EUROPEAN JOURNAL OF ENTOMOLOGY
ISSN (online): 1802-8829
http://www.eje.cz
Eur. J. Entomol. **121**: 341–346, 2024
doi: 10.14411/eje.2024.035
SHORT COMMUNICATION

Morphological allometry of three hymenopteran ectoparasitoids of stored-product insect pests

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Key words. Hymenoptera, Pteromalidae, *Anisopteromalus calandrae*, *Anisopteromalus quinarius*, Braconidae, *Heterospilus prosopidis*, Coleoptera, Chrysomelidae, Bruchinae, *Callosobruchus maculatus*, stabilizing selection, negative allometry, behavior

Abstract. Allometric analysis provides an insight into the function and diversification mechanisms of body parts in organisms. The allometry and variability in size of various body parts of three hymenopteran species, *Anisopteromalus calandrae* (Howard), *Anisopteromalus quinarius* Gokhman & Baur, and *Heterospilus prosopidis* Viereck, which are solitary ectoparasitoids of stored-product insect pests, were studied. The relationship between many of the traits measured and body size is negatively allometric, others were isometric, and none were positively allometric. The two species of *Anisopteromalus* were less variable in size than *H. prosopidis* and there were intersexual differences in both species of *Anisopteromalus*. Although the patterns in these differences are complex and difficult to interpret from an ecological perspective, based on information on their behaviour it is hypothesized that stabilizing selection is associated with the negative allometry of two traits: ovipositor length in at least two species and male leg length in both species of *Anisopteromalus*. This hypothesis is supported by the lower variability in the size of these body parts compared to other body parts examined in this study, which is typical of traits subject to stabilizing selection.

INTRODUCTION

Elucidating the nature and genesis of morphological diversity in organisms is one of the main goals of evolutionary biology. Morphometric approaches are widely used in this pursuit, ranging from traditional methods based on linear measurements to more modern methods based on coordinate matrices, such as geometric morphometrics. Morphological allometry is one characteristic described by morphometric values and refers to the scaling relationship between the size of a given body part and the organism's overall body size. The type of allometry is determined by the slope of a log–log regression of the size of a body part against overall body size; these patterns are classified as positively allometric (slope > 1), isometric (slope = 1), or negatively allometryic (slope < 1) (Futuyma, 2013).

Combined with data on morphological variation, allometry can provide insights into the type and degree of selection acting on traits. For example, in male stag beetles *Lucanus maculifemoratus* Motschulsky, the relationship for head size is significantly positively allometric and its distribution is conspicuously right-skewed. This pattern is interpreted as possibly reflecting the advantage of a large head, including the mandibles, in male–male combat (Tatsuta et al., 2001) and is a typical case of directional selection. In contrast, the relationship for the male genitalia of this beetle is negatively allometric, with an approximately normal size distribution with less variation than recorded for head size; this pattern is interpreted as reflecting stabilizing selection, in this case female requirement for a specific size and shape of male genitalia, which is sexual selection. The relationships in males of the Far Eastern knotweed borer moth *Ostrinia latipennis* (Warren) with the lengths of the forewing, hindwing, fore femur, tibia, mid-femur, and tibia are isometric, for which there is no obvious selection pressure; however, the relationship for its genitalia is negatively allometric and varies less than for somatic traits, likely reflecting stabilizing selection (Ohno et al., 2003). The importance of collecting data on both allometry and variability when estimating the type and degree of selection is highlighted in a review of genital allometry (Eberhard et al., 2009).

