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## Content

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**Comparative study of the Chatham Islands Odonata, II:  
Morphometric and molecular comparison between *Xanthocnemis  
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(Zygoptera: Coenagrionidae)**

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## Abstract

We compared Chatham Island endemic species *Xanthocnemis tuanuii* to its congeners from the New Zealand South Island: *X. zealandica* (newly collected specimens) and *X. sinclairi* (type specimens plus newly collected material). Two independent tests were performed – geometric morphometrics and molecular. Both analyses were consistent in supporting the status of *X. tuanuii* as a good species. Species differed statistically in the following morphological traits: head (dorsal view), male appendages (dorsal, lateral, posterior and ventral views), thorax (dorsal view), and penis (dorsal and lateral view). In addition to the original diagnostic features (mainly shape of the male superior appendages), a new morphological character is suggested here which reliably distinguishes the species based on the shape of the inferior appendages.

There was no statistical support for the species status of *X. sinclairi*. The only feature reported as diagnostic (lower lobe of male superior appendages) was found to be variable and insufficient to warrant the previously proposed taxonomic rank for *X. sinclairi*. Molecular analysis of specimens showing identical appendages to the *X. sinclairi* holotype grouped them with *X. zealandica* specimens. Therefore *X. sinclairi* is synonymised with *X. zealandica*.

## Key words

Chatham Island, New Zealand, South Island, Geometric morphometrics, *Xanthocnemis*, phylogeny

## Introduction

Marinov & McHugh (2010) presented a detailed reference list of all odonatological studies carried out on the Chatham Islands and introduced the taxonomic issues pertaining to the New Zealand endemic genus *Xanthocnemis*. The report noted that the earliest studies on this Pacific archipelago have assigned specimens to both *X. sobrina* (McLachlan, 1873) described as being larger than their counterparts from the New Zealand South Island (Hutton 1898, 1899; Hudson 1904) and to *X. zealandica* (McLachlan, 1873) for the lack of any significant morphological differences between representatives from the two geographical areas (Alfken 1903; Tillyard 1913; Wise 1973; Macfarlane 1979). As became clear from Seehausen et al. (2014), Edmond de Selys Longchamps (1813-1900) had considered raising Chatham Island specimens to a separate species rank, however he had never published this view. Finally Rowe (1981) established *X. tuanuii* sp. nov. for the populations on the main Chatham Island. This study based its conclusion on the shape of the lower lobe of the superior appendages in males (being more sharply pointed upward compare to *zealandica*), presence of lateral flanges on the penis (claimed to be absent in *zealandica*), dark area overarching the metainfraepisternum below the metathoracic spiracle (absent in *zealandica*) and denser coverage of hairs on the body compared to *zealandica*. Rowe (1981) as-



signed older *Xanthocnemis* records (where archived specimens were available for investigation) to the newly-proposed species and proposed it as the only species from the genus found on the island during his study.

However, in the only comparative molecular investigation of New Zealand Odonata, Nolan et al. (2007) reported two haplotypes for *Xanthocnemis* larvae sampled in the Chatham Island. Due to the lack of reliable diagnostic features on the larvae, the species identification in their research was based on allozymes and mitochondrial cytochrome *c*-oxidase subunit I (COI) nucleotide sequences. Chatham Island larvae identified as *X. zealandica* had an allelic composition similar to that found for this species elsewhere in New Zealand. The only larva that deviated from this scheme was identified as “presumably *X. tuanuii*”.

To resolve this apparent conflict between the morphological and molecular analyses, Marinov & McHugh (2010) published the first part of a new study that was initiated on the Chatham Island and New Zealand South Island specimens, with a particular focus on adults that can be reliably differentiated based on the taxonomic features proposed by Rowe (1981). In the first part of this study variation in total body length, abdomen length, hind wing length and wing surface area between the Chatham Island and the South Island *Xanthocnemis* populations were compared. The Chatham Island specimens were found to be statistically larger, with broader wings and a relatively small abdomen relative to body length, than their South Island counterparts. However, differentiation between the two taxa based on other morphological traits were problematic and obscured by overlapping variation between the main diagnostic features reported for *tuanuii* and the corresponding features of *zealandica*. Sharply pointed lower lobes of the superior appendages were noted in *zealandica* specimens from Stewart Island (investigated from museum specimens only and not included in the statistical analysis) as well with a body length approaching the one typical of *tuanuii*. Again Stewart Island as well as other South Island specimens exhibited a dark area on the same region of the thorax as in *tuanuii*, but was very reduced in size. Genitalia and body hairiness were not investigated in that study. Marinov & McHugh (2010) concluded that apart from body size, the *Xanthocnemis* populations inhabiting the Chatham Island and South Island were not significantly different. They suggested that the variability of the proposed diagnostic characters could be attributed to the lower temperatures typical of the Chatham Island environment. The study's results were in congruence with Rowe (1981) who reported just one species for the Chatham Islands. Marinov & McHugh (2010) discussed possible hypotheses for the two haplotypes reported in Nolan et al. (2007) from the same island. They are presented here with slight modifications and additions as three hypotheses: 1) a chance dispersal event by *zealandica* females who were unable to establish a viable population, 2) sympatric evolution on the island with *zealandica* being very localised, and 3) allopatric speciation with both taxa still in the so called “Grey Zone” (De Quiroz 2007) established for daughter lineages that have not fully undergone speciation; only one spe-



cies occurs on the Chatham Island which is indistinguishable from *zealandica* at a molecular level, while the second haplotype (of the specimen identified by exclusion) being a result of a possible contamination of the molecular sample.

The present paper reports the results of the second part of the studies on the Odonata of the Chatham Island – detailed morphometric and molecular comparison between *Xanthocnemis* populations of the Chatham and South Islands.

## Material and Methods

Marinov & McHugh (2010) presented details about the sampling methodology and locations. The present study was carried out on a larger sample size which included new *Xanthocnemis* specimens from the South Island only and the type specimens (holotype and allotype) of *X. sinclairi*. The latter were obtained from the original depository place at Auckland Museum. To preserve intact the type specimens were used for the morphological analysis only. Only body parts that were visible with slight manipulation were included. Therefore, *X. sinclairi* types were excluded from molecular analysis and lateral comparison of the penes. Other specimens obtained for this study from the South Island high country region (Locality 1 below) were identified as *X. sinclairi* and used for the molecular analysis instead.

New *Xanthocnemis* specimens come from the following South Island localities:

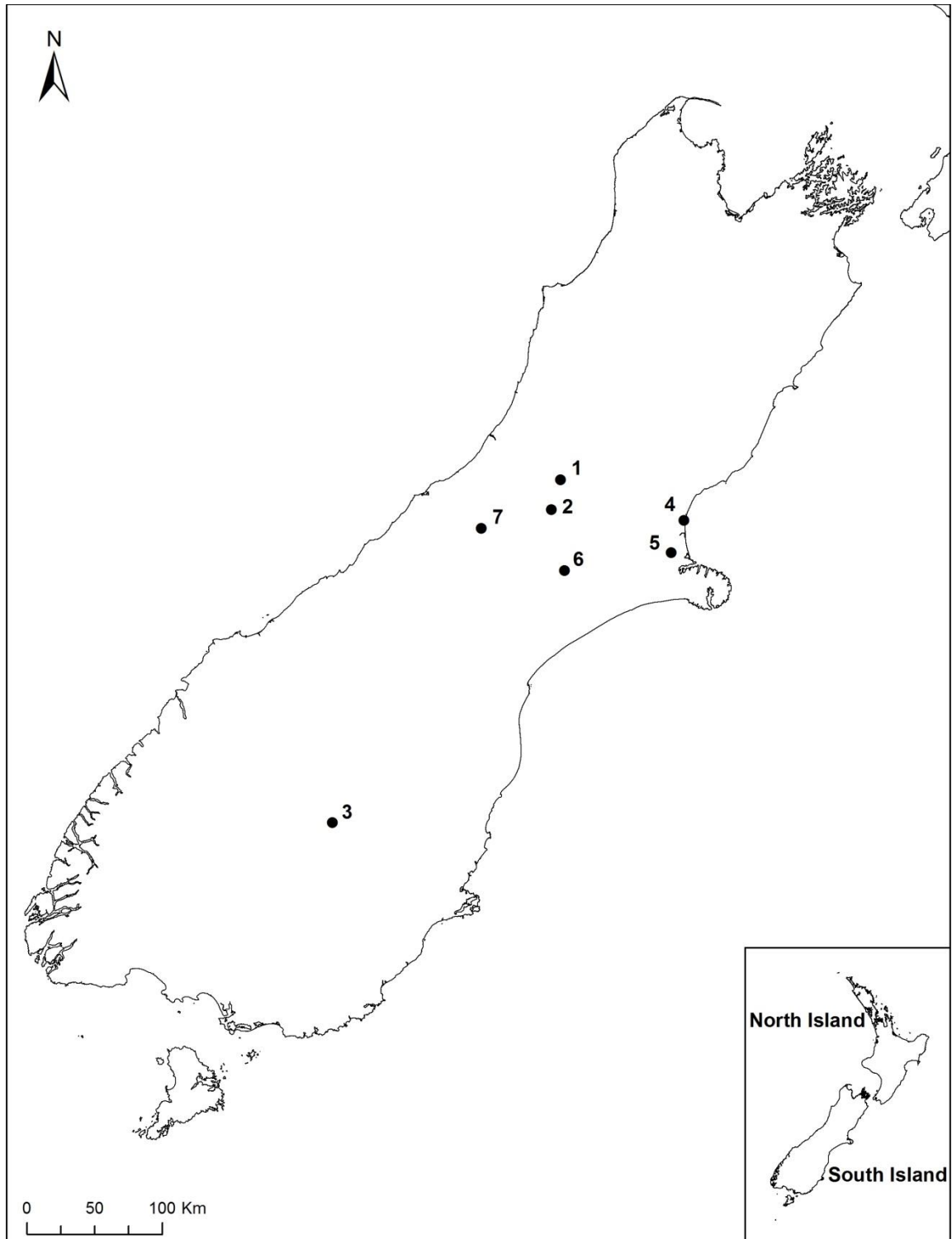
1. Mountains tarns on Bealey Spur Track (43.0316S, 171.5879E; 1,040 m a.s.l.): 06 February 2010.
2. Henrietta Lake (43.2283S, 171.5001E; 550 m a.s.l.): 01 January 2010.
3. Clutha River by Alexandra (45.2565S, 169.38961E; 150 m a.s.l.): 28 December 2009.
4. Wetlands within Pegasus town (43.3086S, 172.7022E; 22 m a.s.l.): 14 February 2010.
5. University of Canterbury, Christchurch campus (43.522726S, 172.582984E; 10 m a.s.l.): 17 November 2011.
6. Irrigation channel NW of Methven (43.633963S; 171.609879E; 320 m a.s.l.): 24 December 2009.

*X. sinclairi*'s type locality is:

7. Headwaters of the Rakaia River, tarns in the valley of the Lauper Stream below Whitcombe pass (43.2167S, 170.9667E; 1,250 m a.s.l.): 11 February 1971.

All new localities and *X. sinclairi* type locality are mapped on Fig. 1.





**Figure 1.** New localities for *Xanthocnemis* species on the New Zealand South Island including the *X. sinclairi* type locality. Numbers correspond to the sampling localities given above.

