

## A description of a new species of *Diasemopsis* (Diptera, Diopsidae) from the Comoro Islands with morphological, molecular and allometric data

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### Abstract

A new species of *Diasemopsis* (Diptera, Diopsidae) from Comoro Islands is described and illustrated for the first time with allometric datasets. *Diasemopsis comoroensis* Carr & Földvári is shown to be genetically close, but morphologically distinct from the widespread Afro-tropical species *D. meigenii* (Westwood); notably a significant divergence in the degree of sexual dimorphism within eye span has occurred between the two species. A revised molecular phylogeny of the genus *Diasemopsis* is presented based on the partial sequences of four genes.

**Key words:** Diopsidae, *Diasemopsis*, Comoro Islands, new species, morphological description, genitalia, eye span allometry, molecular phylogeny

### Introduction

The diopsid stalk-eyed flies are a diverse Schizophoran family, comprising approximately 160 known species. Diopsidae is divided into the Centroncinae, which do not possess eyestalks and the Diopsinae, all of which do. Uniquely amongst dipterans males and females possess eyestalks, at the end of which are located both their eyes and antennae.

Recent molecular and morphological studies have produced a robust phylogeny of the stalk-eyed flies (Baker *et al.* 2001, Meier & Baker 2002, Kotrba and Balke 2006). Emphasis within these studies has been placed on the genus *Diasemopsis*, a group of 51

species found predominantly in sub-Saharan Africa. The composition of the genus *Diasemopsis* has been the source of some debate, with the species *D. meigenii* and *D. minuta* being placed in monotypic genera by Séguy (1955); however later authors have determined, using both morphological and molecular data, that these species can be confidently described as belonging to the genus *Diasemopsis* (Shilito 1971, Baker *et al.* 2001).

Here we describe a previously uncharacterised *Diasemopsis* species collected from the island of Mohéli, Comoro Islands and establish its phylogenetic position within the genus *Diasemopsis*. We compared aspects of its morphology with its sibling species, *D. meigenii*, and highlighted a recent, rapid divergence in sexual dimorphism with respect to eye span.

## Methods and Materials

All allometric and molecular work was performed on individuals taken from the same laboratory population as the morphologically described specimens.

### *Morphology*

*Males:* The specimens were studied with an Olympus (SZ 60) stereoscopic microscope at magnifications of 10–112.5 times. The dissected genital parts (on microscopic slides) were examined with an Olympus (BX40) light microscope attached with a drawing tube. The drawings were made with this drawing tube (“camera lucida”). The original pencil drawings were copied in ink on tracing paper then scanned at 50%. The photographs were made with the SZ60 microscope equipped with an Olympus C 5050Z digital camera.

Male genitalia were examined after treatment with 10% NaOH. In some cases the vials containing the dissected abdomen were heated to reduce the reaction time. After different parts were cleared sufficiently, they were put in lactic acid, 70% alcohol and glycerine. The separated postabdomen was put into a drop of gelatine-glycerine on a microscopic slide. This mixture is solid at room temperature and becomes fluid after careful heating.

After examination and drawing the genital parts were cleaned with NaOH or KOH and then placed in a small plastic vial filled with glycerine and attached to the same pin which supports the mounted specimen.

*Females:* The morphology of internal female genital organs was studied in freshly killed specimens. The organs were removed under a dissecting scope and embedded on a microscope slide in polyvinyl lactophenol with an admixture of chlorazol black E. The preparations were studied and documented in bright field as well as DIC contrast using a Zeiss Axioskop compound microscope equipped with a Zeiss AxioCam digital camera.

### Allometric Comparisons

*D. comoroensis* and *D. meigenii* were reared, in the laboratory, under variable larval density to generate the variance in adult size required for the investigation of allometry (David *et al.* 1998; Cotton *et al.* 2004). Eclosing individuals (*D. comoroensis*: 31 males and 36 females; *D. meigenii*: 41 males and 38 females) were collected, frozen and measured to an accuracy of 0.01 mm using a monocular microscope and the image analysis program NIH Image (Version 1.55). Measurements were taken of eye span (between the outermost lateral edges of the eye bulbs) and body length (from the front of the face to the tips of the wings; Baker & Wilkinson 2001).

Absolute trait size data were non-normally distributed so differences between sexes and species were detected using non-parametric Wilcoxon-tests. Eye span is a highly allometric trait in stalk-eyed flies (Baker & Wilkinson 2001), so we analysed species differences and sexual dimorphism of eye span using General Linear Models (GLMs) containing SPECIES or SEX and BODY LENGTH as fixed factors, their interaction, and an intercept. The significance of each effect (or interaction) was determined via *F*-tests on the change in explained variance upon removal of each term from the full model. A significant interaction implies that the *relationship* between eye span and body length (i.e. the allometric slope of eye span) differs between the groups (species or sexes). An additional model, comprising SPECIES, SEX and BODY LENGTH main effects, plus all interactions, was also constructed. The significance of the three-way SPECIES  $\times$  SEX  $\times$  BODY LENGTH interaction was used to identify a difference in sexual dimorphism between the two species.

### Gene Cloning And Phylogenetic Analysis

Genomic DNA was extracted from individual, laboratory-reared, adults ground in TNES (50mM Tris pH 8.0, 400mM NaCl, 20 mM EDTA, 0.5% SDS), to which Proteinase K (20mg/ml) was added and the mixture incubated at 37°C. 5M NaCl was added and the DNA was precipitated with EtOH. PCR was performed in 50 $\mu$ l volumes (5U Abgene Red Hot DNA Polymerase, 2.5 mM MgCl<sub>2</sub>, 0.4mM dNTP). Two nuclear genes, *white* (*w*) and *wingless* (*wg*), and two mitochondrial genes, *16S ribosomal RNA* (*16S*) and *cytochrome oxidase subunit II* (*COII*), were chosen for the phylogenetic analysis. The primers and annealing temperatures used for each gene are shown in Table 1. PCR was performed over 30 cycles, with a ten minute extension time at 72°C in the final cycle.

All PCR products were ligated into the pGEM-T Easy Vector (Promega) and transformed into Subcloning Efficiency DH5 $\alpha$  Chemically Competent Cells (Invitrogen). Plasmid DNA was extracted using the Qiagen Spin Miniprep kit and sequenced using T7 and SP6 primers (Macrogen Inc, Seoul, Korea). The sequences for each of the genes have been deposited into the GenBank database (Accession numbers DQ054781 and AY910526–AY910528). A concatenated alignment of all four genes was created using the sequences from the other 17 available *Diasemopsis* species in the GenBank database as

well as *Teleopsis dalmanni* (Wiedemann), *T. quinqueguttata* (Walker) and *Sphyracephala beccarii* (Rondani). The alignment was produced by ClustalX (Thompson *et al.* 1997) and then edited by eye.

