Disentangling wing shape evolution in the African mayfly, Teloganodidae (Ephemeroptera)

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Wings are one of the most important structures in the evolution of insects and winged insects are widely accepted as being monophyletic. In Ephemeroptera, wing structure and shape is important for interpreting taxonomic relationships. Morphological variation in wing shape of 14 distinct operational taxonomic units (OTUs) of South African Teloganodidae mayfly was examined using landmark-based geometric morphometric methods and molecular phylogenetics to determine evolutionary shape change and allometry. Fore and hind wing shape data were tested for phylogenetic structure using an independently derived molecular phylogeny, which were then mapped into PCA shape space. The effect of evolutionary allometry as a factor contributing to shape change was examined and quantified. Significant phylogenetic signal was found in fore and hind wing shape, and evolutionary allometry was found to have a significant effect on fore wing but not hind wing shape variation. In the fore wing, evolutionary allometry was removed to explore the non-allometric component of shape variation and discuss possible implications on flight performance. The principal findings of this research show that the relationships between wing shape and size are complex and taxon-specific. We have revealed that evolutionary size changes have a considerable effect on the evolutionary shape changes of Teloganodidae fore wings, however this does not account for all the variation in shape. An increased fore wing size is associated with a broader wing base and narrower, raked wing apex, promoting less-energetically demanding flight, possibly with a higher range of speeds. The smaller species have a relatively narrow wing base and increased wing area at the apex, suggesting a slower flight with more agility, which is more energetically costly. The non-allometric variation involves similar, but less distinct shape changes compared to the allometric component, indicating that other processes must also contribute to the same features of evolutionary shape variation.

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1. Introduction

Winged insects, or Pterygota, are widely accepted as monophyletic. This single origin for wings is supported by the basic structure of wing venation which can be homologized across the insect orders (Wigglesworth 1973; Averof & Cohen 1997; Jockusch & Nagy 1997; Wootton & Kukalová-Peck 2000; Grimaldi & Engel 2005; Medved et al. 2015). Wings can therefore be considered as one of the most important structures in the evolution of insects.
as independent components of wing morphology (Debat et al. 2003; Huey et al. 2000). Flight behaviour may also play an important role in wing shape (Berwaerts et al. 2006; Johansson et al. 2009; DeVries et al. 2010) and so shape should be optimized by selection for flight functionality, especially relating to dispersal, reproduction and predator avoidance (Edmunds & Traver 1954; Johansson et al. 2009; Outomuro & Johansson 2011). It can be expected that a similar wing shape should be seen within certain flight behaviours and at the same time, variation of this shape related to body size (Outomuro et al. 2013).

Ephemeroptera spend the majority of their lives underwater as aquatic nymphs before emerging into short-lived, flying, reproductive adults. They are the only extant insects to have two winged stages, with an immature subimagos (sub-adult) that characteristically has hydrophobic microtrichia present on the wings, which are shed when the subimagos moult into the imago stage (Edmunds & McCafferty 1988). Morphologically linking the imago with the respective nymphal stage is notoriously difficult in mayflies, with a classic example being the family Prospopistomatidae. First discovered in France, Geoffroy (1762) thought that they were Crustacea, and it was not until Joly (1871) reared a nymph through to the example, the nymph of the species described by Crass (1947) as Engel 2005).

Almost every structure in the mayfly group of Gondwanan origin (Edmunds 1975; Craw 1972, 1988; McCafferty & Wang 1997; McCafferty 1999; Sartori et al. 2008; Selvakumar et al. 2014) that vary in size between its genera. In South Africa the largest genus, Ephemereyllina, has a wing length of 8–10 mm with a body length of approximately 7–8 mm, while the smallest genus, Lestogella, has a wing length of 4–5 mm and is approximately 4–5 mm in body length. They are endemic and restricted to pristine, relicual-forested mountain streams in the southern and Western Cape of South Africa (McCafferty & Wang 1997).

