồng và phát triển bền vững", 2021, tr.154–161.