

Phylogeny of Culicomorpha (Diptera)

O. A. SÆTHER

Museum of Zoology, Department of Zoology, University of Bergen, Norway

Abstract. The phylogeny of the families of Culicomorpha and their closest relatives are cladistically reinvestigated adding published information from several sources. A revised data matrix is presented and some characters are discussed. Different outgroups and options used, characters unordered or ordered, the results reweighted or not and the results discussed. Nymphomyiidae in all cladograms, alone or together with Thaumaleidae, forms the sister group of the traditional Culicomorpha and should be included in this infraorder as suggested by others. Superfamily Chironomoidea is not monophyletic as Thaumaleidae or Nymphomyiidae + Thaumaleidae form the sister group to the remaining traditional Culicomorpha. The mutual relationships of Chironomidae, Simuliidae and Ceratopogonidae are variable between cladograms. They form a monophyletic group with Chironomidae and Simuliidae as sister groups when characters are unordered and reweighted or when some characters are ordered, others unordered and the result reweighted. When the characters are ordered, or when a combination of ordered and unordered are used they form a Hennigian comb with the phyletic sequence Chironomidae/Ceratopogonidae/Simuliidae. When characters are ordered and reweighted the phyletic sequence is Chironomidae/Simuliidae/Ceratopogonidae. Culicoidea is monophyletic in all cladograms, but the division into superfamilies is not warranted.

Introduction

Infraorder Culicomorpha includes mosquitoes, black flies and various biting and non-biting midges. According to the most widely accepted phylogenies (Hennig, 1973; Steyskal, 1974; Sæther, 1977; Wood & Borkent, 1989; Oosterbroek & Courtney, 1995; Shcherbakov *et al.*, 1995), Culicomorpha are divided into two superfamilies, Culicoidea and Chironomoidea (Fig. 7A). Culicoidea includes the families Culicidae, Chaoboridae, Corethrellidae and Dixidae, while Chironomoidea includes Simuliidae, Ceratopogonidae, Thaumaleidae and Chironomidae.

Wood & Borkent (1989) provided the most comprehensive recent manual phylogenetic analysis of the nematocerous families of Diptera, while the first numerical parsimony analysis of the relationships of these families recently was performed by Oosterbroek & Courtney (1995). The latter authors gave an extensive literature review, a nearly complete data matrix and discussed all characters used. Their results concerning Culicomorpha and its sister group (Tanyderidae + Ptychopteridae) are identical to those of

Wood & Borkent (1989), but differ in the placement of the other families. Shcherbakov *et al.* (1995) regard Ptychopteridae as the sister group of Culicomorpha, and place Tanyderidae together with Psychodidae in Psychodoidea.

There have been a few attempts to modify Hennig's classification of Culicomorpha. On the basis of cytology, absence of ocelli and wing venation, Hackman & Väisänen (1982) placed Thaumaleidae within infraorder Bibionomorpha. Kovalev (1989, 1990) and Krzeminska *et al.* (1993) placed this family in infraorder Axymyiomorpha. Kalugina (1991) placed Simuliidae in infraorder Ptychopteromorpha.

Michelsen (1996) outlined several important aspects of the skeleto-muscular organization of the adult prothorax and cervix pertaining to the ground pattern of Diptera. This led to the characterization of Neodiptera, a higher level taxon including Brachycera and bibionomorph Nematocera (*sensu* Hennig, 1973). He also gave some preliminary evidence for a sister group relationship between Blepharoceroidea and Culicomorpha and suggested that perhaps Nymphomyiidae should be included with Culicomorpha. However, he regarded Culicomorpha in the accepted sense as a monophyletic group.

Recently, Pawlowski *et al.* (1996) investigated the phylogenetic relationships within the families of the infraorder by using 28S ribosomal RNA gene sequences. All families placed

Correspondence: O. A. Sæther, Museum of Zoology, Department of Zoology, University of Bergen, Muséplass 3, N-5007 Bergen, Norway. E-mail: Ole.Sather@zmb.uib.no

in Culicomorpha were confirmed as clades. However, the phylogenies based on morphological characters were in disagreement with phylogenies based on molecular characters. Their results did not support the generally accepted division of Culicomorpha into two superfamilies, both superfamilies being paraphyletic.

Miller *et al.* (1997), who examined the phylogenetic relationships of Culicomorpha inferred from 18S and 5.8S ribosomal DNA sequences, found that Corethrellidae, Chaoboridae and Culicidae formed a monophyletic group with Chironomidae as their sister group. However, phylogenetic relationships were unresolved for representatives of Dixidae, Simuliidae and Ceratopogonidae.

Pawlowski *et al.* (1996) mentioned that Sæther (1977) accepted the recent classification of Culicomorpha. That is correct only in the sense that the phylogeny within the infraorder was never discussed. However, Sæther (1977) pointed out that the female genitalia of Chironomidae and partly Thaumaleidae were very close to those of a theoretical progenitor of the Diptera. Characters of the female genitalia, however, have never been used in the erection of a scheme of argumentation or a cladogram for Culicomorpha.

Wood & Borkent (1989), as well as Oosterbroek & Courtney (1995), ignored most potential characters mentioned by Sæther (1977, 1979, 1990b). There also are additional characters to be found in the larval mentum (Sæther, 1971), and the important findings of Michelsen (1996) should be included in any parsimony analysis of the relationships between nematocerous families. In order to examine whether the traditional views of the relationships are supported, or if Pawlowski *et al.* (1996) may be correct, these additional characters were incorporated in a data matrix which is mainly in accordance with Wood & Borkent (1989) and Oosterbroek & Courtney (1995). Characters not concerning Culicomorpha or Nymphomyiidae have been excluded. Although I am sceptical of several aspects of such analyses (Sæther, 1983, 1986, 1988, 1990a,b), 'true' phylogenies probably never are completely unparsimonious. Analyses at the family level are also much more 'objective' than doing parsimony analyses performed at the generic or specific level (Sæther, 1990a).

The data matrix of the families included numerous polymorphies, especially among Chironomidae. Thus, the data were also analysed with Chironomidae subdivided into the eleven subfamilies. It would have been advantageous to use the same procedure for Ceratopogonidae, which also show much polymorphism, but the present stage of knowledge precludes such a procedure.

Methods

Parsimony analysis was performed using PAUP 3.1.1 and MacClade 3.06 on a Power Macintosh 8200/120. All searches with only family data were performed using the branch and bound procedure. When Chironomidae was divided into subfamilies, heuristic searches with 1000 replications were performed under different options. The cladograms were compared using MacClade 3.06.