Using independent phylogenetic data (from molecular methods) to determine historical shape change in morphometric data is a developing field (e.g. (Klingenberg et al. 2013)). It allows testing of the phylogenetic signal of morphometric data, by incorporating phylogenetic structure based on morphometric datasets (Klingenberg & Gidaszewski 2010) using Independent Contrasts (IC) (Felsenstein 1985; Rohlf 2001). It is well established that in studies using geometric morphometric data with phylogeny, the effects of allometry on morphological diversification is an important factor that must be considered. Allometry in this context can be defined as the association between shape and centroid size, or the covariation of parts due to the variation in size, and involves a distinction of size and shape (Mosimann 1970; Gould 1966; Klingenup 1996, 1998, 2008). In terms of diversification among taxa, evolutionary allometry is the evolutionary change of shape associated with the evolutionary change of size (Cock 1966; Klingenberg 1996). This size variation seen among different species indicates evolutionary processes that have formed the observed shape (Klingenup & Zimmermann 1992; Drake & Klingenberg 2008). A comparative approach that takes phylogenetic relationships into account is required to investigate evolutionary allometry (Klingenup & Marugan-Lobon 2013) and concerns the covariation of shape change and size among the phylogenetic branches that are derived from a common ancestor (Klingenup 1996).

In this study, morphological variation in the wing shape of 14 operational taxonomic units (OTUs) (including potential new species) of Teloganodidae mayfly was examined in an evolutionary context. As mayfly wings are important for inferring taxonomic relationships, we tested for phylogenetic structure in the wing shape data using an independently derived molecular phylogeny. The effects of evolutionary allometry as a factor contributing to shape change was examined and quantified, then removed to explore the non-allometric component of wing shape variation and possible implications on flight performance, which is discussed.

2. Materials and methods

2.1. Specimen preparation

A total of 71 Teloganodidae individuals were collected in the field as ultimate nymphal instars and allowed to eclose to subimagos or imago stage for positive adult—nymph association. The fore and hind wings were removed and photographed using a Leica M125 dissecting Microscope with a Leica DFC 295 mounted camera. Four
genera and 14 distinct Operational Taxonomic Units (OTUs) were represented in the dataset as *Ephemerellina barnardi* Lestage, 1924, *Lestagella penicillata* (Barnard, 1940), six undescribed *Lestagella* species, *Lithogloea harrisoni* Barnard 1932, *Nadinetella brincki* (Demoulin, 1970), *Nadinetella crassi* (Allen & Edmunds, 1963) and three undescribed species without clear generic classification (Appendix A). Sample size per species was limited by availability of material due to the restricted, mountainous habitat of this mayfly family which meant that obtaining and rearing ultimate instar specimens from some of the genera was difficult (two individuals per genus in some cases). Where possible, a generally equal sex ratio was retained, although sexual size and shape dimorphism occurs in most Ephemeroptera adults (e.g. some Tricyrhyidae genera have much larger females than males [Barber-James 2008] and most Baetidae exhibit sexual dimorphism [Peckarsky et al. 2001, 2002]).

A preliminary examination of the Teloganodidae wing data showed that among-species variation exceeds intra-specific differences between sexes. The right-side wings of male and female specimens were temporarily slide-mounted in an ethanol-based gel and photographed. Wings were then placed into individual vials with the rest of the specimen and are housed at the Albany Museum in Grahamstown (AMGS), South Africa.

### 2.2. Molecular phylogeny for comparative analyses

The comparative analyses were based on a molecular phylogeny of the Teloganodidae obtained from a maximum likelihood analysis using three genes (12S, 16S and COI) and a representative sequence of the Teloganodidae obtained from a maximum likelihood analysis (Stamatakis 2014) which was used via the CIPRES Science Gateway Models for partitions were selected by the RAxML software (Klingenberg 2011). A full Procrustes fit was used to extract the shape information from the landmark coordinates, known as the Procrustes coordinates (Dryden & Mardia 1998). The fore and hind wing datasets were averaged by species and a covariance matrix was generated. The raw data was checked for outliers using a plot of the cumulative distribution of the (Procrustes) distances from the average.

