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Morphometric study of wing venation in the recent Trichoceridae - an application to the fossils?

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Abstarct. The wings of 12 species of the genus *Trichocera* MEIGEN and representatives of the genera: *Diazosma* BERGROTH and *Nothotrichocera* ALEXANDER were measured, 22 characters per wing. Sexual dimorphism in wings was stated and found to coincide with discerning the species and genera. Within the genus *Trichocera*, the male wing characters - in spite of their great similarity - may be used for tracing the inter-species relations. Clustering procedure applied to the male representatives of the genera reflects the generic assignment.

Key words: Trichoceridae, wings, sexual dimorphism, morphometric, principal components analysis.

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INTRODUCTION

Recently numerous materials of fossil Trichoceridae have been collected and are a subject of the monograph in preparation (KRZEMIŃSKI, DAHL, KRZEMIŃSKA- in prep.). Their age varies from the Lower Jurassic to Miocene. The specimens are represented mainly by the imprints of wings, often with clearly visible venation. The materials are so rich that authors face the problem of variation - there is no more a case of "one wing - one species".

This problem is since long known among the specialists of other groups of animals, and it is equally known that the multivariate morphometric techniques can be helpful in grouping the fossil specimens (for a review, see REYMENT, BLACKITH and CAMPBELL 1984). A natural background for studying the fossil specimens should be a sample of their living descendants, as the closest relatives. This work is meant to be such a background for the future study on the fossil Trichoceridae. The sample of extant Trichoceridae is represented only by the wings which will be analysed by the methods that eventually could be applied to the fossils. The genera and species representatives will be re-grouped only

on a basis of the variation their wings provide, by means of these techniques that do not demand previous classification. This should reveal how much the information obtained is biassed if we rely only on the wing venation as a source of this variation. The author hopes also to find out some hints that would help dealing with the morphometric analysis of the fossils.

It must be said, however, that the wing venation does not play a main role in the taxonomy of the recent Trichoceridae. Of the three genera of the family: *Trichocera* MEIGEN, *Nothotrichocera* ALEXANDER and *Diazosma* BERGROTH, only the latter is distinguished by the wing character: long A_2 (Fig.1) (the genus *Paracladura* BRUNETTI is not taken into account in this study, compare a paper: "Paracladurinae - new subfamily", this issue). The wing characters ascribed to the genus *Nothotrichocera* by ALEXANDER (1926) were meant to help discerning this genus from *Paracladura*; otherwise they hold in the genus *Trichocera* and even *Diazosma*. Within the entire genus *Trichocera* (72 species) there is no single species that could be defined by the venation characters. A character: "R_{2+3} shorter than first section of R_2" ascribed to *Trichocera major* by EDWARDS (1921) appeared not to hold in all specimens (EDWARDS 1938).

Thus the task undertaken in the present work seems to be a rather akward one.

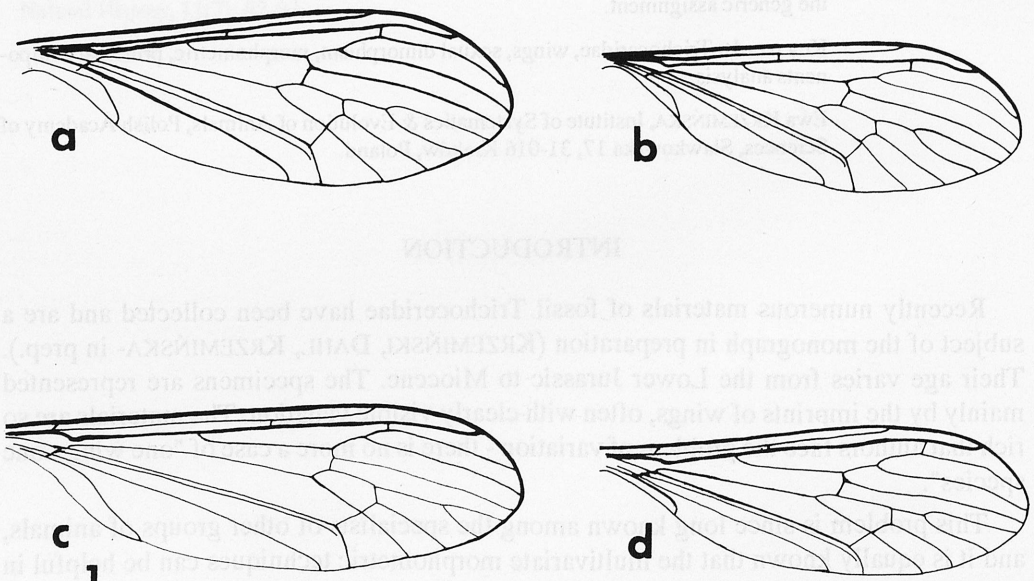


Fig. 1. Wing venation in the genera (Trichoceridae): a - *Trichocera* (*Trichocera*), b - *Trichocera* (*Metatrichocera*) *forcipula*, c - *Diazosma*, d - *Nothotrichocera*.

