Arthropod Structure & Development 46 (2017) 297–303

Contents lists available at ScienceDirect

Arthropod Structure & Development

journal homepage: www.elsevier.com/locate/asd

Ontogenetic trajectory and allometry of *Diplonychus rusticus* (Fabricius), an Oriental aquatic bug (Hemiptera: Belostomatidae) from the Western Ghats of India

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ARTICLE INFO

Article history:
Received 4 October 2016
Accepted 30 December 2016
Available online 28 January 2017

Keywords:
CHAID
Allometric coefficients
Developmental instar
Morphometry

ABSTRACT

Despite being one of the dominant groups in freshwater ecosystems, morphological and ontogenetic studies on aquatic Hemiptera have received little attention in the Oriental region. We present the ontogenetic trajectory and allometry of the widespread Oriental belostomatid species, *Diplonychus rusticus* (Fabricius) for the first time. We have measured nine different morphological variables throughout the growth of the bug using both field captured and laboratory reared specimens. Our results suggest that the developmental instars can be distinguished by the size variables, as seen in the Principal Component Analysis. On the basis of a CHAID (Chi-squared Automatic Interaction Detection) based regression tree, we also show that the characters – total length without head and maximum width – prove to be adequate for effective instar identification. The multivariate allometric growth pattern shows that different body parts exhibit different types of allometry. This is apparent in the allometry exhibited by forelegs and mid and hind legs, which show allometry of opposite polarities. This may be due to the different functions attributed to these body parts. Our results show that the growth pattern in *D. rusticus* is comparable with the New World genus *Belostoma*, suggesting a conserved growth pattern in the family Belostomatidae.

1. Introduction

Allometry is primarily concerned with the association between variation of different traits and the variation of overall size of an organism. Allometric analyses can address variation at different levels with different biological origins of variation and covariation among traits (Klingenberg, 1998). Allometric analyses have been done for a variety of animals (Davies and Brown, 1972; Chabot and Stenson, 2000; Palestrini et al., 2000; Weston, 2003). Hemimetabolous insects such as aquatic Hemiptera have proved to be excellent candidates for studying ontogenetic allometry due to the inextensibility of the involved structures in each instar (Klingenberg and Spence, 1993; Tseng and Rowe, 1999; Iglesias et al., 2008). These bugs, especially representatives of the families Belostomatidae, Nepidae and Notonectidae, are a major component in the aquatic fauna of any water body and most often are the top predators in aquatic communities (Ohba and Nakasuji, 2006).

Nymphal instars of many belostomatids are quite similar in the overall morphology throughout their development and differ only in a few aspects such as the number of antennal segments, the number of tarsal segments and the development of wing pads (Smith, 1974). Life history and laboratory studies giving brief

Abbreviations: TL, Total length without head; MW, maximum width across the first visible abdominal segment; F1, length of forefemur; F2, length of midfemur; F3, length of hindfemur; T1, length of foretibia; T2, length of midtibia; T3, length of hindtibia; RL, length of the rostrum; PCA, Principal Components Analysis; CHAID, Chi squared Automatic Interaction Detection.

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http://dx.doi.org/10.1016/j.asd.2016.12.008
1467-8039/

descriptions of nymphal instars have been done for the New World belostomatid genera, such as *Belostoma* (Torre Bueno, 1906; Cullen, 1969; Schnack, 1971; McPherson and Packauskas, 1986) and *Abedus* (Menke, 1960; Smith, 1974), as well as the North American and Neotropical species of the widespread genus *Lethocerus* (Rankin, 1935; De Carlo, 1962; Cullen, 1969). So far, members of only one new world belostomatid genus – *Belostoma* – have been studied for ontogenetic allometry (Iglesias et al., 2008). Life history studies for the Old World belostomatids are comparatively rare and have been done for only a few species such as *Limnogeton fieberi* (Voelker, 1968), *Lethocerus cordofanus* (Tawfik, 1969), *Diplonychus rusticus* (Su and Yang, 1992) and *Lethocerus indicus* (Nesemann and Sharma, 2013). Most of the life history studies have been conducted on bugs reared in the laboratory, while some are based on field observations. To our knowledge, there are no instances of comparison between the growth patterns for field captured and laboratory reared individuals and no studies providing the ontogenetic trajectories of African or Oriental belostomatids.

