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# Baltic amber fossils reveal early evolution of sexual dimorphism in stalk-eyed flies (Diptera: Diopsidae)

Marion Kotrba*

*Zoologische Staatssammlung München, Münchhausenstr. 21, München D-81247, Germany*

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## Abstract

A cladistic analysis of 16 species of extant and amber fossil stalk-eyed flies of the family Diopsidae places the fossil †*Prosphyracephala succini* (Loew) as the sister group of all other Diopsinae, the subfamily in which eye stalks occur. The study is based on a scoring including five old and 23 new finds of †*P. succini* from Baltic amber, and for the first time allows a morphometric analysis of eye span and various body size parameters in this species. The data indicate that sexual dimorphism of the eye stalks already existed in †*Prosphyracephala*, suggesting that this feature evolved early in the Diopsinae. Contrary to recent views that the ancestral condition of diopsine eye stalks was monomorphic, the new results suggest that sexual selection was involved in the evolution of eye stalks from the very beginning of the lineage.


***Keywords:*** Diptera; Diopsidae; †*Prosphyracephala*; *Cladodiopsis*; Phylogeny

## Introduction

Phylogenetic hypotheses are not only important for proposing natural classifications, but they are also a prerequisite for testing evolutionary hypotheses. The present study of fossil and extant species of stalk-eyed flies (Diopsidae, Fig. 1) exemplifies the importance of including fossil records into such considerations, casting new light on the existing hypotheses regarding the evolution of sexual dimorphism in the family.

In Diopsidae the presence of eye stalks constitutes a shared derived feature of the entire subfamily Diopsinae. Within each species and either sex, eye span ($E$) and body length ($L$) show a close linear correlation, with $E = aL + b$ (e.g. Burkhardt and de la Motte 1987; Wilkinson and Dodson 1997). Sexual dimorphism, present in many species, is reflected in the slopes ($a$) and/or intercepts ($b$) of the regression lines. Only recently has the evolution of sexual dimorphism in Diopsinae been discussed by Baker and Wilkinson (2001). In their well-resolved and well-supported phylogenetic hypothesis, established by cladistic analysis of mitochondrial as well as nuclear gene sequences, monomorphic and dimorphic species show a scattered distribution (Fig. 2). Parsimony analysis suggested monomorphism as the ancestral condition of the Diopsinae, implying the convergent evolution of sexual dimorphism at least four or five times within the subfamily. However, the maximum likelihood approach did not give significant support to monomorphism as opposed to dimorphism at the base of the Diopsinae.

Baker and Wilkinson's (2001) analysis excluded two important basal lines of the Diopsinae: Cladodiopsis

*Tel. +49 89 8107 147.
*E-mail address:* marion.kotrba@zsm.mwn.de (M. Kotrba).

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(see Fig. 2): *Cladodiopsis* Séguy, endemic to Madagascar, and, since it was a molecular approach, the Eocene amber fossil †*Prosphyracephala* Hennig. *Cladodiopsis* had been included in the Sphyracephalini by previous authors (Shillito 1971; Steyskal 1972; Feijen 1989; for major classification see Fig. 2). Hennig (1965) had considered †*Prosphyracephala* as the extinct sister group of all extant Diopsinae, i.e. the Sphyracephalini and Diopsini combined, whereas Shillito (1971), Feijen (1983) and Schumann (1994) regarded the genus as a basal branch of the Sphyracephalini. A phylogenetic analysis by Meier and Hilger (2000), summarizing the published information on diopsid adult morphology with the addition of several new egg characters, did include †*Prosphyracephala* and *Cladodiopsis* but could not resolve their phylogenetic positions.

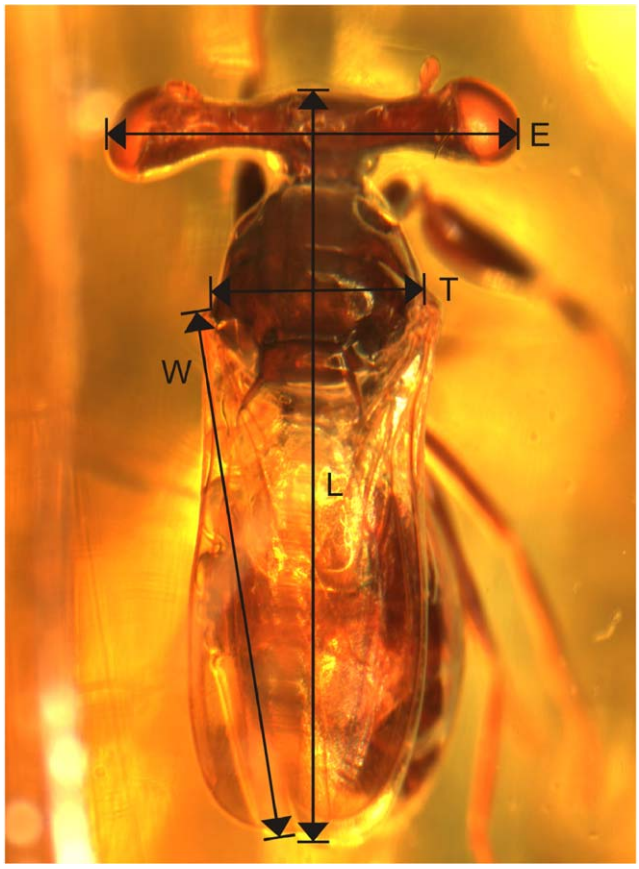


**Fig. 1.** †*P. succini* (Loew) in Baltic amber (Natural History Museum Krakow). Arrows indicate the measurement of eye span (*E*), body length (*L*), thorax width (*T*), and wing length (*W*).

Including the lost type material of †*P. succini*, only seven specimens of †*Prosphyracephala* were known from Baltic amber prior to the present study (Loew 1873; Meunier 1903; Hennig 1965; Schumann 1994). Several new finds, predominantly from private amber collections, now provide the opportunity for a more detailed phylogenetic and morphometric analysis. This paper has the goal to (1) further resolve the phylogeny of the Diopsinae and specifically the phylogenetic position of †*Prosphyracephala*, (2) find out whether sexual dimorphism existed in this earliest known representative of the Diopsinae, and (3) reanalyse the evolution of sexual dimorphism in the basal clades of Diopsinae.

