Title: Performance of Pairwise Shape Dissimilarity Morphometrics on Non-mammalian Taxa (Insecta: Neuroptera: Mantispidae)

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Short title: Pairwise Shape Dissimilarity

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Abstract

Morphometric dissimilarity metrics aim to quantify the variation between compared specimens such that inferences about their relatedness and alpha taxonomy can be made. Recently, the technique has developed metrics that purport to quantify shape dissimilarity between specimens – employing the use of least squares regression analysis. These metrics have been well-applied by studies in the hominin fossil record with an arguably unsubstantiated backing for the technique. Originally postulated was the $\log_{10} se_m$ metric which subsequently led to the standard error test of the null hypothesis (STET) metric. Following this, the standard deviation of logged ratios (S_{LR}) metric arose as a pairwise dissimilarity metric that constrains the regression to a zero-intercept, i.e., a significant development in the robustness of the technique. This metric was tested on extant primates in order to evaluate its effectiveness alongside the two other metrics. It was shown to be the most reliable for comparisons between specimens of primates, but was unable to discriminate between hetero- and con-specific comparisons. Arguably, an alternative model organism with which to compare the technique is lacking. This study considers shape dissimilarity metrics with respect to a group of non-mammalian organisms (mantidflies) and tests the metrics against three lines of evidence (morphology, CO1-DNA and geographic distribution) that can delimit the species-level taxonomy for the group. It is shown that the metrics are unable to discriminate between pairwise comparisons of closely related species, resulting in biologically erroneous groupings, and contradicting the groupings derived from morphological, CO1-DNA and distributional comparisons. It is thus asserted that the technique is unsuitable for use in alpha taxonomy as an additional line of evidence in mantidflies. It is further supposed that morphometrics in general should be employed

with caution in studies of evolutionary history as phylogeny is not the only information contained within morphometric data.

Keywords: standard deviation of logged ratios, log se_m, standard error test of the null hypothesis, mantispid, DNA

Introduction

Morphometrics is a set of methods that use organismal shape variables that can be analyzed in order to deduce ontogenic, evolutionary and/or experimental treatment conclusions for the subject in question (Rohlf and Marcus, 1993). Traditional morphometrics, or multivariate morphometrics, applies multivariate statistical methods to a set of measured distances between defined landmarks on an organism (Rohlf and Marcus, 1993). Nested within morphometrics, shape dissimilarity metrics attempt to quantify the degree of dissimilarity in morphological shape between any two given specimens. This can be used to interpret the degree of relatedness between organisms under the assumption that similar shapes are present in closely related organisms. Thackeray and colleagues (1997a, b) originally postulated that conspecificity between two specimens can be deduced by using a simple morphometric method. The method entails taking consistent measurements of structural homologues between specimens, plotting the measurements on *xy* axes and inferring a linear regression. The standard error of the m-coefficient (slope of linear regression) can then be analyzed to infer relatedness. The standard error of the m-coefficient (se_m) is taken as a test statistic to quantify the probability of conspecificity between compared specimens. This test statistic is ultimately derived from a linear regression associated with the equation of a straight line (Equation 1; see Fig. 1). Once $\log_{10}$ transformed, the $\log_{10} se_m$-statistic is a

measure of the degree of scatter around the regression line. Therefore lower $\log_{10} se_m$-values relate to less variability, or dissimilarity, in shape between compared specimens, and as such generally arise when specimens are of the same species (Thackeray, 2010). Moreover, the metric being based upon standard error of the slope in least squares regression means that it should reflect both geometric and allometric shape variation (Aiello and Collard, 2000). As such, the metric should be somewhat unaffected by differences in the size of compared specimens and should provide a view to the morphometric proportionality between compared specimens.

Equation 1: $y = mx + c$

The technique, ultimately rooted in the conceptual realm of phenetics (Sneath and Sokal, 1973), was developed by Thackeray et al. (1997a, b, 2007) using both vertebrates and invertebrates. The study by Thackeray et al. (1997b) reports that intraspecific comparisons resulted in a central tendency across the $\log_{10} se_m$ values. The 95% confidence parameters for conspecific $\log_{10} se_m$-values were noted as -1.61 ± 0.23, similar to the controversial 'golden ratio' (Thackeray, 1997a, 2007, Livio, 2002). Importantly, however, interspecific comparisons were left unexplored. Nevertheless, the $\log_{10} se_m$-value was hypothesized to potentially serve as a 'biological species constant' and the $\log_{10} se_m$-interval of -1.61 ± 0.23 was consequently regarded as a potential statistical definition of a species (Thackeray, 2007). However, further applications of the method regarding closely related taxa are key to refining the technique (Thackeray et al., 1997b).

The method outlined above has been well-applied in paleoanthropology with regards to hominid taxonomy (Thackeray et al., 1997a, b, Wolpoff and Lee, 2001, Lee and

Wolpoff, 2005, Thackeray et al., 2005, Wolpoff and Lee, 2006, 2007, Thackeray and Prat, 2009, Cofran and Thackeray, 2010, Houghton and Thackeray, 2011, Thackeray and Odes, 2013). However, apart from the initial study conducted by Thackeray et al. (1997a, b), the robustness of the method remains to be tested further on both extant and non-hominid/non-primate organisms. Only one study by Gordon and Wood (2013), has addressed the performance of the method with regards to heterospecific comparisons using non-hominid primates. However, as it stands, no study has tested the technique on non-primates, and information regarding the performance thereof is lacking.

The test statistic ($\log_{10} se_m$) however, has been criticized by a series of authors. The first was the 'regression dilution bias' (although not called thusly by the authors) noted by Wolpoff and Lee (2001). Regression dilution bias is a bias in the regression slope towards the x-axis (Draper et al., 1998, Frost and Thompson, 2000). Thus, different statistics are produced when the same specimens are conversely compared on different axes. This makes the statistic unreliable because one regression can result in a $\log_{10} se_m$-value that may place specimens closer to one another with a higher probability of conspecificity (within the proposed conspecficity interval), while the opposite regression can result in a lower probability of conspecificity (falling outside the interval). Wolpoff and Lee (2001) therefore developed the standard error test of the null hypothesis (STET). The standard error test of the null hypothesis uses the Pythagorean Theorem to combine both variants of the se_m-statistic derived from regressions of *x* on *y* and of *y* on *x* in the form of a hypotenuse of a right-angled triangle; the orthogonal sides of which are analogous to both se_m-statistics (Equation 2). The authors also argued against the $\log_{10}$-transformation of the se_m-statistic, citing the dampening effects of the $\log_{10}$-transformation as undesirable and unjustified. As such, the STET value was left

untransformed. However, the statistic may be multiplied 100-fold to allow for ease of interpretation as the values tend to be smaller than 0.01 which represents highly decimalized, and thus impractical numbers to work with. As with the $\log_{10} se_m$-statistic, lower STET values relative to higher STET values relate to a higher probability of conspecificity.

Equation 2: $STET = [(se_{mx})^2 + (se_{my})^2]^{1/2}$

However, Gordon and Wood (2013) demonstrate that both $\log_{10} se_m$ and STET are flawed in that they incorporate a non-zero intercept within the regression. This poses a problem as the metric produced misrepresents the data by 'fitting' the regression to the data. This would normally not be a problem for a standard regression analysis between a dependent and a predictor variable. However, when comparing two specimens where there is no predictor variable to regress a dependent variable onto, the regression is not being compared to an absolute. This can cause different shapes to be interpreted as similar (see Figure 2 in Gordon and Wood 2013 as an example). Gordon and Wood (2013) concluded that both statistics are therefore unreliable for measuring variation in shape and developed the standard deviation of logged ratios metric as a solution (S_{LR}; Equation 3). The S_{LR}-metric considers *y* and *x* as vectors containing the set of homologous measurements for both specimens respectively. This metric was derived to constrain the regression to a zero-intercept through the origin and thus prevents the metric from misrepresenting the data by allowing the regression to be compared to an absolute (the origin). The metric also incorporates a log-transformation to remedy the problem of greater leverage with larger measurements compared to smaller ones in regressions. In so doing, the log-transformation considers proportional differences in

measurement instead of absolute differences. S_{LR}-values closer to zero (0) represent greater degrees of similarity in shape and measure shape dissimilarity.

Equation 3: $S_{LR} = \sqrt{[VAR\{\log(y/x)\}]}$

Study organism

Mantidflies (Insecta: Neuroptera: Mantispidae) are easily identified by the presence of raptorial forelegs and an elongated pronotum (Welch and Kondratieff, 1991). The taxonomy of the family is unresolved due to their complex morphology and a confusing scientific legacy left in the literature (Snyman et al., 2012). The Afrotropical endemic mantispid genus *Pseudoclimacliella* Handschin, 1960 is no different and is in dire need of taxonomic revision. A robust taxonomy can be achieved by integrating different techniques nested in morphology, biogeography and molecular analysis (Snyman et al., 2012). Without a robust taxonomic foundation, the prospect of novel discoveries in other biological fields, such as conservation or agriculture may remain elusive (Ohl, 2007).