MATERIALS

The wings of the following species were examined: *Trichocera* (*Trichocera*) *annulata* MEIGEN - 34 males + 11 females (Italy); *T.*(*T.*) *dahlae* MENDL - 30 males + 12 females (Germany); *T.*(*T.*) *hiemalis* (DeGEER) - 30 males + 8 females (Poland); *T.*(*T.*) *implicata* DAHL - 12 males (7 from Poland, 5 from Germany); *T.*(*T.*) *japonica* MATSUMURA - 32 males (22 from Poland, 10 from Germany); *T.*(*T.*) *maculipennis* MEIGEN - 32 males + 12 females (Poland); *T.*(*T.*) *major* EDWARDS - 35 males (23 from Poland, 7 from Germany, 5 from France) + 14 females (9 from Poland, 4 from France, 1 from Germany); *T.*(*T.*) *parva* MEIGEN - 39 males (29 from Poland, 10 from France); *T.*(*T.*) *regelationis* (L.) - 40 males + 23 females (Poland); *T.*(*T.*) *saltator* (HARRIS) - 37 males (Poland).

Trichocera (*Metatrichocera*) *candida* DAHL - 11 males + 4 females (Poland); *T.*(M.) *forcipula* NIELSEN - 23 males (18 from France, 5 from Poland) + 7 females (France).

Diazosma hirtipennis SIEBKE: 3 males (1 from USA, 2 from Great Britain), 8 females (6 from USA, 1 from Great Britain, 1 from Switzerland)

Nothotrichocera representatives - males: a specimen of new described species (Chile), *antarctica* EDWARDS (USA), 8 males of undetermined species (New Zealand); females: *antarctica* (2 females from USA), 3 females of undetermined species (New Zealand).

METHODS

The camera pictures of one wing of each specimen was taken (left or right wing, since the pilot study showed no significant differences between left and right wing; however, the specimens showing greater assymetry in wings were excluded). The wing on picture (of size ca. 11-12 cm) was placed in a coordinate system (drawn on the transparency laid on the picture) so that the horizontal axis *x* was tangent to the costal margin of the alula and to the point or part of costal margin most convex, so that no part of wing is over the axis. The perpendicular, vertical axis *y* is tangent to the distal margin of wing (Fig.2). The starting point ("0") is outside the wing. The *x* coordinates of the following landmarks were measured: *size* (alular incision), *h* (humerus), *rcur* (curve of subcosta), *cua* (cross-vein between Cu and A_1), *sc* (end of Sc), *scr* (cross-vein sc-r), *rs* (beginning of Rs), *rr* (cross-vein r-r), *rlen* (length of cell delimited by two last veins, equal arithmetic difference: *rs-rr*), *r2a3* (fork of Rs), *fork* (R_{2+3} fork), *r1* (end of R_1), *ind* (first fork of Mb, inner corner of d cell), *oud* (cross-vein m-m, outer corner of d cell), *dlen* (d cell length equal arithmetic difference: *ind-oud*), *m1a2* (fork of M_{1+2}), *mcu* (cross-vein m-cu), *cu* (end of Cu), *a1* (end of A_1), *a2* (end of A_2).

All coordinates of cross-veins concern their lower, anal end.

Only two *y* coordinates were measured: *yr4* (tip of R_4 vein) and *widt* (greatest width of wing, the *y* coordinate of Cu tip).

The method of measuring was chosen so that it can be applied to the fossil wings and was dictated by the way they are preserved. The anal part of wing is often not retained and the wing may be contracted along its long axis, thus the shape of anal part was not taken

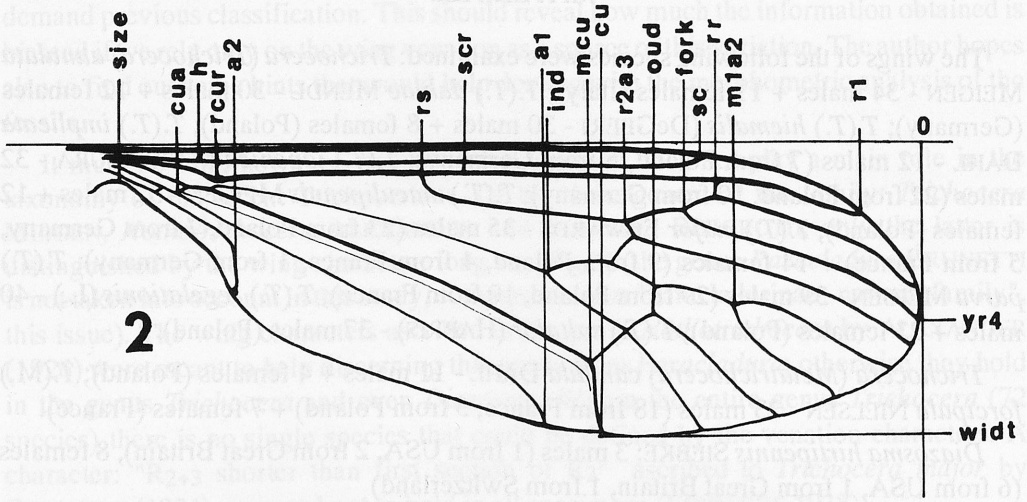


Fig. 2. Measurements used. Further explanations in text.

under the consideration and the *y* coordinates were reduced to only two, as they are most readily falsed in a contracted wing while the respective *x* coordinates keep their position.

