RESEARCH ARTICLE

Intraspecific Sexual Size and Shape Dimorphism in an Australian Freshwater Fish Differs with Respect to a Biogeographic Barrier and Latitude

Clint D. Kelly · Kaila E. Folinsbee ·
Dean C. Adams · Michael D. Jennions

Abstract Geographically structured variation in morphology is a common phenomenon in animals with environmental factors covarying with both latitude and biogeographic barriers having profound impacts on body size and shape. The Pacific blue-eye (*Pseudomugil signifer*) is a freshwater fish that lives along Australia's east coast and occurs on either side of a terrestrial barrier, the Burdekin Gap. By quantifying the size and shape of males and females from 10 populations we found that Pacific blue-eyes are not sexually size dimorphism north of the Burdekin Gap whereas the degree of dimorphism was dependent upon latitude south of the barrier. Rensch's rule was not supported as the degree of male-biased size dimorphism did not increase with increasing population mean body size. Body shape was related to body size and was sexually dimorphic south of the Burdekin Gap but not north of it. Our study represents a rare case of identifying how both body size and shape differ with respect to latitude and a major terrestrial biogeographic barrier and lends further support to the notion that *P. signifer* may comprise two species, or incipient species, that are separated by the Burdekin Gap.

Keywords Body shape · Body size · Sexual selection · Rensch's rule · Bergmann's rule

C. D. Kelly · M. D. Jennions
Research School of Biology, Australian National University,
Canberra, ACT 0200, Australia

C. D. Kelly (✉) · D. C. Adams
Department of Ecology, Evolution and Organismal Biology,
Iowa State University, Ames, IA 50011, USA
e-mail: cdkelly@iastate.edu

K. E. Folinsbee
Department of Anthropology, Iowa State University, Ames,
IA 50011, USA

Introduction

Rensch ([1960](#), pp. 157–159) observed that the magnitude of sexual size dimorphism (SSD) tends to co-vary with mean body size and, as a rule, when males are larger than females, male size diverges more than female size as mean body size increases among related species. Rensch also noted that when females are larger than males (reversed SSD), SSD is negatively related to body size. Several functional mechanisms might explain male-biased SSD, but it is unlikely that any single one is responsible across the broad range of taxa in which it is observed (Fairbairn [1997](#), [2005](#)). One hypothesis suggests that Rensch's rule is driven by sexual selection for large male size with concomitant increases in female size being a genetically correlated response to selection on males, and/or a product of fecundity selection acting directly on females (Fairbairn [1997](#), [2005](#)). Female-biased size dimorphism on the other hand, is predicted to increase with decreasing body size due to stronger direct selection on female size for greater fecundity and direct selection for smaller male size due to greater mobility and agility, or from both processes acting simultaneously (Fairbairn [1997](#); Szekely et al. [2004](#); Blanckenhorn [2005](#); Kelly et al. [2008](#)). Increasing male size in reversed SSD species might be a correlated response to selection on females for greater fecundity.

Rensch's rule was originally formulated to explain interspecific variation in sexual size dimorphism; however, it might also explain population variation in dimorphism within a species (Fairbairn and Preziosi [1994](#); Fairbairn [2005](#); Young [2005](#); Lengkeek et al. [2008](#); Herczeg et al. [2010](#)). Tests for within-species patterns, although rare, are critical to confirm that macroevolutionary patterns arise from equivalent microevolutionary processes (Blanckenhorn et al. [2006](#)). Intraspecific patterns of SSD that support

Rensch's rule can be explained by genetic adaptation to local intensities of sexual selection on males (Fairbairn and Preziosi 1994), but they can also be a product of males having greater phenotypic plasticity in body size than females. Lengkeek et al. (2008) recently suggested that phenotypic plasticity could underlie interpopulation variation in SSD in Mediterranean blennies. For example, if the availability of breeding resources dictates the intensity of male-male competition and the degree of sexual competition, in turn, influences an individual's investment in body size, then interpopulation variation in resource availability could produce differences among populations in male body size.

Although some among-population studies support Rensch's rule (Fairbairn and Preziosi 1994; Fairbairn 2005; Young 2005; Lengkeek et al. 2008; Herczeg et al. 2010), a recent meta-analysis based on a wide array of animal taxa suggests that empirical support is generally lacking (Blanckenhorn et al. 2006). Instead, Blanckenhorn et al. (2006) found support for an intraspecific version of Rensch's rule based on sex-specific latitudinal variation in body size that is independent of which sex is larger. The increase in body size with latitude was steeper for males than females, meaning that among-population variation in body size is generally greater for males than females. Such a clinal pattern of SSD among populations is likely the product of factors that covary systematically with latitude, which potentially include intensities of sexual selection and environmental factors that mediate differential phenotypic plasticity (Blanckenhorn et al. 2006).

Studies of sexual dimorphism nearly always focus on size rather than shape differences (Butler et al. 2007; Berns and Adams 2010). There is no biological reason, however, to suspect that size dimorphism is more important than shape dimorphism. Clearly trait (Kelly and Adams 2010; Outomuro and Johansson 2011) and body (Abell et al. 1999) shape can be direct targets of sexual selection (see also Kaliontzopoulou et al. 2012). Similarly, natural selection can target body shape differently within each sex. For example, male and female guppies (Poecilia reticulata) exhibit a greater difference in body shape in high-predation environments, perhaps because males suffer higher predation than females thus favouring a body shape that maximizes predator-escape performance (Hendry et al. 2006; Burns et al. 2009). Interestingly, predation pressure does not significantly affect body size in guppies (Hendry et al. 2006; but see Burns et al. 2009). That body size and shape can be independently targeted by sex-specific divergent selection (Abell et al. 1999; Butler and Losos 2002) highlights the need to broaden the scope of morphological studies beyond simply measuring size. Sexual shape dimorphism can also vary geographically, and it is equally important to assess whether difference among populations

in shape are based on equivalent microevolutionary mechanisms (see Langerhans and DeWitt 2004).

Here, we examined geographical variation in sexual size and shape dimorphism in 10 populations of the Pacific blue-eye (Pseudomugil signifer), a small (<6 cm standard length) freshwater fish widely distribution across eastern Australia (Allen et al. 2003). P. signifer is sexually dimorphic with males reportedly being larger than females and having longer and more-colourful fins (Hadfield et al. 1979; Wong et al. 2004b). This sexual dimorphism is likely the product of sexual selection for larger males as those with longer and more colourful fins typically achieve greater success in aggressive male–male conflicts over spawning sites (Wong 2004; Wong et al. 2004a). Females also choose mates but male morphology does not appear to play a significant role as females mainly prefer males that court more actively (Wong 2004). Despite the apparent male-biased sexual size dimorphism, male body size and fin length are tremendously variable among populations. Several traits exhibits substantial clinal variation in morphology, increasing in size or number as latitude decreases (i.e. larger fish moving northward towards the equator) (Hadfield et al. 1979; Wong et al. 2004b). Recent molecular phylogenetic studies suggest extensive genetic divergence among populations at a broad geographical scale (McGlashan and Hughes 2002; Wong et al. 2004b) with mitochondrial DNA sequence data identifying populations north and south of the Burdekin Gap as two distinct genetic clades (McGlashan and Hughes 2002; Wong et al. 2004b). Molecular evidence also suggests that populations south of the Burdekin Gap (Fig. 1) are more connected by gene flow than northern populations. The importance of the terrestrial Burdekin Gap as a biogeographical barrier has been shown in phylogeographical studies of several taxa (reviewed in Chapple et al. 2011) including: frogs (Schäuble 2004), marsupials (Brown et al. 2006), and birds (Joseph and Moritz 1994). The gap, located south of Townsville, QLD is approximately 200 km wide, consisting of a dry woodland corridor extending to the coast. Unmack (2001) found that the Burdekin Gap divided two distinct aquatic biogeographic regions, with 13 northern species of freshwater fish reaching their southernmost limit at the Burdekin Gap. Observed population differences in morphology and genetics have led to suggestions that P. signifer located north and south of the Burdekin Gap might be different species (McGlashan and Hughes 2002; Wong et al. 2004b). Mate choice experiments support this hypothesis as females prefer local males as mates to males from geographically distant populations (Wong et al. 2004a).

