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Polyphyly of *Lordiphosa* and its relationships in Drosophilinae (Diptera: Drosophilidae)

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Abstract. The phylogenetic relationships of *Lordiphosa* and some taxa in Drosophilinae were analysed on the basis of a total of forty-one selected drosophilid species. These included eighteen species of five *Lordiphosa* species-groups as the main target, twenty-three species representative of the major drosophiline ingroup taxa and four species of Steganinae as outgroup. Sixty-eight morphological characters of adults were subjected to cladistic analysis. From the results it is concluded that *Lordiphosa* is polyphyletic; the *Lo. tenuicauda* species-group and genus *Nesiodrosophila* form a single monophyletic group; *Lordiphosa* proper (i.e. *Lordiphosa* spp. minus the *tenuicauda* group) comprises another monophyletic group; within *Lordiphosa* proper the *fenestrarum, nigricolor* and *denticeps* groups are all monophyletic, but monophyly of the *miki* group is not strongly supported; genera *Hirtodrosophila* and *Scaptomyza* and subgenus *Sophophora* are all monophyletic; and within Drosophilinae, genus *Scaptodrosophila* is the first to have split from the main lineage, but the branching order of other clades, *Chymomyza*, *Lordiphosa* proper, *Sophophora*, *Hirtodrosophila*, *Nesiodrosophila* + *Lo. tenuicauda* group, *Scaptomyza*, *Dorsilopha* and subgenus *Drosophila*, remains unresolved. The topology of maximum parsimony cladograms suggests that *Lordiphosa* proper lies close to *Sophophora* as proposed previously, although its phylogenetic position could not be determined conclusively. By contrast, bootstrap values tended to contradict another hypothesis that *Lordiphosa* and *Scaptomyza* are sister groups.

Introduction

No consensus has yet been reached on drosophilid phylogeny despite many attempts based on morphological and molecular approaches. Disputes continue over the relationships among even major drosophiline taxa, including genera *Scaptomyza* Hardy and *Idiomyia* Grimshaw (the Hawaiian *Drosophila*), and subgenera *Sophophora* Sturtevant and *Drosophila* of *Drosophila* Fallén. Throckmorton (1975), for example, considered that *Sophophora* has split relatively early from the major drosophiline lineage and forms the sister group of all other drosophiline taxa except *Microdrosophila* Malloch, *Scaptodrosophila* Duda and *Chymomyza* Czerny. In contrast, Grimaldi (1990) identified *Sophophora* as the sister group of subgenus *Drosophila*. He revised drosophilid taxonomy in light of a cladistic analysis and elevated subgenera *Scaptodrosophila*, *Hirtodrosophila* Duda and *Lordiphosa* Basden of *Drosophila* to generic rank. He further revived genus *Idiomyia* to include the Hawaiian *Drosophila* species. His revision indicates that genus *Drosophila* is monophyletic, whereas it is paraphyletic according to Throckmorton's (1975) hypothesis. The same discrepancy exists between hypotheses deduced from molecular data (see Tatarenkov *et al*., 1999, for review concerning this issue). Genus *Lordiphosa*, on which this study focuses, may be a key taxon for resolving these issues of drosophilid phylogeny. Some members of this genus have been included previously in subgenera *Sophophora*, *Hirtodrosophila* or *Drosophila*, suggesting some relationships of *Lordiphosa* to these taxa, but the true phylogenetic position of the genus has not been determined. Okada (1963) and Laštovka & Máca (1978) hypothesized that *Lordiphosa* was most closely related to *Sophophora*, whereas Grimaldi (1990) placed it as the sister group to *Scaptomyza*. Despite its probable importance to drosophilid phylogeny, the monophyly of *Lordiphosa* cannot be guaranteed, as only three

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species of this genus have been subjected to phylogenetic studies: *Lordiphosa denticeps* (Okada & Sasakawa) by Throckmorton (1975), *Lo. fenestrarum* (Fallén) by Grimaldi (1990) and *Lo. andalusiaca* (Strobl) by Pélandakis & Solignac (1993).

Basden (1961) established *Lordiphosa* as a new subgenus of *Drosophila*, designating *Drosophila fenestrarum* Fallén as the type species, and included five other species. Grimaldi (1990), who elevated this taxon to generic rank, is followed here. Currently, five species-groups are recognized in the genus. The *fenestrarum* group was first recognized as a distinctive group within *Drosophila* by Basden (1954), comprising the nominate species and *Dr. forcipata* Collin, although the latter species was later synonymized with *Dr. andalusiaca* Strobl by Basden (1961). As mentioned above, Basden (1961) later erected *Lordiphosa* for this species-group and divided it into two species-subgroups, the *fenestrarum* and *acuminata* subgroups. In a revision of *Lordiphosa*, Laštovka & Máca (1978) recognized two new species-groups, the *nigricolor* and *miki* groups. They established the *nigricolor* group for three species transferred from other subgenera, *Dr. nigricolor* Strobl from *Drosophila*, and *Dr. mommai* Takada & Okada and *Dr. pappi* Okada from *Sophophora*. However, *Dr. pappi* was recently synonymized with *Lo. nigricolor* by Watabe & Watanabe (1993). The *miki* group was established for a single species, *Dr. miki* Duda, transferred from *Sophophora*. The *denticeps* group of two species was originally established within subgenus *Hirtodrosophila* of *Drosophila* by Okada (1967), but its subsequent taxonomic history is somewhat confused. It was first transferred to subgenus *Lordiphosa* and identified as a synonym of the *nigricolor* group (Okada, 1990) and later revived as a valid species-group within genus *Lordiphosa* on the basis of unique morphological characters (Zhang, 1993a). The last species-group, the *tenuicauda* group, was established by Toda (1983) to include *Dr. tenuicauda* Okada, transferred from the *grandis* group of subgenus *Drosophila*, and another species closely related to it. He noted, however, that some characters of this species-group were inconsistent with the subgeneric diagnosis given by Laštovka & Máca (1978) and that it was only remotely related to the other species-groups then recognized in *Lordiphosa*.

Before Okada's (1984) revision, *Lordiphosa* was little known and seemed to be a small taxon of only fifteen species with largely Palearctic distributions. Okada (1984), however, included eight Japanese and Nepalese species previously described in other subgenera, and an additional six new species of the *nigricolor* group from the Oriental Region. Of the eight previously known species, three had been included in the *nipponica* subgroup of the *melanogaster* group of *Sophophora*, which Okada (1984) synonymized with the *miki* group. Two Nepalese species previously included in the *mommai* group of *Sophophora* were transferred to the *nigricolor* group, along with another Nepalese species. The remaining two species were transferred from the *grandis* group of *Drosophila* to the *tenuicauda* group. He conducted a phenetic analysis of twenty selected species, but the result was inconsistent with the classification of species-groups. His revision contains the fundamental diagnosis for *Lordiphosa* and a key to the species examined. He also pointed out, on the basis of internal adult and egg morphology, that the *tenuicauda* group is only distantly related to the other species-groups.

Recently, numerous new *Lordiphosa* species have been described from China and Southeast Asia (Singh & Gupta, 1981; Okada, 1988; Kumar & Gupta, 1990; Zhang & Liang, 1992, 1994; Zhang, 1993a,b; Gupta & De, 1996; Hu *et al*., 1999). In addition, a considerable number of undescribed species have been collected in China (Toda, unpublished). In consequence, ninety-three species (sixty-three described and thirty undescribed) of *Lordiphosa* are now known. These species and plentiful material of *Lordiphosa* offered an opportunity to resolve several long-standing problems in drosophilid phylogeny. Hence, the purpose of this study was (1) to re-examine the monophyly of *Lordiphosa*, (2) establish the phylogenetic position of *Lordiphosa* within Drosophilinae and (3) examine the relationships among the species-groups of *Lordiphosa*.

Materials and methods

A total of forty-one drosophilid species were selected for the present study (Appendix 1). Eighteen species, three or four from each species-group, were selected from *Lordiphosa* to examine the monophyly of the genus and its five species-groups. In addition, twenty-three species representative of the major taxa of Drosophilinae were selected to serve as ingroup in an examination of the phylogenetic position of *Lordiphosa* within the subfamily. Four steganine species were selected as outgroup: the division of Drosophilidae into two subfamilies, Steganinae and Drosophilinae, which are sister groups, is a stable feature of all previous drosophilid phylogenies.

The specimens studied were preserved in 70% ethanol. Characters from all parts of both male and female flies were identified for inclusion in this study. External morphology was examined under a stereoscopic microscope and, where necessary, measurements were made with an ocular micrometer. Mouthparts and male and female terminalia were removed and cleared in a 10% KOH solution at 100 °C for several minutes before being examined in a droplet of glycerol under a compound light microscope. Drawings were made with an ocular mesh micrometer and graph paper, and microscopic photographs were taken of some characters.

Data analysis was performed using PAUP version 3.1.1 (Swofford, 1993) run on a Macintosh Quadra 840Av. Data analysis included two main steps. (1) Maximum parsimony cladograms were generated by heuristic search, in which the addition sequences were set at random and tree-bisection-reconnection (TBR) branch-swapping was performed. The most parsimonious cladogram was obtained after ten replicates of such a search. According to the resulting maximum parsimony cladogram, character optimization was performed using DELTRAN (delayed transformation) and ACCTRAN (accelerated transformation). (2) The confidence value for each clade of the most parsimonious cladograms was derived by a bootstrap analysis with 100 replicates. Each bootstrap replicate was analysed by heuristic search with 'simple' addition sequence and TBR branch-swapping.

Anatomical characters and observations

Character selection was based initially on Grimaldi (1990), but careful re-examination revealed that some of his characters were problematic. Such characters were redefined or omitted from the cladistic analysis. In addition, we found many new potentially informative characters in male and female terminalia. Combining both sources provided sixty-eight characters for analysis (Appendix 2). The characters were coded *a posteriori* so that all characters of the hypothetical ancestor at the root of the resulting cladograms were 0. All transformation series (TS) were assumed to be 'unordered'. Some TS correspond to those of Grimaldi (1990), in which case his code number is indicated in parentheses as (G#) immediately after the corresponding characters of this study listed below. Where Grimaldi's definition was altered, however, his number is followed by an asterisk (G#*).

Head

1. *Supracervical setae*: (0) blunt; (1) tapered, thin and apically curved and sharp. (G2).
2. *Ocellar setae*: (0) inside triangle made by ocelli (Fig. 1A); (1) outside (Fig. 1B).
3. *Eye, longest axis*: (0) nearly rectangular to body axis (Fig. 2A); (1) distinctly oblique (Fig. 2B).
4. *Frons, profile line from base to ptilinal fissure*: (0) convex (Fig. 2A); (1) nearly straight (Fig. 2B).
5. *Setulae on basal lobe of palpus*: (0) absent; (1) present (Fig. 3E).
6. *Cibarium, anterior margin*: (0) not thickened (Fig. 4A,B); (1) thickened, somewhat triangularly protruded in lateral view (Fig. 4C). (G14*).

The apomorphy defined here probably corresponds to the 'heavily sclerotized bulb near the angle' of Grimaldi (1990).