Any data matrix should preferably be analysed using an outgroup which does not contain the sister group of the assemblage to be analysed, in order to exclude possible ingroups. Accordingly, the data matrix was analysed using Tipuloidea as outgroup as suggested by the cladogram of Wood & Borkent (1989), Psychodidae + Trichoceridae + Tipuloidea + Neodiptera as suggested by the results of Oosterbroek & Courtney (1995), Trichoceridae + Tipuloidea + Tanyderidae + Ptychopteridae as suggested by the cladogram of Michelsen (1996), by using all non-culicomorph families as outgroup and by using Lundberg rooting, i.e. a theoretical archetype with character alternative 0 for all characters that essentially equals the outgroup of Mecoptera + Nannochoristidae + Siphonaptera used by Oosterbroek & Courtney (1995). The different outgroup taxa are referred to as OC, WB, M, All and A, respectively.

Neodiptera of Michelsen (1996) was accepted as a monophyletic unit and used in the analyses. However, because of numerous polymorphies this may give different results compared to treating all included families as separate entities. In parsimony analyses with two sister groups both with polymorphies of a character, only the apomorphic character alternative counts if the sister group of both combined shows the apomorphic alternative; only the plesiomorphous alternative if it shows the plesiomorphous character alternative. The purpose of the present study, however, was to examine the relationships between the different families of Culicomorpha.

Also, in Chironomidae there are several polymorphies which influence the outcome of the relationship with other culicomorph families. Thus, the characters were scored also for the different subfamilies of Chironomidae. The additional characters and data matrix for this are given in Sæther (2000). For characters which are unknown for the different subfamilies, but so far appear to be monomorphic, the prevalent character alternative is used.

Data matrix

The characters not mentioned in Hackman & Väisänen (1982), Wood & Borkent (1989), Courtney (1991, 1994), Oosterbroek & Courtney (1995) or Michelsen (1996) were derived from the literature, especially Johannsen (1933, 1935), Thomsen (1937), Nilsen (1951), Sæther (1971, 1977, 1980, 1990b), McAlpine *et al.* (1981), Oosterbroek & Theowald (1991), Nilsson (1997), Papp & Darvas (1997), and by checking available material. For some characters of several non-culicomorph families, however, the scoring of character alternatives may have been somewhat arbitrary or directly erroneous. As the primary purpose of this study was not to evaluate the phylogeny of the Diptera as a whole but of Culicomorpha alone, mistakes in families outside Culicomorpha should not influence the outcome.

Oosterbroek & Courtney (1995) introduced polymorphies 'only if the ground-plan condition for the respective taxon could not be established'. The same scoring was used here for their characters, but for all additional characters each polymorphy was scored.

Characters and character states

Character 6, etc. from Wood & Borkent (1989) is given as WB6, from Oosterbroek & Courtney (1995) as OC6, etc. For scorings of taxa see Table 1.

Imagines

1. *Ocelli*: (0) present; (1) vestigial or absent (WB6, OC63).
2. *Pedicel*: (0) not especially enlarged, male flagellum not markedly plumose nor noticeably different from that of female; (1) enlarged, especially in males; (2) enlarged, male flagellum markedly plumose (WB79, OC65).
3. *Setae of flagellomeres*: (0) arranged haphazardly; (1) in encircling whorls (OC64).
4. *Male antenna*: (0) with more than 15 flagellomeres; (0) with 13–15; (2) with 11 or less.
5. *Female antenna*: (0) with as many flagellomeres as in male; (1) with fewer.
6. *Laterocervical*: (0) without mesal orifice representing the exit of a cervical gland; (1) with (Michelsen, 1996: 94).
Although Michelsen did not examine all families of Diptera, he regards this character as a purported autapomorphy for the 'polyneurans'. Rather than scoring unexamined families with a question mark, the argumentation of Michelsen are followed also for the following three characters.
7. *Origin of episto-dorsocervical muscle*: (0) not transferred to laterocervical; (1) transferred (Michelsen, 1996: 96).
8. *Furcasterno-laterocervical muscle*: (0) not divided into 2 discrete muscles; (1) divided (Michelsen, 1996: 96).
9. *Basal labial palpomere*: (0) not operated by 2 muscles arising from prementum; (1) operated by 2 muscles (Michelsen, 1996: 96).
10. *Frons (vertex)*: (0) without sagittal (coronal) suture; (1) with (Pawłowski *et al.*, 1996).
11. *Costa*: (0) completely surrounds the wing but is strongest along the anterior margin (or wing reduced); (1) costa fades out and becomes absent beyond the insertions of R and M (McAlpine, 1981; OC71).
12. *Posterior veins of wing*: (0) distinct, without marked concentration of anterior veins along costal margin; (1) reduced, anterior veins concentrated along costal margin (OC72).
13. *Wing vein M₃*: (0) present and usually with a discal cell; (1) M₃ and discal cell absent (WB80, OC78).
14. *Radial sector of wing*: (0) with more than 3 branches; (1) with 3; (2) with 2 or less (WB81).
15. *Femora*: (0) uniformly sclerotized, undivided; (1) subdivided by apparently membranous cuticle into short, basal zone and elongate, distal zone (WB15, OC80).
16. *Pulvilli*: (0) present; (1) absent (OC83).
17. *Sperm*: (0) transferred as a liquid or amorphous mass, often by a sperm pump; (1) transferred by a complex, 2-chambered, symmetrical spermatophore formed within the male before or during ejaculation (WB83, OC93).
18. *'True' aedeagus (gonapophyses VIII joined medially)*: (0) well developed; (1) gonapophyses VIII separate; (2) gonapophyses VIII strongly reduced (OC87?).

19. *Gonocoxites*: (0) without basal or apical lobes (volsellae) beyond extreme base, parts and/or appendages of gonapophyses (volsellae) placed basally between gonocoxites; (1) with lobes and/or appendages beyond base.
20. *Gonostylus*: (0) double or deeply furcate; (1) simple.
21. *Sternite IX of male*: (0) well developed; (1) reduced to apodemes; (2) apodemes reduced.
22. *Seminal capsules*: (0) 3 subequal; (1) 3 capsules with one reduced; (2) 2 capsules; (3) one capsule; (4) no capsules (Sæther, 1977; OC97).
23. *Spermathecal ducts*: (0) opens separately; (1) with common opening; (2) partly fused (WB85).
24. *Female gonocoxite VIII*: (0) present; (1) reduced to internally thickened ridges; (2) absent (Sæther, 1977).
25. *Female gonapophysis VIII*: (0) well developed, sometimes divided; (1) reduced and/or fused; (2) absent (Sæther, 1977).
26. *Female gonostylus IX*: (0) present; (1) absent (Sæther, 1977; 1990b).
27. *Notum of female gonapophysis IX*: (0) well developed; (1) reduced; (2) absent (Sæther, 1977).
28. *Rami of female gonapophysis IX*: (0) well developed; (1) reduced; (2) absent (Sæther, 1977).
In simuliids the connection between the notum, the rami and coxosternapodeme IX is particularly well sclerotized forming a 'gonofurca', an autapomorphy for the family. There are, however, more or less membranous or sclerotized connections also in other nematocerous families, but never as in Simuliidae.
29. *Gonocoxite IX in female*: (0) well developed; (1) fused with tergite IX to form a gonotergite; (2) fused with sternite IX to form a gonosternite (Sæther, 1977; 1990b).
30. *Female accessory glands*: (0) 2; (1) one (Sæther, 1977).
31. *Postgenital plate of female*: (0) well developed; (1) reduced or absent (Sæther, 1977).
32. *Labia of female*: (0) well developed; (1) reduced or absent (Sæther, 1977).