In this study, we hypothesized that if Rensch's rule holds true at the intraspecific taxonomic level then the degree of male-biased size dimorphism should be greater with increasing mean body size across the 10 sample

Fig. 1 A map of eastern Australia depicting average-shape (both sexes pooled) TPS grids for each of the 10 sampled populations. *Shading* indicates species distribution
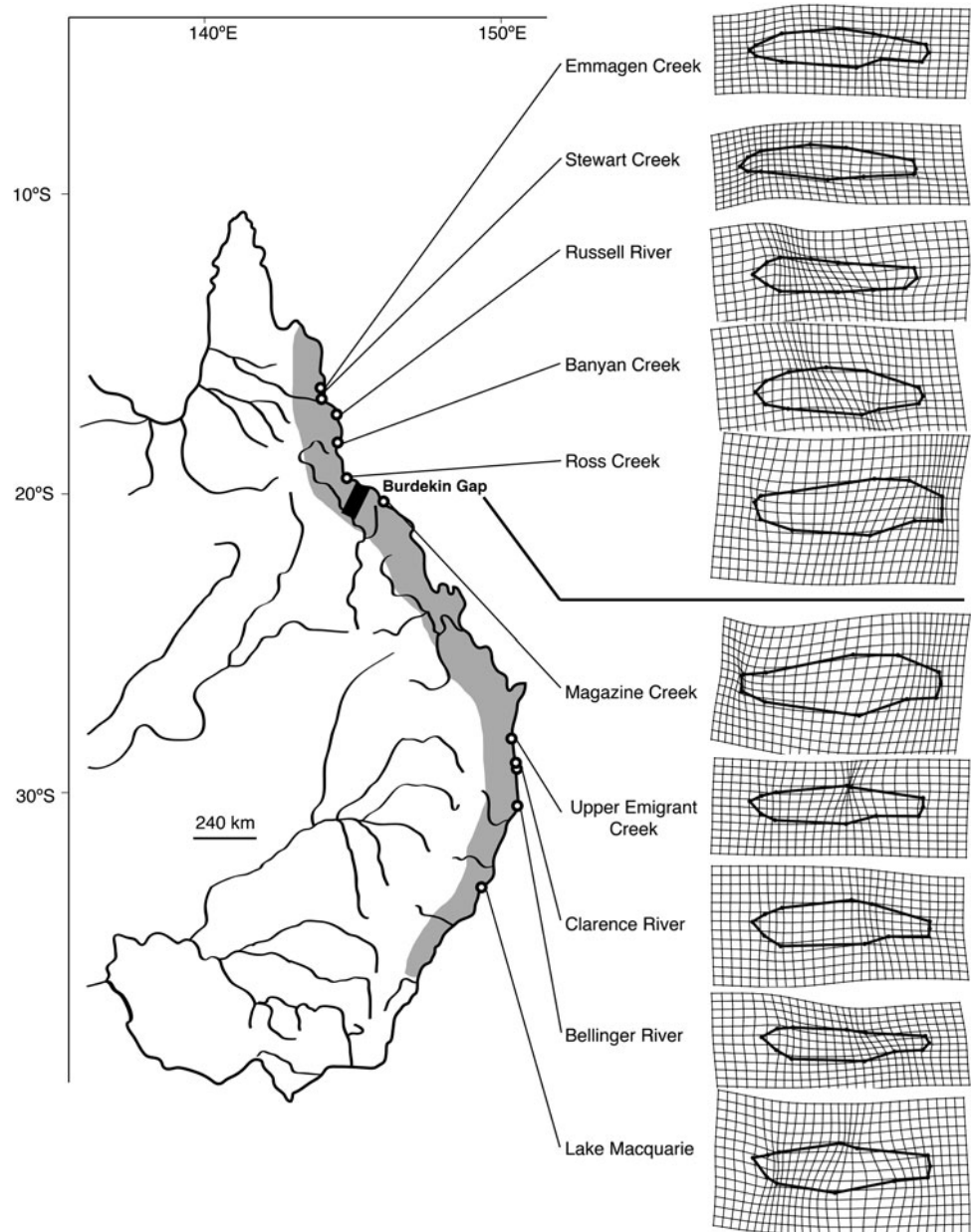
populations. We also examined whether the sexes differed in body shape given that males use their bodies in agonistic encounters and shape could influence these interactions. Finally, we asked whether sexual size and shape dimorphism are geographically dependent by determining whether morphology is related to latitude (fine scale) and location relative to the Burdekin Gap (broad scale). Our study contributes to a small but growing list of intraspecific tests of Rensch's rule (Fairbairn and Preziosi 1994; Fairbairn 2005; Young 2005; Lengkeek et al. 2008; Herczeg et al. 2010) and studies of population differences in sexual shape dimorphism in fish (Langerhans et al. 2004; Hendry et al. 2006). Importantly, however, our study represents a rare case of identifying how both body size and

shape differ with respect to latitude and a major biogeographic barrier.

Methods

Samples

Adult male and female *P. signifer* were field-collected from six populations in Queensland, Australia during December–January 2006 (Table 1). Fish were collected using a beach seine or a dip-net (rarely and only where seining was not possible). The fish were immediately euthanized in clove oil and preserved in absolute ethanol

upon collection for later photography and digitizing. Additional ethanol-preserved samples from four other populations were obtained from the Australian Museum (Table 1). Gaps in our sampling south of the Burdekin Gap were a result of our inability to find fish either in the wild or preserved in museum collections. Our dataset thus comprised five populations located north, and five south, of the Burdekin Gap.

Morphometric Analysis

We used landmark-based geometric morphometric methods to quantify overall body shape (Rohlf and Marcus 1993; Adams et al. 2004; Zelditch et al. 2004). These methods quantify the shape of anatomical objects from the coordinates of homologous locations, after the effects of nonshape variation (i.e. orientation, position and scale) are mathematically held constant. We used 4 semi-landmarks representing curvature and 8 fixed landmarks to quantify body shape. We obtained high-resolution photographs of the left side of each individual using a digital camera and then digitized the locations of the 12 homologous landmarks on each photograph (Fig. 2).

Once all specimens were digitized, we aligned them using a generalized Procrustes superimposition (Rohlf and Slice 1990). During this procedure, semilandmarks were permitted to slide along their tangent directions (see e.g. Bookstein 1997; Bookstein et al. 1999) to minimize the Procrustes distance between specimens (see e.g. Rohlf 2010; Serb et al. 2011). A set of 24 Procrustes shape coordinates were obtained; we retained only the first 20
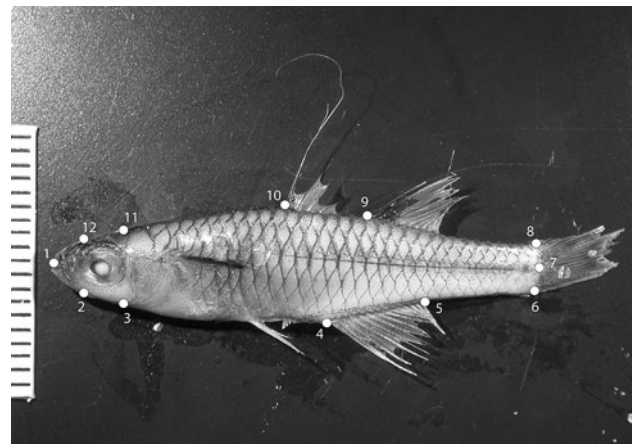
Fig. 2 The landmarks placed on digital photographs of *P. signifer* for use in geometric morphometric analysis of body shape. The marks to the left of the fish are 1 mm apart and were used to standardize landmark distances to the same absolute scale across all images. Landmarks 2, 3, 11 and 12 were semi-landmarks; all others were fixed

dimensions from a principal components analysis of these for use as shape variables in subsequent statistical analyses because only these contained the shape variation (Zelditch et al. 2004). We estimated the body size of each fish as its $\log_{10}$-transformed centroid size (Bookstein 1986) which is the square root of the sum of the squared distances from each landmark to the location on the fish that minimizes that sum (i.e. the fish's centroid).