7. *Hypopharynx, apodeme*: (0) absent or almost flat (Fig. 4A); (1) expanded in anterior portion (Fig. 4B,C). (G15).
8. *Cibarium, number of medial sensilla*: (0) 11 or fewer per side (Fig. 4A,D,F); (1) 20–26 (Fig. 4B,E); (2) 35 or more (Fig. 4C). (G27*).
9. *Cibarium, position of anterior sensilla*: (0) situated before or on anterior margin of hypopharynx (Fig. 4D); (1) behind (Fig. 4B,C,E).
10. *Cibarium, dorsal wall*: (0) oval or oblong, medially without constriction (Fig. 4D); (1) pear-shaped, with posterior portion oval (Fig. 4E); (2) medially constricted, with posterior portion parallel-sided (Fig. 4F). (Partly G31).
11. *Cibarium, anterior end*: (0) not dilated laterad (Fig. 1C); (1) more or less dilated (Fig. 1D).
12. *Cibarium, anterolateral corners*: (0) distinctly protruded (Fig. 4A,C,E); (1) only slightly protruded (Fig. 4B,D).
13. *Cibarium, dorsal surface*: (0) nearly flat in lateral view (Fig. 4A); (1) anteriorly slightly convex (Fig. 4C); (2) anteriorly strongly convex (Fig. 4B).
14. *Labellum, number of pseudotracheae*: (0) 6 or more (Fig. 3A); (1) 5 (Fig. 3B); (2) 4 (Fig. 3C,D).
15. *Labellum, widths of pseudotracheae*: (0) nearly the same (Fig. 3A–C); (1) distinctly varied (Fig. 3D).
16. *Arista, number of ventral branches excluding terminal fork*: (0) 2 or more; (1) only one. (G49).
17. *First flagellomere, number of internal organ(s)*: (0) only one (Fig. 3F); (1) 2 (Fig. 3G).
18. *Prementum, ventral surface*: (0) nearly flat or slightly swelling at distal end (Fig. 4G); (1) forming discrete bump (Fig. 4H). (G17*).

Thorax

19. *Prescutellar setae*: (0) present; (1) absent. (G95*).
20. *Acrostichal setulae, number of rows*: (0) 6 or more; (1) 4; (2) 2. (G98*).
21. *Acrostichal setulae in line with and anterior to dorsocentrals*: (0) one or a few thicker and longer than other acrostichals; (1) the same size as others. (G101).

One or a few setulae just before the transverse suture are usually thicker and longer than the others. The innermost one is situated just lateral to the dorsocentral line but is not included in this TS.

22. *Dorsalmost seta of a group or row of small setae ventral to and between 2 prominent katepisternals*: (0) nearly the same size as others (Fig. 2B); (1) longer than others but shorter than anterior katepisternal (Fig. 2A); (2) as long as or longer than anterior katepisternal. (G102*).
23. *Anterior dorsocentral setae, position*: (0) situated close to posteriors, dcp = (distance between ipsilateral dorsocentrals)/(cross distance between anterior dorsocentrals) < 0.4; (1) $0.4 \leqslant \text{dcp} < 0.6$; (2) far anterior to posteriors, $0.6 \leqslant \text{dcp}$. (G105*).

Wing

24. *Bm-cu crossvein*: (0) absent; (1) present. (G114).

Legs

25. *Mid and hind tarsi, cuneiform setulae*: (0) 2 rows; (1) one row; (2) absent. (Partly G119*, G120*).
26. *Male fore tarsus, sex comb(s)*: (0) absent; (1) present. (G121*).

Male terminalia

27. *Epandrium, pubescence*: (0) covering nearly entirely or partly (Fig. 5B,C); (1) absent (Fig. 5A).
28. *Epandrium, setae*: (0) present not only on ventral portion but also on other portions (Fig. 5B,C); (1) only on ventral portion (Fig. 5A). (G177).
29. *Surstylus, fusion to epandrium*: (0) separated but articulated (Fig. 5B); (1) fused (Fig. 5A,C).

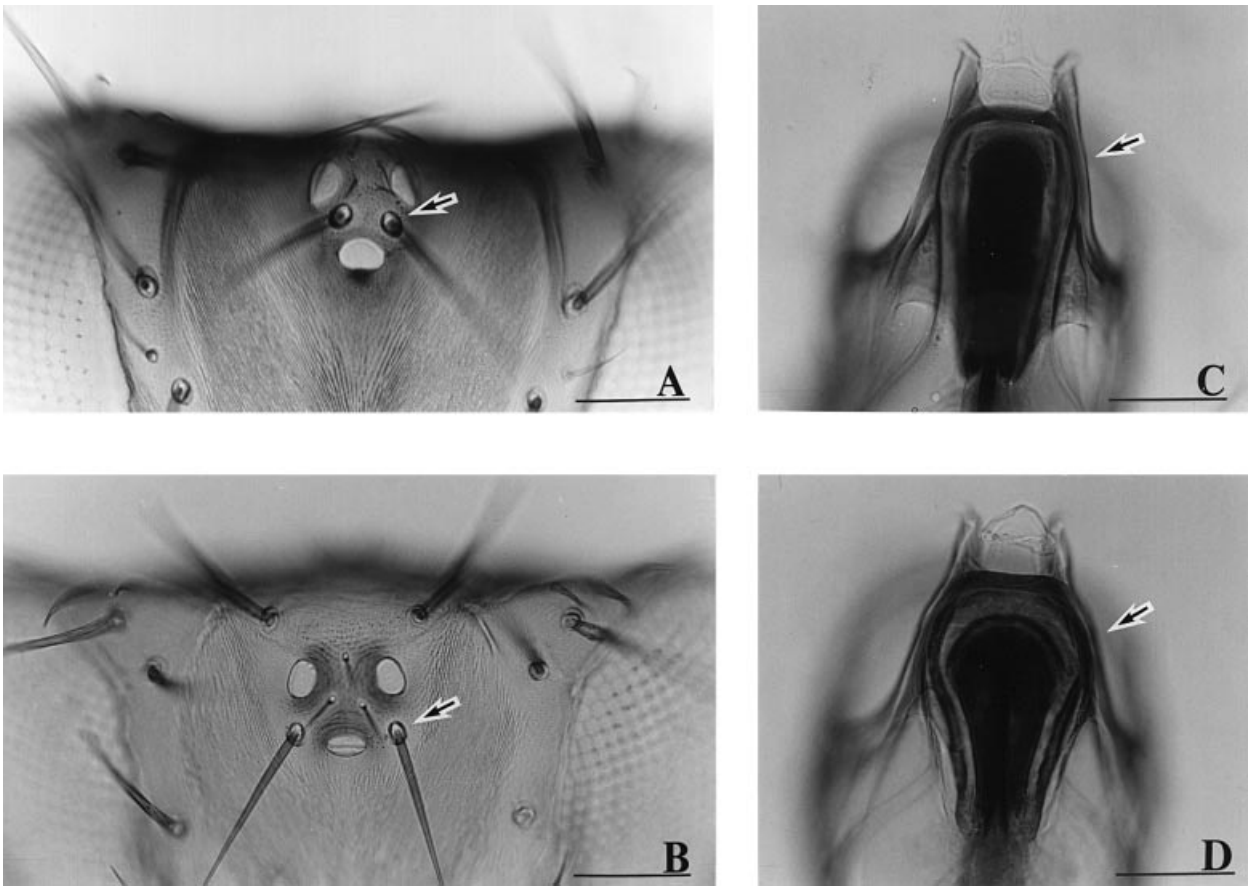

Fig. 1. A,B, Vertices; C,D, anterior part of cibarium (dorsal views). A, *Hirtodrosophila quadrivittata*; B, *Nesiodrosophila sakagamii*; C, *Lordiphosa fenestrarum*; D, *Drosophila* (*Drosophila*) *hydei*. Scales = 0.1 mm.

30. *Surstylus, comb(s) of peg-like prensisetae*: (0) absent; (1) one set arranged linearly (Fig. 5A–C); (2) 2 sets each arranged linearly (Fig. 5D). (Partly G155*, G178*, G186*, G187*, G200*).
31. *Surstylus, stout or peg-like setae on outer mesal surface*: (0) absent (Fig. 5B,D); (1) present (Fig. 5A,C).
32. *Cercus, pubescence*: (0) covering nearly entirely or partly (Fig. 5B,C,E,F); (1) absent (Fig. 5A,G).
33. *Cercus, fusion to epandrium*: (0) separated (Fig. 5A–C); (1) fused.
34. *Membrane between cercus and epandrium*: (0) pubescent; (1) not pubescent.
35. *Cercus, shape of ventral portion*: (0) broad, round (Fig. 5B,G); (1) tapering or elongated, with setae at tip (Fig. 5A,E,F); (2) pointed or elongated, without any setae at tip (Fig. 5C). (Partly G141, G183).
36. *Cercus, stout spines or pegs on ventral portion*: (0) absent (Fig. 5C); (1) a tuft or row of small, stout spines near caudoventral corner (Fig. 5E); (2) a row of large, stout spines on ventral to anteroventral margin (Fig. 5B); (3) large, peg-like spines on apex to caudoventral margin (Fig. 5A,G); (4) only one, very large, somewhat plate-like peg at ventral apex (Fig. 5F). (Partly G193, G195, G202).
37. *Hypandrium, paramedian setae*: (0) present (Fig. 6C); (1) absent (Figs 6D; 7B).
38. *Hypandrium, caudolateral processes*: (0) absent; (1) pair of elongated processes (Fig. 6B,D); (2) pair of flaps (Fig. 6C). (G148*, G174*).

Grimaldi (1990) referred to a pair of long processes flanking the parameres as gonopods in *Chymomyza* (G148-1) and *Scaptomyza* (G174-1). However, those organs are not gonopods but a part of the hypandrium (see below for the definition of gonopods), and may or may not be homologous between the two genera.

39. *Paramere, fusion to hypandrium*: (0) separated; (1) fused (Fig. 6C).

The parameres are a pair of processes usually articulated with the aedeagal base throughout Diptera, but the terminology is very confused (see McAlpine, 1981, for review). Drosophilid taxonomists have traditionally called each an anterior paramere, but Grimaldi (1987) used the term paraphysis. Here, as elsewhere (Zhang & Toda, 1992), we

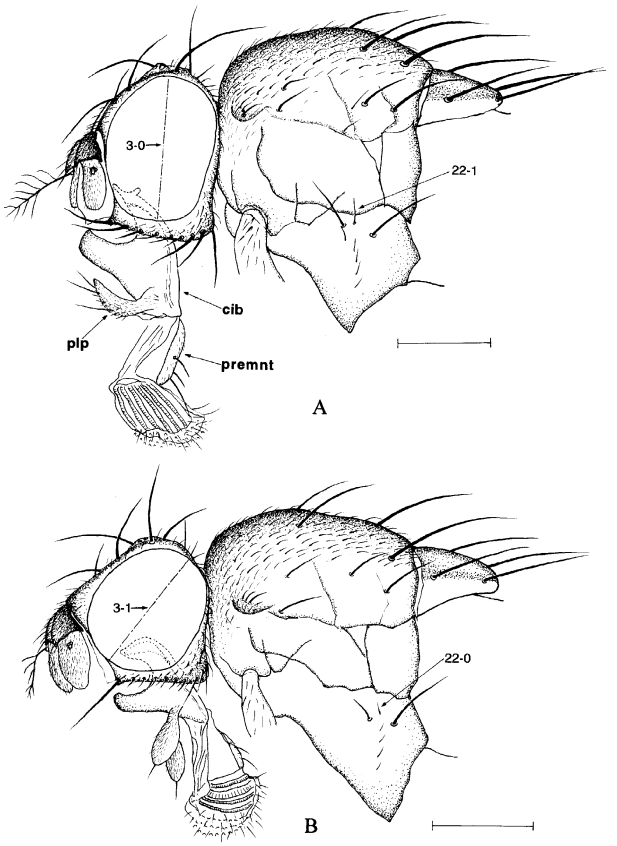

Fig. 2. Heads and thoraces. A, *Drosophila* (*Sophophora*) *melanogaster*; B, *Nesiodrosophila sakagamii*. Scales = 0.25 mm. plp = palpus, cib = cibarium, premnt = prementum.

apply the term paramere (McAlpine, 1981) to conform to standard terminology among dipterists and to emphasize the homology of the structure throughout Diptera.

