Phylogeny of Drosophilinae (Diptera: Drosophilidae), with comments on combined analysis and character support

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Abstract

Drosophilidae (Diptera) is a diverse, cosmopolitan family of flies. Here, we present a combined analysis phylogeny of Drosophilinae, one of the two subfamilies of Drosophilidae, based on data from six different data partitions, including both molecular and morphological characters. Although our data show support for the monophyly of the Hawaiian Drosophilidae, and the subgenus *Sophophora*, neither the genus *Drosophila* nor the subgenus *Drosophila* is monophyletic. Partitioned Bremer support (PBS) indicates that morphological data taken from Grimaldi's monograph (Grimaldi, 1990a), as well as sequences from the mitochondrial (mt) 16S rDNA and the nuclear *Adh* gene, lend much support to our tree's topology. This is particularly interesting in the case of Grimaldi's data, since his published hypothesis conflicts with ours in significant ways. Our combined analysis cladogram phylogeny reflects the catchall designation that the name *Drosophila* has become, in that the cladogram does not support the monophyly of either the genus or subgenus *Drosophila*. © 2002 Elsevier Science (USA). All rights reserved.

1. Introduction

The dipteran family Drosophilidae includes over 3000 described species that are distributed all over the world (Wheeler, 1986). One of these, *Drosophila melanogaster*, has become a model organism for a wide range of biological disciplines. Indeed, with the extensive research emphasis on developmental genetics and the now complete *Drosophila* genome project (Rubin et al., 2000), one can argue that *D. melanogaster* is the most widely studied eukaryote. However, despite its frequent use in biological research, many investigators are unaware that the family Drosophilidae contains approximately 65 genera, of which *Drosophila* is only one.

The family Drosophilidae is comprised of two subfamilies, the Drosophilinae and the Steganinae. Morphological variation within Drosophilidae is truly impressive. Several genera display high degrees of sexual dimorphism, including hypercephaly (Grimaldi and Fenster, 1989), extensive foreleg and mouthpart modification (Carson et al., 1970), and wing patterning. The Hawaiian Drosophilidae, which comprises approximately one sixth of the diversity in this family, is perhaps the most impressive group (Hardy, 1965). In addition to its size, the morphological variation within this group is nearly equal to that in the remaining species in the family. Several Hawaiian drosophilid clades possess males with highly modified mouthparts and tarsi that are used during courtship (Spieth, 1966). These diverse modifications have no direct parallel in any other family of Diptera.

The variety of ecological niches occupied by Drosophilidae is nearly as impressive as their morphological diversity. Species which breed in decaying plant and fungal matter are most common (Carson, 1971; Heed, 1968, 1971; Throckmorton, 1975). However, others, like the genus *Cladochaeta* and some members of the genus *Drosophila*, are parasitic (Carson, 1974; Grimaldi and Nguyen, 1999). Some drosophilid larvae are predatory, feeding upon bee larvae, scale insects, and even frog and spider embryos (Ashburner, 1989). Given this variety, the family has understandably attracted the attention of numerous systematists, ecologists, and taxonomists over the years.

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Morphological studies have led to three major phylogenetic hypotheses for the family Drosophilidae. Probably the most cited is Throckmorton's (1975) study. He used internal morphological characters from several studies to infer the relationships among most of the major drosophilid groups (Throckmorton, 1962, 1966, 1975). Okada (1989) used phenetic analyses of a relatively small set of characters to propose tribes and subtribes within Drosophilidae. Both of these studies lack explicitly stated methods or measures of support for each relationship.

Recently, Grimaldi (1990a) has used cladistic methodology to infer higher level relationships within Drosophilidae. He generated a massive data set of 208 characters for about 160 taxa. He also reanalyzed Throckmorton's (1975) and Okada's (1989) data sets using maximum parsimony so a direct comparison among the three studies could be made. Based on these analyses, Grimaldi (1990a) divided the Drosophilidae into higher level clades: tribes, subtribes, and genus groups. His results indicated that several taxa, *Hirtodrosophila*, *Lordiphosa*, and *Scaptodrosophila*, previously considered to be subgenera within *Drosophila*, should be elevated to generic rank.

Grimaldi (1990a) also proposed major changes in the relationships of the endemic Hawaiian Drosophilidae. Throckmorton (1966), based on his morphological analyses, had considered species of *Drosophila* and *Scaptomyza* from Hawai'i to be sister taxa. He postulated one, or possibly two, initial colonizations to account for the diversity in this group (Throckmorton, 1966). Grimaldi's cladistic analyses suggested that *Scaptomyza* and Hawaiian *Drosophila* were not monophyletic. Instead, he placed all Hawaiian *Drosophila* in the genus *Idiomyia* (Grimshaw), a name he resurrected. Idiomyia was placed as the sister taxon to a clade of mycophagous genera in the *Zygothrica* Genus Group. *Scaptomyza* was placed in the *Scaptomyza* Genus Group, a clade within the large *Drosophila* Genus Complex. Based on this phylogeny, *Scaptomyza* was actually more closely related to the genus *Drosophila* than either was to the Hawaiian *Drosophila*.

Although Grimaldi's (1990a) study is the most comprehensive in terms of sampling and number of morphological characters, it disagrees at several points with all previously published molecular data (reviewed in DeSalle and Grimaldi, 1991, 1992). While the placement of *Scaptodrosophila*, *Hirtodrosophila*, and *Lordiphosa* as genera are supported by molecular studies (DeSalle, 1992, however see Katoh et al., 2000; Remsen and DeSalle, 1998), molecular data support the notion that the Hawaiian Drosophilidae are monophyletic and the sister group of the genus *Drosophila* (see Powell, 1997; Powell and DeSalle, 1995). This incongruence is likely due to the methodology that Grimaldi (1990a) employed to analyze his data. Because of constraints on memory

and processor speed at this time of this study, he only selected 1000 equally parsimonious trees upon which to perform successive weighting. When these data are reanalyzed using a faster computer and more efficient algorithms one obtains over 30,000 equally parsimonious trees, the strict consensus of which, while largely unresolved, is also not incongruent with respect to the molecular characters. Successive weighting of only a subset of the possible trees apparently led to a bias in the results of Grimaldi's (1990a) study and the incorrect impression of incongruence between the two types of characters.

Even considering some of its analytical problems, Grimaldi's study set a standard of taxon sampling which, to date, no molecular study has achieved, in spite of the widespread use of DNA sequence data in drosophilid molecular systematics. In Fig. 1, we summarize the findings of the major morphological and molecular systematics studies of Drosophilidae. The majority of molecular systematic studies within Drosophilidae have targeted the species group (e.g., Barrio and Ayala, 1997; O'Grady, 1999) or subgenus (O'Grady and Kidwell, 2002; Pelandakis et al., 1991; Tamura et al., 1995) level. Those studies aimed at higher level relationships within this family have not sampled extensively across the major clades of Drosophilidae (e.g., DeSalle, 1992; Kwiatowski and Ayala, 1999; Kwiatowski et al., 1994; Pelandakis and Solignac, 1993; Thomas and Hunt, 1993). Furthermore, investigations willing to take advantage of the potentially fruitful use of existing morphological data in combination with DNA sequences have been almost completely lacking (however, see Remsen and DeSalle, 1998).