The Mantispinae exhibit an interesting life history. Hypermetamorphic ontogeny occurs inside a spider egg-sac where the mantispine larva feeds exclusively on spider eggs (Redborg, 1998). A small and mobile first-instar larva develops into an immobile and grub-like third-instar larva before pupating. Spider eggs are a rare food source and the larva must take full advantage of the available eggs (Redborg and Macleod, 1985, Redborg, 1998). Additionally, the larva can produce an allomone that slows the development of the eggs. However, if only a small number of eggs are available to the larva, it will pupate sooner. The resulting adult may therefore differ up to a 100% in size compared with siblings which had access to a larger food source (Redborg and

Macleod, 1985, Redborg, 1998). Since the above mentioned method claims to be immune to differences in size, it may prove to be extremely valuable for organisms such as mantispines with an ontogeny that may result in major intraspecific size and shape differences.

Two species of *Pseudoclimaciella* were chosen for the study. *Pseudoclimaciella tropica* (Westwood, 1852) and *P. loanga* (Navás, 1909) are two species that exhibit large intraspecific size variation and crypsis. Such potential for size variation and cryptic species in *Pseudoclimaciella* will test the performance of the technique's accuracy. Additionally, the wide distributions in the chosen test species (Fig. 2) bring to light the possibility of a species complex within each species. If true, this will likewise serve to test the sensitivity of the method with respect to crypsis. Furthermore, being non-mammalian and non-primate, the taxa are ideal models for testing the technique on novel taxa.

In summary, the study aims to:

1. Test the shape dissimilarity metrics as a tool for delimiting non-primate, non-mammalian species.
2. Test the robustness, sensitivity and response of the shape dissimilarity metrics to monophyly, closely related species and crypsis.

Materials and Methods

Morphological comparisons

Twenty *Pseudoclimaciella tropica* (Westwood, 1852) and eleven *Pseudoclimaciella loanga* (Navás, 1909) were used in this study (Table 1). Specimens used in this study are housed in the following collections: South African National Collection of Insects (SANC), Agricultural Research Council, Roodeplaat, RSA; Musee Royal de l'Afrique Centrale (MRAC), Tervuren, Belgium; Museum für Naturkunde (ZMB), Berlin, Germany and Mr. L.P. Snyman's personal collection at the University of Pretoria, South Africa. Database accessions of specimens used (Table 1) refer to records contained in the Palpares Relational Database (Mansell and Kenyon, 2002).

The same specimens used in the morphometric analysis were used for morphological comparison. Each specimen was prepared by following a similar method to that which was used by Lambkin (1986). Hereafter, the genitalia was dissected out and then transferred into an excavated glass slide filled with glycerine for visual comparison under a Leica M165C microscope. Photographs were taken using a Canon 550D equipped with a 100mm macro Canon lens with three lens extensions for small structures. All photos were stacked using Helicon focus (version 5.3.7.2) and enhanced when necessary using Corel Paintshop Pro 4X. From these visual comparisons, *sensu strictu* species delimitations were made based on genitalic morphology. Species are treated as *senu lato* and *sensu strictu* in the context of this study. It is not the goal of this paper to describe new species, however, these delimitations may prove useful for such work in the future. A combination of Lambkin's (1986) and Hoffman's (1992) terminology for genitalia was used. Subsequent to the study, prepared genitalia and

terminalia were labeled and stored in glycerine in 2mm Eppendorf tubes attached to the specimens' pin to allow for future study.

Molecular analysis

Extraction, amplification and precipitation: The mitochondrial protein coding gene Cytochrome Oxidase I (COI) was chosen for genetic characterization as it has a relatively high mutation rate and is therefore suitable for low-level species classification (Simon et al., 1994). For the extraction of genomic DNA from tissue, NucleoSpin® Tissue kits were used as per manufacturer protocol (Macherey-Nagel, Düren, Germany). Primers used for amplification were C1-J-2183 and TL2-N-3014 (Simon et al., 1994), using the following parameters: Initial denaturation of 95°C (5 min); 33 cycles of 93°C (20 s), 50±2°C (40 s), 72°C (2 min); final elongation of 72°C for 5 min. Polymerase chain reaction (PCR) was performed in a final volume of 50ul containing approximately 50 – 100 ng of genomic DNA, 2.5 mM $MgCl_2$, 20 pmol of each primer, 10 mM dNTP's, 1 X buffer in the presence of one unit of *taq* (Super-Therm®, Separation Scientific SA (PTY) LTD, South Africa). All purifications were done using NucleoSpin® Gel and PCR Clean-up kits (Macherey-Nagel, Düren, Germany), following the manufacturers specifications. Cycle-sequencing reactions were prepared using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and a standard sodium acetate/ethanol precipitation protocol was followed for precipitation of the products.

Analysis: All sequences generated were assembled and edited in CLC Bio Main Workbench version 7 (http://www.clcbio.com). The sequences obtained were aligned using the online version of MAFFT version 7 with default parameters

(http://mafft.cbrc.jp/alignment/server/index.html). Aligned sequences were viewed and checked manually in Mega version 6.06 (Tamura et al., 2013). Edited sequences were submitted to GenBank (for accession numbers see Table 1). Data-display networks (also called neighbor-networks) were constructed with SplitsTree4 (Huson and Bryant, 2006). Parsimony uninformative sites were included and p-distances uncorrected. The use of network graphs is particularly useful on species level where the evolutionary history might not always be tree-like. In many such cases, more than one tree is needed to explain the relationship among studied specimens whereas one network may suffice (Huson and Bryant, 2006).

Species included: The genus *Pseudoclimaciella* Handschin, 1960 is currently undergoing revision, as such an unpublished key (being prepared as part of a thesis) was used for the identification of the two study species (key available on request from the corresponding authors). The key is based on the key published by Handschin (1960). The diagnosis for the species in this paper is as follows: Both *P. tropica* and *P. loanga* have a yellow vertex and frons as opposed to the general reddish head capsule found in several other *Pseudoclimaciella* species. *P. loanga* has a black occiput followed by a semi-circular transverse black band on the posterior margin of the vertex. *P. loanga* has a maximum of two hyaline costal cells between the pterostigma and the pigmentation on the wing apices. The apical pigmentation is small and generally comprises more than five cells. *P. tropica* has a black occiput followed by a straight transverse black band on the posterior margin of the vertex. *P. tropica* has a minimum of three hyaline costal cells between the pterostigma and the pigmentation on the wing apices. The apical pigmentation is small and generally comprises two to four cells.

Due to the uncertainty of the status of the rest of the *Pseudoclimaciella* species, names were excluded in the phylogenetic analysis and species were referred to as sp. 1, sp. 2 etc. No *P. loanga* species from west Africa were suitable for molecular work and were subsequently excluded. Four specimens of *Pseudoclimaciella* sp 2, two specimens of *P.* sp. 1 and *P. tropica* and a single *P.* sp. 3 were sequenced and included in the analysis.

Morphometric analysis

All specimens were mounted and 21 linear measurements (mm) were taken of various homologous morphological structures that define shape using a digital caliper (Table S1). In a previous study, the S_{LR} statistic was demonstrated to be reliable for sets of 21 variables (Figure 4 in Gordon and Wood, 2013). The morphological structures measured were kept robust in order to represent the overall shape of the insect in line with communication with co-authors of the initial study (Thackeray et al., 1997b). Only males were used to control for the potential of minor morphometric sexual dimorphism. The measurements of each specimen were arranged in vectors and the $Log_{10}se_m$-, STET- and S_{LR}-metrics for each pairwise comparison was calculated in R (version 3.0.1) according to the equations above. Additionally, suspicions of allometry (non-isometric body scaling) in the dataset prompted using log-transformed measurements to make pairwise regressions with the slope taken as a measure for allometry (slopes that differ from one will reflect a presence of non-isometric scaling in the measurements analysed). From this, datasets of values for each metric in each pairwise comparison were created, and subsequently the values for each comparison were grouped into comparison types that comprised inter- and intra-specific species by species comparisons for both *sensu lato* and *sensu strictu* species conceptions (Table S2). Additionally, the above analyses were repeated excluding a single variable for each

comparison to assess the effect of each individual variable on the metric produced (Table S2). Exclusion of a single variable was chosen because selecting one variable to run the analysis on yielded no results. The mean ± 1 standard deviation was calculated for each comparison type and the grouped values were used to calculate 95% confidence intervals.

Results

Morphology

Upon inspection of the *Pseudoclimaciella tropica* and *P. loanga* genitalia two distinct types emerged for both species complexes; the west African and the southern African type. This could potentially split both *P. tropica* and *P. loanga* into two species each, in accordance with the allopatry displayed in the distributional map (Fig. 2).

Pseudoclimaciella tropica – The west African specimens (Fig. 3) exhibited a short pseudopenis and a median process with a rectangular distal tip, whereas the southern African specimens (Fig. 4) exhibited a long pseudopenis and a median process with a pointed distal tip. The southern African specimens' genitalia were on average 1.5 mm in length while the west African specimens' genitalia were on average 0.7 mm in length. Moreover, both genitalic types also corresponded to a seemingly consistent difference in adult body size with the southern African specimens larger on average in comparison to the west African specimens.

Pseudoclimaciella loanga – Both west and southern African specimens' genitalia exhibited similar overall size (approx. 1 mm), but the west African specimens (Fig. 5) exhibited a sclerotized process, perpendicular to the mediuncus, extending from the basal area of the mediuncus past the basal margin of the mediuncus, while the southern African specimens (Fig. 6) did not exhibit such a process. The southern African specimens exhibited a wider apex of the mediuncus relative to the west African specimens, which exhibited a more slender and isosceles triangular-like apex of the mediuncus. The overall body size of both southern and west African specimens were similar, unlike the *P. tropica* complex.

