

Short communication

The systematic position of *Cladodiopsis* Séguy, 1949 and the origin of sexual dimorphism in stalk-eyed flies (Diptera: Diopsidae) inferred from DNA sequence data

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1. Introduction

Considerable interest in the phylogeny of stalk-eyed flies (Diopsidae) has been triggered by their remarkable lateral elongation of the head into stalks distally carrying the eyes and antennae. These head modifications are often sexually dimorphic, with males carrying longer eye stalks than females. The eye stalks have been shown to play an important role in male combat and female mate choice, making Diopsidae a useful and popular study system to address a variety of evolutionary questions (e.g., Baker and Wilkinson, 2001; Kotrba, 2004; Wilkinson et al., 2003; Wolfenbarger and Wilkinson, 2001, and references therein).

Diopsidae contains about 160 species currently assigned to 11 genera. Recent cladistic analyses of the family were presented by Meier and Hilger (2000, adult and egg morphology), Baker et al. (2001, mtDNA and nDNA), Meier and Baker (2002, the two previous data sets combined), and Kotrba (2004, morphology, focus on †*Prospyracephala*). Diopsidae is divided into the Centrioncinae, which do not possess eye stalks and the Diopsinae, all of which do. Diopsinae comprises the tribes Sphyracephalini and Diopsini. According to most authors Sphyracephalini include *Sphyracephala* Say, 1828 (including *Pseudodiopsis* Hendel, 1917) and—arguably—*Cladodiopsis* Séguy, 1949, while the Diopsini comprise all other extant Diopsinae genera (Feijen, 1989; Shillito, 1971; Steyskal, 1972). †*Prospyracephala*

Hennig, 1965 was considered to represent the sister group of all other known Diopsinae (Hennig, 1965; Kotrba, 2004).

In the molecular studies of Diopsidae nuclear genes such as *white* and *wingless* achieved very good resolution of intrafamilial relationships, which concur well with morphological findings. However, four diopsid genera, *Cladodiopsis*, *Sinodiopsis* Feijen, 1989, *Cobiopsis* Feijen, 1989, and *Diopsina* Curran, 1928 (Meier and Baker, 2002) were not available for molecular studies previously. Of these, the phylogenetic placement of *Cladodiopsis*, a genus endemic to the Madagascan Region, has been thought to be of crucial importance for understanding the relationships and character evolution within the Diopsidae in general (Meier and Hilger, 2000). Meier and Hilger (2000) could place *Cladodiopsis* within Sphyracephalini, in agreement with Feijen (1989); Shillito (1971); and Steyskal (1972) but alternatively also found *Cladodiopsis* in ‘many positions within the lower Diopsinae.’ Kotrba (2004) most recently placed *Cladodiopsis* at the base of the Diopsini, albeit with weak support.

Fresh specimens of *Cladodiopsis seyrigi* Séguy, 1949 were collected at Mandrake (Madagascar) in December 2004 by one of the authors (M.B.). From these we obtained tissue samples suitable for DNA sequencing. The goals of this paper are to (i) clarify the systematic position of *Cladodiopsis* building on the extensive DNA sequence data set of Baker et al. (2001), as well as a combination of the molecular with morphological data presented by Kotrba (2004), (ii) study the presence of sexual dimorphism in *Cladodiopsis*, and (iii) discuss our results with respect to eye stalk evolution.

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2. Materials and methods

2.1. DNA extraction, sequencing, and data editing

Total DNA was nondestructively extracted from an individual fly using the DNeasy tissue kit (Qiagen, Hilden, Germany). Three mtDNA and two nDNA gene regions previously used by Baker et al. (2001) were amplified to add the *Cladodiopsis* sequences to an existing data set. We failed to sequence elongation factor one alpha which was included by Baker et al. (2001).

Using standard PCR protocols and double-stranded sequencing, we sequenced 3' ends of the 16S rRNA, the entire tRNA_{Leu} and a part of the 3' of ND1 (primers M14, M223), the 3' end of Cytochrome *c* oxidase II (COII; primers COIIF and George), and a central fragment of 12S rRNA (12S; primers 12Sai, 12Sbi) (Simon et al., 1994). Nuclear gene fragments sequenced were the *white* fragment with the primers as used by Baker et al. (2001), and a fragment of *wingless*, amplified and sequenced using the Lepidoptera primers Lep wg1a and Lep wg2a (Brower and Egan, 1997).