40. *Paramere, ventrobasal articulation with hypandrium*: (0) articulated (Fig. 7A,B,D); (1) not articulated.
41. *Paramere, fusion to aedeagus*: (0) articulated with aedeagus; (1) fused to aedeagus; (2) fused to aedeagal guide.
42. *Paramere, shape*: (0) elongate (Figs 6A; 7A,C–E); (1) oblong, plate-like; (2) large, broad, plate-like; (3) small, somewhat conical (Fig. 6D); (4) small, club-shaped (Fig. 6B); (5) small, somewhat oval plate; (6) basally broad, apically acutely pointed (Fig. 7B).
43. *Paramere, pubescence*: (0) short, partly covering (Figs 6A; 7 A); (1) absent (Figs 6B–F; 7B,D,E); (2) long, distally covering (Fig. 7C). (Partly G201*).
44. *Paramere, sensilla*: (0) minute, arranged nearly longitudinally (Figs 6F; 7A–C); (1) small, in a patch (Figs 6C,D; 7D); (2) long, at apex (Figs 6B,E; 7E); (3) only one; (4) absent; (5) each on peg-like tooth (Fig. 6A). (Partly G145*).
45. *Paramere, ventral recurved process*: (0) absent; (1) present (Fig. 7C).
46. *Paramere, caudal process*: (0) absent; (1) present (Fig. 7B,C).
47. *Gonopods, fusion and shape*: (0) fused, forming plate or vertical rod situated dorsally to aedeagus (Figs 6A,B,E; 7B,D,F,H); (1) separated (Figs 6F; 7G); (2) fused, forming bridge connecting caudal ends of hypandrium (Fig. 7I); (3) almost degenerated. (Partly G199*).

There has been much confusion for all endopterygote orders in the interpretation and naming of processes on the posterior margin of sternite IX, i.e. hypandrium (McAlpine, 1981). In drosophilid taxonomy, too, no consistent term or interpretation has been established for this organ, although it is conventionally called the posterior paramere. Gonopods are defined here as the structures articulating anteriorly with the hypandrium and posteriorly with the 10th sternite, and bridging the caudal ends of the hypandrium (see Zhang & Toda, 1992; Chen & Toda, 1994).

48. *Aedeagal apodeme*: (0) mostly rod-like (Figs 6D–E; 7A–E); (1) laterally flat in muscle-attaching portion (Fig. 6A–C); (2) horizontally flat. (Partly G153).
49. *Aedeagus, fusion to aedeagal apodeme*: (0) articulated (Figs 6A,E,F; 7A–D); (1) fused (Figs 6B–D; 7E).
50. *Aedeagus, basal processes*: (0) absent (Figs 6B,C; 7D); (1) simple rod-like or plate-like, apically articulated with gonopod (Fig. 6A); (2) fused to gonopod, forming together an elongate flap without any serrations or finger-like processes (Fig. 6F); (3) fused to gonopod, forming elongate flap with serrations or numerous, small, conical processes, or both, in tuft (Fig. 6E); (4) connected to gonopod with membrane bearing numerous finger-like or conical processes (Fig. 7A,B); (5) free from gonopod (Fig. 7C,E).
51. *Aedeagal guide*: (0) present (Fig. 6A–C); (1) almost absent (Figs 6E; 7A–E).
52. *Aedeagus, connection to gonopod*: (0) connected with membrane (Fig. 6A); (1) free from gonopod, or gonopod absent; (2) fused (Fig. 7D).
53. *Aedeagus, sclerotization*: (0) more or less sclerotized; (1) pale, membranous (Fig. 7A–D).

Female terminalia

54. *Tergite VII, mid-dorsal constriction or separation*: (0) mid-dorsally broad (Fig. 8A); (1) constricted (Fig. 8B); (2) separated into 2 lateral plates completely or connected with each other only by narrow membrane (Fig. 8C).
55. *Tergite VII, pubescence*: (0) covering largely (Fig. 8A,B); (1) covering only anteroventral portion (Fig. 8C); (2) absent.
56. *Sternite VII, shape*: (0) nearly quadrate (Fig. 8D); (1) shallowly notched caudomedially, somewhat quadrate (Fig. 8E); (2) deeply notched caudomedially, more or less V-shaped (Fig. 8F).
57. *Sternite VII, pubescence*: (0) covering nearly entirely (Fig. 8D,E); (1) not covering caudal portion (Fig. 8F).
58. *Tergite VIII, shape*: (0) simple plate, not elongated below (Fig. 8A); (1) elongated below, apically articulated with

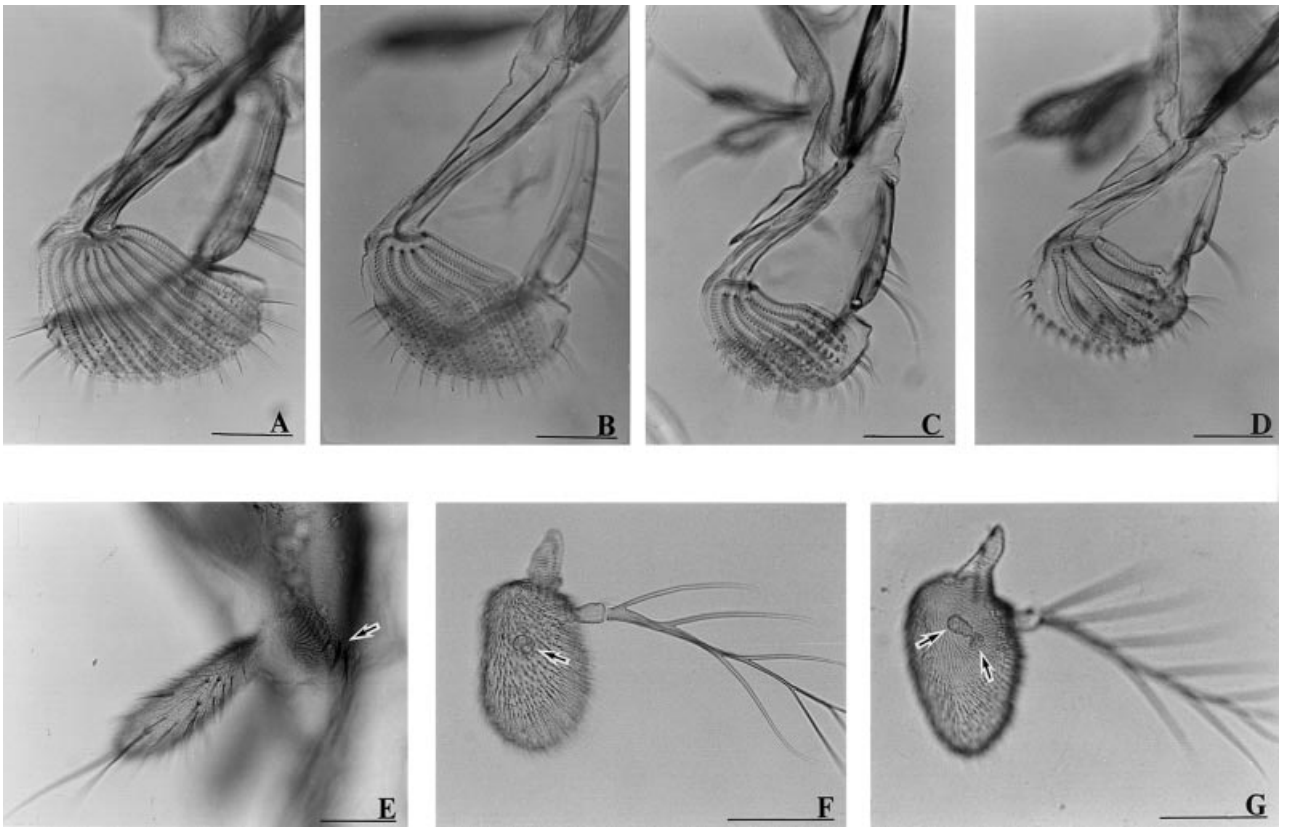

Fig. 3. A–D, Distal part of proboscises; E, palpus and its basal lobe; F,G, 1st flagellomeres. A,E, *Drosophila* (*Drosophila*) *hydei*; B, *Lordiphosa collinella*; C,F, *Lordiphosa tenuicauda*; D, *Nesiodrosophila sakagamii*; G, *Drosophila* (*Sophophora*) *melanogaster*. Scales = 0.1 mm.

oviscapt, forming arch surrounding epiproct and hypoproct (Fig. 8B,C).

59. *Tergite VIII*; *pubescence*: (0) absent entirely (Fig. 8C); (1) absent on tergite VIII, but present on membrane between tergite VIII and epiproct; (2) covering dorsally (Fig. 8 A,B).
60. *Cerci*: (0) present (Fig. 8A); (1) absent (Fig. 8B,C). (G207*).
61. *Epiproct, pubescence*: (0) present (Fig. 8A,B); (1) absent (Fig. 8C).
62. *Hypoproct, pubescence*: (0) covering nearly entirely (Fig. 8G); (1) covering partly (Fig. 8H); (2) absent (Fig. 8I).
63. *Oviscapt (sternite VIII)*: (0) simple plate resembling the other sternites (Fig. 9A,B); (1) nearly entirely divided into 2 lateral lobes connected by anteroventral bridge (Fig. 9C,D). (G211*).
64. *Oviscapt, pubescence*: (0) absent (Figs 8B,C; 9C,D); (1) covering at least partly (Fig. 9A,B).
65. *Oviscapt, ovisensilla*: (0) only trichoid ones (Fig. 9A,B); (1) peg-like ones present (Figs 8B,C; 9C,D). (G212).
66. *Oviscapt, lateral ovisensilla on mesal surface*: (0) present (Figs 8B; 9A–D); (1) absent (Fig. 8C).
67. *Oviscapt, apical ovisensillum*: (0) neither so robust nor the largest among marginal ones (Fig. 8B,C); (1) robust and the largest, but not so distinguishable from the others (Fig. 9C); (2) very robust, distinguishable from the others (Fig. 9D). (G213*).

In addition to Grimaldi's (1990) apomorphy (corresponding to our state 2), we recognized an intermediate apomorphy for state 1 in the *tenuicauda* group.

68. *Spermathecal capsule, apical indentation*: (0) absent; (1) present.