In this study we present a phylogenetic hypothesis for 41 taxa of Drosophilinae, based upon data from Grimaldi's morphological data sets mentioned above in combination with DNA sequences from five different genes. Four representatives of subfamily Steganinae are used as outgroup taxa. We examine the results in light of both previous morphological and molecular hypotheses of the relationships among these flies. We find that portions of our cladogram agree with certain aspects of these studies but also contain some surprising differences. The data corroborate the monophyly of the Hawaiian Drosophilidae as hypothesized by numerous investigators, but we also conclude that the genus *Drosophila* is essentially useless if we are to use it to designate any biologically meaningful clade.
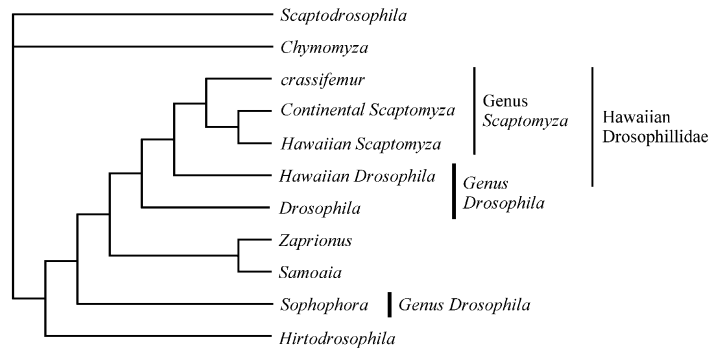
2. Materials and methods

2.1. Specimens

DNA used for sequencing was extracted from drosophilids contained in the American Museum of
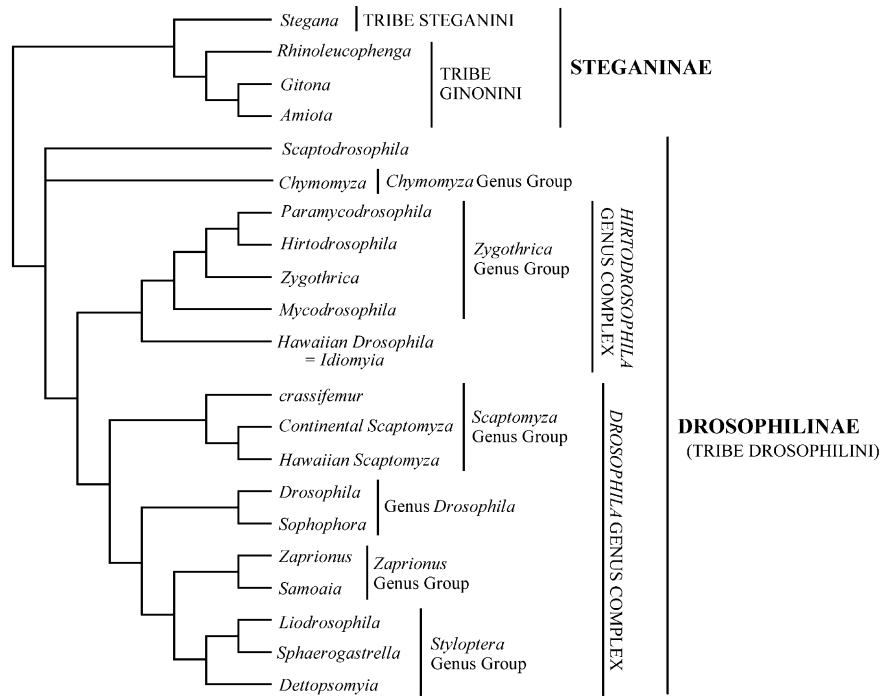
Fig. 1. Summary of results from several systematic studies of the family Drosophilidae. The upper cladogram summarizes Grimaldi's (1990) monograph based on morphological characters and the lower cladogram summarizes several molecular studies based on DNA sequence analysis.

Natural History's (AMNH) frozen collection, from field caught individuals, or from live cultures obtained from the National *Drosophila* Species Resource Center (Tucson, AZ). Some live cultures are maintained at 25 °C at AMNH. Appendix A lists the species collected during the course of this study, along with the relevant collection information. Vouchers of all field collected material are available in the collections of the AMNH.

2.2. Data matrix

Appendix B contains information on how the different partitions were assembled into a single data matrix. Since different researchers have used different exemplar taxa to represent the same higher-level taxonomic groups (species groups or genera), it was sometimes necessary for us to use placeholder taxa. For example, Grimaldi (1990a) selected *Stegana acutangula* to repre-

sent the genus *Stegana* in his study. We were only able to obtain *Stegana flavimana* for sequencing, however. To increase taxon sampling and reduce the amount of missing data, we are equating the three *Stegana* representatives, even though they are clearly not a single species. Of course, this approach assumes that these taxa are more closely related to one another than they are to the other species in this study. We feel that this is justified given (1) the vast amount of data supporting the monophyly of the various species groups and genera we have examined, (2) that the goal of this study is a higher-level phylogeny, not species-level questions, and (3) such an approach has been employed in other studies (e.g., Gatesy, 1997).

2.3. Grimaldi's data

Morphological characters used in this study were those of Grimaldi (1990a). To present a cladistic hypothesis of phylogenetic relationships in Drosophilidae based on morphological data, we reanalyzed Grimaldi's data matrix. When Grimaldi analyzed his matrix, he excluded several taxa because of constraints with computer power. Grimaldi had originally included a number of ordered characters but after an initial search, he arbitrarily defined a number of these as most homoplasious and unordered some, but not all, of them (Grimaldi, 1990a, 1999). Grimaldi's search strategy was as follows: (1) The m*h;bb algorithm of Hennig86 (Farris, 1988) was employed. The number of random addition replicates performed was not specified. (2) Because of the large numbers of taxa analyzed, only 100 equal length trees were saved for evaluation. (3) Successive weighting was used to reduce this number. The number of iterations of successive weighting used or the number of resultant trees obtained was not specified. (4) Hand revisions were then made to the tree, linking taxa that shared apomorphies. A criterion for these revisions was not stated.

We show a cladogram in this study that is constructed from a subset of Grimaldi's large data set. Repeated PAUP* runs conducted on Grimaldi's full taxon sample did not go to completion, but yielded tens of thousands of equally parsimonious trees (data not shown). The consensus of such a number of trees would likely yield minimal resolution; thus, we present the tree constructed from the subset of the data here.

2.4. DNA manipulation

In the present study, we employ sequences from five genes. These are portions of the mitochondrial 16S ribosomal DNA (rDNA), the nuclear 28S rDNA, the nuclear Alcohol dehydrogenase (*Adh*) coding region, and most of the coding regions of the nuclear Superoxide dismutase (*Sod*) and the mitochondrial cyto-chrome oxidase subunit II (COII) genes. For the first four genes, the primers used in PCR amplification were the same as those employed in Remsen and DeSalle (1998). For COII, the primers used were from Beckenbach et al. (1993) with modifications after O'Grady et al. (1998).

2.5. Amplifications were performed in Perkin–Elmer DNA thermal cyclers

The following cycling parameters were used: denaturation at 94 °C for 1 min, annealing at 45–60 °C for 1 min, and extension at 72 °C for 2.5 min. Either 25 or 30 cycles of amplification served well for all fragments we amplified. Wherever possible, we have sequenced PCR-amplified DNA fragments directly. However, in some cases, the only way to obtain sequence of high quality was by cloning the amplified fragments. For this, we used the TA Cloning Kit (Invitrogen) according to the manufacturer's instructions. Three to five clones from each reaction were sequenced to reduce errors which may be incorporated during cloning. Sequencing reactions for automated sequencing were prepared with the ABI Prism DNA Sequencing Kit (Perkin–Elmer), and sequencing reactions were run on an ABI Model 377 Automated DNA Sequencer in the AMNH Molecular Systematics Laboratory. DNA sequences generated for this study are being deposited in the GenBank database under the following accession numbers: for mitochondrial sequences, AF479792-479812; for 28S (domain 1), AY081358-081379; for 28S (domain 2), AY081380-AY081393; for 28S (domain 3), AY081403-AY081432; for *Adh*, AY081438-081443; for COII, AF478412-478440; and for *Sod*, AY081433-AY081437.