In this study, the allometry and variability of various body parts of three species of Hymenoptera, *Anisopteromalus calandrae* (Howard, 1881) and *Anisopteromalus quinarius* Gokhman & Baur, 2014 of family Pteromalidae and *Heterospilus prosopidis* Viereck, 1910 of family Braconidae were recorded and compared for different sexes and among species. These species are solitary ectoparasitoids of concealed hosts, are easily maintained on stored-product pests under laboratory conditions (in this study, *Callosobruchus* bean beetles) and have been used as model species in various studies. Many allometric studies on Hymenoptera have assessed single species (often single traits; e.g., Melin et al., 2019), considered only phylogenetically related species (e.g., Janzon, 1986), or compared allometric patterns between castes in eusocial species (e.g., Feener, 2009). In contrast, this study is unique in focusing on parasitoids of the same host species and includes a phylogenetically distant parasitoid species. Thus, the

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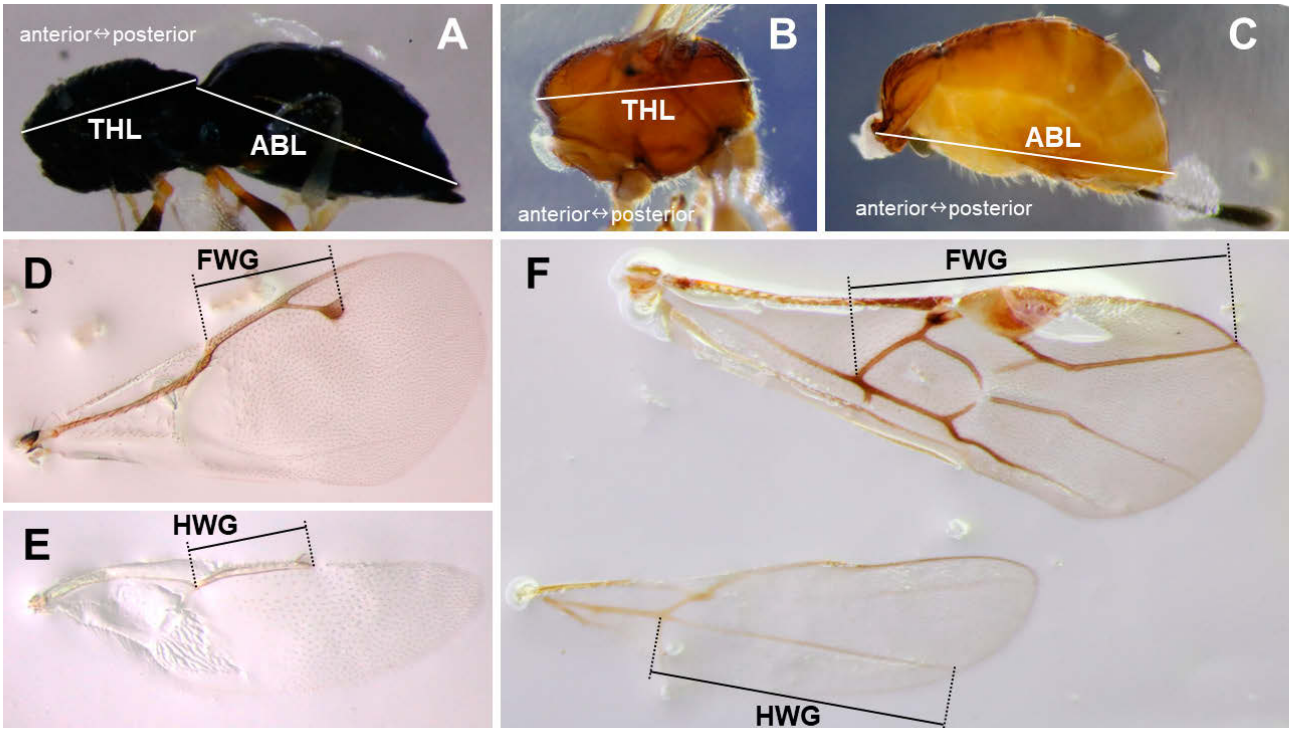