Pupae

33. *Metathoracic leg sheath*: (0) extending beyond wing sheath, parallel to sheaths of other 2 legs; (1) metathoracic leg sheath bent in an S-shape, concealed beneath wing sheath, ending beside apex of mesothoracic leg (WB76, OC60).
34. *Apex of abdomen*: (0) terminating in a pair of immovable lobes or projections or in terminal discs or spines; (1) terminating in paddle-like but not articulated anal lobes; (2) terminating in pair of articulated, membranous paddles, each with supporting midrib (WB77, OC62).

Larvae

35. *Frontoclypeal apotome*: (0) triangularly V-shaped; (1) broadly U-shaped (OC1).

49. *Pharyngeal filter*: (0) present; (1) absent (WB62, OC27).
50. *Antenna*: (0) slender, tapering apically, and usually short; (1) large and stout, and one-segmented (WB64, OC14).
51. *Antenna*: (0) slender or stout; (1) markedly reduced (OC15).
52. *Antenna*: (0) capable of relatively little movement, terminating in several straight setae; (1) prehensile, terminating in claw-like setae, capable of holding prey captured by the mandibles as well as of grasping the substrate (WB65, OC16).
53. *Mentum (postmentum, hypostomium)*: (0) a separate plate; (1) with posterior/lateral margins fused to postgenae (OC31).

The mentum is extensively discussed by Oosterbroek & Courtney (1995; chs 29–31). Their interpretations of structural transformations are in agreement with Sæther (1971). However, they fail to mention that the most fully separate and isolated ventromedial sclerite or mentum is found in Tanypodinae, where the mentum (Fig. 1F) has moved in front of the genae and its function apparently has been taken over by the sclerotized and toothed ligula consisting of fused glossae of the prementum. Also in Aphroteniinae the mentum appears fully isolated. In *Anaphroteniella tenuicornis* Brundin (Fig. 1E) it is fused with the submentum forming a postmentum. In *Paraphrotenia cf. excellens* Brundin (Fig. 1D) it appears fully covered by the fused genae. As shown by Sæther (1971, 1980), a mostly indistinct division line separates the mentum from the submentum in some chironomids, and by a difference in sclerotization in most chironomids. The mentum in chironomids may be displaced forward and isolated, a single more or less isolated plate, a completely double plate (Fig. 1I), or the ventromentum may be reduced to ventromental plates, which may be prominent, weak or vestigial, and may be bare, have setae or striations (Sæther, 1971, 1980; Fig. 1G–L).

54. *Mentum (postmentum, hypostomium)*: (0) with numerous teeth; (1) with few teeth; (2) without teeth (OC30).

According to Oosterbroek & Courtney the mentum is toothed in Dixidae. However, it consists of a simple unadorned plate both in *Dixa nebulosa* Meigen (Fig. 1C) and *Dixella aestivalis* (Meigen). There are teeth exactly underneath on hypopharynx which may be confused with teeth on the mentum.

55. *Mentum*: (0) simple or absent; (1) a double-walled plate (ventromentum and dorsomentum) (Sæther, 1971, 1980).

In most cases outside Chironomidae, the ventromentum or ventromental plates are not indicated in drawings. Simuliidae, for instance, appear to always have a completely double-walled mentum but the ventromentum is weak and not extending much past the margins of the dorsomentum. The mentum is completely double-walled, at least in Simuliidae (Fig. 1B), and several Chironomidae, while in Thaumaleidae (Fig. 1A) and most Chironomidae it is at least partly double-walled. It appears to be simple or nearly so in Ceratopogonidae and in Dixidae (Fig. 1C).

56. *Mentum*: (0) well developed; (1) reduced; (2) absent.
57. *Ventromental plates*: (0) without setae (beard) underneath; (1) with.

In some Orthoclaadiinae and all Prodiamesinae of Chironomidae there is a fringe of setae either on the medial,

membranous part of the cardo and/or the dorsal, inner surface of the ventromental plate. In Simuliidae at least *Metacnephia* (*Byssodon*) *maculata* (Meigen) has setae both underneath the ventromental plates and on the posteromedian part of the cardo (Fig. 1B). Also in *Dixa* there is a corresponding setal fringe on the cardo.

58. *Prementum and hypopharynx*: (0) distinctly separated; (1) fused into complex, prementohypopharyngeal complex (OC33).
59. *Labiopharynx*: (0) not connected to pharyngeal phragma; (1) dorsolateral corner of labiopharynx connected via cibarial bar to paraclypeal phragma (OC34).
60. *Abdominal segments*: (0) 9 or 10; (1) 8 or fewer (OC35).
61. *Prothoracic parapods*: (0) absent; (1) present in first-instar larva; (2) present in all instars, crowned apically with rows of hooklets (WB66, OC38).

This character previously was considered to be a primary synapomorphy for Chironomoidea. However, Borkent & McKeever (1990) found a prothoracic parapod in the first instar larva of a *Corethrella appendiculata* Grabham.

62. *Abdominal segments I–VII*: (0) without parapods; (1) each of these segments with a pair of eversible, crochet-tipped parapods (WB8, OC39).
 63. *Last abdominal segment*: (0) without parapods; (1) with crochet-tipped, anal parapods (OC40).
 64. *Anterior thoracic spiracle*: (0) present; (1) absent (WB67, OC49 in part).
 65. *Thoracic segments*: (0) distinct, not appreciably wider than first abdominal segment; (1) enlarged and fused (WB68, OC37).
 66. *Abdominal spiracles*: (0) absent or when present flush with surface; (1) on elevated siphon (WB69, OC51).
 67. *Posterior abdominal spiracle*: (0) situated laterally on abdominal segment; (1) situated dorsally on segment, or at end of siphon; (2) situated at end of last abdomen segment (OC50).
 68. *Posterior abdominal spiracles*: (0) absent, or when present surrounded by 2 pairs of flaps; (1) posterior pair of flaps reduced, elevated to apex of siphon (WB70, OC52).
 69. *Procerci*: (0) absent; (1) present (WB71).
- As mentioned by Oosterbroek & Courtney, the homology of the procerci may be questionable. However, here Wood & Borkent's hypothesis, based on the assumption that in thaumaleids the 8th and 9th segments are fused, is followed.
70. *Lobes surrounding abdominal spiracles*: (0) immovable or absent; (1) movable (WB72, OC48).
 71. *Anal papillae*: (0) retractable; (1) non-retractable (WB73, OC43).
 72. *Last abdominal segment*: (0) without fan-like row of long setae; (1) with (WB74, OC41).
 73. *Pigment of adult eye*: (0) appearing in pupa, after larval stage; (1) developed precociously, becoming conspicuous as early as second instar (WB75, OC28).
 74. *Malpighian tubules*: (0) 4 or less; (1) 5 (OC44).
 75. *Gastric caeca*: (0) absent; (1) 4 or 8 short and small caecae arranged around circumference of anterior end of midgut; (2) 2 or 3 large caecae present at anterior end of midgut;