Results

Maximum parsimony and monophyletic groups

The heuristic search for a maximum parsimony solution resulted in two cladograms, which were different from each ther only in the relationships between *Dr.* (*Dr.*) *immigrans* Sturtevant and *Dr.* (*Dorsilopha*) *busckii* Coquillett. A strict consensus of these two cladograms (length = 245, CI = 0.437, RI = 0.765) was rooted so that the outgroup species formed a basal polytomy with the ingroup (Figs 10–12). Apomorphies are indicated on each branch of this cladogram, based on the results of character optimization. Although DELTRAN and ACCTRAN came to the same result in most TS, different results were obtained in TS7, 8, 20, 21, 44, 47, 48, 49, 54, 65 and 68. Synapomorphies not identified by both DELTRAN and ACCTRAN are ignored in the following

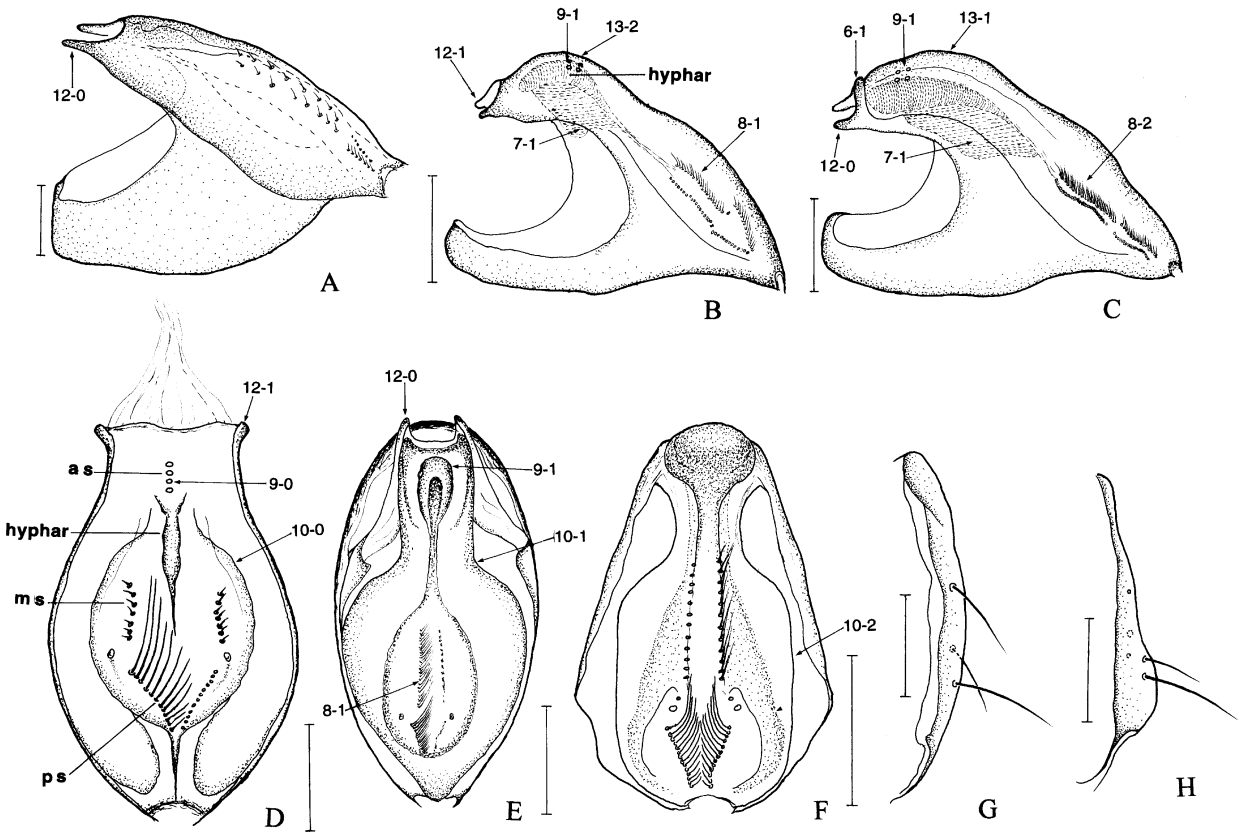

Fig. 4. A–F, Cibaria, hypopharynges (hyphars) and cibarial sensilla (A–C from dorsolateral views and D–F from dorsal views); G,H, prementa (lateral views). A,G, *Amiota* (*Amiota*) *albilabris*; B, *Lordiphosa tenuicauda*; C,H, *Lordiphosa fenestrarum*; D, *Stegana* (*Steganina*) *longifibula*; E, *Lordiphosa kurokawai*; F, *Nesiodrosophila raridentata*. Scales = 0.1 mm. a s = anterior sensilla, m s = medial sensilla, p s = posterior sensilla.

discussion. Figure 13 shows the bootstrap value, in percent, for the drosophilines at each node of the cladogram. A bootstrap value of 50% was taken as a provisional criterion for monophyly.

The monophyly of Drosophilinae was strongly supported by the following autapomorphies: tapered, thin and apically curved and sharp supracervical setae (ch. 1-1); anterior cibarial sensilla situated behind the anterior margin of hypopharynx (ch. 9-1); arched tergite VIII of female (ch. 58-1); absence of cerci in female (ch. 60-1); nearly entirely bilobed oviscapt (sternite VIII) (ch. 63-1); caudomedially notched sternite VII of female (ch. 56-1,2). Okada (1989) and Grimaldi (1990) pointed out some other synapomorphies for this monophyletic subfamily. Further discussion of these features is beyond the aim of this paper.

Scaptodrosophila was identified as the sister group to all the remaining drosophilines. The monophyly of the latter was supported by two synapomorphies: absence of prescutellar setae (ch. 19-1); anteriorly convex dorsal margin of cibarium (ch. 13-1,2).

Lordiphosa was shown to be polyphyletic. The *tenuicauda* species-group was placed as the sister group to *Nesiodrosophila* Wheeler & Takada, these two groups together forming a monophyletic group based on the following synapomorphies: apical ovisensillum robust and the largest among the marginal ones (ch. 67-1,2); female hypoproct at least partly nonpubescent (ch. 62-1,2; the latter, the complete lack of pubescence, probably having evolved independently in *Nesiodrosophila sakagamii* Toda and *Sophophora*); ocellar setae situated outside triangle made by ocelli (ch. 2-1; homoplasies seen in *Dr.* (*Sophophora*) *melanogaster* Meigen, *Scaptomyza graminum* (Fallén) and *Dr.* (*Dr.*) *transversa* Fallén); cibarium only slightly protruded at anterolateral corners (ch. 12-1; regarded as having been shortened independently from steganines). The monophyly of the *tenuicauda* group was supported by an autapomorphy, cercus ventrally pointed or elongated but without any setae at tip (ch. 35-2), and the monophyly of *Nesiodrosophila* by the following synapomorphies: cibarium parallel-sided in posterior portion (ch. 10-2); longest axis of eye distinctly oblique to the body axis (ch. 3-1); frons very flat (ch. 4-1); pseudotracheae in the labellum nearly the same in width (ch. 15-1) (these three characters seen in *Stegana longifibula* Takada as homoplasies); paramere fused to the hypandrium (ch. 39-1; homoplasies seen in *Lo. cyanea* (Okada) and some lineages of subgenus *Drosophila*); mid-dorsally separated tergite VII of female (ch. 54-2; regarded as having evolved independently in many lineages of Drosophilidae). The remaining *Lordiphosa* species

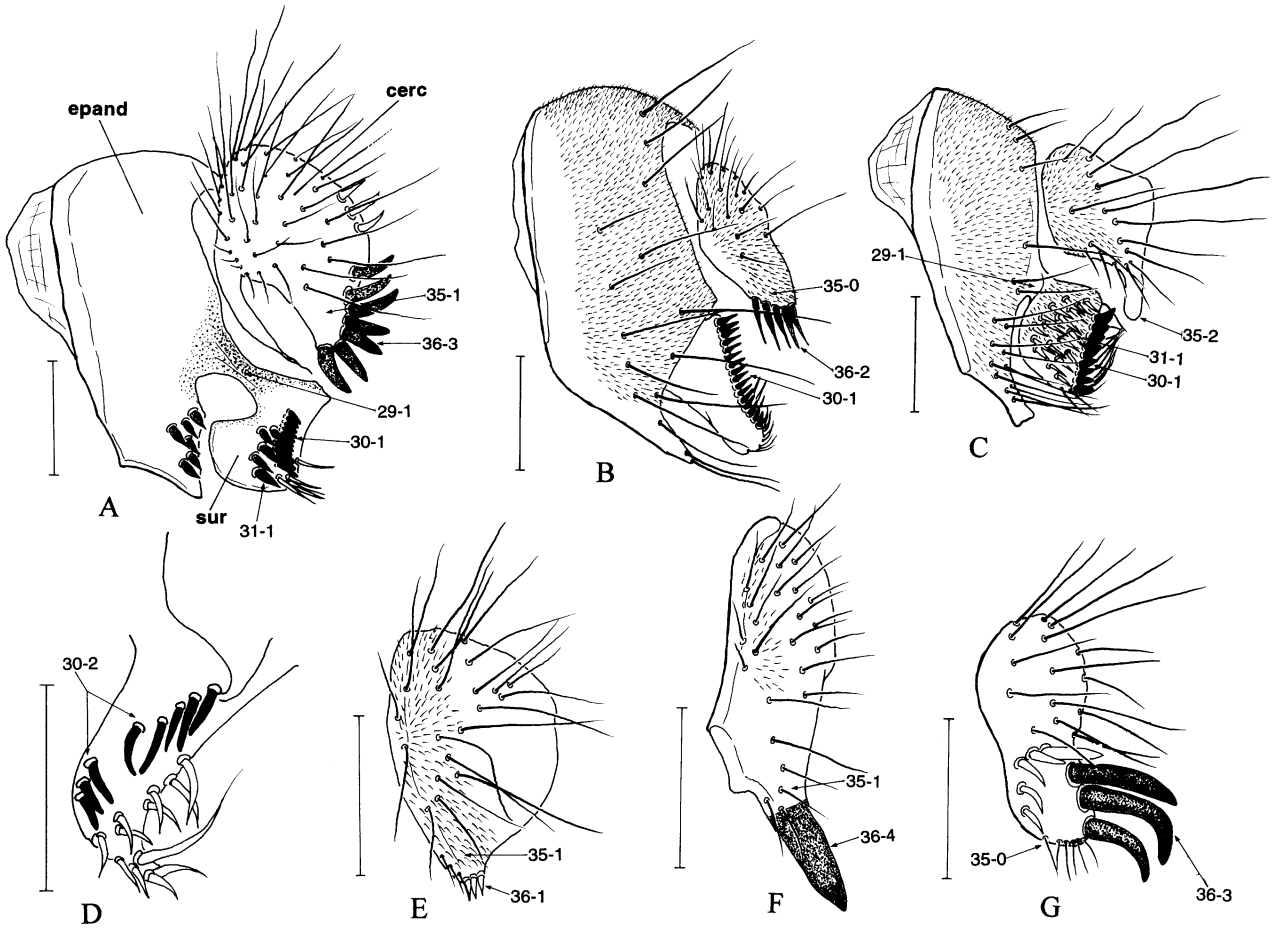

Fig. 5. A–C, Periphallic organs; D, surstylus; E-G, male cerci (lateral views). A, *Drosophila* (*Drosophila*) *funebris*; B, *Lordiphosa kurokawai*; C, *Lordiphosa tenuicauda*; D, *Drosophila* (*Sophophora*) *melanogaster*; E, *Lordiphosa nigricolor*; F, *Lordiphosa magnipectinata*; G, *Drosophila* (*Sophophora*) *auraria*. Scales = 0.1 mm. epand = epandrium, sur = surstylus, cerc = cercus.

comprised another monophyletic group based on the following synapomorphies: pale, membranous aedeagus (ch. 53-1); anteriorly expanded hypopharyngeal apodeme (ch. 7-1; homoplasies seen in *Dr.* (*So.*) *melanogaster*, subgenus *Drosophila* and the clade of *Nesiodrosophila* + *tenuicauda* group); five pseudotracheae in the labellum (ch. 14-1; homoplasies seen in *St. longifibula, Hirtodrosophila nokogiri* (Okada), *Lo. acutissima* (Okada) and *Lo. cyanea*); surstylus separated from the epandrium (ch. 29-0; regarded as having secondarily reversed from the surstylus fused to the epandrium as in *Ne. sakagamii and Sc. pallida* (Zetterstedt)); lack of paramedian setae on the hypandrium (ch. 37-1; homoplasies seen in some steganines, genera *Chymomyza* and *Scaptomyza,* and *Dr.* (*So.*) *auraria* Peng); paramere ventrobasally articulated with the hypandrium (ch. 40-0; regarded as having evolved independently from similar structures seen in some steganines).