### *Geometric morphometrics*

A total of 37 *Xanthocnemis* specimens from both the Chatham and South Islands were photographed using a Leica M125 microscope with digital camera and Leica Application Suite V4.3., University of Canterbury. The following body parts were specifically targeted for macrophotography: head (dorsal and frontal views), thorax (dorsal and



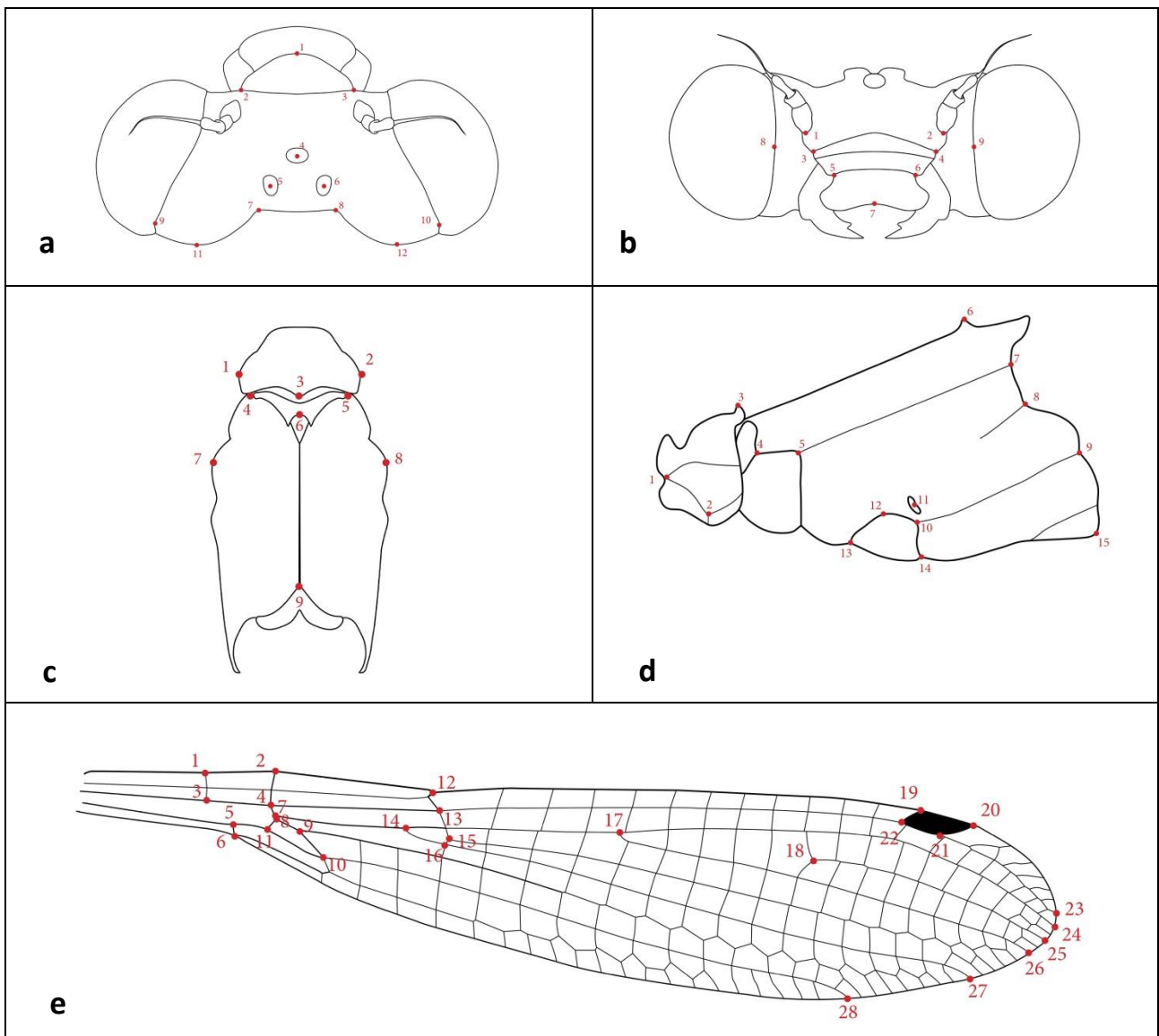


lateral views), hind wing, male appendages (dorsal, lateral, posterior and ventral views), penis (dorsal and lateral views) and female ovipositor (lateral view). The ovipositor was later excluded since only one female was obtained from the Chatham Islands which was not sufficient to run a statistical analysis.

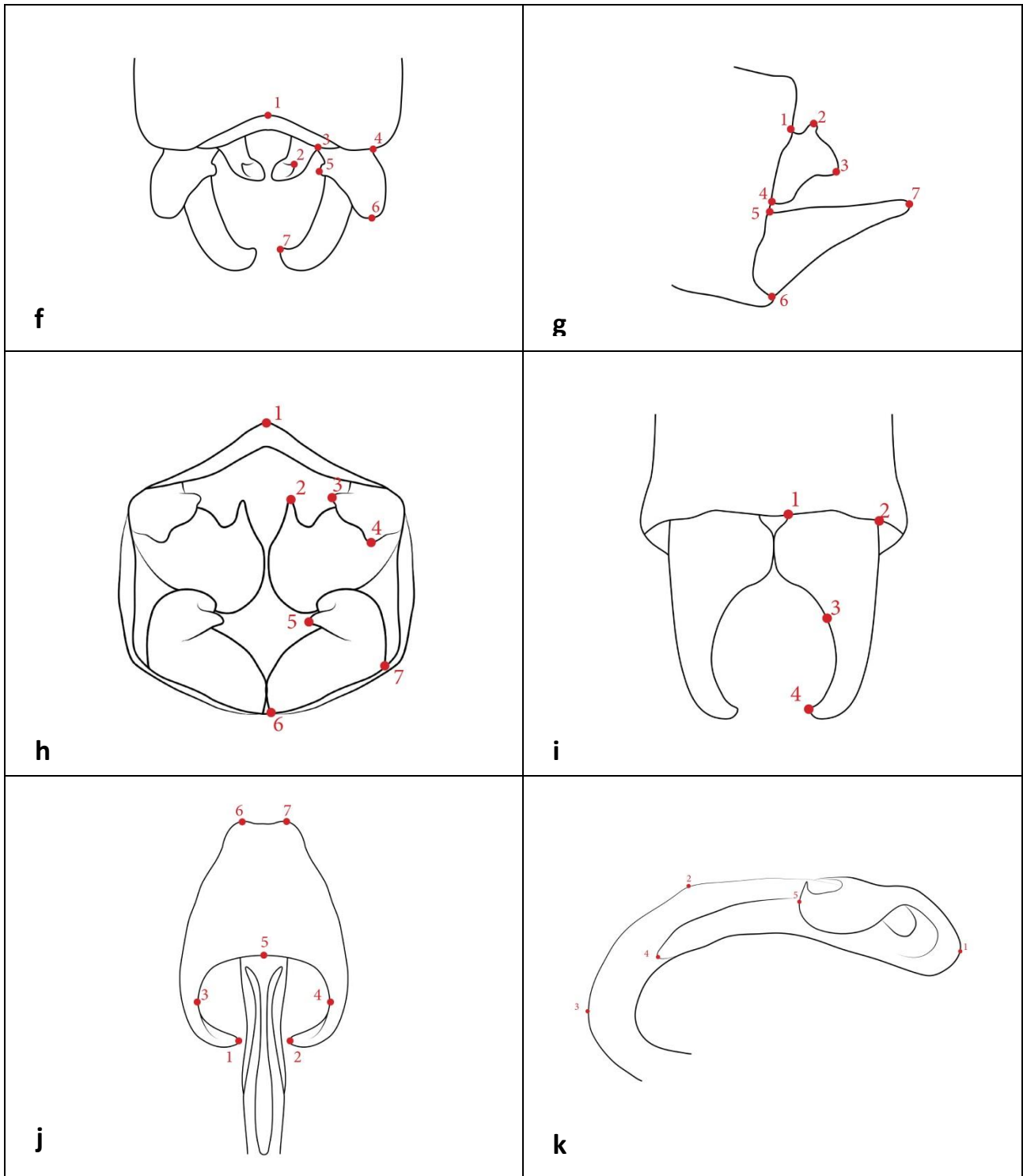
*X. sinclairi* holotype was compared to all other populations for these characters, except the lateral view of the penis as it was impossible to be obtained without damaging the type specimen.

An additional analysis was run on 64 wings of Chatham Island specimens which were photographed in the field (following Marinov & McHugh 2010) and released at the sampling locations. This comparison was performed to investigate any possible intra island morphological variations between the localities on the Chatham Island.

Landmark-based methods were used which are particularly useful when structures such as wings have easily identifiable homologous landmarks which can be reliably identified among specimens. A number of body parts were included in the analysis in







**Figure 2.** Body landmarks assigned to *Xanthocnemis* specimens: a-b) head dorsal and frontal views; c-d) thorax dorsal and lateral views; e) wing; f-i) male appendages dorsal, lateral, posterior and ventral views; j-k) penis dorsal and lateral views.

order to assess their reliability in studies of closely related organisms, such as the three *Xanthocnemis* species targeted in this research. For this study, between 4 and 28 (Fig. 2) landmarks were digitised using the software tpsDig2 (Rohlf 2008) depending on the structure. These digitised landmark data were subjected to generalized least squares (Procrustes) superimposition using tpsRelW (Rohlf 2008) to normalize the position, ori-



entation, and scale of all specimens reducing the data to a series of relative warp scores (RWS). This also allowed us to visualize the shape variation of each trait as shape deformations between locations (Holwell et al. 2010). The percentage of shape variation attributed to each of these warp scores for all traits was also determined (Rohlf 2013). As a large proportion of the total shape variation was attributed to the first four warp scores (cf. Results), a MANOVA analysis was performed on RWS 1-4 for each trait to determine the effect of location on morphological variation for each structure. The key hypothesis being tested was that Chatham Island specimens of *Xanthocnemis* were significantly different in shape to those on the mainland of New Zealand.

### *Molecular analysis*

Total nucleic acid from the *Xanthocnemis* samples was extracted using the prepGEM Insect kit (ZyGEM Corporation Ltd., New Zealand) following the manufacturer's instructions. 4µl of the extract was used as a template for PCR using KAPA 2G Robust DNA polymerase (Kapa Biosystems, USA) with the the *cytochrome c-oxidase I* (COI) primers CO1-2F (5 –TYG AYC CID YIG GRG GAG GAG ATC C-3) and CO1-2R (5 –GGR TAR TCW GAR TAW CGN CGW GGT AT-3 ) described by Otto & Willson (2001) and used in Nolan et al. (2007) in the comparative study of the New Zealand *Xanthocnemis* species (cf. above). The following thermal cycling protocol was utilised: 95°C for 3 minutes; then 30 cycles of 95°C (15 sec), 55°C (15 sec), and 72°C (15 sec); final elongation after the last cycle 72°C for 1 minutes. The ~650nt amplicons were resolved on a 1% agarose gel stained with SYBR® Safe (Life Technologies, USA). The amplicons were cloned into pGEM-T easy vector (Promega, USA) and the resulting plasmid obtained from a single transformed *E. coli* colony was isolated and sequenced at Macrogen Inc (Korea) for each sample (GenBank accession: KM106822 - KM106850).

Sequences were aligned with references sequences available in GenBank using MUSCLE (Edgar 2004) implemented in MEGA5 (Tamura et al. 2011). All pairwise identities (p-distances with pairwise deletion of gaps) were calculated using SDTv 1.0 (Muhire et al. 2013). Maximum likelihood (ML) phylogeny of the sequenced *cytochrome c-oxidase I* gene sequences was inferred using PHYML (Guindon et al. 2010) with GTR+G nucleotide substitution model chosen as the best fit model by jModelTest (Posada 2009) with 1000 bootstrap replicates. Branches in ML phylogenetic tree with less than 70% branch support were collapsed using Mesquite v2.75 (<http://mesquiteproject.org/>). Therefore, only two Odonata species used in Nolan et al. (2007) remained as outgroups to *Xanthocnemis* in the present study – *Austrolestes colenisonis* (White, 1846) (Zygoptera) and *Aeshna brevistyla* Rambur, 1842 (Anisoptera).

## **Results**

Both geometric morphometric and molecular analyses were congruent in grouping Chatham Island specimens in a distinct cluster opposed to the specimens from the South Island.