Diplonychus rusticus (Fabricius) is a very common belostomatid inhabiting a variety of lentic habitats, especially large, permanent ponds with profuse aquatic vegetation. It is a moderately sized bug, about 15–16 mm in length. This is a typical hemimetabolous species with five developmental instars. Originally described from India (see Fabricius, 1781), *D. rusticus* is widespread in the Indian Subcontinent, Indochina and Southeast Asia (Polhemus and Polhemus, 2013). The potential of this species as an efficient predator of mosquito larvae has been well documented (Venkatesan and Sivaraman, 1984; Saha et al., 2007, 2010) and its role as the natural biological control agent of some rice pests has been speculated (Yano et al., 1981). The predatory and foraging tactics of adults as well as of the nymphal instars have been worked out in detail (Cloarec, 1989, 1990a,b; Cloarec, 1991). There are also studies on the structural details of the male reproductive system, mating behaviour, egg laying and laboratory rearing of this bug (Venkatesan, 1983; Jawale and Ranade, 1988, 1989; Su and Yang, 1992). Though many aspects of the biology of *D. rusticus* are known, no studies have yet focused on its postembryonic growth pattern.

For this paper, we studied the postembryonic growth in both field captured and laboratory reared individuals. Here we show that the developmental instars of *D. rusticus* can be distinguished on the basis of size. We present a standardised method for rapid identification of instars by taking the minimum required measurements and show that biometry can be used effectively for determining the instar. We provide the ontogenetic trajectory of *D. rusticus* based on the morphometric analysis of nine different variables measured throughout the growth. We also compare the growth patterns as shown by ontogenetic allometry for field captured individuals and exuviae of the laboratory reared individuals.

2. Materials and methods

2.1. Field site

A permanent pond with floating and emergent vegetation, situated approximately 35 km north of Pune (N 18° 42′ 53.57″, E 73° 41′ 12.55″), Maharashtra, western India was chosen for collection of *D. rusticus* due to the presence of a stable population throughout the year.

2.2. Laboratory rearing and measurements

One encumbered male (Fig. 1A) was collected from the field site and was maintained in a glass tank with aged tap water and *Pistia* sp. for support and chironomid larvae as prey, till the eggs hatched. Twenty hatchlings were separately maintained in another glass tank containing aged tap water with *Pistia* sp. for support and chironomid larvae as prey. After each moult, the exuviae of all the individuals were collected and stored in 70% ethanol.

Measurements were taken with a calibrated stereoscope. Twenty exuviae of each of all five instars were considered for morphometry. Nine different variables were measured for each exuvia. The variables considered for morphometry are as follows: Total length without head (TL), maximum width across the first visible abdominal segment (MW), length of forefemur (F1), length of midfemur (F2), length of hindfemur (F3), length of foretibia (T1), length of midtibia (T2), length of hindtibia (T3) and length of the beak or rostrum (RL). All measurements are in millimetres. The head was excluded from the measurement of total length to avoid the increase in error due to positional effect. All the morphological variables which were measured are illustrated in Fig. 1C–G.

2.3. Field collection and measurements

Nymphs were collected from the same pond as mentioned above. Sampling was carried out with the help of a handheld net (30 cm × 24 cm, mesh size: 1 mm) and all the collected nymphs were preserved in 70% ethanol with a drop of glycerol to prevent them from hardening. Thirty nymphs of each of the five instars were considered for morphometry. All the aforementioned variables were measured for each nymph.

2.4. Statistical analyses

Principal Components Analysis (PCA) was carried out for both the datasets containing the morphometry data of all five instars for the exuviae and the field captured individuals to understand whether the instars formed distinct groups. First two principal components were plotted to obtain the scatter of the data in the morphospace corresponding to each instar. Scree plot was generated to understand contribution of each principal component extracted by PCA.

To predict the developmental instar of the individuals collected on field using biometric characters, a regression tree was constructed using exhaustive CHAID (Chi-squared Automatic Interaction Detection) algorithm for the dataset of the exuviae. This algorithm chooses the independent variable (predictor) that has the strongest interaction with the dependent variable at each step. Categories of each predictor are merged if they are not significantly different with respect to the dependent variable (Kass, 1980). In CHAID analysis, because larger variables are extracted first, masking the effects of smaller variables, we performed the analysis multiple times by removing the variable that was extracted in the earlier run. As a result, CHAID was performed four times: (1) with all the variables; (2) excluding variable TL that got separated in the first run; (3) with only three femora (F1–F3) lengths; and (4) with only three tibiae (T1–T3) lengths.

