Rostral Geometric Morphometrics in a Hippolytid Shrimp: Are There Elements That Reflect the Homozygous/Heterozygous State of Its Morphotypes?

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Abstract: Geometric morphometry has been widely used in decapods’ studies for taxonomic needs, and for eco-morphological adaptation and intraspecific variations recordings. Among the 40 species of the genus Hippolyte, the Mediterranean endemic Hippolyte sapphica is the only one with two distinct conspecific morphotypes, without intermediate forms: morph-A with a long, dentate and morph-B with a very short, toothless rostrum. Previous studies have shown that the “rostral loss” in morph-B seems to be controlled by a single pair of alleles, with a complete dominance of allele b, expressed in morph-B. We aim to elucidate morphotypes’ rostral pattern in relation to size, sex, and season. Shrimps were collected during two different (dry/wet) seasons from two sites: s.1 with a mixed (morph-A and B) and s.2 with a pure, unmixed (morph-A) species populations. After morph and sex identification, individuals were photographed and geometric morphometric analysis of rostrum was carried out on a set of landmarks. The data suggest that only morph-A rostral shape seems to be influenced by shrimp’s size, sex, and time of the year. Interestingly, two distinct morph-B clusters appear, which probably correspond to the homozygous and heterozygous state (BB and BA) of the gene site that controls the species morphotypes’ phenology.

Keywords: Hippolytidae; rostral dimorphism; morphotypes; geometric morphometrics; Mediterranean

1. Introduction

Throughout the study of shape, various approaches have been proposed for the analysis and the quantification of form patterns in biological systems. Geometric Morphometrics (GM) emerged as powerful technique to compare organisms’ shape and identify its causes [1–4]. Particularly in freshwater and marine crustaceans, morphometric analyses are widely used for the study of intra- and inter species population variability and asymmetries [5–11].

Hippolytid species are included in one of the oldest genera, with interesting taxonomic history and a worldwide distribution with the exception of Antarctica waters [12,13] (Figure 1, Table 1). Almost 208 years ago Leach established the new genus Hippolyte Leach, 1814 with the monotypic H. varians. Rafinesque (1814) [14] followed with Carida viridis, which probably corresponded to H. inermis Leach, 1815 [15] and Hippolyte coerulescens (Fabricius, 1775) [16] was then described as Astacus coerulescens [12]. The first complete genus revision in Atlanto-Mediterranean region with a catalogue of the world species was presented by d’Udekem d’Acoz (1996) [12]. Till now, the genus comprises 40 species [17–19] (Figure 1, Table 1), many of them with considerable rostral variation. Rostral structure has a strong taxonomic value [20,21], assists in the buoyancy of the body [9], and eliminates predation [22–24]. Its variability is usually related to environmental conditions [25], to sexual dimorphism [9,12,26], and to reproductive maturation [27]. A remarkably high
rostral variability in shape and dentition has been observed in many hippolytid shrimps (Table 1). For example, *H. garciarasoi*, *H. leptocerus*, and *H. varians* are the species with obvious types in rostral shape and dorso-ventral dentition, while *H. inermis*, *H. niezabitowskii* and *H. prideauxiana* are variable only in the meristic characters and the position of the rostral dentition (Table 1). Usually, the observed variability is continuous with intermediate forms or morphotypes, which are dispersed along the species’ distributional ranges. The only species of the genus with the most characteristic sharp dimorphic rostral system is *H. sapphica*.

Figure 1. World map indicating the geographical distribution of *Hippolyte* species as a percentage % to the total number (species number/total number), according to the main zoogeographic regions. PA: Palaearctic, NA: Nearctic, NT: Neotropical, AT: Afrotropical, OR: Oriental, AU: Australasian, PAC: Pacific Oceanic Islands, ANT: Antarctic. Modified by [28].
### Table 1. Zoogeographical distribution, habitat characteristics, rostral formula and body lengths for *Hippolyte* species. Rostral formula: a(b)/c(d), a: dorsal teeth, b: postrostral teeth, c: ventral teeth, d: usual number of ventral teeth. TL: total length, CL: carapace length, RL: rostral length, DD: data deficient.