2.6. Phylogenetic analysis

The number of taxa for which character data were available varied for each of our six data partitions. Table 1 lists the partitions and the number of taxa that were coded for each partition. Taxa for which no character data were available were coding as missing for that partition.

Sequences for phylogenetic analysis were aligned using the Clustal alignment algorithm contained in Megalign Version 1.02 by DNASTAR with refinements of

Table 1
Number of taxa coded (out of 45) for each data partition in this study

Partition	Number of taxa
1. Grimaldi's morphology	33
2. 16S rDNA	45
3. 28S rDNA	45
4. *Adh*	26
5. *Sod*	24
6. COII	37

the resulting alignments made by eye. These refinements consisted solely of correcting obvious alignment anomalies introduced by Clustal; for example, where identical segments of sequence failed to align due to adjacent gaps. Aligned sequences were imported into PAUP* (Phylogenetic Analysis Using Parsimony Version 4.0b8; Swofford, 2001) and heuristic searches were performed using 100 replicates of random addition of taxa. All characters were weighted equally.

Decay indices and partitioned Bremer support (PBS; Baker and DeSalle, 1997) values for all nodes of the combined analysis tree were calculated using PAUP*, following the methods described in the references cited.

2.7. Incongruence length difference (partition homogeneity) tests

To test for congruence between and among data partitions, we used 200 replicates of the partition homogeneity test (PHT) (essentially the ILD test of Farris et al., 1994, 1995) as contained in PAUP* Version 4.0b8 (Swofford, 2001).

3. Results

3.1. Phylogenetic hypothesis

In Fig. 2, we present the strict consensus of two equally parsimonious trees that result from 100 random heuristic searches of PAUP* performed upon our complete data set. The node numbers indicated in Fig. 2 (in small print to the right of each node) are those referred to in Table 2. The length of the tree is 5759 steps; it has a consistency index of 0.399 and a retention index of 0.357. Decay indices are indicated above each branch point and bootstrap values (where these exceed 50%) below each branch point. The tree has been rooted with subfamily Steganinae (represented by *Amiota*, *Stegana*, *Rhinoleucophenga*, and *Gitona*) as the outgroup. A basal clade of *Liodrosophila/Sphaerogastrella/Dettopsomyia* that appears here is also supported by Grimaldi's analysis (Grimaldi, 1990a).

The genus *Drosophila* is not monophyletic in the present analysis. Not surprisingly, *Scaptodrosophila* falls outside the genus *Drosophila*. This placement is similar to that obtained in Grimaldi's monograph (Grimaldi, 1990a), and prompted him to elevate *Scaptodrosophila* to full generic status. Interestingly, however, the subgenus *Sophophora* also appears to be well outside what is typically considered to be the genus *Drosophila*. Our representatives of this subgenus (the *melanogaster*, *obscura*, *willistoni*, and *saltans* species groups) appear as a monophyletic assemblage with rather weak support (decay index = 2).

The Hawaiian Drosophilidae is monophyletic in our study, as they are in Remsen and DeSalle (1998). Their

sister groups are an assemblage of taxa including the *virilis* and *repleta* species groups, the subgenus *Siphlodora*, and others. In the Remsen and DeSalle study, four of the groups in this assemblage (the *virilis*, *repleta*, *robusta*, and *melanica* groups) formed a clade that appeared as sister to the Hawaiian taxa. The other clades included in the present assemblage were not sampled in the earlier study.

Grimaldi proposed that the *Zygothrica* Genus Group was the sister clade of the Hawaiian Drosophilidae (Grimaldi, 1990a). In the present study, these genera (represented by *Hirtodrosophila*, *Mycodrosophila*, and *Paramycodrosophila*) do not appear in this relationship with the Hawaiians. Rather, they appear with *Samoaia* in a clade that is sister to a large assemblage of other species groups. The relationship of *Samoaia* with these mostly Neotropical genera is somewhat perplexing. However, several representatives of the *Zygothrica* Genus Group are also endemic to Samoa (Grimaldi, 1990b; Wheeler and Kambysellis, 1966).

3.2. Partitioned bremer support

It can be useful, when evaluating the strength of nodes in a phylogenetic tree, to know how each of the data sets used in constructing that tree are contributing to the support for each node. We have used the partitioned Bremer support (PBS; Baker and DeSalle, 1997) as a measure of assessing the relative contributions of the six data partitions to the combined analysis tree seen in Fig. 2. The PBS contributed by each partition for each node is shown in Table 2, and the node numbers shown in this table correspond to those given in Fig. 2. Positive PBS values indicate that the partition lends support to a given node, negative values indicate that the partition lends conflict to a given node, and PBS values of zero indicate that the partition in question lends neither support nor conflict to a given node.

Two data partitions (*Sod* and COII) have PBS values that sum to a negative total. What is of interest, however, is not merely that these totals are negative, but how the negative PBS values are distributed on the combined analysis tree. Are they confined to a few nodes, or many? An examination of Table 2 shows that negative values are scattered widely throughout the tree, suggesting that some of these data may be strongly favoring an alternative tree topology. The negative value of the *Sod* partition may be caused by missing sequences from a number of taxa for that partition (although other partitions with missing sequences, like *Adh*, appear to be lending strong support to the combined analysis tree). The COII gene is a rapidly evolving mitochondrial locus, perhaps not the best suited for species group-level analysis, and this may be the main factor contributing to its negative PBS value.