For both the datasets of field captured individuals and exuviae, multivariate allometry method based on Jolicoeur (1963) and Kowalewski et al. (1997) was employed to derive the allometric coefficients (AC) for all the nine variables. Ninety five percent confidence intervals around the coefficients were estimated by bootstrapping, with 2000 bootstrap replicates. The ninety five percent confidence intervals around the allometric coefficients were estimated in order to determine the type of allometry viz. hyperallometry, isometry or hypoallometry (terminology follows Iglesias et al., 2008), exhibited by each variable.

All the statistical analyses were performed using the following freeware: Tanagra (Rakotomalala, 2005) for CHAID analysis and

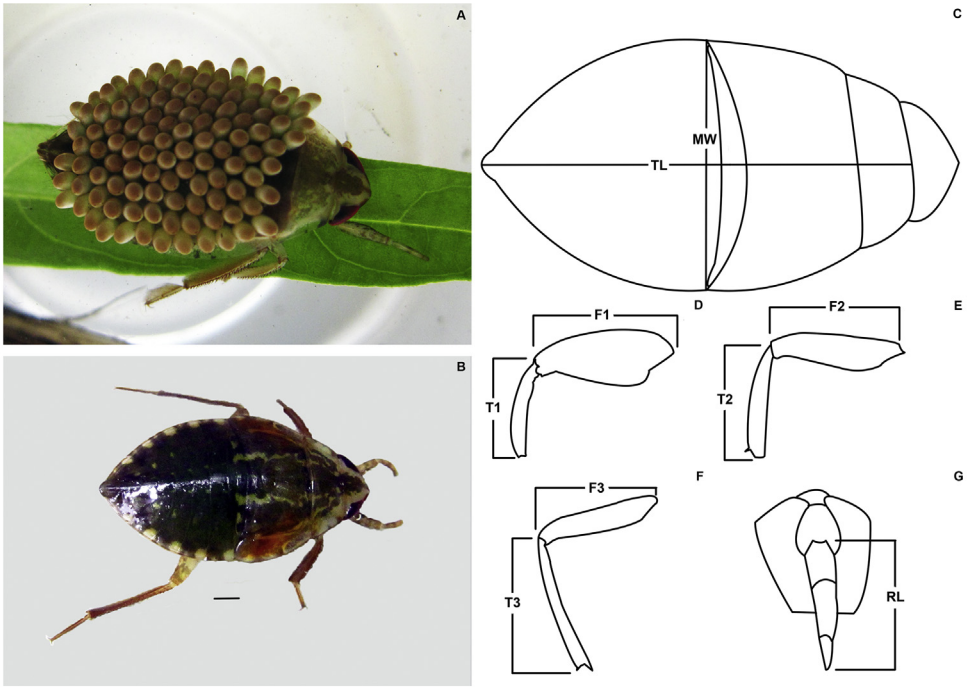

Fig. 1. Morphological and morphometric characters of *Diplonychus rusticus* (Fabricius): A-Live encumbered male, B-5th instar nymph (scale – 1 mm), C–G: Measurements taken – C-Total length without head (TL) and Maximum Width (MW), D-Length of forefemur (F1) and Length of foretibia (T1), E-Length of midfemur (F2) and Length of midtibia (T2), F-Length of hindfemur (F3) and Length of hindtibia (T3) and G-Length of the rostrum (RL).

PAST 3.13 and PAST 2.17 (Hammer et al., 2001), respectively, for the Principal Components Analysis and multivariate allometry.

3. Results

3.1. Diagnostic characters of the nymphs of D. rusticus

Nymphs of all the instars are ovate (Fig. 1B) and morphologically very similar. They can be distinguished primarily based on size. The first through fourth instar have two-segmented antennae and one segment is added in the fifth instar and the adult, respectively. Rostrum is three-segmented from the first instar to the adult. Fore tarsi are one-segmented from the first instar to the adult, while the mid and hind tarsi are two-segmented in the nymphal instars and add a segment in the adult. Wing pads begin to appear in the second instar and increase in length throughout the successive instars. Ventrally, the metapleural episterna are expanded around the hind coxae and supposedly function as a protective covering for basal spiracles (Menke, 1979). Some of the notable changes occurring during each instar are summarised in Table 1.