## Material and methods

Twenty-eight specimens of †*P. succini* from Baltic amber (including Bitterfeld amber) were investigated, including the material described by Meunier (1903 as †*P. breviata*, but synonymized with †*P. succini* by Hennig 1965) and Schumann (1994). The type material of †*P. succini* (Loew, 1873) could not be located and is probably lost (Hennig 1965; Schumann 1994). The majority of the investigated material came from private collectors (listed in the Acknowledgements).

Besides the fossil species, 15 extant diopsid species representing all major clades of the family were included in the phylogenetic analysis, as well as representatives of the four other families of Diopsoidea sensu McAlpine (1997), i.e. Psilidae, Nothybidae, Gobryidae, and

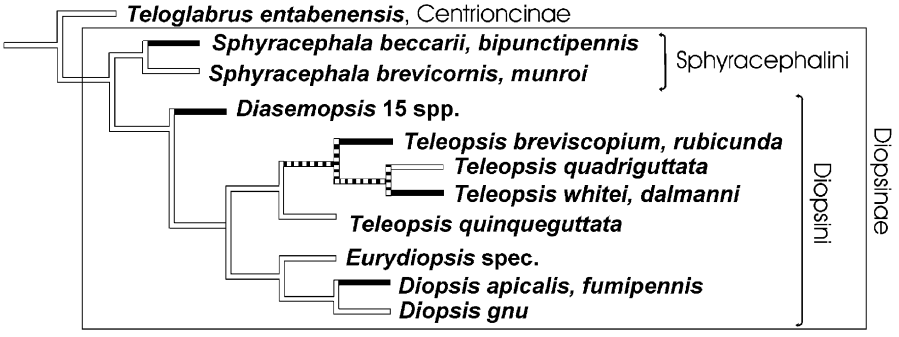


**Fig. 2.** Phylogeny of Diopsidae proposed by Baker and Wilkinson (2001) based on cladistic analysis of mitochondrial and nuclear genes. Terminal clades with congruent character states collapsed. White lines indicate eyestalk monomorphism, black lines eyestalk dimorphism, dotted lines indicate an ambiguous condition.

**Table 1.** Dataset for cladistic analysis of Diopsidae

| Character number | 0000000001 | 1111111112 | 2222222223 | 3333333334 | 4444444445 | 5555555556 | 666666 |
|---|---|---|---|---|---|---|---|
| Taxon | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 1234567890 | 123456 |
| Psilidae | 000**AA**00000 | 010000**B**00- | 00**1**00-0-00 | ???-??1100 | 00000?0-00 | -000**002--0** | **1B21-0** |
| Gobryidae | 0120201001 | 1000010010 | 1101-101-0 | ???1??0000 | 0001?????? | ????**002--0** | **0121-0** |
| Nothybidae | 0000001001 | 0100000000 | 00**BB**000030 | ???0??1100 | 0001-0??-1 | --0-**002--0** | **012?-0** |
| Syringogastridae | 0110001001 | **BBB**0110101 | 11**B**0111110 | 1101000011 | 1000-00--0 | --0-**002--1** | **012100** |
| *Teloglabrus entabenensis* | 0111010100 | 0011110010 | 0-01001101 | 1121000011 | 1110000-00 | -000**001110** | **000020** |
| *Sphyracephala beccarii* | 1112011110 | 1111101010 | 0001100020 | 0011110011 | 1110111--0 | --0-**102000** | **102121** |
| *Sphyracephala brevicornis* | 1112010110 | 1111101010 | 0001100020 | 0011110011 | 1110111--0 | --0-**102000** | **102120** |
| *Sphyracephala bipunctipennis* | 1122011110 | 1111101010 | 0001100020 | 0011110011 | 11101120-0 | 0-0-**102100** | **102121** |
| *Diasemopsis longipedunculata* | 1112111111 | 1211101110 | 0001100010 | 0011110011 | 1110001-00 | -000**102220** | **012-21** |
| *Diasemopsis conjuncta* | 1112110111 | 1211101110 | 0011100010 | 0011110011 | 1110002000 | 0000**102220** | **012-21** |
| *Diasemopsis meigenii* | 1112111111 | 1211101110 | 0011100010 | 0011110011 | 1110001-00 | -000**101221** | **012-21** |
| *Teleopsis whitei* | 1112111111 | 1211100110 | 0101100110 | 0011110011 | 1**2**10002110 | 1000**111221** | **012-11** |
| *Teleopsis quinqueguttata* | 1112111111 | 1211101110 | 01**0**1100110 | 0011110011 | 1**2**10002110 | 1000**111221** | **012-10** |
| *Teleopsis quadriguttata* | 1112111111 | 121110111? | 0101-00?10 | 00111100-1 | 1**2**10002110 | 1000**111221** | **012-10** |
| *Eurydiopsis* spec. | 1112111111 | 1211101110 | 0101100010 | 0011110011 | 1**2**10002101 | 1100**1C2221** | **002-10** |
| *Diopsis apicalis* | 1112111111 | 1211101110 | 0101100010 | 0011110011 | 1**2**10001-01 | -100**102221** | **012-11** |
| *Diopsis circularis* | 1112111111 | 0211101110 | 0-01100010 | 0011110011 | 1**2**10001-01 | -11?**101221** | **012-1?** |
| *Diopsina africana* | 1112110111 | 1211101100 | 0101100010 | 0011110011 | 1**2**10?????? | ????**102221** | **012-10** |
| *Cladodiopsis seyrigi* | 111201**C**11**1** | 1111101010 | 0**00**1200020 | 0011110011 | 1110?????? | ????**101210** | **012121** |
| †*Prosphyracephala succini* A | 1112010111 | 111111010?? | ???**0**?????**0** | ???????00**11** | ?**110**?????? | ????**010111** | **001021** |
| †*Prosphyracephala succini* B | 111201?111 | 121111010?? | ???**0**?????**0** | ???????00**11** | ?**110**?????? | ????**010111** | **001021** |

Revisions and additions with respect to the matrix of Meier and Hilger (2000) in boldface. $A=0+1+2$; $B=0+1$; $C=0/1$. Characters 2 and 3 included in the analysis alternatively; character 66 excluded during generation of the phylogenetic hypothesis.