All computations were performed by SYSTAT package (Systat, Inc., Evanston, IL).

RESULTS

1. **Sexual dimorphism within the genus** *Trichocera*.

The sexual dimorphism of the wings in the genus *Trichocera* was the first result stated and appeared to influence all further approach. The phenomenon was expressed to a various degree in all eight species in which the females were available.

a). wing size (Table I, variable: *size*): female wings are significantly larger than those of males in the species: *maculipennis, major, regelationis* of the subgenus *Trichocera*. In *annulata* and *dahlae* the difference is not significant though observed, in *hiemalis* this effect is not observed, as well as in the examined species of the subgenus *Metatrichocera*: *candida* and - especially - *forcipula*.

b). shape: Fig. 3. presents the male and - overlaid - female wing of the same size, in the position they were examined. All the measurements discussed below and presented in Table I are standardized to wing length (expressed in % of wing length, i.e. *size*). Female wing is wider (variable *widt*, Fig.4) and its distal part is more "heavy", expanded, drooping sooner from the axis *x* than the male wing (a good measure of this feature is variable *yr4* - Fig. 5). Generally, the landmarks of female wing are shifted to the proximal end when compared to the male. Thus almost all measurements taken from female wing are statistically larger than those of male. The smallest differences are observed again in two *Metatrichocera* representatives: *candida* and *forcipula*. Within the subgenus *Trichocera*

Table I

Means of standardized variates for males, below the differences (* - significant) observed in females, in 8 species (*size* in 0.1 mm).

Species	No.	Sex	Variables																				
			h	*rcur*	*cua*	*sc*	*scr*	*rs*	*rr*	*rlen*	*r2a3*	*fork*	*r1*	*ind*	*oud*	*dlen*	*m1a2*	*mcu*	*cu*	*a1*	*a2*	*yr4*	*widt*	*size*
annulata	34	♂	90.0	87.8	91.9	24.2	54.5	63.3	21.2	42.1	35.3	26.9	7.3	43.4	29.9	13.5	21.2	38.1	35.8	43.5	84.9	17.6	32.3	57.6
	12	♀	+0.1	0.0	+0.2	+1.7	+1.7*	+2.2*	+3.1*	-0.9	+1.5*	+2.4*	+0.2	2.0*	2.2*	-0.4	+0.8*	+1.8*	+2.2*	+2.1*	+1.2*	+3.8*	+2.0*	+3.6
dahlae	30	♂	91.5	89.8	93.3	24.4	55.2	64.3	22.3	42.0	38.1	27.7	8.5	45.6	31.3	14.5	23.2	39.4	35.8	44.2	86.6	15.2	31.2	69.4
	12	♀	+1.3*	+1.2	+1.0*	+2.3*	+3.7*	+4.5*	+3.4*	+1.2	+2.5*	+3.8*	-0.7	+3.8*	+2.8*	+0.8*	+2.8*	+3.1*	+1.8*	+2.1*	+2.9*	+4.7*	+2.7*	+6.2
hiemalis	30	♂	89.7	87.9	92.3	24.2	54.3	64.4	22.2	42.3	35.7	27.9	11.5	41.5	27.5	14.1	18.1	37.5	35.5	44.0	85.4	15.0	32.0	57.2
	9	♀	+1.1*	+1.2	+0.5	+2.6*	+3.8*	+4.9*	+2.9*	+2.2*	+2.3*	+2.6*	-0.4	+3.3*	+2.5*	+0.7*	+2.7	+3.2*	+2.7*	+2.7*	+1.2*	+5.5*	+2.1*	+1.0
maculi-pennis	32	♂	90.9	89.1	92.7	24.0	54.2	62.2	22.6	39.5	35.5	26.8	8.5	42.5	29.7	12.8	19.9	38.5	35.6	43.6	85.8	16.0	31.2	63.1
	12	♀	+1.0*	+1.0	+0.9	+2.0	+3.9*	+2.3*	+2.5*	-0.1	+3.3*	+2.8*	-0.3	+4.3*	+4.3*	-0.1	+4.5*	+4.0*	+2.7*	+2.3*	+1.7*	+4.8*	+2.8*	+14.6*
major	35	♂	91.3	89.4	93.1	26.4	55.6	67.5	23.7	43.9	39.1	32.4	10.4	46.2	32.8	13.9	23.0	42.4	37.7	44.9	86.0	19.3	33.2	76.0
	14	♀	+0.7	+0.6	+0.2	+0.2	-1.3	+2.5*	+2.2*	+0.2	+0.4	+1.1*	-0.5	+1.9*	+1.2*	+0.2	+2.0*	+1.6*	+1.3*	+1.3*	+1.2*	+3.6*	+1.3*	+9.9*
regela-tionis	40	♂	90.6	88.6	92.5	24.6	53.7	62.8	23.6	39.2	36.3	27.7	9.2	43.5	30.0	13.7	20.6	38.8	35.5	43.4	85.0	17.0	32.2	59.8
	23	♀	+0.9*	+0.9	+0.5	+0.9	+3.7*	+2.5*	+1.6*	+0.9	+1.4*	+1.8*	-0.8	+2.2*	+1.8*	+0.2	+2.0*	+2.4	+2.3*	+2.2*	+2.2*	+3.8*	+1.9*	+6.5*
candida	12	♂	91.6	89.7	93.1	25.9	59.2	68.7	23.9	44.7	37.1	30.3	8.0	44.4	30.6	13.7	18.8	39.7	36.7	43.9	85.1	18.6	32.1	62.3
	4	♀	+0.3	+0.5	+0.1	+0.6	-0.6	+1.2	+0.6	+0.8	+2.8*	+0.8	+0.3	+3.5*	+2.1*	+1.5*	+4.4*	+2.4*	+0.9	+1.6*	+2.4*	+3.2*	+2.6*	+2.3
forcipula	23	♂	89.7	87.5	92.1	29.9	59.0	69.1	28.0	41.1	42.8	34.8	11.9	50.7	35.5	15.2	27.2	43.5	41.7	48.6	86.1	21.1	35.2	47.9
	7	♀	+1.2*	+1.6	+0.7	+2.5*	+0.5	+2.4*	+0.4	+2.0*	+1.0*	+2.1	+1.5	+1.2*	+0.5	+0.7	+1.8	+1.4*	+1.7*	+1.3	+1.7*	-0.5	+0.9	-1.5