PBS analysis suggests that Adh, 16S rDNA, and Grimaldi's morphological partitions lend the most support to our phylogenetic tree. Each of these parti-
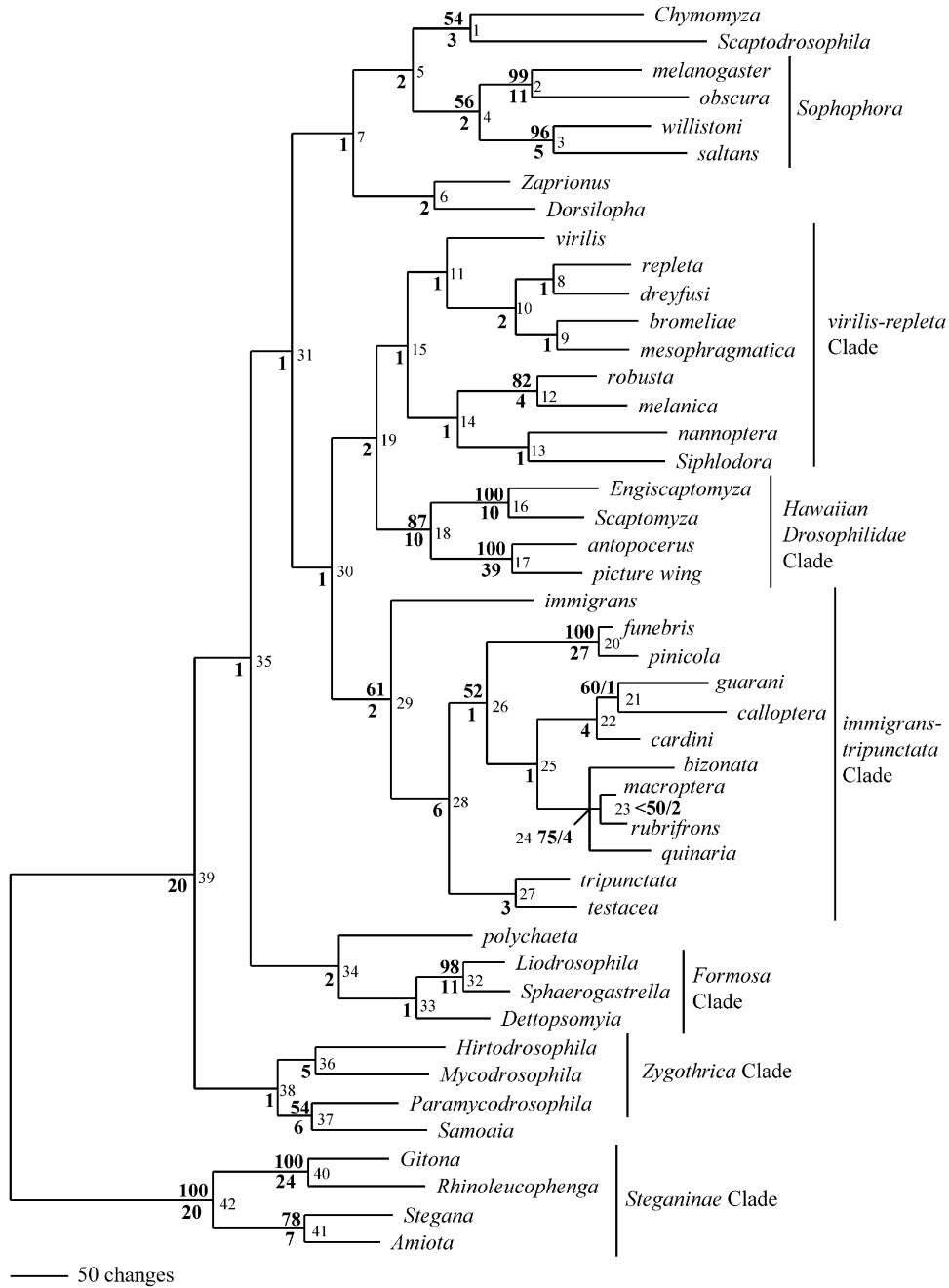
Fig. 2. Strict consensus of two equally parsimonious trees obtained when all data in this study are analyzed simultaneously. The node numbers (in small print to the right of each node) are those referred to in Table 2. Bootstrap values (where >50%) are indicated above each node and Bremer support values below each node.

tions, when analyzed separately, yields multiple equally parsimonious trees. The consensus of these trees is shown in Fig. 3 for Grimaldi's data, in Fig. 4 for the 16S data, and in Fig. 5 for the *Adh* data. Examined individually, these partition trees have few, if any, clades in common. (For example, Hawaiian drosophilids are monophyletic only in the *Adh* partition tree). However, combined with the other data, their influence on the final tree topology is considerable.

3.3. Congruence analyses

Our PHT test results (Tables 3 and 4) reveal some heterogeneity between and among our data partitions. As in Remsen and DeSalle (1998), we have chosen the phylogenetic tree generated from the combination of all our partitions as the best estimate of phylogeny we can offer at this time, as the molecular and morphological data partitions are not revealed to be signifi-

Table 2
Partitioned Bremer support for all nodes in the total evidence tree (Fig. 2)

Node	Partitions[a]						
	A	B	C	D	E	F	G
1	−3	12	1	2	−5	−4	3
2	−2.5	10.5	2.5	5.5	−2	−3	11
3	9	2	0	10	−6	−10	5
4	1.4	−0.5	4.6	4.2	−4.5	−3.2	2
5	1	0	4.4	4.3	−4.6	−3.1	2
6	0.85	0.54	2.85	5.85	−4.31	−3.77	2
7	2.3	0.83	−1.8	7.4	−3.7	−4	1
8	3	0	−2	8	−4	−4	1
9	5	3	−4	2	−6	1	1
10	2.3	−1.1	3.2	5.4	−5.2	−2.6	2
11	2	1	−1	6	−3	−4	1
12	2	12.5	2.5	1	−4	−10	4
13	2.7	1.1	−2.1	6.6	−4	−3.3	1
14	2.7	1.1	−2.1	6.6	−4	−3.3	1
15	3.4	1	−2.8	6.8	−4.4	−3	1
16	5	−0.25	8	4.25	−4.5	−2.5	10
17	8	2	7	18	−2	6	39
18	2.25	−2.75	1	13.75	−1.75	−2.5	10
19	3	3	−13	10	−6	5	2
20	1.5	22.8	11.5	9.7	−4.5	−14	27
21	2.33	0.83	−1.83	7.34	−3.67	−4	1
22	5.75	0.75	3.63	5.25	−5.38	−6	4
23	5	1	−1	2	−6	1	2
24	0.67	11.3	0	4.36	−0.33	−12	4
25	3.5	−1.25	−0.25	3.75	−5	0.25	1
26	2.33	0.83	−1.83	7.33	−3.67	−4	1
27	2	2.17	1.83	9	−4.5	−7.5	3
28	6.86	4.43	8.71	−15.28	−4.29	5.57	6
29	5	−5.5	7	4	−4.5	−4	2
30	2.8	0	−1.2	5.9	−4.2	−2.3	1
31	3.5	−1.25	−0.25	3.75	−5	0.25	1
32	1.67	21.5	−9.83	6	−5.17	−3.17	11
33	2.6	0.8	−2.2	7.6	−3.8	−4	1
34	1.86	1.14	−3.29	8.86	−4.43	−2.14	2
35	5	3	−4	2	−6	1	1
36	−4	2	−2	−3	0	12	5
37	0	−3.2	12.3	3	−2.31	−3.8	6
38	3.5	−1.25	−0.25	3.75	−5	0.25	1
39	18	8.3	−5.2	3.5	−4.8	0.2	20
40	12	6	9	2	−6	1	24
41	2	3	2	0	0	0	7
42	18	7	1	2	−6	−2	20
Total	152	130	32.09	210.5	−173.5	−101.7	250

[a] A = Grimaldi's morphology, B = 16S, C = 28S, D = *Adh*, E = *Sod*, F = COII, G = Total Bremer support.

cantly heterogeneous ($p = 0.340$; Table 4). The same is true of the mtDNA and morphological partitions ($p = 0.860$; Table 4). However, heterogeneity does exist in any PHT test in Table 4 in which nuclear gene partitions are included.

A series of PHT tests performed on all pairs of data partitions (Table 3) suggests that the likely sources of this heterogeneity are the *Adh* and *28S* data partitions. *Sod* (also a nuclear gene) produces nonsignificant *p* values when tested against any of the other individual partitions in the data matrix; *Adh*

produces significant *p* values when tested against the 16S and 28S rDNA data partitions ($p = 0.005$ for both of these tests). The 28S partition shows significant conflict with Grimaldi's morphological data ($p = 0.005$). These results are particularly interesting when one considers the significant support the *Adh* and Grimaldi partitions confer on the combined analysis tree.

4. Discussion

4.1. The present phylogenetic hypothesis: is it surprising?

In some ways, our combined analysis tree displays results that are difficult to reconcile with other phylogenetic hypotheses (e.g., Grimaldi, 1990a). However, in other cases it concurs quite strongly with previously published results. There is support for *Sophophora* being a representative of a radiation basal to most of the other *Drosophila* (Throckmorton, 1975), the *immigrans–tripunctata* and *virilis–repleta* radiations within the subgenus *Drosophila* (Throckmorton, 1975), and the monophyly of the Hawaiian Drosophilidae (Baker and DeSalle, 1997; DeSalle, 1992; Remsen and DeSalle, 1998; Russo et al., 1995; Thomas and Hunt, 1991).

Perhaps the most interesting grouping is that of *Samoaia* with several mycophagous genera of drosophilids. However, considering the low Bremer support for this node, this relationship should probably best be viewed with caution, as further collection of character data may overturn this relationship.

