



Short Communication

Polyphyly of the *Zaprionus* genus group (Diptera: Drosophilidae)

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ABSTRACT

The *Zaprionus* genus group comprises three drosophilid genera (*Zaprionus*, *Phorticella* and *Samoia*) that are thought to be related to the *Drosophila immigrans* species group. We revised the phylogenetic relationships among the three genera and their placement within the subfamily Drosophilinae using one mitochondrial (*COII*) and one nuclear (*Amyrel*) gene. The Bayesian tree inferred from concatenated amino acid sequences of the two genes strongly suggests the polyphyly of the *Zaprionus* genus group and of each of the genera *Zaprionus* and *Phorticella*. Paraphyly of the *D. immigrans* species group was also shown here; the *quadrilineata* subgroup formed the sister clade to the genus *Samoia*. These results suggest the necessity of taxonomic revisions for some relevant genera and species groups included within the genus *Drosophila*.

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1. Introduction

The drosophiline genera *Zaprionus* Coquillett and *Phorticella* Duda are characterized by the presence of silvery white longitudinal stripes on the frons and the mesonotum. These stripes have long been hypothesized to be homologous between the two genera so that *Phorticella* was once considered a subgenus of *Zaprionus* (Sturtevant, 1927). Wilson et al. (1969) considered both genera along with two others (*Chaetodrosophillela* Duda and *Samoia* Malloch) to be “fairly certainly related to the *immigrans*” species group of the genus *Drosophila*. Throckmorton (1975) suggested that “the entire cluster [i.e. the *immigrans* group and the allied genera] is, in reality, a close-knit lineage little justifying the nomenclatural industry expanded on it.” Okada and Carson (1983) apparently resolved the taxonomic confusions between *Zaprionus* and *Phorticella*, showing each to be a separate genus comprising two subgenera. Currently, the genus *Zaprionus* consists of 56 species, of which 46 belong to the Afrotropical subgenus *Zaprionus sensu stricto* and 10 to the Oriental-Australasian subgenus *Anapriponus* Okada. The genus *Phorticella* consists of 11 species, of which seven belong to the subgenus *Phorticella s.s.* and four to the subgenus *Xenophorticella*. All *Phorticella* species are found in the

Oriental-Australasian region, except *P. (X.) madagascariensis* Chasagnard & McEvey which is endemic to Madagascar. Okada and Carson (1983) suggested, however, *Phorticella s.s.* to be close to *Scaptodrosophila* Duda, a relatively distant genus from *Zaprionus* and *Drosophila s.s.*, in light of the morphology of male genitalia.

Grimaldi (1990) erected many genus groups to include drosophilid genera that were believed to be monophyletic in light of morphological characters. One of these groups was the *Zaprionus* genus group containing three genera that share a single synapomorphy: a broad facial carina that is extended to oral margin, prominent and rounded on edge. These genera were *Zaprionus* with its two subgenera, *Phorticella* and *Samoia*. *Zaprionus* and *Phorticella* were believed to be monophyletic in light of their bulbous clypeus, longitudinal stripes and epandrial setation. However, Grimaldi (1990) used, as a representative species for *Phorticella*, the species *P. argentostrata* (Bock), and for the subgenus *Anapriponus*, the species *Z. multistriatus* Sturtevant. Both species are junior synonyms of *Z. bogoriensis* Mainx (Wynn and Toda, 1988). Later molecular phylogenetic studies have failed to reconfirm the sister relationship of *Zaprionus* and *Samoia*, placing the latter closer, basally to the clade including the *Drosophila immigrans* group (Pélandakis and Solignac, 1993; Tatarenkov et al., 2001; Robe et al., 2005; O’Grady and DeSalle, 2008; Yassin et al., 2008). Furthermore, *Zaprionus* appears to be more related to the *D. repletoides* group [Yassin et al., 2008; cited as the *D. tumiditarsus* group: however, *D. tumiditarsus* Tan, Hsu & Sheng, 1949 was synonymized with *D. repletoides* Hsu, 1943 by Wheeler (1981)], a monotypic

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group of unclear phylogenetic position. The *D. immigrans* group has been shown to be polyphyletic with respect to other *Drosophila* s.s. groups and some drosophiline genera (Kato et al., 2007).