**TABLE 1.** List of PCR primers.

Gene	Sequence Coordinate or Primer Name	Annealing Temperature (°C)	Reference
16S	12727–12747(S)	50.0	Baker <i>et al.</i> (2001)
	13270–13290(A)		
COII	A3772	50.8	Brower (1994)
	S3291		
w	11404–11426(S)	58.6	Baker <i>et al.</i> (2001)
	11975–11997(A)		
wg	<sup>5</sup> GTTAGAACATGTTGGATGCG <sup>3</sup>	53.3	Adapted from Baker <i>et al.</i> (2001)
	<sup>5</sup> CGTTCAACGACAATGACCTC <sup>3</sup>		

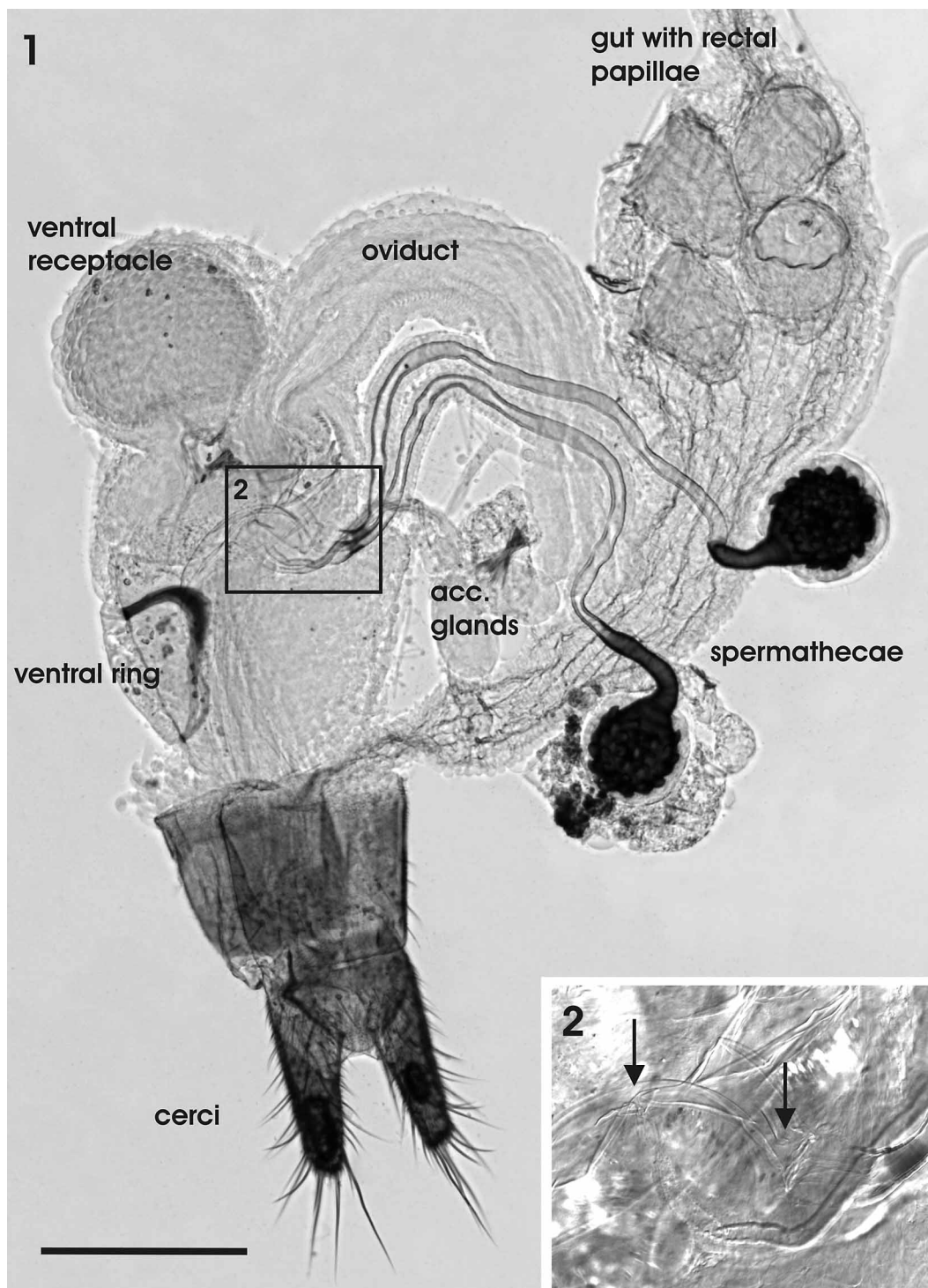
The alignment was analysed in Modeltest 3.7 (Posada and Crandall 1998), which determined that a GTR+I+ $\Gamma$  model, with a four-category gamma distribution, was the most appropriate for phylogenetic analysis. A Maximum Likelihood (ML) tree was produced using PAUP 4.0b10 (Swofford 2002), using the parameters determined by Modeltest 3.7. The ML tree was bootstrapped with 1000 replicates and branches with values of less than 50% were collapsed. A Bayesian phylogeny was produced using MrBayes 3.1.1 (Ronquist & Huelsenbeck 2003), also using a GTR+I+ $\Gamma$  model. The MCMC analysis ran with four chains for 1,000,000 generations. For both tree-creating methods the *Diasemopsis* sequences were rooted using the *Teleopsis* and *Sphyracephala* sequences.