| Taxa                          | Distribution                      | Depth Range (m) | Habitat                        | TL (mm) | RL/CL | Rostral Formula | Reference(s) | Rostral Variability |
|-------------------------------|-----------------------------------|----------------|--------------------------------|---------|-------|----------------|--------------|--------------------|
| *Hippolyte acuta* (Stimpson, 1860) | Pacific Ocean (N, S Japan, Korea) | 2 to 5         | eelgrass bed                    | DD      | 1.03–1.36 | 1(0)/0–4 (usually 1–2) | no           | [12,29]         |
| *Hippolyte australiensis* (Stimpson, 1860) | Australia                         | 0 to 15        | tufted algae                    | 18 to 25 | 1     | 0(0)/4–6 (rarely 3) | no           | [12,30]         |
| *Hippolyte bifidirostris* (Miers, 1876) | New Zealand                       | 18 to 36       | DD                             | DD      | 1     | rostrum very long, strongly dentate, with bifid/trifid rostral apex | no           | [12,30,31]      |
| *Hippolyte californiensis* Holmes, 1895 | Northeastern Pacific Ocean         | Intertidal     | seagrass, gorgonians           | 38      | 1.16  | 3(0)/4–5       | no           | [32,33]         |
| *Hippolyte caradina* Holthuis, 1947 | Pacific Ocean                     | DD             | DD                             | DD      | 2(1)/1 | no             | [12,30]      |
| *Hippolyte cataphracta d’Udekem d’Acoz, 2007* | S. Africa                         | 6 to 8         | *Tropiometra carinata*          | 22      | 0.9   | 1(0)/2–3       | yes, males with slender rostrum, rostral formula: 3(0)/0–1 | no           | [34]            |
| *Hippolyte chacei* Gan & Li, 2019 | Gulf of Guinea, tropical E Atlantic Ocean | 34 to 37       | *Tanacetipathes* spinescens, *Antipathella* wollastonii, *Muriceopsis* tuberculata | DD      | 1     | 3(0)/2        | yes, male rostral formula: 1(0)/4 | [31]            |
| *Hippolyte clarki* Chace, 1951 | Hainan Island, NS China Sea       | 1 to 3         | Sargassum sp.                   | DD      | 0.9   | 0(0)/4         | yes, male rostral formula: 1(0)/4 | [35]            |
| *Hippolyte coerulescens* (J.C. Fabricius, 1775) | NE Pacific Ocean                 | Intertidal to 30 | seagrass, gorgonians           | 28      | 0.8 to 1.4 | 3(0)/4        | no           | [38]            |
| *Hippolyte commensalis* Kemp, 1925 | Atlantic Ocean                   | Sublittoral    | Drifting substrates, mud-sand flats, Sargassum natans | 16.5    | 0.7–0.9 | 1(0–2)(0)/1(3) | no           | [12]            |
| *Hippolyte edmondsoni* Hayashi, 1981 | Indo-Pacific Ocean               | 0.5 to 30      | *Xeniia* sp.                    | DD      | 0.7   | 0(0)/1        | no           | [36]            |
| *Hippolyte dossea* (Marin et al., 2011) | Izu Islands, Japan, Ball, and N Great Barrier Reef of Australia | 5 to 8         | *Steneopeltisa japonica*, *Eflatounaria* sp. | DD      | 0.5   | 0(0)/1        | no           | [36]            |
| *Hippolyte edmondsoni* Hayashi, 1981 | Indo-Pacific Ocean, Hawaiian Islands | DD             | DD                             | 10.3    | <0.5  | 0(1)/0        | no           | [12,37]         |
| Taxa                        | Distribution                      | Depth Range (m) | Habitat                                                                 | TL (mm) | RL/CL | Rostral Formula | Rostral Variability                      | References |
|-----------------------------|-----------------------------------|-----------------|-------------------------------------------------------------------------|---------|-------|-----------------|------------------------------------------|------------|
| Hippolyte garciarasoi d’Udekem d’Acoz, 1996 | Atlantic Ocean, Mediterranean Sea | 0 to 15         | photophilous algae, Posidonia oceanica, Deep photophile algae, Coralligen, marine caves, coastal detritical bottoms Posidonia oceanica, Cymodocea nodosa, Zostera marina, Zostera noltii, and photophilous algae (Ulva spp.) | 15      | 0.6–0.8 | 2(1–3)(1)/1–4    | yes, in shape and dentition               | [12]       |
| Hippolyte holthuisi Zariquiey Alvarez, 1953 | Mediterranean Sea                 | 7 to 50         | Deep photophile algae, Coralligen, marine caves, coastal detritical bottoms Posidonia oceanica, Cymodocea nodosa, Zostera marina, Zostera noltii, and photophilous algae (Ulva spp.) | 19      | 0.9    | 2(0)/2          | no                                       | [38,39]    |
| Hippolyte inermis Leach, 1815 | Atlantic Ocean, Mediterranean Sea | 1 to 30         | Posidonia oceanica, Cymodocea nodosa, Zostera marina, Zostera noltii, and photophilous algae (Ulva spp.) | Atlantic: to 50.1 Mediterranean: to 39.5 | 1.1    | 0–1(2)(0)/2–3(0–6) | no                                      | [12]       |