Syringogastridae (Table 1). Taxon sampling was designed to closely match previous analyses (Meier and Hilger 2000; Baker et al. 2001; Baker and Wilkinson 2001; Meier and Baker 2002). The taxonomy of *Diasemopsis meigenii* (Westwood), *Teleopsis whitei* (Curran) and *T. quinqueguttata* (Walker) follows Meier and Baker (2002).

Specimens were studied using a Leica MZ12 stereoscope with drawing tube and a Zeiss AxioCam digital camera. Some amber pieces were temporarily submerged in water to reduce distortion effects without altering the specimen. Nevertheless, a few of the morphometric data are approximations rather than exact measurements. Amber specimens differ in which characters are observable or hidden due to their particular position in the stone or to inclusions of air, particles, swirls, etc. Therefore, owing to partial lack of data, some specimens could be included in one morphometric analysis but not another. In some specimens the terminalia were not clearly visible but the sex could be safely inferred from the shape of the abdominal tip. Where the tip of the abdomen was entirely lost the sex could not be determined.

The character matrix for the phylogenetic analysis comprises 52 binary and 14 multistate characters (see Appendix A and Table 1). It is based on the matrix of Meier and Hilger (2000), respective character numbers identical, see there regarding the alternative inclusion of characters 2 and 3). The matrix of Meier and Hilger has been revised and augmented with several additional characters (printed in boldface in Table 1). Some characters discussed by Hennig (1965) and/or Schumann (1994), but excluded by Meier and Hilger (2000), could be included in the present analysis due to more detailed study. For a detailed general description of diopsid morphology see Feijen (1989).

Phylogenetic analyses were carried out using the programs Hennig86 (Farris 1988; 'ie') and PAUP* (4.0b10, Swofford 2002; 'branch and bound') in connection with Nexus Data Editor (0.5.0, Page 2001) and WinClada (Nixon 1999) as shell programs. All characters were weighted equally. Multistate characters were treated as unordered. Sexual dimorphism (character 66) was excluded from the phylogenetic analysis, because the resulting cladogram was subsequently used to analyse the evolution of sexual dimorphism. In order to assess the level of nodal support on the most parsimonious trees, bootstrap values were calculated in PAUP* (branch and bound search, 1000 replications). Moreover, Bremer support (Bremer 1988) was calculated using AutoDecay (Eriksson 1998) in connection with PAUP* (branch and bound search).

To assess the presence of sexual dimorphism in the fossil specimens, the following parameters were measured (see Fig. 1) and recorded together with the sex of the specimen: eye span $E$ (distance between the lateral margins of the eyes), eye stalk diameter $D$ (measured at the stalk's narrowest part), body length $L$ (distance from face to posterior end of abdomen or to tips of folded wings, whichever could be determined), thorax width $T$, and wing length $W$. 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). Residues were tested for normal distribution using the Kolmogorov–Smirnov test.

The evolution of sexual dimorphism (character 66) was analysed by coding this character as binary (present–absent) and optimizing it a posteriori onto the new phylogenetic hypothesis in WinClada and McClade (Maddison and Maddison 1992).

## Results

All fossil specimens included in this study were classified as belonging to the monotypic genus †*Prosphyracephala*, based on the genus concept of previous authors (Loew 1873; Hennig 1965; Schumann 1994), i.e. similar to *Sphyracephala* but with slightly longer eye stalks, longer scutellar spines, apically wide scutellum, notopleural bristle reduced, smaller anal lobe, shorter anal cell, and with the anal vein forming a straight line with the posterior margin of the anal cell.

Within the sample some morphological variation was found, specifically regarding the visibility of the anal vein and wing pattern. While the anal vein is clearly visible in some specimens, it is faint or not distinguishable from a fold in others — a problem also encountered by other authors (Feijen 1989, and pers. comm.). Likewise, a wing pattern is clearly discernible in some specimens, whereas it is faint or completely absent in others. In both cases intermediates between the extremes were observed, and it was not possible to define discrete character states. To rule out the possibility that cryptic species in the sample could influence the results of the following analyses, both conditions of the anal vein (character 12) were accounted for and coded as two separate taxa (†*P. succini* A: anal vein present; †*P. succini* B: anal vein absent).

### Phylogenetic analysis

The 66 morphological characters and their coding for a total of 21 taxa are listed in Appendix A and Table 1, respectively. Some characters of the wing base, such as the sizes of alula and anal lobe, the shape of the anal cell, and the course of the anal vein, had been misinterpreted for †*Prosphyracephala* in the past (Hennig 1965; Feijen 1989; Schumann 1994). For clarification, comparative illustrations of the wings of representatives for the major clades of Diopsidae were scaled to the same wing length (Fig. 3).

The alula of †*Prosphyracephala* is much reduced compared to *Teloglabrus* and *Sphyracephala*, and thus much more resembles the conditions in *Cladodiopsis* and *Diasemopsis*. The anal lobe of †*Prosphyracephala* is narrow compared to that of *Sphyracephala*, more