## Geometric morphometrics

### ♦ *Xanthocnemis* from Chatham Island vs. South Island

There was a significant correlation by geographic location of the variation in shape for a number of the traits examined in specimens collected in the Chatham and South Island Islands. Specifically, we found significant variation on the head (dorsal view), male appendages (dorsal, lateral, posterior and ventral views), thorax (dorsal view), and penis (dorsal and lateral view) (Table 1). No significant shape differences were revealed for the head (frontal view), thorax (lateral view) and wings between specimens from the two geographic areas. The variation in the shape of the investigated morphological traits are plotted as ordinations of relative warp scores 1 vs. 2 in Fig. 3 (see Appendix) together with the corresponding mean consensus shape of the Chatham Island and the South Island specimens as thin plate spline deformations. Table 2 presents the numerical values of shape variations. No significant intra-island variations were revealed for the wings of the Chatham Island specimens (Fig. 4).

**Table 1. MANOVA statistics for the effect of geographic population (South Island and Chatham Islands) on the different morphological structures.**

Structures	Population effect (shape only)		
	Wilk's $\lambda$	<i>F</i>	<i>P</i>
Appendages dorsal view	0,1577	9,111	<0.001
Appendages lateral view	0,2461	6,094	<0.001
Appendages posterior view	0,0613	12,9	<0.001
Appendages ventral view	0,448	4,693	<0.005
Head dorsal view	0,6573	2,334	<0.05
Head frontal view	0,8776	1,012	>0.4
Penis dorsal view	0,3696	3,87	<0.005
Penis lateral view	0,3632	2,803	<0.05
Thorax dorsal view	0,4837	4,232	<0.001
Thorax lateral view	0,7859	1,237	>0.3
Wings	0,796	1,249	>0.2
Wings Chatham Island	0,7631	0,7645	>0.7

### ♦ Molecular analysis of *Xanthocnemis tuanuii*

DNA sequences obtained for this study were compared to the isolates from Nolan et al. (2007). In respect to *Xanthocnemis* Fig. 5 shows two clusters only, one for each island investigated. All five isolates from the adult *X. tuanuii* were similar to that which Nolan et al. (2007) established as “presumably *tuanuii*” based on their work on Odonata larvae and group them in one cluster. On the other hand all South Island specimens grouped well into a second cluster with pairwise identities at and above 96% (Fig. 6). One specimen only (MX04) showed consistently lower identities to the rest of the South Island specimens (between 96%-98%). However, these low range variations



**Table 2. Percentages of shape variation for each structure explained by relative warp scores 1-4.**

were found to be insignificant for the final conclusions made in the Discussion. Moreover the same specimen shared 98% identity to MX23 collected from a lowland locality while it had lower identity to other specimens from its own locality (MX01-MX10).

♦ *Xanthocnemis sinclairi*

*X. sinclairi* types fell clearly within the South Island group of specimens for all morphological traits examined including the dorsal view of the penis (Fig. 3j). Close examination of the holotype revealed no differences in colouration compared to other specimens sampled from the South Island and included in this analysis. This confirms that the only morphological trait for species separation remains the shape of the lower lobes of the male superior appendages as reported in the original species description (Rowe, 1987). This was given as “presence of a sclerotized subapical tooth”. Figure 7 compares the lower lobes of the holotype to three specimens used in this study: South Island specimens (including material from both high and low elevation) and Chatham Island specimens. High country specimens had lower lobes identical to *sinclairi* and were thus identified as that species, while lowland specimens were similar to typical *zealandica* for this trait. All 13 other high country specimens (Locality 1) included in both morphological and molecular analyses had lower lobes partially or totally identical to *sinclairi*. This trait was found to be highly variable with almost every specimen exhibiting its own characteristic shape and varying even between the left and right appendages. Fig. 8 shows some examples where the subapical tooth occurs on the right hand side appendage and is absent on the left hand side

Structures	Relative Warps				Total	RWS1				RWS2				RWS3				RWS4			
	S1	S2	S3	S4		$\chi^2$	SE	$\chi^2$	SE	$\chi^2$	SE	$\chi^2$	SE	$\chi^2$	SE	$\chi^2$	SE				
Appendages dorsal view	49,57%	17,20%	11,12%	8,32%	86,21%	1,25E-05	0,22	-1,13E-05	0,01	1,63E-05	0,01	1,33E-05	0,009								
Appendages lateral view	60,33%	13,24%	10,29%	5,87%	89,73%	2,00E-05	0,0152	9,17E-06	0,0071	-7,04E-06	0,0062	1,67E-06	0,0047								
Appendages posterior view	38,34%	25,49%	15,61%	10,36%	89,81%	0,001149	0,02025	-0,004423	1,59E-02	-0,00842	0,01291	0,000113	0,01048								
Appendages ventral view	46,83%	43,31%	9,15%	2,71%	100%	0,001234	0,014981	0,0024604	0,01388	-0,000177	0,00664	0,00087	0,003494								
Head dorsal view	50,53%	18,73%	14,07%	3,88%	87,22%	-0,004051	0,00825	0,00078491	0,0052	-0,001588	0,00459	6,33E-05	0,002331								
Head frontal view	72,20%	7,10%	5,93%	4,12%	89,35%	0,01274	0,013942	-0,00030626	0,00341	0,003677	0,00395	-6,70E-04	0,003704								
Aedeagus dorsal view	48,36%	17,51%	16,16%	6,26%	88,29%	1,38E-03	0,00963	0,000139555	0,00572	-0,00046	0,00551	-3,66E-04	0,003451								
Aedeagus lateral view	36,53%	30,96%	16,16%	10,44%	94,09%	0,00031	0,007370808	0,001202174	6,67E-03	0,00475	0,00052	0,001415	0,00366								
Thorax dorsal view	39,68%	22,20%	17,39%	6,74%	86,01%	-3,35E-03	0,009806438	-0,00012741	0,00682	0,001213	0,00517	-1,18E-03	0,003958								
Thorax lateral view	35,12%	19,06%	11,28%	7,68%	73,13%	-6,62E-04	0,005892783	-0,00147794	0,0047	0,000441	0,00351	-2,63E-03	0,002433								
Wings	33,56%	18,89%	13,73%	9,64%	75,82%	-8,84E-05	0,002470261	4,38E-05	1,84E-03	0,000555	0,00159	0,000312	0,001339								
Wings Chatham Island	33,09%	17,86%	13,53%	11,12%	75,60%	4,22E-04	1,53E-03	0,000105341	0,00116	3,70E-06	0,00102	0,000106	0,000915								





one, while other specimens had more than one tooth. Interestingly one lowland specimen had the apical end of its lower lobe divided giving an appearance of a presence of a subapical tooth.

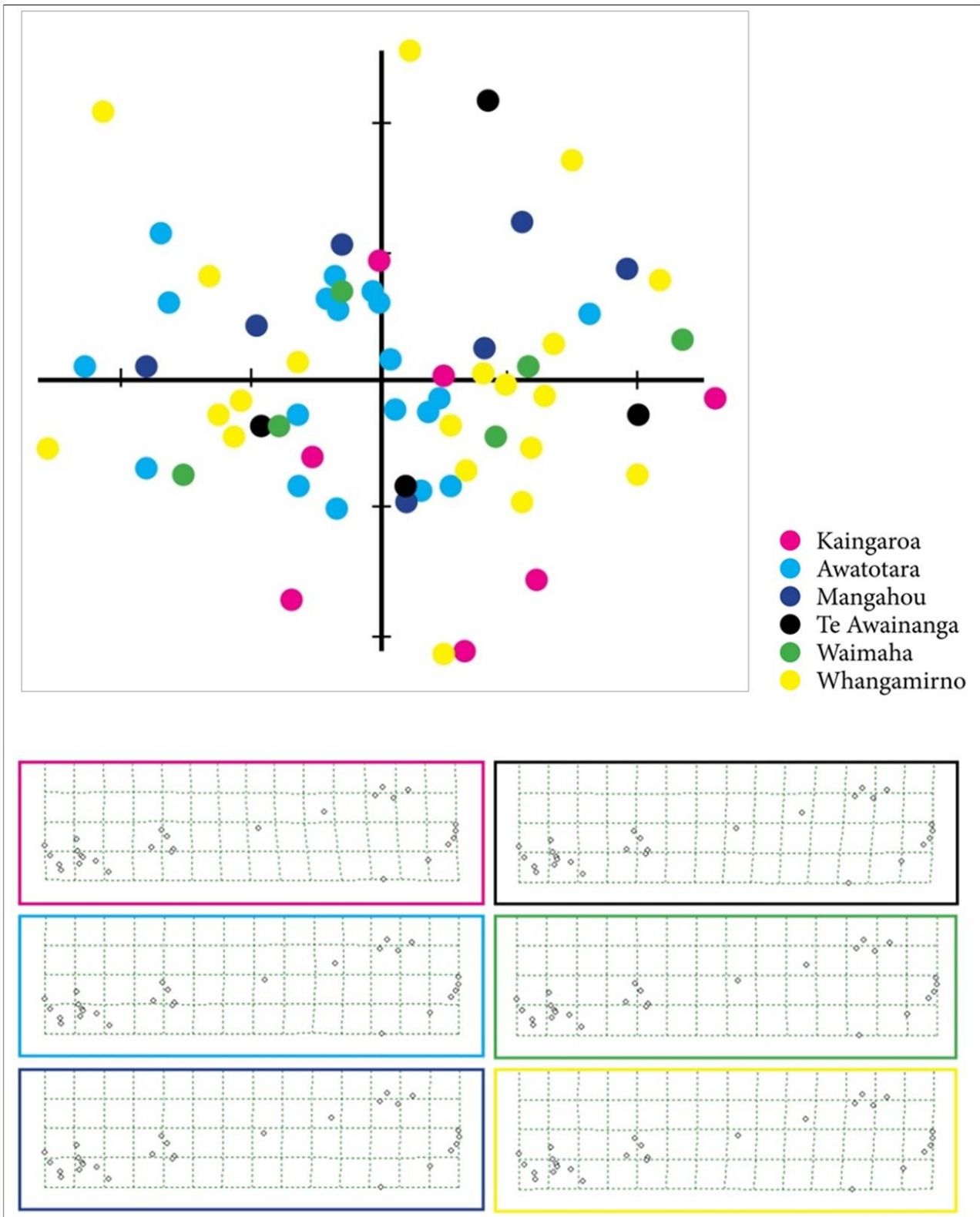


Figure 4. Wing shape variations for individuals from six local populations on the Chatham Island (see Marinov & McHugh (2010) for details about the sampling locations): x axis RWS1; y axis RWS2.





**Figure 5.** Phylogenetic tree of *cytochrome c-oxidase I* gene region of *Xanthocnemis* specimens collected in the Chatham Islands and the South Island of New Zealand studied here (coded as “M” and “MX”) and results of Nolan et al. (2007) which include North Island specimens as well.

## Discussion

DeSalle et al. (2005) introduced the so called taxonomic circle to deal with intra- and interspecific variation in morphological traits or molecular loci. The taxonomic circle postulates that species description should be regarded as a multistep process which is represented as a circle with specific nodes. They are formed by the interrelations bet-



ween various disciplines that add evidence to assist in species delimitation, such as morphology, DNA, geography, reproduction and ecology. The nodes are distributed evenly around the circumference of the taxonomic circle giving each of them equal weight. The taxonomists may enter the circle at whatever node they feel confident with, but do not have to leave it until they have successfully tested at least three nodes that arguably give enough confidence for establishing a new species name.