Fig. 1. Measurements of the thorax, abdomen, forewing, and hindwing. (A) Left lateral view of an *Anisopteromalus calandrae* female with head removed; (B) left lateral view of the thorax of a *Heterospilus prosopidis* female; (C) left lateral view of the abdomen of an *H. prosopidis* female; (D) forewing of an *Anisopteromalus quinarius* female, (E) hindwing of an *A. quinarius* female; (F) forewing (upper) and hindwing (lower) of an *H. prosopidis* female. Abbreviations: ABL – abdomen length at longest part; FWG – measured part of forewing; HWG – measured part of hindwing; THL – thorax length at longest part. Landmarks for forewing and hindwing measurements differ between *Anisopteromalus* and *Heterospilus* (see Material and Methods).

results will provide new insights into the morphological diversity and allometric patterns in Hymenoptera.

MATERIAL AND METHODS

The following parasitoid strains were used: *A. calandrae* strain "ja", *A. quinarius* strain "ka", and *H. prosopidis* strain "AZ2007." These strains have been used in many studies; their histories are reported by Sasakawa et al. (2012, 2013) and Amemiya & Sasakawa (2021). The two species of *Anisopteromalus*, which are morphologically similar, were identified based on their chromosomes by A.V. Timokhov (Moscow State University, Moscow, Russia) and confirmed morphologically based on a comprehensive taxonomic study by Baur et al. (2014). Specimens were reared on larvae of the bean beetle *Callosobruchus maculatus* (Fabricius) infesting cowpea *Vigna unguiculata* (Linnaeus) Walpers, in an incubator maintained at 29°C under a 16L : 8D photoperiod. For each species, specimens were obtained from a single rearing event. Freshly emerged adults were killed by immersion in absolute ethanol and preserved in 70% ethanol until dissection. Each wasp was dissected on a glass slide into 10 parts: head, thorax, abdomen, antennae, forewings, hindwings, forelegs, middle legs, hindlegs and ovipositor. The dissected parts were mounted on slides in Euparal, and scaled digital images were taken of the head in full-face view, the wings and ovipositor in dorsal view and the leg parts in lateral view. Based on these images, the following measurements were obtained with an accuracy of 1 µm, using ImageJ software version 1.50i (Rasband, 2016): head length, measured from the vertex to apex of clypeus (HL); head width at the widest part (HW1); head width, measured as the distance between the eyes at the narrowest part (HW2); antennal length (AL); thorax length at the longest part, as shown in Fig. 1 (THL); abdomen length at the longest part, as shown in Fig. 1 (ABL); forewing size, measured as the distance between two landmarks, as shown in Fig. 1 (FS); hindwing size, measured as the distance between two landmarks, as shown in Fig. 1 (HS); lengths of the forefemur (FE1), foretibia (TB1), and fore tarsus (TA1); lengths of the mid-femur (FE2), mid-tibia (TB2), and mid-tarsus (TA2); lengths of the hind femur (FE3), hind tibia (TB3) and hind tarsus (TA3); and ovipositor length (OL). The positions of these measurement are shown in Fig. 1, and those of head measurements are illustrated in Brazidec et al. (2024, fig. 1). Wing size measurements were based on a partial structure of the wing vein, because the membranous part was occasionally destroyed during dissection and therefore not consistently measurable from base to tip, as is in many studies. Moreover, due to differences in wing vein structures, different landmarks were used in *Anisopteromalus* and *H. prosopidis*. For some (particularly small) specimens, some traits were destroyed during dissection and no measurements were obtained. Such specimens were excluded from the analysis of those traits, leading to different sample sizes among traits, even within the same species and sex. The resulting sample sizes was 35–51 for each trait.