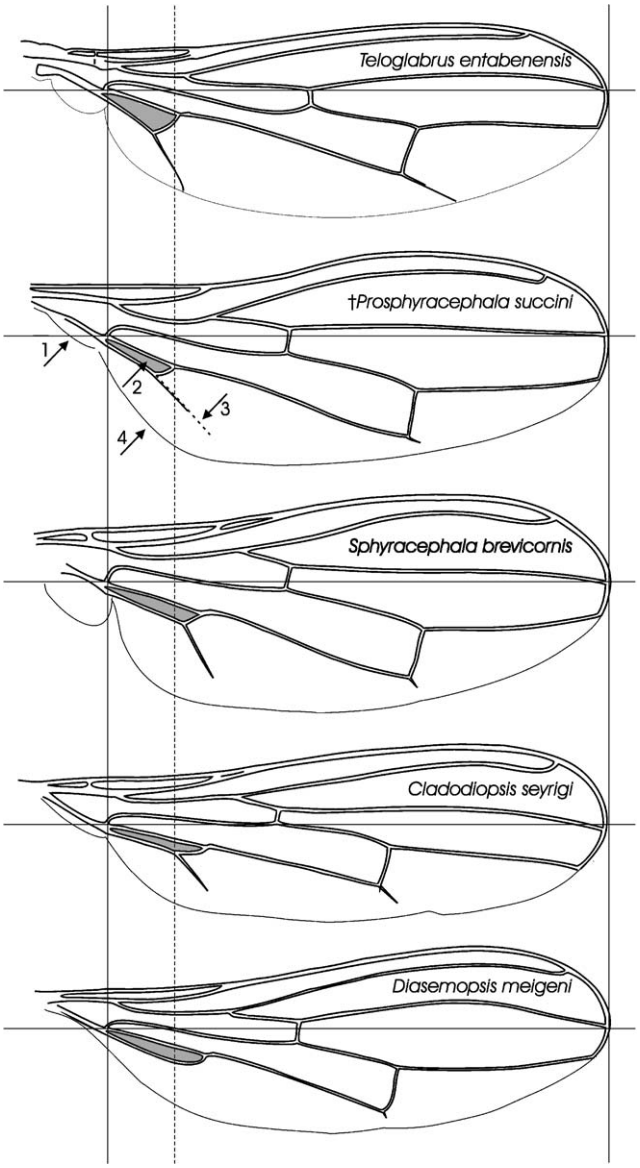


**Fig. 3.** Evolution of wing venation in Diopsidae. Drawings from digital images; reference lines added to facilitate comparison. Arrows: 1 = alula; 2 = anal cell (highlighted); 3 = anal vein; 4 = anal lobe.

resembling that of *Teloglabrus*, *Cladodiopsis* and *Diasemopsis*. The anal cell (grey in Fig. 3) of †*Prosphyracephala* is narrowed but not elongate with respect to *Teloglabrus*, whereas it is narrowed and elongate in all other Diopsinae. Where the anal vein of †*Prosphyracephala* is discernable it runs in almost straight continuation of the posterior margin of the anal cell.

The cladistic analysis resulted in 16 most parsimonious trees with length 135, CI = 0.67, and RI = 0.77. The strict consensus tree is shown in Fig. 4. Regarding the family, subfamily and genus levels the tree topology largely agrees with the results of Meier and Hilger

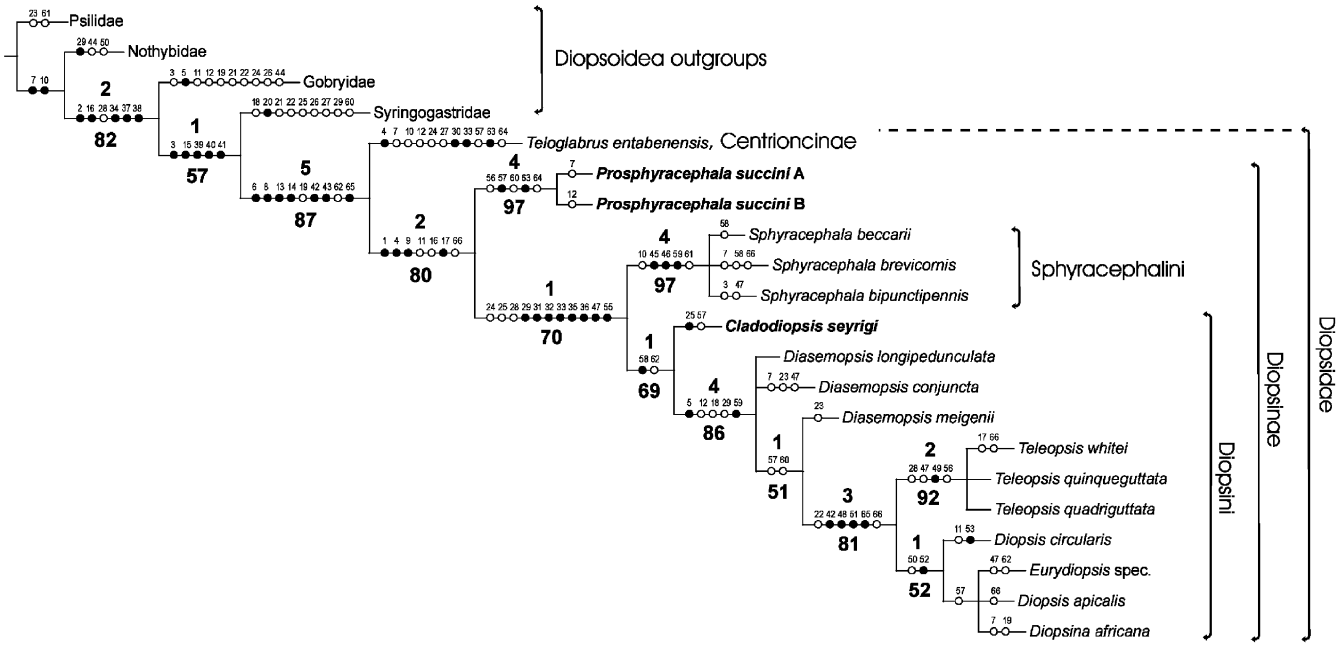


**Fig. 4.** Phylogeny of Diopsidae based on cladistic analysis of 65 morphological characters (strict consensus from 16 most parsimonious trees, length = 136; CI = 0,66; RI = 0,76; slow optimization). Solid circles: unique character changes, open circles: homoplastic changes, character numbers above circles; Bremer support values given above the branches, bootstrap values > 50% below.

(2000), Baker et al. (2001), and Meier and Baker (2002). However, the positions of †*Prosphyracephala* and *Cladodiopsis* are now unambiguously resolved, with †*Prosphyracephala* emerging at the base of the Diopsinae and *Cladodiopsis* emerging at the base of the Diopsini. On the other hand, much of the terminal resolution in *Sphyracephala*, *Diasemopsis*, *Teleopsis*, and the *Diopsis* + *Eurydiopsis* clade is lost. Successive approximative weighting does not change the topology of the strict consensus tree, but resolves the relationships in *Sphyracephala* in agreement with Meier and Hilger (2000), though not with Baker et al. (2001) or Meier and Baker (2002).