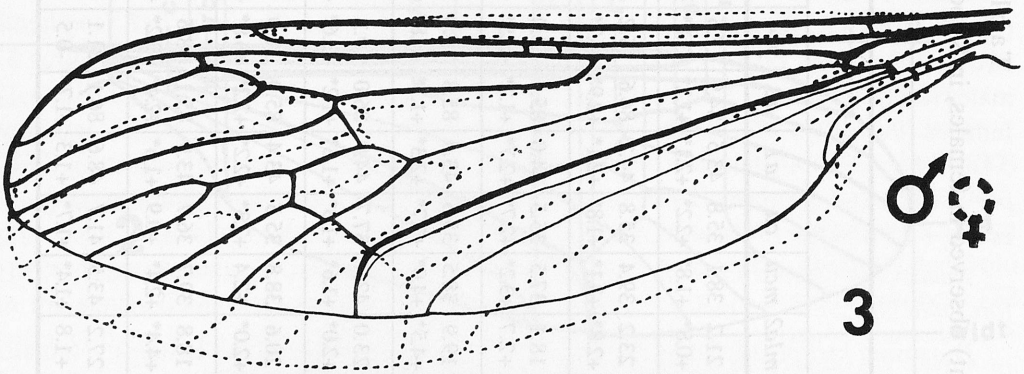


Fig. 3. Male and female wing of the same size: bold line - male, broken line - female (note the shift of all the landmarks to the proximal part in the female wing).

