

BIVARIATE DISCRIMINANT ANALYSIS FOR THE IDENTIFICATION OF  
*NIHONOTRYPAEA JAPONICA* AND *N. HARMANDI*  
(DECAPODA: THALASSINIDEA: CALLIANASSIDAE)

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A B S T R A C T

For two callianassid shrimps, *Nihonotrypaea japonica* (Ortmann) and *N. harmandi* (Bouvier), morphometric character variations [carapace length (CL), eyestalk width (EsW), cornea width (CoW), and rostrum angle (RA)] were examined based on the materials collected from sandflats in western Kyushu, Japan. Allometric relationships were established between each combination of two morphometric characters for each species, in which CL was fixed on the abscissa as a reference variable. The values of EsW and CoW linearly increase with the CL value, with the slopes similar for EsW between the two species and a steeper slope for CoW in *N. harmandi*. By using the combination of (CoW/EsW) ratio vs. CL and that of RA vs. CL, bivariate discriminant analyses were run to establish an appropriate function separating the two species in each combination. The relatively smaller cornea size in *N. japonica* and more acute rostrum angle in *N. harmandi* were confirmed as the two effective characters that separate the two species. On the basis of the overall misclassification probabilities, the linear discriminant function using (CoW/EsW) ratio and CL was regarded as best to correctly separate the two species for practical use.

Callianassid ghost shrimps are one of the most common members of the marine macrofauna worldwide, occurring from sublittoral to estuarine intertidal sediments. In western Kyushu, Japan, the second author (AT) and his colleagues have studied various aspects of the biology of a callianassid species, originally identified as *Callianassa japonica* Ortmann, inhabiting an intertidal sandflat (called the Tomioka Bay sandflat) in the estuarine system ranging from Ariake Sound (estuary) through Tachibana Bay (intermediate waters) to the East China Sea (open sea) (see Figs. 1 and 2 in Tamaki and Miyabe, 2000), including the species' population ecology and bioturbating effects on the benthic community structure (e.g., Tamaki *et al.*, 1997, 1999; Tamaki and Miyabe, 2000; Flach and Tamaki, 2001). Until recently, it was believed that two species of *Callianassa* commonly occurred in intertidal habitats in Japan: *C. japonica* Ortmann, 1891, and *C. petalura* Stimpson, 1860 (see Sakai, 1969). However, in their taxonomic revision of these species, Manning and Tamaki (1998) revealed that Sakai's "*C. japonica*" was in fact a mixture of *C. japonica* and *C. harmandi* Bouvier, 1901. This was based on the description by de Man (1928) who recognized the distinction of the two

species but assigned the wrong (i.e., reverse) name to each of them. Manning and Tamaki (1998) had access to the type specimens of the two species to solve the taxonomic confusion. They also proposed a new genus, *Nihonotrypaea*, to include the three Japanese species. Now all the material studied on the Tomioka Bay sandflat has proven to be *N. harmandi* (Tamaki *et al.*, 1999).

Based on a small set of specimens, Manning and Tamaki (1998) stated that *Nihonotrypaea harmandi* and *N. japonica* could be distinguished by the size of their cornea alone (see Fig. 1): in *N. harmandi* the cornea is relatively large, at least half the width of the eyestalk, whereas in *N. japonica* the cornea is much smaller, one-third to one-fifth the width of the eyestalk. These characteristics could be used during the extensive survey of the distribution of the two species in the above-mentioned estuarine system [see Fig. 8 in Tamaki *et al.* (1999) and Fig. 2 in Tamaki and Miyabe (2000)]. It was revealed that adults of *N. harmandi* mainly occurred in the coastal waters of the East China Sea plus Tachibana Bay plus the outermost one-third part of Ariake Sound, while those of *N. japonica* occurred in the middle one-third part of Ariake Sound; there was a little overlap