The rationale of the introduction of the taxonomic circle to the modern taxonomy lies in the observed boost of molecular techniques and the need to be reconciled with classical taxonomy based on species' morphology and anatomy (DeSalle et al. 2005). Damm et al. (2010) applied the taxonomic circle to the African representatives of genus

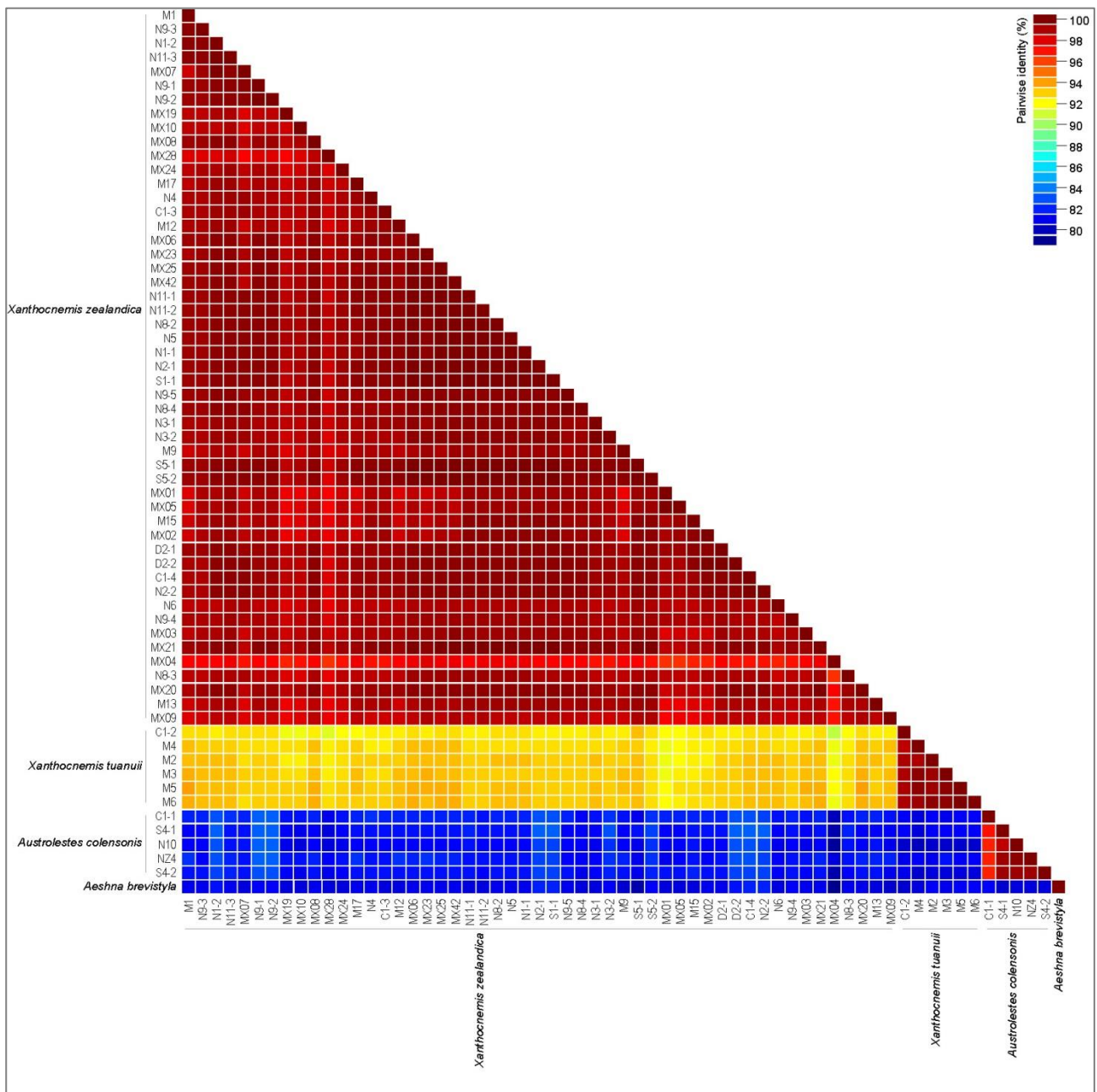


Figure 6. Percentage pairwise identity matrix of the *Xanthocnemis* cytochrome *c-oxidase I* gene region of various samples.





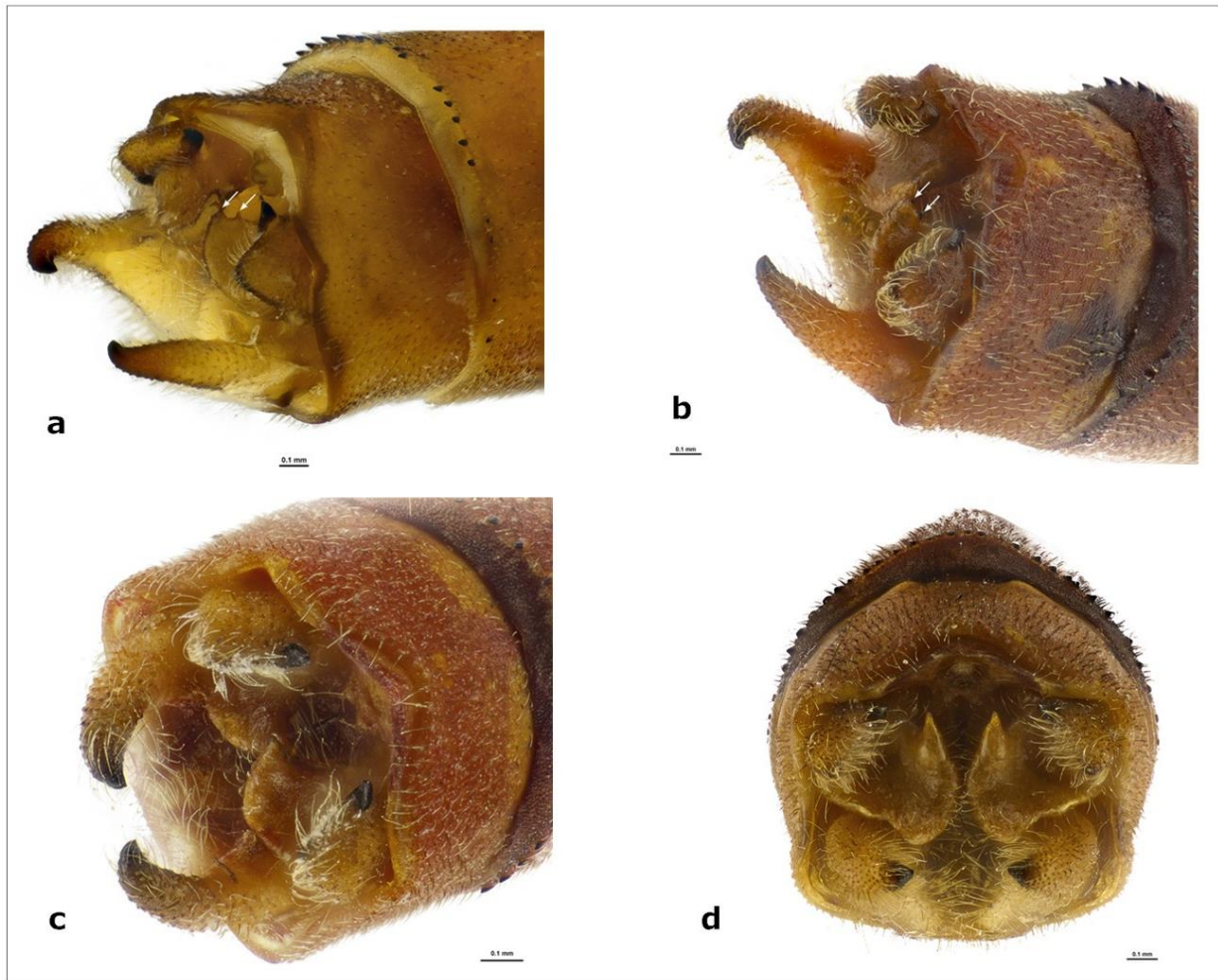


Figure 7. Comparison between the male appendages of the *Xanthocnemis* specimens: a) holotype of *X. sinclairi*; b) South Island the high country; c) South Island lowlands; d) Chatham Island. Arrows point out to the teeth on the lower lobe of the superior appendages.

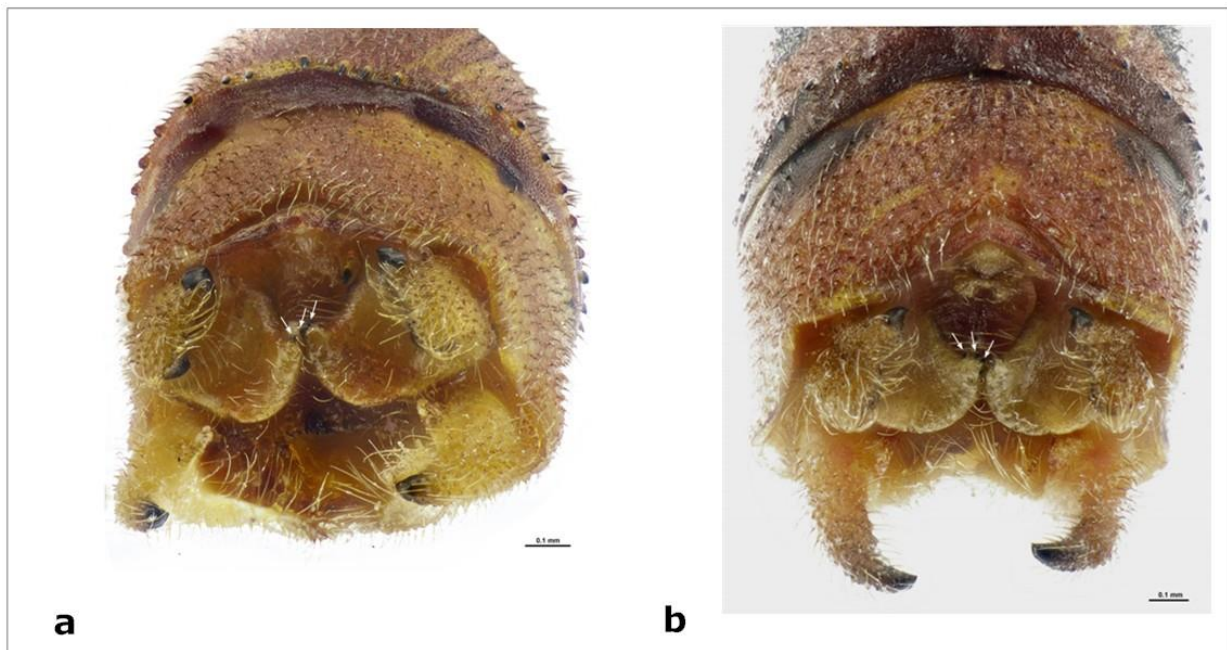


Figure 8. Variations in the teeth position and numbers in *Xanthocnemis* specimens collected for this study: a) subapical tooth present on the right hand side appendage and absent on the left one; b) two subapical teeth present on the left hand side appendage.



*Trithemis* (Odonata: Libellulidae). Molecular analysis of *Trithemis* specimens from East and South Africa revealed three distinct genetic clades that were later supported by a detailed morphological comparison and assessment of ecological traits.

Morphological variation between species is frequently subtle and difficult to quantify with simple linear measurements as demonstrated in Kohli et al. (2014) for the genus *Boyeria* (Odonata: Aeshnidae). Geometric morphometric analyses have in recent decades revolutionised our ability to quantify shape variation in biological structures (Adams et al 2004). The use of geometric morphometrics in species delimitation is now commonly employed (Mutanen & Pretorius 2007; Ludoški et al 2008), and is useful for a variety of applications to study the evolution of morphological shape (Holwell 2008; Holwell & Herberstein 2010; Holwell et al 2010). Geometric morphometric analysis either focuses on variation in the relative position of well-defined landmarks, or on variation in the outlines of structures. These approaches have been successfully applied to a number of entomological studies including Odonata (Hassal et al., 2008; Sadeghi et al. 2009).