The relatively poor consistency and retention indices are partly due to much homoplasy in the outgroups — a common problem when dealing with acalyptrate phylogenies at the family level. Exclusion of all four outgroups does not alter the topology of the strict consensus tree, but raises the index values to CI = 0.76 and RI = 0.84.

## Morphometric analysis

Fig. 5a illustrates the eye span $E$ of the investigated amber specimens as a function of their body length $L$ and sex. Because measurements of the body length are often inexact, due to different degrees of body flexion between thorax and abdomen and the different shapes of male and female terminalia, the eye span was also computed against the wing length $W$ and thorax width $T$ as alternative indicators of body size (Fig. 5b, c). Finally, in order to provide a character entirely independent of body size, the relative slenderness of the eyestalks was determined as the ratio of eyestalk length ($E/2$) to eyestalk diameter $D$ (Fig. 5d).

Table 2 lists the results of the regression analyses and ANCOVAs for $E$ in relation to $L$, $T$, and $W$.

The results show a highly significant correlation between $E$ and all three indices of body size. The correlation is described by the formula $E = ax + b$, with $a$ being the slope of the regression line, $x$ the indicator of body size, and $b$ the intercept of the regression line with the $y$-axis. For all three measurements, significant to highly significant sex-related differences ($\Delta$) were found regarding the slopes and/or the intercepts of the regression lines. Likewise, a $t$-test revealed a highly significant difference between the male and female $E/2D$ values ($p < 0.01$).

The statistical analysis is based on the assumption that all investigated amber specimens are conspecific. To account for the yet unexplained variation of the anal vein, its visibility is indicated by different symbols in Fig. 5a–d. Sexual dimorphism is evident in both, the specimens with well discernible anal vein (solid symbols) and those in which the anal vein could not or hardly be discerned (open and grey symbols). The occurrence of intermediates argues against two discrete groups (i.e.

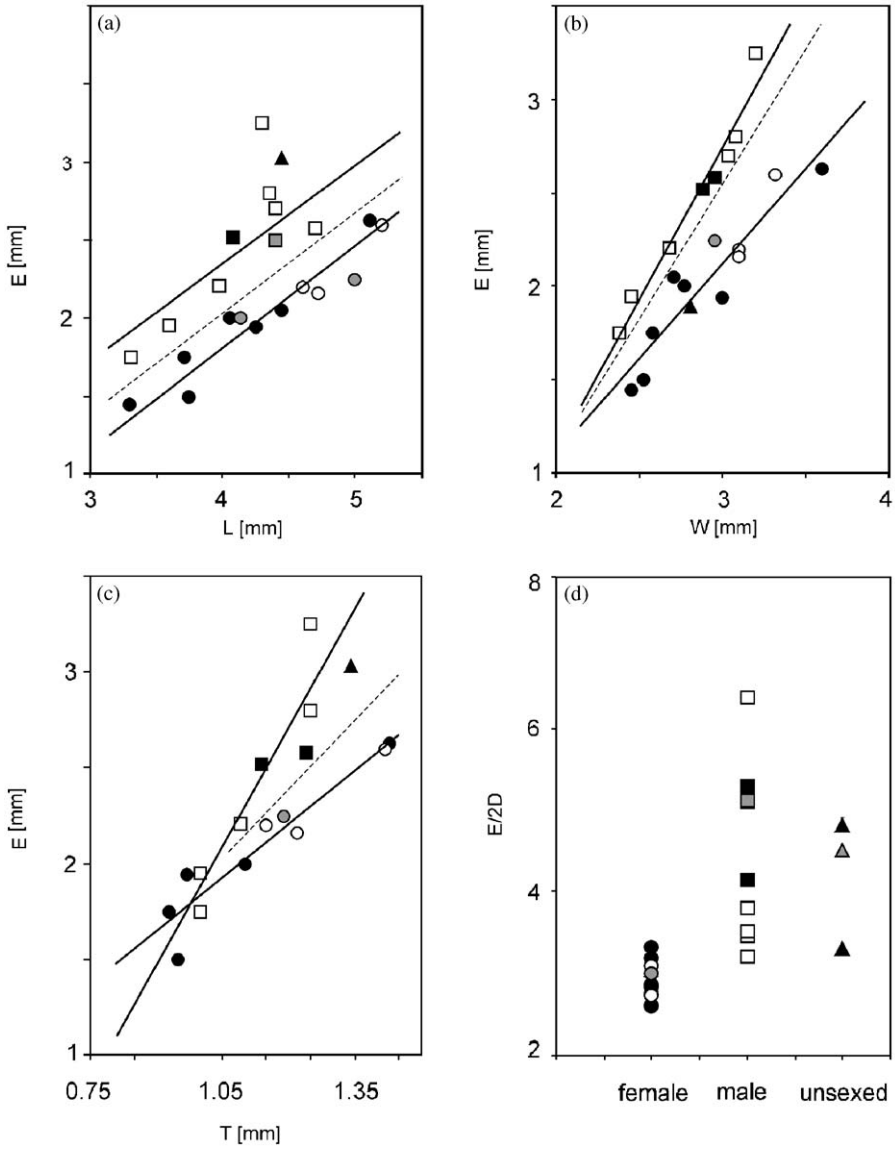


**Fig. 5.** Morphometry in †*P. succini*. (a) Eye span as a function of body length; (b) eye span as a function of wing length; (c) eye span as a function of thorax width; (d) ratio of eye stalk length to eye stalk diameter. Squares = males, circles = females, triangles = unsexed specimens; open symbols = anal vein not discernible, solid symbols = anal vein visible, grey symbols = condition of anal vein uncertain. Regression lines solid, dotted lines separate data on males and females.

species), and the allometric similarity between the two morphs supports the assumption of a single species. In any case, not even the potential presence of an additional species within the sample affects the evidence for presence of sexual dimorphism in †*Prosphyracephala*.