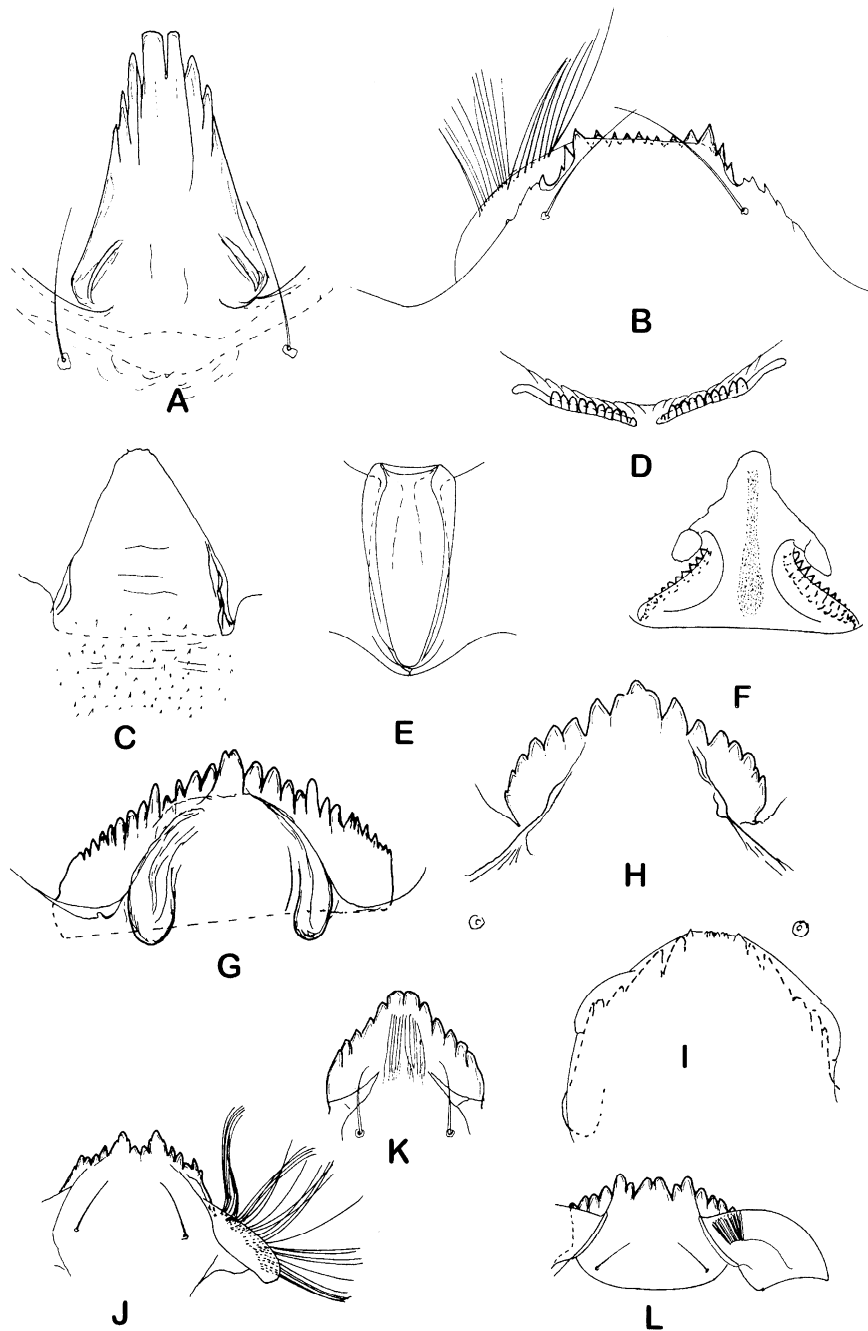


Fig. 1. Mentum of fourth-instar larva, ventral view except D dorsal view. A, *Thaumalea* sp. (Thaumaleidae); B, *Metacnephia* (*Byssodon*) *maculata* (Meigen) (Simuliidae); C, *Dixa nebulosa* Meigen (Dixidae); D, *Paraphrotenia* cf. *excellens* Brundin (Aphroteniinae, Chironomidae); E, *Anaphroteniella tenuicornis* Brundin (Aphroteniinae, Chironomidae); F, *Anapopynia plumipes* (Fries) (Tanypodinae, Chironomidae); G, *Lasiodiamesa* sp. (Podonominae, Chironomidae); H, *Telmatogeton remani* Remmert (Telmatogetoninae, Chironomidae); I, *Pagastia* sp. (Diamesinae, Chironomidae); J, *Prodiamesa olivacea* (Meigen) (Prodiamesinae, Chironomidae); K, *Eukiefferiella claripennis* (Lundbeck) (Orthoclaudiinae, Chironomidae); L, *Microtendipes pedellus* (de Geer).

- (3) 4 usually large caecae at the anterior end of midgut (OC46).
76. *Anastomoses*: (0) 10 or 11 anastomoses present; (1) at least dorsal anastomoses 1–3, usually 1–9 absent, anastomoses 10 present; (2); dorsal anastomoses 4–10, 3–10 or 2–10 absent (OC53).
77. *Ganglionic tracheae*: (0) first 3 forming midventral anastomoses; (1) all 3 midventral anastomoses absent (OC54).
78. *Lateral longitudinal trunk*: (0) complete; (1) reduced beyond region of first or second transverse connective (OC55).
79. *Anal papillae*: (0) with tracheae; (1) without (1) (OC56).

Cytology

80. *Meiosis in male*: (0) 'normal' (i.e. chiasmata formed in autosomal bivalents); (1) no chiasmata formed (White, 1949; Hackman & Väisänen, 1982).
81. *Sex chromosomes*: (0) distinguishable; (1) not distinguishable (White, 1949; Hackman & Väisänen, 1982).

Although character data are lacking for many of the families, the suggestions of White (1949) and Hackman & Väisänen (1982) about four main assemblages are followed, rather than introducing a number of question marks. The first assemblage includes Tipuloidea minus Limoniidae, and Ptychopteridae, Tanyderidae and Trichoceridae; the second Limoniidae, Psychodidae and Culicomorpha minus Thaumaleidae; the third and fourth the remaining groups. The character data for Nymphomyiidae, however, are regarded as unknown.