### 2.4. Comparative phylogeny

A Principal Component Analysis (PCA) using the covariance matrix of the Procrustes coordinates was used to examine wing shape variation between species. Principal component (PC) scores were mapped onto the phylogeny to reconstruct and visualize evolutionary shape changes, which is done by projecting the phylogeny into the shape space where it was drawn onto PC score scatterplots of multivariate ordinations of the species means. The bootstrap iterations was used for further comparative analyses with the geometric data (Fig. 2). Branch lengths were used to weight the analyses.

### 2.3. Morphometric data features

A set of 16 landmarks were placed on distinctive points along the main veins on the fore wings and 13 landmarks placed on the hind wings (Fig. 1) using TPSUtil v1.58 (Rohlf 2015) and TPSDig v2.17 (Rohlf 2015). Landmark points along main veins were chosen so that the configurations were repeatable, corresponding for each wing and were chosen to cover the wing shape as completely as possible. A warped outline drawing estimating mean shape of a Teloganodidae wing was made in TPSDig, for visualisation of shape changes.

All geometric morphometric analyses were conducted using MorphoJ v1.06d (Klingenberg 2011). A full Procrustes fit was used to extract the shape information from the landmark coordinates, known as the Procrustes coordinates (Dryden & Mardia 1998). The fore and hind wing datasets were averaged by species and a covariance matrix was generated. The raw data was checked for outliers using a plot of the cumulative distribution of the (Procrustes) distances from the average.
shapes corresponding to the internal nodes of the phylogeny were reconstructed by squared-change parsimony (Maddison 1991; Rohlf 2001; Klingenberg & Gidaszewski 2010), this analysis minimizes the length of the tree in Procrustes distance units and maps the morphometric data onto the phylogenetic tree (Klingenberg & Ekau 1996; Klingenberg & Gidaszewski 2010). Phylogenetic signal is the association between phylogenetic relationship and phenotypic similarity; a strong phylogenetic signal exists if closely related taxa tend to be more similar to each other than remotely related taxa (Cole et al. 2002; Cardini & Elton 2008; Klingenberg & Gidaszewski 2010). If there is signal, then the comparative analyses of the data need to consider the phylogenetic structure. A permutation approach using 10,000 random permutations was used (Klingenberg & Gidaszewski 2010) to test for phylogenetic signal in the datasets, with a null hypothesis of no phylogenetic signal.

Phylogenetic independent contrasts (IC) of shape variation were generated as weighted differences for sister nodes, therefore adjusted for phylogeny (Felsenstein 1985; Rohlf 2001) and the IC residuals account for species relationships. Independent contrasts were used as units of these analyses to determine the overall evolutionary processes (Klingenberg & Marugán-Lobón 2013). Using the shape information from the terminal taxa, this reconstruction gives an idea of how specific clades diversified and dispersed through morphometric variable space (e.g. (Klingenberg & Ekau 1996; Monteiro & Nogueira 2010; Klingenberg & Gidaszewski 2010; Meloro & Jones 2012; Klingenberg et al. 2012; Figueirido et al. 2013, 2010)).

As Teloganodidae differ in size between genera, the degree to which wing shape variation among species is related to the evolutionary change in wing size (evolutionary allometry; Gould 1966; Klingenberg 1996, 2016; Pélabon et al. 2014) was examined. The effect of evolutionary allometry and its significance across the datasets was investigated using a multivariate regression while accounting for the phylogenetic relationships among species, which was done using phylogenetic independent contrasts of shape against the independent contrasts for centroid size (Monteiro 1999; Klingenberg & Marugán-Lobón 2013). For regression analyses, statistical significance was assessed using 1000 permutations.