Unfortunately, none of the previous morphological or molecular revisions have included *Phorticella* species, because these species are rarely captured by standard *Drosophila* collection techniques and are difficult to culture in the laboratory (unlike *Zaprionus* s.s., some *Anaprionus*, *Samoaia* and many *Drosophila* species). Nothing is known about their life history traits or chromosomal configurations. The recent discovery of many *Anaprionus* and *Phorticella* species in Malaysia (Kondo and Toda, in prep.) and Taiwan (Prigent, in prep.), and the rare species *P. (X.) madagascariensis* Chassagnard & McEvey in Madagascar (Yassin and David, in prep.), has prompted us to conduct the present molecular phylogenetic revision of the genus group using DNA sequences of one mitochondrial (mtDNA) gene, cytochrome oxidase subunit 2 (*COII*) and one nuclear gene (*Amyrel*). The results strongly suggest the polyphyly of the *Zaprionus* genus group, as well as of both genera *Zaprionus* and *Phorticella*. Based on the results, we discuss some taxonomic and nomenclatural revisions that should be implemented.

2. Materials and methods

2.1. Sampled species

The list of the species used in this study is given in Table 1. Only exemplars of genera and groups relevant to this study were sampled in light of previous phylogenetic studies: i.e. all the genera and subgenera of the *Zaprionus* genus group, the genus *Scaptodrosophila*, *Drosophila melanogaster* Meigen (the type species of the subgenus *Sophophora* Sturtevant), *D. funebris* (Fabricius) (the type species of the genus *Drosophila*), *D. repletoides*, and four subgroups of the *D. immigrans* group. Only two species were sampled from the subgenus *Zaprionus*, as the phylogeny of its nearly 50 species has recently been revised using molecular and morphological characters (Yassin et al., 2008). The subgenus *Anaprionus* has usually been represented in previous molecular phylogenetic investigations (Pélandakis and Solignac, 1993; Da Lage et al., 2007) by two species: *Z. bogoriensis* and another species erroneously identified as

Z. lineosus (Walker). Yassin et al. (2008) considered the misidentified *Z. lineosus* to be a newly cryptic one but further morphological examination revealed that it was also *Z. bogoriensis*. All these taxa belong to the subfamily Drosophilinae, and thus the genus *Leucophenga* Mik of the subfamily Steganinae was taken as an outgroup.

2.2. Molecular phylogenetic analysis

DNA extraction, amplification of the two genes (*COII* and *Amyrel*) and sequencing were conducted using the same protocols as in Yassin et al. (2008). Nucleotide sequences were viewed and manually edited using MEGA ver. 4 program (Kumar et al., 2008). They were deposited in GenBank with accession numbers given in Table 1. Multiple alignments were performed using ClustalW (Thompson et al., 1994) under the MEGA default parameters. Stationarity of base composition was tested using the ENCprime software package (November, 2002). Because of the observed significant departure from stationarity in the *Amyrel* sequences, DNA of both genes was translated into amino acid sequences. Phylogenetic relationships were inferred using Bayesian analysis as implemented in MrBayes ver. 3.1.1 program (Ronquist and Huelsenbeck, 2003), under the JTT + Γ + F substitution model, as proposed by the ProtTest program (Abascal et al., 2005). Posterior probability was estimated as clade support for each internal node, after a run of 2,000,000 generations with a sample frequency of 100 generations and a burn-in period of 5000 generations.