3.2. Principal components analysis

The PCA plot of the dataset of all the nine morphological variables for the nymphs as well as the exuviae clearly show five distinct areas in the morphospace which represent each of the five instars (Fig. 2). The clusters corresponding to each instar for the dataset of the exuviae overlapped with those for the dataset of the field-captured nymphs. The first principal component (PC1) accounts for 98.2% of total variation for the dataset containing the data for both the exuviae and the nymphs. Within-group variation is much less for the dataset of the exuviae as compared to the dataset of the field-captured nymphs, as shown by the minimal spread of the data in the morphospace (Fig. 2). The axis loadings of all the variables are given in the Appendix.

3.3. Instar prediction using biometric characters

The regression tree constructed using exhaustive CHAID algorithm to predict the developmental instar of the nymphs collected on field showed that the characters total length without head (TL) and maximum width (MW) were sufficient to distinguish between

Table 1
Comparative account of morphological changes during the growth of *D. rusticus*.

Character	1st instar	2nd instar	3rd instar	4th instar	5th instar	Adult
Antennal segments	2	2	2	2	3	4
Tarsal segments	F:1, M:2, H:2	F:1, M:2, H:2	F:1, M:2, H:2	F:1, M:2, H:2	F:1, M:2, H:2	F:1, M:3, H:3
Swimming hair on hind-tarsi	Indistinct	Very short	Short	Moderately developed	Well developed	Long and dense
Extent of wing pads	Absent	Midpoint of metanotum	2/3rd of metanotum	4/5th of metanotum	Base of metanotum	Macropterous
Extent of metapleural episterna	First three abdominal sternites	Half of the 4th abdominal sternite	Apex of the 4th abdominal sternite	Half of the 5th abdominal sternite	Apex of the 5th abdominal sternite	–

F = Foretarsus, M = Midtarsus, H = Hindtarsus.

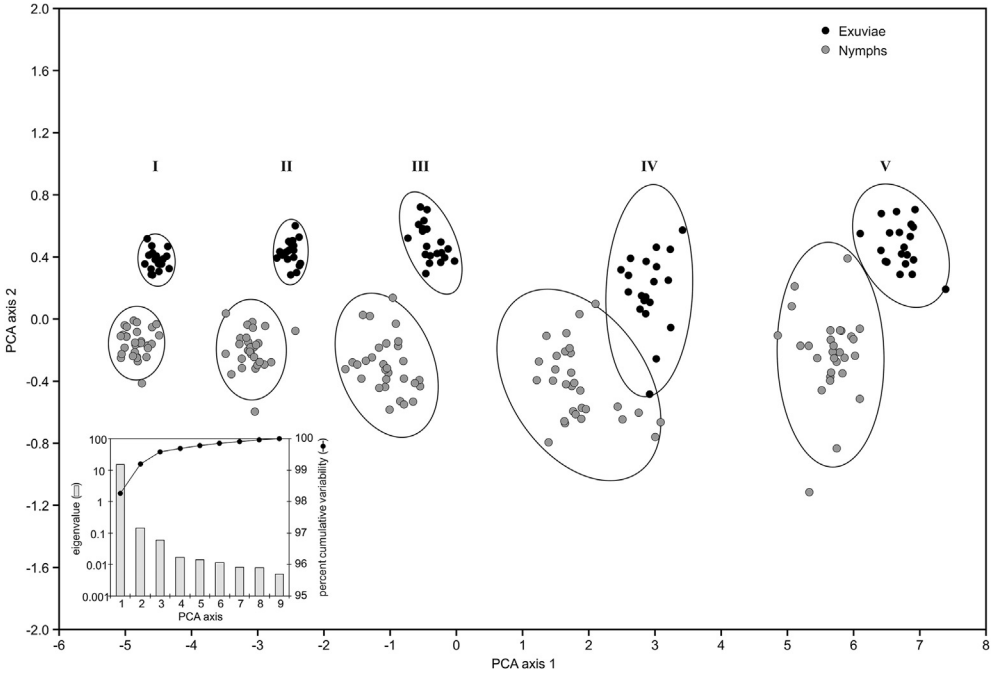

Fig. 2. Principal Components Analysis: PC1 vs. PC2 plot of all five instars (1–5) of *Diplonychus rusticus* using the data of all the nine variables for exuviae and nymphs. **I**-1st instar, **II**-2nd instar, **III**-3rd instar, **IV**-4th instar and **V**-5th instar. Scree plot showing percent variation explained by each PCA axis is provided in inset.