## Phylogenetic examination of the evolution of sexual dimorphism

The finding of sexual dimorphism in †*Prosphyracephala* and the new results regarding the phylogeny of the Diopsidae call for a new analysis of the evolution of sexual dimorphism in this family. To this end, the presence of sexual dimorphism (character 66) was optimized a posteriori onto the strict consensus tree (Fig. 6a). Dimorphism in *Cladodiopsis seyrigi* was established by the present author (unpublished data). Monomorphism in *Diopsina africana* has been shown by Feijen (1989). The number of steps required on the strict consensus tree is five. This number is identical to that on all most parsimonious trees. Alternatively, the presence of sexual dimorphism was optimized onto the phylogenetic hypothesis of Baker and Wilkinson (2001), with terminal clades having congruent character states

**Table 2.** Morphometric analysis (ANCOVA) of eye span in relation to three indicators of body size in †*Prosphyracephala succini* (Loew)

| | *N* | $r^2$ | | *a* | Δ*a* | *b* | Δ*b* |
|---|---|---|---|---|---|---|---|
| *L* | 12♀, 9♂ | 0.80 | | 0.65 | — | –0.78 | 0.58 |
| | | | *s* | 0.09 | — | 0.40 | 0.10 |
| | | | *p* | 0.00*** | — | 0.07* | 0.00*** |
| *T* | 9♀, 7♂ | 0.89 | | 1.87 | 2.34 | –0.03 | –2.32 |
| | | | *s* | 0.32 | 0.69 | 0.37 | 0.79 |
| | | | *p* | 0.01*** | 0.01*** | 0.93 | 0.01*** |
| *W* | 11♀, 8♂ | 0.93 | | 1.01 | 0.57 | –0.91 | –1.10 |
| | | 0.12 | *s* | 0.21 | 0.35 | 0.59 | |
| | | 0.00*** | *p* | 0.01*** | 0.02** | 0.08* | |

*L* = body length; *T* = thorax width; *W* = wing length; *a* = slope of regression line; *b* = intercept of regression line with *y*-axis; Δ = sex-related difference. Significance levels: * = 0.1, ** = 0.05, *** = 0.01.

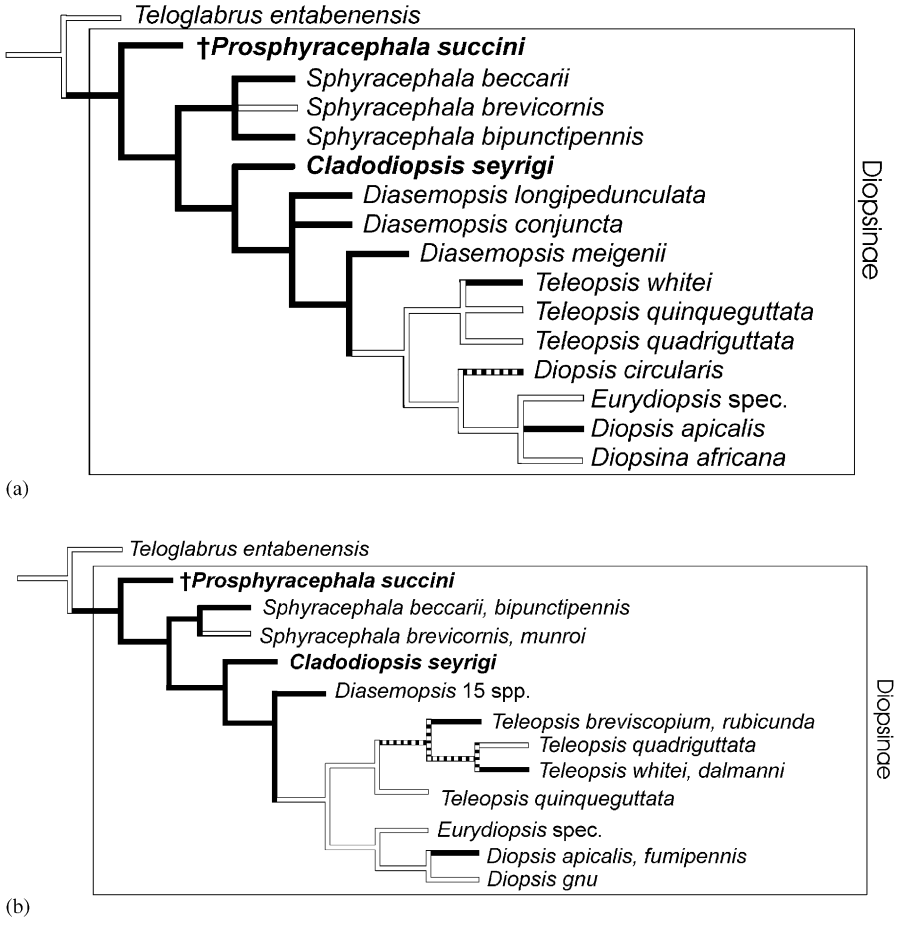


**Fig. 6.** Evolution of sexual dimorphism in Diopsidae. White lines indicate eyestalk monomorphism, black lines eyestalk dimorphism, dotted lines indicate an ambiguous condition. (a) Strict consensus derived from the present analysis; (b) phylogenetic hypothesis of Baker and Wilkinson (2001) with †*Prosphyracephala* and *Cladodiopsis* added. Terminal clades with congruent character state collapsed. Both cladograms support presence of dimorphism at the base of Diopsinae, with both slow and fast optimization.

collapsed, and with †*Prosphyracephala* and *Cladodiopsis* added (Fig. 6b). In both cladograms monomorphism is identified as the ancestral state at the base of the Diopsinae by both fast and slow optimization.

## Discussion

Eye stalks have evolved several times independently in higher Diptera (e.g. Grimaldi and Fenster 1989;

Wilkinson and Dodson 1997). They generally constitute a sexually dimorphic feature which occurs exclusively in males. Only in Diopsinae do the females have eye stalks as well, but in many species these are much shorter than those of the males. Behavioural studies show that the size of dipteran eye stalks is positively correlated with success in male–male contest and female choice (e.g. Burkhardt and de la Motte 1987; Grimaldi and Fenster 1989; Wilkinson and Dodson 1997; Panhuis and Wilkinson 1999). Accordingly, the most common and plausible hypothesis for eye stalk evolution in Diptera involves inter- and intrasexual selection on a sexually dimorphic feature (e.g. Grimaldi and Fenster 1989; Wilkinson and Dodson 1997).