Sequences were edited using the Sequencher 4.1 software package (GeneCodes Corp.). Alignment was conducted by eye against the original matrix of Baker et al. (2001) for consistency, with the alignment editor Se-Al (Rambaut, 1996). Alignment was trivial as ambiguously aligned characters of 12S and 16S, and introns of *white* and *wingless* were removed by Baker et al. (2001). The newly obtained sequences were submitted to GenBank (DQ317403–DQ317407). Specimens studied were deposited at Zoologische Staatssammlung Munich. Duplicates of the same series of specimens will be repatriated to the University of Antananarivo.

2.2. Data analysis

We conducted two parsimony analyses—one with molecular data alone (*Cladodiopsis* sequences and data submitted to GenBank by Baker et al., 2001), and one combining these with morphological data from Kotrba (2004). The latter data set included also the fossil species †*Prospyracephala succini* (Loew, 1873). In all analyses *Teloglabrur entabensis* (Feijen, 1983) was chosen as the outgroup.

Our DNA data matrix from 34 ingroup and two outgroup species was analyzed running equally weighted parsimony searches with PAUP* version 4.0b10 (Swofford, 2002) using TBR heuristic searches (keep multiple trees), 1000 random addition sequences, and gaps coded as a fifth character state (Giribet and Wheeler, 1999). We assessed confidence in the detected topology by applying (1) bootstrapping with 1000 pseudoreplicates and 100 random additions per pseudoreplicate (Felsenstein, 1985), (2) 1000 parsimony jackknife replications (Farris et al., 1996) with 30 addition sequence replicates and deletion of 33% of the data, and (3) calculation of the total branch support (Bremer, 1988, 1994) as well as partitioned Bremer support (Baker and DeSalle, 1997) using TreeRot v.2c (Sorenson,

1996). The combined data set consisting of 37 taxa and 3303 characters was analyzed using the same procedures.

Bayesian analyses of the molecular data were conducted with MrBayes 3.04 (Huelsenbeck and Ronquist, 2001), using a GTR+I+ Γ model (the optimal model explaining our data as estimated with Modeltest; Posada and Crandall, 1998). We used the default priors (uniform probabilities) starting with random trees, and ran the three heated and one cold Markov chains for 3,000,000 generations, sampled at intervals of 1000 generations. To determine the point at which the Markov chains reached stationarity, the log-likelihood scores were plotted against generation time, and visually determined when the log-likelihood values reached a stable equilibrium. Trees (once burn-in samples were discarded, the first 100,000 generations) were combined in a single majority consensus topology, and the percentage of the nodes were taken as a posteriori probabilities (Huelsenbeck and Ronquist, 2001). As these are posterior probabilities of the clades under the assumed models, we consider values of 95% or greater to be significantly supported (Rannala and Yang, 1996).

The evolution of sexual dimorphism was analyzed by coding this character as binary (present–absent) and optimizing it a posteriori onto the new phylogenetic hypothesis in MacClade (Maddison and Maddison, 2000).

2.3. Morphometry

To test for the presence of sexual dimorphism in *C. seyrigi* the eye span *E* (distance between the lateral margins of the eyes) and the body length *L* (distance between face and posterior end of the abdomen) was measured in 15 male and 11 female specimens. The data were analysed by a *t* test based on a general linear regression model (ANCOVA) computed with the statistics program S+ (Venables and Ripley, 1999). The residues were tested for normal distribution using the Kolmogorov–Smirnov Test.

We could not assess sexual dimorphism in the other two species of *Cladodiopsis*: *C. sicardi* Séguéy, 1949 is only known from the female holotype which has relatively short and slender eye stalks; *C. leptophylla* Séguéy, 1949 is only known from two male types, whose long and slender eye stalks resemble those of male *C. seyrigi*. We have seen these types which very well agree with Séguéy's (1949) drawings. Feijen (1989, p. 63) described *C. sicardi* as “..rather *Sphyracephala*-like.”, which is however only superficially the case.