Molecular data

Analysis was based on CO1-DNA sequences approximately 675 basepairs long. The data-display network (Fig. 7) indicated the presence of four distinct species groups. *P. tropica* forms a distinct cluster comprising two lineages, one from west Africa and one from southern Africa. Bootstrap support was high for all nodes, and importantly, bootstrap support for the two *P. tropica* lineages both amount to 100 (Fig. 7).

Morphometric data

Log_{10} se_m – The comparisons for both *sensu strictu* and *sensu lato* species produced $\log_{10} se_m$ values that were between -1.8390 and -1.5664 for both hetero- and con-specific comparisons (Tables 2,3). No prominent patterns in the 95% confidence intervals were found that might indicate separate species in both *sensu strictu* and *sensu lato* species comparisons.

In the *sensu lato* species comparisons, the *P. tropica* - *P. tropica* comparisons showed the greatest amount of variation, while the *P. loanga* - *P. loanga* comparisons showed the least (Fig 8). Conspecific comparisons produced $\log_{10} se_m$ values that were between -1.7375 and -1.6170 (Table 2). Heterospecific comparisons produced $\log_{10} se_m$ values that were between -1.6723 and -1.6420 (Table 2).

Within the *sensu strictu* species comparisons, the southern *P. tropica* - western *P. tropica* comparisons showed the greatest amount of variation, while the southern *P. loanga* - west *P. loanga* comparisons showed the least (Fig. 8). Conspecific comparisons produced $\log_{10} se_m$ values that were between -1.8390 and -1.5891 (Table 3). Heterospecific comparisons produced $\log_{10} se_m$ values that were between -1.7542 and -1.5617 (Table 3).

STET – The comparisons for both *sensu strictu* and *sensu lato* species produced STET values that were between 0.0208 and 0.0536 for both hetero- and con-specific comparisons (Tables 4,5). No prominent patterns were noted in the 95% confidence intervals that might indicate separate species in both *sensu strictu* and *sensu lato* species comparisons.

In the *sensu lato* species comparisons, the *P. tropica* - *P. tropica* comparisons showed the greatest amount of variation, while the *P. loanga* - *P. loanga* comparisons showed the least (Fig. 8). Conspecific comparisons produced STET values that were between 0.0269 and 0.0408 (Table 4). Heterospecific comparisons produced STET values that were between 0.0356 and 0.0394 (Table 4).

Within the *sensu strictu* species comparisons, the western *P. loanga* - western *P. tropica* comparisons showed the greatest amount of variation, while the southern *P. loanga* - southern *P. loanga* comparisons showed the least (Fig. 8). Conspecific comparisons produced STET values that were between 0.0208 and 0.0403 (Table 5). Heterospecific comparisons produced STET values that were between 0.0262 and 0.0536 (Table 5).

S_{LR} – The comparisons for both *sensu strictu* and *sensu lato* species produced S_{LR} values that were between 0.1045 and 0.1867 in the 0.1 order of magnitude (Tables 6,7). No prominent patterns in 95% confidence intervals were found that might indicate separate species in both *sensu strictu* and *sensu lato* species comparisons.

In the *sensu lato* species comparisons, the *P. tropica* - *P. tropica* comparisons showed the greatest amount of variation, while the *P. loanga* - *P. loanga* comparisons showed the least (Fig. 8). Conspecific comparisons produced S_{LR} values that were between 0.1045 and 0.1373 (Table 6). Heterospecific comparisons produced S_{LR} values that were between 0.1449 and 0.1539 (Table 6).

Within the *sensu strictu* species comparisons, the southern *P. tropica* - west *P. tropica* comparisons showed the greatest amount of variation, while the southern *P. loanga* - west *P. loanga* comparisons showed the least (Fig. 8). Conspecific comparisons produced S_{LR} values that were between 0.1045 and 0.1867 (Table 7). Heterospecific comparisons produced S_{LR} values that were between 0.1272 and 0.1788 (Table 7).

Allometry scores (slope of logged regressions) – The comparisons for both *sensu strictu* and *sensu lato* species produced allometry scores that were between 0.9782 and 0.9937 for both hetero- and con-specific comparisons (Tables 8,9).

In the *sensu lato* species comparisons, the *P. tropica* - *P. tropica* comparisons showed the greatest amount of variation in allometry scores, while the *P. loanga* - *P. loanga* comparisons showed the least (Fig. 8). Conspecific comparisons produced allometry scores that were between 0.9796 and 0.9874 (Table 8). Heterospecific comparisons produced allometry scores that were between 0.9814 and 0.9836 (Table 8).

Within the *sensu strictu* species comparisons, the western *P. loanga* - western *P. tropica* comparisons showed the greatest amount of variation in allometry scores, while the southern *P. loanga* - southern *P. loanga* comparisons showed the least (Fig. 8). Conspecific comparisons produced allometry scores that were between 0.9798 and 0.9937 (Table 9). Heterospecific comparisons produced allometry scores that were between 0.9769 and 0.9859 (Table 9).

Individual variables analysis – No significant differences in 95% confidence intervals were found between comparison metrics when removing a single variable from the analysis, except in the fore- and hind-wing lengths. In comparisons where the fore- and hind-wing measurements were omitted, the $\log_{10} se_m$, STET and SLR metrics were significantly different – having non-overlapping 95% confidence intervals as compared with comparisons where other variables were omitted (Table S2). Additionally, the allometry scores were significantly lower (farther from one) in comparisons where fore- and hind-wing measurements were omitted.

Discussion

Lambkin (1986) and Hoffman (1992) note in their respective revisions of the Australian and north American Mantispidae, that variation in the morphology of the median process, mediuncus and pseudopenis of male genitalia correspond to different species.

In the *P. tropica* and *P. loanga* complexes, the genitalic differences point to two potentially separate species because such differences (mainly genitalic size for *P. tropica*) should maintain a pre-zygotic barrier to mating as per the lock-and-key hypothesis (Shapiro and Porter, 1989). What remains to be studied however, is the functional morphology of the male genitalia and terminalia with the female genitalia and terminalia as Oswald (1993) has done with the Psychopsidae (Neuroptera). Such a study may provide valuable insights into the species-level boundaries of the *Pseudoclimaciella* and Afrotropical Mantispidae in general. Nevertheless, whilst *Pseudoclimaciella* taxonomy is out of the scope of this study, as we do not wish to erect new species with this information, the results from the morphological comparisons do point to two potentially separate species within each *sensu lato* species. This, in addition to the apparent allopatry in geographic distribution, serves as a baseline for comparison to test the shape dissimilarity morphometrics technique.

Our results show an inability for the shape dissimilarity metrics to discriminate between species at both the *sensu lato* and *sensu strictu* species level. In general, hetero- and con-specific comparisons fell within an overlapping 95% confidence interval all in the same order of magnitude, and for the few comparisons that do not have overlapping 95% confidence intervals, the resulting classification would be erroneous and biologically irrelevant. This finding is in accordance with Gordon and Wood's (2013) result, which showed that the method is unable to discriminate between hetero- and con-specific comparisons. Similarly, their results produced values in an overlapping range with both types of comparisons (see Gordon and Wood, 2013 Table 4). However, a second result from their study shows that the method is, in fact, able to discriminate between hetero- and con-generic comparisons with hetero-generic comparisons

producing values an order of magnitude greater (0.1) than con-generic comparisons (0.01) (see Gordon and Wood, 2013 Table 3 – STET and S_{LR}). This can be interpreted to show that the method is only sensitive to significant differences in morphology, possibly differences so obvious that morphometric analyses are unnecessary. In light of this, one interesting result from our study is that the comparisons between mantispid species produced S_{LR} values more comparable to that of the mixed genera by Gordon and Wood (2013) being in the order of magnitude 0.1. An explanation of this result is that mantispid species might show a higher degree of shape variability than the primate species measured by Gordon and Wood (2013). This is supported by the process of their developmental biology which governs their adult size (Redborg, 1998). Larval mantispids are generalist predators of spider eggs, which means that they may feed on an assortment of food quality and quantity. When one considers this in concert with the fact that their adult size is directly dependent on the quality and quantity of their larval food source, support can be garnered to assert that they show greater degrees of shape variability than primates. The pairwise shape dissimilarity metrics might then be indicative of shape variability as a result of ontogeny, but not necessarily shape variability that is applicable to taxonomy or phylogeny.

Interestingly, the slopes of the logged regressions show that allometry is indeed present in the dataset. This result makes sense given the ontogeny of the mantispids. Our results showed the greatest variability in the shape dissimilarity metrics when *P. tropica* - *P. tropica* (for *sensu lato* species) and west *P. tropica* - southern *P. tropica* (for *sensu strictu* species) were compared (Fig. 8). Concordantly, the allometry scores for these comparisons are also widely variable. This suggests that allometry promoted greater variability in the metrics produced. This is probably due to non-isometric scaling in the

bodies of the insects. This result is interesting because it provides some quantitative support for the ontogeny of the mantispids based on shape dissimilarity metrics.