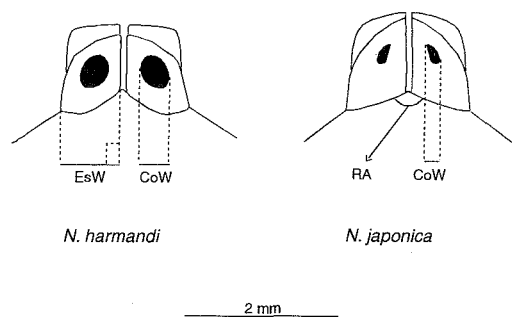


Fig. 1. Positions for the morphometric character measurement concerning the eyestalk width (EsW), cornea width (CoW), and rostrum angle (RA) in *Nihonotrypaea harmandi* and *N. japonica*.

in their distribution margins. Later, however, our examination of a greater number of specimens has revealed that the ratios of cornea width to eyestalk width of some specimens fall between one-third and a half. Thus, it became necessary to identify those “gray” specimens correctly, especially for those collected from the sandflats in the overlapped distribution range of the two species. We have also become aware that the above-mentioned ratio varies with body size. Therefore, in order to convincingly discriminate between the two species in a sample containing specimens of different body sizes, any information about a developmental allometry between the ratio of cornea width to eyestalk width and body size is required. Any other allometric relationships could also be useful. In the present paper, we first seek for these relationships and establish appropriate bivariate discriminant functions separating the two species. The final goal is to evaluate the statement of Manning and Tamaki (1998) on the basis of the misclassification probability associated with the discriminant function using the ratio of cornea width to eyestalk width and body size.

#### MATERIALS AND METHODS

As a prerequisite for the establishment of discriminant functions, we needed to obtain sufficient specimens of *Nihonotrypaea harmandi* and *N. japonica* beforehand. The justification for our *a priori* identification of the specimens of the two species collected from each main distribution range in the target estuarine system in western Kyushu comes from several lines of evidence, especially from (1) their larval abundance patterns and (2) their benthic population characteristics. Tamaki and Miyabe (2000) have revealed that (1) with the advancement of larval stages, larvae of *N. japonica* are retained only within Ariake Sound, disappearing from Tachibana Bay, and (2) throughout development, larvae of *N. harmandi* are re-

tained mainly in Tachibana Bay, never transported beyond the innermost margin of the distribution range of adults in Ariake Sound. An example of the difference in the benthic population parameters is the period of occurrence of ovigerous females in the year, from February to November for *N. japonica* (Wardiatno and Tamaki, unpublished data) and from June to October for *N. harmandi* (Tamaki *et al.*, 1997). For the present study, 540 benthic specimens of *N. harmandi* and *N. japonica* were collected on the Tomioka Bay sandflat in Tachibana Bay and the (Shirakawa plus Arao) sandflats in Ariake Sound, respectively; the Shirakawa and Arao sandflats are located just eastward of plankton-sampling stations Stn H-8 and Stn H-3 in Fig. 1 of Tamaki and Miyabe (2000), respectively. This separate collection of the benthic specimens ensured the noncontamination of the two species at each location. Collections were made by means of “yabby pumps” during low tide. All shrimps were immediately fixed in 10% buffered sea water-Formalin.

In the laboratory, all observations, drawings, and measurements [to nearest 0.1 mm (length) or 0.1° (angle)] of the specimens were made using a light stereomicroscope equipped with a *camera lucida*. Eyestalk width (EsW) and cornea width (CoW) were defined as the length of the base of the eyestalk and the length of the cornea, after projected on a line perpendicular to the inner side-line of either eyestalk (Fig. 1); data from both eyestalks were taken from each individual. Two other constituent parts of the body were selected for morphometric variations and their dimensions measured, as follows: (1) Carapace length (CL) = length along mid-dorsal curvature from tip of rostrum to posterior margin of carapace. The length of the curve was measured by a computer-linked digitizer. (2) Rostrum angle (RA) = angle at rostrum as indicated in Fig. 1. The character (2) was chosen from our impression of wider RA for *N. japonica*. In fact, this feature had properly been sketched in de Man (1928) but was not adopted as a convincing character by Manning and Tamaki (1998) (see their Fig. 1).