Results

When all characters are unordered and OC outgroup is used, sixty-two equally parsimonious cladograms each of 330 steps and with a consistency index (CI) of 0.77, a retention index (RI) of 0.59 and a rescaled consistency index (RC) of 0.45 are obtained. The strict consensus and the majority rule cladograms are shown in Fig. 2A. Reweighting the sixty-two most parsimonious cladograms according to RC gives five cladograms (Fig. 2B).

When all characters are ordered and OC outgroup is used, sixteen equally parsimonious cladograms each of 367 steps and with CI of 0.73, RI of 0.56 and RC of 0.41 are obtained. The main differences compared with the reweighted cladogram in Fig. 2B consist in making Chaoboridae and Corethrellidae sister families, Nymphomyiidae and Thaumaleidae sister families, and including Ptychopteridae near Tipuloidea.

However, some characters obviously should be ordered, whereas others obviously cannot be ordered and some are more doubtful. With the multistate characters 18, 23, 29, 34, 54, 56, 67 and 75 unordered and the remaining multistate trends ordered 145 equally parsimonious cladograms each of 355 steps, CI of 0.74, RI of 0.58 and RC of 0.43 are obtained. The most parsimonious cladograms are essentially identical to

those obtained when all characters are ordered, except that Ptychopteridae now, as when all characters are unordered, form the sister groups of the clade Culicomorpha + Nymphomyiidae. Reweighting these cladograms according to RC gives five cladograms with Chaoboridae and Corethrellidae sister families but otherwise identical.

Using WB, M, All or A as outgroup with all characters unordered gives the same internal Culicomorpha configuration, lengths and fit, always with Nymphomyiidae as the sister group of the remaining Culicomorpha. Reweighting according to RC gives Corethrellidae as sister group of Culicidae + Chaoboridae, Ceratopogonidae as sister group of Simuliidae + Chironomidae and Tipuloidea as sister group of Tanyderidae + Trichoceridae.

When all characters are ordered, Thaumaleidae and Nymphomyiidae become sister families and the sister group of the remaining culicomorph families with all different taxa as outgroup. Simuliidae and Chironomidae in most cladograms become sister families, forming one branch of a trichotomy with Culicoidea and Ceratopogonidae as the other two branches. With reweighting according to RC, Ceratopogonidae becomes the sister group of Simuliidae + Chironomidae.

With some multiple characters unordered and some ordered the internal results in most cladograms are the same as for when all characters are ordered and reweighted. With reweighting according to RC there is one internal configuration for all outgroup taxa, differing from the above in having Corethrellidae as the sister group of Culicidae + Chaoboridae (Fig. 3A).

Substituting Chironomidae by its subfamilies usually did not result in any change within Culicomorpha. If, however, the preferred cladogram of the subfamily relationship from Sæther (2000) is used as a constraint, the sister group relationship between Nymphomyiidae and Thaumaleidae is replaced by a phyletic sequence, and Ceratopogonidae becomes the sister group of Culicoidea, not of Simuliidae + Chironomidae (Fig. 3B). In several instances, Trichoceridae change places with Tipuloidea in the cladograms, but not when the above-mentioned constraint is used.

Discussion

The cladograms of Wood & Borkent (1989) and Oosterbroek & Courtney (1995) (Fig. 4A), Pawlowski *et al.* (1996) (Fig. 4B), and the cladograms most commonly obtained by the different parsimony runs (Fig. 4C,D) were compared in MacClade 3.06 with some characters ordered, others unordered. Pawlowski *et al.* do not include Nymphomyiidae and Ptychopteridae, and the relationships of Fig. 4A have been assumed also for this cladogram. Making Nymphomyiidae the sister group of Thaumaleidae will shorten their cladogram by four steps.

One of the two shortest cladograms obtained here is shown in Fig. 4C. A cladogram making Nymphomyiidae + Thaumaleidae the sister group of Chironomidae + Simuliidae