Testing the effect of individual measurements on the metrics provided support to show that no single morphometric variable had a significant effect on the metric produced – this is with exception of the hind- and fore-wings however. When the hind- and fore-wings were omitted from the analysis, the allometry scores were significantly lower (farther from one) and the 95% confidence intervals were significantly narrower – showing a greater degree of allometry when the wings are excluded from analysis. This provides reason to support that the wings of mantispids are in all probability not subject to non-isometric scaling as the rest of the body is, and as such, the wings are more similar across all the mantispids studied. Wing morphology is probably determined by physical and mechanical restrictions in aerodynamics (Snodgrass, 1935, Kingsolver and Koehl, 1985, Kingsolver and Koehl 1994, Liu, 2009), thus allometric changes due to ontogeny might yield an insect that is unable to fly. If this is true, the wing size and shape will be under influence by more restricted and strenuous selection, forcing them to stabilize phenotypically, and thus limiting allometry.

It is important to note that selection of measurements used for the morphometric analysis could potentially influence the result. Broad-scale measurements, as used here, might not be adequately sensitive to differences between species, whereas fine-scale measurements may prove useful in this regard. As such, another interpretation of the morphometric results obtained from this study is that the measurements selected were simply not refined enough for species discrimination within the genus. A worthwhile endeavor for future refinement of this method may then be to test the use of finer measurements and contrast the results with broad-scale measurements. Nevertheless, the

present results point toward the conclusion that the metric is not entirely informative for discriminating between these species – particularly when using broad-scale body measurements.

Even though classification informative conclusions can be seldom made using data-display networks, other conclusions can be made which may underpin conclusions for classification. The ambiguities (separation) between the two lineages (*P. tropica* and *P. loanga*) are indicative of independently evolving linages with limited gene flow between the populations from the two geographic regions. The molecular analysis therefore supports the morphological conclusions but contradicts the morphometric data. As such, *P. tropica* can be viewed as either two distinct *sensu strictu* species or as two sub-species. Since no molecular data for *P. loanga* could be generated for analysis, but similar genitalic differences were noted, we assume that the same is true for *P. loanga.*

In conclusion, there are two main findings that we can take from this study. In the first place, these results provide evidence to show that the use of pairwise shape dissimilarity metrics in delimiting species taxa, as suggested by Thackeray (1997a,b, 2010) and applied by many other authors, is certainly not applicable in Mantispidae species. Moreover, we caution that this may potentially prove to be the case in several other taxa as well – insect taxa in particular, but also fossil taxa where shape dissimilarity morphometrics has been often used. This is in line with the arguments against the use of these methods in Gordon and Wood (2013).

A second issue in the use of this method lies in its initial premise. The underlying premise of the method assumes that specimens of similar shape would be more closely

related to one another. While this might be true, it is not without complication – and it is this complication which undermines the method's utility. This assumption is too simplified, as many other factors can influence the shape of an organism besides phylogeny. In the present study on mantispids, quantitative evidence was found for one life history trait that is important for ontogeny – being, adult size as derived from food source. The metrics which our comparisons produced were greatly variable. This likely owes to the fact that the size and shape of the mantispids in our study were under the influence from various different sources of food across Africa – which may contribute to allometry in the body of the adult. As such, the mantispid shape variation is not only a function of phylogeny, but also of ontogeny. Thus, we generally caution the use of this method in isolation – and especially on fossils. Fossil data is usually collected from small sample sizes where intraspecific variation might be difficult to determine. While the variation might prove to infer some degree of relatedness (phylogeny), the complicating factors to organismal shape (such as ontogeny) cannot be quantified with inadequate sample sizes. The strength of this morphometric method lies in its ease of application and simplicity. However, it seems as though the method is in fact oversimplified by relying too heavily on an unsound assumption. As such, we assert that the morphometric method tested in this study is unsuitable for deriving taxonomic conclusions, and will be better used in order to study the shape variation in organisms as a result of other factors (such as ontogeny or experimental treatment). Beyond this, the use of many fine-scale measurements, as in geometric morphometrics, may be preferred and could yield substantially more robust results – provided it can tease apart phylogeny from ontogeny.

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Table 1: Table showing study, database and GenBank accession numbers for specimens measured in this study. Database accessions refer to records contained in the Palpares Relational Database (Mansell and Kenyon, 2002)

Species	Study Accession	Database Accession	Collection	GenBank Name/Accession
P. tropica	MAN Gen Prep 15	NEUR50011	SANC	-
	MAN Gen Prep 20	NEUR50106	L.P. Snyman	-
	MAN Gen Prep 21	NEUR50105	L.P. Snyman	-
	MAN Gen Prep 28	NEUR50001	SANC	-
	MAN Gen Prep 29	NEUR50108	L.P. Snyman	-
	MAN Gen Prep 34	NEUR50104	L.P. Snyman	-
	MAN Gen Prep 36	NEUR50103	L.P. Snyman	-
	MAN Gen Prep 39	NEUR50117	MRAC	-
	MAN Gen Prep 60	NEUR50114	ZMB	-
	MAN Gen Prep 61	NEUR50115	ZMB	-
	MAN Gen Prep 62	NEUR50115	ZMB	-
	MAN Gen Prep 63	NEUR50109	SANC	-
	MAN Gen Prep 48	NEUR50122	MRAC	-
	MAN Gen Prep 50	NEUR50111	SANC	-
	MAN Gen Prep 51	NEUR50112	SANC	-
	MAN Gen Prep 65	NEUR50113	L.P. Snyman	-
	MAN Gen Prep 66	NEUR50113	L.P. Snyman	-
	MAN Gen Prep 67	NEUR50113	L.P. Snyman	-
	MAN Gen Prep 68	NEUR50113	L.P. Snyman	-
	MAN Gen Prep 69	NEUR50113	L.P. Snyman	-
	…	NEUR50125	L.P. Snyman	Pseudo108/KP901401
	…	NEUR50118	L.P. Snyman	Psivy/KP901404
P. loanga	MAN Gen Prep 41	NEUR50116	MRAC	
	MAN Gen Prep 42	NEUR50110	MRAC	-
	MAN Gen Prep 46	NEUR50121	MRAC	-
	MAN Gen Prep 52	NEUR50027	SANC	-
	MAN Gen Prep 53	NEUR50027	SANC	-
	MAN Gen Prep 54	NEUR50027	SANC	-
	MAN Gen Prep 56	NEUR50027	SANC	-
	MAN Gen Prep 57	NEUR50027	SANC	-
	MAN Gen Prep 58	NEUR50027	SANC	-
	MAN Gen Prep 43	NEUR50118	MRAC	-
	MAN Gen Prep 45	NEUR50119	MRAC	-
Pseudoclimaciella spp.	*P.* sp1	NEUR50127	L.P. Snyman	Pseudo32/KP901402
	P. sp1	NEUR50129	L.P. Snyman	Pseudo29/KP901403
	P. sp2	NEUR50125	L.P. Snyman	Pseudo40/KP901396
	P. sp2	NEUR50128	L.P. Snyman	Pseudo72/KP901397
	P. sp2	NEUR50123	L.P. Snyman	Pseudo35/KP901398
	P. sp2	NEUR50119	L.P. Snyman	Pseudo18/KP901399
	P. sp4	NEUR50120	L.P. Snyman	Pseusp/KP901400

Table 2: Table showing results of $\log_{10} se_m$ comparisons for all variables in *Sensu lato* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by ^{a,b,c} and are compared across rows.

	P. loanga	*P. tropica*
P. loanga	-1.7114 ± 0.0867[a] -1.7375 – -1.6854 n=45	-1.6571 ± 0.1094[b] -1.6723 – -1.6420 n=200
P. tropica	-1.6571 ± 0.1094[b] -1.6723 – -1.6420 n=200	-1.6380 ± 0.1291[b] -1.6563 – -1.6170 n=190

Table 3: Table showing results of $\log_{10} se_m$ comparisons for all variables in *Sensu strictu* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by [a,b,c] and are compared across rows.

	P. loanga S	*P. loanga* W	*P. tropica* S	*P. tropica* W
P. loanga S	-1.7994 ± 0.0710[a] -1.8390 – -1.7598 n=15	-1.6574 ± 0.0490[b] -1.6780 – -1.6367 n=24	-1.7345 ± 0.0803[c] -1.7542 – -1.7147 n=66	-1.6210 ± 0.1093[b] -1.6508 – -1.5912 n=54
P. loanga W	-1.6574 ± 0.0490[a] -1.6780 – -1.6367 n=24	-1.7077 ± 0.0582[a] -1.7688 – -1.6466 n=6	-1.6310 ± 0.0898[a,b] -1.6582 – -1.6037 n=44	-1.6015 ± 0.1039[b] -1.6366 – -1.5664 n=36
P. tropica S	-1.7345 ± 0.0803[a] -1.7542 – -1.7147 n=66	-1.6310 ± 0.0898[b] -1.6582 – -1.6037 n=44	-1.7377 ± 0.0740[a] -1.7576 – -1.7177 n=55	-1.5879 ± 0.1315[b] -1.6142 – -1.5617 n=99
P. tropica W	-1.6210 ± 0.1093[a] -1.6508 – -1.5912 n=54	-1.6015 ± 0.1039[a] -1.6366 – -1.5664 n=36	-1.5879 ± 0.1315[a] -1.6142 – -1.5617 n=99	-1.6232 ± 0.1011[a] -1.6574 – -1.5891 n=36

Table 4: Table showing results of STET comparisons for all variables in *Sensu lato* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by [a,b,c] and are compared across rows.