In the allometric analysis, which measurements should be used as indices of overall body size remains a matter of debate. Indices used for Hymenoptera vary among studies, ranging from total body weight (e.g., Danforth & Desjardins, 1999; Godfrey et al., 2021) to total body length (e.g., Tschinkel et al., 2003; Žikić et al., 2009), thorax width (e.g., Feener, 2009; Melin et al., 2019) and forewing length (e.g., Rabieh & Afkhami, 2021). In this study, thorax length + abdomen length (BL) was used as an index of overall body size for the following reasons: it covers a larger area of the body than partial parts such as thorax width and forewing length; unlike total body weight, wings and appendages were not

Table 1. Percentage variance and factor loadings for the first two principal components (PC1 and PC2) of a principal component analysis based on a variance–covariance matrix. These analyses were performed only for specimens with no missing data, using the "prcomp" function in the *stats* package in R. Trait abbreviations are defined in Material and Methods.

Trait	*Anisopteromalus calandrae*				*Anisopteromalus quinarius*				*Heterospilus prosopidis*			
	Females (*n* = 28)		Males (*n* = 36)		Females (*n* = 26)		Males (*n* = 38)		Females (*n* = 38)		Males (*n* = 45)	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Variance (%)	84.2	7.1	95.8	1.2	78.1	11.5	93.9	2.3	96.2	1.1	97.3	0.6
HL	0.127	–0.038	–0.140	0.001	–0.134	0.025	–0.127	–0.006	–0.163	–0.069	–0.171	–0.047
HW1	0.121	–0.029	–0.147	0.000	–0.120	0.023	–0.119	0.001	–0.185	–0.007	–0.191	–0.001
HW2	0.119	–0.022	–0.146	–0.005	–0.102	0.015	–0.116	0.000	–0.176	–0.004	–0.188	–0.012
AL	0.118	–0.015	–0.139	0.003	–0.111	0.023	–0.107	0.010	–0.173	–0.005	–0.196	0.011
FS	0.108	–0.008	–0.167	–0.012	–0.119	0.022	–0.093	0.007	–0.177	0.005	–0.185	0.003
HS	0.097	0.029	–0.154	0.018	–0.112	–0.004	–0.102	0.000	–0.202	0.008	–0.196	0.003
FE1	0.120	0.043	–0.166	0.003	–0.137	–0.063	–0.111	0.011	–0.187	–0.003	–0.205	0.010
TB1	0.125	0.069	–0.161	0.009	–0.143	–0.120	–0.122	0.002	–0.198	–0.008	–0.201	0.017
TA1	0.110	0.068	–0.164	0.016	–0.146	–0.081	–0.107	0.023	–0.155	0.004	–0.154	0.011
FE2	0.117	0.008	–0.162	0.008	–0.126	0.032	–0.111	0.012	–0.185	0.005	–0.216	0.006
TB2	0.113	0.000	–0.158	0.007	–0.110	0.057	–0.102	0.013	–0.200	0.006	–0.212	–0.001
TA2	0.125	–0.004	–0.162	0.016	–0.122	0.046	–0.124	0.006	–0.142	0.004	–0.118	0.011
FE3	0.118	–0.019	–0.159	0.004	–0.123	0.015	–0.111	0.008	–0.197	0.005	–0.215	0.007
TB3	0.122	–0.005	–0.160	0.000	–0.116	0.001	–0.111	0.000	–0.194	–0.002	–0.207	0.001
TA3	0.118	–0.007	–0.165	–0.004	–0.110	0.008	–0.110	0.008	–0.158	0.009	–0.154	0.009
OL	0.080	0.002	–	–	–0.085	0.055	–	–	–0.180	0.000	–	–
BL	0.118	–0.061	–0.176	–0.060	–0.140	0.013	–0.162	–0.063	–0.223	0.035	–0.230	–0.023

included, in order to address the issue of independence between response and explanatory variables; and principal component analysis of the variance–covariance matrix showed that the thorax length + abdomen length had the highest loading in four of six species × sex combinations (Table 1). A standardized major axis regression was done using the "slope.test" and "sma" functions in the R package *smatr* (Warton et al., 2012). The "slope.test" function tests whether slopes differ from isometric (slope = 1.0). The "sma" function tests differences in allometric slopes among groups; differences between males and females were analysed for the same species and among species within the same sex.