This scenario was seemingly disproved by the analysis of Baker and Wilkinson (2001), who proposed monomorphism as the most likely ancestral state for diopsine eye stalks (Fig. 2). However, the situation changes with the inclusion of two critical taxa, †*Prosphyracephala* and *Cladodiopsis*, in the present analysis (Figs. 5 and 6). The phylogenetic placement of †*Prosphyracephala* at the base of the Diopsinae is in concordance with Hennig (1965), contrary to Feijen (1989) and Schumann (1994), who placed the fossil genus at the base of the Sphyracephalini. The placement of *Cladodiopsis* in the Sphyracephalini by previous authors is now contradicted by evidence supporting its inclusion in the Diopsini. In both †*Prosphyracephala* and *Cladodiopsis* the eye stalks are sexually dimorphic.

A re-evaluation of the distribution of sexual dimorphism in the Diopsidae now places sexual dimorphism at the base of the Diopsinae (black lines in Fig. 6), along with the evolution of the eye stalks themselves. It thus supports the scenario of sexual selection as a driving force in the evolution of diopsid eye stalks, as appears to be the case for all other stalk-eyed flies. It also implies that sexual dimorphism must have been secondarily reduced (and sometimes re-evolved) repeatedly throughout diopsid evolution, e.g. in *Diasemopsis* (Baker and Wilkinson 2001) and *Teleopsis* (Feijen 1998). This suggests that the maintenance of eye stalks in 'higher' diopsids is not entirely due to sexual selection, but also serves important visual needs. The relatively rapid evolutionary changes in this remarkable trait constitute a rewarding field of ongoing and future research.

In spite of the new discoveries, the early phylogeny of Diopsidae is still far from being resolved. Preliminary studies show considerable intrageneric variation within *Cladodiopsis*. More detailed investigation is required concerning both the monophyly of the genus and its phylogenetic position. Other urgently needed studies involve the genera *Sphyracephala* and *Pseudodiopsis* (which are synonymized by some authors, e.g. by Feijen 1989). Future research on the phylogeny of Diopsidae therefore primarily depends on the collecting of fresh material of various species of *Cladodiopsis*, *Sphyracephala* and *Pseudodiopsis* for further morphological study, as well as molecular analyses, preferably involving nuclear genes (see Baker et al. 2001).

Another important issue will be the continued close collaboration with amber collectors. Every new find of fossil Diopsidae may add to our understanding of the early history of the family. For example, the observed differences regarding the visibility of the anal vein in †*Prosphyracephala* have not been explained. They could be due to different preservation, intraspecific variation, or to the existence of more than a single species in the sample. Because Baltic amber was formed over a possible time span of several million years during the late Eocene, such specimens are not necessarily contemporaneous, i.e. they also could show different evolutionary stages. Last but not least, if †*Prosphyracephala* is the sister group of recent Diopsinae, the direct ancestor of the subfamily might still be found in the fossil record.

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## Appendix A. List of characters

At the end of each character entry the corresponding values for length, consistency index and retention index are given; uninformative/autapomorphic characters are denoted by [U].

1. Eye stalk: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
2. Inner vertical bristle: (0) present; (1) absent (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
3. Fronto-orbital bristles: (0) several; (1) one; (2) none (see Meier and Hilger 2000); [$L = 3$, CI = 66, RI = 50].
4. Arista: (0) plumose; (1) micropubescent; (2) glabrous (see Meier and Hilger 2000); [$L = 2$, CI = 100, RI = 100].
5. Arista: (0) tripartite; (1) bipartite; (2) unsegmented (see Meier and Hilger 2000); [$L = 2$, CI = 100, RI = 100].
6. Funiculus/pedicellus: (0) funiculus projecting into pedicellus; (1) pedicellus projecting into funiculus (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
7. Prosternum: (0) basiliform or reduced; (1) precoxal bridge complete (see Meier and Hilger 2000); [$L = 5$, CI = 20, RI = 20]. The condition of the prosternum shows intraspecific variability in *C. seyrigi*.
8. Scutellar spines: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
9. Metapleural spines: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
10. Alula: (0) well developed; (1) reduced; [$L = 3$, CI = 33, RI = 50]. Meier and Hilger (2000) coded the condition of the alula in *Cladodiopsis* as well as in *Sphyracephala* and Centrioncinae as 'present'. However, the alula of *Cladodiopsis* is strongly reduced compared to that of *Sphyracephala* and Centrioncinae, and much more resembles the (absent) condition in †*P. succini* and the Diopsini (Fig. 3).
11. Cu–$A_1$ (vein 5): (0) reaching wing margin; (1) not reaching wing margin (see Meier and Hilger 2000); [$L = 3$, CI = 33, RI = 33].
12. $A_1$–$CuA_2$ (vein 6): (0) reaching wing margin; (1) continued beyond anal cell but not reaching wing margin; (2) not continued beyond anal cell (see Meier and Hilger 2000, and Fig. 3); [$L = 4$, CI = 50, RI = 71].
13. Cells bm and dm: (0) separate; (1) united (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
14. Fore femur: (0) slender; (1) incrassate (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
15. Fore femur: (0) without tubercles; (1) with tubercles (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
16. Tubercles on hind femur: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 50].
17. Subapical bristles on mid tibia: (0) one; (1) two (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 75].
18. Third abdominal tergite: (0) separate from $t1 + t2$; (1) fused with $t1 + t2$ (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 88].
19. Suture on syntergum: (0) present; (1) lost (see Meier and Hilger 2000); [$L = 3$, CI = 33, RI = 33].
20. Female abdominal spiracle 1: (0) in membrane; (1) in tergite (see Meier and Hilger 2000); [U].
21. Female abdominal spiracle 6: (0) in membrane; (1) in tergite (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 0].
22. Female abdominal spiracle 7: (0) in membrane; (1) in tergite (see Meier and Hilger 2000); [$L = 3$, CI = 33, RI = 71].
23. Number of spermathecae: (0) three; (1) two; [$L = 3$, CI = 33, RI = 0]. As opposed to the coding of Meier and Hilger (2000), the author has found three spermathecae to be present in *C. seyrigi* and *T. quinqueguttata*.
24. Male tergite 6: (0) almost as long as tergite 5; (1) about half as long as tergite 5 (see Meier and Hilger 2000); [$L = 3$, CI = 33, RI = 33].
25. Male abdominal sternite 6: (0) undivided; (1) divided; (2) absent (see Meier and Hilger 2000); [$L = 3$, CI = 66, RI = 50].
26. Male abdominal spiracle 6: (0) in membrane; (1) in sclerite (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 0].
27. Male abdominal sternite 7: (0) not forming ventral band; (1) forming ventral band (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 0].
28. Male abdominal spiracle 7: (0) in membrane; (1) in sclerite (see Meier and Hilger 2000); [$L = 3$, CI = 33, RI = 50].
29. Male abdominal sternite 8: (0) enlarged; (1) normal; (2) very small; (3) absent (see Meier and Hilger 2000); [$L = 4$, CI = 75, RI = 75].
30. Surstylus: (0) bare or with bristles; (1) with tubercles and spinous bristles (see Meier and Hilger 2000); [U].
31. Inner gonostylus: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
32. Phallapodeme: (0) of normal size; (1) reduced (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
33. Phallapodeme: (0) separate from hypandrium; (1) linked via process; (2) 2/3 fused (see Meier and Hilger 2000); [U].
34. Aedeagus: (0) long; (1) short (see Meier and Hilger 2000); [U]
35. Aedeagus: (0) without basal ring; (1) with basal ring (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
36. Postgonites: (0) simple; (1) complex (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].