| Hippolyte jarvinensis Hayashi, 1981 | Central Pacific Ocean, Jarvis and Line Islands, Solomon Islands St. Helena in the tropical South-Central Atlantic Ocean | DD DD | Macrorhynchia filamentos, Plumaphyes pennacea Zostera capensis, Thalassodendron ciliatum, Halodule uninervis, Thalassia hemprichii, Halodule wrightii | 8       | 0.7    | 1(0)/1          | no                                       | [12,37]    |
| Hippolyte karenae Fransen & De Grave, 2019 | Atlantic Ocean, Mediterranean Sea | 15 to 20.4      | Photophilic algae: Laurencia pinnatifida, Gelidium sesquipetale Photophilic algae: small seagrasses, Posidonia oceanica, Leptometa phalangium, L. celtica Muriceopsis tuberculata | DD     | <1     | 3(0)/2          | yes, males with slender rostrum, rostral formula: 1–3(0)/0–1 | [17]       |
| Hippolyte kraussiana (Stimpson, 1860) | Indo-Pacific Ocean, Mozambique   | 50              | Zostera capensis, Thalassodendron ciliatum, Halodule uninervis, Thalassia hemprichii, Halodule wrightii | DD      | DD     | DD              | DD                                      | [40]       |
| Hippolyte lagarderei d’Udekem d’Acoz, 1995 | Atlantic Ocean                   | intertidal      | Photophilic algae: Laurencia pinnatifida, Gelidium sesquipetale Photophilic algae: small seagrasses, Posidonia oceanica, Leptometa phalangium, L. celtica Muriceopsis tuberculata | 22      | 0.67 to 0.78 | 0–2(0)/0–3    | yes, in shape inclination               | [12]       |
| Hippolyte leptocerus (Heller, 1863) | Atlantic Ocean, Mediterranean Sea | intertidal to 30 | Posidonia oceanica, Leptometa phalangium, L. celtica Muriceopsis tuberculata | Atlantic: 17.7 to 22.4 Mediterranean: 11 to 15 | 0.4–0.5 | 2–3(1–6)/1–4(0–4) | yes, in shape and dentition               | [12]       |
| Hippolyte leptometrae Ledoyer, 1969 | Atlantic Ocean, Mediterranean Sea | 95 to 130       | Leptometa phalangium, L. celtica Muriceopsis tuberculata | 18      | 1.4    | 2(0)/2          | no                                       | [12,34]    |
| Hippolyte longiallex d’Udekem d’Acoz, 2007 | NE Atlantic Ocean                | 35 to 40        | Leptometa phalangium, L. celtica Muriceopsis tuberculata | 8       | 0.7    | 2–3(0)/1–2      | no                                       | [34]       |
| Taxa                              | Distribution                          | Depth Range (m) | Habitat                        | TL (mm) | RL/CL | Rostral Formula | Rostral Variability | References |
|----------------------------------|---------------------------------------|-----------------|--------------------------------|---------|-------|-----------------|---------------------|------------|
| Hippolyte multicolorata          | Yaldwyn, 1971 Pacific Ocean           | intertidal      | algae                          | 8.5     | 1.1   | 0(0)/4–9, trifid apex | no                  | [41]       |
| Hippolyte nanhaiensis            | Gan & Li, 2019 Xisha Islands, South China Sea | 1 to 3          | Galaxaura sp., Halimeda sp.    | DD      | 0.7   | 2(0)/1          | no                  | [31]       |
| Hippolyte ngi                    | Gan & Li, 2017 Subar Laut Island, St. John’s Island and Hainan Island, NS China Sea | 1 to 5          | Sargassum sp.                  | DD      | 0.73  | 1(0)/2          | no                  | [18]       |
| Hippolyte nicholsoni             | Chace, 1972 Caribbean Sea              | 2 to 12         | Pseudopterogorgia acerosa       | DD      | 0.3–0.5 | 1–2(0)/1–3     | no                  | [12]       |
| Hippolyte nizabitowskii d’ Acoz, 1996 | Mediterranean Sea                     | 0.5 to 5        | sheltered meadows, seagrasses  | 10 to 20| 0.8   | 0–2(0–4)(0)/0–4 | yes, in dorsal dentition | [12]       |
| Hippolyte obliquimanus            | Dana, 1852 Antigua, Carriacou, Tobago, Guadeloupe, Curacao, Puerto Rico, Venezuela, Brazil | intertidal      | Thalassia testudinum, Syringodium filiforme | 15      | 1     | 3–4(0)/4, bifid apex | yes, shape and dentition | [42,43]  |
| Hippolyte orientalis             | Heller, 1861 Red Sea, Suez Canal, Gulf of Aden | intertidal      | DD                             | DD      | 1     | 1(0)/3          | no                  | [44]       |
| Hippolyte palliola               | Kensley, 1970 Atlantic Ocean          | Intertidal to 25| amongst algae on bottom with shells and hydroids, turtle-grass | 10      | 0.3   | 1(0)/0          | no                  | [12]       |
| Hippolyte pleuracanthus          | (Stimpson, 1871) W Atlantic Ocean     | 0.4 to 0.8      | flatsmuddy substrate with T. testudinum, Zostera, Diplanthera | 12 to 18| 0.5   | 2(0)/1          | no                  | [12,45,46] |
| Hippolyte prideauxiana           | Leach, 1817 (in Leach, 1815–1875) Atlantic Ocean, Mediterranean Sea | intertidal to 60| Antedon bifida and Antedon mediterranea | 10.4 to 21.7| 0.6   | 0(0)/1–7        | yes, in ventral dentition | [12]       |