Scaptodrosophila is placed basal to the genus *Drosophila* in our tree. This is in agreement with previous molecular and morphological results (DeSalle, 1992; Grimaldi, 1990a), in which *Scaptodrosophila* was removed from *Drosophila* and elevated to generic status.

Several aspects of the tree topology are similar to that observed in the combined analysis tree presented in Remsen and DeSalle (1998). The genus *Drosophila* is not monophyletic in either cladogram. The subgenus *Sophophora* appears outside the remainder of genus *Drosophila*; in addition, the Hawaiian taxa are embedded within subgenus *Drosophila*. We do not observe a sister-group relationship between the Hawaiian Drosophilidae and the *Zygothrica* Genus Group, as Grimaldi (1990a) did. However, we do observe monophyly of the Hawaiian Drosophilidae.

Our partitioned Bremer support analyses have implications for the importance of including data from widely disparate sources in a phylogenetic analysis of this type. For example, Grimaldi's morphological data, the 16S rDNA and *Adh* sequences, when analyzed individually, do not produce cladograms that
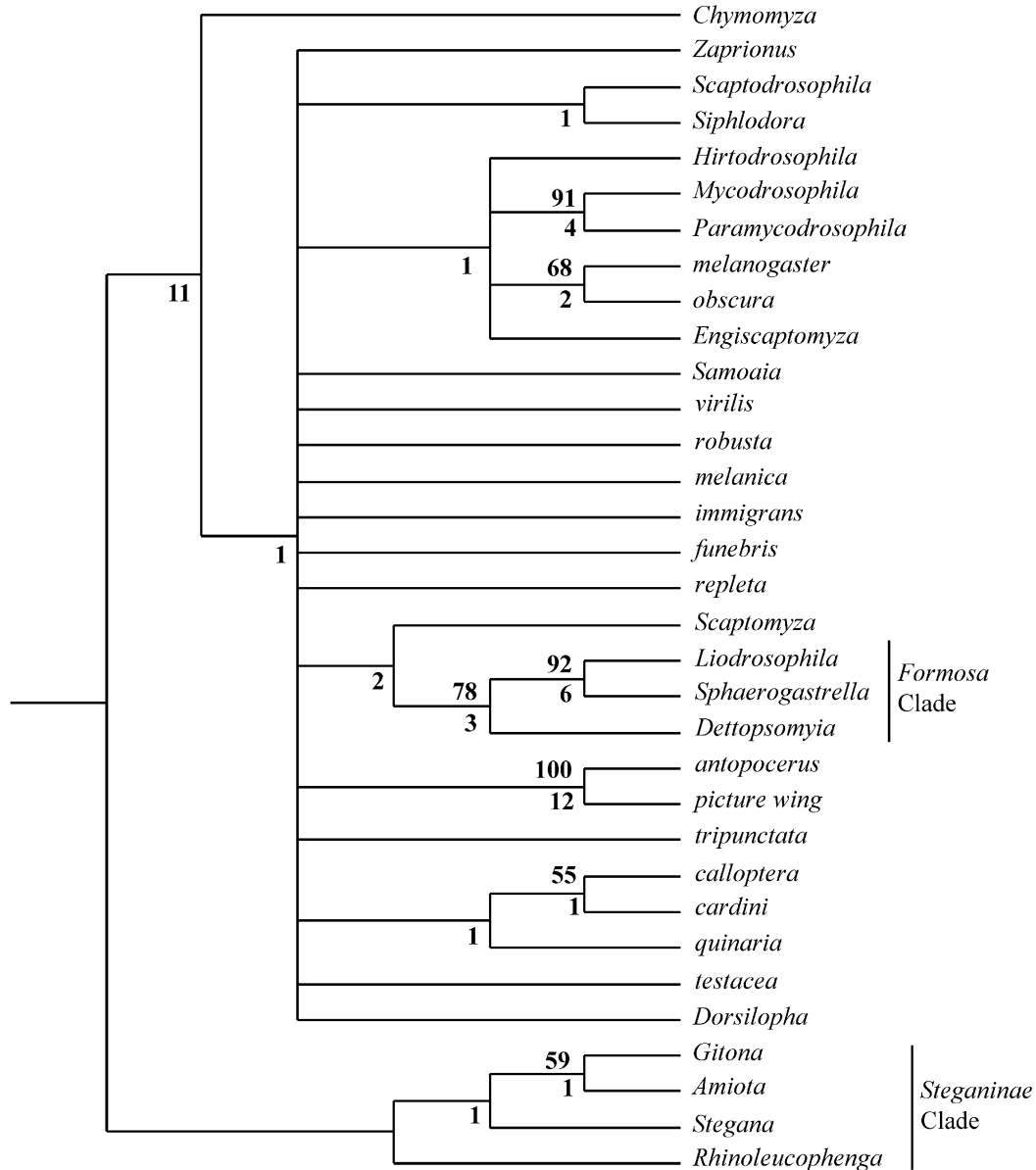
Fig. 3. The strict consensus of 49 equally parsimonious trees obtained when Grimaldi (1990a) morphological data are analyzed for taxa contained in the present study. The trees are length 270, *CI* = 0.462, *RI* = 0.626. Bootstrap and Bremer support values are given as in Fig. 2.

support many of the groupings apparent in our combined analysis tree. (Compare the combined analysis tree in Fig. 2 with the individual partition trees for those three data partitions in Figs. 3–5.) However, the sum of the PBS values for these partitions (Table 2) reveals that they are the largest contributors of support to the combined analysis. It appears that there is some phylogenetic signal not present in the individual analyses that emerges only in the simultaneous analysis of all the data.