3. Results

Fig. 1 shows the Bayesian phylogenetic tree of the sampled taxa, with posterior probability value at each internal node. The subgenus *Phorticella* appears as the earliest branching lineage, followed by the genus *Scaptodrosophila*, and then *D. melanogaster*, the sole representative of the subgenus *Sophophora* of the genus *Drosophila* in this study. The remaining taxa form a clade, *Drosophila* s.l., containing the subgenus *Drosophila* and its allied genera, i.e. *Phorticella*, *Samoaia* and *Zaprionus*. The clade includes the second subgenus

Table 1
List of taxa used in this study with GenBank accession numbers for the *COII* and *Amyrel* genes.

Subfamily	Genus	Subgenus	Group	Subgroup	Species	<i>COII</i>	<i>Amyrel</i>				
Drosophilinae	<i>Drosophila</i>	<i>Drosophila</i>	<i>funebris</i> <i>immigrans</i>	<i>funebris</i> <i>hypocausta</i> <i>immigrans</i> <i>nasuta</i> <i>quadrilineata</i>	<i>funebris</i>	EU390744	AF335557				
					<i>hypocausta</i>	—	AY733043				
					<i>neohypocausta</i>	EU493720	—				
					<i>immigrans</i>	AF478424	AF491632				
					<i>nasuta</i>	EU493719	AY733059				
					<i>quadrilineata</i>	GQ352273	—				
					<i>nigrilineata</i> -like	GQ352274	GQ352262				
					<i>tetravittata</i>	GQ352272	GQ352266				
					<i>repletoides</i> <i>melanogaster</i>	<i>Sophophora</i> <i>Phorticella</i>	<i>repletoides</i> <i>melanogaster</i>	<i>melanogaster</i>	<i>repletoides</i>	EU161098	AY736500
									<i>melanogaster</i>	U37541	AF022713
									<i>singularis</i>	GQ352267	GQ352256
									sp.7	GQ352269	—
									<i>madagascariensis</i>	GQ352270	—
									sp.K1	GQ352271	GQ352257
									<i>leonensis</i>	AF478438	EU161100
									<i>coracina</i>	—	GQ352263
									<i>latifasciaeformis</i>	AY847765	GQ352255
									<i>bogoriensis</i>	EF453720	AY736516
					<i>obscuricornis</i> <i>pyinoolwinensis</i> -like sp.K1	<i>Scaptodrosophila</i>	<i>obscuricornis</i> <i>pyinoolwinensis</i> -like sp.K1	<i>obscuricornis</i> <i>pyinoolwinensis</i> -like sp.K1	<i>obscuricornis</i>	GQ352276	GQ352259
<i>pyinoolwinensis</i> -like	GQ352277	GQ352260									
sp.K1	GQ352275	GQ352261									
<i>indianus</i>	EF453709	EF458322									
<i>tuberculatus</i>	EF453719	AY736524									
<i>maculata</i>	—	DQ021939									
<i>albofasciata</i>	EU493701	—									
Steganinae	<i>Leucophenga</i>			<i>maculata</i>	—	DQ021939					
				<i>proxima</i>	—	—					
					—	—					