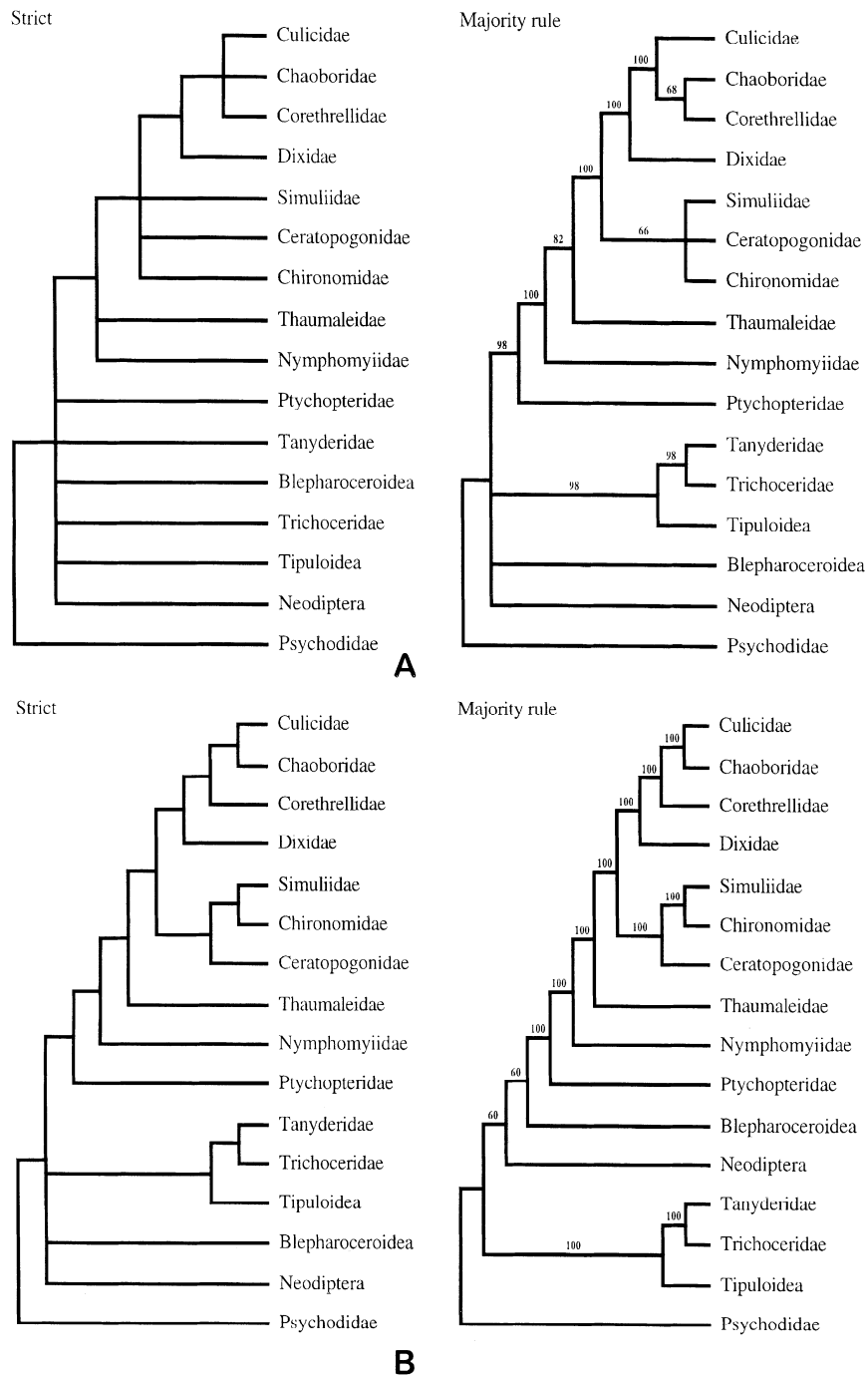


Fig. 2. A, Strict consensus and majority rule cladograms obtained with all characters unordered and OC as outgroup; B, the same reweighted according to the rescaled consistency index (RC).

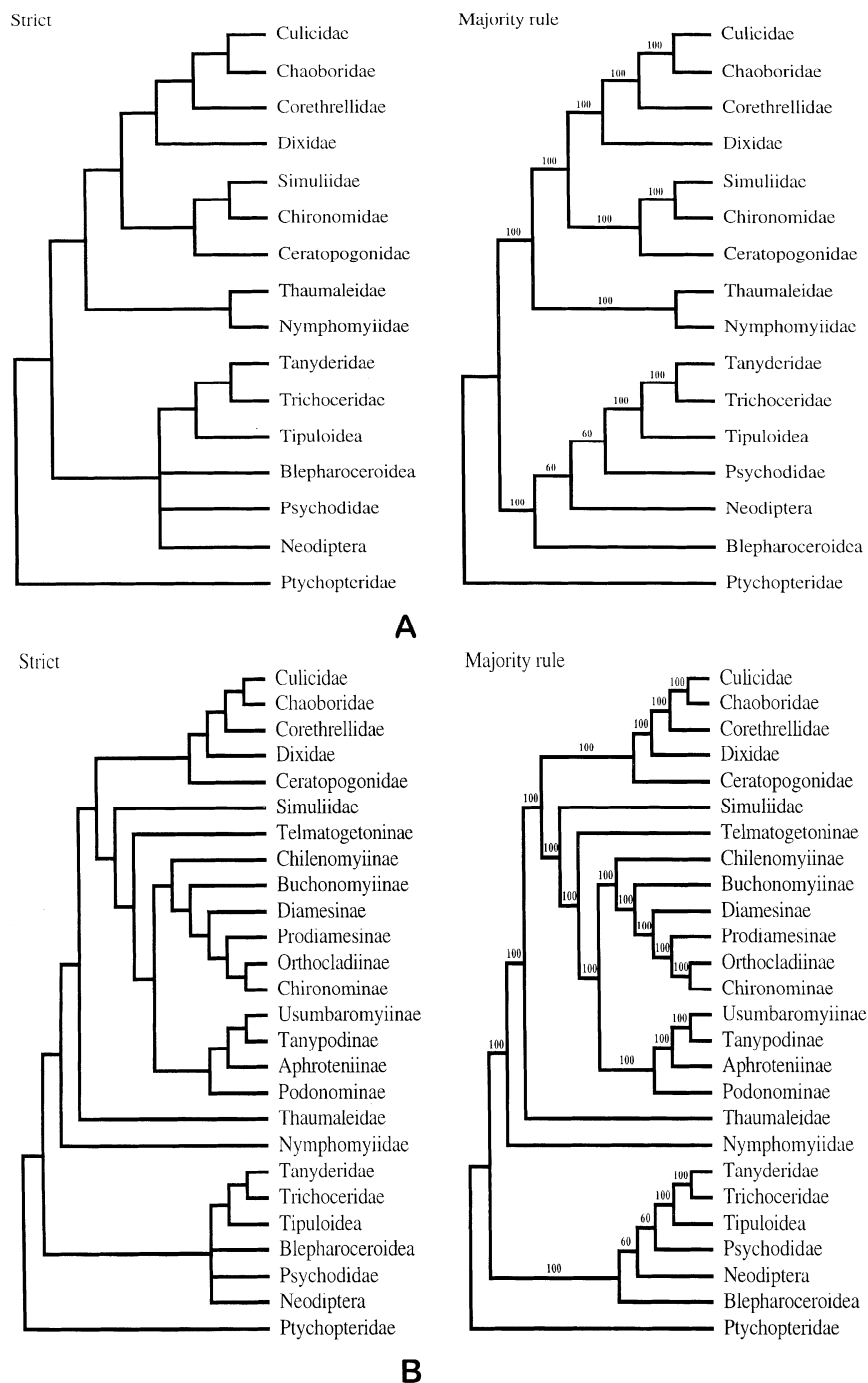


Fig. 3. A, Strict consensus and majority rule cladograms obtained with some characters ordered, others unordered, All as outgroup, and the results reweighted according to RC; B, the same with Chironomidae substituted by its subfamilies and the preferred cladogram of subfamily relationships from Sæther (2000) used as constraint.