Within *Lordiphosa* proper (i.e. *Lordiphosa* minus the *tenuicauda* group), three species-groups were each identified as monophyletic. The monophyly of the *fenestrarum* group was based on the anterior dorsocentral seta situated far anterior to the posterior, $0.6 \leqslant$ dcp (ch. 23-2; homoplasies seen in genus *Scaptomyza*, *Dr.* (*Dr.*) *funebris* (Fabricius) and *Dr.* (*Dr.*) *virilis* Sturtevant), and paramere partly covered with short pubescence (ch. 43-0; regarded as having secondarily reversed to the similar state seen in some steganines and *Scaptodrosophila* as in *Lo. acutissima, Lo. cyanea, Sc. graminum, Dr.* (*Dr.*) *virilis* and *Dr.* (*Dr.*) *transversa*) as synapomorphies. The monophyly of the *nigricolor* group was supported by acrostichal setulae in line with and anterior to the dorsocentrals the same size as others (ch. 21-1; regarded as having appeared independently in a number of lineages of Drosophilidae) and male cercus with a tuft or row of small, stout spines near caudoventral corner (ch. 36-1; homoplasies seen in *Hi. quadrivittata* (Okada) and *Dr.* (*Dr.*) *immigrans*) as synapomorphies. Male cercus bearing a row of large, stout spines on the ventral to anteroventral margin (ch. 36-2), aedeagus fused to the gonopod (ch. 52-2) and pubescent membrane between the cercus and the epandrium (ch. 34-0; regarded as a secondary reversal also seen in some other drosophiline species) as synapomorphies supported the monophyly of the *denticeps* group. On the other hand, the bootstrap value for the clade of the three *miki* group species studied was too low (39%) to support their monophyly, although *Lo.*

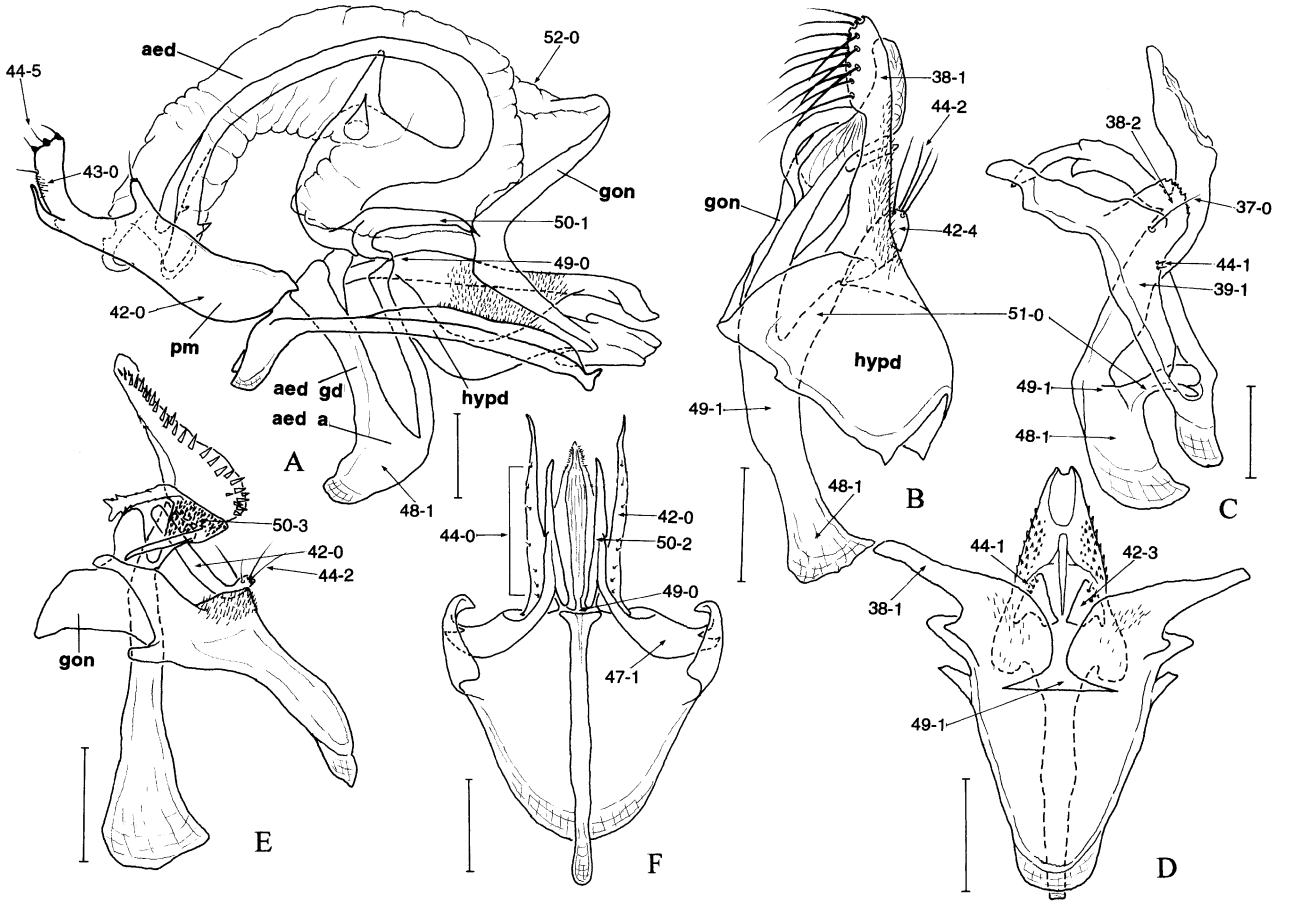

Fig. 6. Phallic organs (A from ventrolateral view; B,C,E from lateral views; D from ventral view; F from dorsal view). A, *Amiota* (*Phortica*) *kappa*; B, *Chymomyza fuscimana*; C, *Drosophila* (*Drosophila*) *immigrans*; D, *Scaptomyza* (*Parascaptomyza*) *pallida*; E, *Drosophila* (*Sophophora*) *melanogaster*; F, *Drosophila* (*Sophophora*) *obscura*. Scales = 0.1 mm. hypd = hypandrium, pm = paramere, aed gd = aedeagal guide, gon = gonopod, aed a = aedeagal apodeme, aed = aedeagus.

magnipectinata (Okada) and *Lo. stackelbergi* (Duda) comprised a monophyletic group based on the following synapomorphies: male cercus bearing a single, very large, somewhat plate-like peg at the ventral apex (ch. 36-4); pubescence absent from the female tergite VIII but present on the membrane between the tergite VIII and the epiproct (ch. 59-1; a homoplasy seen in *Lo. fenestrarum*). As for relationships among these groups, the *denticeps* group was identified as the sister group to the other groups: the monophyly of the latter was supported by thirty-five or more medial cibarial sensilla (ch. 8-2), prementum forming a discrete bump at the distal end of ventral surface (ch. 18-1) and male cercus tapering below, with setae at tip (ch. 35-1; similar homoplastic morphologies seen in *Lo. denticeps, Hi. nokogiri, Sc. pallida and Dr.* (*Dr.*) *funebris*). Relationships between the *fenestrarum* and *nigicolor* groups and the three species of the *miki* group could not be determined because the bootstrap values for the relevant clades were too low.

The monophyly of *Sophophora* was strongly supported by the following synapomorphies: two internal organs seen in the 1st flagellomere (ch. 17-1); largely or entirely nonpubescent tergite VII (ch. 55-1,2) and caudally nonpubescent sternite VII (ch. 57-1) of female; setulae on the basal lobe of palpus (ch. 5-1; a homoplasy seen in the lineage leading to genus *Scaptomyza* and subgenera *Drosophila* and *Dorsilopha* Sturtevant); dorsalmost seta of a group of small setae ventral to and between two prominent katepisternals longer than others but shorter than anterior katepisternal (ch. 22-1; homoplasies seen in the clades of *Lo. mommai* + *Lo. coei* (Okada) and *Lo. acutissima* + *Lo. cyanea* and the lineage leading to genus *Scaptomyza* and subgenus *Drosophila*); lack of pubescence on the epandrium (ch. 27-1; a homoplasy seen in the clade of *Dr.* (*Dr.*) *transversa* + *Dr.* (*Dr.*) *funebris*); lack of pubescence on the male cercus (ch. 32-1; homoplasies seen in *St. longifibula* and the clade of *Dr.* (*Dr.*) *transversa* + *Dr.* (*Dr.*) *funebris*); aedeagal basal process fused to the gonopod, forming together an elongate flap (ch. 50-2,3; a homoplasy seen in *Scaptodrosophila*); female tergite VII separated into two lateral plates (ch. 54-2; see above for homoplasies); lack of pubescence on the female tergite VIII (ch. 59-0; regarded as a secondary reversal); lack of pubescence on the epiproct (ch. 61-1; a homoplasy seen in *St. longifibula*); lack of pubescence

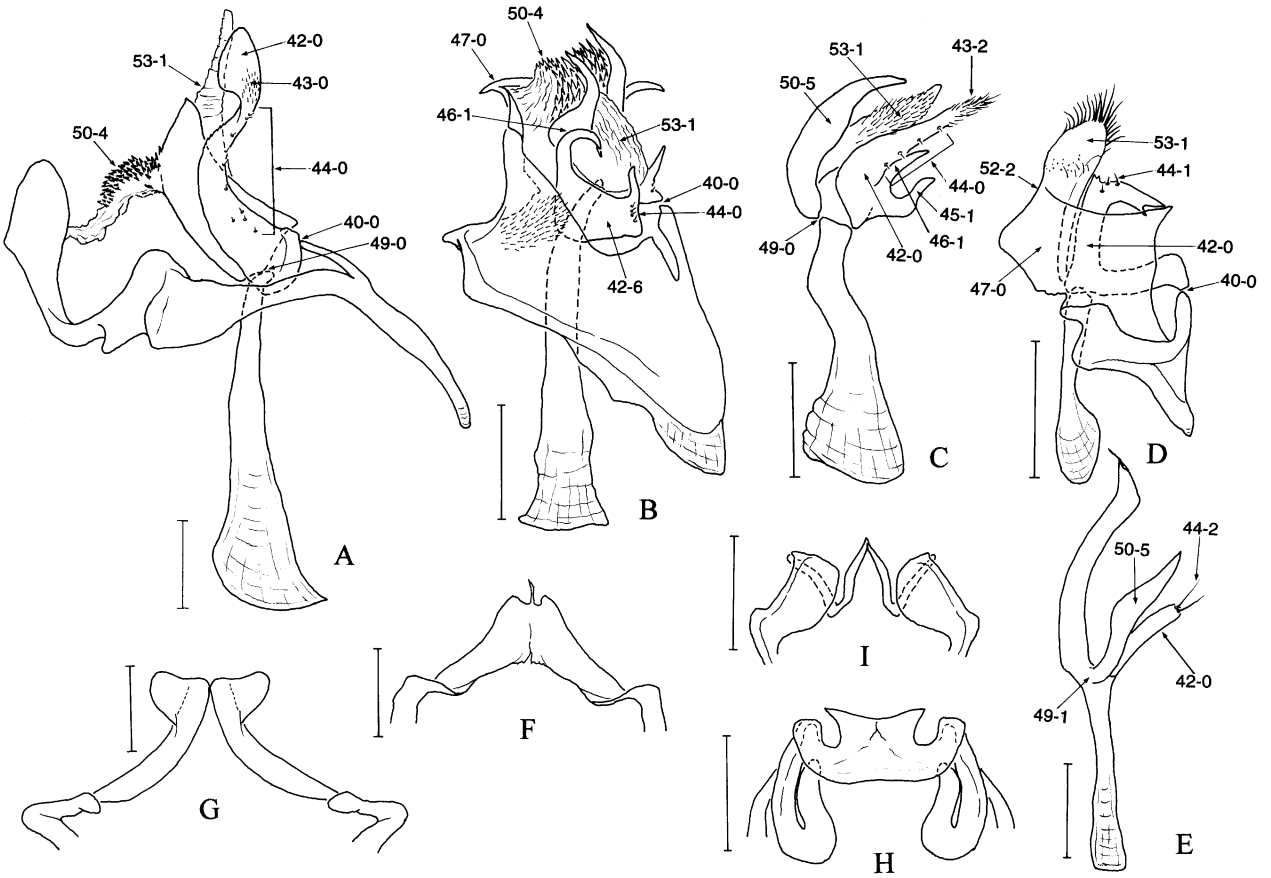

Fig. 7. A–E, Phallic organs (lateral views); F–I, gonopod(s) (ventral views, except for G from dorsal view). A,G, *Lordiphosa fenestrarum*; B, *Lordiphosa nigricolor*; C, *Lordiphosa mommai*; D, *Lordiphosa kurokawai*; E, *Lordiphosa tenuicauda*; F, *Lordiphosa magnipectinata*; H, *Drosophila* (*Drosophila*) *transversa*; I, *Hirtodrosophila quadrivittata*. Scales = 0.1 mm.

on the hypoproct (ch. 62-2; see above for the homoplasy). Although *Sophophora* was identified as the sister group to *Lordiphosa* proper in the maximum parsimony cladograms, the bootstrap value was too low (34%) to support the monophyly of these two taxa.