Nedelkovich et al. (2013) used an integrative approach to reveal new species of the genus *Chrysotoxum* (Diptera: Syrphidae). They employed geometric morphometrics, assigning landmarks to the wings and surstylus of these flies and linked their results to data obtained by sequencing the ITS2 region. A similar approach is applied here for the first time to Odonata. To the best of our knowledge so far only Gyulavári et al. (2011) and Vega-Sánchez et al. (2010) have combined morphometric studies with molecular evidence to study the differences between populations of two *Chalcolestes* and three *Hetaerina* species respectively. However, morphological characters in the first study were measured by digital callipers or using an ocular micrometer for microscopic features such as anal appendages and no landmarks assigned to the homologous morphological features. The second study incorporated landmarks from the wings only.

To achieve more accurate and comprehensive results for the study reported here, 110 body landmarks were applied to 10 morphological traits. The variation between the Chatham Island and the South Island specimens was shown to be significant. Reliable diagnostic features other than male appendages were sought for facilitating identification in the field without the need to kill specimens. This method has some apparent disadvantages associated with the state of maturity of the specimen prior to killing, and the preservation method. While wing structures allow for an easy and more reliable comparison in a near two-dimensional plane, structures like head and thorax cannot always be positioned or compressed to achieve comparison in the same planes, nor is there a way to compensate for any distortions. The latter inevitably happens in teneral or immature specimens in which the cuticle is not hard enough and deforms after death. Also, it is not possible to arrange individual features of every specimen after killing, in order to achieve the same relative position/distance between each other. Struc-



tures with movable joints change their situation and thus the leading edge of the labrum or the tip of the inferior male anal appendages, for example, may fix at various levels in different specimens resulting in small changes of the relative positions of the landmarks assigned for these body parts.

In spite of these constraints, our test of the workability of this methodology (using the entire body rather than selected parts) gave satisfactory results in morphologically very close species from the genus *Xanthocnemis*. The statistical comparison gave the same level of significance ( $P < 0.001$ ) for the differences between the Chatham and South Island specimens in the male anal appendages (dorsal, lateral and posterior views). Ventral views of the same structure were still significantly different, but with lower support ( $P < 0.005$ ). This is perhaps because superior appendages are not visible from the ventral side. Therefore in ventral view the landmarks were assigned to the inferior appendages only, which had never been used in *Xanthocnemis* species differentiation before, but proved to be a valid diagnostic, although apparently less reliable than superior appendages. Figure 9 compares inferior appendages of *X. zealandica* and *X. tuanuii*. It adds to species diagnostic showing *X. tuanuii*'s appendages being stouter. Frontal views of the head, on the other hand, were found not significantly different between the two islands ( $P > 0.4$ ) in spite of the possible post-mortem distortions. Similar  $P$ -values were achieved for two other tests, lateral view of the thorax ( $P > 0.3$ ) and wings ( $P > 0.2$ ) which was unexpected giving the preliminary results in morphological measurement reported in Marinov & McHugh (2010). These authors found a significant difference ( $P < 0.001$ ) between *Xanthocnemis* specimens of the Chatham and South Island in all body measurements taken in that study (body length, abdomen length, wing length and wing surface) as well as all ratios except for wing:body length ratio. They concluded that the longer bodies of the Chatham Island populations must have been due to evolutionary changes affecting the front part of the body (head and thorax). That might have resulted in an enlarged thorax to correspond to the wing load associated with the larger wing area. All those changes were attributed to the local climatic conditions of strong winds blowing on the island as having a significant effect.

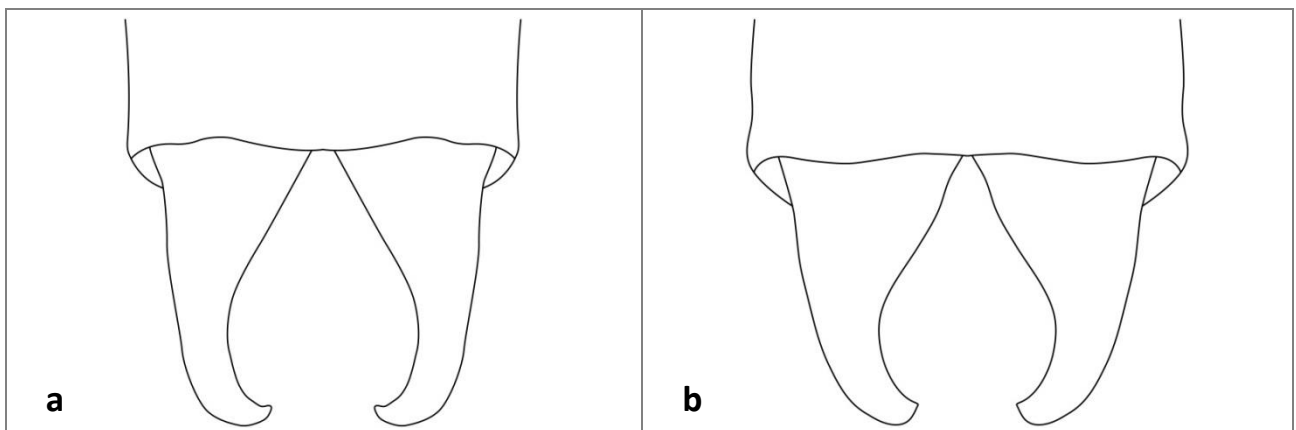


Figure 9. Comparison between male inferior appendages (ventral views) of: a) *Xanthocnemis zealandica*; b) *X. tuanuii*.





The new data here support the increased thorax width for Chatham Island specimens which resulted in significantly different results for dorsal views when comparing specimens from the two investigated islands. Possibly the enlargement of the wing area and thorax length has changed proportionally over evolutionary time, so the relative positions of the homologous body parts have remained the same.

Molecular results for adult *Xanthocnemis* specimens are in full congruence with geometric morphometric data. Haplotypes derived from the earlier study of Nolan et al. (2007) were compatible to our results. The phylogenetic tree clusters Chatham Island adults, and shows them as a distinct species that is sister to all South Island specimens investigated here. These include the ones sampled from high country areas that were identified as *X. sinclairi* based on the shape of the lower lobe of the superior appendages. Therefore, as a result of both morphological and molecular tests *X. sinclairi* is proposed to be removed from species rank. Although *X. sinclairi* indeed shows morphological differences from typical *X. zealandica*, these seem to be insufficient to warrant a full specific status because: 1) overall body dimensions and proportions overlap with those of *zealandica*, 2) no molecular support is evident for differentiation between the two taxa, and 3) no visual colour marks/pattern present on *sinclairi* body that would suggest intraspecies sexual recognition was required to facilitate reproductive isolation. There are, however, ecological and geographical features that could eventually put *sinclairi* back into the taxonomic circle. All specimens sampled for this study as well as all previously reported data on *sinclairi* come from mountain tarns at around and above 1,000 m a.s.l. The close resemblance to lowland typical *zealandica* suggests an origin from a common ancestor with the lowland populations that rose up with the tectonic uplift. It is possible that the high country individuals have developed in some isolation from their lowland counterparts and have acquired small morphological changes, however, at the molecular level they are still indistinguishable. A larger sample size from a gradient of altitudes would be necessary in order to validate this view. This should include specimens from other mountain ranges to test if the observed small morphological change is a local phenomenon in the Central Southern Alps of New Zealand's South Island or is commonly observed trend for all populations living at high altitudes.

In conclusion to the main question about the phylogenetic relationships between *Xanthocnemis* populations inhabiting Chatham Island and New Zealand's South Island, the new analysis unequivocally confirms the separate specific status of the specimens from the two sampled areas. Male superior appendages (especially the shape of their lower lobe) was found to be the most reliable diagnostic feature of the external morphology. Inferior appendages can also be used as diagnostic. The molecular distances between the established haplotypes show that both *X. tuanuii* (endemic to the Chatham Island) and *X. zealandica* (endemic to North, South and Stewart Islands of New Zealand) have originated from a common ancestor. The third hypothesis suggested in the introduction is accepted as the most plausible, so allopatric speciation is proposed



for both taxa. The inferred contamination of the molecular sample in Nolan et al. (2007) is omitted as improbable as the haplotype of the Chatham Island larva was found to be identical to imagines collected in this study. The question therefore remains regarding the occurrence of a second haplotype on the Chatham Islands which Nolan et al. (2007) found to be identical to *X. zealandica*. Unfortunately the material for that study is untraceable for verification and detailed morphological comparison of the larvae (I. Hogg, per. comm.). Accidental occurrence of *X. zealandica* specimens on the Chatham Island during the field study of Nolan et al. (2007), even though very unlikely, is not to be excluded.

This is the first Odonata study to use homologous landmarks over the entire body for a morphological comparison between closely related species. This source of data appears to be a useful addition to dragonfly taxonomy. Perhaps, once this methodology had been applied to other species using the same set of landmarks, a species diagnostic system similar to what had been developed in forensic science for identity check using finger prints could be developed for identifying species.

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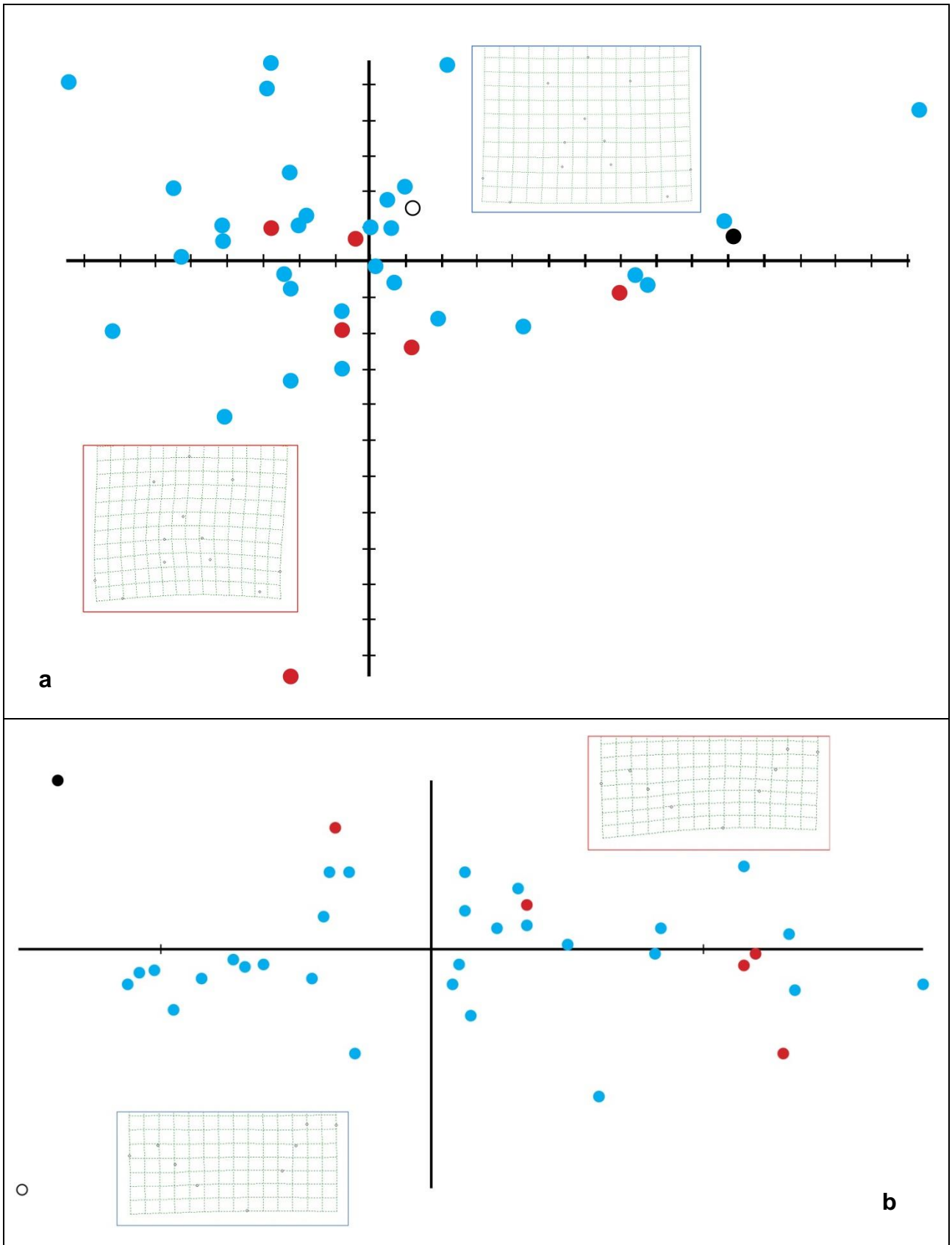