Size correction based on the regression of the IC was used to eliminate the effect of evolutionary allometry on the data. Firstly, a regression of shape on size is computed using independent contrasts (as described above) and a vector of regression coefficients obtained. This was used to compute the residual component of variation in the taxa, i.e. size-corrected shape scores (species values and not contrasts) free of evolutionary allometry, which were used in a PCA to visualise shape change. Any large differences between the size-corrected and uncorrected analyses would indicate that evolutionary allometry plays a role in wing shape evolution in Teloganodidae. A PCA of the complete variation in the IC and a PCA considering the allometric effects was then computed. The eigenvectors and eigenvalues can be interpreted in the same way as for PCA in other contexts.

Angular comparisons between vectors were used to quantify the shape changes visualised from the PCA and regression analyses. For example, if allometry accounts for most of the variation, then the

| Table 1 | Tree lengths and phylogenetic signal permutation test results for the species means for the right-side fore and hind wings. |
|---|---|---|---|
| | Fore wing | Hind wing |
| | Species Means | Species Means | |
| Tree length | .003783 | .02440 |
| Phylogenetic signal (P-value) | <.00001 | 0.0116 |

Bold indicates a significant result, and rejection of the null hypothesis of no phylogenetic signal.
allometric regression vector from a shape on size regression may be expected to coincide with the PC1. The angles between vectors quantitatively assess the similarity of two vectors in shape space (e.g., (Klingenberg & Zimmermann 1992; Klingenberg et al. 1998, 2001; 2003; Klingenberg & Zaklan 2000)). The smaller the angle, the more similar the vectors; the P-values presented are tested against the null hypothesis that the vectors have random directions in shape tangent space (see Li 2011 for details on methods). Klingenberg & Marugán-Lobón (2013) provide details of assessing these angles, with brief equations and comparison of this method with work done by other authors.

3. Results

3.1. Comparative phylogenies

The maximum likelihood phylogeny based on three mitochondrial genes (COI barcoding region, 16S and 12S) was constructed and used for comparative analyses (Fig. 2). The genera that hold more than one species were shown to be monophyletic. All nodes showed good support (bootstrap >70), except for E. barnardi which seems to have an uncertain relationship with the Species C/Species B clade and the other genera.

The permutation tests for phylogenetic signal in the fore and hind wing shape data were significant (Table 1), indicating that the data has clear phylogenetic structure, reflected in the shape space (Figs. 3 and 4). Projection of the phylogenetic tree into the PC plots for the fore (Fig. 3) and hind (Fig. 4) wings at species level shows a small amount of branch crossing (a visual indication of homoplasy), especially within Lestagella. The genera are clearly separated in the wing shape space and closely related species groups are clustered together, a “starburst” pattern (clusters at the end of a single branch) can be seen for Lestagella and the Nadinetella/Lithogloea group for both the fore and hind wings. A general trend includes a consistent direction of shape variation within the first PC of both the fore and hind wings, from one extreme side of the plot (Ephemereillina) to the other (Lestagella).

3.1. Fore wing shape projection onto phylogeny

The PCA of the variation of fore wing shape between taxa, uncorrected for size, showed that 74.9% of the total variation can be explained by the first three PCs (PC1 = 39.9%; PC2 = 21.5%; PC3 = 13.6%). These three PCs clearly separate the genera and most species in the shape space (Fig. 3). The shape changes associated with the first three PCs show some of the main features of wing variation. PC1 separates a relatively broader wing base, narrow and downward-curving (rake-tipped or raked) wing apex and distal shift in medial and radial veins and branch intersections (Ephemereillina) from a narrower wing base, broader wing apex and proximal shift in medial and radial veins and branch intersections (Lestagella). PC2 separates a broader wing, particular in the cubitus region and anterior margin, that slightly shorter in length (Lithogloea and Nadinetella) from a slightly longer, narrower shape (Lestagella). PC3 separates a relatively narrower wing with a distal shift in medial veins and intersections and proximal shift in the radial branch intersection and shift in the cubital cross vein down.