Variation in size was evaluated using the coefficient of variation (CV) for each trait. Two comparisons were performed. First, interspecies and intersexual differences in the CV were determined. Using the "glmer" function of the R package *lme4* (Bates et al., 2015), a generalized linear mixed model (GLMM) was constructed with a Gamma error structure and log link function, in which CV values of all response variables of the allometric regression were included as the response variable. Species, sex, and their interaction were included as fixed effects of the explanatory variables, and trait identity was included as a random effect for the intercept. To further examine the possible association between male leg length and courtship behaviour, which was revealed by the allometric analysis (see Discussion), the CV of male legs among species were compared. A GLMM was constructed in which the error distribution, link function and random effect were identical to those in the first analysis and included the presence or absence of male courtship behaviour (present in *Anisopteromalus*, absent in *Heterospilus*) and species nested within male courtship behaviour as fixed effects. The raw data are available in Table S1.

RESULTS

In *A. calandrae*, the head width, antenna length and forewing size in both males and females, mid-tibia and hind femur lengths in males and ovipositor length were negatively allometric, where-

Table 2. Slopes of the allometric relationships and coefficients of variation (CV) for each trait. The statistical significance of each slope was tested using an isometric relationship (slope = 1) as the null hypothesis (*P < 0.05). Underlined slope values indicate a significant intersexual difference in slope (α < 0.05). Trait abbreviations are defined in Material and Methods.

Trait	*Anisopteromalus calandrae*				*Anisopteromalus quinarius*				*Heterospilus prosopidis*			
	Females		Males		Females		Males		Females		Males	
	Slope	CV (*n*)	Slope	CV (*n*)	Slope	CV (*n*)	Slope	CV (*n*)	Slope	CV (*n*)	Slope	CV (*n*)
HL	0.91	11.7 (51)	0.90	17.0 (49)	0.83*	12.6 (49)	0.74*	12.6 (49)	0.83*	18.4 (47)	0.76*	18.0 (50)
HW1	0.96	11.8 (51)	0.85*	16.5 (49)	0.78*	12.0 (49)	0.68*	11.5 (49)	0.81*	17.9 (45)	0.83*	19.3 (50)
HW2	0.79*	10.1 (51)	0.85*	16.4 (49)	0.76*	11.7 (49)	0.66*	11.2 (49)	0.81*	18.4 (47)	0.82*	19.4 (50)
AL	0.81*	11.4 (35)	0.84*	15.8 (44)	0.81*	11.6 (35)	0.62*	10.2 (45)	0.77*	17.5 (42)	0.86*	19.7 (48)
FS	0.80*	10.3 (51)	0.80*	15.4 (50)	0.70*	10.7 (49)	0.56*	9.5 (50)	0.79*	18.1 (49)	0.80*	18.4 (49)
HS	0.92	11.5 (46)	0.94	16.7 (44)	0.82*	12.6 (48)	0.64*	10.5 (49)	0.90*	20.1 (48)	0.84*	19.4 (49)
FE1	0.93	11.3 (49)	0.97	18.4 (48)	0.88	13.9 (44)	0.66*	11.3 (46)	0.85*	19.1 (48)	0.89*	20.4 (50)
TB1	1.06	13.0 (48)	0.92	17.5 (48)	1.04	17.2 (44)	0.74*	12.6 (46)	0.85*	19.5 (48)	0.88*	20.5 (50)
TA1	1.03	13.1 (48)	0.94	17.9 (48)	0.96	15.8 (42)	0.67*	11.4 (46)	0.70*	16.1 (48)	0.68*	15.7 (50)
FE2	0.85	10.9 (51)	0.91	17.6 (49)	0.80*	12.0 (46)	0.67*	11.0 (49)	0.82*	18.5 (49)	0.94	21.5 (49)
TB2	0.89	11.5 (51)	0.88*	16.9 (49)	0.88	13.3 (46)	0.64*	10.9 (49)	0.89*	20.1 (49)	0.92*	21.0 (49)
TA2	0.88	11.2 (51)	0.94	17.2 (48)	0.93	14.1 (44)	0.72*	12.1 (49)	0.63*	14.6 (49)	0.54*	12.5 (49)
FE3	0.86	10.9 (51)	0.91*	17.2 (50)	0.79*	11.9 (48)	0.65*	11.0 (49)	0.89*	20.2 (49)	0.93	22.0 (49)
TB3	0.91	11.7 (51)	0.93	17.7 (50)	0.81*	12.2 (48)	0.72*	11.8 (48)	0.89*	20.4 (49)	0.93	21.8 (49)
TA3	0.86	11.0 (51)	0.94	18.1 (50)	0.71*	11.0 (47)	0.69*	11.1 (48)	0.71*	16.4 (49)	0.67*	15.6 (49)
OL	0.72*	9.5 (48)	–	–	0.72*	11.6 (47)	–	–	0.80*	18.2 (49)	–	–