The following two genera were each recognized as monophyletic: *Hirtodrosophila* based on medially not constricted dorsal wall of cibarium (ch. 10-0; regarded as a secondary reversal), only one ventral branch of arista (ch. 16-1; homoplasies seen in the clade of *Lo. denticeps* + *Lo. neokurokawai* (Singh & Gupta) and genus *Scaptomyza*) and paramere fused to the aedeagus (ch. 41-1; a homoplasy seen in *Ne. raridentata* (Okada & Chung)); and *Scaptomyza* based on only one ventral branch of arista (ch. 16-1; see above for homoplasies), anterior dorsocentral seta situated far anterior to the posterior, $0.6 \leqslant$ dcp (ch. 23-2; see above for homoplasies), lack of paramedian setae on the hypandrium (ch. 37-1; see above for homoplasies), one pair of caudoventral processes of the hypandrium (ch. 38-1; similar processes regarded as having derived independently in *Chymomyza*) and small, somewhat conical paramere (ch. 42-3; a homoplasy seen in *Ne. sakagamii*). Although subgenera *Drosophila* and *Dorsilopha* comprised a monophyletic group, the bootstrap value was too low (16%) to support it. Within subgenus *Drosophila,* however, *Dr. funebris, Dr. transversa* and *Dr. testacea* von Roser were regarded as constituting a monophyletic group based on stout or peg-like setae on the outer mesal surface of surstylus (ch. 31-1; a homoplasy seen in the clade of *Lo. tenuicauda* + *Lo. pseudotenuicauda* (Toda)) and large, broad, plate-like paramere (ch. 42-2; a homoplasy seen in the clade of *Lo. acutissima* + *Lo. cyanea*). The relationships among *Chymomyza*, *Lordiphosa* proper, *Sophophora*, *Hirtodrosophila*, *Nesiodrosophila* + *Lo. tenuicauda* group, *Scaptomyza*, *Dorsilopha* and subgenus *Drosophila* remain unclear because of low bootstrap values.

Discussion

The phylogenetic position of *Lordiphosa* in Drosophilidae has not been confirmed yet. Okada (1963) compared about eighty species of the major drosophilid genera, subgenera and species-groups, including *Lordiphosa* (the *fenestrarum* group), for eleven morphological characters of adults, larvae and eggs, and mentioned that *Lordiphosa* was best placed near *Sophophora*. This hypothesis was supported by Laštovka &

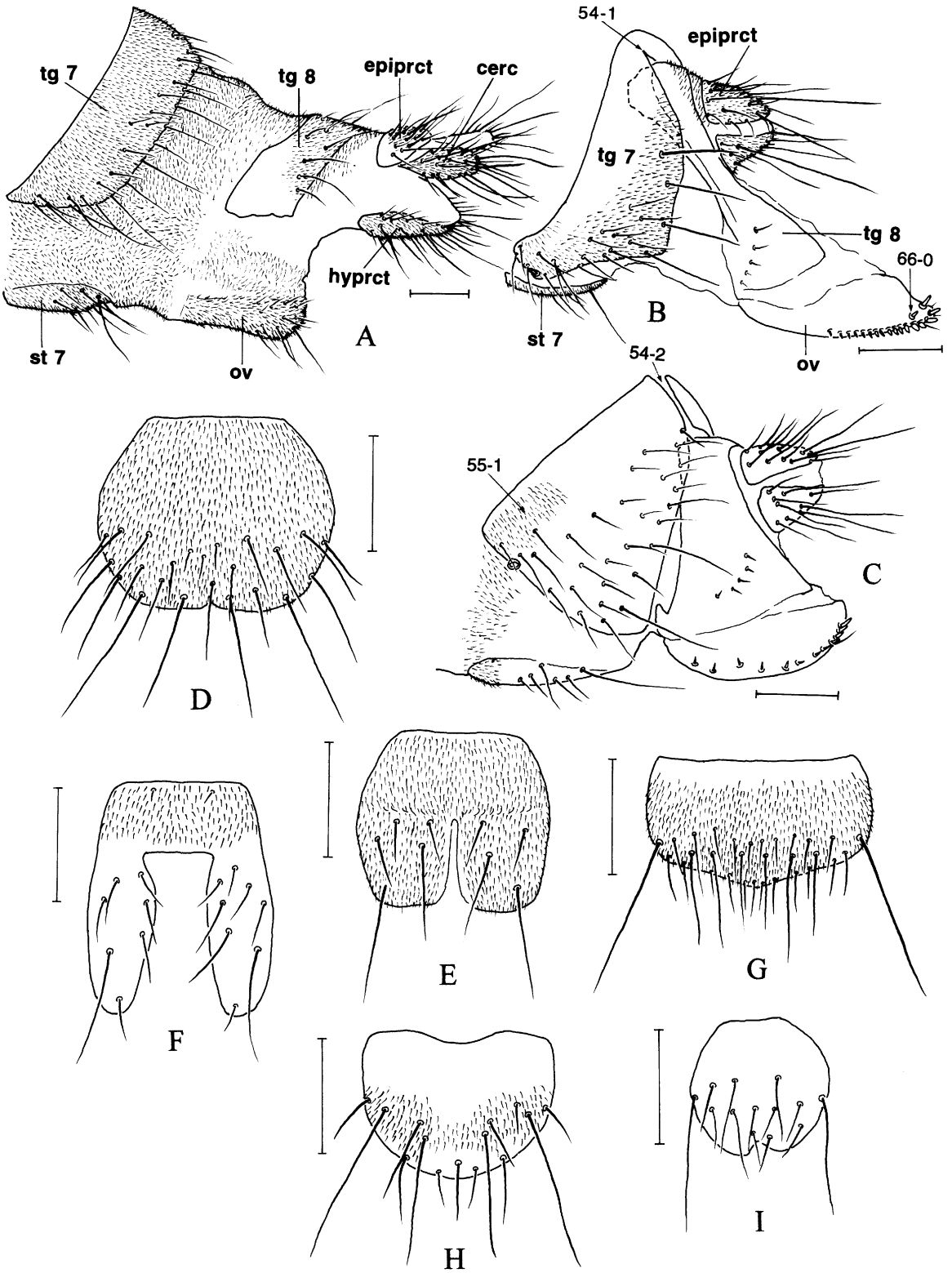

Fig. 8. A–C, Female terminalia (lateral views); D–F, sternite VII (ventral views); G–I, hypoproct (ventral views). A,D, *Amiota* (*Phortica*) *kappa*; B,E, *Scaptodrosophila coracina*; C,F, *Drosophila* (*Sophophora*) *melanogaster*; G, *Stegana* (*Steganina*) *longifibula*; H, *Lordiphosa acutissima*; I, *Nesiodrosophila sakagamii*. Scales = 0.1 mm. tg7 = tergiteVII, tg8 = tergiteVIII, st7 = sterniteVII, ov = oviscapt (sternite VIII), epiprct = epiproct, cerc = cercus, hyprct = hypoproct.

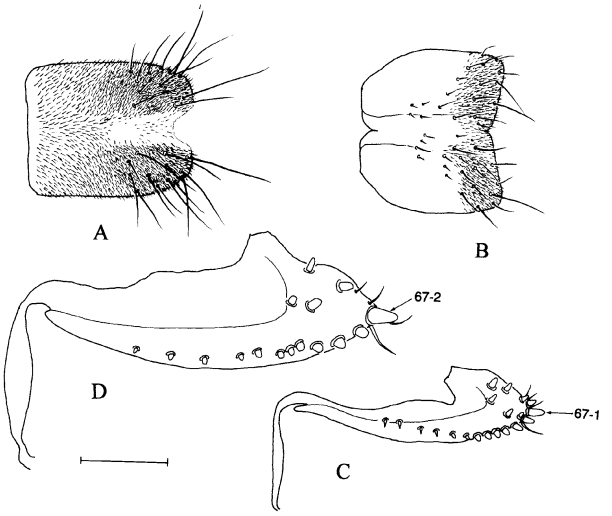

Fig. 9. Oviscapt (A,B from ventral views; C,D from lateral views). A, *Amiota* (*Phortica*) *kappa*; B, *Stegana* (*Steganina*) *longifibula*; C, *Lordiphosa tenuicauda*; D, *Lordiphosa acutissima*. Scale = 0.1 mm.

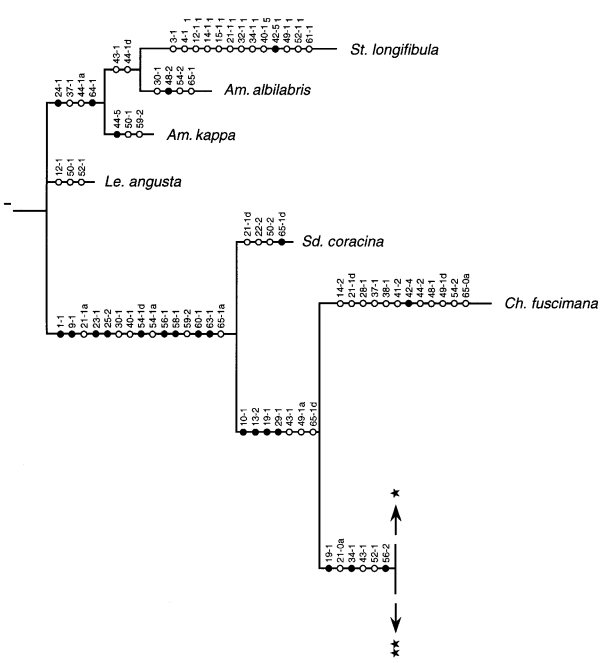

Fig. 10. The strict consensus of two maximum parsimony cladograms resulting from heuristic search using PAUP 3.1.1, with indication of apomorphies (● = occurring only once, ○ = including homoplasies; different results from ACCTRAN and DELTRAN are marked with 'a' and 'd', respectively). Continued to Figs 11, 12.