In the case of the morphological data, the rather strong support lent to our combined analysis by Grimaldi's data is significant, since our tree differs drastically from Grimaldi's at several points. These data provide the second highest amount of support to our combined analysis hypothesis, but only account for seven percent of our total number of characters. This illustrates that even in data sets, which include large numbers of molecular characters, the addition of morphological data may impart valuable information which would not be available otherwise, and that contrary to some theorists, molecular data may not always "swamp" the phylogenetic signal from smaller numbers of morphological characters.
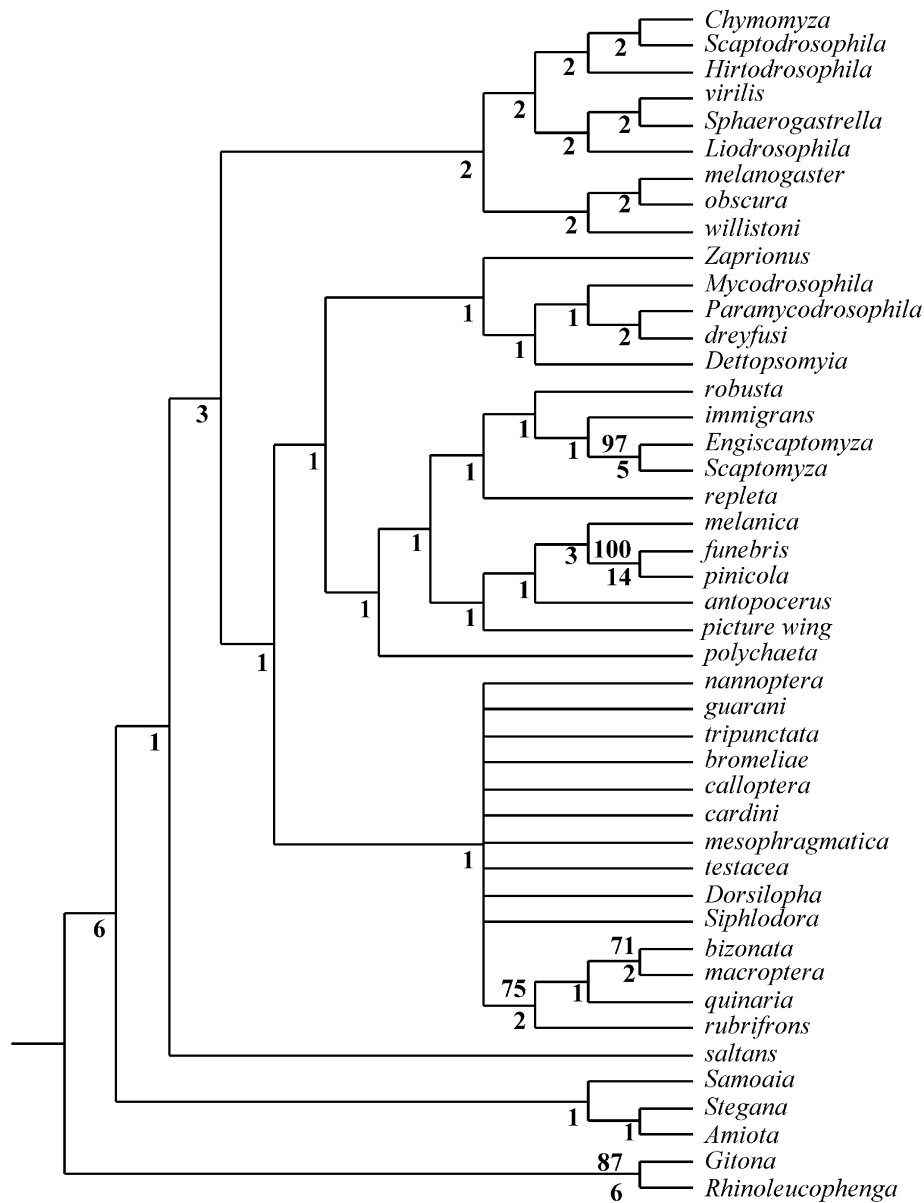
Fig. 4. The strict consensus of four equally parsimonious trees obtained when 16S rDNA sequence data are analyzed for the taxa contained in this study. The trees are length 1007, *CI* = 0.382, *RI* = 0.405. Bootstrap and Bremer support values are given as in Fig. 2.

4.2. Drosophilinae clades defined by our phylogenetic analysis

Although the utility of the genus *Drosophila*, as currently defined, is called into question by our combined analysis cladogram, a number of clades may be readily identified on our tree. These groupings include the following:

(1) The Steganinae Clade as includes the most recent common ancestor of *Gitona, Rhinoleucophenga, Stegana, Amiota*, and all of their descendants. Although our sampling strategy adequately represents the diversity

within Steganinae, additional sampling will be required before we can propose groupings within this clade.

(2) The three taxa we examined from Grimaldi's *Styloptera* Genus Group are also monophyletic (Grimaldi, 1990a). However, we are not using the name "Styloptera" for two reasons. First, Grimaldi's concept of this "genus group" includes several genera in addition to the exemplars in this study. One could imagine that sampling additional taxa might support a clade which includes the same genera as the *Styloptera* Genus Group (sensu Grimaldi, 1990a), but which has a most recent common ancestor different from that of the *Det-*
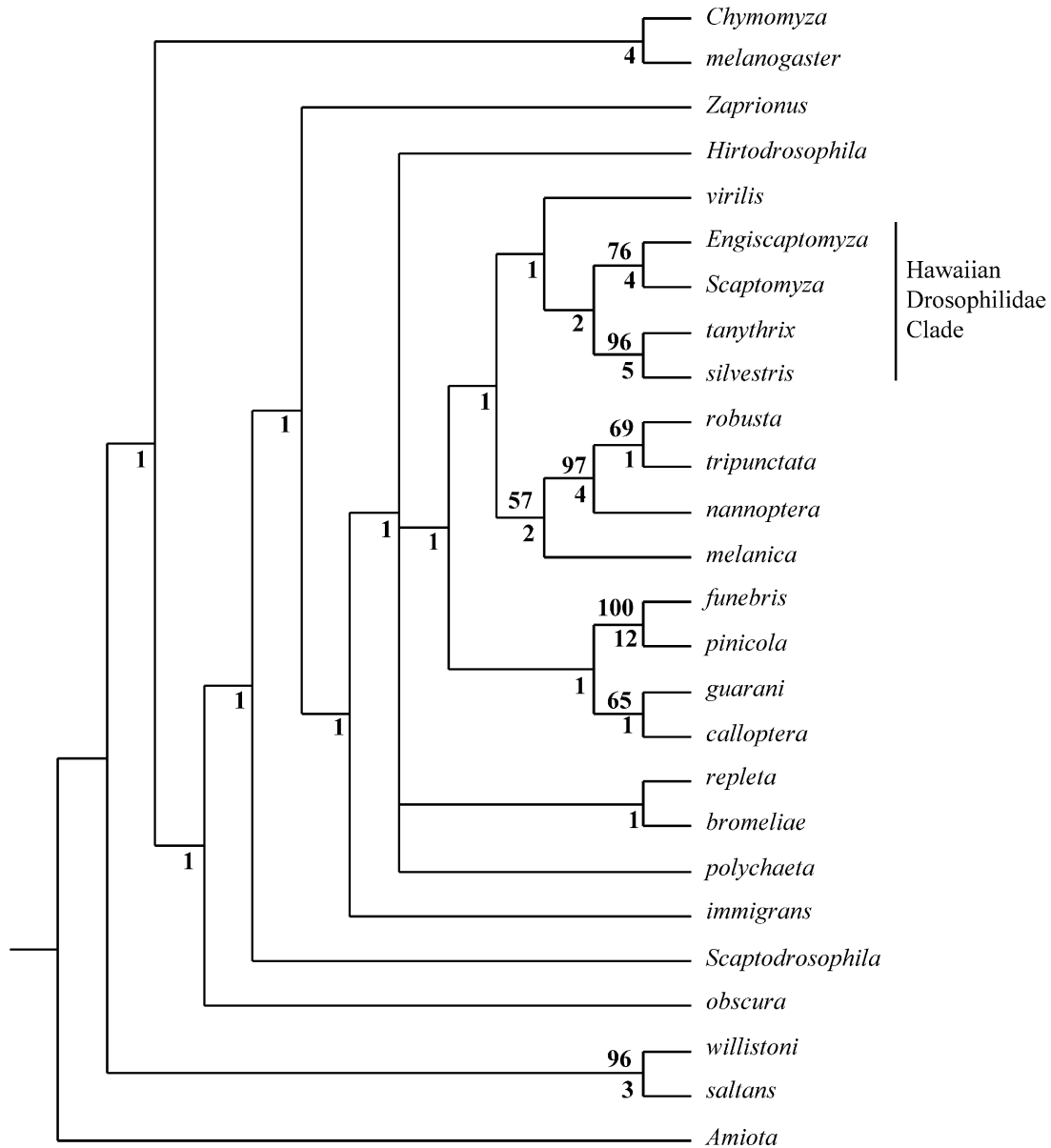
Fig. 5. The strict consensus of two equally parsimonious trees obtained when *Adh* sequence data are analyzed for the taxa contained in this study. The trees are length 827, *CI* = 0.424, *RI* = 0.491. Bootstrap and Bremer support values are given as in Fig. 2.

topsomyia–Liodrosophila–Sphaerogastrella clade. This would only lead to confusion concerning the taxa included within this clade. Second, the name "Styloptera" would best be used to refer to a monophyletic genus *Styloptera*, rather than a more inclusive group. This follows the proposal that, given several possible names

Table 3
p Values resulting from ILD tests between data partitions used in this study

	Grim	16S	28S	*Adh*	*Sod*	COII
Grim	—	0.015*	0.005*	0.415	0.795	0.805
16S		—	0.005*	0.005*	0.550	0.015*
28S			—	0.005*	0.975	0.005*
Adh				—	0.065	0.550
Sod					—	1.00
COII						—

* Significant.