We performed all digitizing and morphometric analyses in R 2.15.0 (R Development Core Team, 20011) using routines written by Claude (2008) and routines in the package 'geomorph' (Adams and Otarola-Castillo 2012, 2013).

Table 1 Identity, latitude, longitude, location relative to the Burdekin Gap and sample size for each of the 10 *Pseudomugil signifer* populations examined in this study

Population identity	Location relative to Burdekin Gap	Population	Latitude	Longitude	Sample size	
					Males	Females
1	North (QLD)	Emmagen Creek	16° 2′ 23″	145° 27′ 27″	20	20
2	North (QLD)	Stewart Creek	16° 19′ 1″	145° 19′ 24″	20	5
3	North (QLD)	Russell River[a]	17° 25′ 34″	145° 56′ 52″	11	10
4	North (QLD)	Banyan Creek	17° 55′ 51″	145° 55′ 53″	13	12
5	North (QLD)	Ross Creek	19° 18′ 30″	146° 48′ 38″	20	19
6	South (QLD)	Magazine Creek	20° 0′ 47″	148° 15′ 38″	19	10
7	South (NSW)	Upper Emigrant Ck.[a]	28° 51′ 51″	153° 31′ 25″	9	10
8	South (NSW)	Clarence River[a]			12	10
		Mororo Creek	29° 25′ 18″	153° 14′		
		Mororo Creek	29° 21′ 51″	39″ 153° 18′ 40″		
9	South (NSW)	Bellinger River[a]	30° 26′ 59″	153° 0′ 9″	8	11
10	South (NSW)	Lake Macquarie[a]	33° 4′ 24″	151° 36′ 30″	9	10

Australian Museum specimen numbers: Russell R. (I22035-002), U. Emigrant Ck (I39093-001), Clarence R. (Thorny Ck.: I41518-068; Mororo Ck.: I41516-076), Bellinger R. (I19012-004) and Lk. Macquarie (I43150-001)

[a] Denotes sample from Australian Museum

Statistical Analysis

Our interest was in identifying whether sexual size and shape dimorphism were related to latitude and whether these patterns differed either side of the Burdekin Gap. To address these issues we analyzed our univariate and multi-variate morphological data using both fully factorial and nested analyses (population nested within region), however, since both approaches gave qualitatively similar results we present the former only. A linear model with the dependent variable body size (log centroid size) and the independent variables region, sex, and latitude tested whether the sexes are sexually size dimorphic, whether SSD differs with regard to the Burdekin Gap, and whether size dimorphism follows the latitudinal version of Rensch's rule. We ran a full model with body size as the dependent variable and the predictor terms sex, region and latitude and their interactions. We found the minimal adequate model by eliminating non-significant terms from the full model beginning with the three-way interaction between sex, region and latitude. Interaction terms including 'region' suggest that patterns of morphology differ either side of the Burdekin Gap and preclude interpretation of main effects; in these cases we examined the data separately for each region. A significant sex × latitude interaction with respect to body size suggests that SSD is related to latitude.

We describe how SSD scales with body size by using reduced major axis regression (RMA) to regress log male (centroid) size on log female (centroid) size. If the RMA slope is greater than unity and males are larger than females then SSD increases with increasing body size (i.e. male size diverges more rapidly than female size) but if females are larger then SSD decreases with increasing size. Statistical significance of the RMA slope was tested by examining the 95 % confidence limits.

We performed a number of statistical analyses to examine how region, latitude, sex and size affect body shape. First, to assess variation in body shape in relation to region, sex, latitude, and body (log centroid) size we used multivariate analysis of covariance (MANCOVA). Log_{10}-transformed centroid size was included in the model to ensure that both isometric and allometric effects of body size were accounted for in the analyses. We analyzed the data separately for each region if the interaction terms region × latitude × sex × size or region × latitude × sex are significant in the minimal adequate model. Minimal adequate models were found by eliminating non-significant terms from the full model beginning with interaction terms.

We also expressed the observed sexual shape dimorphism within each population as the Euclidean distance between male and female phenotypic means using the full set of 20 shape variables (Adams and Collyer 2009). Multivariate vectors for sex were statistically evaluated

using a residual randomization procedure, which entailed comparing the observed vector's magnitude with an empirically generated random distribution (from 9,999 iterations) obtained by permuting residuals from a reduced model that contained only main effects (Collyer and Adams 2007; Adams and Collyer 2007, 2009). For factorial designs, this procedure has superior statistical power to alternative randomization procedures (Anderson and ter Braak 2003). We first tested whether there was a regional difference in the degree of sexual shape dimorphism ($MD_{North, South}$) and then performed pairwise comparisons to determine which populations differed. Whether pairs of populations differed more than expected by chance was evaluated by using the residual permutation procedure described above. We accounted for the proportion of significant results that are in fact type I errors ('false discoveries') in our pairwise comparisons by implementing false discovery rate control (Verhoeven et al. 2005). Patterns of shape variation were graphically depicted in a principal component plot (only PC1 and PC2 depicted), with vectors of sexual shape dimorphism included. The first two principal components (PC1 = 37.2 % and PC2 = 16.3 %) accounted for 53.5 % of overall shape variation.

Finally, multivariate patterns of shape change with respect to body size were visualized using scores from a multivariate regression of sex-specific body shape versus log(centroid size) to facilitate population-level comparisons (Drake and Klingenberg 2008; Adams and Nistri 2010). Thin-plate spline (TPS) deformation grids were produced for phenotypic means of males and females and compared with a consensus configuration representing the average across all specimens to facilitate biological interpretation of the observed shape differences. All statistical analyses were performed in R 2.15.0 (R Developmental Core Team, 2011).

Results

Sexual Size Dimorphism and Rensch's Rule

The minimal adequate model showed significant region × sex (Estimate = 0.157 ± 0.044, t = 3.56, df = 251, $p = 0.0004$) and region × latitude (Estimate = 0.159 ± 0.064, t = 26.26, df = 251, $p < 0.0001$) interactions, which suggests that the effect of sex and latitude on body size differs either side of the Burdekin Gap, a pattern that is clearly seen in Fig. 3. We therefore analyzed body size within each region separately. A separate within-region minimal adequate model for the north retained the only the term 'latitude' and showed that body size decreased with increasing latitude (Estimate = −0.156 ± 0.007, t = 23.32, df = 148, $p < 0.0001$). On the contrary, the minimal adequate model for the southern
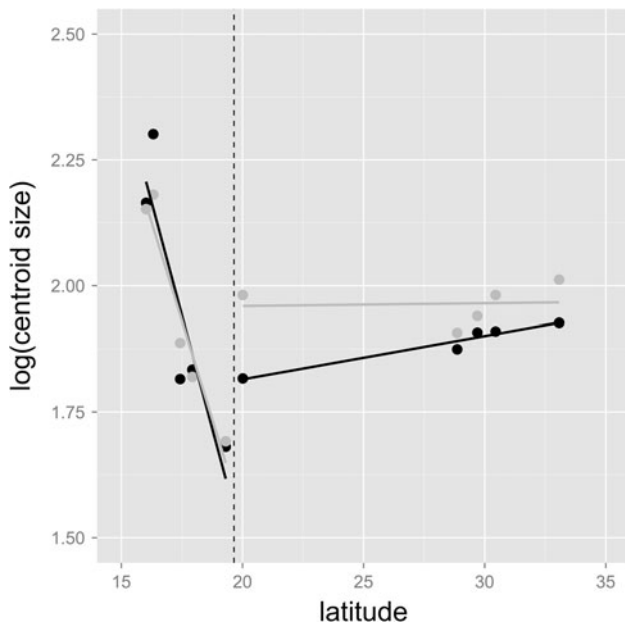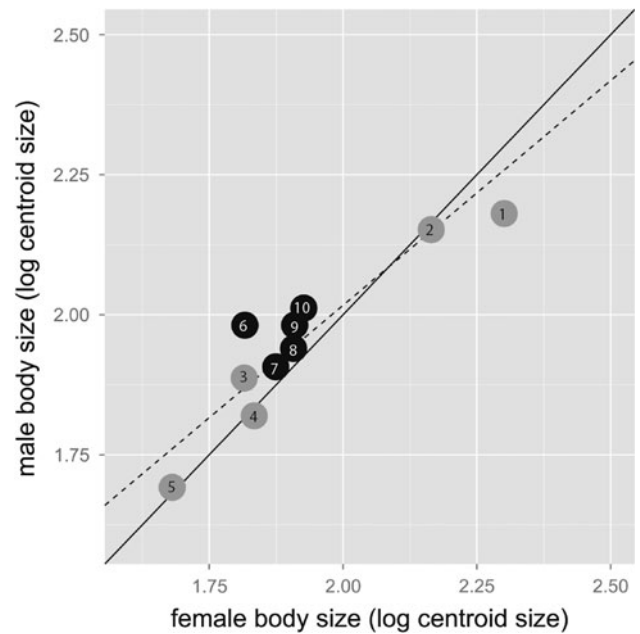
Fig. 3 *Plot* of latitude versus population-mean body (log centroid) size. *Dashed line* represent the location of the Burdekin Gap. *Grey circles* and *line* represent males; *black* represents females