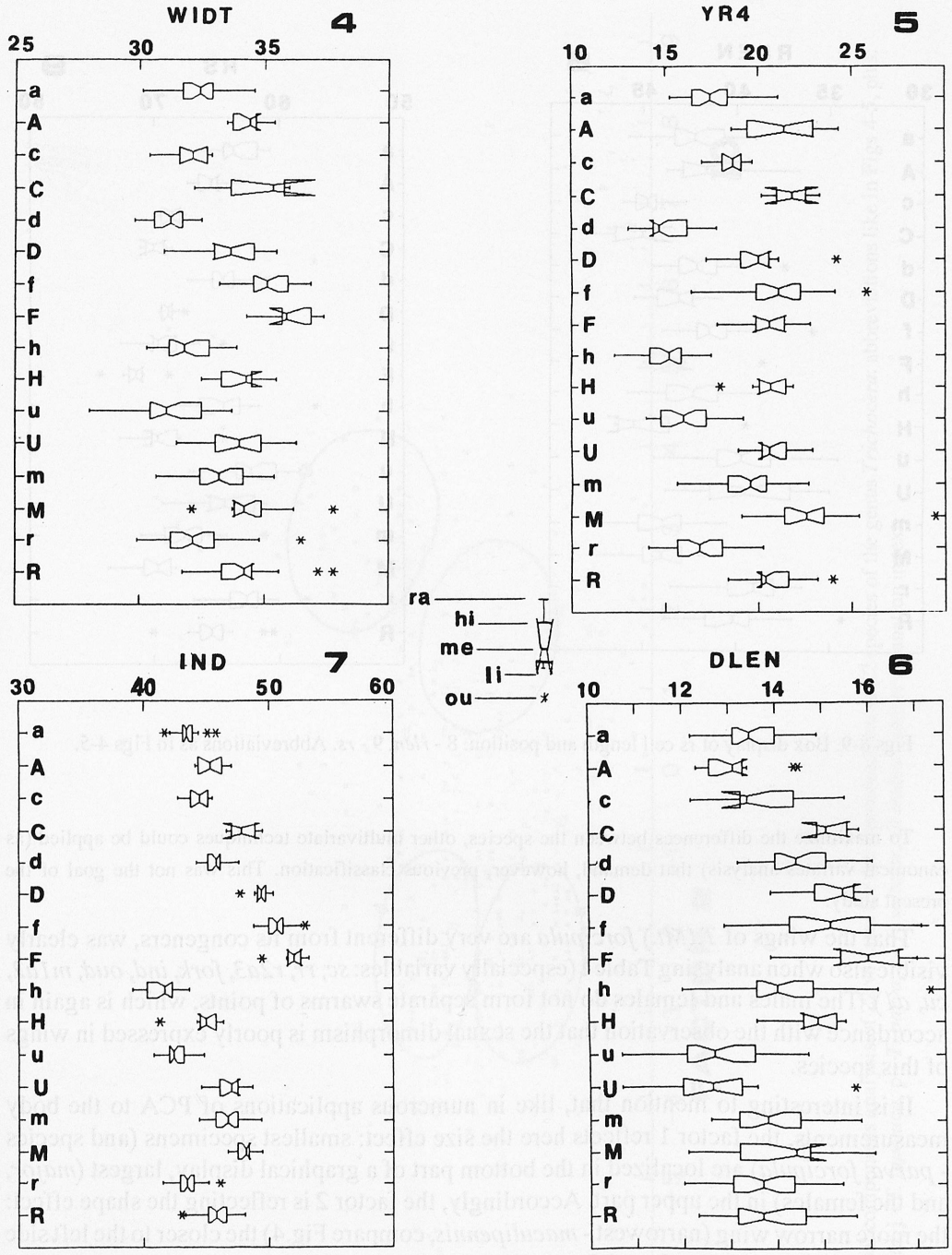
- in *T. major*. A very stable character is the position of h vein. Length of both cells: d cell and rs cell does not differ significantly, although both cells are transferred to the inside of the wing, which is indicated by the landmarks delimiting these cells: *ind, oud* and *rs, rr* - respectively (Figs 6-7, 8-9).

2. **Principal components analysis** (PCA) (Fig.10) was performed to check whether the species differences of wing venation allow to discern them, and how this process will be disturbed by sexual dimorphism. The analysis was performed on raw measurements of all variables beside *size* (in 0.1mm) collected from representatives of 12 *Trichocera* species. The covariance matrix was factored, first component loadings (sorted) are as follows:

h - 10.703, *ar* - 10.611, *rcur* - 10.441, *a2* - 10.070, *rs* - 7.780, *scr* - 6.199, *ind* - 5.601, *mcu* - 5.246, *a1* - 5.191, *rlen* - 4.938, *r2a3* - 4.572, *cu* - 4.364, *oud* - 4.059, *widt* - 3.837, *fork* - 3.698, *m1a2* - 3.129, *rr* - 2.845, *sc* - 2.833, *yr4* - 2.618, *dlen* - 1.540, *r1* - 0.875. Percent of variance explained by first and second component is: 96.847% and 1.289%, respectively.

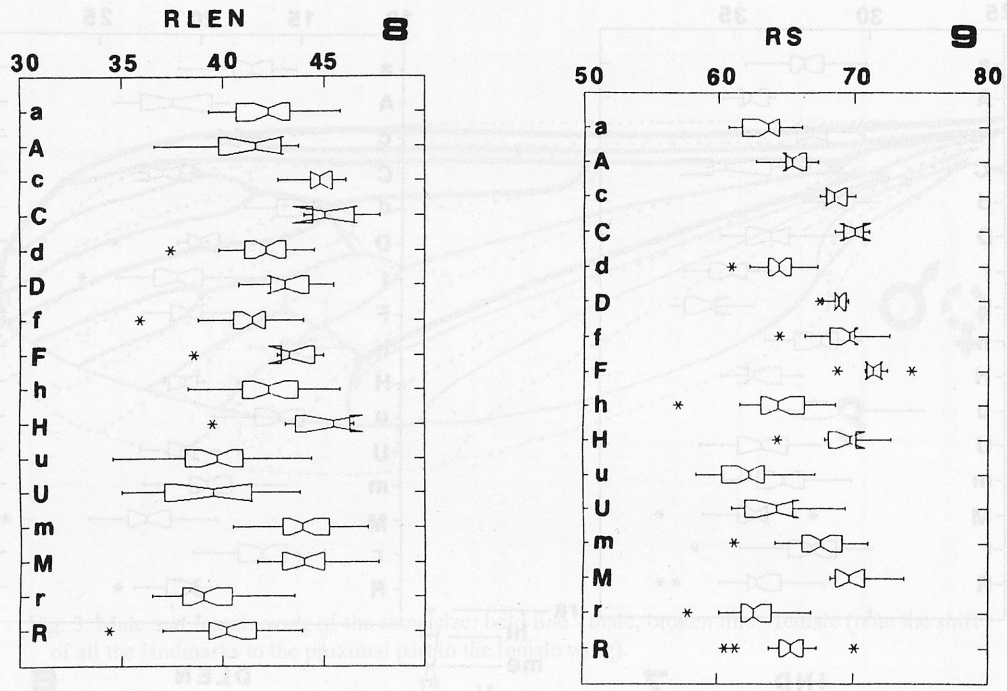
The points are distributed between two swarms: one is formed by all representants of the subgenus *Trichocera* + *T.(Mt.) candida*, while the other - exclusively by *T.(Mt.) forcipula*.