Fig. 3. Mapped distribution of the total fore wing shape variation onto the phylogeny of Teloganodidae species. PC scores for the mean shape of each species were mapped onto the phylogenetic tree from Fig. 2, using squared-change parsimony. Scatter points are labelled with the OTU names and colour-coded by genus. The wing overlays at each PC axis indicate the wing shapes for the extreme scores for the respective PC, the colour of the wing outline corresponds to the genus. Negative scores are always indicated by a “full” or “closed” landmark circle and positive scores are always indicated by an “empty” or “open” landmark circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
towards the posterior margins (*Lestagella* sp.7) from the rest of the *Lestagella* species that show a broader wing.

### 3.1.2. Hind wing shape projection onto phylogeny

The PCA of the variation of hind wing shape between taxa, uncorrected for size, showed 82.7% of the total variation can be explained by the first three PCs (PC1 = 42.12%; PC2 = 29.73%; PC3 = 10.89%). Branches in the phylogenetic map show more crossing within genera, indicating more homoplasy within the shape variation in the hind wing (Fig. 4). PC1 separates a much broader, shorter wing (*Ephemerellina*) from an oval shaped, narrow and slender wing shape (*Lestagella*). PC2 separates a relatively broader wing with a proximal shift of the costal projection (Landmark 3), larger cubital region that tapers towards the wing apex.

Fig. 4. Mapped distribution of the total hind wing shape variation onto the phylogeny of *Teloganodidae* species. PC scores for the mean shape of each species were mapped onto the phylogenetic tree from Fig. 2, using squared-change parsimony. Scatter points are labelled with the OTU names and colour-coded by genus. The wing overlays at each PC axis indicate the wing shapes for the extreme scores for the respective PC, the colour of the wing outline corresponds to the genus. Negative scores are indicated by a “full” or “closed” landmark circle and positive scores are indicated by an “empty” or “open” landmark circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
and a distal shift in the radial vein intersections (Species A) from a narrower wing with a more proximal shift of vein intersections (Species C). PC3 separates a relatively broader wing apex on the anterior margin with shorter MP veins (Species A) from a narrower wing with longer MP branches (Ephemerellina).

### 3.2. Evolutionary allometry

The multivariate regression of independent contrasts of fore wing and hind wing shape on independent contrasts of centroid size accounts for 28.2% and 8.8% of the variation in shape respectively. The permutation test shows that this allometry is significant for the fore wing ($P = 0.0005$) but not the hind wing ($P = 0.3210$), indicating that there is clear allometry in the fore wing dataset and no further analyses were conducted for the hind wing dataset.

A larger fore wing size is associated with a relative wider wing base, enlarged cubitus region and raked (narrow and downward-curving) wing apex, while a smaller wing size is associated with a relatively narrow wing base, smaller cubitus region and a broader wing apex (Fig. 5). These wing shape differences also affect the relative venation patterns, where the Cubital (CuA) Media (MA$_2$ and MA$_3$) and Radial (iRS) veins along with their branch intersections shift towards the wing apex with increasing size.

Wing shape among Teloganodidae appears to be characterised by limited variation in limited directions. A PCA of the shape scores that are free of the effects of allometry (the residual component of variation in species means) reveals that 72.8% of the allometry-corrected shape variation among species is concentrated in only three dimensions (Fig. 6). PC1 in the size-corrected analysis accounts for slightly less than the uncorrected shape, while PC2 and PC3 accounts for slightly more in the size-corrected shape. The shapes associated with the first three PCs of the size-corrected PCA is similar to the PCA for total shape variation, but not as pronounced for PC1. PC1 is still associated with a shorter and narrower wing with a raked apex, but the wing base is not as broad (Ephemerellina) and the Lestagella wing shape is associated with a broader posterior margin and similar shift in venation. PC2 shows a similar shape change to the PC3 for total shape variation (between Lestagella sp.7 and all other Lestagella species). PC3 now separates a narrower, longer wing with a more curved and shorter CuA vein (Species B) from a broader, shorter wing with a longer CuA vein and slightly raked-apex (N. crassii).