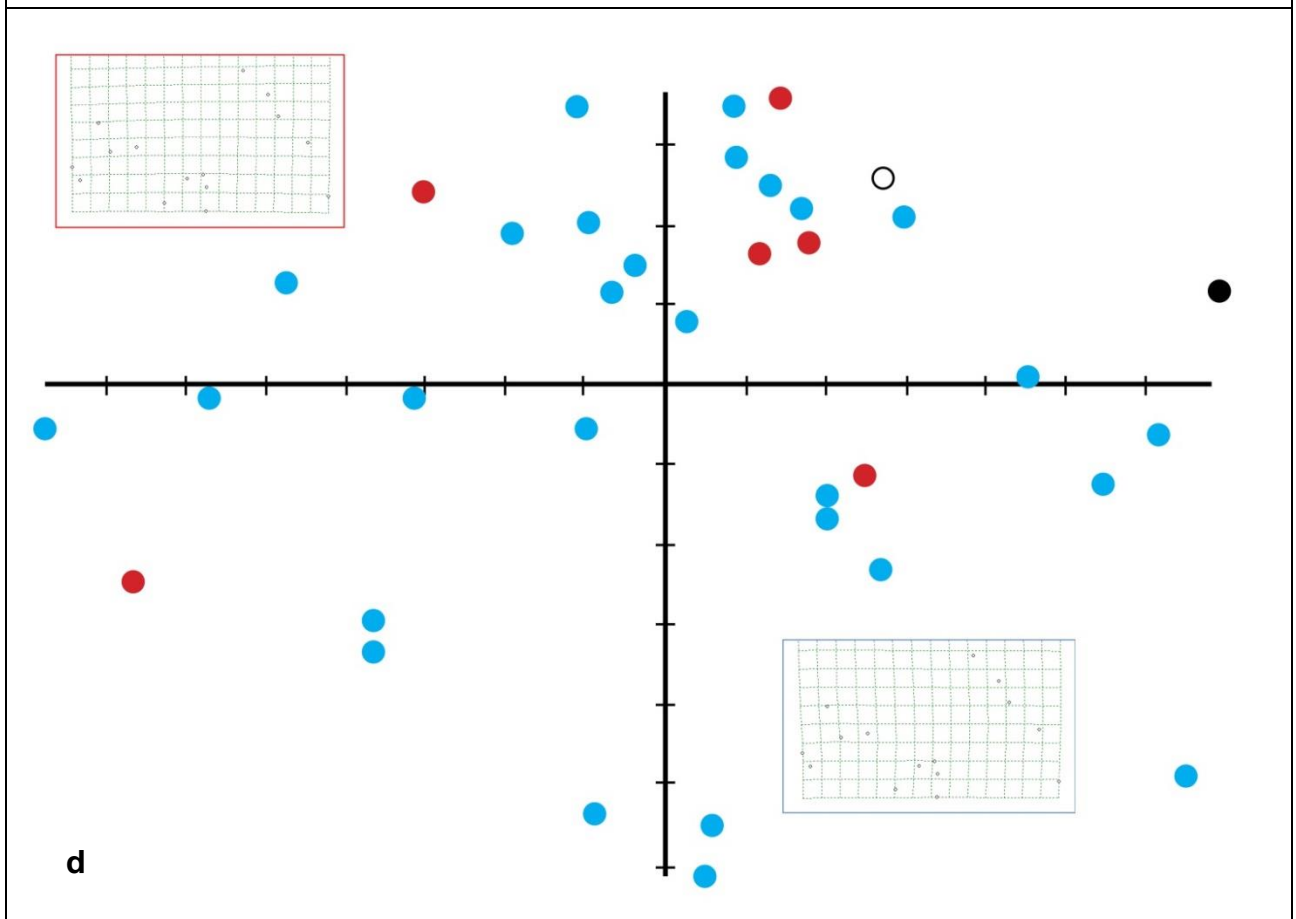
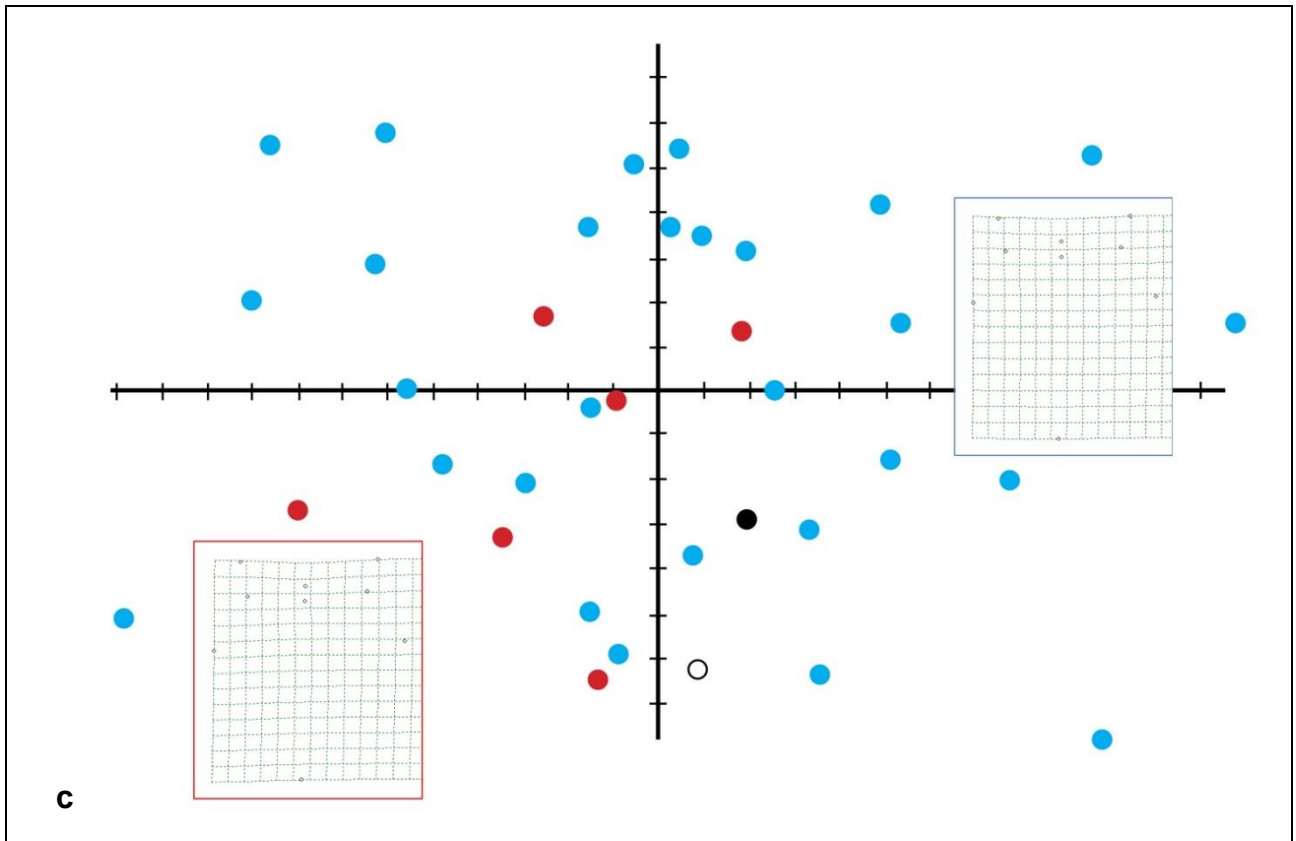