3. Results

3.1. Phylogenetic analysis

The aligned DNA sequence data set consisted of 3236 characters (Baker et al., 2001). Parsimony analysis revealed 1 tree (cost 4135; 2007 constant and 991 informative characters), *ci* = 0.4397 and *ri* = 0.6871. The topology agrees with Baker et al. (2001) and Meier and Baker (2002).

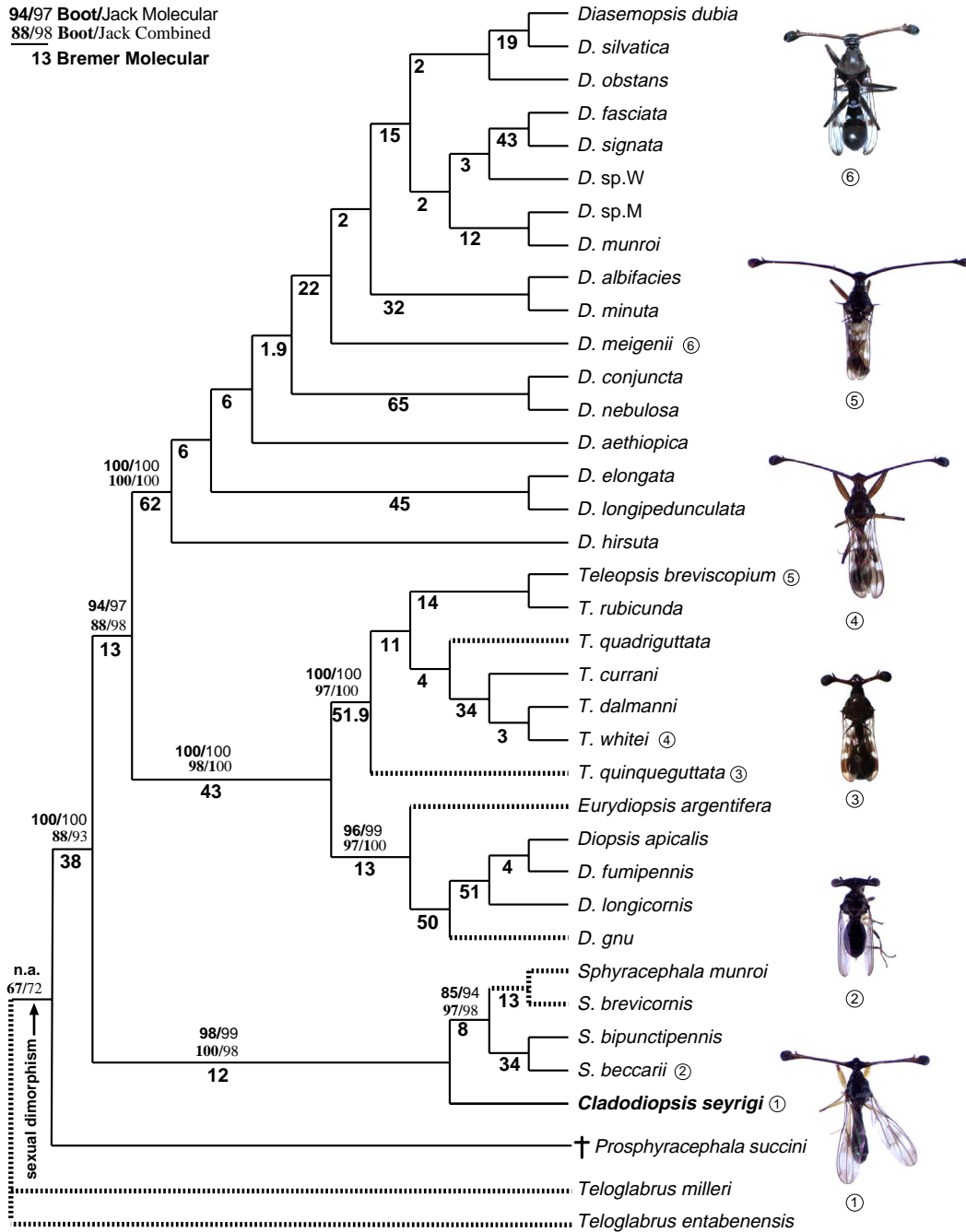


Fig. 1. The most parsimonious topology found: As molecular characters alone and “molecular + morphology” revealed the same topology (†*Prospyracephala* added in the combined), both results are depicted in the same figure. Numbers above branches are bootstrap/jackknife percentages for the crucial deeper nodes, below branches are Bremer support values for the molecular analysis. Classification follows Meier and Baker (2002). Solid lines indicate presence of sexual dimorphism of eye stalks, dotted lines indicate monomorphism of that character (optimized using MacClade). Habitus photographs illustrate morphological diversity of Diopsideae.

Cladodiopsis was unambiguously placed as the sister group of *Sphyracephala* (bootstrap and jackknife percentages of 98/99; Bremer support 12 steps) within the Sphyracephalini (Fig. 1). This result is consistent with that of Meier and Baker (2002) and previous authors (Feijen, 1989; Shillito, 1971; Steyskal, 1972). It contradicts Kotrba’s (2004) morphological analysis which supported the inclusion of *Cladodiopsis* in the Diopsini albeit with very low bootstrap

value (69) and Bremer support (1). With respect to the morphological characters analyzed by Kotrba (2004) the inclusion of *Cladodiopsis* in the Sphyracephalini requires only two additional steps (increase 1.5%). Calculation of partitioned Bremer support values revealed that *white* (PBS 6), *wingless* (PBS 6), and 16S (PBS 4) support the sister group relationship of *Cladodiopsis* and *Sphyracephala*, 12S (PBS –4) contradicts it and COII (PBS 0) does not support it. Separate