### *Diasemopsis comoroensis* Carr & Földvári, new species

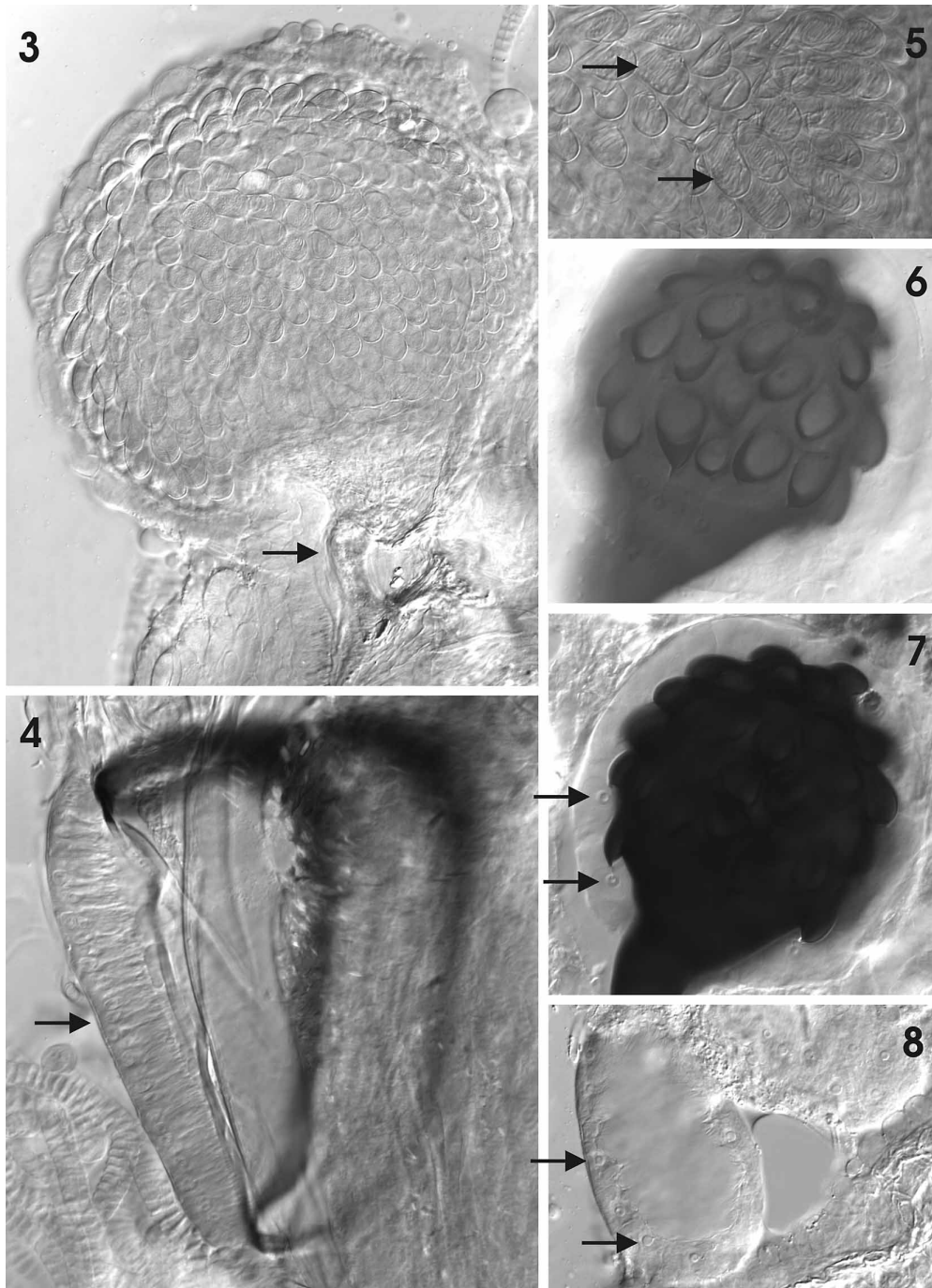
(Figs 1–9, 11, 13–16)

#### *Description*

**Type material:** Holotype, male (Natural History Museum, London). Paratypes 4 males, 5 females (Natural History Museum, London), 5 males, 5 females (Zoologische Staatssammlung, München), 5 males, 5 females (Hungarian Natural History Museum, Budapest), 2 males, 2 females (Centre National de Documentation et de Recherche Scientifique, Moroni, Comoro Islands). All type specimens (dried, double mounted, excellent condition) taken from a laboratory culture housed at University College, London in May 2005. Parent specimens collected at Comoro Islands, Mohéli, creek uphill from Hoani on way toward Chalet St. Antoine, leg. M. Kotrba 21.iv.2002.



**FIGURES 1-2:** *D. comoroensis* internal female genital organs. 1. *D. comoroensis* female reproductive tract (ovaries omitted). Scale bar 250  $\mu\text{m}$ . 2. Detail of genital papilla. Arrows indicate the separate openings of spermathecal ducts (right) and accessory gland ducts (left).



**FIGURES 3–8:** 3: Multichambered ventral receptacle. Arrow indicates the tubular entrance. 4: Ventral sclerotized ring. Arrow indicates specialized epithelium. 5: Detail of ventral receptacle. Arrows indicate coiled spermatozoa within the cuticular chambers. 6: Spermatheca with denticles. 7: Spermatheca. Arrows indicate cuticular end apparatus of epithelial gland cells. 8: Accessory gland. Arrows indicate cuticular end apparatus of epithelial gland cells.

**Head.** Completely black (including eye stalks) and covered with minute pale hairs. Facial teeth smaller than half of the width of the eye stalks in the middle. Outer vertical bristles at least as long as width of the eye stalk in the middle. Inner vertical bristles short, but distinct, as long as  $1/3^{\text{rd}}$  of the width of the eye stalk in the middle.

**Thorax.** Uniformly grayish pollinose, except the surface of the meron. Metapleural spine dark brown to black, at most the tip can be yellow. The spine is curved upwards (dorsally) in anterior view. Scutellar spines 2.5–3 times as long as the scutellum.

**Wing Fig. 9.** Completely hyaline, except for three infuscated brownish bands. The apical band is as broad as  $1/20^{\text{th}}$  of the wing length and is restricted to the space between M and  $R_{2+3}$  veins (not reaching these veins). The central band is darkest around  $R_{4+5}$  and it is situated between  $R_{2+3}$  and Cu slightly extended towards the anterior cross vein (R–M). The proximal band is more an infuscated brownish spot below the cell cup.

**Legs Fig. 11.** Front coxae are shiny posteriorly and also with a lateral shiny spot at proximal  $1/3^{\text{rd}}$  of the coxae. Legs are yellow–brown, brown in general; tarsi 3–5 on the front leg are paler and yellow. Front femora incrassate (length/width approximately 4), bearing on their ventral side two longitudinal rows of 3–5 prominent bristles each and between these two rows of 18–22 much shorter peg-like tubercles each.

**Preabdomen.** Subshining black except for the following silver pollinose areas: an uninterrupted band along posterior margin of tergite 1, lateral triangles at posterior margin of tergite 2, distal half of tergite 3, and subsequent tergites.