Allometric relationships were established between each combination of two morphometric characters for each species, in which CL is fixed on the abscissa as a reference variable; for EsW and CoW, data from both sides of a specimen were plotted against the same CL value. A total of 200 specimens for *Nihonotrypaea harmandi* (99 males and 101 females) and 340 specimens for *N. japonica* (190 males and 150 females) were used. All statistical tests were performed with SPSS statistical packages (Norusis, 1994a, b). Firstly, the linear regression equations between each of EsW and CoW *versus* CL were established for each species, and the significance of the differences between the two species (concerning slopes and intercepts) was tested by analysis of covariance (ANCOVA). Secondly, using each combination of the following two variables, (1) (CoW/EsW) ratio vs. CL and (2) RA vs. CL, bivariate discriminant analysis was run to establish an appropriate function separating the two species. The equality of the group covariance matrices was the criterion to determine the optimum classification rule (i.e., the choice of either a linear discriminant function or a quadratic discriminant function—we used the squared Mahalanobis distance to centroid for the latter case). However, the use of the linear discriminant function may not be severely affected by unequal group covariance matrices, provided that large samples are used (e.g., Norušis, 1994b; Swan and Sandilands, 1995). Thus, even if the optimum classification rule was the squared

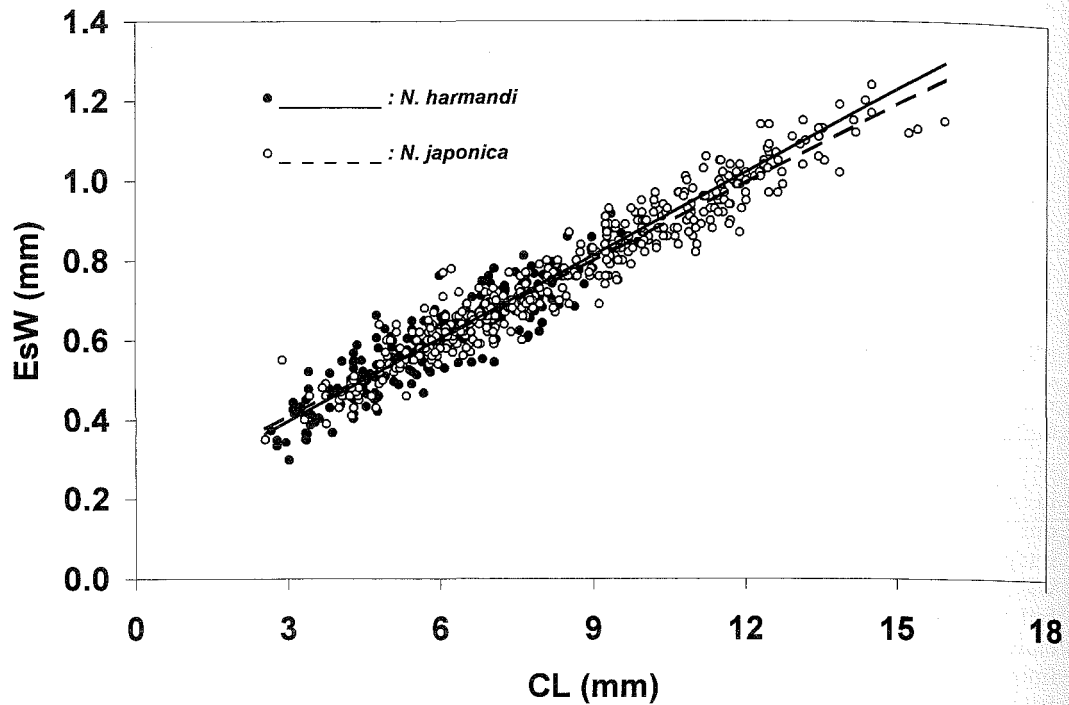


Fig. 2. EsW (eyestalk width) vs. CL (carapace length) relationships in *Nihonotrypaea harmandi* [solid circle dots ( $n = 400$ ) and solid line] and *N. japonica* [open circle dots ( $n = 680$ ) and dashed line]. The linear regression equations for the lines are given in Table 1.