Table 4
p Values resulting from ILD tests among data partitions used in this study

Comparison	*p* Value
1. All partitions	0.005*
2. Nuclear DNA vs. morphology	0.035*
3. Morphology vs. molecules	0.340
4. Mitochondrial DNA vs. morphology	0.860
5. Mitochondrial vs. nuclear DNA	0.005*

* Significant.

for a clade, one of lowest taxonomic rank should often be chosen (Bryant, 1996). We are defining the Formosa Clade as containing the most recent common ancestor of *Dettopsomyia*, *Liodrosophila*, *Sphaerogastrella*, and all of their descendants. This name is based on the name of the oldest type in this clade, *Dettopsomyia formosa*.

(3) There is increasing evidence to suggest that the subgenus *Sophophora* is quite distinct from the rest of the genus *Drosophila*. Some studies have supported the notion that this group is the sister of the subgenus *Drosophila* (DeSalle, 1992; Grimaldi, 1990a). However, as additional taxa have been added to the phylogeny, the placement of this taxon has changed significantly. Recent analyses (Remsen and DeSalle, 1998) indicate that this group is actually quite basal within the Drosophilidae. Interestingly, Throckmorton (1975) also placed this group in the "Sophophoran radiation," basal to many other groups in *Drosophila*. *Sophophora* is here defined as the most recent common ancestor of *melanogaster*, *obscura*, *saltans*, and *willistoni* and all of their descendants; the monophyly of these subordinate clades has been discussed elsewhere (Gleason et al., 1997; O'Grady et al., 1998; O'Grady, 1999). Three species groups placed within *Sophophora* by taxonomists (*dispar*, *fima*, and *dentissima*—Burla, 1954; Mather, 1954; Tsacas, 1979, 1980) have not been sampled or tested for monophyly. Additional work needs to be done on these to ascertain their placement.

(4) Several of the groups Throckmorton (1975) recognized, including the *immigrans–tripunctata* and *virilis–repleta* "radiations," are monophyletic in this study. We are defining the *immigrans–tripunctata* clade as the most recent common ancestor of the *immigrans* and *tripunctata* species groups and all of their descendants. The *virilis–repleta* clade includes the common ancestor of *virilis* and *repleta* and all of their descendants.

(5) The Hawaiian Drosophilidae forms a monophyletic group in this study. This clade is defined as the most recent common ancestor of *Scaptomyza*, the various *Drosophila* species groups endemic to Hawaii, and all their descendants. Throckmorton recognized two main lineages within the Hawaiian Drosophilidae, the Hawaiian *Drosophila* and *Scaptomyza* (Throckmorton, 1975). These are supported by several phylogenetic analyses (Baker and DeSalle, 1997; DeSalle, 1992; Kambysellis et al., 1995; Thomas and Hunt, 1991; this study). Additional clades within the Hawaiian Drosophilidae could be named, but it is best to wait until more taxonomically inclusive studies can be done. Grimaldi's (1990a) placement of the Hawaiian *Drosophila* lineage is not supported in the present study and should be considered questionable given his analytical methodology (see section on Grimaldi's data above).

(6) Grimaldi (1990a) proposed the *Zygothrica* Genus Group to include several mycophagous Neotropical genera, including *Hirtodrosophila*, *Mycodrosophila*, and *Paramycodrosophila*. Our analyses suggest that *Samoaia* is more closely related to this group than previously believed. This is perhaps not completely surprising given the distribution of some *Zygothrica* Genus Group species (Grimaldi, 1990b). However, we are not proposing to include *Samoaia* here or proposing a name for this clade until further studies with more extensive taxon sampling can be done. We do propose the Carinata clade to include the most recent common ancestor of *Hirtodrosophila* and *Mycodrosophila* and all their descendants, based on the relationships proposed in this study and others (Grimaldi, 1987, 1990a, 1990b).

5. Conclusion

In this study, we have presented a combined analysis phylogeny of 41 taxa of Drosophilinae based on data from six different partitions. We have shown support for the monophyly of the Hawaiian Drosophilidae and the subgenus *Sophophora*. However, both the genus *Drosophila* and subgenus *Drosophila* are not monophyletic according to the present phylogenetic tree.

Morphological data taken from Grimaldi's monograph (Grimaldi, 1990a), as well as sequences from the 16S rDNA of mitochondria and the *Adh* nuclear gene, lend much support to our tree's topology, as revealed by PBS analysis. Analysis of these data partitions alone yields partition trees that are topologically different from our combined analysis tree and having few if any nodes in common. This illustrates the importance of exploring data by simultaneous analysis as well as separate analysis. Interesting results from simultaneous analyses can be produced even if the constituent data partitions display heterogeneity, as is evident here from use of the PHT test.

Portions of our tree do not contain high Bremer support values. Thus, our cladogram should be viewed as a preliminary venture into constructing larger-scale Drosophilinae phylogenies with multiple data partitions, and thus may be strengthened or overturned by the addition of more character data.

However, we can conclude from our results that the taxonomic definition of the genus *Drosophila* is so broad and inclusive that its utility should be called into question. Strictly applied, this "genus" contains fully half of the 3500 species in the family Drosophilidae. If our results are supported by further investigations, taxonomic revisions may be necessary to reflect more correctly the evolutionary history of this group.

Acknowledgments

Appendix A. Collection information for some species used in this study

Species	Collection Information
Amiota picta	9♂, Oversite Canyon, Huachuca Mountains, AZ; 15.v.1995; O'Grady
Gitona bivisualis	29♂♀, Empire-Cienega Riparian Reserve, Sonoita, AZ; 10-11.xi.1996; O1.3, O'Grady
Rhinoleucophenga obsea	Chamela, Mexico; W. Etges & G. Huckins
Stegana flavimana	1♂, Jatun Sacha Biological Station, Tena, Ecuador; 5.viii.1997, O28.12, O'Grady
Dettopsomyia nigrovittata	M. Watada Collection
Drosophila (Dorsilopha)	
D. busckii	24♂♀, San Quintin, Baja California Norte, Mexico; xi.1995; O'Grady
Drosophila (Drosophila)	
D. bizonata	M. Watada Collection
D. immigrans	20♂, Tucson, AZ; 12.ii.1995; O'Grady
D. macroptera	11♂, Heed Cabin, Mt. Lemmon, AZ 25.viii.1997; O29.3, Heed, Babcock, Dyreson, Silva, & O'Grady
D. falleni	23♂, Trenton Falls Gorge, Trenton Falls, NY; vii.1997; O'Grady
D. quinaria	3♂, Whitesboro, NY; 1.viii.1997; O'Grady
D. nannoptera	W. B. Heed Laboratory Stock
D. hydei	W. B. Heed Laboratory Stock
D. rubifrons	3♂, Mt. Bigelow, AZ; ix.1996; O'Grady 43♂♀, Heed Cabin, Mt. Lemmon, AZ; 25.viii.1997; O29.2, Heed, Babcock, Dyreson, Silva, & O'Grady
D. tripunctata	10♂, Athens, GA, iv.1994; O'Grady & Dyreson
Drosophila (Siphlodora)	
D. flexa	25♂♀, El Caracol, Sonora, Mexico 1-2.v.1997; O19.1&O20.8, Babcock, Silva, & O'Grady
Drosophila (Sophophora)	
D. obscura	M. Radak Collection
Mycodrosophila sp?	8♂, Mapledale Road, Barneveld, NY; vii.1997; O24.1, O'Grady
Paramycodrosophila sp?	2♂, Jatun Sacha Biological Station, Tena, Ecuador; 5.viii.1997; O28.3, O'Grady