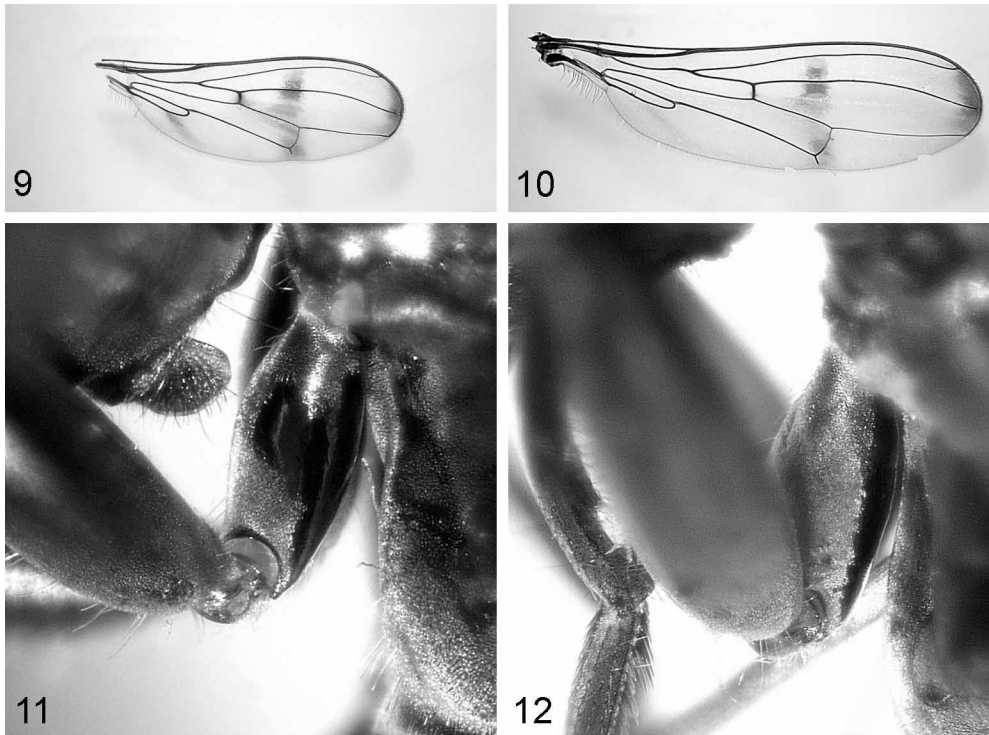
**Postabdomen (male) Figs 13–16.** In ventral view (Fig. 13) the connection of the hypandrium to the aedeagal apodeme is clearly visible. The membranous tip of the hypandrium is divided into two lobes anteriorly. There are two thick hairs on the medial inner surface of the hypandrium, the bilobed surstyli have numerous short, distinct hairs and the gonopods bear minute hairs as well (Fig. 14). In lateral view the aedeagal apodeme is curved (more than that of *D. meigenii*) and not broadening on anterior half. The ligament connecting to the hypandrium joins in the middle of the aedeagal apodeme (Fig. 15). The epandrium and cerci have long, dispersed hairs along their surface. Hairs on the hypandrium are more restricted to the ventral part and are shorter than those of *D. meigenii*. The distal half of the paramere is broadening towards the tip (Fig. 16).

**Postabdomen (female) Fig. 1.** The internal female genital organs of *D. comoroensis* are most similar to those of *D. meigenii* as described and depicted by Kotrba (1995).

The tubular vagina is surrounded by a thick layer of muscles particularly in its anterior region. It is anteriorly connected to the common oviduct, which descends from the paired ovaries and lateral oviducts. The posterior end of the vagina is attached to the vulva behind sternum 8. From the ventral anterior portion of the vagina emanates the voluminous roundish ventral receptacle, which is composed of more than 300 tubular chambers, each with a diameter of about 15–20  $\mu\text{m}$  (Fig. 3). In mated females these chambers house individual tightly coiled spermatozoa (Fig. 5). The ventral receptacle is connected with the lumen of the vagina via a narrow, tubular duct. A dense structure dorsal of the entrance

may be part of a valve mechanism. Opposite the entrance of the ventral receptacle a pouch-like dorsal evagination of the vaginal lumen receives the spermathecal ducts and, ventrolateral of that, the ducts of the accessory glands (Fig. 2). Posterior to this a ring-shaped ventral sclerite is embedded in the ventral wall of the vagina. Part of the vaginal musculature inserts on this ring-shaped sclerite, thus sparing a cushion of specialized epithelium within its centre (Fig. 4). Two spherical spermathecae are present, one with a diameter of about 100 µm, the other slightly larger. Their strongly sclerotized, dark brown capsules are ornamented with short hollow denticles (Fig. 6). Each of these denticles is connected to the end apparatus of an epithelial gland cell (Fig. 7). The base of the spermathecae is not telescoped but drawn out into a tubular portion which merges smoothly with the apical ends of the long spermathecal ducts. The bases of the spermathecal ducts are slightly sclerotized as well next to their opening into the vagina. Like the spermathecae, the membranous ovoid reservoirs of the accessory glands are surrounded by epithelial gland cells with cuticular end apparatuses (Fig. 8). The delicate ducts of the accessory glands are only about 1/3<sup>rd</sup> as long as those of the spermathecae.

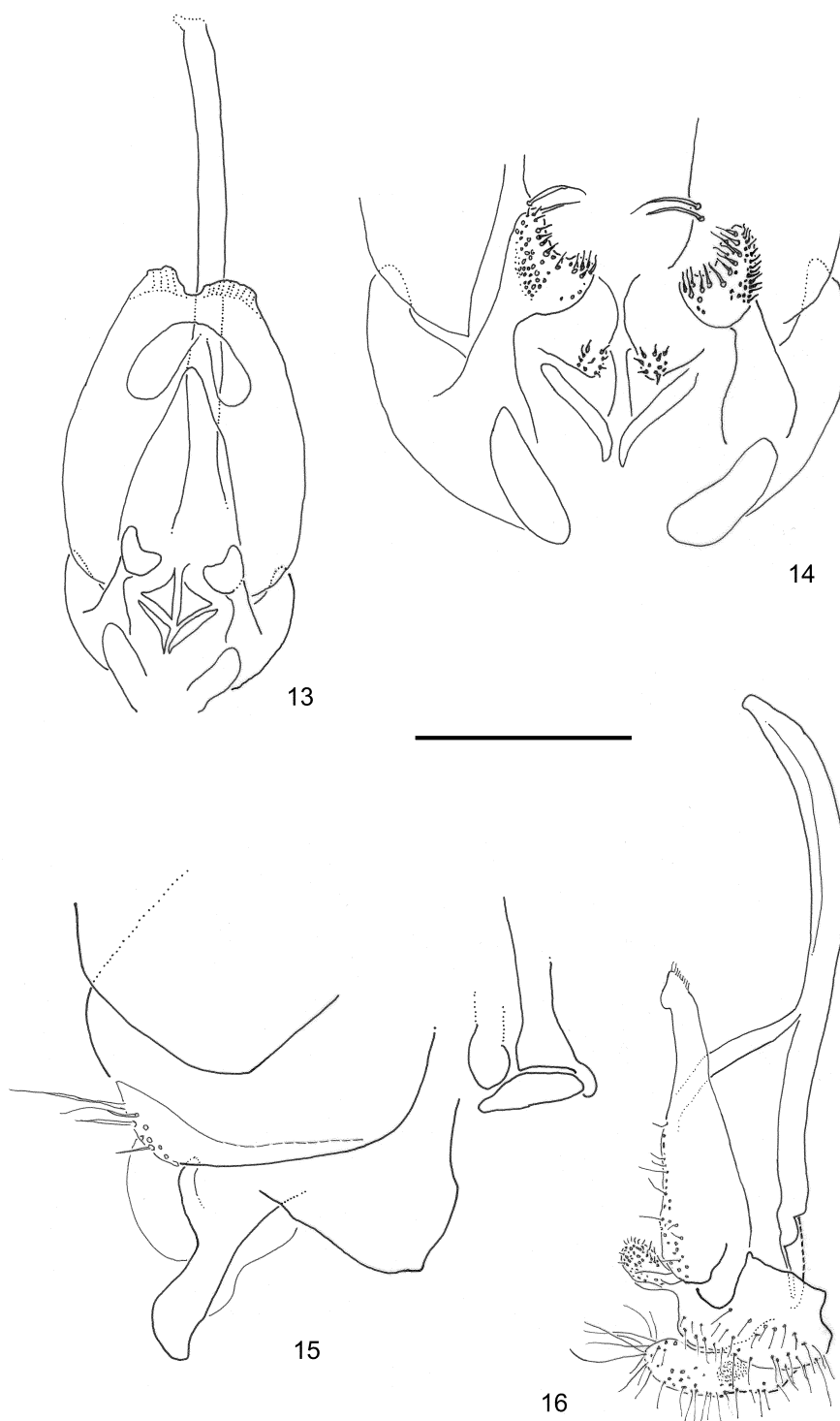
A description of the entire reproductive system of another stalk eyed fly, *T. whitei* (Curran) (detailed under its former name *Cyrtodiopsis whitei*), was given by Kotrba (1993) including further details as well as physiological and functional aspects.