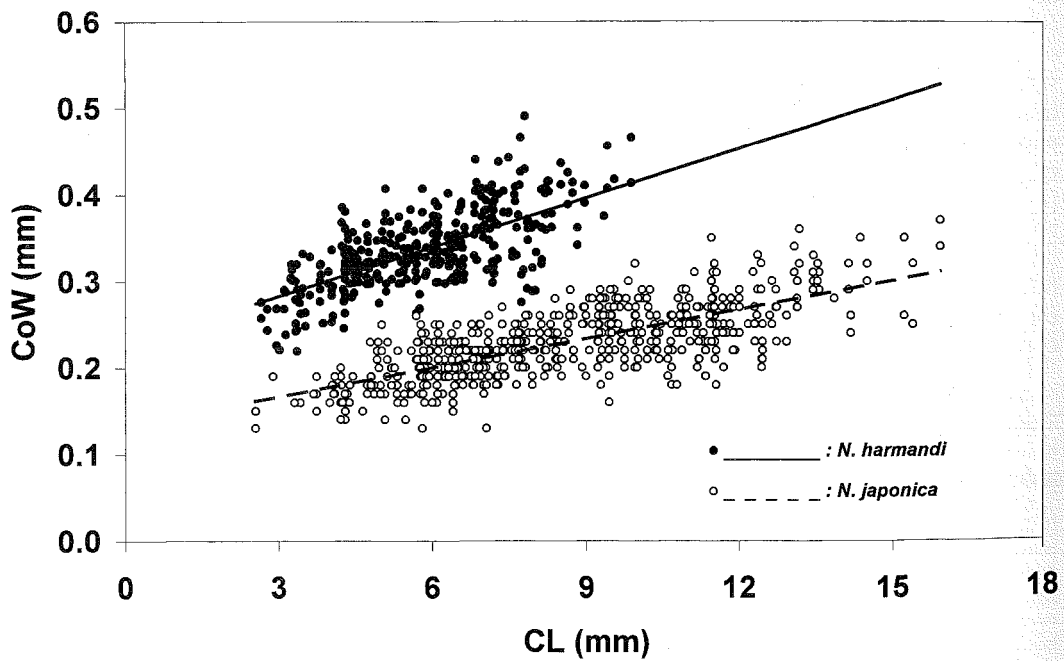


Fig. 3. CoW (cornea width) vs. CL (carapace length) relationships in *Nihonotrypaea harmandi* [solid circle dots ( $n = 400$ ) and solid line] and *N. japonica* [open circle dots ( $n = 680$ ) and dashed line]. The linear regression equations for the lines are given in Table 1.

Table 1. Linear regression equations for each of two morphometric variables, eyestalk width (EsW) and cornea width (CoW), versus carapace length (CL) in *Nihonotrypaea harmandi* ( $n = 400$ ) and *N. japonica* ( $n = 680$ ), with the analysis of covariance (ANCOVA) results for the differences between the two species.

Relation between	Species	$y = ax + b$	$r^2$	ANCOVA	
				Slopes (a)	Intercepts (b)
EsW and CL	<i>N. harmandi</i>	EsW = 0.0694 CL + 0.188 ( $P < 0.001$ )	0.83	non-parallel ( $0.01 < P < 0.05$ )	-
	<i>N. japonica</i>	EsW = 0.0648 CL + 0.211 ( $P < 0.001$ )	0.94		
CoW and CL	<i>N. harmandi</i>	CoW = 0.0191 CL + 0.225 ( $P < 0.001$ )	0.44	non-parallel ( $P < 0.001$ )	-
	<i>N. japonica</i>	CoW = 0.0112 CL + 0.132 ( $P < 0.001$ )	0.55		

Mahalanobis distance, we also evaluated the appropriateness of the linear discriminant function on the basis of the probability of misclassification. This is because a linear discriminant function would be simpler for practical use.