	P. loanga	*P. tropica*
P. loanga	0.0285 ± 0.0055[a] 0.0269 - 0.0302 n=45	0.0375 ± 0.0135[b] 0.0356 - 0.0394 n=200
P. tropica	0.0375 ± 0.0135[a] 0.0356 - 0.0394 n=200	0.0388 ± 0.0144[a] 0.0367 - 0.0408 n=190

Table 5: Table showing results of STET comparisons for all variables in *Sensu strictu* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by [a,b,c] and are compared across rows.

	P. loanga S	*P. loanga* W	*P. tropica* S	*P. tropica* W
P. loanga S	0.0229 ± 0.0039[a] 0.0208 - 0.0250 n=15	0.0319 ± 0.0036[b] 0.0304 - 0.0335 n=24	0.0274 ± 0.0055[a] 0.0262 - 0.0288 n=66	0.0444 ± 0.0134[c] 0.0407 - 0.0480 n=54
P. loanga W	0.0319 ± 0.0036[a,b] 0.0304 - 0.0335 n=24	0.0291 ± 0.0040[a] 0.0248 - 0.0333 n=6	0.0355 ± 0.0069[b] 0.0334 - 0.0376 n=44	0.0480 ± 0.0165[c] 0.0424 - 0.0536 n=36
P. tropica S	0.0274 ± 0.0055[a] 0.0262 - 0.0288 n=66	0.0355 ± 0.0069[b] 0.0334 - 0.0376 n=44	0.0276 ± 0.0050[a] 0.0262 - 0.0290 n=55	0.0456 ± 0.0154[c] 0.0425 - 0.0487 n=99
P. tropica W	0.0444 ± 0.0134[a] 0.0407 - 0.0480 n=54	0.0480 ± 0.0165[a] 0.0424 - 0.0536 n=36	0.0456 ± 0.0154[a] 0.0425 - 0.0487 n=99	0.0371 ± 0.0094[b] 0.0339 - 0.0403 n=36

Table 6: Table showing results of S_{LR} comparisons for all variables in *Sensu lato* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by ^{a,b,c} and are compared across rows.

	P. loanga	*P. tropica*
P. loanga	0.1307 ± 0.0217[a] 0.1242 - 0.1373 n=45	0.1495 ± 0.0324[b] 0.1449 - 0.1539 n=200
P. tropica	0.1495 ± 0.0324[b] 0.1449 - 0.1539 n=200	0.1160 ± 0.0209[a] 0.1045 - 0.1275 n=190

Table 7: Table showing results of S_{LR} comparisons for all variables in *Sensu structu* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by ^{a,b,c} and are compared across rows.

	P. loanga S	*P. loanga* W	*P. tropica* S	*P. tropica* W
P. loanga S	0.1160 ± 0.0209^{a} 0.1045 - 0.1275 n=15	0.1406 ± 0.0178^{b} 0.1331 - 0.1482 n=24	$0.1339 \pm 0.0270^{a,b}$ 0.1272 - 0.1405 n=66	0.1556 ± 0.0335^{b} 0.1464 - 0.1647 n=54
P. loanga W	$0.1406 \pm 0.0178^{a,b}$ 0.1331 - 0.1482 n=24	0.1276 ± 0.0175^{a} 0.1093 - 0.1460 n=6	$0.1539 \pm 0.0302^{b,c}$ 0.1447 - 0.1631 n=44	0.1631 ± 0.0323^{a} 0.1522 - 0.1740 n=36
P. tropica S	0.1339 ± 0.0270^{a} 0.1272 - 0.1405 n=66	0.1539 ± 0.0302^{b} 0.1447 - 0.1631 n=44	0.1342 ± 0.0312^{a} 0.1258 - 0.1427 n=55	0.1702 ± 0.0427^{b} 0.1617 - 0.1788 n=99
P. tropica W	0.1556 ± 0.0335^{a} 0.1464 - 0.1647 n=54	0.1631 ± 0.0323^{a} 0.1522 - 0.1740 n=36	0.1702 ± 0.0427^{a} 0.1617 - 0.1788 n=99	0.1747 ± 0.0355^{a} 0.1627 - 0.1867 n=36

Table 8: Table showing results of regression slopes (allometry scores) from logged comparisons for all variables in *Sensu lato* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by [a,b,c] and are compared across rows.

	P. loanga	*P. tropica*
P. loanga	0.9858 ± 0.0053[a] 0.9842 - 0.9874 n=45	0.9825 ± 0.0079[b] 0.9814 - 0.9836 n=200
P. tropica	0.9825 ± 0.0079[b] 0.9814 - 0.9836 n=200	0.9810 ± 0.0098[b] 0.9796 - 0.9824 n=190

Table 9: Table showing results of regression slopes (allometry scores) from logged comparisons for all variables in *Sensu strictu* species. For each comparison (top-to-bottom): mean ± 1 standard deviation, 95% confidence interval and n=number of comparisons is shown. Significant differences are denoted by [a,b,c] and are compared across rows.

	P. loanga S	*P. loanga* W	*P. tropica* S	*P. tropica* W
P. loanga S	0.9880 ± 0.0047[a] 0.9854 - 0.9906 n=15	0.9837 ± 0.0051[a] 0.9816 - 0.9859 n=24	0.9839 ± 0.0068[a] 0.9823 - 0.9856 n=66	0.9829 ± 0.0077[a] 0.9808 - 0.9850 n=54
P. loanga W	0.9837 ± 0.0051[a,b] 0.9816 - 0.9859 n=24	0.9886 ± 0.0048[a] 0.9836 - 0.9937 n=6	0.9807 ± 0.0081[b] 0.9782 - 0.9831 n=44	0.9817 ± 0.0096[a,b] 0.9785 - 0.9850 n=36
P. tropica S	0.9839 ± 0.0068[a] 0.9823 - 0.9856 n=66	0.9807 ± 0.0081[a,b] 0.9782 - 0.9831 n=44	0.9834 ± 0.0073[a] 0.9814 - 0.9854 n=55	0.9791 ± 0.0110[b] 0.9769 - 0.9813 n=99
P. tropica W	0.9829 ± 0.0077[a] 0.9808 - 0.9850 n=54	.9817 ± 0.0096[a] 0.9785 - 0.9850 n=36	0.9791 ± 0.0110[a] 0.9769 - 0.9813 n=99	0.9827 ± 0.0088[a] 0.9798 - 0.9857 n=36

Figure Legends

Fig. 1: Linear regression model depicting standard error of slope error (se_m). Reversing the axes i.e. regressing specimen A onto B (opposite to what is shown) results in a different se_m value. These two values are used to obtain the mean $\log_{10} se_m$ and STET test statistics described in the text.

Fig. 2: African map showing the localities of the *Pseudoclimaciella* specimens used in this study. Triangles represent the southern African (white) and west African (black) *P. loanga* complex specimens respectively. Points represent the southern African (black) and west African (white) *P. tropica* complex specimens respectively. The two *sensu lato* species may each be split across the geographic gradient by distributional, CO1-DNA and genitalic evidence, resulting in south-eastern and west variants for each complex.

Fig. 3: Habitus (A), genitalia (ventral, B; dorsal, C) of west African *P. tropica*. Scale bar applies to B and C only. Median process with a rectangular distal tip (i), short pseudopenis (ii) and small genitalia size are diagnostic characters in the context of this study. Specimen details: Ivory Coast, Comoe nat. park, 08 44'N 03 49 W, 21.xi.1999, K. Mody; MAN Gen Prep 65.

Fig. 4: Habitus (A), genitalia (ventral, B; dorsal, C) of southern African *P. tropica*. Scale bar applies to B and C only. Median process with a pointed distal tip and long pseudopenis are diagnostic characters in the context of this study.

Specimen details: South Africa, Limpopo, Wolkberg Wild. Area, 24°02'S 30°05'E, 15.xii.2005, M. Potgieter; MAN Gen Prep 50.

Fig. 5: Habitus (A), genitalia (dorsal, B; ventral, C) of west African *P. loanga.* Scale bar applies to B and C only. A slender, isosceles triangular-like apex of the mediuncus (i) and the presence of a basal mediuncal process (ii) extending past the basal margin of the mediuncus are diagnostic characters in the context of this study. Specimen details: Côte d'Ivoire [Ivory Coast], Bingerville, vi- 1962, J. Decelle; MAN Gen Prep 42.

Fig. 6: Habitus (A), genitalia (ventral, B; dorsal, C) of southern African *P. loanga.* Scale bar applies to B and C only. A broad apex of the mediuncus relative to the west African specimens and the lack of a basal mediuncal process found in the west African specimens are diagnostic characters in the context of this study. Specimen details: South Africa, Tvl, Soutpan, Pretoria Dist., 25.24S 28.06E. 9.xii.1987. M.W. Mansell; MAN Gen Prep 58.

Fig. 7: Data-display network of *Pseudoclimaciella* CO1-DNA data. Bootstrap values are shown. *P. tropica* highlighted in green.

Fig. 8: Boxplots of variation in shape dissimilarity metrics. *Sensu strictu* and *Sensu lato* comparisons denoted by s.s. and s.l. respectively. Green boxplots indicate comparisons between same species and blue boxplots represent comparisons between different species – with reference to *sensu strictu* and *sensu lato* species conceptions.