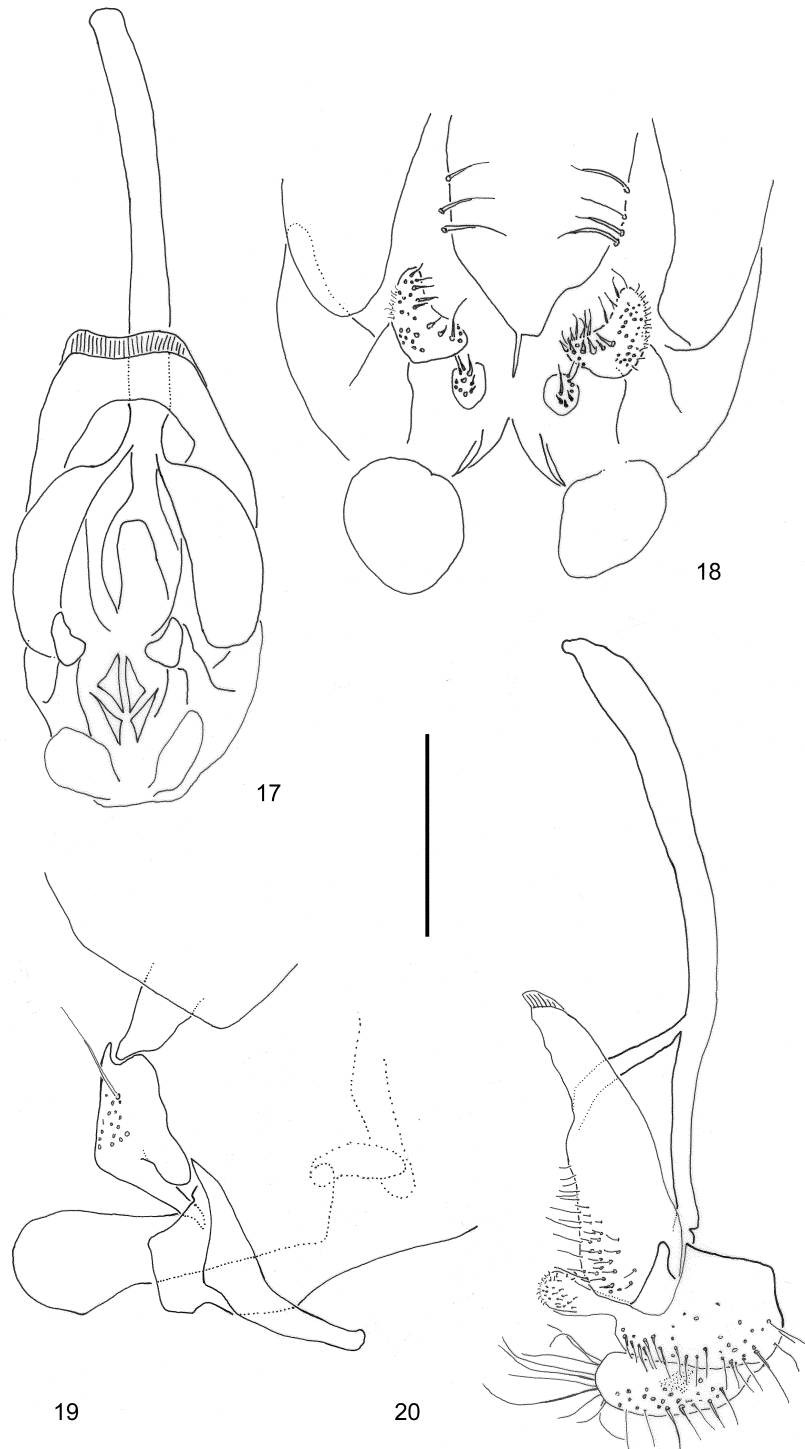
**FIGURES 9 & 10:** *D. comoroensis* (9) and *D. meigenii* (10) wings. The larger apical band is clearly visible in the *D. meigenii* wing, spanning between the M and R2+3 veins.

**FIGURES 11 & 12:** *D. comoroensis* (11) and *D. meigenii* (12) first coxae.





**FIGURES 13–16:** *D. comoroensis* male genitalia. 13: ventral view, 14: detailed ventral view with surstyli, gonopods, cerci (no hairs on cerci drawn), 15: detailed lateral view with gonopod and paramere, 16: lateral view with aedeagal apodeme, hypandrium and epandrium. Scale bar 0.4 mm for Fig 13, 16, 0.2 mm for Fig 14 and 0.1 mm for Fig 15.



**FIGURES 17–20:** *D. meigenii* male genitalia. 9: ventral view, 10: detailed ventral view with surstyli, gonopods, cerci (no hairs on cerci drawn), 11: detailed lateral view with gonopod and paramere, 12: lateral view with aedeagal apodeme, hypandrium and epandrium. Scale bar 0.4 mm for Fig 17, 20, 0.2 mm for Fig 18 and 0.1 mm for Fig 19.