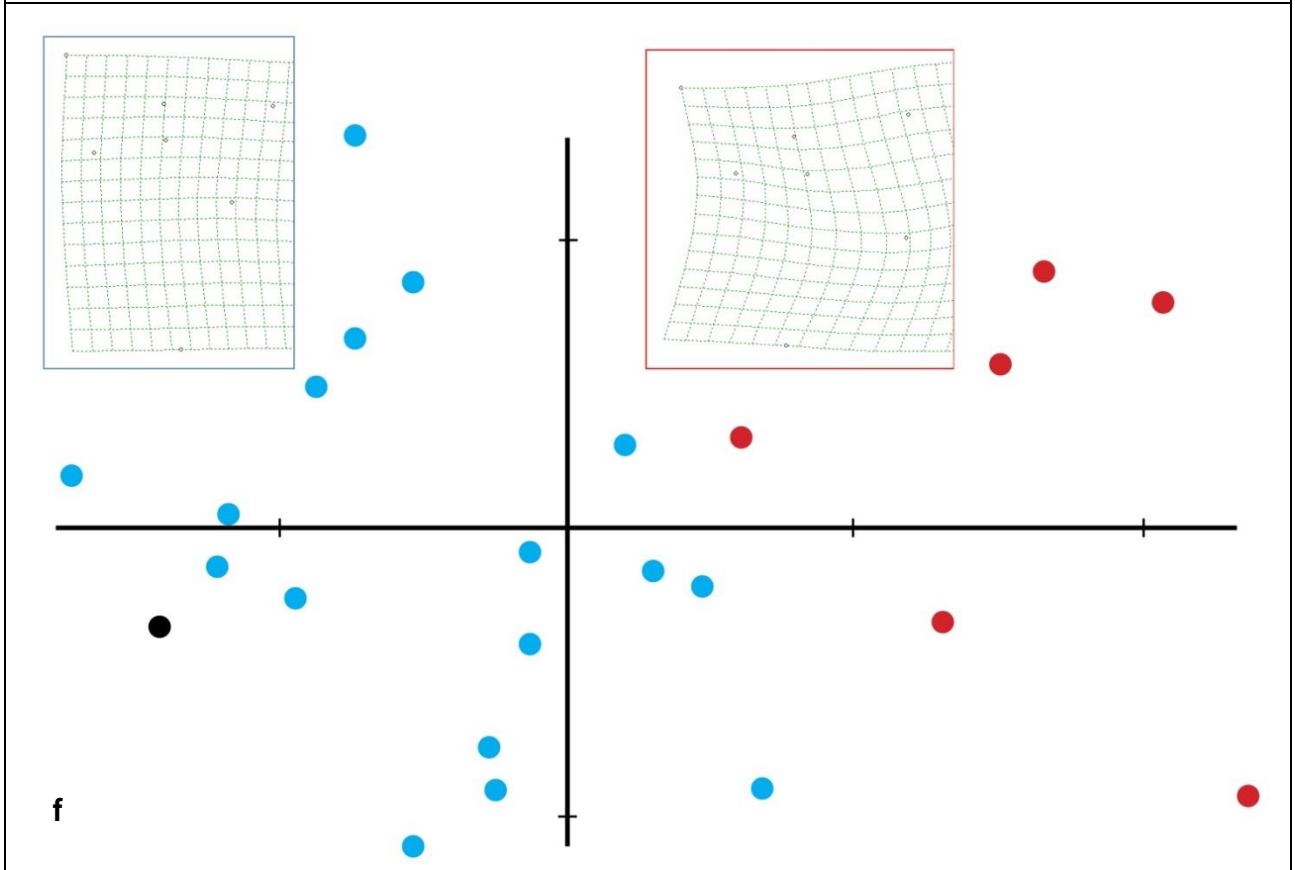
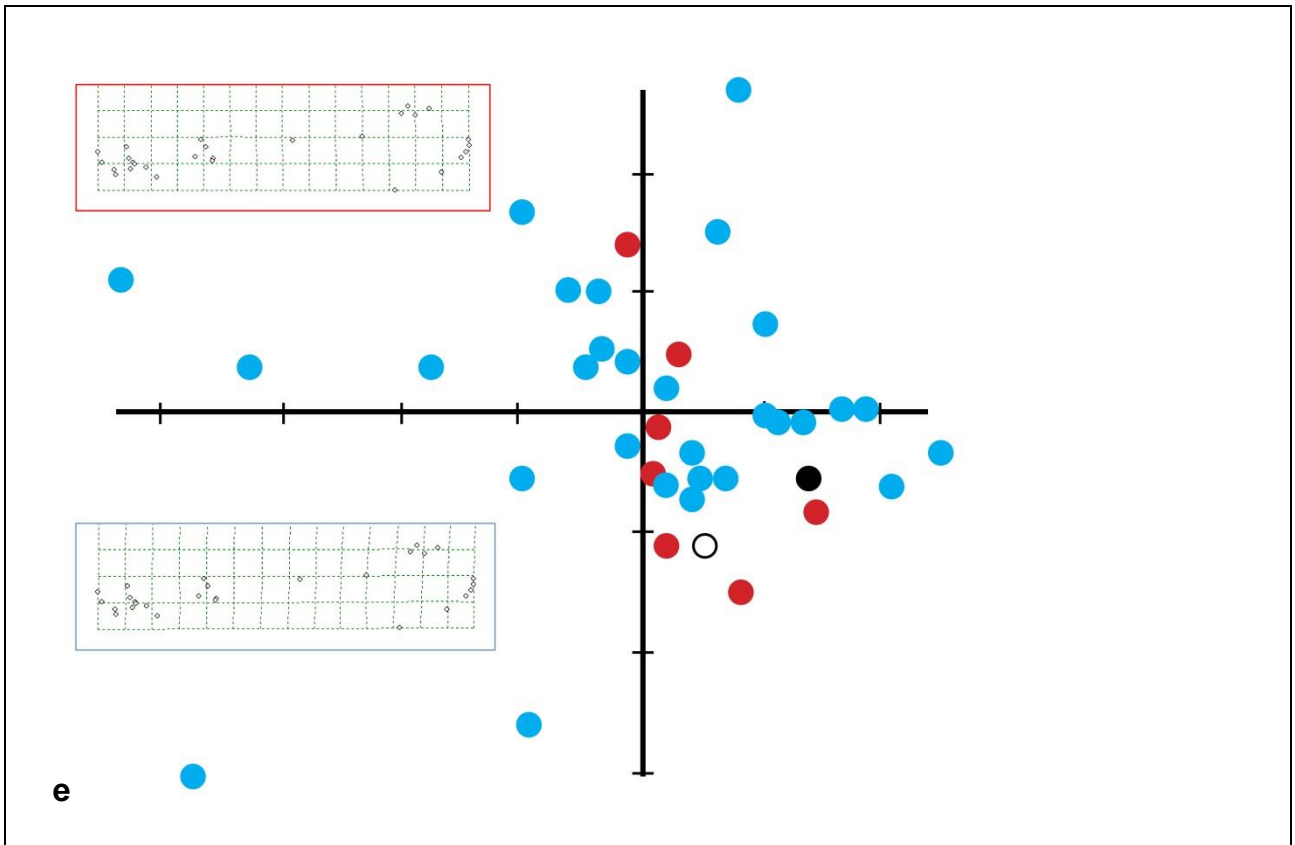
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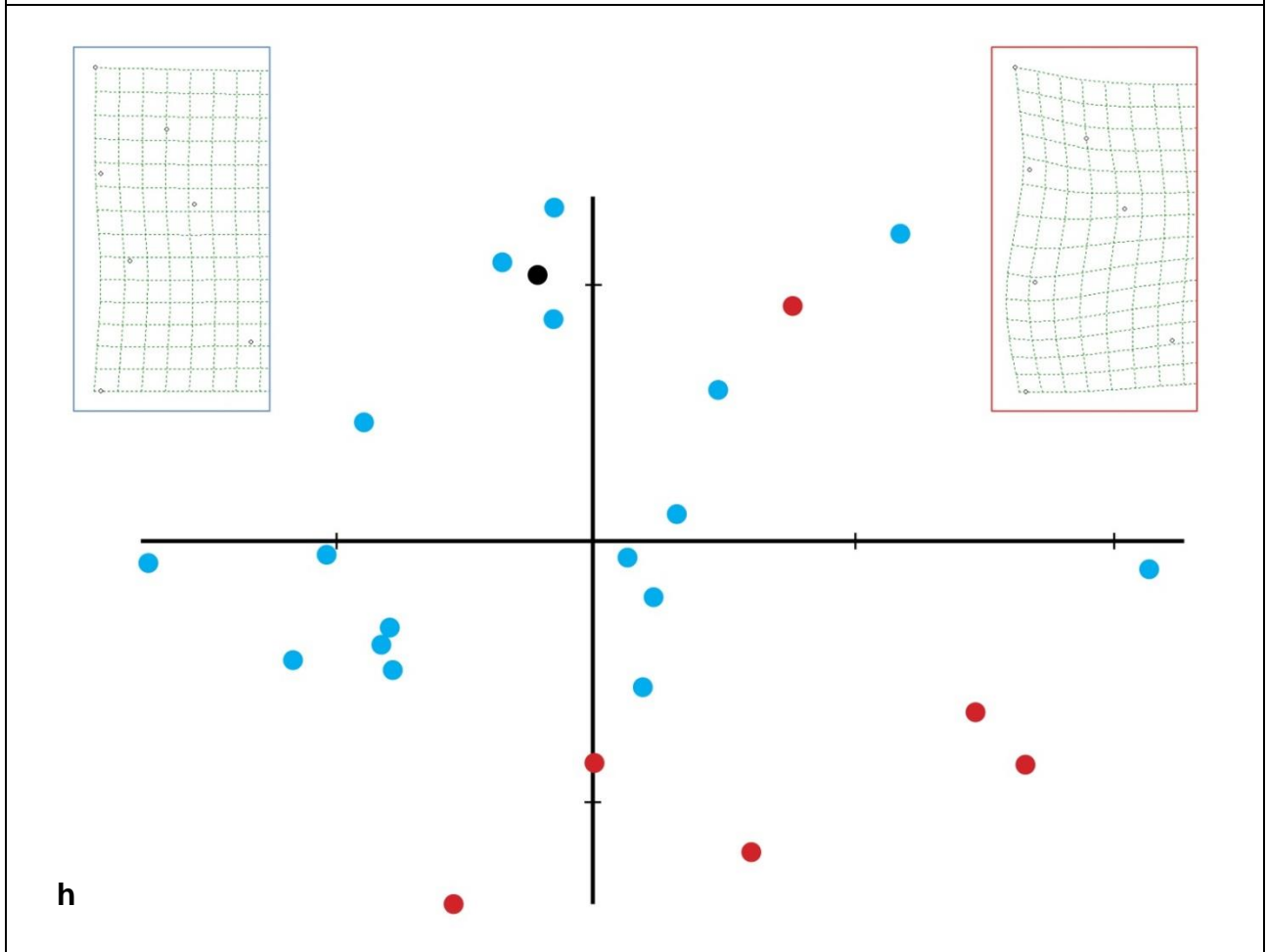
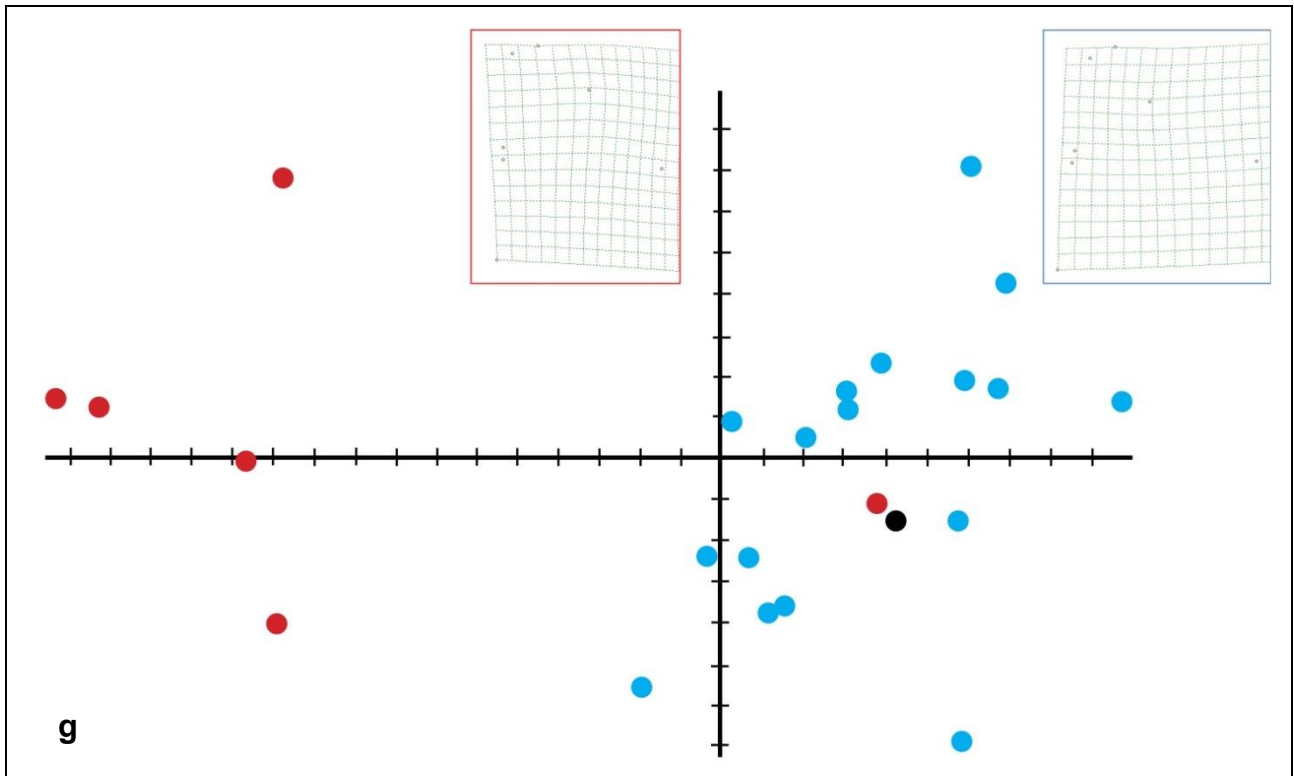