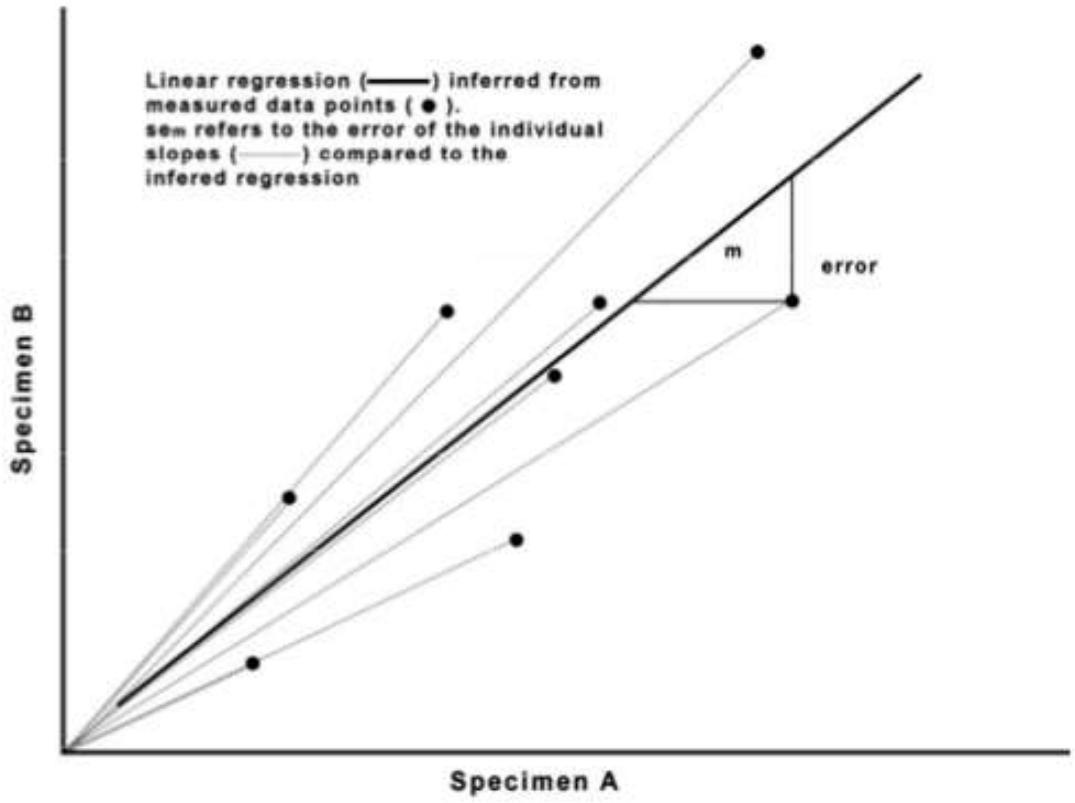

Linear regression (——) inferred from measured data points (●).
se_m refers to the error of the individual slopes (——) compared to the infered regression
m
error
Specimen B
Specimen A