analysis of mtDNA suggested *Cladodiopsis* was the sister group of *Sphyracephala bipunctipennis* + *S. beccarii* (bootstrap 66) and *S. munroi* and *S. brevicornis* (boot 80) the sister clade of these three which is not plausible according to evidence from morphology and/or nDNA; however, nDNA alone supported the combined topology (boot 100) and was also in agreement with morphological evidence.

The Bayesian analysis (likelihood -24259.967) revealed the same overall topology with minor changes within *Diopsis* and *Diasemopsis*. Most nodes had posterior probabilities of 100. *Cladodiopsis* was retrieved as the sister of *Sphyracephala* likewise with a posterior probability of 100.

The combined analysis with morphological characters included revealed 1 tree (4213 steps, $ci=0.4446$, $ri=0.6875$), with †*Prosphyracephala* at the base of Diopsinae (see Kotrba, 2004), and the remaining topology being in agreement with the analysis of molecular characters alone (Fig. 1). We did not assess PBS or BS values for the combined analysis because of the numerous missing data in the morphological matrix, but the resampling measures suggest a moderately stable position of †*Prosphyracephala*.

3.2. Evolution of sexual dimorphism of eye stalks

The eye span E generally shows a positive linear correlation with the body length L in Diopsidae. In *C. seyrigi* this is hardly recognizable in females but very distinct in males (Fig. 2). The correlation is described by $E = aL + b$, with ‘ a ’ being the slope of the regression line and ‘ b ’ the intercept of the regression line with the y -axis. Highly significant gender-related differences were found regarding both slope ‘ a ’ and intercept ‘ b ’. Sexual dimorphism is strong in large specimens, but hardly evident in small specimens, whose body length is close to where the male and female regression lines intersect (Fig. 2). Tracing the presence/absence of sexual dimorphism on our molecular topology suggests that convergent loss or possession of this character within the Diopsinae are equally parsimonious explanations (6 steps). However, incorporating the fossil *Prosphyracephala*, sister to all other Diopsinae, suggests evolution of sexual dimor-

phism at the basis of Diopsinae and subsequent loss (6 steps, while the opposite case would require 7 steps).