Appendix B. Species used to represent terminal taxa in this study, with relevant literature citations

	morphology[1]	16S	28S	*Adh*	COII	*Sod*
Steganinae						
Amiota	*picta*	*picta*[2]	*picta*[2]		*picta*[2]	
Gitona	*sp?*	*bivisualis*[2]	*bivisualis*[2]		*bivisualis*[2]	
Rhinoleucophenga	*sp?*	*obesa*[2]	*obesa*[2]		*obesa*[2]	
Stegana	*sp?*	*flavimana*[2]	*flavimana*[2]			
Drosophilinae						
Chymomyza	*amoena*	*amoena*[3]	*bicolor*[4,5]	*amoena*[5]	*amoena*[2]	*amoena*[6]
Dettopsomyia	*formosa*	*nigrovittata*[2]	*nigrovittata*[2]			
Drosophila[a] (***Dorsilopha***)						
D. busckii[a]	*busckii*	*busckii*[2]	*busckii*[2]		*busckii*[2]	
Drosophila[a] (***Drosophila***)						
bizonata group		*bizonata*[2]	*bizonata*[2]		*bizonata*[2]	
bromeliae group		*bromeliae*[2]	*bromeliae*[2]	*bromeliae*[2]	*bromeliae*[2]	
calloptera group	*calloptera*	*ornatipennis*[2]	*ornatipennis*[2]	*ornatipennis*[2]	*ornatipennis*[2]	*ornatipennis*[2]
cardini group	*cardini*	*cardini*[2]	*cardini*[2]		*dunni*[2]	
dreyfusi group		*camargoi*[2]	*camargoi*[2]		*camargoi*[2]	
funebris group	*funebris*	*funebris*[3]	*funebris*[4,5]	*funebris*[5]	*funebris*[2]	*funebris*[5]
guarani group		*guarani*[3]	*guarani*[2,4]	*guarani*[2]	*griseolineata*[2]	
immigrans group	*immigrans*	*immigrans*[3]	*immigrans*[2,4]	*immigrans*[7]	*immigrans*[2]	*immigrans*[5]
macroptera group		*macroptera*[3]	*macroptera*[2]			
melanica group	*melanica*	*melanica*[3]	*melanica*[2,4]	*melanica*[5]	*micromelanica*[2]	*melanica*[5]
Mesophragmatica group		*guacha*[3]	*gaucha*[2,4]		*gaucha*[2]	
nannoptera group		*nannoptera*[3]	*nannoptera*[2]	*nannoptera*[2]	*nannoptera*[2]	*nannoptera*[2]
pinicola group		*pinicola*[3]	*pinicola*[5]	*pinicola*[5]		*pinicola*[5]
polychaeta group		*polychaeta*[3]	*polychaeta*[2,8]	*polychaeta*[2]	*polychaeta*[2]	
quniaria group	*quinaria*	*falleni*[2]	*falleni*[2]		*quniaria*[2]	*quniaria*[2]
repleta group	*repleta*	*mulleri*[3]	*hydei*[2,8]	*hydei*[9]	*hydei*[2]	*hydei*[10]
robusta group	*colorata*	*robusta*[3]	*robusta*[5]	*robusta*[5]		*robusta*[5]
rubifrons group		*rubifrons*[2]	*rubifrons*[2]			
testacea group	*testacea*	*putrida*[2]	*putrida*[2]		*putrida*[2]	*putrida*[2]
tripunctata group	*tripunctata*	*tripunctata*[2]	*mediopictoides*[8]			
tripunctata[2]	*tripunctata*[2]	*tripunctata*[2]				
virilis group	*virilis*	*virilis*[5]	*virilis*[5,8]	*americana*[11]		*virilis*[10]
Drosophila[a] (***Siphlodora***)						
Siphlodora	*flexa*	*flexa*[2]	*flexa*[2]		*flexa*[2]	*flexa*[2]
Drosophila[a] (***Sophophora***)						
melanogaster group	*melanogaster*	*melanogaster*[3]	*melanogaster*[4,5]	*melanogaster*[12]	*melanogaster*[13]	*teisieri*[6]
obscura group	*affinis*	*pseudoobscura*[3]	*pseudoobscura*[4,5]	*ambigua*[14]	*obscura*[2]	*pseudoobscura*[5]

Appendix B (*continued*)

	morphology[1]	16S	28S	*Adh*	COII	*Sod*
saltans group		*austrosaltans*[2]	*austrosaltans*[2]	*prosaltans*[15]	*prosaltans*[15]	*saltans*[6]
willistoni group		*willistoni*[3]	*willistoni*[4,5]	*willistoni*[16]	*willistoni*[2]	*willistoni*[6]
Hawaiian *Drosophila*						
antopocerus group	*adunca*	*tanythrix*[5]	*tanythrix*[5]	*tanythryx*[17]	*tanythrix*[2]	*tanythrix*[5]
picture wing group	*hanaulae*	*silvestris*[5]	*silvestris*[5]	*silvestris*[18]	*silvestris*[17]	*silvestris*[5]
Hirtodrosophila	*duncani*	*pictiventris*[3]	*pictiventris*[5]	*pictiventris*[5]	*pictiventris*[2]	*pictiventris*[6]
Liodrosophila	*onchopyga*	*aerea*[3]	*aerea*[2]		*aerea*[2]	
Mycodrosophila	*dimitada*	*sp?*[2]	*sp?*[2]		*sp?*[2]	
Paramycodrosophila	*sp?*	*sp?*[2]	*sp?*[2]		*sp?*[2]	
Samoaia	*ocellaris*	*leonensis*[2]	*leonensis*[2]		*leonensis*[2]	
Scaptodrosophila	*lurida*	*stonei*[3]	*lebanonensis*[5]	*lebanonensis*[19]	*brooksae*[2]	*lebanonensis*[6]
Scaptomyza						
crassifemur group	*crassifemur*	*crassifemur*[5]	*crassifemur*[5]	*crassifemur*[20]	*crassifemur*[2]	*crassifemur*[5]
other *Scaptomyza*	*albovittata*	*sp?*[5]	*pallida*[4,5]	*sp?*[5]	*sp?*[2]	*palmae*[5]
Sphaerogastrella		*sp?*[2]	*sp?*[2]			
Zaprionus	*vittiger*	*tuberculatus*[3]	*tuberculatus*[4,5]	*tuberculatus*[18]	*tuberculatus*[2]	*tuberculatus*[6]

[a] *Dorsilopha* is a monotypic subgenus. Source of characters.
[1] Grimaldi (1990b).
[2] This study.
[3] DeSalle (1992).
[4] (Pelandakis et al., 1991).
[5] Remsen and DeSalle (1998).
[6] Kwiatowski et al. (1994).
[7] Albalat et al. (1994).
[8] Pelandakis and Solignac (1993).
[9] Mennotti-Raymond et al. (1991).
[10] Kwiatowski et al. (1994).
[11] Nurminsky et al. (1996).
[12] Kreitman (1983).
[13] de Bruijn (1983).
[14] Marfany and Gonzalez-Duarte (1991).
[15] O'Grady et al. (1998).
[16] Anderson et al. (1993)
[17] Baker and DeSalle (1997).
[18] Thomas and Hunt (1993).
[19] Marfany and Gonzalez-Duarte (1993).
[20] Thomas and Hunt (1991).

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