the nymphs of different instars. The regression tree constructed using the dataset of the lengths of all the femora revealed that the 1st and 5th instars can be separated on the basis of the length of the forefemur (F1) alone, while 2nd, 3rd and 4th instars can be defined by a combination of the lengths of fore (F1) and midfemur (F2). Similarly, the regression tree constructed using the dataset of the lengths of tibiae showed that the 5th instar can be separated from the rest by the length of the midtibia (T2) alone, while the remaining four instars can be separated on the basis of the combination of the lengths of the mid (T2) and hind tibiae (T3). It was seen that the rostrum length (RL) is not a character of importance for separating the instars. Four decision rules that separate the nymphs belonging to different instars are as shown in Fig. 3A–D. These decision rules were used to assign instar to the field-captured individuals and all four rules equivocally grouped the instars corresponding to their grouping in the PCA analysis.

3.4. Ontogenetic allometry

The nine different morphological variables considered for multivariate allometric analysis exhibit different types of allometry. In field-captured individuals, total length without head and the rostrum show isometry, while maximum width shows a strongly positive allometry or hyperallometry. Mid and hind legs exhibit moderate hyperallometry, while forelegs are clearly hypoallometric. In exuviae, both the total length without head and maximum width show hyperallometry. Mid and hind legs show moderate hyperallometry, while rostrum and forelegs show hypoallometry. All the allometric coefficients (AC) along with the 95% confidence intervals are summarised in Table 2.

4. Discussion

Although the taxonomy (Lauck and Menke, 1961; De Carlo, 1964; Polhemus, 1994; Perez Goodwyn, 2006) and the general aspects of biology (Smith, 1997 and references therein) of the family Belostomatidae have been widely studied, the avenue of morphology- and biometry- based studies remains comparatively unexplored. In this study, we have used a set of externally measurable morphological characters in order to shed light on the growth pattern of *D. rusticus*. We started with the principal components analysis (PCA) to determine whether there is a clear separation of data, which corresponds to the developmental instar for both the datasets of the exuviae and the field-captured nymphs, which indeed was the case. The clusters formed by the data for each instar of the exuviae and the field-captured nymphs overlapped partially and were separated only on the second axis, but the variation explained by this axis is much less (Fig. 2, inset). This separation is expected, as the dataset for the exuviae represents maximum growth in each instar for a clutch from a single pair, while the field data are likely to have more variation as they might represent individuals born from different egg clutches. This also explains why there is low within-group variation observed in the dataset for the exuviae as compared to the field data.

The first principal component in the PCA accounts for more than 98% of the variance for both nymphs and exuviae, providing an excellent fit of the simple ontogenetic allometry model to these datasets. This pattern has also been shown in various earlier studies on aquatic Hemiptera and other insects (Davies and Brown, 1972; Klingenberg and Zimmermann, 1992; Klingenberg and Spence, 1993; Iglesias et al., 2008). Klingenberg (1996) suggests that this dominance of the first principal component in explaining the total variance of a dataset is indicative of "*a well integrated ontogeny and constrained phenotypic variation of growth trajectories*", which is confirmed by the current study as well.

Studies using the exhaustive CHAID analysis have been conducted in some vertebrates like the spotted owlets for determining the age of the nestlings (Pande et al., 2011) or for species prediction of stone loaches (Raghavan et al., 2013). We could not find such studies for any invertebrate taxon. A set of regression rules that determined the instar of the exuviae successfully separated the