Table 3. Interspecies differences in allometric slopes. Different letters indicate significant differences among species (α < 0.05). Trait abbreviations are defined in Material and Methods.

Trait	Result
Female: TA1, TA2	*A. calandrae* a *A. quinarius* a *H. prosopidis* b
Male: HW1, HW2, AL, FS, HS, FE1, FE2, TB2, FE3, TB3	*A. calandrae* a *A. quinarius* b *H. prosopidis* a
Male: TA3, TA1	*A. calandrae* a *A. quinarius* b *H. prosopidis* b
Male: TA2	*A. calandrae* a *A. quinarius* b *H. prosopidis* c

as that of other body parts was isometric (Table 2). In *A. quinarius*, all body parts were negatively allometric, except for the forefemur, foretibia, fore tarsus, mid-tibia and mid-tarsus lengths in females, which were isometric. In *H. prosopidis*, all body parts were negatively allometric, except for the mid-femur, hind femur and hind tibia lengths in males, which were isometric.

Standardized major axis regression detected intersexual slope differences in antennal length, wing size, and the lengths of all foreleg parts and the mid-tibia and hind femur in *A. quinarius* and mid-femur length in *H. prosopidis* (Table 2). Interspecies differences in slope were detected for head width and lengths of some leg parts (Table 3).

The CV values for all traits are listed in Table 2. The GLMM of interspecies and intersexual differences in CV (Table 4) indicate that, with *H. prosopidis* females as the reference group, *A. calandrae*, *A. quinarius*, and the interaction between males and *A. quinarius* had negative effects on the CV, whereas the interaction between males and *A. calandrae* had a positive effect; no effect of sex was detected (Table 4). These results indicate that, overall, the two species of *Anisopteromalus* were less variable in size than *H. prosopidis* and that, when comparing males and females of the same species, females and males were less variable in size in *A. calandrae* and *A. quinarius*. The GLMM of male leg length (Table 4) revealed that, when *H. prosopidis* with no courtship behaviour was used as the reference group, courtship behaviour had a significant negative effect on the CV, indicating that in species with male courtship the males were less variable in leg length than in species in which the males do not court the females. The term *A. calandrae* nested within courtship behaviour had a positive effect, indicating that the size of *A. quinarius* is less variable than that of *A. calandrae*.

DISCUSSION

The results indicate that the allometric relationship and variability in size, evaluated using the CV, differ between species, sexes and traits. Although the patterns in these differences are complex, overall, many traits are negatively allometric, others are isometric, and no traits are positively allometric. Allometry is generally associated with ecological, physiological and mechanical factors (Eberhard et al., 2009; Futuyma, 2013) and should be interpreted with care. In studies that use laboratory-maintained strains, as in the present study, it is also necessary to confirm whether long-term rearing influences the results. Nevertheless, it is likely that some of the results have ecological implications when combined with the recorded variability in size and information on the behavioural characteristics of the target species. In particular, the ovipositor and male leg length results provide insights into the type of selection acting on these traits.