Fig. 4 Allometric relationship for body size (centroid size) of male and female *P. signifer*. The *dashed line* represents the reduced major axis regression model (slope = 0.80, intercept = 0.41, r^2 = 0.84, 95 % C.I. = 0.58–1.11, p = 0.01). The *solid line* is the line of unity and represents no sexual size dimorphism. *Grey circles* indicate populations located north of the Burdekin Gap and *black circles* indicate southern populations. *Numbers within the symbols* represent population identity from Table 1

region showed that sexual size dimorphism became increasingly male-biased with decreasing latitude (i.e. moving north) below the Burdekin Gap (latitude × sex interaction: Estimate = -0.060 ± 0.018, t = 3.30, df = 104, p = 0.001) due to females getting smaller rather than males getting larger (Fig. 3).
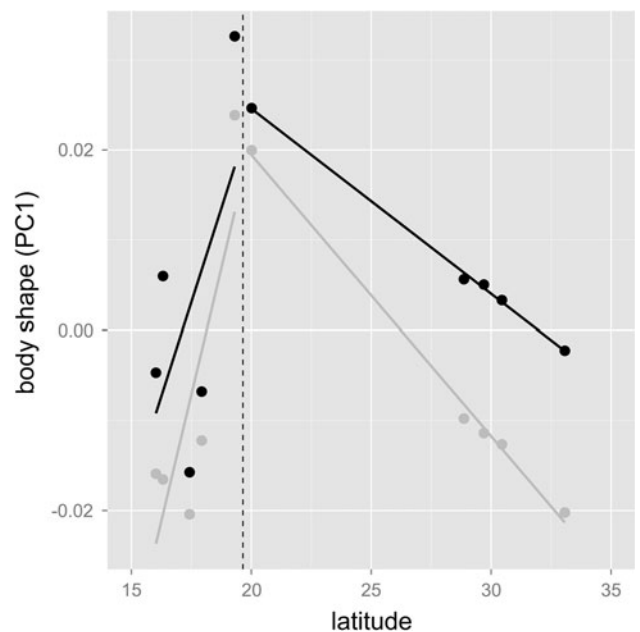
Within-region major axis regression slopes did not differ significantly from 1 (North: slope = 0.815 with 95 % confidence limits of 0.559 and 1.900; South: slope = 0.939 with 95 % confidence limits of 0.242 and 3.635) and so all 10 populations across the latitudinal range were pooled. Male and female body size was significantly related across the 10 populations (r = 0.837, n = 10, p = 0.002; Fig. 4) but the slope of reduced major axis regression was not significantly >1 (slope = 0.879 with 95 % confidence limits of 0.850 and 1.355). This suggests that SSD is not related to average body size across populations within or among regions.

Sexual Shape Dimorphism

Significant four-way and three-way interactions in a full MANCOVA using the full set of 20 shape variables suggest that the effect of sex, latitude, and body (log centroid) size on body shape differs either side of the Burdekin Gap (region × latitude × sex × size: Approximate $F_{20,223}$ = 1.72, p = 0.031, Pillai's trace = 0.13; region × latitude × sex: Approximate $F_{20,223}$ = 8.93, p < 0.0001, Pillai's trace = 0.44; Fig. 5). We therefore analyzed body shape differences within each region separately. A significant latitude × sex × size interaction in the North suggests that the degree of sexual shape dimorphism changes with latitude and body

Fig. 5 *Plot* of latitude versus body shape (PC1). *Dashed line* represent the location of the Burdekin Gap. *Grey circles* and *line* represent males; *black* represents females

(log centroid) size (Table 2) but this does not appear to be the case in the South (latitude × sex × size interaction from full model: Approximate $F_{20,81}$ = 1.29, p = 0.21, Pillai's

Table 2 Results from MANCOVA of different effects on body shape in populations North and South of the Burdekin Gap. Only parameters from minimal adequate model are shown. *Df* for F_{approx} are 20 and 123, and 20 and 81, for North and South analyses respectively

Model parameter	Pillai's trace	F_{approx}	p
North			
Latitude	0.8483	34.387	<0.00001
Sex	0.4588	5.214	<0.00001
Size	0.4925	5.967	<0.00001
Latitude × sex	0.3159	2.840	0.00022
Latitude × size	0.6439	11.122	<0.00001
Sex × size	0.2257	1.793	0.028
Latitude × sex × size	0.2310	1.847	0.022
South			
Latitude	0.8292	20.1498	<0.00001
Sex	0.6914	9.2976	<0.00001
Size	0.3604	2.3380	0.0038
Latitude × sex	0.3690	2.4264	0.0027
Latitude × size	0.2950	1.7367	0.0432

trace = 0.25). In the South, the degree of sexual shape dimorphism was related to latitude and size-related changes in body shape were dependent on latitude (Table 2). Our graphical depictions of body shape confirm these findings as sexual shape changed with body size in all populations except for Ross Ck. males, whose shape showed no relationship with body size (Fig. 6). The effect of increasing body size on shape was more similar for the sexes across the southern populations (i.e. both sexes generally show positive relationships) whereas the sexes tended to converge in their body shape with increasing size in the northern populations (i.e. sexes within populations show positive and negative relationships; Fig. 6) as suggested by significant latitude × sex × size interaction in North-specific MANCOVA but not in the South-specific analysis. We also found that a change in body shape with increasing body size was more pronounced in the North than in the South (Fig. 7).

