

## A MOLECULAR ANALYSIS OF THE AFROTROPICAL BAETIDAE

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### Abstract

Recent work on the Afrotropical Baetidae has resulted in a number of important taxonomic changes: several polyphyletic genera have been split and more than 30 new Afrotropical genera have been established. In order to test their phylogenetic relevance and to clarify the suprageneric relationships, we reconstructed the first comprehensive molecular phylogeny of the Afrotropical Baetidae. We sequenced a total of *ca.* 2300 bp from nuclear (18S) and mitochondrial (12S and 16S) gene regions from 65 species belonging to 26 genera. We used three different approaches of phylogeny reconstruction: direct optimization, maximum parsimony and maximum likelihood. The molecular reconstruction indicates the Afrotropical Baetidae require a global revision at a generic as well as suprageneric level. Only four of the 12 genera were monophyletic when represented by more than one species in the analysis. Historically, two conflicting concepts of the suprageneric classification of Afrotropical Baetidae were proposed. One was based on the gathering of sister genera into complexes and the other on the division of the family into a restricted number of subfamilies. According to our reconstruction, neither is completely satisfactory: the major complexes of genera present in Africa are either paraphyletic or polyphyletic and the division of the Afrotropical Baetidae into two subfamilies is probably too simplified.

Key words: Ephemeroptera; Baetidae; Africa; Madagascar; molecular phylogeny; systematics.

### Introduction

Taxonomic knowledge of the Afrotropical mayfly fauna has increased substantially in the past two decades. Descriptions of new taxa and several revisions at the supraspecific level have led to a considerable increase in the number of described species and genera (Elouard 2001). This is particularly true for the Baetidae.

Prior to 1980, most African Baetidae species were assigned to Palaearctic genera. South Africa is a good illustration of the case: its fauna has been relatively well known since the middle of the twentieth century, but almost all the species were attributed to a restricted number of European genera, based primarily on imaginal similarities (McCafferty and de Moor 1995). Waltz and McCafferty (1987) were the first to create new African genera to accommodate species that present important modifications in larval morphology. Thereafter, Gilles (Gillies 1985, 1988, 1990, 1991, Gillies and Elouard 1990, Gillies et al. 1990, Gillies and Wuillot 1997), Elouard (Elouard et al. 1990), Wuillot (Wuillot and Gillies 1993a, 1994) and Lugo-Ortiz and McCafferty (1996a, 1996b, 1996c, 1997a, 1997b, 1997c, 1997d, 1997e, 1997f, 1997g, 1998a, 1998b, 1998c, 1999, Lugo-Ortiz and de Moor 2000) greatly contributed to a clarification of the systematics of the Afrotropical Baetidae. In 1990, Gillies transferred all the species attributed to the Palaearctic genus *Centroptilum* to the new genus *Afroptilum*. Gillies' concept of *Afroptilum* was rapidly shown to be polyphyletic and numerous genera were created to accommodate the different species groups (Wuillot and Gillies 1993a, 1993b, 1994, Lugo-Ortiz and McCafferty 1996a, 1996b, 1996c, 1997a, 1997d, 1998a, Barber-James and McCafferty 1997, McCafferty et al. 1997, Lugo-Ortiz et al. 2001). Species previously assigned to *Baetis*, *Acentrella* and *Pseudocloeon* were reassigned to other genera by Lugo-Ortiz and McCafferty (1997c, 1997f, 1998b) and several new genera were described. In total, more than 30 new Afrotropical genera have been established in the last 25 years.

The reported low diversity of Baetidae in most areas of Africa results, at least partially, from a lack of data and comprehensive analysis of material collected by systematists. It is clear that when intensive sampling is performed over large geographical areas, numerous new taxa are discovered and distribution ranges are greatly extended. For example, West Africa was well investigated by the ORSTOM team during the 1970s to 1980s leading to a large increase in taxonomic knowledge of the mayfly fauna. Previous to this project, the West African Baetidae fauna was virtually unknown. Based on the collected material, about 20 new species and seven new genera of Baetidae were described; many of them were endemic.