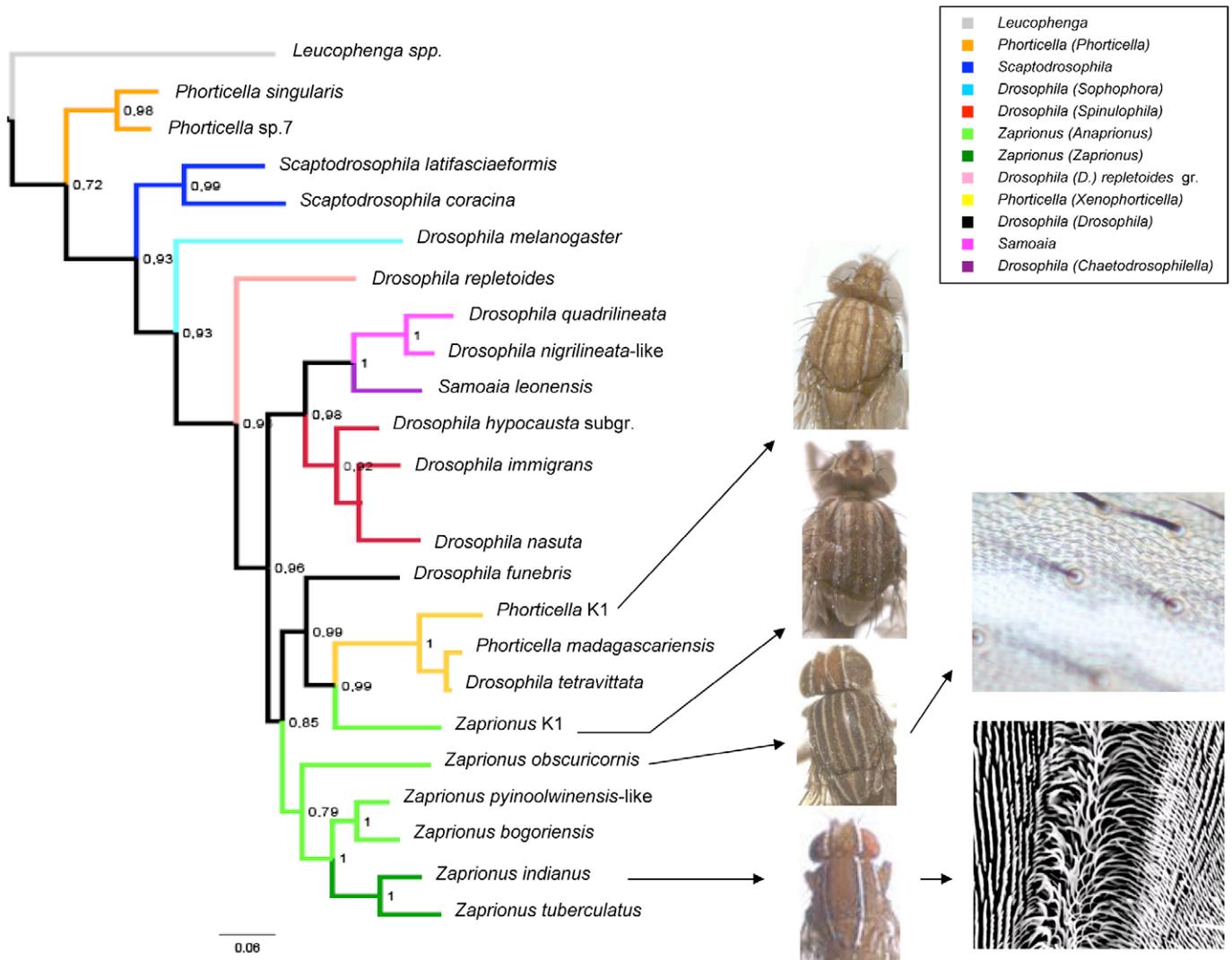


Fig. 1. Bayesian phylogenetic tree of the *Zaprionus* genus group inferred from concatenated amino acid sequences of *COII* and *Amyrel* genes. Posterior probability value is given beside each node. Generic and subgeneric nomina are shown in colors. For *Zaprionus* and *Xenophorticella* species, photomicrographs of patterns and fine structure of longitudinal stripes are given.

Xenophorticella of *Phorticella*, showing the polyphyly of the genus *Phorticella*.

Drosophila repletoides is the first group to have branched off the *Drosophila s.s.* clade. It is then followed by two sister clades. The first clade contains the *D. immigrans* species group and the genus *Samoia*. This clade is further subdivided into two clades, the first containing the *immigrans*, *hypocausta* and *nasuta* subgroups, and the second the *quadrilineata* subgroup and the genus *Samoia*. In sum, the *immigrans* group appears paraphyletic with respect to *Samoia*. The second clade contains *D. funebris*, the genus *Zaprionus* and the subgenus *Xenophorticella*. The subgenus *Xenophorticella* is monophyletic. Although Takada and Momma (1975) described *D. tetravittata* as a species of the *D. quadrilineata* subgroup of the *D. immigrans* group, its morphological characteristics strongly suggest that this species should be transferred to the subgenus *Xenophorticella* of *Phorticella*. The present molecular phylogenetic analysis supports this suggestion: *D. tetravittata* is placed within the clade of *Xenophorticella*. On the other hand, the genus *Zaprionus* is polyphyletic. A single new species of the subgenus *Anaprius*, *Z. sp.K1*, is placed as the sister to the *Xenophorticella* clade, and they together formed the sister clade to *D. funebris*. As the sister group to the clade comprising *Z. sp.K1*, *Xenophorticella* and *D. funebris*,