37. Postocular bristle: (0) absent or small; (1) enlarged (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
38. Prothoracic spiracle: (0) slit-like; (1) subcircular, not covered by hairs (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
39. Supraalar carina: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
40. Cylindrical posterior extension of metathorax: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
41. Tarsal sawline at least on midtarsal element: (0) absent; (1) present (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
42. Position of postalar bristle: (0) normal; (1) dorsomedially displaced; (2) absent (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100]. In addition to the normal (0) and displaced (1) conditions coded in Meier and Hilger (2000), the complete reduction of the postalar bristle in *Teleopsis*, *Eurydiopsis* and *Diopsis* was coded as (2).
43. Dorsal seam on pedicellus: (0) present; (1) absent (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
44. Ptilinal fissure: (0) long, with descending lateral arms; (1) short, without arms (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 0].
45. Chorion sculpturation: (0) mostly striated; (1) no striation, hexagonal pattern (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
46. Fine structure of micropyle: (0) with tube and surrounding struts; (1) without tube and struts (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
47. Plastron opening: (0) chorion without externally visible openings; (1) chorion broken up into open hexagons on dorsal side; (2) open hexagons in single band across full length of egg (see Meier and Hilger 2000); [$L = 5$, CI = 40, RI = 57].
48. Chorion on either side of open hexagon band: (0) not elevated; (1) forming elevated ridge (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
49. Distribution of ridges: (0) ridges evenly distributed; (1) ventral ridges far apart (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
50. Egg length: (0) $< 0.95$ mm; (1) $> 1.2$ mm (see Meier and Hilger 2000); [$L = 2$, CI = 50, RI = 66].
51. Number of open hexagons along band of plastron openings: (0) less than 40; (1) more than 80 (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
52. Ridge termination: (0) terminating at egg pole or fusing; (1) terminating also freely on the chorion (see Meier and Hilger 2000); [$L = 1$, CI = 100, RI = 100].
53. Position of micropyle: (0) central; (1) strongly shifted toward ventral (see Meier and Hilger 2000); [U].
54. End section of ridges: (0) uninterrupted; (1) broken up into stubs (see Meier and Hilger 2000); [U].
55. Bristles on pedicel: (0) short or at least not constituting a fringe; (1) constituting a fringe; [$L = 1$, CI = 100, RI = 100]. In all Diopsinae except †*P. succini* the pedicel bears a long, regular fringe of delicate setae along its distal margin.
56. Clypeus: (0) small; (1) large; [$L = 2$, CI = 50, RI = 75]. The clypeus of †*P. succini* is large in comparison to most other Diopsidae.
57. Scutellum: (0) apically wider than long; (1) apically as wide as long; (2) apically narrower than long; [$L = 5$, CI = 40, RI = 75].
58. Scutellar spines: (0) shorter than scutellum; (1) about as long as scutellum; (2) longer than scutellum (see Hennig 1965); [$L = 2$, CI = 100, RI = 100].
59. Apical bristles: (0) longer than scutellar spines; (1) about as long as scutellar spines; (2) shorter than scutellar spines (see Hennig 1965); [$L = 2$, CI = 100, RI = 100].
60. Posterior notopleural bristle: (0) distinct; (1) reduced (see Hennig 1965); [$L = 3$, CI = 33, RI = 77]. In a few specimens of †*Prosphyracephala* an extremely tiny hair in the position of the posterior notopleural bristle could be discovered under highest magnification.
61. Anal lobe: (0) not enlarged; (1) enlarged (see Fig. 3 and text); [$L = 2$, CI = 50, RI = 66].
62. Wing base: (0) evenly covered with microtrichia; (1) with bald areas without microtrichia; [$L = 3$, CI = 33, RI = 66]. In *Teloglabrus*, †*P. succini*, *Sphyracephala* and *Eurydiopsis*, the wing base is evenly covered with microtrichia, whereas in all other Diopsidae and the outgroups it has bald areas without microtrichia.
63. Anal cell: (0) triangular; (1) narrowed; (2) narrowed and elongate (see Fig. 3 and text); [$L = 2$, CI = 100, RI = 100].
64. Continuation of $A_1$–$CuA_2$ (vein 6) beyond anal cell: (0) almost straight; (1) at a distinct angle (see Fig. 3 and text); [$L = 2$, CI = 50, RI = 50].
65. Tubercles on fore femur: (0) in single row; (1) in double row; (2) in double row plus two rows of spinous bristles; [$L = 2$, CI = 100, RI = 100]. In the ground plan condition of Diopsidae the fore femora are ventrally adorned with a longitudinal double row of short tubercles which are flanked on either side by a row of spinous setae. In the clade comprising *Diopsina*, *Diopsis*, *Eurydiopsis* and *Teleopsis*, the spinous setae are reduced while the tubercles become elongated into spines themselves.
66. Sexual dimorphism of eye stalks: (0) absent; (1) present (see Baker and Wilkinson 2001, and text); [$L = 5$, CI = 20, RI = 55].

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