In all species, the relationship with ovipositor length was negatively allometric. Allometry of ovipositor length is reported for several species of Hymenoptera, with some studies reporting a positive allometric relationship (e.g., Rudoy et al., 2022), isometric relationship (e.g., Rabieh & Afkhami, 2021) and a negative allometric one (e.g., Yu et al., 2018). The negative allometric relationship detected in this study may be associated with the oviposition behaviour of the three species studied. In these species, females paralyze the host and then lay eggs on its surface. Moreover, females of *A. calandrae* use their ovipositor to destroy the eggs laid by other females of the same species (Gokhman et al., 1999). In this behaviour, individuals with ovipositors that are either too short or too long will be at a disadvantage in terms of reaching the host and accurately manoeuvring the host and eggs. This indicates that the negative allometry detected in this study may be associated with stabilizing selection for a particular ovipositor length. Indeed, the ovipositor lengths of the two species of *Anisopteromalus* were less variable than other traits (lowest in *A. calandrae*, and third lowest in *A. quinarius*; Table 2), which is typical of traits subject to stabilizing selection. The importance of ovipositor length in parasitoids and the resulting local adaptation of ovipositor length are reported for other parasitoid wasps (e.g., Žikić et al., 2010; Yu et al., 2018; Hernández-López et al., 2021), which suppors this hypothesis.

In both of the species of *Anisopteromalus* included in this study, more leg parts of males had a negative allometric relationship than in females, which contrasts with the findings for *H. prosopidis*. Considering the information on the behaviour of these two taxa, this difference may be associated with mating behaviour. In both species of *Anisopteromalus* the males mount females and perform an elaborate courtship on the female's back (e.g., Van den Assem, 1974; Yoshida & Hidaka, 1979; Gokhman et al.,

Table 4. Generalized linear mixed model results for interspecies and intersexual differences in CV and for the effects of male courtship and species on the CV of male legs. The reference groups for intercept analyses were *H. prosopidis* females for the former model and *H. prosopidis* for the latter model. SE – standard error.

Model and explanatory variables	β (mean ± SE)	Student's *t*	*P*
Model for interspecies and intersexual differences			
Intercept	2.91 ± 0.03	104.88	< 0.001
Male	0.03 ± 0.03	1.01	0.313
A. calandrae	–0.48 ± 0.03	–16.09	< 0.001
A. quinarius	–0.36 ± 0.03	–12.10	< 0.001
Male × *A. calandrae*	0.38 ± 0.04	8.77	< 0.001
Male × *A. quinarius*	–0.16 ± 0.04	–3.70	< 0.001
Model for male legs			
Intercept	2.94 ± 0.04	75.16	< 0.001
Courtship behaviour	–0.5 ± 0.05	–10.09	< 0.001
A. calandrae (nested within courtship behaviour)	0.43 ± 0.05	8.69	< 0.001

1998), whereas males of *H. prosopidis* do not exhibit this type of behaviour. Given the constraint that males of *Anisopteromalus* must walk carefully on the narrow back of a female, large or otherwise ungainly legs may not be favoured by selection. This, together with the lower degree of variability in the leg parts of males in both species of *Anisopteromalus* than in *H. prosopidis*, may indicate that the negative allometric relationship recorded for male legs in species of *Anisopteromalus* indicates the size has been subject stabilizing selection. Future studies on the relationship between male leg size and male reproductive success will provide new insights into this issue.

ACKNOWLEDGEMENTS. We thank three anonymous reviewers for their helpful comments. This study was partly supported by JSPS KAKENHI to KS (no. 22K06386).

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Received February 24, 2024; revised and accepted September 17, 2024

Online supplementary file:
S1 (http://www.eje.cz/2024/035/S01.xlsx). Table S1. The raw measurements (µm) of body parts.