| Hippolyte proteus                | (Paulson, 1875) Red Sea, Suez Canal   | DD              | DD                             | 13      | 1.1   | 2(1–4)(0)/2(1–4) | no                  | [12]       |
| Taxa                                      | Distribution              | Depth Range (m) | Habitat                                  | TL (mm) | RL/CL | Rostral Formula                  | Rostral Variability | References |
|------------------------------------------|---------------------------|-----------------|------------------------------------------|---------|------|-----------------------------------|--------------------|------------|
| *Hippolyte sapphica* d’ Udekem d’Acoz, 1993, “forma A” d’ Udekem d’ Acoz, 1996 | Mediterranean Sea          | 0 to 1.5        | *Zostera marina, Cymodocea nodosa*       | 12 to 27 | 1.1  | 2(1–3)(1–2)/2–3(1–4)              | sharp dimorphic    | [12,47]    |
| *Hippolyte sapphica* d’ Udekem d’ Acoz, 1993, “forma B” d’ Udekem d’ Acoz, 1996 | Mediterranean Sea          | 0 to 1.5        | *Zostera marina, Cymodocea nodosa, Cystoseira spp.* | 15      | 0.25 | 0(1)/0                           | sharp dimorphic    | [12,47]    |
| *Hippolyte singaporensis* Gan & Li, 2017 | Singapore                 | 0 to 1.5        | *Enhalus acoroides, Sargassum spp., Padina spp.* | DD      | 1    | 0(0)/1                           | no                 | [18]       |
| *Hippolyte varians* Leach, 1814          | Atlantic Ocean            | 7 to 60 (mainly 20 to 40) | deep photophile algae, Coralligen, marine caves, coastal detritical bottoms | 20.1 to 32.2 | 0.8  | 2(0)/2(0–4)                      | yes in dentition  | [12]       |
| *Hippolyte ventricosa* H. Milne Edwards, 1837 (in H. Milne Edwards, 1834–1840) | Red Sea, Suez Canal, Indian Ocean | 1 to 3         | *Thalassia sp., Sargassum sp.*           | 13 to 24 | 1.1  | 1–3(0)/1–5                       | no                 | [12,31,37] |
| *Hippolyte williamsi* Schmitt, 1924      | E Pacific Ocean           | Intertidal      | *Sargassum sp.* sublittoral, soft substrata, turfe-grass flats, *T. testudinum, Halodule wrightii, Syringodium filiforme* | 20      | 1    | 3(0)/4                           | no                 | [12,42]    |
| *Hippolyte zostericola* (Smith, 1873)    | W Atlantic Ocean, Pacific Ocean | 0.5 to 1.5    |                                           | DD      | 0.5–0.7 | 2(0)/3                           | no                 | [12,45]    |
Hippolyte sapphica includes two morphotypes, morph-A with a long, dentate rostrum and morph-B with juvenile-like, short rostrum [12] (Figure 2). Ntakis et al. (2010) [48] confirmed the conspecific status of the two distinct morphotypes. Morph-B is distributed only in Central Mediterranean (Amvrakikos Gulf, Greece and Venice Lagoon, Italy), whereas its sympatric morph-A has a wider distribution in Ionian, Aegean, and Black Seas [12,13,39,49,50]. The state of the “rostral loss” in morph-B was subjected to the parsimonious hypothesis that there is a single pair of alleles, with a complete dominance of allele b expressed in morph-B. Indeed, Liasko et al. (2015) [49] confirmed this hypothesis through the analysis of lab-reared offspring, where morph-A females had proportions of morphs in their offspring close to either 1:1 (all-A, or all-B) and morph-B females had offspring either all-B or offspring close to 3:1 [49]. Additionally, Liasko et al. (2017) [9] showed that the rostrum in morph-A follows a strict isometrical growth, so it could serve as a growth and/or age marker of the species and that is sexually dimorphic with the male individuals bearing narrower rostra. Moreover, the hypothesis that morph-B females develop some compensatory morphological traits such as enlargement of the body somites, scaphocerite, and telson, substituting the “rostral loss”, has also been confirmed by the same study. Although carapace structure was subjected to geometric morphometric analysis in H. sapphica morphotypes, this information is lacking for the rostral phenotype. The purpose of the present study is to investigate, by means of GM, possible rostral morphological shifts and correlations with body size, sex, and season. Till now, the rostral shortening and its functions have been associated with sexual maturity and mating in some penaeid and aristeid species [27] and references herein. However, the “rostral loss” as a phenomenon is unique and is presented only in hippolytid shrimps and especially in H. sapphica morphotypes. This fact, combined with our previous studies on the species, makes the current contribution very important, completing the morphological puzzle of the rostral diversity and answering various questions, related to possible occurrence of the phenomenon, and life history adaptations of the species.