4. Discussion

The molecular and combined data unambiguously place *Cladodiopsis* in the Sphyracephalini as the sister group of *Sphyracephala* and not at the base of Diopsini as suggested by Kotrba (2004). Thus, long and slender eye stalks are no longer unique to the Diopsini tribe. Actually, Meier and Hilger (2000) suggested that according to a parsimonious mapping of eye stalk morphology onto their cladogram, the stocky and short eye stalks of most *Sphyracephala* (Fig. 1) constitute a derived feature of that genus. Our results are consistent with the main conclusion of Kotrba (2004) i.e., the presence of sexual dimorphism already at the base of the Diopsinae. According to the taxon sampling available now, sexual dimorphism was the ancestral state and it was completely lost five times during the evolution of Diopsinae. In addition to this, it was reduced to some degree five to eight times (depending on parameters used in a linear parsimony reconstruction) according to Baker and Wilkinson (2001). Despite the early and widespread occurrence of sexual dimorphism in diopsids, assessing the ancestral state remains sampling sensitive. We conducted simulations with MacClade by which we successively added imaginary monomorphic taxa at different branches of the tree to address the potential influx on the reconstruction of the ancestral state. Ambiguity or reversal of the ancestral state towards monomorphic condition might occur adding monomorphic taxa basal to the *Teleopsis*–*Diopsis* clade and/or *Diasemopsis*. Prospective candidates are those genera not included in the present analysis. *Diopsina* does have monomorphic species (Feijen, 1989). However, this genus is probably part of the *Diopsis*–*Eurydiopsis* clade, so its addition would not reverse the ancestral state (Kotrba, 2004). In *Sinodiopsis* and *Cobiopsis* the presence or absence of sexual dimorphism cannot be assessed, as they are both rare and only known from females (Feijen, 1989). *Cobiopsis* is likely positioned subordinated within the *Diasemopsis* clade (Kotrba, unpublished), judged from the number and morphology of the spermathecae depicted by Feijen (1989). The position of *Sinodiopsis* remains unknown.

The secondary reduction of a male trait, that obviously must once have been favored by selection when it evolved, may seem contrainuitive on first glance. However, this phenomenon has been shown to be generally widespread (Wiens, 2001) and specifically to occur also within Diopsidae, e.g., in *Diasemopsis* (Baker and Wilkinson, 2001) and *Teleopsis* (Feijen, 1988). According to Saunderson (1993) from a stochastic perspective a higher frequency of losses than of gains is to be expected for a character that evolved early in a large clade. This theory would apply, if sexual dimorphism evolved right at the base of the Diopsidae, as suggested by our studies. However, things are more complicated in Diopsidae. In some *Teleopsis* species, female preference for males with particularly long eye stalks serves to

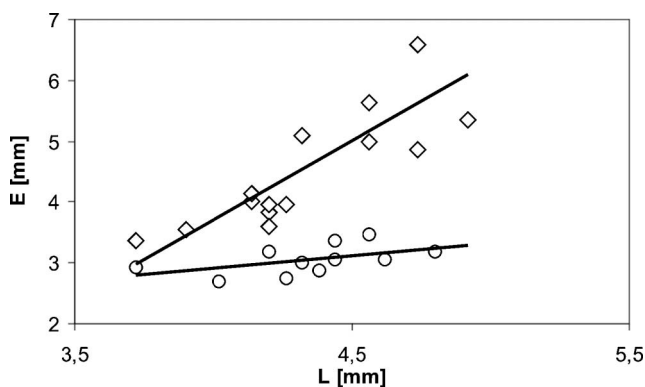


Fig. 2. Correlation of eye span, E and body length, L in *Cladodiopsis seyrigi* (diamonds, males; circles, females), including regression lines for both sexes.

avoid the effects of a selfish (meiotic) driver gene, which is passed on by males with shorter eye stalks (Pennisi, 2003; Wilkinson et al., 1998). Reduction of female preference and subsequent reduction of sexual dimorphism might be the result of overcoming the driver gene mechanism as suggested by Pennisi (2003).

Once more, Diopsidae emerge as a superb study group for tracking down the actual mechanisms of evolution. A robust phylogeny, whose few major gaps will hopefully be closed soon, will allow us to reliably trace the concerted changes of the remarkable genetic, morphological, physiological, and ethological characters of this family and to interpret the intricate causal connections between these character changes.

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