the remaining species of *Zaprionus* form a clade, within which species belonging to the subgenus *Anaprius* are paraphyletic with respect to the monophyletic subgenus *Zaprionus*.

4. Discussion

4.1. Taxonomic consequences of the polyphyly of the *Zaprionus* genus group

The *Zaprionus* genus group was erected to include three genera: *Zaprionus*, *Phorticella* and *Samoia* (Grimaldi, 1990). Both *Zaprionus* and *Phorticella* are characterized by the presence of silvery white stripes on the frons and the mesonotum, and each is subdivided into two subgenera. They have both a paleotropical distribution. The genus *Samoia* includes seven species that are all endemic to the islands of Samoa in the Pacific Ocean and its monophyly has been determined using polytene chromosomes (Ellison, 1968). In this paper we have revised the phylogenetic relationships between the three genera and their placement within the subfamily Drosophilinae using mitochondrial and nuclear markers. The major conclusion is that the genus group, as well as the genera *Phorticella* and *Zaprionus*, is polyphyletic. Consequently, the *Zaprionus* genus group

is no longer justified, and the genera *Phorticella* and *Zaprionus* should be taxonomically revised, along with their related taxa such as the *D. immigrans* species group and *D. reptoides*, in accordance with their phylogeny.

4.2. Evolution of longitudinal white stripes in the Drosophilinae

The results show that the presence of longitudinal white stripes has evolved independently in different lineages of the subfamily Drosophilinae. In the subgenus *Phorticella*, this pattern is the result of white (or a lack of) pigmentation. On the other hand, [Walt and Tobler \(1978\)](#) studied the fine structure of such stripes in the subgenus *Zaprionus* and showed that the white stripes consisted of long and protruding trichomes each bearing two grooves and surrounded by shorter trichomes with a prominent crest. The stripes of *Xenophorticella* resemble those of *Zaprionus s.s.*, whereas only crest-bearing short trichomes are found in *Anaprionus*. In light of the molecular phylogeny, we are inclined to believe there has been parallel evolution of the stripes with similar or identical fine structure in *Zaprionus s.s.* and *Xenophorticella*. Unlike in *D. melanogaster* ([Calleja et al., 2002](#)), the developmental genetic basis of mesonotal patterning has not yet been investigated in these taxa, though this could provide insight into the assessment of homology of their longitudinal stripes ([DeSalle and Grimaldi, 1994](#)).

4.3. Division of the genus *Phorticella*

The results clearly reveal that the genus *Phorticella* is polyphyletic, though the exact phylogenetic positioning in the subfamily Drosophilinae of its two monophyletic subgenera cannot be assessed here due to the small taxon sampling. As pointed out by [Okada and Carson \(1983\)](#), the subgenus *Phorticella* resembles some *Scaptodrosophila* species, such as the *subtilis*, *victoria* and *ruffrons* groups, in morphology, especially of the male genitalia. The phylogenetic position of *Phorticella s.s.* should be investigated, including related *Scaptodrosophila* species, in further studies based on molecular and morphological data. The subgenus *Xenophorticella* is included in the large, heterogeneous *Drosophila s.l.* clade. To solve the polyphyly of the genus *Phorticella*, *Xenophorticella* should be upgraded to the genus rank, but only after confirming the phylogenetic position of its type species, *Zaprionus flavipennis* Duda, which was not included in the present analysis.