The primary axis of variation (PC1) contributes 43.2% of the total variation and mainly describes changes in the relative positions of internal radial and medial vein intersections and the shape of the wing apex. A negative change from the mean results in a raked wing apex, narrower wing and shift of internal vein intersection landmarks and bottom margin landmarks towards the wing apex (E. barnardi, Fig. 6). A positive change from the mean shape describes a broader wing at the apex and cubital portion and a shift of the internal intersection landmarks towards the wing base (Lestagella, Fig. 6). PC2 contributes 16% of the total variation and mainly describes subtle changes in the broadness of the wing base and landmark positions on internal vein intersections and hind margin of the wing (observed between species of Lestagella, L. sp7 and L. sp1/sp2/sp6). PC3 contributes 13.6% of the total variation and mainly describes the wing length and broadness, with a slight shift in vein/margin landmarks. A negative change results in a longer, narrower wing with a narrow base and a slight shift of CuA towards the wing base (Species B, Fig. 6). A positive change describes a broader and shorter wing with vein/margin landmarks slightly shifted towards the wing apex (Nadinetella, Fig. 6). More branch crossing (homoplasy) is seen after the effects of evolutionary allometry were removed. The genera clearly cluster together and closely related species groups form a “starburst” pattern (clusters at the end of a single branch) can be seen for Lestagella and the Nadinetella/Lithogloea group in the fore wings.

### 3.3. Evolutionary variation

To examine the patterns of evolutionary variation in the fore wing shape, the covariance matrix of IC was examined with a PCA.

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**Fig. 5. Regression plots showing evolutionary allometry of the fore wing**. A warped outline of the fore wing represents the large and small extremes of wing size. The regression reveals that size accounts for 28.2% of fore wing shape variation among species. Diagrams superimposed show the shape changes from the mean shape expected for the extreme left and right of the plots (centroid size extremes). Extreme shape scores are illustrated as coloured outlines. Negative scores are indicated by a “full” or “closed” landmark circle and positive scores are indicated by an “empty” or “open” landmark circle.
The shape changes associated with the first three PCs clearly resembles the corresponding PCs in the analysis of species means (Fig. 3, Table 2), although the variation for PC1 and PC2 are significant and PC3 is not (Fig. 7a). The shape change for the PC1 of independent contrasts is very similar to the shape change associated with the allometric regression vector (angle 15.5°, P < 0.00001). PC1 takes up 39.9% of the total variation of IC of shape and the allometric regression accounts for 28.1%, indicating that evolutionary allometry is a significant contributing factor in forewing shape diversification. To characterize the patterns of evolutionary change without allometric effects, a PCA of the residuals from the regression of IC of shape on the IC of centroid size was used (Fig. 7b). There is a clear resemblance between the shape change for the PC1 for the size-corrected contrasts (Fig. 7b, Table 3) and PC2 of the uncorrected contrasts (Fig. 7a, Table 3) and PC2 of the uncorrected contrasts (Fig. 7a, Table 3) and vice versa, while the PC3s for both size corrected contrasts and for uncorrected contrasts have a clear resemblance (Table 3, Fig. 7).

4. Discussion

In this study, the evolutionary changes in wing shape were explored for 14 OTUs of Teloganodidae mayflies with the aim of determining the effects of phylogeny and evolutionary allometry. Phylogenetic structure was found to be significant in both the fore and the hind wings.