**Etymology:** The name refers to the type locality, Comoro Islands.

**Distribution:** Mohéli, Comoro Islands

***Diasemopsis meigenii* (Westwood, 1837)**

(Figs 10, 12, 17-20)

syn. *Diopsis breviseta* Bezzi, 1908: 167, Type locality: Ethiopia (Eritrea)

Séguy (1955) established the new genus *Chaetodiopsis* for *D. meigenii* (Westwood, 1837, originally in *Diopsis*). However, *Chaetodiopsis* was treated as a junior synonym of *Diasemopsis* by the Catalogue of the Diptera of the Afrotropical Region (Crosskey 1980) as well as by Meier & Baker (2002), based on sound molecular and morphological analysis. *Chaetodiopsis* is embedded deeply within *Diasemopsis*, i.e. its recognition would render *Diasemopsis* polyphyletic. Here we follow the revised classification suggested by Meier & Baker (2002).

**Material studied:** 5 males, 5 females taken from a laboratory culture housed at University College, London in May 2005. This culture was derived from a culture in the laboratory of G. S. Wilkinson, University of Maryland at College Park, which was originally founded from flies caught near Pietermaritzburg, South Africa in December 1994 by M. Kotrba. The dried, double-mounted specimens are deposited in the Hungarian Natural History Museum, Budapest.

Other material: 3 males, 3 females, Lourenço marques, Mozambique, *Diopsis meigeni* Westw. Det Lindner. Cameroun: 2 males, 3 females, Yaounde Obili, 20–2–1963, coll. L. Segers: 1 male, same data, 3–1–1963 (all Zoologische Staatssammlung, München); 1 female, Nkolbisson, Dept. Nyong-Sanaga, IX. [19]68, L.G. Segers leg., van Schuytbroek det. 19 [no year given], *Chaetodiopsis meigeni* (Royal Museum for Central Africa). Republic of Congo: 1 male, Mayambé: Kiniati, 7–VI–1911, R. Mayné; 1 male Ikengé, IX–1912, R. Mayné, 5 males, Congo da Lemba, R. Mayné; dates: V–1912, V–1912, I–II–1913, III–1913, IV–1913; 1 male, Lemfu, P. Vandereijst; 1 male, Mandungu, 25–XI–1912, R. Mayné (all Royal Museum for Central Africa).

**Head.** Completely covered with minute pale hairs, generally black, but the eye stalks are brown. Facial teeth 1.5 times longer than width of eye stalks in the middle. Outer vertical bristles at least as long as width of the eye stalk in the middle. Inner vertical bristles minute and weak, shorter than 1/3<sup>rd</sup> of width of the eye stalk in the middle.

**Thorax.** Uniformly grayish pollinose, except the surface of the meron. Metapleural spine yellow or orange yellow and straight in anterior view. Scutellar spines 2.5–3 times as long as the scutellum.

**Wing Fig. 10.** Completely hyaline, except for three infuscated brownish bands. The apical band is as broad as 1/14<sup>th</sup> of the wing length and is restricted to the space between M and R<sub>2+3</sub> veins (the band reaches these veins). The central band is darkest around R<sub>4+5</sub> and

it is situated between  $R_{2+3}$  and Cu slightly extended towards the anterior cross vein (R–M). The proximal band is more an infuscated brownish spot below the cell cup.

**Legs Fig. 12.** Front coxae are only shiny on the posterior surface. Legs are yellow-brown in general, tibiae and first two tarsi of the front leg are black, tarsi 3–5 on the front leg are whitish yellow (clearly contrasting the other tarsi). Front femora incrassate (length/width approximately 4), bearing on their ventral side two longitudinal rows of 2–4 prominent bristles each and between these two rows of 24–28 much shorter peg-like tubercles each.

**Preabdomen.** Subshining black except for the following silver pollinose areas: an uninterrupted band along posterior margin of tergite 1, lateral triangles at posterior margin of tergite 2, distal half of tergite 3, and subsequent tergites.

**Postabdomen (male) Figs 17–20.** The hypandrium is connected to the aedeagal apodeme and the membranous tip of the hypandrium is continuous, not divided into two lobes anteriorly (Fig. 17). There are 3(–4) thick hairs on the medial surface of the hypandrium, the bilobed surstyli have numerous short, distinct hairs and the gonopods bear minute hairs (Fig. 18). In lateral view the aedeagal apodeme is more straight (that of *D. comoroensis* is curved) and also broadening towards tip. The ligament connecting to the hypandrium joins at basal 1/3<sup>rd</sup> of the aedeagal apodeme (Fig. 20). The epandrium and cerci have long, dispersed hairs along their surface. Hairs on the hypandrium are reaching to the lateral part and are longer than those of *D. comoroensis*. The distal half of the paramere is slightly narrowing towards the tip (Fig. 19).

**Distribution:** Widespread in Afro-tropical regions.

#### Allometric differences between *D. comoroensis* and *D. meigenii*

*D. comoroensis* was significantly smaller than its congener for both eye span and body length (Table 2; Wilcoxon tests; male eye span  $\chi^2 = 40.50$ , male body length  $\chi^2 = 28.46$ , female eye span  $\chi^2 = 48.67$ , female body length  $\chi^2 = 34.05$ ; all d.f. = 1, all  $P < 0.001$ ). Male *D. comoroensis* had a significantly shallower eye span allometry than male *D. meigenii* (Fig. 21; Table 2; SPECIES  $\times$  BODY LENGTH interaction terms in Table 3). However, female *D. comoroensis* and *D. meigenii* did not differ significantly in the slope of their eye span allometry (Fig. 21; Table 2; SPECIES  $\times$  BODY LENGTH interaction term in Table 4).

Within species, females had longer body lengths than males (Table 2; Wilcoxon tests; *D. comoroensis*  $\chi^2 = 3.78$ , d.f. = 1,  $P = 0.05$ ; *D. meigenii*  $\chi^2 = 10.81$ , d.f. = 1,  $P = 0.001$ ). However, males from both species had significantly larger eye span than females before and after controlling for body length variation (Table 2; absolute eye span Wilcoxon tests: *D. comoroensis*  $\chi^2 = 18.18$ , d.f. = 1,  $P < 0.001$ , *D. meigenii*  $\chi^2 = 27.20$ , d.f. = 1,  $P < 0.001$ ; SEX terms in Table 5 and 6). There was a small, but significant, difference in the slope of eye span allometry between male and female *D. comoroensis*, with males having a slightly

steeper scaling relationship (Fig. 21; Table 2; SEX × BODY LENGTH interaction term in Table 5). Eye span allometry was significantly steeper in male *D. meigenii* than in their female conspecifics (Fig. 21; Table 2; SEX × BODY LENGTH interaction term in Table 6). However, the degree of sexual dimorphism in *D. comoroensis* was significantly weaker than that observed in *D. meigenii* (Fig. 21; Table 2; full model SPECIES × SEX × BODY LENGTH interaction term  $F = 12.22$ , d.f. = 1,138,  $P < 0.001$ ).