A similar progression occurred within the Malagasy Baetidae fauna. Until the mid 1990s, very few taxa were known from Madagascar. Between 1997 and 2004, more than 50 new species and eight new genera were described as part of an aquatic biodiversity project led by ORSTOM (presently IRD (Institut de Recherche pour le Développement, France) (Elouard et al. 2003). One of the results of the increased knowledge of the Malagasy fauna was a strengthened recognition of the importance of affinities between African and Malagasy faunas. This implied that the old concept of an endemic Malagasy fauna evolving separately since the breakup of Gondwanaland is only one of several factors likely to explain the present composition, and that other factors such as colonization and dispersal must be taken into account (Gattolliat and Sartori 2003).

For the present study, we used nuclear and mitochondrial gene sequences from 65 taxa to reconstruct the first comprehensive molecular phylogeny of the Afrotropical Baetidae (Monaghan et al. 2005). The aim of this reconstruction is 1) to clarify the suprageneric relationships, 2) to test the phylogenetic relevance of the recent taxonomic work and 3) to investigate the relative roles of dispersal and vicariance in forming the present-day Malagasy fauna. Only the first two points are developed in this paper; the unravelling of the origin of the Malagasy fauna will be discussed elsewhere (Monaghan et al. 2005).

### Material and Methods

Sixty-five species, belonging to 26 different genera of Baetidae, were sequenced for this study (18 of the 24 Malagasy genera and 18 of the 42 Subsaharan genera). Of the 26 included genera, seven were collected only in Madagascar, 10 genera were collected both in Madagascar and Africa, six were collected only in Africa and three were collected in Afrotropical areas and Europe (Switzerland) and/or Asia (Borneo and New Guinea). For the outgroups, four species from the family Tricorythidae (from Madagascar and Africa) were sequenced.

Samples were collected in May–June 2003 in Madagascar and South Africa by the authors and other specimens were taken from collections of the Museum of Zoology in Lausanne or given by different collectors (see acknowledgements). DNA was extracted and four gene fragments (mitochondrial 12S and 16S and two fragments of nuclear 18S, total length 1444–1449 bp) were amplified using methods detailed in Monaghan et al. (submitted). To avoid errors due to contamination or mislabelling, additional individuals of 38 ingroup species were sequenced. All specimens were given a unique number for the study and extracted DNA is stored at the Natural History Museum, London in the frozen collection database (BMNH#s 704056-704136 and 704632-704678).

For phylogeny reconstruction, three different approaches were used: direct optimization as implemented in POY v. 3.0.11 (Gladstein and Wheeler 1999); maximum parsimony with PAUP\* v. 4.0b10 (Swofford 2002) and maximum likelihood under a GTR+I+G model (selected in Modeltest 3.06, Posada and Crandall 1998) using PhyML (Guindon and Gascuel 2003). For details of the analysis, see Monaghan et al. (2005).

To test the support of individual clades, Bremer support was calculated using a heuristic procedure implemented in POY on the output tree obtained by direct optimisation and data were bootstrapped (1000 replicates) with PAUP\*.

### Results

Both direct optimization and parsimony approaches produced a single tree (Fig. 1). The two reconstructions differed only by a single node within the *Cloeon* lineage (Fig. 1 Clade B). Seven well-supported clades (Fig. 1 Clades A, B, C, D, E, F, G) were identified. The relationships between these seven clades were difficult to