For all PCAs of the fore and hind wing, PC1 takes up a substantial amount of the variation, far more than any other PC, indicating that the shape variation in each data set is highly concentrated in a single
direction. Also, the shape changes associated with the first PCs are consistent across the levels of analysis. This suggests that a common process may be channelling the phenotypic variation in a single direction. Because the wing shape variation within-taxa is concentrated considerably in a single dimension of the shape space, evolutionary changes are also most likely in that direction. It is possible that the PCs act as “lines of least resistance”, where this direction of variation controls or limits evolutionary change (Schluter 1996; Arthur 2001; Hunt 2007; Klingenberg 2010), although keeping in mind that there is no single explanation that can account for all variation across taxa (Efroni et al. 2010). Changes in body size and wing shape can also arise from random evolutionary processes such as genetic drift, the founder effect and population bottlenecks (Brown & Lomolino 1998; Legendre & Makarenkov 2002; Heads 2005; Whittaker and Fernandez-Palacios, 2007).

The limited sample size and low number of species within the Teloganodidae family make it difficult to see clustering patterns in the PCAs, however the Nadinetella/Lithogloea group and the Lestagella genus show a characteristic “starburst” pattern that has been described for caecilians (Sherratt et al. 2014). These clusters at the ends of a single branch suggest that the ancestral lineages have crossed the shape space and evolved novel wing shapes and have subsequently undergone more local shape evolution, and suggests that wing shape variation is associated with species divergence. There is evidence of convergent evolution or homoplasy in the wing shape, where evolutionary divergence can be seen among closely related taxa, which is common among Ephemeroptera (Edmunds 1972; McCafferty & Wang 1997, 2000). The homoplasy (crossing of branches) among some of the species also implies that extrinsic pressures have also played a role in Teloganodidae wing evolution. This divergence among closely related taxa could reflect the plasticity of the wings to evolve, for instance, in response to selection on functional morphology or in response to the environment or habitat.

Fig. 7. Patterns of evolutionary diversification in fore wing shape, showing changes associated with the PCs of phylogenetically independent contrasts for shape. (a) PCA for independent contrasts for complete shape variation and (b) PCA for the residuals from the regression of independent contrasts of shape on independent contrasts of centroid size. Extreme shape scores are illustrated as coloured outlines. Negative scores are indicated by a “full” or “closed” landmark circle and positive scores are indicated by an “empty” or “open” landmark circle. Please note that the mapped PCA for PC1 and PC3 (7b) shows the colour outline inverse of the other mapped PCAs, the sign (+/-) of the contrasts is arbitrary and every data point can equivocally be rotated by 180° (Garland et al. 1992), colours for outlines have been switched so that they are visually comparable with other figures. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
Our analyses showed that evolutionary allometry plays a considerable and significant role on fore wing shape, where an increased size is associated with a broader wing base and narrower, raked wing apex compared to the smaller species with a relatively narrow wing base and increased wing area at the apex. The raked apex of the larger fore wings allows for a passive pitch-angle, reducing induced-drag which influences the creation of lift while flying, which may assist in increasing speed and control in flight despite the high aspect ratio (e.g. as in dragonflies (Norberg 1972, 1973). The increased concentration of wing area towards the wing base in larger wings not only promotes less-energetically demanding flight (Weis-Fogh 1973; Ellington 1984), it also results in a higher range of flight speeds (Wootton 1992) as has been shown for Odonata (Outomuro et al. 2013) and may be beneficial for avoiding predators (Hedenström et al. 2001; Combes et al. 2010). This is especially applicable to the larger genus Ephemerellina as it has been described as “very solidly built, plump and high” (Harrison 1951) making it more conspicuous for predators than the smaller species. The increased wing area towards the base is also important for wing loading in the larger, heavier mayflies (Edmunds & Traver 1954; Edmunds 1972; Wootton & Kukalova-Peck 2000; Norberg 2002).

The wing shape associated with the smaller size (Lestagella), where wing area is increased towards the apex is expected to be relatively more energetically costly (Outomuro et al. 2013), suggesting a slower flight with more agility. Swarms of the smallest mayfly, Lestagella, have been observed in the field to exhibit horizontal patrolling (Edmunds 1972), flying upstream to oviposit (V. Ross-Gillespie pers. comm. 2013; Ross-Gillespie 2014), thus more agile flight may be useful for this behaviour. The study highlights the importance of detailed ecological and life history data on taxa as limited information regarding the ecology and life history of Teloganodidae was found in the literature.