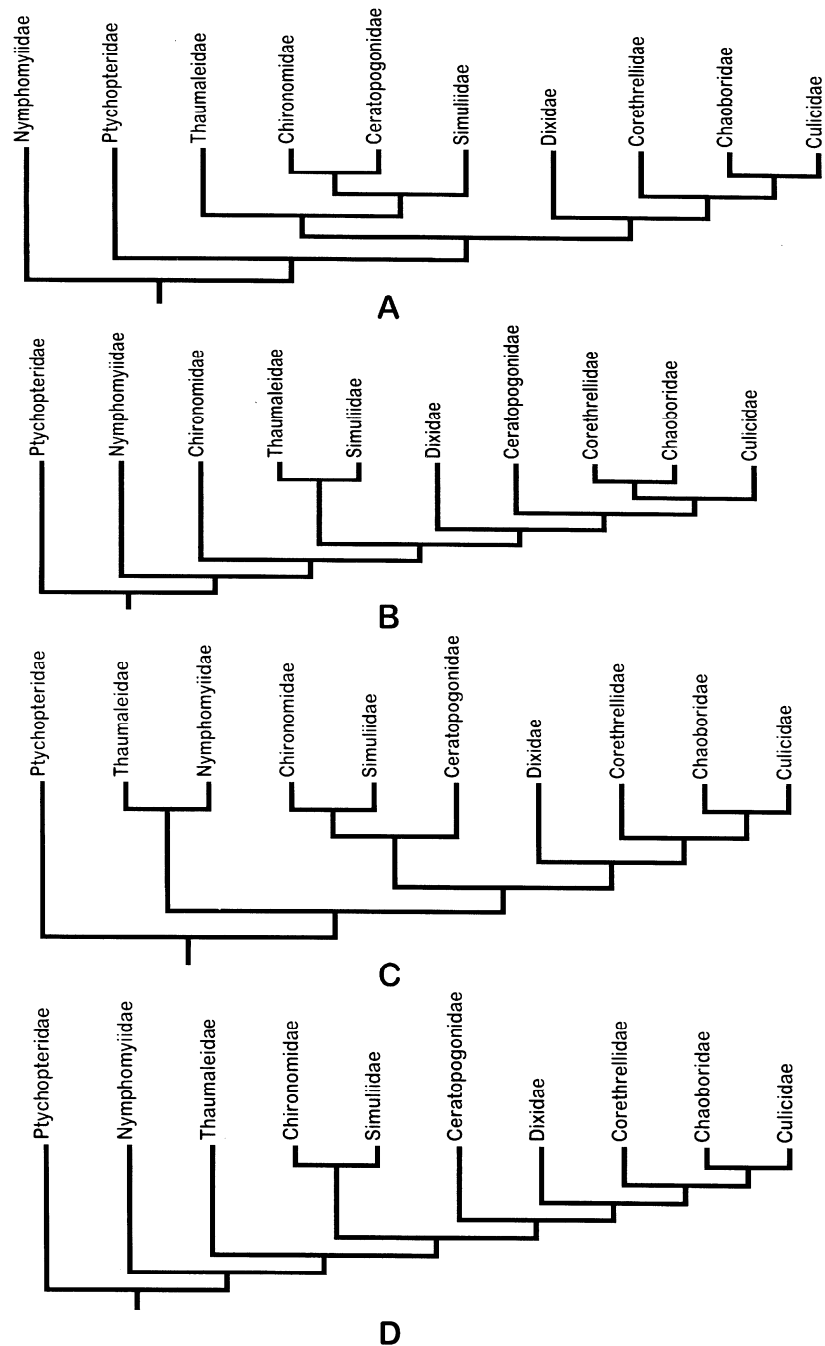


Fig. 4. A, The relationships of Culicomorpha according to Wood & Borkent (1989) and Oosterbroek & Courtney (1995); B, the maximum parsimony phylogram in Pawlowski *et al.* (1996) with Nymphomyiidae and Ptychopteridae added in the same position as in A; C,D, the most common cladograms obtained here for the relationships of Culicomorpha.

+ Ceratopogonidae instead of all other Culicomorpha is equally short. Also having Simuliidae and Ceratopogonidae changing place does not alter the number of steps, but this configuration was never obtained. The cladogram shown in Fig. 4D is two steps longer, but only one step longer if Nymphomyiidae is regarded as the sister of Thaumaleidae.

The relationship of culicomorph families of Wood & Borkent (1989) and Oosterbroek & Courtney (1995) is four steps longer than the shortest cladogram, but only three steps longer if Thaumaleidae is regarded as the sister group of all other Culicomorpha. Making Chaoboridae and Corethrellidae sister groups does not change the length of any cladogram.

The maximum parsimony phylogram in Pawlowski *et al.* (1996) is fourteen steps longer than the shortest cladogram, ten steps if Nymphomyiidae is regarded as the sister group of Thaumaleidae. Their neighbour-joining cladogram is eighteen steps longer than the shortest cladogram, fourteen if Nymphomyiidae is regarded as the sister group of Thaumaleidae. Figure 6 in Pawlowski *et al.* (1996) indicates that very small mistakes in base interpretation are needed to obtain a different cladogram length and probably a different cladogram configuration. In both Ceratopogonidae and in Chironomidae they have chosen apomorphic (highly derived) and apomorphous (showing many relative apomorphies) representatives, without evaluating the variation and without including more basal genera.

The results of the present analysis differ from those of Wood & Borkent (1989) and Oosterbroek & Courtney (1995) in some important aspects. In all cladograms, without exception, Nymphomyiidae or Nymphomyiidae + Thaumaleidae form the sister group of the remaining Culicomorpha. When Nymphomyiidae alone forms the sister group to the rest, Thaumaleidae in all cladograms is the sister family of the remaining Culicomorpha, leaving Chironomoidea in its present sense paraphyletic. Culicoidea, however, is monophyletic. Corethrellidae and Chaoboridae are sister families in all analyses when the results not are reweighted, while Chaoboridae and Culicidae are together when the results are reweighted according to the rescaled consistency index. The relations between Chironomidae, Ceratopogonidae and Simuliidae are highly uncertain. The two most commonly obtained configurations are shown in Fig. 4C,D. Much of the difficulties in estimating the relative phylogenetic position of these families by parsimony analysis are a result of the many polymorphies in Ceratopogonidae and Chironomidae.

There are very few unique synapomorphies. The apomorphous character alternatives 34 and 73 appear to be uniquely derived, i.e. objective synapomorphies in the sense of Sæther (1983), for Culicidae and Chaoboridae. Chaoboridae + Corethrellidae also show two apparent objective synapomorphies (in chs 18, state 2, and 52).

There are two objective synapomorphies for Culicoidea in the reduced gonapophyses VIII of the female (ch. 25, states 1 and 2) and in the large and stout, one-segmented antenna (ch. 50).

There is one apparent unique synapomorphy for Simuliidae + Chironomidae, the absence of most dorsal anastomoses (ch. 76, state 2). However, this is a character with unknown states for several families.

For Culicomorpha in the traditional sense there are two unique synapomorphies, the shape of the leg sheaths in the pupa (ch. 33) and the lack of midventral anastomoses (ch. 77). However, none of these are universal, as there are secondary transformations of the first in Chironomidae, and the character alternative for several families are unknown for the second.

There are no uniquely derived synapomorphies which do not show outside parallelism or inside reductions for any other combinations of culicomorph families. Neither does there appear to be any morphological support for the clade Simuliidae + Thaumaleidae, which appears to be one of the better supported in Pawlowski *et al.* (1996).

In conclusion, Culicomorpha is monophyletic; Nymphomyiidae should be included in the infraorder, where they form, alone or most likely together with Thaumaleidae, the basal group of Culicomorpha; and the division into superfamilies is not warranted.