## RESULTS

The scatter plots for each pair of two variables in the two species, in which CL is fixed on the abscissa, are shown in Fig. 2 (for EsW) and Fig. 3 (for CoW). The ranges of CL were 2.7–9.9 mm for *Nihonotrypaea harmandi* and 2.6–15.9 mm for *N. japonica*; a much larger body size is attained in *N. japonica*. The minimum mature body sizes of *N. harmandi* (both sexes) are at around a 4.5-mm CL (Tamaki *et al.*, 1997), whereas those of *N. japonica* (both sexes) seem to occur in the range of 4.8–6.0-mm CL (Wardiatno and Tamaki, unpublished data). Thus, the specimens used for the two species spanned from immature to full-grown individuals. In Figs. 2 and 3, the values of the ordinate variables linearly increase with the CL value, and their linear regression equations are summarized in Table 1.

The results of ANCOVA for each pair of two variables between the two species are also summarized in Table 1. The slopes of the linear regression lines (vs. CL) are regarded as non-parallel for both EsW (Fig. 2) and CoW (Fig. 3). Although the line slope in the EsW vs. CL relationship is greater for *Nihonotrypaea harmandi*, the difference is not so clearly visible at least for the overlapped CL values. By contrast, the steeper slope in the CoW vs. CL relationship for *N. harmandi* is obvious, meaning its allometric larger cornea size with body size.

The scatter plots for CoW/EsW ratio vs. CL (as the reference variable) in the two species are shown in Fig. 4. For each species, the ratio decreases with CL ( $r = -0.68$ ,  $P < 0.001$  for *Nihonotrypaea harmandi*;  $r = -0.64$ ,  $P < 0.001$  for *N. japonica*). As expected, the ratio values are greater in *N. harmandi* over the overlapped CL values, with the range and mean  $\pm$  SD being 0.41–0.82 and  $0.58 \pm 0.09$  for *N. harmandi*, and 0.18–0.43 and  $0.31 \pm 0.05$  for *N. japonica*. Inconsistent with the statement of Manning and Tamaki (1998), 16.8% of the *N. harmandi* specimens and 27.2% of the *N. japonica* specimens were out of the range of the ratio values described therein, i.e., less than a half for *N. harmandi* and either more than one-third or less than one-fifth for *N. japonica*. In the discriminant