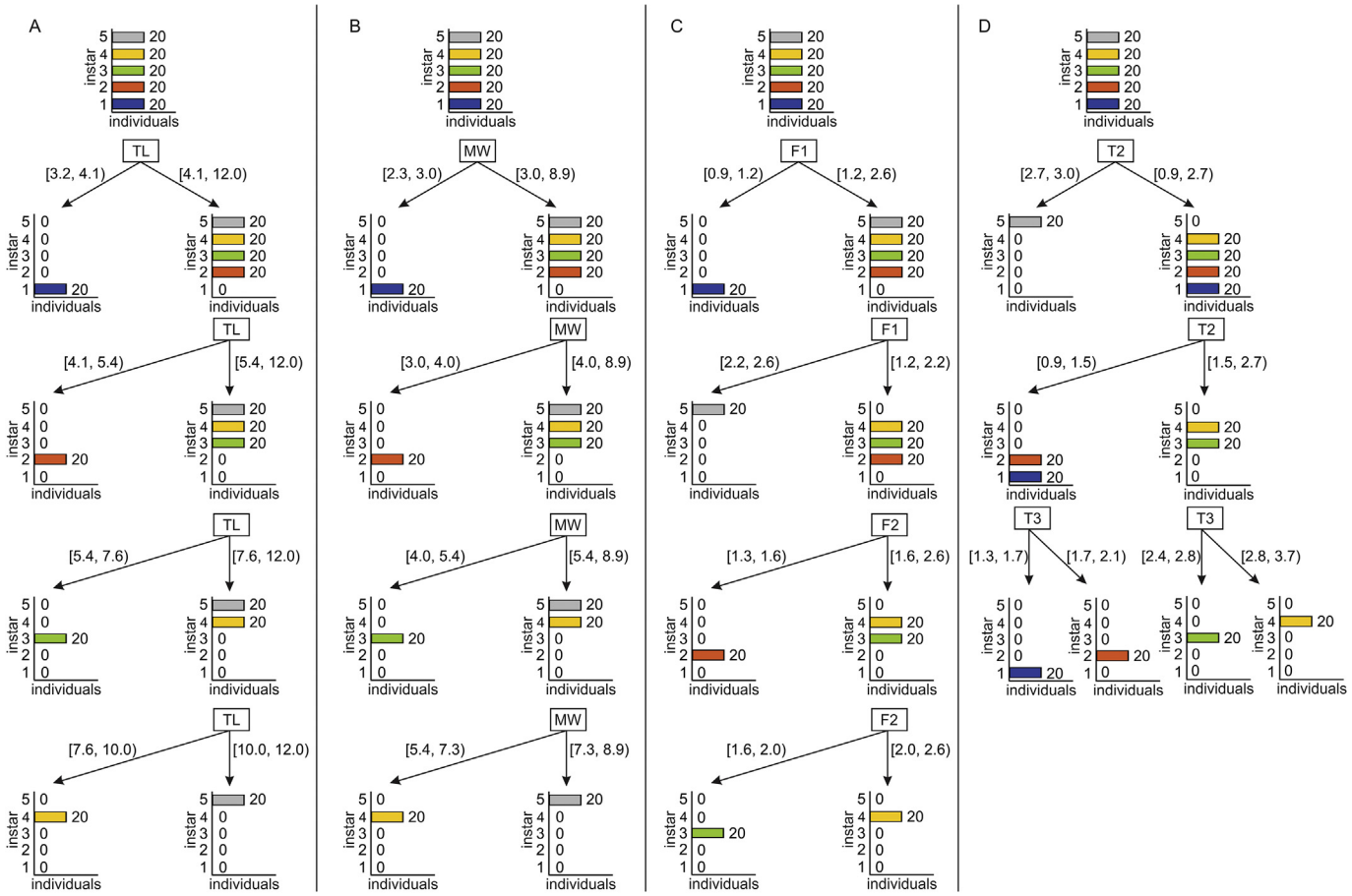

Fig. 3. Decision trees based on exhaustive CHAID algorithm: Bar diagrams show the number of individuals in each nymphal instar (1–5) of *Diplonychus rusticus*. Numbers over each group indicate size range for the said character for each instar. A-Total length without head (TL), B-Maximum width (MW), C-Lengths of femora (F1 and F2), D-Lengths of tibiae (T2 and T3).

Table 2
Multivariate allometry in *D. rusticus*: Allometric Coefficients (AC) with ninety five percent confidence intervals (95% CI).