Our phenotypic trajectory analysis using the full set of 20 shape variables showed that Northern populations have significantly smaller vectors (suggesting smaller degree of sexual shape dimorphism) than Southern populations (suggesting greater degree of sexual shape dimorphism) (mean difference in trajectory magnitude, $MD_{North, South} = 0.0076$, $p_{size} = 0.0245$; Fig. 5). Pairwise comparisons showed that the Northern populations Banyan Ck. and Russell R. were significantly less sexual shape dimorphic than most Southern populations after correcting for multiple tests (Table 3 and Fig. 8). Vectors for all but two populations occupy the same range of values along PC1 but group by region along PC2; the two populations nearest the Burdekin Gap in the North (Ross Ck.) and South (Magazine Ck.) have similar values along both PC axes (Fig. 8). Examination of thin-plate spline deformation grids (Fig. 1) also show
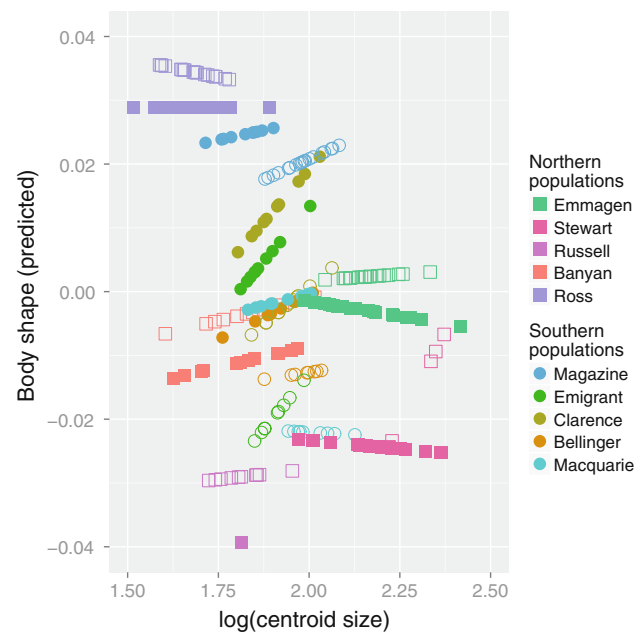
Fig. 6 Predicted values of body shape ($\hat{Y}$) from population- and sex-specific regressions versus log(centroid size) for regions north and south of the Burdekin Gap (males *filled symbols*; females *open symbols*)
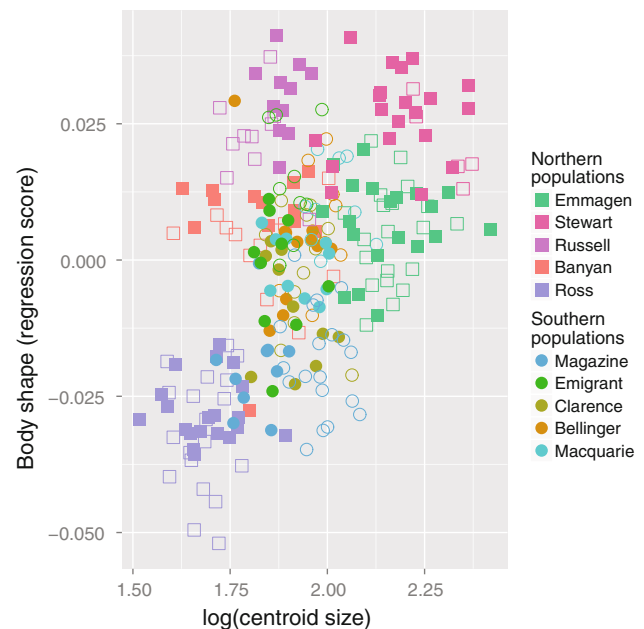
Fig. 7 Regression scores of body shape versus log(centroid size) for populations located within regions north and south of the Burdekin Gap (males *filled symbols*; females *open symbols*))

a striking similarity in population average body shape (males and females combined) for these two populations.

Examination of thin-plate spline deformation grids shows that the sexes are more similar to each other in body shape than they are to other populations (Fig. 9). Sex differences in shape were marginally more pronounced in

Table 3 Pairwise population comparisons of sexual shape dimorphism vectors. *P*-values were generated after 10, 000 random permutations and are given below the diagonal whereas vector magnitudes are above

Population	Emmagen	Stewart	Russell	Banyan	Ross	Magazine	Emigrant	Clarence	Bellinger	Macquarie
Emmagen		0.0097	0.0088	0.0084	0.0031	0.0009	0.0051	0.0060	0.0070	0.0098
Stewart	0.0584		0.0185	**0.0181**	0.0128	0.0088	0.0046	0.0037	0.0027	0.0001
Russell	0.0722	0.0153		0.0004	0.0057	0.0097	**0.0139**	**0.0148**	**0.0158**	**0.0186**
Banyan	0.0960	**0.0057**	0.8817		0.0053	0.0093	**0.0135**	**0.0144**	**0.0154**	**0.0182**
Ross	0.5759	0.0708	0.3096	0.2757		0.0040	0.0082	0.0091	0.0101	0.0129
Magazine	0.8715	0.2902	0.0584	0.0830	0.4800		0.0042	0.0051	0.0061	0.0089
Emigrant	0.3026	0.5809	**0.0029**	**0.0054**	0.1222	0.3565		0.0009	0.0019	0.0047
Clarence	0.2189	0.6724	**0.0005**	**0.0025**	0.0826	0.2560	0.5241		0.0010	0.0038
Bellinger	0.1824	0.8056	**0.0005**	**0.0027**	0.0681	0.1940	0.4943	0.5585		0.0028
Macquarie	0.0692	0.9910	**0.0006**	**0.0002**	0.0230	0.1149	0.2810	0.2670	0.2692	

Bold values are statistically significant after controlling for the false discovery rate
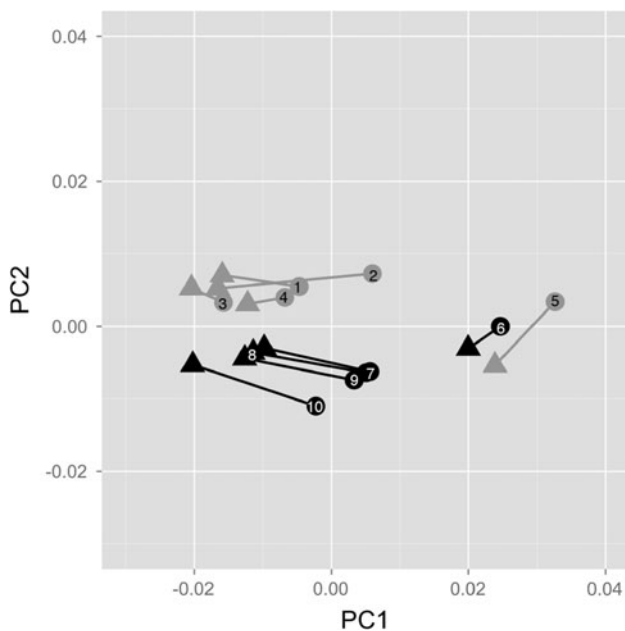
Fig. 8 Vectors of sexual shape dimorphism for each population located north (*grey*) and south (*black*) of the Burdekin Gap. Vectors of body shape are projected onto the first two principal components (PC) with vector length representing the relative degree of shape difference between the sexes (males *triangles*; females *circles*). Population identities are given in Table 1

populations south of the Burdekin Gap with males generally having have a deeper body, and dorsal and adipose fins that are inserted more anterior than in females (Fig. 9).

Discussion

Pacific blue-eyes display striking sex differences in morphology with latitude as well as relative to the Burdekin Gap: fish are more sexually shape and size dimorphic in the South than those in the North. Specifically, we found that fish from populations south of the Burdekin Gap (higher latitudes) become increasingly more size dimorphic (males larger) with decreasing latitude (i.e. moving north) whereas fish north of the Burdekin Gap (lower latitudes) increase in average size with decreasing latitude but are not sexually size dimorphic. Body shape sexually dimorphic in fish south, but not north, of the Burdekin Gap and changes with latitude in both regions.