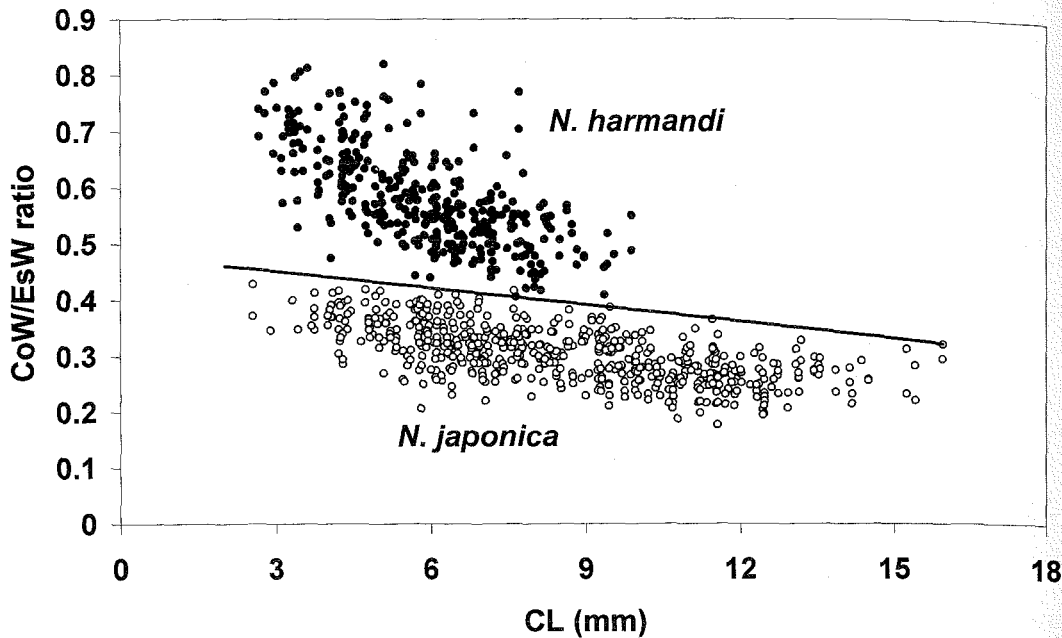


Fig. 4. Scatter plots for CoW (cornea width)/EsW (eyestalk width) ratio vs. CL (carapace length) in *Nihonotrypaea harmandi* [solid circle dots ( $n = 400$ )] and *N. japonica* [open circle dots ( $n = 680$ )], with the discriminant line established with the use of the two variables [line for  $z = 0$  in equation (1) in Results].

analysis, Box's  $M$  test showed the significantly unequal group covariance matrices ( $P < 0.05$ ), pointing to the squared Mahalanobis distance as the optimum classification rule. However, the linear discriminant function could also be established, as follows:

$$z = 0.182\text{CL} + 18.222(\text{CoW/EsW ratio}) - 8.767, \quad (1)$$

with  $z > 0$  for *N. harmandi* and  $z < 0$  for *N. japonica*.

Actually, the linear discriminant function gave the overall smaller misclassification probabilities: for *N. harmandi*—0% (Linear) vs. 2.5% (= 10/400 for either eye) and 0.5% (= 2/400 for both eyes) (Mahalanobis); and for *N. japonica*—0.1% (= 1/680 for either eye; Linear) vs. 0% (Mahalanobis).

The scatter plots for RA vs. CL (as the reference variable) in the two species are shown in Fig. 5. As expected, the RA values are greater in *Nihonotrypaea japonica* over the overlapped CL values. For *N. harmandi*, regardless of variances, RA appears to be rather "constant" against CL (range = 64.5–130.0°; mean  $\pm$  SD = 99.9  $\pm$  12.0°;  $r = 0.05$ ;  $P > 0.05$ ), but it slightly increases with CL for *N. japonica* (range = 118.0–154.0°; mean  $\pm$  SD

= 133.4  $\pm$  6.6°;  $r = 0.26$ ;  $P < 0.001$ ). In the discriminant analysis, Box's  $M$  test showed the significantly unequal group covariance matrices ( $P < 0.001$ ). However, the linear discriminant function could also be established, as follows:

$$z = 0.073\text{CL} + 0.108\text{RA} - 13.571, \quad (2)$$

with  $z < 0$  for *N. harmandi* and  $z > 0$  for *N. japonica*.

Actually, the linear discriminant function gave the far smaller misclassification probability for *N. harmandi* [0.5% (= 1/200; Linear) vs. 6.5% (= 13/200; Mahalanobis)] but the slightly higher misclassification probability for *N. japonica* [1.2% (= 4/340; Linear) vs. 0.6% (= 2/340; Mahalanobis)].

## DISCUSSION

Of the studies on morphometric character variations for crustaceans, only a few have been done for callinassid shrimps. For example, Felder and Lovett (1989) used morphometric characters (i.e., carapace length, chela width, and chela height) to estimate maturation size of male and female *Lepidophthalmus louisianensis* (Schmitt, 1935)