Field captured nymphs			Exuviae		
Variable	AC (95% CI)	Allometry	Variable	AC (95% CI)	Allometry
TL	0.98 (0.96–1.00)	=	TL	1.11 (1.10–1.13)	+
MW	1.10 (1.08–1.12)	+	MW	1.17 (1.15–1.19)	+
F1	0.83 (0.81–0.86)	–	F1	0.83 (0.80–0.86)	–
F2	1.06 (1.03–1.10)	+	F2	1.01 (0.99–1.03)	=
F3	1.04 (1.00–1.07)	+	F3	0.99 (0.98–1.01)	=
T1	0.83 (0.78–0.87)	–	T1	0.83 (0.80–0.87)	–
T2	1.07 (1.04–1.10)	+	T2	1.07 (1.05–1.09)	+
T3	1.07 (1.05–1.10)	+	T3	1.05 (1.03–1.07)	+
RL	1.02 (0.98–1.08)	=	RL	0.93 (0.89–0.97)	–

TL = Total Length without head, MW = Maximum Width, F1 = length of forefemur, F2 = length of midfemur, F3 = length of hindfemur, T1 = length of foretibia, T2 = length of midtibia, T3 = length of hindtibia, RL = length of the rostrum, '=' Isometry, '+' Hyperallometry, '–' Hypoallometry.

field-captured nymphs into instars predicted in PCA. Thus, our study suggests that it is possible to predict the instars in the field if we devise a regression tree for laboratory reared individuals. Such methods can help in field studies for quick identification of the developmental stages and to understand ontogenetic assemblages in their natural habitat. For the exhaustive CHAID analysis, the dataset of the exuviae was chosen as it represents the maximum growth for each instar. The regression tree based on exhaustive CHAID algorithm shows very clearly that only two characters, i.e. total length without head (TL) and maximum width (MW), are sufficient to determine the instar of the nymphs (Fig. 3). This might be very helpful for rapid segregation of instars in field-based studies. The lengths of leg segments, i.e. femur and tibia also proved to be equally adequate for instar determination (length of forefemur and lengths of mid and hind tibiae for instar 1; lengths of fore and mid femora and lengths of mid and hind tibiae for instars 2, 3 and 4; lengths of forefemur and midtibia for instar 5 respectively), but these characters cannot be used for field-based studies, as they cannot be measured on field. Similar studies for other belostomatid genera are required to confirm whether or not the decision rules obtained for *D. rusticus* are applicable throughout the family Belostomatidae.

It is seen from the multivariate allometric analysis that the different body parts present different types of allometry. Similar trends have been observed for antennal segments (Matsuda, 1960; Iglesias and Crespo, 2003, 2008) and for rostral segments (Iglesias et al., 2008) in other aquatic hemipterans. This is very apparent in the case of allometries exhibited by the leg segments. Both forefemur and foretibia exhibit negative allometry in field-captured nymphs as well as exuviae, while segments of mid and hind legs experience moderately positive allometry (see Table 2). This may be due to the different functions attributed to these body parts. Mid and hind legs are used for swimming, while forelegs are essential for capturing and holding the prey (Menke, 1979) and are required to develop rapidly in the younger instars in order to hunt and survive. Characters such as the segments of forelegs are directly linked to predation success and may face stronger selection pressures than other characters, as suggested by Ohba et al. (2006). Such differences in allometric patterns are useful in predicting the functionality of the involved structures and their developmental variation (Iglesias et al., 2008). Our results corroborate the results obtained in an earlier study on the New World genus *Belostoma* (Iglesias et al., 2008), suggesting that there might be a conserved ground plan of development in the family Belostomatidae. The allometric patterns of closely related species are very useful for inferring the phylogenetic relationships between them and, as shown for members of the gerrid genus *Limnoporus*, correspond quite well with the hypothesized phylogeny of the genus (Klingenberg and Spence, 1993).

The overall pattern of growth as seen from the allometric coefficients is similar for both the laboratory-reared and field-captured specimens. It is previously noted for the members of the family Gerridae that the allometric pattern is only weakly affected by the differences in laboratory and the field conditions (Klingenberg and Zimmermann, 1992). But the differences between the allometric coefficients and hence the type of allometry shown by the same variables in some cases (see Table 2), such as the total length without head (TL), lengths of mid and hind femora (F2 and F3 respectively) and rostrum length (RL) for the two datasets of field-captured nymphs and exuviae, cannot be satisfactorily explained in the current study. However, the effect of limited sample size and the fact that exuviae represent maximum growth at moulting could have contributed to these variations. Further, it is also essential to note that exuviae were collected from the nymphs belonging to a single egg clutch, while field data are likely to have come from individuals born from different egg clutches. Therefore, it might be pertinent to consider the allometric coefficients obtained from the field data to be better indicators of the growth pattern in *D. rusticus*, as they represent a stable natural population.