4.4. Division of the genus *Zaprionus*

The genus *Zaprionus* has also been revealed to be polyphyletic: the studied species are divided into two groups, *Z. sp.K1* and the others. *Zaprionus sp.K1* is placed as the sister to the *Xenophorticella* clade. This species morphologically resembles some species of the subgenus *Anaprionus*, such as *Z. lineosus* (Walker) (the type species of *Anaprionus*), *Z. aungsani* Wynn & Toda, *Z. grandis* (Kikkawa & Peng), *Z. multistriatus* (Duda), *Z. orissaensis* (Gupta) and *Z. spinilineosus* Okada & Carson, for example in having two pairs of paramedian setae on the hypandrium and the unornamented tarsi of male forelegs; these characters are shared also by *Xenophorticella* species. Although all the above known species were not included in the present analysis, due to unavailability of their fresh specimens for DNA analysis, if further studies confirm that they form a clade together with *Z. sp.K1* as the sister to the *Xenophorticella* clade, *Anaprionus* should be upgraded to the genus rank or be transferred as a subgenus of the genus “*Xenophorticella*”.

The remaining species of the subgenus *Anaprionus* have also been divided into two groups, *Z. obscuricornis* (de Meijere) and the others. *Zaprionus obscuricornis* is certainly the most morphologically diverged species in the genus. Among its most remarkable characters, is the presence of a few bristles on the anepisternum.

The absence of these bristles is a diagnostic character of the family Drosophilidae. Two other exceptions for the presence of anepisternal setation also occur in the subfamily Drosophilinae: *Scaptodrosophila merdae* (Bock) and *Mycodrosophila heterothrix* (McEvey & Bock), but no material of these species was available to us for DNA analysis. The high wing costal index in *Z. obscuricornis* is another particular characteristic (reaching 5.6, although it usually ranges between 2.7 and 3.4 in other *Zaprionus* species). The male genitalia (especially the surstyli) are also different from the general plan found in the genus. All these exceptional characters (anepisternal bristles, high costal index and hypertrophied surstyli) are also present in another *Zaprionus* (*Anaprionus*) species, i.e. *Z. (A.) silvestriatus* (Bock & Baimai), which appears to be closely related to *Z. (A.) obscuricornis* ([Okada and Carson, 1983](#)). If the paraphyletic relationship of these *Anaprionus* species is true as shown here, a proper taxonomic solution would be to establish a new subgenus of *Zaprionus* for *Z. obscuricornis* (and probably *Z. silvestriatus* as well) and to include the other group in the subgenus *Zaprionus*, or to establish two new subgenera for the two groups of *Anaprionus*. In any case, we need more crucial evidence, from both molecular and morphological analyses before taking either solution.

4.5. Implications for the current nomenclatural problem considering the phylogenetic revision of the genus *Drosophila*

Our results also reconfirmed the long-recognized problem of the parphyly of the genus *Drosophila* in spite of the small taxonomic sampling of the study. The current situation of this problem is somewhat complicated by the proposal of [van der Linde et al. \(2007\)](#) to the International Commission on Zoological Nomenclature. In order to preserve the binomen *Drosophila melanogaster* for this very important model organism even under any taxonomic revisions to be taken place in the future, they submitted an application asking the Commission to use its plenary power to set aside all previous type fixations for *Drosophila* Fallén and designate *Drosophila melanogaster* Meigen (presently, the type species of the subgenus *Sophophora* Sturtevant) as the type species of *Drosophila* Fallén. This issue is still under debate among world *Drosophila* researchers (e.g., [McEvey et al., 2008](#); [O’Grady et al., 2008](#); [Prigent, 2008](#); [Yassin, 2008](#); [Dalton, 2009](#)) and consideration of the Commission. For the sake of stability, we urge caution in regards to any nomenclatural changes to the current *Drosophila s.s.*, before the Commission decides to accept or reject the application. Notwithstanding, our present results provide promising guidelines for future phylogenetic and taxonomic revisions of *Drosophila s.l.* and even across the whole subfamily Drosophilinae.

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