References

- Borkent, A. & McKeever, S. (1990) First instar larvae of *Corethrella appendiculata* Graham have a prothoracic proleg (Diptera: Corethrellidae). *Entomologica Scandinavica*, **21**, 219–223.
- Courtney, G.E. (1991) Phylogenetic analysis of the Blephariceromorpha, with special reference to mountain midges (Diptera: Deuterophlebiidae). *Systematic Entomology*, **16**, 137–172.
- Courtney, G.E. (1994) Biosystematics of the Nymphomyiidae (Insecta: Diptera): life history, morphology and phylogenetic relationships. *Smithsonian Contributions to Zoology*, **550**, 1–41.
- Hackman, W. & Väisänen, R. (1982) Different classification systems in the Diptera. *Annales Zoologici Fennici*, **19**, 209–219.
- Hennig, W. (1973) Ordnung Diptera (Zweiflüger). *Handbuch der Zoologie*, **4**(2), 1–337.
- Johannsen, O.A. (1933) Aquatic Diptera. Part I. Nemocera, exclusive of Chironomidae and Ceratopogonidae. *Memoirs of Cornell University Agricultural Experiment Station*, **164**, 1–71, pls I–XXIV.
- Johannsen, O.A. (1935) Aquatic Diptera. Part II. Orthorrhapha-Brachycera and Cyclorhapha. *Memoirs of Cornell University Agricultural Experiment Station*, **177**, 1–62, pls I–XII.
- Kalugina, N.S. (1991) Novye mezozoiskiye Simuliidae i Leptoconopidae i proiskhozhdenie krovososanya y nizhikh dvukrylykh nasekomykh. (New Mesozoic Simuliidae and Leptoconopidae and the origin of bloodsucking in the lower dipteran insects.) *Paleontologicheskii Zhurnal*, **1991**(1), 69–80.
- Kovalev, V.G. (1989) Geological history and the systematic position of the family Thaumaleidae (Diptera). *Entomologicheskoe Obozrenie*, **69**, 798–808 (in Russian).
- Kovalev, V.G. (1990) Flies. Muscidae. *Late Mesozoic Insects of Eastern Transbaikalia* (ed. by A. P. Rasnitsyn), pp. 123–177. Trudy Paleontologicheskogo Instituta, 239.
- Krzeminska, E., Blagoderov, V. & Krzeminski, W. (1993) Elliidae, a new fossil family of the infraorder Axymyiomorpha (Diptera). *Acta zoologica cracoviensis*, **35**, 581–591.
- McAlpine, J.F. (1981) Morphology and terminology – adults. *Manual of Nearctic Diptera 1* (ed. by J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth and D. M. Wood), pp. 9–63. Research Branch, Agriculture Canada, Monograph 27, Ottawa.
- Michelsen, V. (1996) Neodiptera: new insights into the adult morphology and higher level phylogeny of Diptera (Insecta). *Zoological Journal of the Linnean Society*, **117**, 71–102.

- Miller, B.R., Crabtree, M.B. & Savage, H.M. (1997) Phylogenetic relationships of the Culicomorpha inferred from 18S and 5.8S ribosomal DNA sequences (Diptera: Nematocera). *Insect Molecular Biology*, **6**, 105–114.
- Nilsen, A. (1951) Contributions to the metamorphosis and biology of the genus *Atrichopogon* Kieffer (Diptera, Ceratopogonidae). With remarks of the evolution and taxonomy of the genus. *Det Kongelige Danske Videnskabernes Selskab, Biologiske Skrifter*, **6**(6).
- Nilsson, A. (ed.) (1997) *Aquatic Insects of North Europe. A Taxonomic Handbook, Vol. 2 Odonata – Diptera*. Apollo Books, Stenstrup.
- Oosterbroek, P. & Courtney, G. (1995) Phylogeny of the nematocerous families of Diptera (Insecta). *Zoological Journal of the Linnean Society*, **115**, 267–311.
- Oosterbroek, P. & Theowald, B. (1991) Phylogeny of the Tipuloidea based on characters of larvae and pupae (Diptera, Nematocera) with an index to the literature except Tipuloidea. *Tijdschrift voor Entomologie*, **134**, 211–267.
- Papp, L. & Darvas, B. (eds) (1997) *Contributions to a Manual of Palaearctic Diptera (with Special Reference to Flies of Economic Importance), Vol. 2. Nematocera and Lower Brachycera*. Science Herald, Budapest.
- Pawlowski, J., Szadziewski, R., Kmiecik, D., Fahrni, J. & Bittar, G. (1996) Phylogeny of the infraorder Culicomorpha (Diptera: Nematocera) based on 28S RNA gene sequences. *Systematic Entomology*, **21**, 167–178.
- Sæther, O.A. (1971) Notes on general morphology and terminology of the Chironomidae (Diptera). *Canadian Entomologist*, **103**, 1237–1260.
- Sæther, O.A. (1977) Female genitalia in Chironomidae and other Nematocera: morphology, phylogenies, keys. *Bulletin of the Fisheries Research Board of Canada*, **197**, 1–211.
- Sæther, O.A. (1979) Hierarchy of the Chironomidae with special emphasis on the female genitalia (Diptera). *Entomologica scandinavica Supplement*, **10**, 17–26.
- Sæther, O.A. (1980) Glossary of chironomid morphology terminology (Chironomidae: Diptera). *Entomologica scandinavica, Supplement*, **15**, 1–51.
- Sæther, O.A. (1983) The canalized evolutionary potential – inconsistencies in phylogenetic reasoning. *Systematic Zoology*, **32**, 343–359.
- Sæther, O.A. (1986) The myth of objectivity – post-Hennigian deviations. *Cladistics*, **2**, 1–13.
- Sæther, O.A. (1988) On the limitations of parsimony in phylogenetic analysis. *Quaestiones Entomologicae*, **24**, 45–50.
- Sæther, O.A. (1990a) Midges and the electronic Ouija board. The phylogeny of the *Hydrobaenus* group revised (Chironomidae, Diptera). *Zeitschrift für Zoologische Systematik und Evolutionsforschung*, **28**, 107–136.
- Sæther, O.A. (1990b) Phylogenetic trends and their evaluation in chironomids with special reference to orthoclads. *Acta Biologica Debrecina. Oecologica Hungarica, Supplementum*, **2**, 53–75.
- Sæther, O.A. (2000) Phylogeny of the subfamilies of Chironomidae. *Systematic Entomology*, in press.
- Shcherbakov, D.E., Lukashovich, E.D. & Blagoderov, V.A. (1995) Triassic Diptera and initial radiation of the order. *International Journal of Dipterological Research*, **6**, 75–115.
- Steyskal, G. (1974) Recent advances in the primary classification of the Diptera. *Annals of the Entomological Society of America*, **67**, 513–517.
- Thomson, L.C. (1937) Aquatic Diptera. Part V. Ceratopogonidae. *Memoirs of Cornell University Agricultural Experiment Station*, **210**, 57–80, pls X–XVIII.
- White, M.J.D. (1949) Cytological evidence on the phylogeny and classification of the Diptera. *Evolution*, **3**, 252–261.
- Wood, D.M. & Borkent, A. (1989) Phylogeny and classification of the Nematocera. *Manual of Nearctic Diptera*, Vol. 3 (ed. by J. F. McAlpine and D. M. Wood), pp. 1333–1370. Research Branch, Agriculture Canada, Monograph 32, Ottawa.

Accepted 12 May 1999