We found an increase in body size with decreasing latitude in northern populations of Pacific blue-eyes whereas body size covariance with latitude in populations south of the Burdekin Gap depended on sex. Bergmann's rule states that body size increases with increasing latitude (or cooler climate) (Bergmann 1847; Angilletta and Dunham 2003). Our observed pattern is the converse to Bergmann's rule, a pattern frequently observed in ectotherms (e.g. Belk and Houston 2002; Blanckenhorn and Demont 2004; Blanckenhorn et al. 2006; Adams and Church 2008, 2011). Endler (1995) documented how environmental variation has dramatically affected phenotypic evolution, including body size, in the Trinidadian guppy (*Poecilia reticulata*). Since body size can covary with environmental factors in fishes (see Endler 1995), the observed body size cline in Northern populations of *P. signifer* presumably stems from environmental factors that covary with latitude only in populations north of the Burdekin Gap. For example, ecological conditions can favour faster growth and greater survival such as: warmer temperatures, longer growing periods due to decreased seasonality, stable water chemistry, slower stream flow, more or higher-quality food items, or reduced predation pressure on larger individuals. In addition, Pusey et al. (2004) report that *P. signifer* in the wild live for 1–2 years and have indeterminate growth (albeit slowing considerably
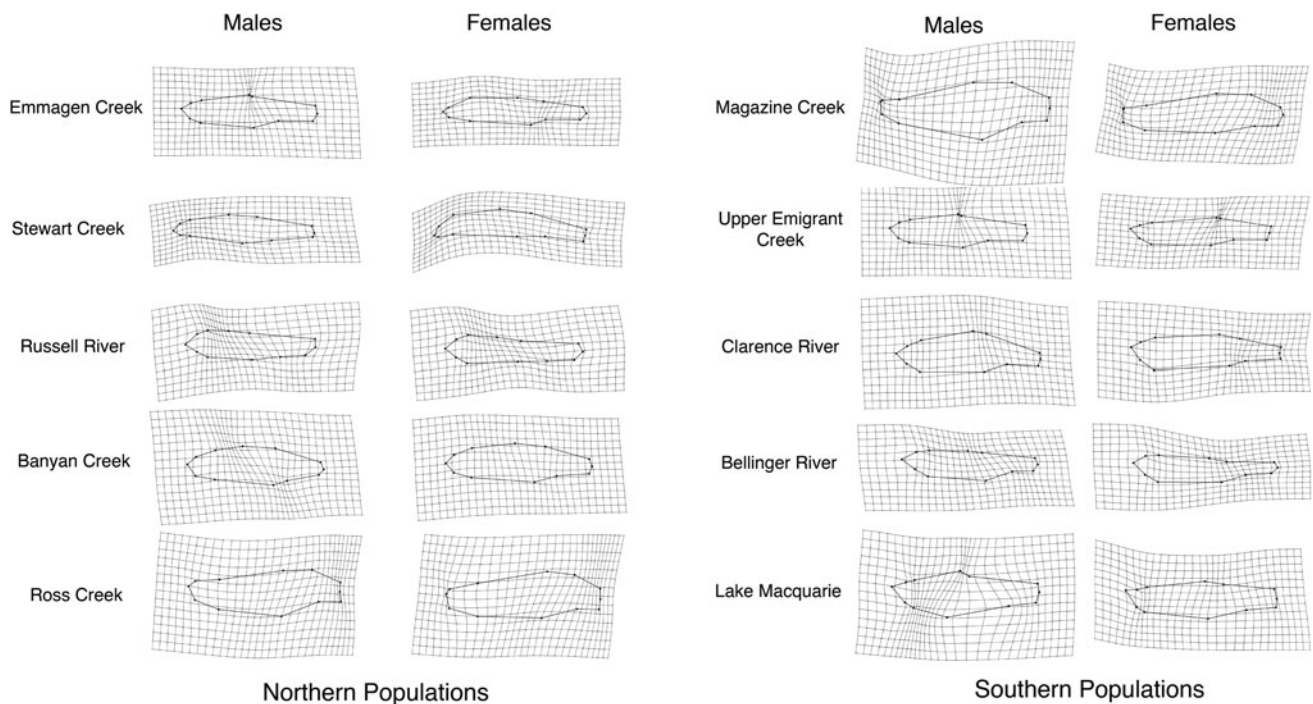
Fig. 9 Thin-plate spline deformation grids by sex for each of the 10 sampled populations within regions located north and south of the Burdekin Gap

after maturation). If there is latitudinal variation in lifespan above the Burdekin Gap with more-northern fish living longer than more-southern ones then Northern fish may simply have more time to grow to a larger body size. Revealing the causal mechanism underlying the clinal variation in body size of *P. signifer* populations north of the Burdekin Gap would be a fruitful avenue of research.

Our results do not support Rensch's rule as the RMA regression of male on female body size did not differ significantly from isometry. This pattern suggests that SSD is independent of mean body size, consistent with the general conclusion of a broad taxonomic meta-analysis by Blanckenhorn et al. (2006). That said, our finding contrasts with the general pattern across many insect taxa (Teder and Tammaru 2005) and several fish species (Lengkeek et al. 2008; Herczeg et al. 2010). These conflicting results hint that perhaps the intraspecific version of Rensch's rule cannot be generalized across taxa as readily as the interspecific version. We supported our prediction that SSD systematically covaries with latitude with populations becoming more male-biased in size moving north; however, this pattern was observed only among populations south of the Burdekin Gap (Northern populations were not sexually size dimorphic). Interestingly, this latitudinal pattern appears to be the result of females decreasing in size with decreasing latitude. Perhaps, females at more southern latitudes experience stronger fecundity selection, decreased predation pressure on larger individuals or live longer and thus achieve larger body size.

Alternatively, female body size might exhibit a significantly greater plastic response to a latitudinal covariable than male size. Whether this pattern is an artefact of Magazine Ck. or generally applies to southern populations will require further morphometric study of populations between Magazine Ck. and Upper Emigrant Ck.

Population level studies that offer support for Rensch's rule typically indicate that sexual selection for larger body size operates more strongly on males in some populations (Szekely et al. 2004; Fairbairn 2005). Our lack of support of Rensch's rule does not, however, mean that sexual selection on male size is absent from all *P. signifer* populations. In fact, sexual selection is arguably (i.e. if one assume no change in the relative strength of natural selection on each sex) stronger south of the Burdekin Gap given the significant male bias in body size in those populations. Sexual selection has also been reported in some Northern populations despite their apparent size monomorphism (e.g. Ross River): larger males accrue greater reproductive success because they are more likely to win intense battles for spawning sites (Wong 2004). Assuming sexual selection for larger size in males, a lack of size dimorphism among populations north of the Burdekin Gap could result from concomitantly strong fecundity selection on females.

Our multivariate analyses showed that Pacific blue-eyes exhibit different patterns of sexual shape dimorphism in relation to latitude either side of the Burdekin Gap. By

analyzing shape variation within each region separately, we found that shape is related to latitude in both regions with the phenotypic trajectory analysis suggesting greater sexual shape dimorphism in the South. Shape differences relative to the Burdekin Gap, however, might be driven primarily by Russell R. and Banyan Ck. differing significantly from most southern populations. Why would shape dimorphism have this apparent relationship with geography? One possible explanation is that the relationship between body shape and geography is driven less by latitude or sex than it is by body size. We found that body shape is significantly related to body size in both geographic regions with southern fish showing similar sex-specific relationships between size and sex compared with sex differences in the North (see Fig. 6). Thus, males and females in the North may have a similar shape within populations simply because they are monomorphic in size whereas southern fish are sexually size dimorphic within populations and therefore have different shapes. In other words, fish in northern populations vary more in body size among populations than between the sexes and so have greater shape variation among populations than between the sexes while the opposite is true for fish in the South. Given the dependence of shape on size, the wider range of body sizes observed among populations in the North may account for the greater change in shape with respect to size in that region compared with the South where relatively little shape-change occurs across the more-limited range of body sizes (i.e. males across southern populations are similar in shape and females across southern populations are similar in shape but males and females within a southern population differ).

That the degree of sexual shape dimorphism covaries with latitude in the South may simply be an indirect consequence of sexual size dimorphism covarying with latitude in that region. It is, however, possible that body shape is a plastic response in blue-eyes and is affected by an underlying environmental variable correlated with latitude. Several studies have reported on environmental factors eliciting sex differences in body shape. For example, in an experimental study on zebrafish (*Danio rerio*) temperature had a significant effect on body shape but affected the sexes differently (Georga and Koumoundouros 2010). Similarly, Fairbairn (2005) showed that male waterstriders have a greater response to rearing temperature than females. In addition, Burns et al. (2009) found that water flow is an important determinant of the degree of sexual shape dimorphism in Trinidadian guppies. Females underwent a greater change in body shape relative to males when reared under benign lab conditions than in fast-flowing natural streams.