Removing the effects of allometry gives a better indication of the shape variation between the lineages. The non-allometric variation involves similar, but less distinct shape changes to the allometric component. Once the allometric component was removed, shape changes were more apparent and enhanced across the genera and not just for the largest (Ephemerellina) and smallest (Lestagella) species. For example, Fig. 6 includes more extreme shape changes from Nadinetella and Species B within the first three PCs, which was not observed in the uncorrected mapped phylogeny (Fig. 3).

Wing shape is generally assumed to be more stable due to strong developmental and phylogenetic constraints, and so significant variations are expected mostly among species and can be used for taxonomy (Sturtevant 1942; Edmunds 1972; McCafferty & Wang 1997; Kluge 1998; Elouard et al. 2003; Zikić et al. 2017). However, it has been shown in some groups that shape is genetically variable within a species (e.g. Diptera: Drosophila, Huey et al., 2000; Gilchrist & Partridge, 2001; Debat et al., 2003) and can be attributed to environmental stress. Thus, geometric morphometric analyses could be a valuable tool for the analysis of geographic variation in winged insect populations.

5. Conclusions

In this study we have shown that the relationships between fore wing shape and size are complex and taxon-specific, at least for Teloganodidae. We have revealed that evolutionary size has a considerable effect on the evolutionary shape of Teloganodidae fore wings, however this does not account for all of the variation in shape. Other processes (such as environmental, functional, developmental and/or behavioural) must also contribute to the same features of evolutionary shape variation. The shape of the fore wings is a strong driver in evolution of the flying characteristics and capabilities of these particular Ephemeroptera taxa. This raises an interesting question of what other factors (such as behaviour, mate recognition, nuptial flight, oviposition, habitat requirements and avoidance of predators to name a few) contribute to the evolution of mayfly wings, especially as the reproductive stage does not feed and so has limited time and energy resources in which to reproduce and disperse in its environment. Further studies investigating possible selective pressures and contributing factors are needed to support this preliminary study showing the importance of wing size and shape on their evolution, and that differences in wing shape morphology relate to ecological differences among clades.

Declarations of interest

None.

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### Table 2

Angular comparisons between PCA for independent contrasts for complete shape variation and size corrected PCA for independent contrasts, to characterise for patterns of evolutionary variation in wing shape.

<table>
<thead>
<tr>
<th>Species means</th>
<th>Independent Contrasts</th>
<th>Angle</th>
<th>P-value</th>
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<tr>
<td>Fore wing:</td>
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</tr>
<tr>
<td>PC1</td>
<td>PC1</td>
<td>33.7°</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>PC2</td>
<td>PC2</td>
<td>45.5°</td>
<td>.00002</td>
</tr>
<tr>
<td>PC3</td>
<td>PC3</td>
<td>83.4°</td>
<td>.55187</td>
</tr>
</tbody>
</table>

Permutation test against the null hypothesis that the vectors have random directions.

### Table 3

Angular comparisons between PCA for species means for complete shape variation and independent contrasts, to characterise for patterns of evolutionary variation without allometric effects.

<table>
<thead>
<tr>
<th>Size corrected</th>
<th>Uncorrected</th>
<th>Angle</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fore wing:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC1</td>
<td>PC2</td>
<td>28.8°</td>
<td>&lt;.00001</td>
</tr>
<tr>
<td>PC2</td>
<td>PC1</td>
<td>40.1°</td>
<td>&lt;.00001</td>
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<tr>
<td>PC3</td>
<td>PC3</td>
<td>16.7°</td>
<td>&lt;.00001</td>
</tr>
</tbody>
</table>

Permutation test against the null hypothesis that the vectors have random directions.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcz.2019.02.005.

References


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