**TABLE 2.** Mean trait size (mm ± S.D.) eye span, body length and allometric slope (± S.E.) of males and females from each species. Allometric slope is the least-squares regression coefficient of eye span on body length.

Species	Sex	Eye span	Body Length	Allometric Slope
<i>D. comoroensis</i>	Male	5.27 ± 0.45	6.58 ± 0.34	1.70 ± 0.07
	Female	4.84 ± 0.32	6.70 ± 0.38	0.82 ± 0.03
<i>D. meigenii</i>	Male	7.19 ± 0.96	7.31 ± 0.55	1.21 ± 0.08
	Female	6.10 ± 0.52	7.62 ± 0.62	0.80 ± 0.04

**TABLE 3.** General Linear Model of body length and species effects on eye span in male *Diasemopsis*.

Factor	SS	d.f.	MS	F-ratio	Prob > F
BODY LENGTH	97.588	1	97.588	2231.58	<0.001
SPECIES	6.098	1	6.098	139.44	<0.001
SPECIES × BODY LENGTH	0.658	1	0.658	15.06	<0.001
ERROR	2.974	68	0.0437		
<b>Total</b>	107.319	71			

**TABLE 4.** General Linear Model of body length and species effects on eye span in female *Diasemopsis*.

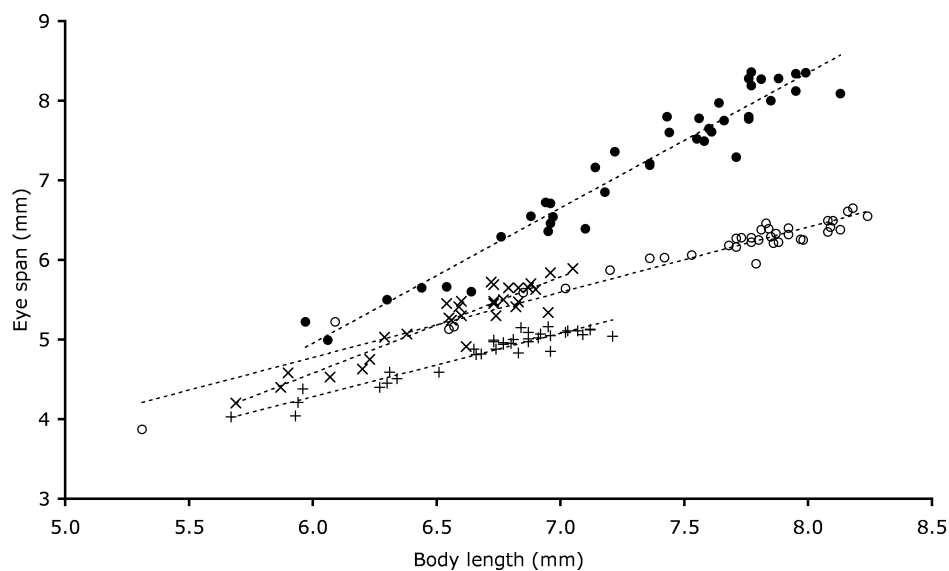
Factor	SS	d.f.	MS	F-ratio	Prob > F
BODY LENGTH	39.406	1	39.406	2989.54	<0.001
SPECIES	2.699	1	2.699	204.82	<0.001
SPECIES × BODY LENGTH	0.002	1	0.002	0.13	0.72
ERROR	0.993	70	0.0132		
<b>Total</b>	43.031	73			

**TABLE 5.** General Linear Model of body length and sex effects on eye span in *D. comoroensis*.

Factor	SS	d.f.	MS	F-ratio	Prob > F
BODY LENGTH	6.263	1	6.263	376.70	<0.001
SEX	4.967	1	4.967	298.73	<0.001
SEX × BODY	0.355	1	0.355	21.34	<0.001
LENGTH					
ERROR	1.048	63	0.0166		
<b>Total</b>	12.633	66			

**TABLE 6.** General Linear Model of body length and sex effects on eye span in *D. meigenii*.

Factor	SS	d.f.	MS	F-ratio	Prob > F
BODY LENGTH	23.292	1	23.292	613.20	<0.001
SEX	39.254	1	39.254	1033.42	<0.001
SEX × BODY	5.059	1	5.059	133.18	<0.001
LENGTH					
ERROR	2.849	75	0.038		
<b>Total</b>	90.585	78			

**FIGURE 21:** Eye span allometry in *D. comoroensis* and *D. meigenii*. × = male *D. comoroensis*, + = female *D. comoroensis*, ● = male *D. meigenii*, ○ = female *D. meigenii*. Least-squares regression lines are given for heuristic purposes.

Gene Sequencing and Phylogenetic Analysis

BLAST searching showed that sequences from *D. meigenii* had the highest similarity to the four sequenced partial *D. comoroensis* genes. Nucleotide identities between the four genes in the two species ranged from 96.7–99.8%, with both nuclear genes sharing 99.8% identity within their coding sequences, showing that the two species must share a very recent common ancestry.