**Figure 2.** Left side of the rostrum of Hippolyte sapphica morphotypes A and B with the configuration of the 8 and 5 landmarks, respectively for each morphotype (modified by [12]). Morph-A, 1: the rear-end of orbital margin, 2: the ventral upper rostral point, 3: the ventral posterior dentition end-point, 4: the ventral anterior dentition end-point, 5: the anterior most rostral tip, 6: the dorsal anterior dentition end-point, 7: the dorsal posterior dentition end-point, 8: the postorbital tooth. Morph-B, 1: the rear-end of orbital margin, 2: the ventral lowest rostral point, 3: the ventral upper rostral point, 4: the anterior most rostral tip, 5: the postorbital tooth.
2. Material and Methods

Shrimp samples were collected during early November and late February 2013 from two sites in the Ionian Sea (Central Mediterranean): s.1, Louros River estuary in Amvrakikos Gulf (39°13'961" N, 020°45'971" E) with a mixed H. sapphica population (morphs A and B) and s.2, Sagiada Lagoon in the Ionian coast, NW Greece (39°62'605" N, 020°18'105" E) with a pure, unmixed species population (morph-A). Samples were collected by means of a hand net, with a frame of 30 cm × 35 cm and a mesh size of 2 mm and preserved in situ in 4% formaldehyde solution. In laboratory, morphotypes, and sexes were identified by stereomicroscopic observation of the rostrum and the second pleopod of shrimps, respectively [12]. Individuals were first photographed and morphometric analysis of the rostrum was carried out on a set of landmarks (Figure 2) defined on the digital photos of shrimps. Coordinates were determined by using image-analyzing software (NIKON Digital Sight DS-L2-Image Pro Plus 7.0; Media Cybernetics, Rockville, MD, USA) and carapace lengths measured with the image analysis system ZEN 2012. The coordinates were submitted to a full Procrustes fit, which project the data to a tangent space by orthogonal projection. After the Procrustes’ fit, landmarks coordinates are abstract units, which reflect the relative distance landmark. The Procrustes coordinates were used in the subsequent analyses. Landmarks topography was selected with the following criteria: in order to detect possible shifts in shape according to the position of dorsal and ventral rostral dentition, the position of postorbital tooth and the rostral points, which indicate the slenderness/wideness of the rostral structure. Only adults with a fully formed rostra were used for morphometric analysis, to avoid possible differences that can be attributed to maturity stage or other factors. Statistical analyses included the study of rostral shape and landmarks’ shift were estimated by MANOVA. As original variables, the Procrustes coordinates of landmarks were used in MANOVA analysis, which allowed an estimation of the overall variability of the carapace form for the dependent or independent factors. Pairwise comparisons were applied in order to reveal significant differences among population types and sexes. Discriminant analysis was also performed in order to test if there is any seasonal classification of Hippolyte sapphica morphotypes, between sexes for the two sampling periods. We also used cluster analysis, in order to access whether there exists a significant underlying variation in morph-B rostra, regardless of any known factor. All statistical analyses were performed by SPSS 23 software and Geometric morphometrics by MorphoJ free software [51].