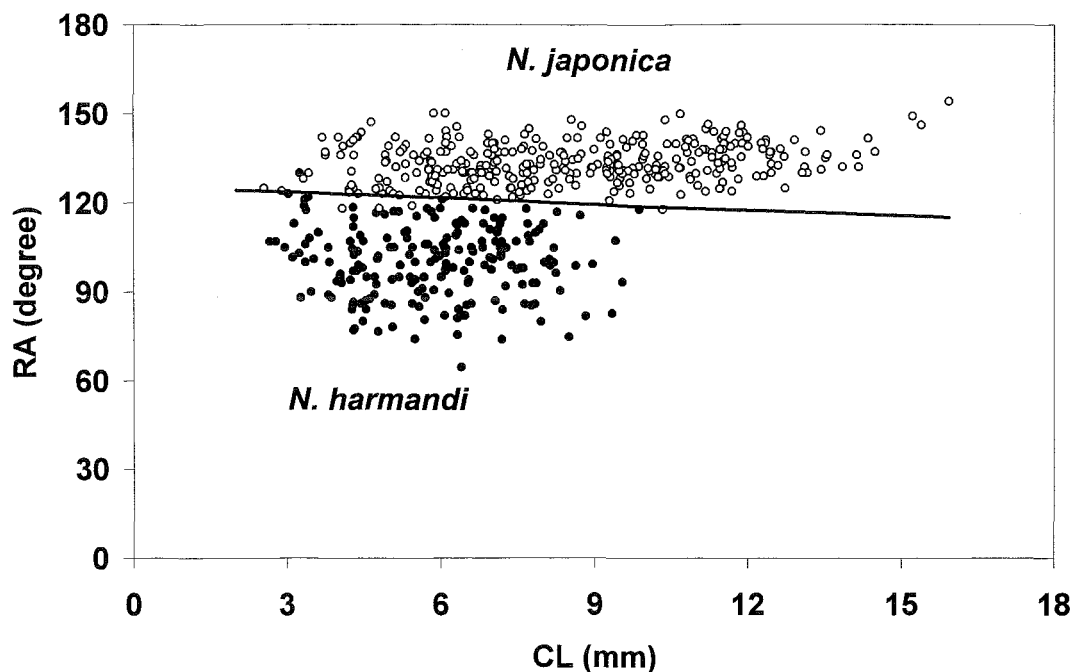


Fig. 5. Scatter plots for RA (rostrum angle) vs. CL (carapace length) in *Nihonotrypaea harmandi* [solid circle dots ( $n = 200$ )] and *N. japonica* [open circle dots ( $n = 340$ )], with the discriminant line established with the use of the two variables [line for  $z = 0$  in equation (2) in Results].

(originally as *Callianassa louisianensis*). Labadie and Palmer (1996) conducted a morphometric analysis of cheliped size and shape variation in the strikingly heterochelous, northeastern Pacific ghost shrimp *Neotrypaea californiensis* (Dana, 1854) to obtain a better understanding of the cheliped function and ontogeny. Dworschak (1998) made some morphometric character comparisons between sexes of *Callianassa tyrrhena* (Petagna, 1792) and *C. candida* (Olivi, 1792) collected from tidal flats in the northern Adriatic Sea. His biometric analysis showed a sexual dimorphism in both species, especially in the size of the propodus of the major cheliped.

A recent example of the use of bivariate discriminant analyses in morphometric research of crustaceans can be seen in Sampeiro *et al.* (1999). They have successfully established two discriminant functions, based on right cheliped length and carapace length, and right cheliped height and carapace length, respectively, for males of the spider crab, *Maja squinado* (Herbst, 1788). Both functions allowed the differentiation between juveniles and adults of the males, with correct assignment of over 99%.

From the two discriminant analyses in the present study, both the relatively smaller cornea size (smaller CoW/EsW ratio) for *Nihonotrypaea japonica* and the more acute rostrum angle (smaller RA) for *N. harmandi* were confirmed, as expected (de Man, 1928; Manning and Tamaki, 1998). On the basis of the overall misclassification probabilities, the use of the (CoW/EsW ratio vs. CL) relationship is apparently better to correctly separate the two species. Furthermore, in this relationship, it is also apparent that the use of the linear discriminant function [equation (1) in Results] is preferred to the squared Mahalanobis distance, in spite of the result of Box's *M* test showing that the optimal choice should be the latter. In other words, with the result of only a single case for the misclassification of the total of 1,080 (= 400 + 680) cases using the linear discriminant function (Fig. 4), the linear function should be adopted for practical use. In conclusion, with a little extension of the ranges of the (CoW/EsW ratio) value for the larger number of specimens, the statement of Manning and Tamaki (1998) that *N. harmandi* and *N. japonica* could be distinguished by the size of their cornea alone

was statistically improved by the present bivariate analysis.

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