We need greater insight into how body shape affects the functional performance (e.g. swimming ability) of blue-eyes and knowledge of how shape, sex, size and performance are ultimately affected by the environment. Moreover, because our current analyses of blue-eye morphology suggest only that body shape is correlated with body size, we need to experimentally identify whether shape and size are independently targeted by selection or whether body shape is a by-product of direct selection on body size.

Our shape analyses also reveal that blue-eyes fall into three general groups with the most northern and southern populations forming two groups while populations located on the immediate northern (Ross Ck.) and southern (Magazine Ck.) sides of the Burdekin Gap form a third group (Figs. 5, 6, 8). The similar shapes of fish from Ross and Magazine Cks. might be a signature of population connectivity. That Wong et al. (2004b) found an extensive genetic break between populations located on either side of the Burdekin Gap, however, counters this possibility. A more likely explanation is that fish from Ross and Magazine Cks. are more similar in body shape because they share a more similar environment than they do with populations from their respective regions. Further work on the environmental characteristics of blue-eye populations is clearly needed.

Biogeographic barriers are predicted to be a major driver of speciation (Wiley 1988). *P. signifer* is widespread along Australia's east coast, and tolerates both marine and freshwater conditions (Pusey et al. 2004), so we might predict fish migrate readily between populations. However, a molecular analysis (Wong et al. 2004b) and our current study suggest that Northern and Southern populations are rather distinct. Populations of Pacific blue-eyes north of the Burdekin Gap exhibited greater variation in body size and shape than Southern populations. The morphological pattern in the North might reflect genetic adaptation to local selection within each river (sensu Fitzpatrick 2012), which is then reinforced by reduced connectivity and genetic exchange (Wong et al. 2004b). On the other hand, the morphological similarity of fish in Southern populations might be a product of high connectivity and gene flow among populations facing similar environments and selection pressures (see Wong et al. 2004b); or parallel evolution among populations if there is no gene flow between Southern rivers. Because gene flow between populations can lead to the appearance of a correlation between phenotype and environment (Felsenstein 2002), Felsenstein and colleagues (Felsenstein 2002; Stone et al. 2011) advocate calculating migration matrices to correct for correlation due to gene flow rather than similar selective pressures. To test this hypothesis, we need to measure migration rate between populations, and determine if gene flow and/or local environmental conditions affect body shape and size.

In summary, our population level study of Pacific blue-eyes supported the converse of Bergmann's rule above the Buredkin Gap because we found larger individuals at lower

latitudes. Our findings did not support Rensch's rule as there was no relationship between mean body size and the extent of sexual size dimorphism. However, we did find that the sexual size dimorphism south, but not north, of the Burdekin Gap varied systematically with latitude (males larger with decreasing latitude) and that these fish were also sexually shape dimorphic. Together our findings suggest that populations south of a major terrestrial biogeographic barrier display significantly greater consistency in body size, as well as a greater degree of sexual size and shape dimorphism than Northern populations. Our morphological analysis—together with evidence of genetic divergence between Northern and Southern populations and female preference for local over foreign males—lends further support to the notion that *P. signifer* might comprise two species, or incipient species, that are separated by the Burdekin Gap.

References

Abell, A., Cole, B., Reyes, R., & Wiernasz, D. (1999). Sexual selection on body size and shape in the western harvester ant, *Pogonomyrmex occidentalis cresson*. *Evolution, 53*, 535–545.

Adams, D. C., & Church, J. O. (2008). Amphibians do not follow Bergmann's rule. *Evolution, 62*, 413–420.

Adams, D. C., & Church, J. O. (2011). The evolution of large-scale body size clines in *Plethodon* salamanders: Evidence of heat-balance or species-specific artifact? *Ecography, 34*, 1067–1075.

Adams, D. C., & Collyer, M. L. (2007). Analysis of character divergence along environmental gradients and other covariates. *Evolution, 61*, 510–515.

Adams, D. C., & Collyer, M. L. (2009). A general framework for the analysis of phenotypic trajectories in evolutionary studies. *Evolution, 63*, 1143–1154.

Adams, D. C., & Otarola-Castillo, E. (2012). Geomorph: Software for geometric morphometric analyses. R package version 1.1-0. http://cran.r-project.org/web/packages/geomorph/index.html.

Adams, D. C., & Otarola-Castillo, E. (2013). Geomorph: An R package for the collection and analysis of geometric morphometric shape data. *Methods in Ecology and Evolution* (in press).

Adams, D. C., & Nistri, A. (2010). Ontogenetic convergence and evolution of foot morphology in European cave salamanders (Family: Plethodontidae). *BMC Evol Biol*, 10.

Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: Ten years of progress following the "revolution". *Italian Journal of Zoology, 71*, 5–16.

Allen, G., Midgley, S., & Allen, M. (2003). *Field guide to the freshwater fishes of Australia*. Collingwood, Vic: CSIRO Publishing.

Anderson, M., & ter Braak, C. (2003). Permutation tests for multifactorial analysis of variance. *Journal of Statistical Computation and Simulation, 73*, 85–113.

Angilletta, M., & Dunham, A. (2003). The temperature-size rule in ectotherms: Simple evolutionary explanations may not be general. *The American Naturalist, 162*, 332–342.

Belk, M. C., & Houston, D. D. (2002). Bergmann's rule in ectotherms: A test using freshwater fishes. *The American Naturalist, 160*, 803–808.

Bergmann, C. (1847). Über die verhältnisse der wärmeökonomie der thiere zu ihrer grösse. *Göttinger Studien, 3*, 595–708.

Berns, C. M., & Adams, D. C. (2010). Bill shape and sexual shape dimorphism between two species of temperate hummingbirds: Black-chinned hummingbird (*Archilochus alexandri*) and ruby-throated hummingbird (*A. colubris*). *The Auk, 127*, 626–635.

Blanckenhorn, W. (2005). Behavioral causes and consequences of sexual size dimorphism. *Ethology, 111*, 977–1016.

Blanckenhorn, W. U., & Demont, M. (2004). Bergmann and converse Bergmann latitudinal clines in arthropods: Two ends of a continuum? *Integrative and Comparative Biology, 44*, 413–424.

Blanckenhorn, W. U., Stillwell, R. C., Young, K. A., Fox, C. W., & Ashton, K. G. (2006). When Rensch meets Bergmann: Does sexual size dimorphism change systematically with latitude? *Evolution, 60*, 2004–2011.

Bookstein, F. L. (1986). Size and shape spaces for landmark data in two dimensions. *Statistical Science, 1*, 181–222.

Bookstein, F. L. (1997). Landmark methods for forms without landmarks: Morphometrics of group differences in outline shape. *Medical Image Analysis, 1*, 225–243.

Bookstein, F., Schäfer, K., Prossinger, H., Seidler, H., Fieder, M., Stringer, C., et al. (1999). Comparing frontal cranial profiles in archaic and modern homo by morphometric analysis. *Anatomical Record Part B, New Anatomist, 257*, 217–224.

Brown, M., Cooksley, H., Carthew, S. M., & Cooper, S. J. B. (2006). Conservation units and phylogeographic structure of an arboreal marsupial, the yellow-bellied glider (*Petaurus australis*). *Australian Journal of Zoology, 54*, 305–317.

Burns, J. G., Di Nardo, P., & Rodd, F. H. (2009). The role of predation in variation in body shape in guppies Poecilia reticulata: A comparison of field and common garden phenotypes. *Journal of Fish Biology, 75*, 1144–1157.

Butler, M. A., & Losos, J. (2002). Multivariate sexual dimorphism, sexual selection, and adaptation in Greater Antillean *Anolis* lizards. *Ecological Monographs, 72*, 541–559.