Within the former swarm it can be observed that the procedure discerns rather sexes that the species, although the males of some species (as: *dahlae* and *annulata*), encircled in Fig. 10, do not overlap. If we omit the sex influence (by excluding the females) the output is not much different, no distinct species clouds are formed (not illustrated).



Figs 4-5: Box* display of variables reflecting the differences between males and females wings of 8 species: 4 - *widt*; 5 - *yr4*: a - *T.(T.) annulata*, c - *M. candida*, d - *T. dahlae*, f - *M. forcipula*, h - *T. hiemalis*, u - *T. maculipennis*, m - *T. major*, r - *T. regelationis*. Upper case letters mean females, respectively. (* - WILKINSON 1990a: me - median, hi - hinge, ra - range, li - confidence limits).

Figs 6-7: Box display of d cell length and position: 6 - *dlen*, 7 - *ind*. Abbreviations as in Figs 4-5.



Figs 8-9. Box display of rs cell length and position: 8 - *rlen*, 9 - *rs*. Abbreviations as in Figs 4-5.

To maximize the differences between the species, other multivariate techniques could be applied (as canonical variates analysis) that demand, however, previous classification. This was not the goal of the present study.

That the wings of *T.(Mt.) forcipula* are very different from its congeners, was clearly visible also when analysing Table I (especially variables: *sc, rr, r2a3, fork, ind, oud, m1a2, cu, a1*). The males and females do not form separate swarms of points, which is again in accordance with the observation that the sexual dimorphism is poorly expressed in wings of this species.

It is interesting to mention that, like in numerous applications of PCA to the body measurements, the factor 1 reflects here the size effect: smallest specimens (and species - *parva, forcipula*) are localized in the bottom part of a graphical display, largest (*major*, and the females) in the upper part. Accordingly, the factor 2 is reflecting the shape effect: the more narrow wing (narrowest - *maculipennis*, compare Fig.4) the closer to the left side is localized. The widest wings (also the females) occupy the right side.

The factoring of covariance matrix gave the best results. If Pearson coefficients matrix was computed, much worse plot was obtained than that presented (the cloud of *forcipula* was not separate) nor was the size/shape effect so distinct. On the other hand, if only standardized data were taken, the percent of variance explained by factor 1 and 2 together