3. Results and Discussion

A total of 170 morph-A and 99 morph-B individuals of H. sapphica were subjected to geometric morphometric analysis. Morph-A rostral shape varies in relation to carapace length (Wilks’ lambda = 0.26; p < 0.001). In Figure 3A, the landmarks displacements are given per 5 units of size in a total size range of 11–37 units. Rostrum and carapace are solid connected structures, which follow similar growth patterns, as expected, indicating their important function they serve. Our results revealed that, after the regression of Procrustes coordinates versus carapace length for the morphotype A, morph-A rostra, both in the mixed and unmixed populations of H. sapphica, are characterized by isometrical growth pattern. Liasko et al. (2015, 2017) [9,49] showed also the strict isometry in morph-A rostra, proposing that this structure could be also used in the growth or age determination of the species instead of carapace. Similarly, rostral morphology, after the removal of the general allometric tendency, using the residuals of the regression, shows a statistically significant correlation with the sampling station (Wilks’ lambda = 0.74; Partial Eta² = 0.26; p < 0.001), as well as with the sex of the individuals (Wilks’ lambda = 0.62; Partial Eta² = 0.38; p < 0.001) (Figure 3B–E). Additionally, the performed discriminant analysis for morph-A males at different annual timepoint (February and November), showed a good recognition. More specifically, males of the mixed population found statistically significant difference in November (Wilks’ lambda = 0.44; p < 0.01) (Table 2, Figure 3F). Between sexes, male individuals found to bear rostra with shorter rostral width (Figure 3C,E), a fact that is also confirmed in H. sapphica morphs [9]. Slender rostra have been reported also as a
male individuals found to bear rostra with shorter rostral width \([12]\). All these characters are present in the larvae of \(H. sapphica\) bear wider rostra, wider abdominal somites, and wider carapace heights \([9]\) in comparison to the male ones. The robustness of the rostral structure diminishes the turbulent water flows behind the shrimps’ body and helps shrimp’s buoyancy especially for the heavier females. As has been shown by Liasko et al. (2015) \([49]\), the morph-A individuals have a propensity to become females, while the morph-B ones the opposite. Thus, the rostral morphological morph-A pattern could demonstrate variations according to the sex ratio and/or the sampling period during the population dynamics of the species. Recent studies have shown that rostral plasticity in shape and dentition has been evaluated highly in response to environmental conditions and spatial boundaries \([55,56]\).