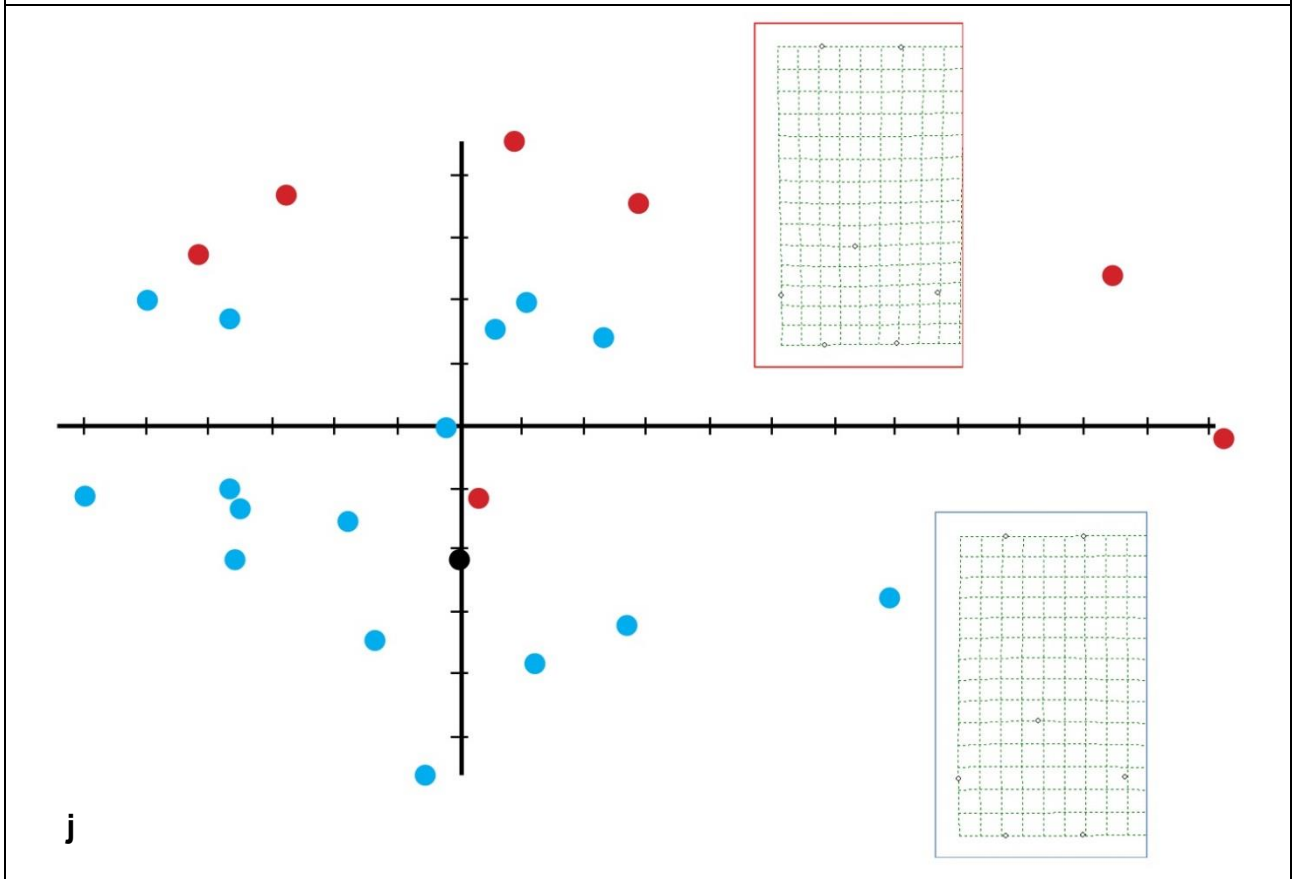
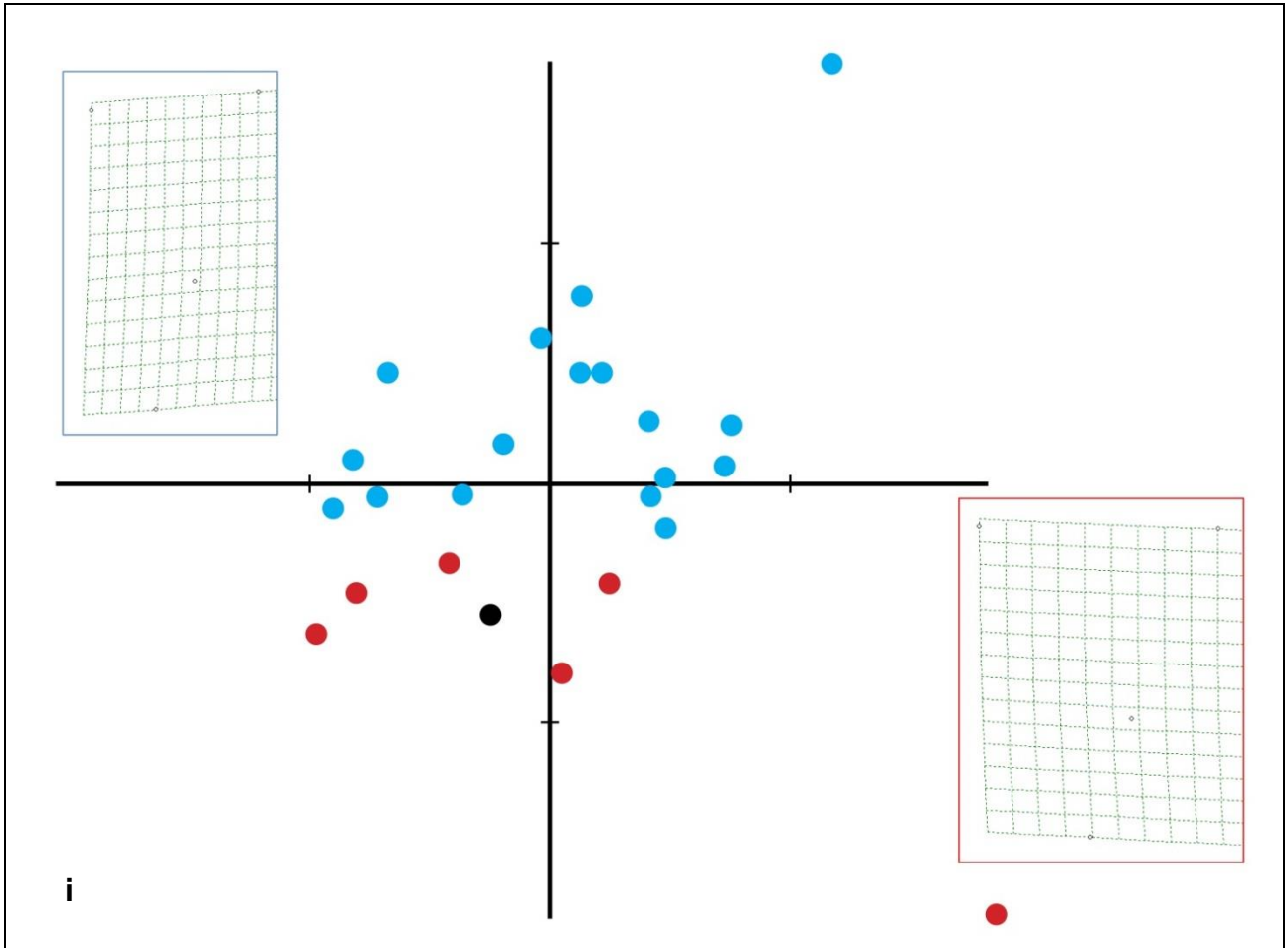
Appendix











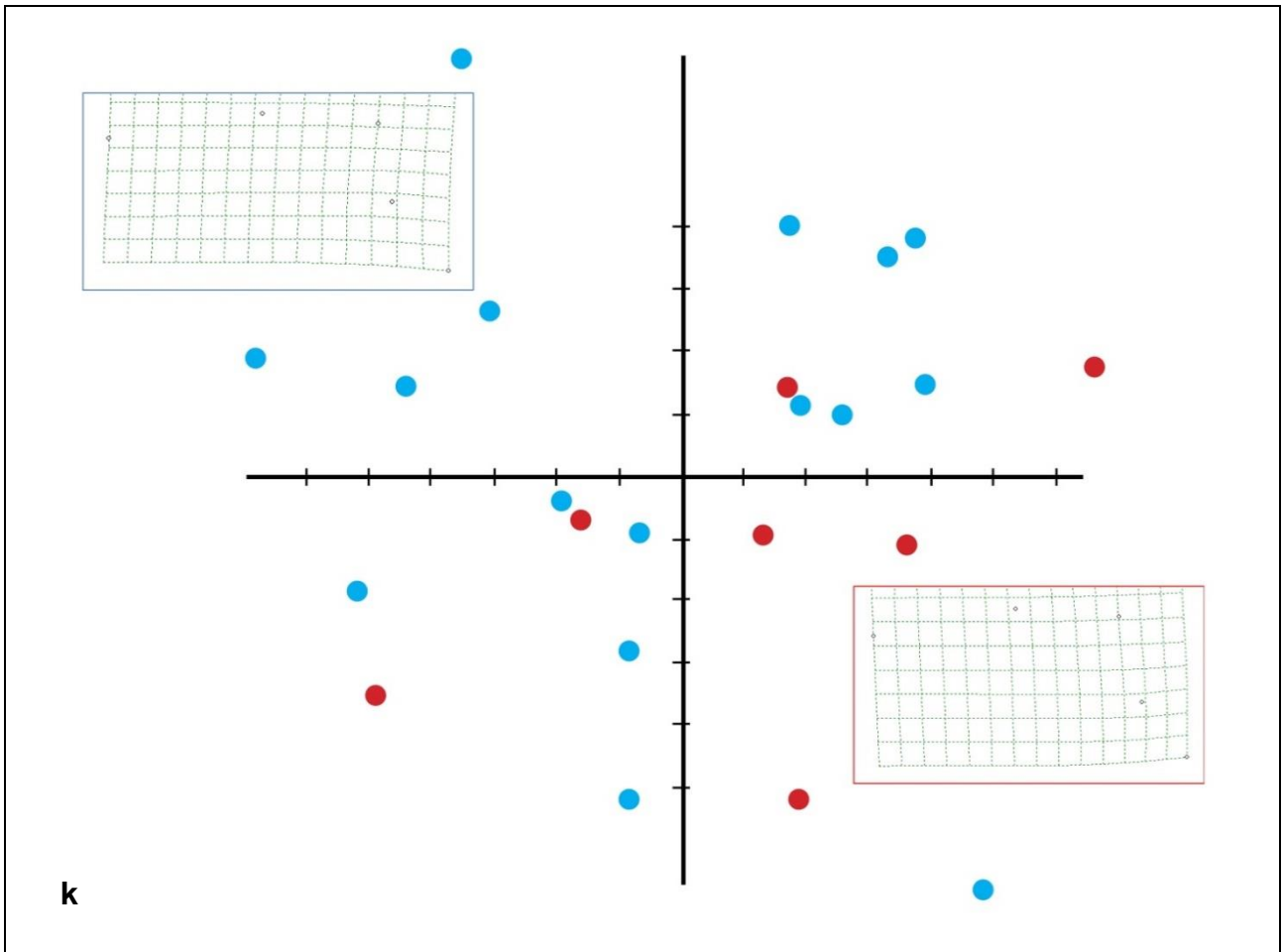


Figure 3. Shape variations in the investigated morphological traits (see Tables 1 and 2 for the percentage of shape variation explained by RWS 1 and 2 and their statistical comparison among populations): a-b) head dorsal and frontal views; c-d) thorax dorsal and lateral views; e) wing; f-i) male appendages dorsal, lateral, posterior and ventral views; j-k) penis dorsal and lateral views. Legend for all figures: x axis RWS1; y axis RWS2; red dot - Chatham Island; blue dot - South Island; black solid dot - *X. sinclairi* holotype; black outline - *X. sinclairi* allotype.







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80	2011	Villanueva, Reagan, Philippinen	Odonata of the Catanduanes-Island, The Philippines
81	2012	Villanueva, Reagan, Philippinen	Odonata of Dinapigue, The Philippines
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84		Marinov, Milen, Christchurch	Odonata of Solomon Islands
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86	2012	Do Manh, Cuong, Hanoi, Vietnam	Mau Son Mountain Odonata, Vietnam
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89	2013	Vincent Kalkman/A.B. Orr, The Netherlands/Australia	Field guide New Guinea Zygoptera
90	2013	Oleg Kosterin, Russia	Progress study Cambodia 2013
91	2013	Dejan Kulijer, Bosia & Herzegovina	Odonata fauna of karst streams and rivers of South Herzegovina (Bosnia and Herzegovina, West Balkan)
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95	2013	Garrison, Rosser/von Ellenrieder, Natalia, Sacramento, USA	The genus <i>Argia</i> in Costa Rica
96	2013	Villanueva, Reagan, Philippinen	Odonata of Surigao del Sur, Philippines
97	2014	Dawn, Prosenjit, Kolkata, India	Rheophilous Odonata diversity of protected areas of Chhattisgarh, India
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