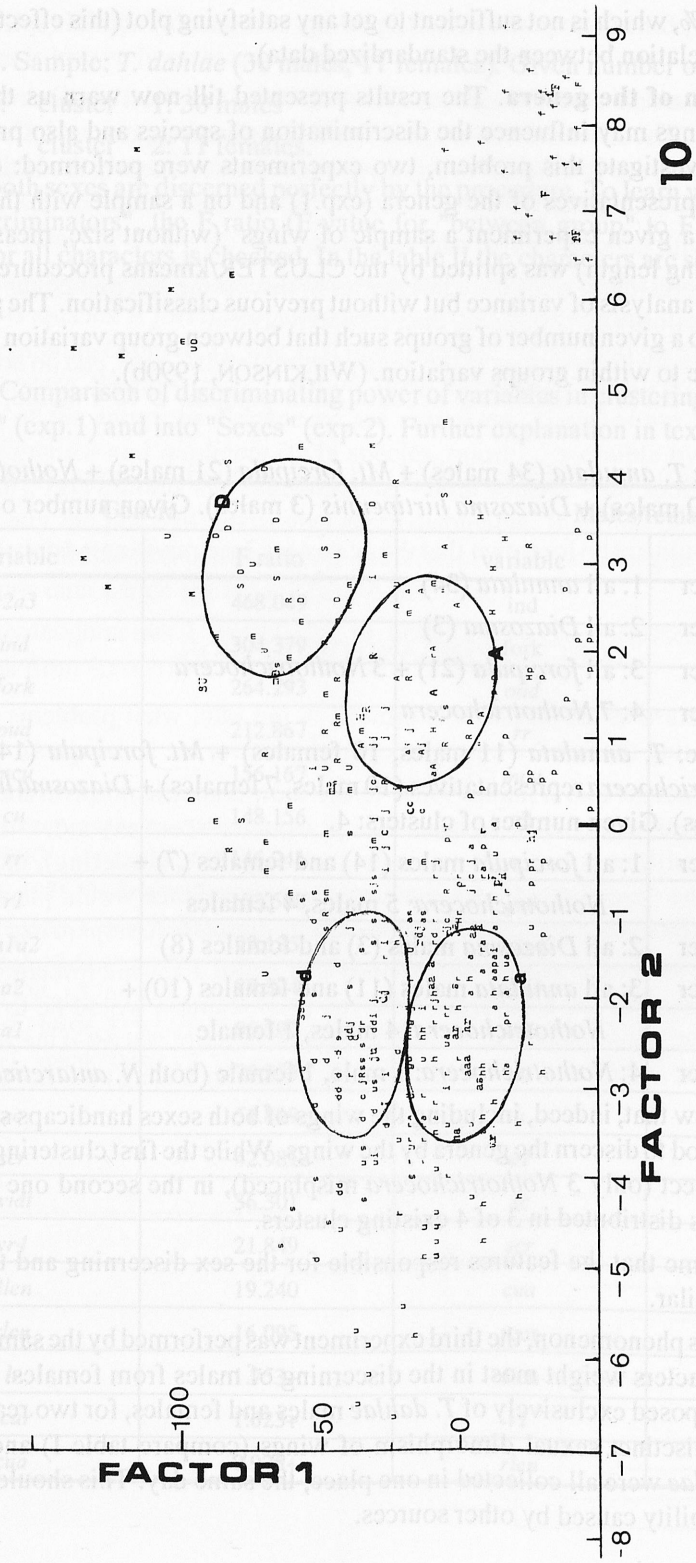


Fig. 10. Principal components analysis of 22 variates for the representatives of 12 species of the genus *Trichocera*: abbreviations like in Figs 4-5, plus: i - *T. implicata*, j - *T. japonica*, p - *T. parva*, s - *T. saltator*, all males. Further exlanation in text.

reached barely 50%, which is not sufficient to get any satisfying plot (this effect is caused by the loss of correlation between the standardized data).

3. **Comparison of the genera**. The results presented till now warn us that sexual dimorphism of wings may influence the discrimination of species and also probably of the genera. To investigate this problem, two experiments were performed: only on a sample of male representatives of the genera (exp.1) and on a sample with the females added (exp.2). In a given experiment a sample of wings (without size, measurements standardized to wing length) was splitted by the CLUSTER/kmeans procedure, working like a multivariate analysis of variance but without previous classification. The procedure splits the cases into a given number of groups such that between group variation is as large as possible relative to within groups variation. (WILKINSON, 1990b).

Exp.1. Sample: *T. annulata* (34 males) + *Mt. forcipula* (21 males) + *Nothotrichocera* representatives (10 males) + *Diazosma hirtipennis* (3 males). Given number of clusters: 4.

Result: cluster 1: all *annulata* (34)
cluster 2: all *Diazosma* (3)
cluster 3: all *forcipula* (21) + 3 *Nothotrichocera*
cluster 4: 7 *Nothotrichocera*

Exp. 2. Sample: *T. annulata* (11 males, 10 females) + *Mt. forcipula* (14 males, 7 females) + *Nothotrichocera* representatives (10 males, 7 females) + *Diazosma hirtipennis* (3 males, 8 females). Given number of clusters: 4.

Result: cluster 1: all *forcipula* males (14) and females (7) + *Nothotrichocera*: 5 males, 4 females
cluster 2: all *Diazosma* males (3) and females (8)
cluster 3: all *annulata* males (11) and females (10) + *Nothotrichocera*: 4 males, 1 female
cluster 4: *Nothotrichocera*: 1 male, 1 female (both *N. antarctica*).

The results show that, indeed, including the wings of both sexes handicaps severly the ability of the method to discern the genera by the wings. While the first clustering is almost perfect in this aspect (only 3 *Nothotrichocera* misplaced), in the second one the genus *Nothotrichocera* is distributed in 3 of 4 existing clusters.

One can presume that the features responsible for the sex discerning and the genera discerning are similar.

To examine this phenomenon, the third experiment was performed by the same method, to state what characters weight most in the discerning of males from females. A sample was this time composed exclusively of *T. dahlae* males and females, for two reasons: this species exhibits disctinct sexual dimorphism of wings (compare table I) and also the specimens of *dahlae* were all collected in one place, the same day. This should diminish the possible variability caused by other sources.

Exp. 3. Sample: *T. dahlae* (30 males, 11 females). Given number of clusters: 2.

Result: cluster 1: 30 males

cluster 2: 11 females.

Thus both sexes are discerned perfectly by the procedure. To learn what characters are "best discriminators", the F ratio (F value for "between group" to F value for "within group") for all characters is checked. In the table II the characters are set according to the

Table II

Comparison of discriminating power of variables in clustering into "Genera" (exp.1) and into "Sexes" (exp.2). Further explanation in text.

Genera		Males/females	
variable	F ratio	variable	F ratio
r2a3	468.049	ind	213.585
ind	304.379	fork	182.745
fork	264.293	*oud*	166.662
oud	212.867	*rr*	149.076
mcu	156.167	*mcu*	148.149
cu	148.156	a2	118.043
rr	146.241	*rs*	109.512
r1	103.588	*yr4*	100.226
m1a2	99.135	*m1a2*	96.409
a2	97.524	*r2a3*	77.771
a1	86.597	*widt*	66.898
rs	76.355	*cu*	49.138
sc	51.829	*h*	48.839
scr	42.981	*a1*	48.392
widt	36.301	*sc*	43.279
yr4	21.849	*scr*	37.557
dlen	19.240	*cua*	35.578
rlen	16.095	rcur	33.394
h	1.753+	dlen	8.008+
rcur	1.623+	*r1*	5.126+
cua	0.925+	*rlen*	5.001+

+ - P>0.05

diminishing F ratio, for two experiments: I (splitting into genera) and III (splitting into sexes).