Fig. 1

Fig. 2

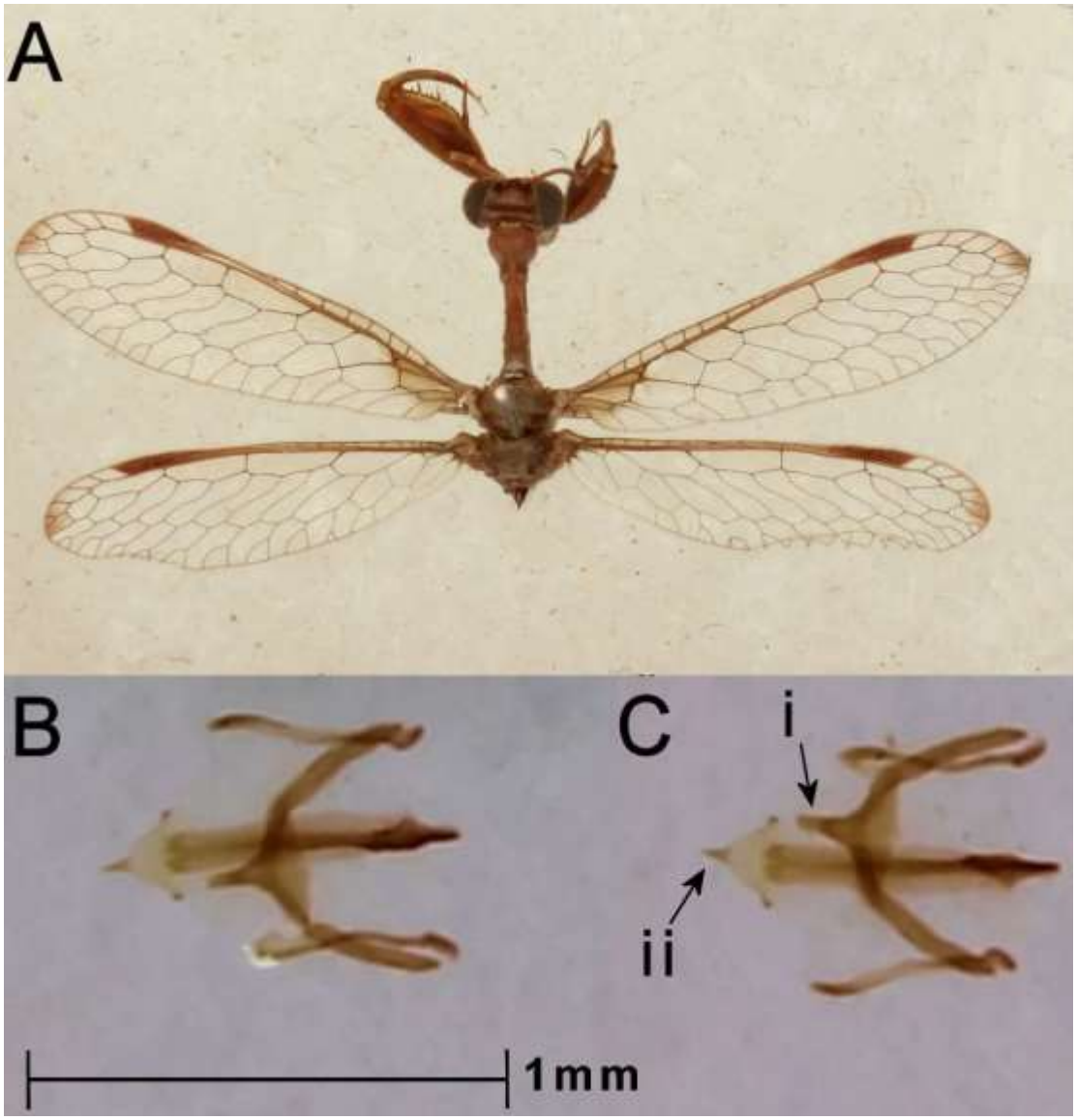


Fig. 3

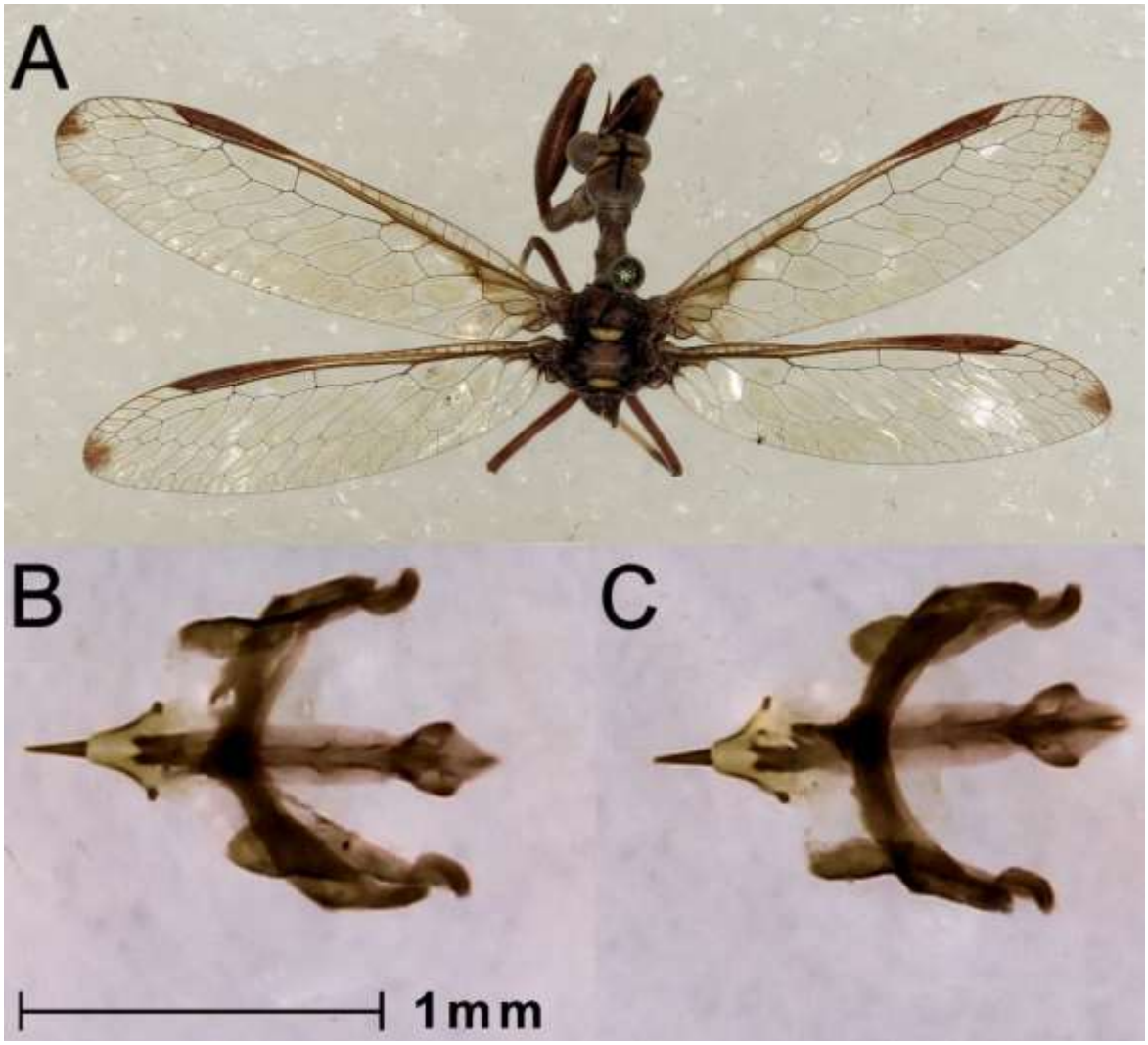


Fig. 4

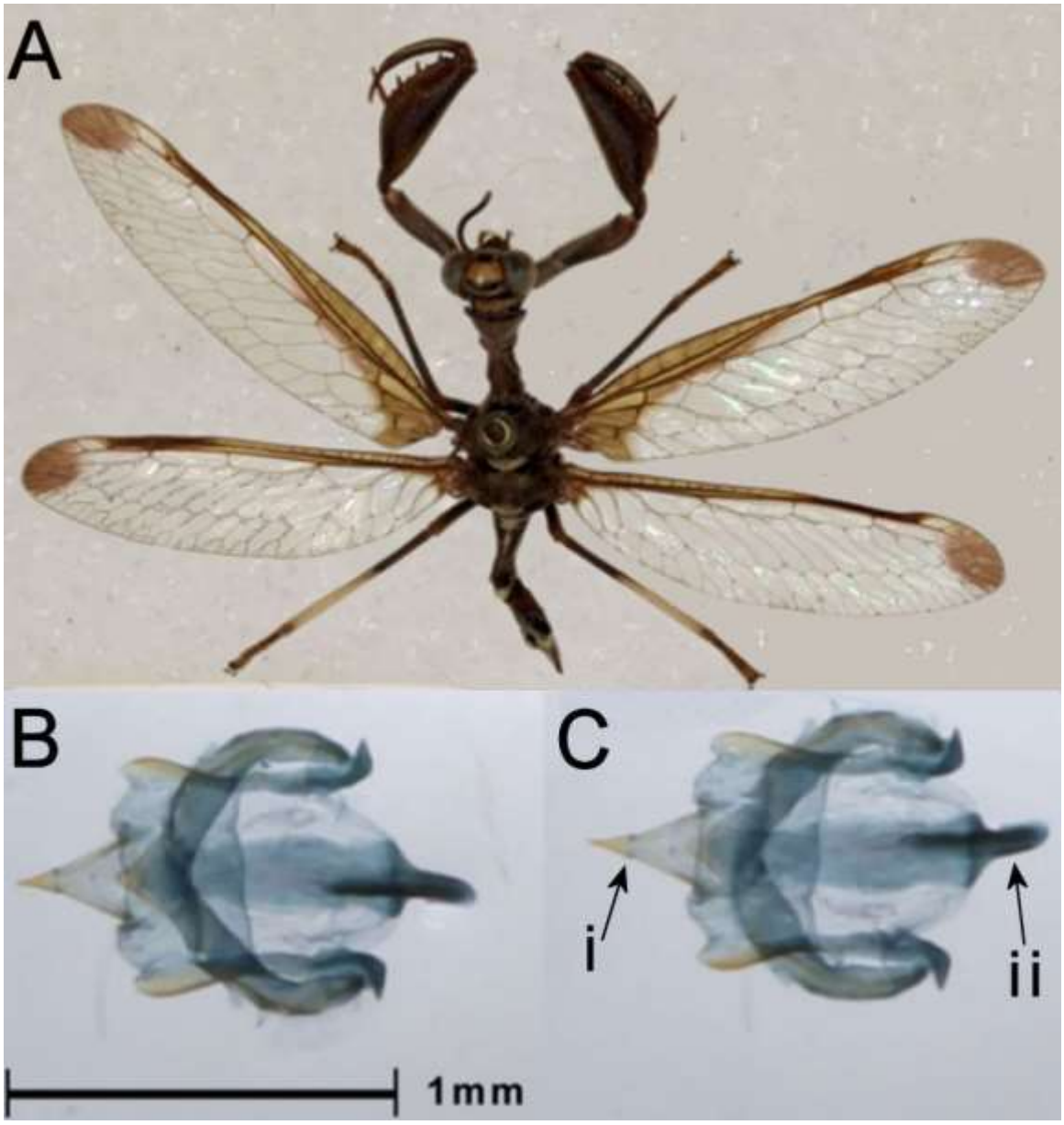


Fig. 5

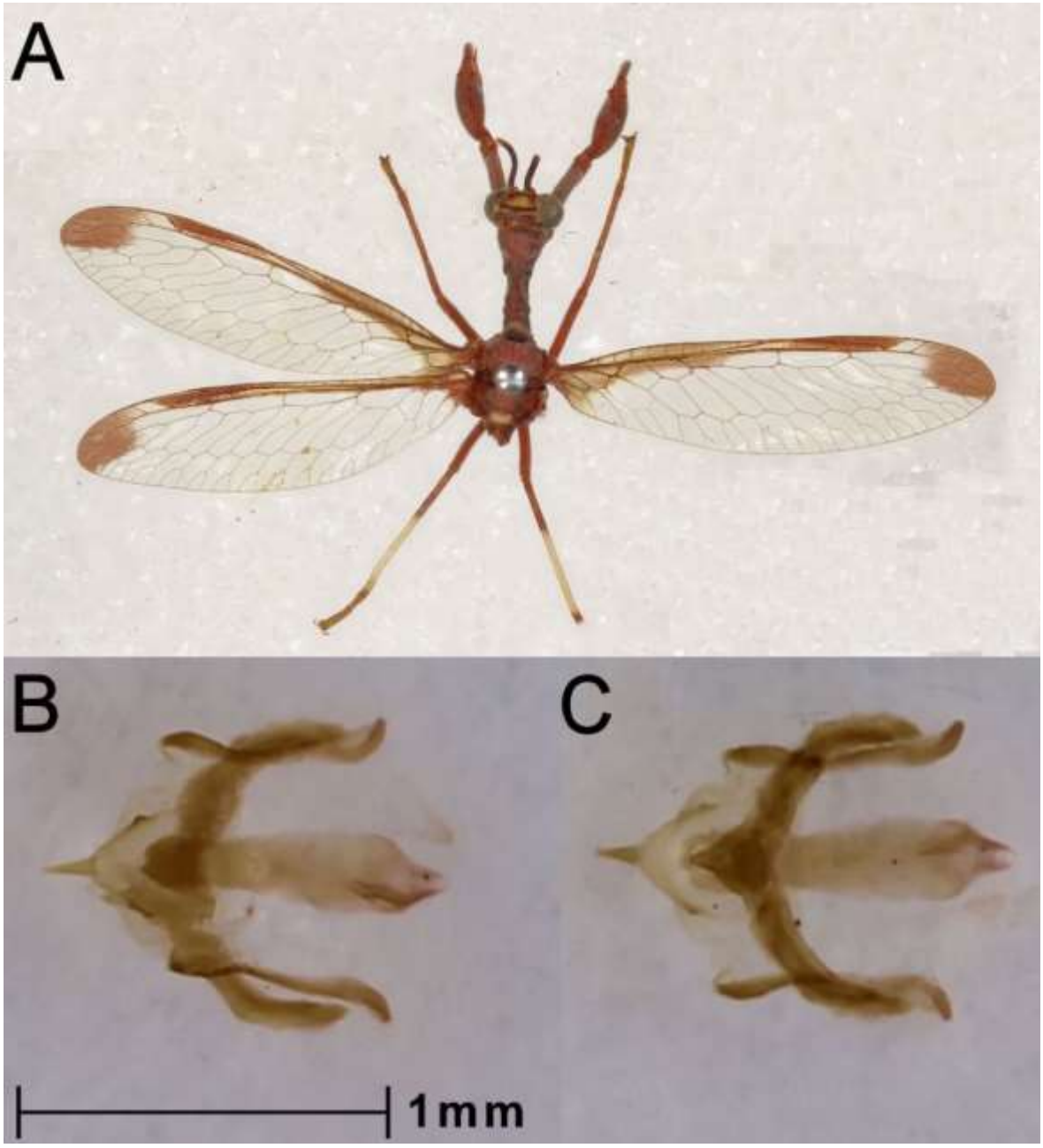


Fig. 6

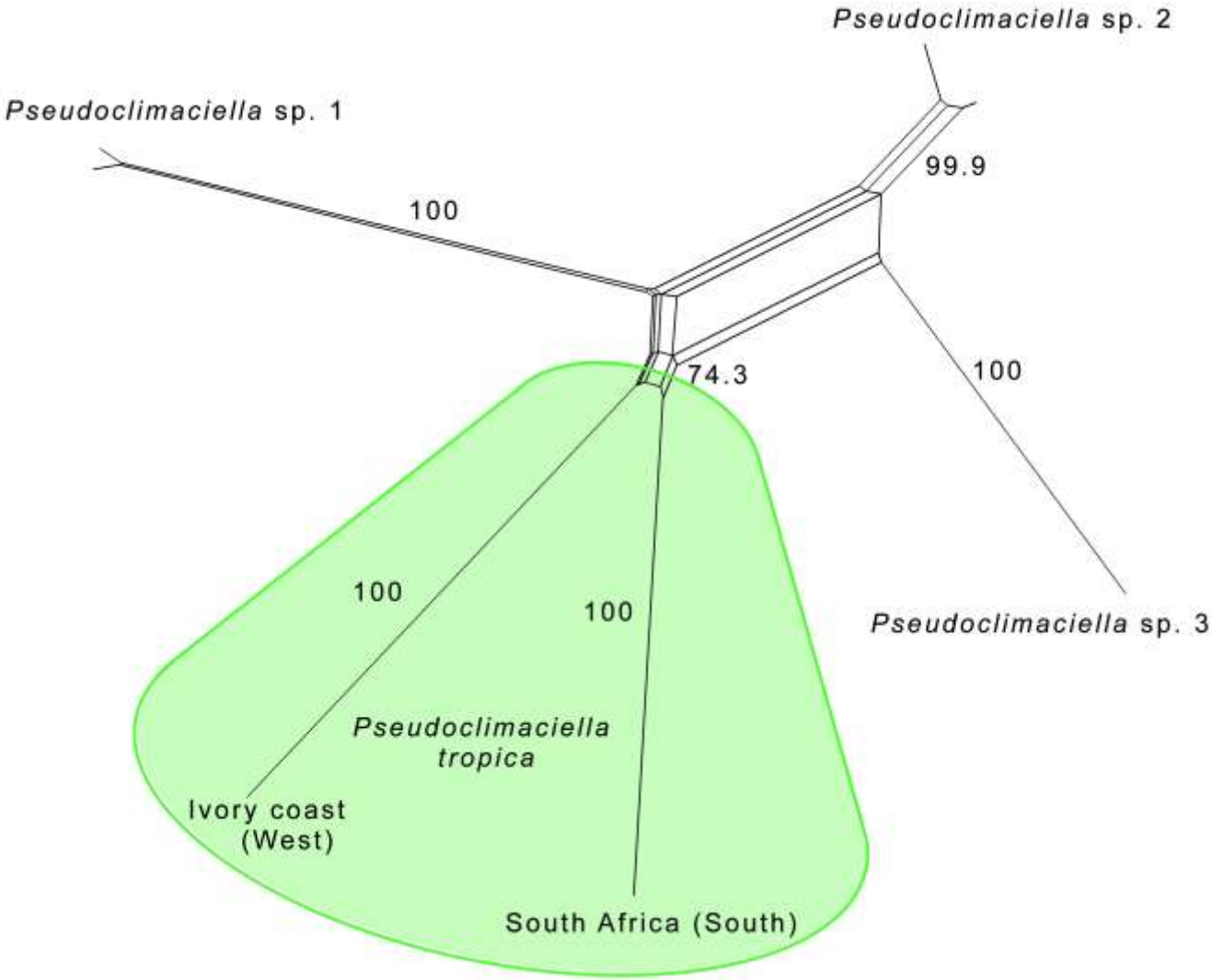
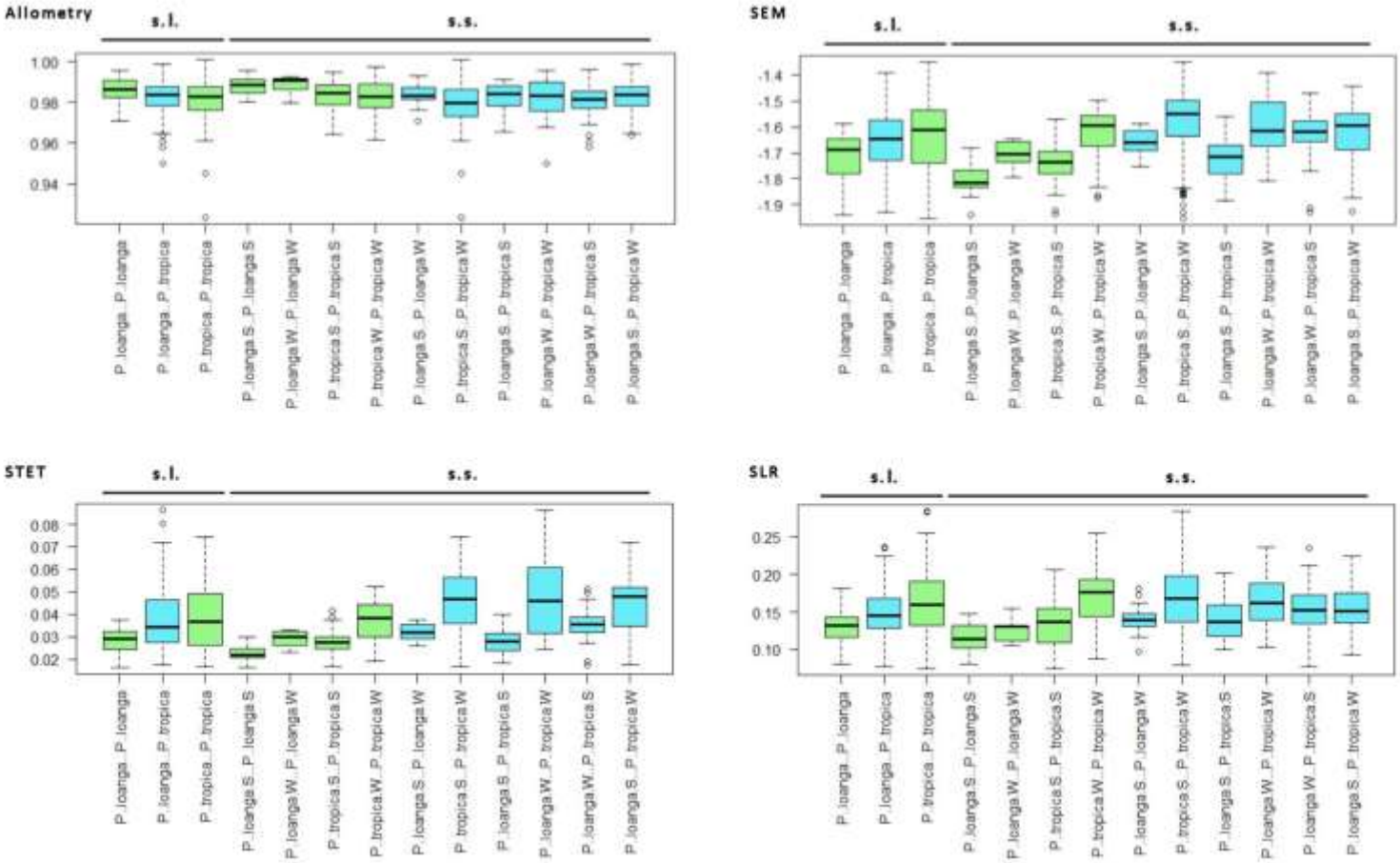

Pseudoclimaciella sp. 2
Pseudoclimaciella sp. 1
99.9
100
74.3
100
100
100
Pseudoclimaciella sp. 3
Pseudoclimaciella tropica
Ivory coast (West)
South Africa (South)

Fig. 7


Allometry
s.l.
s.s.
1.00
0.98
0.96
0.94
P. loanga_P. loanga
P. loanga_P. tropica
P. tropica_P. tropica
P. loanga S_P. loanga S
P. loanga W_P. loanga W
P. tropica S_P. tropica S
P. tropica W_P. tropica W
P. loanga S_P. loanga W
P. tropica S_P. tropica W
P. loanga S_P. tropica S
P. loanga W_P. tropica W
P. loanga W_P. tropica S
P. loanga S_P. tropica W
SEM
s.l.
s.s.
-1.4
-1.5
-1.6
-1.7
-1.8
-1.9
P. loanga_P. loanga
P. loanga_P. tropica
P. tropica_P. tropica
P. loanga S_P. loanga S
P. loanga W_P. loanga W