Butler, M. A., Sawyer, S. A., & Losos, J. B. (2007). Sexual dimorphism and adaptive radiation in *Anolis* lizards. *Nature, 447*, 202–205.

Chapple, D. G., Hoskin, C. J., Chapple, S. N., & Thompson, M. B. (2011). Phylogeographic divergence in the widespread delicate skink (Lampropholis delicata) corresponds to dry habitat barriers in eastern Australia. *BMC Evolutionary Biology, 11*, 191.

Claude, J. (2008). Morphometrics with R. Springer Verlag.

Collyer, M. L., & Adams, D. C. (2007). Analysis of two-state multivariate phenotypic change in ecological studies. *Ecology, 88*, 683–692.

Drake, A. G., & Klingenberg, C. P. (2008). The pace of morphological change: Historical transformation of skull shape in St Bernard dogs. *Proceedings of the Royal Society B: Biological Sciences, 275*, 71–76.

Endler, J. (1995). Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution, 10*, 22–29.

Fairbairn, D. J. (1997). Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics, 28*, 659–687.

Fairbairn, D. J. (2005). Allometry for sexual size dimorphism: Testing two hypotheses for Rensch's rule in the water strider Aquarius remigis. *The American Naturalist, 166*, S69–S84.

Fairbairn, D. J., & Preziosi, R. (1994). Sexual selection and the evolution of allometry for sexual size dimorphism in the water

strider, *Aquarius remigis*. *The American Naturalist, 144*, 101–118.

Felsenstein, J. (2002). Quantitative characters, phylogenies and morphometrics. In N. MacLeod & P. Forey (Eds.), *Morphology, Shape and Phylogeny* (pp. 27–44). Bova Raton, FL: CRC Press.

Fitzpatrick, B. M. (2012). Underappreciated consequences of phenotypic plasticity for ecological speciation. *International Journal of Ecology, 2012*, 1–12.

Georga, I., & Koumoundouros, G. (2010). Thermally induced plasticity of body shape in adult zebrafish Danio rerio (Hamilton, 1822). *Journal of Morphology, 271*, 1319–1327.

Hadfield, A., Ivantsoff, V., & Johnson, P. (1979). Clinal variation in electrophoretic and morphological characters between two nominal species of the genus *Pseudomugil* (Pisces: Atheriniformes: Pseudomugilidae). *Marine & Freshwater Research, 30*, 375–386.

Hendry, A., Kelly, M. L., Kinnison, M. T., & Reznick, D. N. (2006). Parallel evolution of the sexes? Effects of predation and habitat features on the size and shape of wild guppies. *Journal of Evolutionary Biology, 19*, 741–754.

Herczeg, G., Gonda, A., & Merilä, J. (2010). Rensch's rule inverted—female-driven gigantism in nine-spined stickleback Pungitius pungitius. *Journal of Animal Ecology, 79*, 581–588.

Joseph, L., & Moritz, C. (1994). Mitochondrial DNA phylogeography of birds in eastern Australian rainforests: First fragments. *Australian Journal of Zoology, 42*, 385–403.

Kaliontzopoulou, A., Adams, D. C., Meijden, A., Perera, A., & Carretero, M. A. (2012). Relationships between head morphology, bite performance and ecology in two species of Podarcis wall lizards. *Evolutionary Ecology, 26*, 825–845.

Kelly, C. D., & Adams, D. C. (2010). Sexual selection, ontogenetic acceleration, and hypermorphosis generates male trimorphism in Wellington tree weta. *Evolutionary Biology, 37*, 200–209.

Kelly, C. D., Bussiere, L. F., & Gwynne, D. (2008). Sexual selection for male mobility in a giant insect with female-biased size dimorphism. *The American Naturalist, 172*, 417–423.

Langerhans, R., & DeWitt, T. (2004). Shared and unique features of evolutionary diversification. *The American Naturalist, 164*, 335–349.

Langerhans, R., Layman, C., Shokrollahi, A., & DeWitt, T. (2004). Predator-driven phenotypic diversification in Gambusia affinis. *Evolution, 58*, 2305–2318.

Lengkeek, W., Didderen, K., Cote, I. M., van der Zee, E. M., Snoek, R. C., & Reynolds, J. D. (2008). Plasticity in sexual size dimorphism and Rensch's rule in Mediterranean blennies (Blenniidae). *Canadian Journal of Zoology, 86*, 1173–1178.

McGlashan, D., & Hughes, J. (2002). Extensive genetic divergence among populations of the Australian freshwater fish, Pseudomugil signifer (Pseudomugilidae), at different hierarchical scales. *Marine Freshwater Research*.

Outomuro, D., & Johansson, F. (2011). The effects of latitude, body size, and sexual selection on wing shape in a damselfly. *Biological Journal of the Linnean Society, 102*, 263–274.

Pusey, B., Kennard, M., & Arthington, A. (2004). *Freshwater fishes of North-Eastern Australia*. Collingwood, Vic: Csiro Publishing.

Rensch, B. (1960). *Evolution above the species level*. New York: Columbia University Press.

Rohlf, F. J. (2010). tpsRelw: Relative warps analysis.

Rohlf, F. J., & Marcus, L. (1993). A revolution in morphometrics. *Trends in Ecology & Evolution, 8*, 129–132.

Rohlf, F. J., & Slice, D. E. (1990). Extensions of the procrustes method for the optimal superimposition of landmarks. *Systematic Zoology, 39*, 40–59.

Schäuble, C. (2004). Variation in body size and sexual dimorphism across geographical and environmental space in the frogs *Limnodynastes tasmaniensi*s and *L. peronii*. *Biological Journal of the Linnean Society, 82*, 39–56.

Serb, J. M., Alejandrino, A., Otarola-Castillo, E., & Adams, D. C. (2011). Morphological convergence of shell shape in distantly related scallop species (Mollusca: Pectinidae). *Zoological Journal of the Linnean Society, 163*, 571–584.

Stone, G. N., Nee, S., & Felsenstein, J. (2011). Controlling for non-independence in comparative analysis of patterns across populations within species. *Philosophical Transactions of the Royal Society B: Biological SciencesB, 366*, 1410–1424.

Szekely, T., Freckleton, R., & Reynolds, J. (2004). Sexual selection explains Rensch's rule of size dimorphism in shorebirds. *Proceedings of the National Academy of Sciences of the United States of America, 101*, 12224–12227.

Teder, T., & Tammaru, T. (2005). Sexual size dimorphism within species increases with body size in insects. *Oikos, 108*, 321–334.

Unmack, P. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography, 28*, 1053–1089.

Verhoeven, K. J. F., Simonsen, K. L., & McIntyre, L. M. (2005). Implementing false discovery rate control: Increasing your power. *Oikos, 108*, 643–647.

Wiley, E. (1988). Parsimony analysis and vicariance biogeography. *Systematic Zoology, 37*, 271–290.

Wong, B. (2004). Superior fighters make mediocre fathers in the Pacific blue-eye fish. *Animal Behaviour, 67*, 583–590.

Wong, B., Keogh, J., & Jennions, M. D. (2004a). Mate recognition in a freshwater fish: Geographical distance, genetic differentiation, and variation in female preference for local over foreign males. *Journal of Evolutionary Biology, 17*, 701–708.

Wong, B., Keogh, J., & McGlashan, D. (2004b). Current and historical patterns of drainage connectivity in eastern Australia inferred from population genetic structuring in a widespread freshwater fish *Pseudomugil signifer* (Pseudomugilidae). *Molecular Ecology, 13*, 391–401.

Young, K. A. (2005). Life-history variation and allometry for sexual size dimorphism in Pacific salmon and trout. *Proceedings of the Royal Society of London. Series B: Biological Sciences, 272*, 167–172.

Zelditch, M., Swiderski, D., Sheets, H., & FINK, W. (2004). *Geometric morphometrics for biologists*. London: Academic Press.