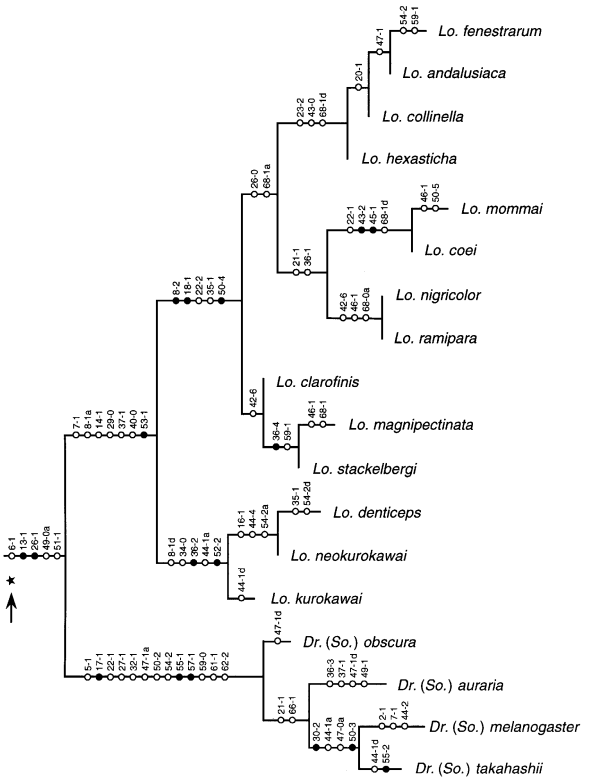

Fig. 11. Continued from arrow and star (→*) in Fig. 10.

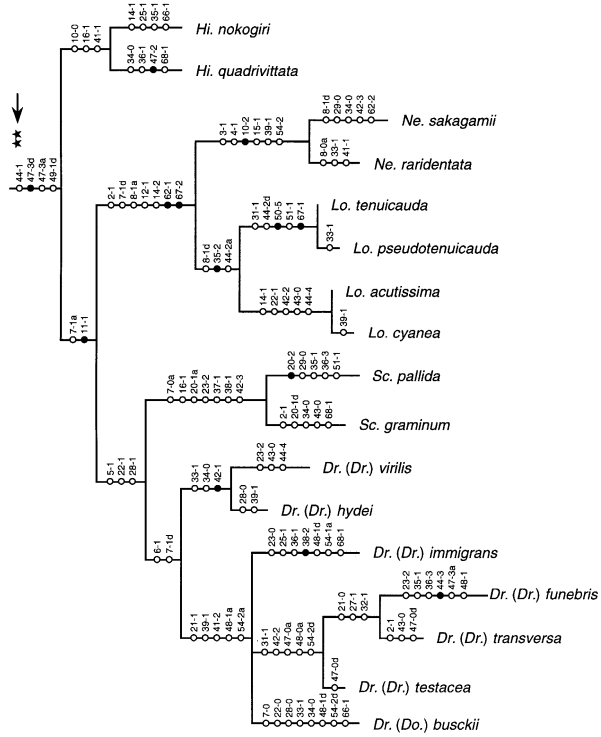

Fig. 12. Continued from arrow and double stars (→**) in Fig. 10.

Máca (1978). They thoroughly revised the taxonomy of *Lordiphosa* and inferred that *Lordiphosa*, particularly *Lo. nigricolor* and *Lo. miki*, were most closely related to *Sophophora* based on many characters of the male terminalia (paramere, aedeagus and hypandrium), antenna, carina and

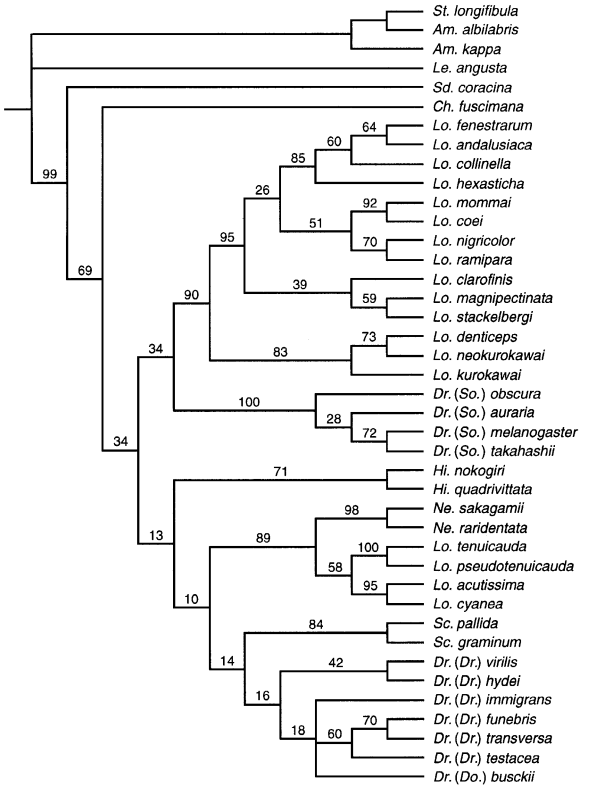

Fig. 13. Bootstrap value (%) for each clade of drosophilines on the strict consensus cladogram.

pigmentation of the abdomen. They also pointed out that *Lordiphosa* shared some characters with other genera, such as the low number of acrostichal setae and the shape of the wing with *Scaptomyza*, and the form of the ventral receptacle and ejaculatory apodeme with *Chymomyza*, but regarded these characters as being so simple or alternative that convergence cannot be excluded.

Throckmorton (1975) included only the *denticeps* group of *Lordiphosa* in his monograph on the evolution of drosophilids. He placed it in the *immigrans-Hirtodrosophila* radiation and mentioned that the *immigrans*, *denticeps* and *pinicola* groups, of which the first and third belong to subgenus *Drosophila*, made up a complex as the core of higher drosophilines.

Grimaldi (1990) placed *Lordiphosa* close to *Scaptomyza*, based on the result of cladistic analysis, but included only *Lo. fenestrarum* in his study. He pointed out the following characters as supporting the monophyly of *Lordiphosa* + *Scaptomyza*: (1) postocular part of gena thick; (2) number of interfrontal setulae reduced to two to six; (3) acrostichals generally reduced to four rows; (4) acrostichals in front of dorsocentrals enlarged; (5) tergite VII in male present; (6) epandrium devoid of setae except on ventral portion; (7) prensisetae in short row on surstylus, especially when only in proximal half of surstylus; (8) paramere elongate, sclerotized. However, these characters are questionable in definition and homology, or specific to *Lo. fenestrarum* and not to *Lordiphosa* as a whole, for the following reasons. (1) It was difficult to measure accurately the relative thickness of the postgena to the gena, and this character varied continuously among the species examined in this study. (2) It was difficult to define objectively the apomorphic state in the number of interfrontal setulae because of continuous variation among the species studied. (3) Although the arrangement of acrostichal setulae in four or two rows was regarded as a synapomorphy supporting the monophyly of *Scaptomyza* in the result of ACCTRAN, the four-row arrangement seen in three species of the *fenestrarum* group, *Lo. fenestrarum*, *Lo. andalusiaca* and *Lo. collinella* (Okada), was regarded as having evolved independently in this lineage. (4) The enlarged acrostichals in front of the dorsocentrals were seen not only in *Lordiphosa* and *Scaptomyza* but also in many other taxa, including even some steganines, and furthermore the *nigricolor* group species lost this character. (5) The male tergite VII was present, even if reduced to vestigial, lightly sclerotized strip or patch attached to the anterior margin of the epandrium, in all the drosophiline species examined, but absent in the four steganines. (6) Although Grimaldi mentioned that the epandrium devoid of setae, except on the ventral portion, was a synapomorphy for *Scaptomyza*, and that this character occurred also in other taxa including *Lordiphosa*, the code for this TS in his original data matrix indicates the plesiomorphic state (0) in *Lo. fenestrarum* (we confirmed the correctness of the latter code, i.e. *Lo. fenestrarum* having several setae on the lateral to dorsal portion of the epandrium, and furthermore that all the species of *Lordiphosa* studied retained the plesiomorphic state). (7) According to the cladogram resulting from the Grimaldi's analysis, the short row of prensisetae only on the dorsal part of distal margin of the surstylus is not a synapomorphy for the clade of *Lordiphosa* + *Scaptomyza*, occurring only in some *Scaptomyza* species (within *Lordiphosa*, too, this character was seen only in some of the species studied, *fenestrarum*, *andalusiaca*, *nigricolor*, *mommai*, *coei*, *denticeps* and *neokurokawai*). (8) Although Grimaldi regarded the parameres of all the *Scaptomyza* studied by him as being long, those are small, conical or club-shaped, much shorter than the aedeagus, according to his Figs 466–475 (we confirmed this in both *Sc. pallida* (Fig. 6D) and *Sc. graminum*.). Thus, all these characters are meaningless for supporting the monophyly of *Lordiphosa* + *Scaptomyza*. Grimaldi, moreover, referred to Hackman's work as follows: 'Hackman (1982) stated that *Lordiphosa* is most closely related to *Scaptomyza*, in particular to the subgenus *Bunostoma* …, and he presented about 12 characters in support of his hypothesis.' This reference is, however, incorrect. Although Hackman (1982) did state, 'The *Bunostoma* species show some external similarity to the *Drosophila* subgenus *Lordiphosa*…', he concluded, after the precise comparison of some characters, including internal ones, that '*Lordiphosa* is distinctly separated from *Scaptomyza*', but made no additional comments about the phylogenetic position of *Lordiphosa*.

Molecular data concerning the phylogenetic position of *Lordiphosa* are available for only one species, *Lo. andalusiaca*. Pélandakis & Solignac (1993) analysed rRNA sequences of seventy-two drosophilid species, including *Lo. andalusiaca*, but could not determine its phylogenetic position: 'the

phylogenetic position of *Dr.* (*Lordiphosa*) *andalusiaca* is not stable. In the various analyses, this species was found close to the subgenus *Sophophora*, close to the *Drosophila* clade, or as a sister group of the *obscura-melanogaster* clade…'.

The present study revealed the polyphyly of *Lordiphosa*: the *tenuicauda* group is distantly related to the other members of *Lordiphosa* as suggested by Toda (1983) and Okada (1984), but is the sister group to genus *Nesiodrosophila,* whereas the remaining *Lordiphosa* species form another monophyletic group. The polyphyly of this genus was documented by a molecular phylogeny for the *Adh* (alcohol dehydrogenase) gene (Katoh, 1999), although *Nesiodrosophila* was not included in the study. As a result, a taxonomic revision of the *tenuicauda* group, including all the known species reviewed by Hu *et al*. (1999) and related taxa, will be made elsewhere.

Although the present study could not determine the phylogenetic position of *Lordiphosa* proper, other than the *tenuicauda* group, within Drosophilinae because of the low bootstrap value, the topology of the maximum parsimony cladograms suggested that it is the sister group of *Sophophora.* This was also supported by the phylogeny for the *Adh* gene: *Lordiphosa* proper was placed as the sister group to the monophyletic clade of two Neotropical sophophoran species-groups, the *willistoni* + *saltans* groups, which were not included in the present study, but the relationships to the main body of other sophophorans that formed another monophyletic group remained unresolved (Katoh, 1999). These findings seem to support, if not conclusively, the hypothesis of Okada (1963) and Laštovka & Máca (1978) that *Lordiphosa* is most closely related to *Sophophora*. By contrast, Grimaldi's (1990) hypothesis, the monophyly of *Lordiphosa* + *Scaptomyza*, was not supported by the 0 bootstrap value: the clade of *Lordiphosa* proper + genus *Scaptomyza* never appeared in the bootstrap analysis with 100 replicates.