P. tropica S_P. tropica S
P. tropica W_P. tropica W
P. loanga S_P. loanga W
P. tropica S_P. tropica W
P. loanga S_P. tropica S
P. loanga W_P. tropica W
P. loanga W_P. tropica S
P. loanga S_P. tropica W
STET
s.l.
s.s.
0.08
0.07
0.06
0.05
0.04
0.03
0.02
P. loanga_P. loanga
P. loanga_P. tropica
P. tropica_P. tropica
P. loanga S_P. loanga S
P. loanga W_P. loanga W
P. tropica S_P. tropica S
P. tropica W_P. tropica W
P. loanga S_P. loanga W
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P. loanga S_P. tropica S
P. loanga W_P. tropica W
P. loanga W_P. tropica S
P. loanga S_P. tropica W
SLR
s.l.
s.s.
0.25
0.20
0.15
0.10
P. loanga_P. loanga
P. loanga_P. tropica
P. tropica_P. tropica
P. loanga S_P. loanga S
P. loanga W_P. loanga W
P. tropica S_P. tropica S
P. tropica W_P. tropica W
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P. tropica S_P. tropica W
P. loanga S_P. tropica S
P. loanga W_P. tropica W
P. loanga W_P. tropica S
P. loanga S_P. tropica W

Fig. 8