Indeed, it can be said that the "best genera discriminators" and "best sex discriminators" are much the same. The main difference is in the discriminating power of *r2a3*, which plays the most important role in discerning the genera, and occupies only the 10th position in the "sex discriminators" list.

In accordance with table I, we also may note that:

- the length of cells (*dlen, rlen*) does not matter in discerning sex and genus although the position of cells does (*rs, rr* and *ind, oud*, respectively);

- three landmarks positioned close to the wing proximal end: *h, rcur, cua* do not provide any variability that could be taken into account (their position is very stable, compare Table I);

- wing width (*widt*) and the shape of distal part (*yr4*) are more important for discerning the sex than the genus.

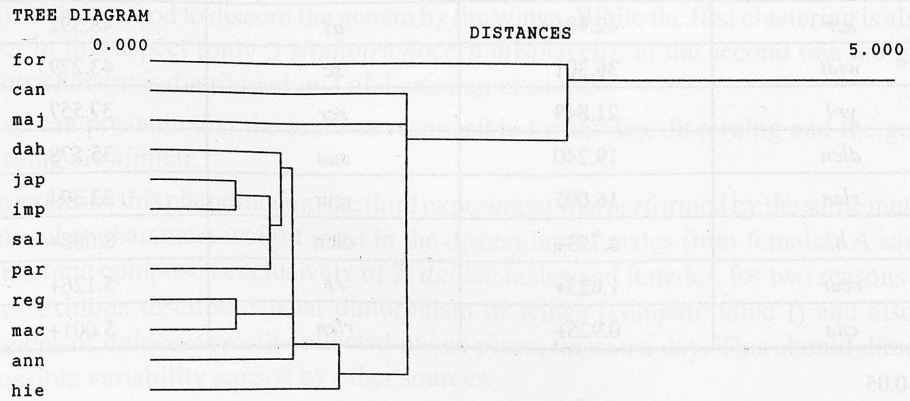
Of course, it should not be forgotten that the genera are here represented only by males.

CONCLUSIONS AND DISCUSSION

Now I would return to the question expressed in the title: what problems are to be expected when treating the fossil wings by means of multivariate techniques?

1. First of all, it seems that it makes sense to use morphometric multivariate analysis without preclassification only in cases when the eye cannot discern any more: if we force too much evidently different cases into a single analysis (PCA, clustering), the result is much worse than the "eye method": no one with some experience would place into the same group the *Nothotrichocera* representatives and *T. annulata* (compare exp. 2).

2. It is obvious now, that sexual dimorphism can cause a misinterpretation if very similar species/specimens are compared. Thus the ideal solution wolud be to take into account only the specimens of one sex, while defining the limits of taxons, if the abundance of material allows it, and then to try to link males and females basing on other characters



(as shapes of cells?). This method can be quite often executed, since the sex of the fossil specimens is known in many cases.

3. The results of this work show that the groupings made on the basis of one sex sample are very reliable (much more than the "eye method"). To show how much are they reliable, a dendrogram is presented, based on a sample of all males of the genus *Trichocera*. The means of standardized variates are introduced (procedure CLUSTER/join, Euclidean distance, nearest neighbor method).

The dendrogram shows the existing relations between the species very well: the first splitted off species is *forcipula* (undoubtedly the most diverging species, also when the genital organs are compared). The next one - *candida* - is the next member of the subgenus *Metatrichocera*, the third one - *T. major*, again the species that is very different when the members of the subgenus *Trichocera* are compared. Together are linked very similar species: *japonica* and *implicata* (twin species, poorly discernible by the genitalia from each other), *regelationis* and *maculipennis* - both species with spotted wings and very similar. Only the place of *parva* may seem disputable. It should be stressed once more than the standardized variates are much more helpful and revealing in clustering analyses than the raw data which are too much size-oriented.

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REFERENCES

ALEXANDER Ch.P. 1926. The Trichoceridae of Australia (Diptera). Proc. Linn. Soc. N.S. Wales (Sydney), **60**(3):298-304.

EDWARDS F. 1921. On British Limnobiidae. Trans. Ent. Soc. Lond., **69**: 229.

EDWARDS F. 1938. Key of British short-palped crane-flies. Trans. Soc. Brit. Ent., **5**:151-157.

REYMENT R.A., R.E. BLACKITH, N.A. CAMPBELL. 1984. Multivariate morphometrics. Academic Press Inc. (London) Ltd., pp.166-173.

WILKINSON L. 1990a. SYSTAT: The System for Graphics. Evanston, IL: SYSTAT, Inc. pp. 166-171.

WILKINSON L. 1990b. SYSTAT: The System for Statistics. Evanston, IL: SYSTAT, Inc. pp. 66-89.