There is no consensus in hypotheses so far proposed for the relationships among major drosophiline genera and subgenera. Most hypotheses, such as those of Throckmorton (1975) based on morphological characters, Beverley & Wilson (1982) based on LHP (larval haemolymph protein), Pélandakis & Solignac (1993) based on 18S rRNA (the large subunit of nuclear ribosomal RNA) gene sequences, Thomas & Hunt (1993), Russo *et al*. (1995), Tamura *et al*. (1995) and Katoh (1999) based on *Adh* gene sequences, Kwiatowski *et al*. (1994) based on *Sod* (Cu, Zn superoxide dismutase) gene sequences, Kwiatowski *et al*. (1997) based on *Gpdh* (glycerol-3-phosphate dehydrogenase) gene sequences, and Tatarenkov *et al*. (1999) based on *Ddc* (dopa decarboxylase) gene sequences, suggest the polyphyly or paraphyly of genus *Drosophila*, i.e. subgenus *Sophophora* separated from the major drosophiline lineage early. By contrast, Grimaldi (1990) proposed the hypothesis that subgenera *Drosophila* and *Sophophora* constitute a monophyletic group. Of the molecular studies, only DeSalle's (1992) hypothesis based on mtDNA sequences supports Grimaldi's hypothesis. However, Remsen & DeSalle (1998) recently addressed this issue, focusing especially on the monophyly of Hawaiian drosophilids, by analysing simultaneously combined multiple datasets from morphology, mtDNA and nuclear DNA, and concluded that genus *Drosophila* was not monophyletic. Although the present study could not resolve this problem, the bootstrap value for the monophyly of *Drosophila* (the clade of the three subgenera *Sophophora* + *Dorsilopha* + *Drosophila* not realized in the maximum parsimony cladograms) was very small, only 8%. Thus, most of the current evidence supports the paraphyly of genus *Drosophila*. To remove this paraphyly from Drosophilinae by retaining *Sophophora* in genus *Drosophila*, most genera, except for a few such as *Scaptodrosophila* that represent early lineages, should be downgraded to subgenera of *Drosophila*, as suggested by Kwiatowski *et al*. (1997) and Tatarenkov *et al*. (1999). Alternatively, *Sophophora* should be elevated to generic rank. Either change will have a major impact on studies of drosophilids: a considerable number of secondary homonyms will be produced in the former case, and *Drosophila melanogaster* will have to be referred to as *Sophophora melanogaster* in the latter case. To solve this problem, and eventually achieve a consensus on the drosophilid phylogeny, additional studies, including more taxa of the family and both morphological and molecular approaches, are needed.

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Appendix 1. A list of species studied.

Subfamily	Genus	Subgenus	Species-group	Species
Steganinae (outgroup)	*Stegana*	*Steganina*	*coleoptrata*	*longifibula* Takada, 1968
	Leucophenga	*Leucophenga*	*mutabilis*	*angusta* Okada, 1956
	Amiota	*Amiota*		*albilabris* (Roth, 1860)
	Amiota	*Phortica*		*kappa* Máca, 1977
Drosophilinae (ingroup)	*Scaptodrosophila*		*coracina*	*coracina* (Kikkawa & Peng, 1938)
	Chymomyza		*fuscimana*	*fuscimana* (Zetterstedt, 1838)
	Hirtodrosophila		*hirticornis*	*nokogiri* (Okada, 1956)
	Hirtodrosophila		*quadrivittata*	*quadrivittata* (Okada, 1956)
	Nesiodrosophila			*sakagamii* Toda, 1989
	Nesiodrosophila			*raridentata* (Okada & Chung, 1960)
	Scaptomyza	*Parascaptomyza*		*pallida* (Zetterstedt, 1847)
	Scaptomyza	*Scaptomyza*		*graminum* (Fallén, 1832)
	Drosophila	*Dorsilopha*		*busckii* Coquillett, 1901
	Drosophila	*Drosophila*	*funebris*	*funebris* (Fabricius, 1787)
	Drosophila	*Drosophila*	*repleta*	*hydei* Sturtevant, 1921
	Drosophila	*Drosophila*	*virilis*	*virilis* Sturtevant, 1916
	Drosophila	*Drosophila*	*immigrans*	*immigrans* Sturtevant, 1921
	Drosophila	*Drosophila*	*testacea*	*testacea* von Roser, 1840
	Drosophila	*Drosophila*	*quinaria*	*transversa* Fallén, 1823
	Drosophila	*Sophophora*	*obscura*	*obscura* Fallén, 1823
	Drosophila	*Sophophora*	*melanogaster*	*melanogaster* Meigen, 1830
	Drosophila	*Sophophora*	*melanogaster*	*takahashii* Sturtevant, 1927
	Drosophila	*Sophophora*	*melanogaster*	*auraria* Peng, 1937
	Lordiphosa		*fenestrarum*	*fenestrarum* (Fallén, 1823)
	Lordiphosa		*fenestrarum*	*andalusiaca* (Strobl, 1906)
	Lordiphosa		*fenestrarum*	*collinella* (Okada, 1968)
	Lordiphosa		*fenestrarum*	*hexasticha* (Papp, 1971)
	Lordiphosa		*nigricolor*	*nigricolor* (Strobl, 1898)
	Lordiphosa		*nigricolor*	*ramipara* (Zhang & Liang, 1992)
	Lordiphosa		*nigricolor*	*mommai* (Takada & Okada, 1960)
	Lordiphosa		*nigricolor*	*coei* (Okada, 1966)
	Lordiphosa		*miki*	*clarofinis* (Lee, 1959)
	Lordiphosa		*miki*	*magnipectinata* (Okada, 1956)
	Lordiphosa		*miki*	*stackelbergi* (Duda, 1935)
	Lordiphosa		*denticeps*	*denticeps* (Okada & Sasakawa, 1956)
	Lordiphosa		*denticeps*	*kurokawai* (Okada, 1971)
	Lordiphosa		*denticeps*	*neokurokawai* (Singh & Gupta, 1981)
	Lordiphosa		*tenuicauda*	*tenuicauda* (Okada, 1956)
	Lordiphosa		*tenuicauda*	*pseudotenuicauda* (Toda, 1983)
	Lordiphosa		*tenuicauda*	*acutissima* (Okada, 1956)
	Lordiphosa		*tenuicauda*	*cyanea* (Okada, 1988)

Appendix 2. Data matrix for forty-one drosophilid species and sixty-eight morphological characters used in the cladistic analysis.

	12345	1 67890	11111 12345	11112 67890	22222 12345	22223 67890	33333 12345	33334 67890	44444 12345	44445 67890	55555 12345	55556 67890	66666 12345	666 678
St. longifibula	00110	00000	01011	00000	10010	00000	01010	01001	05110	00010	01000	00000	10010	000
Le. angusta	00000	00000	01000	00000	00000	00000	00000	00000	00000	00?01	01000	00000	00000	000
Am. albilabris	00000	00000	00000	00000	00010	00001	00000	01000	00110	00200	00020	00000	00011	000
Am. kappa	00000	00000	00000	00000	00010	00000	00000	01000	00050	00001	00000	00020	00010	000
Sd. coracina	10000	00010	00000	00000	12102	00001	00000	00001	00000	00002	00010	10121	00101	000
Ch. fuscimana	10000	00011	00220	00010	10102	00111	00000	01101	24120	00110	00020	10121	00100	000
Lo. fenestrarum	10000	11211	00110	00111	02202	00001	00011	01000	00000	01004	11120	20111	00101	001
Lo. collinella	10000	11211	00110	00111	02202	00001	00011	01000	00000	00004	11110	20121	00101	001
Lo. andalusiaca	10000	11211	00110	00111	02202	00001	00011	01000	00000	01004	11110	20121	00101	001
Lo. hexasticha	10000	11211	00110	00110	02202	00001	00011	01000	00000	00004	11110	20121	00101	001
Lo. mommai	10000	11211	00110	00110	11102	00001	00011	11000	00201	10005	11110	20121	00101	001
Lo. coei	10000	11211	00110	00110	11102	00001	00011	11000	00201	00004	11110	20121	00101	001
Lo. nigricolor	10000	11211	00110	00110	12102	00001	00011	11000	06100	10004	11110	20121	00101	000
Lo. ramipara	10000	11211	00110	00110	12102	00001	00011	11000	06100	10004	11110	20121	00101	000
Lo. clarofinis	10000	11211	00110	00110	02102	10001	00011	01000	06100	00004	11110	20121	00101	000
Lo. magnipectinata	10000	11211	00110	00110	02102	10001	00011	41000	06100	10004	11110	20111	00101	001
Lo. stackelbergi	10000	11211	00110	00110	02102	10001	00011	41000	06100	00004	11110	20111	00101	000
Lo. denticeps	10000	11111	00110	10010	00102	10001	00001	21000	00140	00000	12120	20121	00101	000
Lo. kurokawai	10000	11111	00110	00010	00102	10001	00000	21000	00110	00000	12110	20121	00101	000
Lo. neokurokawai	10000	11111	00110	10010	00102	10001	00000	21000	00140	00000	121??	?????	??1??	???
Dr. (So.) obscura	10001	10011	00100	01010	01102	11011	01010	00001	00100	01002	11021	21101	12101	000
Dr. (So.) auraria	10001	10011	00100	01010	11102	11011	01010	31001	00100	01012	11021	21101	12101	10?
Dr. (So.) melanogaster	11001	11011	00100	01010	11102	11012	01010	00001	00120	00003	11021	21101	12101	100
Dr. (So.) takahashii	10001	10011	00100	01010	11102	11012	01010	00001	00110	00003	11022	21101	12101	100
Hi. nokogiri	10000	00010	00210	10010	00101	00011	00011	00001	10110	03010	01010	20121	00101	100
Hi. quadrivittata	10000	00010	00200	10010	00102	00011	00000	10001	10110	02010	01010	20121	00101	001
Ne. sakagamii	11110	01112	11221	00010	00102	00001	00000	00011	03110	03010	01020	20121	02101	020
Ne. raridentata	11110	01012	11221	00010	00102	00011	00110	00011	10110	03010	01020	20121	01101	020
Lo. tenuicauda	11000	01111	11220	00010	00102	00011	10012	00001	00120	03015	11010	20121	01101	010
Lo. acutissima	11000	01111	11210	00010	01102	00011	00012	00001	02040	03010	01010	20121	01101	020
Lo. pseudotenuicauda	11000	01111	11220	00010	00102	00011	10112	00001	00120	03015	11010	20121	01101	010
Lo. cyanea	11000	01111	11210	00010	01102	00011	00012	00011	02040	03010	01010	20121	01101	020
Sc. pallida	10001	00011	10200	10012	01202	00101	00011	31101	03110	03010	11010	20121	00101	000
Sc. graminum	11001	00011	10200	10011	01202	00111	00000	01101	03010	03010	01010	20121	00101	001
Dr. (Dr.) virilis	10001	11011	10200	00010	01202	00111	00100	00001	01040	03010	01010	20121	00101	000
Dr. (Dr.) hydei	10001	11011	10200	00010	01102	00011	00100	00011	01110	03010	01010	20121	00101	000
Dr. (Dr.) immigrans	10001	11011	10200	00010	11001	00111	00010	10211	20110	03110	01010	20121	00101	001
Dr. (Dr.) funebris	10001	11011	10200	00010	01202	01111	11011	30011	22130	03110	01020	20121	00101	000
Dr. (Dr.) testacea	10001	11011	10200	00010	11102	00111	10010	00011	22110	00010	01020	20121	00101	000
Dr (Dr.) transversa	11001	11011	10200	00010	01102	01111	11010	00011	22010	00010	01020	20121	00101	000
Dr. (Do.) busckii	10001	10011	10200	00010	10102	00011	00100	00011	20110	03110	01020	20121	00101	100