![Figure 3](image-url)

**Figure 3.** Rostral morphological patterns of *Hippolyte sapphica* morphotypes. (A) to the total number of morph-A individuals (pool), and after removal of allometry (B) morph-A females in mixed population, (C) morph-A males in mixed population, (D) morph-A females in unmixed population, (E) morph-A males in unmixed population, (F) morph-A males in mixed population, seasonal (typical point shifts for November), (G) morph-B 1st cluster, (H) morph-B 2nd cluster, NI: number of individuals.

**Table 2.** Seasonal classification table of *Hippolyte sapphica* morph-A species, according to discriminant analysis.

| Belong to  | Classified in February | Classified in November | Total |
|-----------|------------------------|------------------------|-------|
| February  | 22                     | 2                      | 24    |
| November  | 4                      | 16                     | 20    |

In morph-B, the rostral morphology does not change significantly as a function of carapace length, after regression of Procrustes coordinates vs. CL \((p > 0.05)\) and does not show a significant correlation with sex (MANOVA; Wilks’ lambda = 0.9; \(p > 0.05)\). Probably, its hypoplastic, neotenic character influences its morphogenesis. Neotenic characters have been also recorded in the benthic *Hippolyte inermis* and in the pelagic *Hippolyte coerulescens*, such as tooth in the pleuron of 5th pleonite, unusual disposition of dorsolateral telson spines,
long scaphocerite tooth [12]. All these characters are present in the larvae forms [57–60] and this general morphological heterochrony is usually ontogenetically and evolutionary driven. However, two-step cluster analysis (log-likelihood distance measure; BIC clustering criterion) revealed the existence of two distinct groups in morph-B rostra (MANOVA; Wilks’ lambda = 0.29; p < 0.001): 1st cluster with a robust, short rostra and 2nd cluster with extensive, elongated rostra (Figure 3G,H). These two clusters probably correspond to the homozygous and heterozygous state of the gene site (BB and BA) that controls the species morph-B phenology.

In conclusion, rostral shape in morph-A seems to be influenced by many factors, such as the size of the individual, sex, and time of year, which proves its biological usefulness and a possible complex interaction of genetic and epigenetic mechanisms. On the other hand, the rostral shape in morph-B does not show significant allometry or correlation with sex. Under these conditions, the biological mechanisms by which the B allele manages to be preserved in H. sapphica mixed populations become interesting and worth studying.

Author Contributions: Conceptualization, C.A. and R.L.; methodology, R.L.; software, I.L.; validation, C.A., R.L. and I.L.; formal analysis, R.L.; investigation, A.A.K.; resources, A.A.K.; data curation, R.L.; writing—original draft preparation, C.A.; writing—review and editing, C.A.; visualization, R.L. and A.A.K.; supervision, C.A.; project administration, C.A.; funding acquisition, I.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Elli-Christina Kourmouli, who was undergraduate student during this study, for her assistance to the samplings and material assessment.

Conflicts of Interest: The authors declare no conflict of interest.

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