We could not generalise the trends obtained in this study due to limited resources and logistical constraints in carrying out similar studies on other belostomatids. We have also not looked at the allometric basis of the sexual size dimorphism, as previously studied in the genera *Belostoma* and *Lethocerus* (Iglesias et al., 2012). However, this work is one of the few studies done towards understanding the heterochronic and allometric changes taking place during the postembryonic growth in the family Belostomatidae and a lot still needs to be done to elucidate the underlying pattern of growth in these bugs.

Funding

This work was partially supported by the Junior Research Fellowship given by the University Grants Commission (UGC), New Delhi, India to SVP.

Author contributions

SVP designed the study and helped to generate and analyse the data. SVP reared the insects in the laboratory and provided the exuviae for morphometry. DD did the fieldwork and morphometry. RM provided laboratory facilities to DD. SVP, SMP and ND did the statistical analysis. SVP, SMP, ND and RM wrote the paper and all authors approved it.

Acknowledgments

DD and RM thank the Principal and the authorities of Prof. Ramkrishna More Arts, Commerce and Science College, Akurdi, Pune, India for laboratory facilities and support. SVP and SMP thank Hemant Ghate, Modern College, Shivajinagar, Pune, India for his suggestions and help with the morphometry. SVP thanks Anand Padhye and the Head of the department, Department of Zoology, Abasaheb Garware College, Pune, India for laboratory facilities, help and encouragement. SVP is thankful to the Head of the Department of Biodiversity and the Principal, Abasaheb Garware College, Pune, India for their support. SVP acknowledges University Grants Commission, India for providing the funds (junior research fellowship). SMP acknowledges Sanjay Molur, Wildlife Information Liaison Development Society, Coimbatore, India. Authors are thankful to Sayali Sheth, Nikhil Modak, Mihir Kulkarni and Shriraj Jakhalekar for their help with laboratory rearing of the bugs. Authors acknowledge Shriraj Jakhalekar for providing the photographs of the live male specimen. Authors also thank the two anonymous reviewers who provided constructive comments on an earlier version of the manuscript. Authors are grateful to Felipe Moreira (Instituto Oswaldo Cruz, Rio de Janeiro, Brazil), David Redei (Nankai University, Tian Jin, China) and Jing-Fu Tsai (National Museum of Natural Science, Taichung, Taiwan) for their help with the literature.

Appendix

PCA axis loadings for all the measured morphological variables (TL = Total length without head, MW = maximum width, F1 = length of forefemur, F2 = length of midfemur, F3 = length of hindfemur, T1 = length of foretibia, T2 = length of midtibia, T3 = length of hindtibia and RL = length of the rostrum).

Variable	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 6	Axis 7	Axis 8	Axis 9
TL	0.6920	−0.4574	0.5499	−0.0615	−0.0278	−0.0414	−0.0196	0.0510	0.0159
MW	0.5501	−0.0972	−0.7575	0.1989	−0.2594	0.0024	0.0645	−0.0513	0.0238
F1	0.1248	0.3482	0.1498	−0.1214	−0.1454	0.1877	0.6519	−0.1817	0.5583
F2	0.1794	0.4310	0.1155	−0.0736	−0.2243	0.0275	0.3299	0.3413	−0.6978
F3	0.2179	0.5311	0.1228	−0.1867	−0.2887	−0.3292	−0.5232	−0.388	0.0667
T1	0.0836	0.2049	0.1546	0.4546	−0.0814	0.7725	−0.3393	0.0261	0.0346
T2	0.1759	0.2352	−0.1353	−0.3325	0.2535	0.0191	−0.2462	0.7272	0.3617
T3	0.2727	0.1784	−0.1197	−0.201	0.7754	0.1909	0.0470	−0.3848	−0.2260
RL	0.1050	0.2512	0.1285	0.7375	0.3240	−0.4694	0.0997	0.1309	0.1141

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