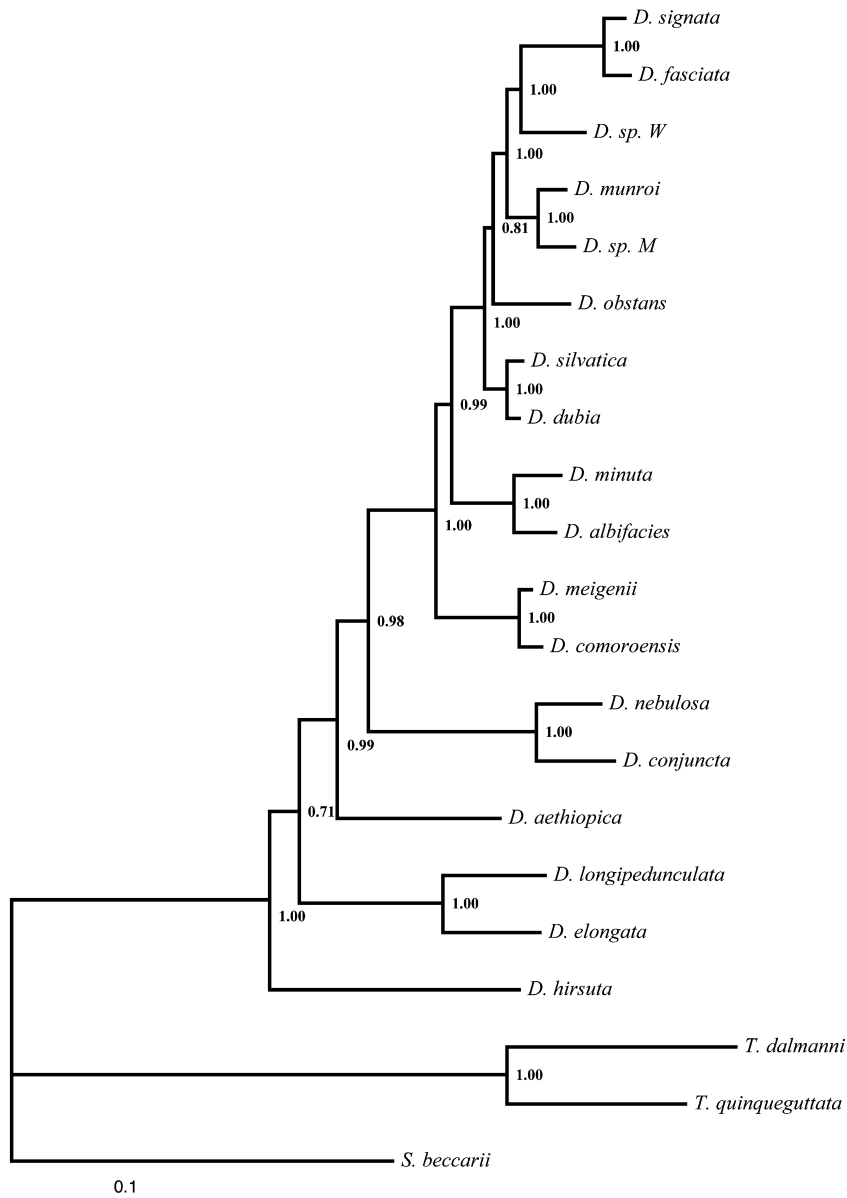


FIGURE 22: Bayesian phylogeny of *Diasemopsis* species, rooted with *Teleopsis* and *Sphyracephala* species. Posterior probabilities are given for each branch.

In both the Bayesian tree (Fig. 22) and ML tree the *D. meigenii* and *D. comoroensis* sequences clustered together on a long branch, deeply embedded within the *Diasemopsis* genus (posterior probability = 1.00 and bootstrap support = 100%). Phylogenies for each of the individual genes all show this close relationship between the two species (data not shown). Thus all four sequenced genes, using two different tree-creating methods, show a very close relationship between *D. comoroensis* and *D. meigenii*. Despite the lower number of taxa and gene sequences used, the phylogenies created were otherwise identical to those of Baker *et al.* (2001) and Meier & Baker (2002), indicating that this is a robust phylogeny of the *Diasemopsis* genus.

### Diagnosis for the two species

**External differences:** *D. comoroensis* has a shorter eye span compared to the body length, exhibiting only mild sexual dimorphism (large eye span, strong dimorphism in *D. meigenii*). The facial teeth are shorter (long in *D. meigenii*) and the inner vertical bristles are more distinct (only minute in *D. meigenii*). The first coxae have shiny spots on the lateral surface at the basal 1/3<sup>rd</sup> of the coxae (missing in *D. meigenii*). Metapleural spines are dark and in anterior view upcurved (yellow and straight in *D. meigenii*).

**Differences in the male genitalia:** *D. comoroensis* has the aedeagal apodeme more curved in lateral view and also the width is even along its length (straight and broadening in *D. meigenii*). The linking structure from the hypandrium attaches the aedeagal apodeme in the middle (at basal 1/3<sup>rd</sup> in *D. meigenii* Figs 16, 20) and the parameres are broadening towards tip (slightly narrowing in *D. meigenii* Figs 15, 19). The hypandrium is bilobed anteriorly in *D. comoroensis*.

### Comments

Both morphological similarity and a phylogenetic approach have placed *D. comoroensis* as a sister species to *D. meigenii*. The species are sufficiently closely related for inter-specific mating to occur under forced laboratory conditions. However such mating attempts do not produce any offspring (Cotton & Carr, unpublished data).

As is the case with a number of species in the current *Diasemopsis* molecular phylogeny, *D. meigenii* and *D. comoroensis* are placed at the end of a long branch. It is unknown what proportion of *Diasemopsis* species have been scientifically described, however relatively few described species have been characterised from a molecular perspective; therefore the isolated position of *D. meigenii* and *D. comoroensis* within the tree is likely to be a result of missing taxa. Greater sampling of *Diasemopsis*, both on mainland Africa and the Indian Ocean islands, may add additional undescribed taxa to the



known 51 *Diasemopsis* species. The current tree topology is ladder-like, it is likely that further gene sequences will provide greater resolution to the *Diasemopsis* phylogeny and produce a more bush-like topology. Preliminary studies of collection specimens of other *Diasemopsis* species suggest there will be numerous new species to be described which may have a strong influence on the tree.

*D. comoroensis* and *D. meigenii* exhibit very different head morphologies in terms of the absolute difference between the two species, and within-species differences (i.e. sexual dimorphism). *D. comoroensis* is mildly sexually dimorphic for eye span, both before and after controlling for body length variation, with males having a larger trait. Male eye span allometry is also slightly steeper than that of females in *D. comoroensis*. However, it is significantly less steep (in both absolute terms and relative to females) than the eye span allometry of male *D. meigenii*. It is noteworthy that the extent of dimorphism in *D. comoroensis* may be partially dependent on the estimate of body size, as the use of an alternative body size proxy (thorax length) produces an outcome of no significant sexual dimorphism in this species (Cotton, unpublished data; sexual dimorphism remains strong in *D. meigenii* with this additional measure).

The nature of selection on eye span in *D. comoroensis* is difficult to discern without further study, but differences between male and female eye span may have resulted from current or past episodes of sexual selection; all sexually-selected diopsids studied thus far exhibit an elevated male eye span allometry (Burkhardt & de la Motte 1985; Wilkinson & Dodson 1997; Baker & Wilkinson 2001), as predicted if eye span is an evolved signal of body size, or correlate thereof (Green 1992; Petrie 1992, Cotton *et al.* 2004). For example, in *D. meigenii*, there is strong sexual selection through female mate preference for large male eye span (Cotton *et al.* 2006). In contrast, the slope of eye span allometries is much shallower in, and does not differ between, female *D. comoroensis* and female *D. meigenii*, suggesting that females from both species share similar eye span scaling optima that are less sensitive to variation in body size.

All species from the genus *Diasemopsis* examined in Baker & Wilkinson (2001) exhibited some degree of sexual dimorphism with respect to their eye span. It is demonstrated here that *D. comoroensis* exhibits only very mild dimorphism, relative to its sister species *D. meigenii*. As these species only recently diverged the observed differences in eye span sexual dimorphism must have evolved rapidly. Loss of sexually selected traits is an increasingly well-observed phenomenon (reviewed in Wiens 2001) and has previously been recorded in an island population (Griffith *et al.* 1999). The colonising of the Comoro Islands is likely to have resulted in new environmental challenges, such as resource availability, novel predators, a reduced population size and isolation from the ancestral population, any of which may have altered the selection pressure on increased male eye span.

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