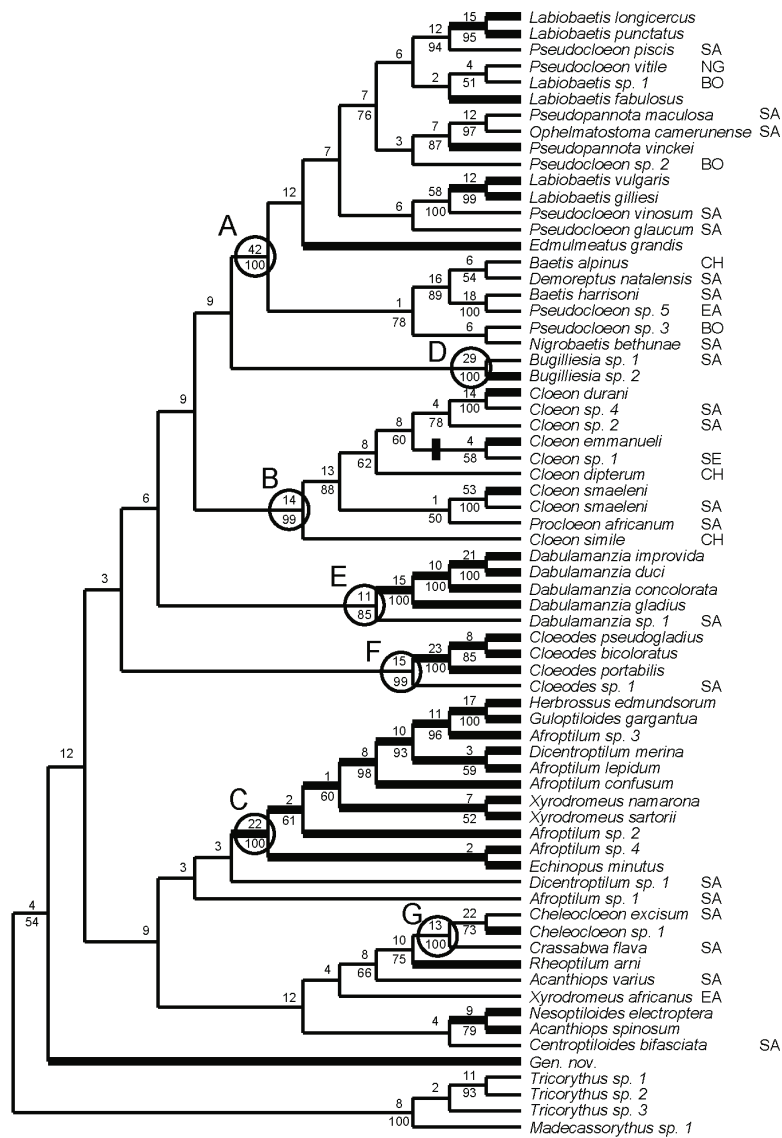


Figure 1. Phylogenetic reconstruction of Afrotropical Baetidae based on the single resulting tree from direct optimization of 12S, 16S and two 18S rRNA gene regions using POY. The vertical bar indicates the alternate placement of *Cloeon sp. 2* using parsimony reconstruction. Values above branches indicate Bremer Support and values below branches indicate parsimony bootstrap percentage (if above 50%). Letters A–G indicate well-supported lineages (see text). Branches are thickened for Malagasy species. Material from other origins: BO = Borneo; CH = Switzerland; EA = East Africa; NG = New Guinea; SA = South Africa; SE = Seychelles.

establish because of poor support of the deeper nodes. The same seven clades resulted from the likelihood reconstruction, with only minor changes occurring within the different clades (Fig. 2). The likelihood topology differs from the direct optimisation and parsimony tree at deeper nodes, notably by separating Baetidae into two basal sister groups (Fig. 2). Only four genera appeared monophyletic; eight were para- or polyphyletic. The monophyly of the 14 others could not be tested as only one species per genus was included in the analysis (Table 1).

### Discussion

Historically, two conflicting concepts of the suprageneric classification of the Afrotropical Baetidae have been proposed. Gillies (1991) considered the African Baetidae to consist of two subfamilies: Baetinae and Cloeoninae (sensu Kazlauskas 1972). Lugo-Ortiz and McCafferty gathered genera in five complexes (*Baetis* complex (Lugo-Ortiz and McCafferty 1998b), *Bugilliesia* complex (Lugo-Ortiz and McCafferty 1996a), *Centroptiloides* complex (Lugo-Ortiz and McCafferty 1998a), *Cloeodes* complex (Lugo-Ortiz and McCafferty 1998c) *Indobaetis* complex (Lugo-Ortiz and de Moor 2000)). Many other genera were not attributed to a complex, as no formal and global reconstruction was undertaken.

*Subfamilies.* The Baetinae are characterized at the imaginal stage by a forewing with paired intercalaries, and at the larval stage by the absence of setae between the prostheca and mola of the right mandible and a single row of denticles on the tarsal claws. The Baetinae subfamily corresponds to the clade A of our reconstruction (Figs. 1 and 2). The Cloeoninae are characterized at the imaginal stage by a single intercalary in the forewing and, at the larval stage, by a row of setae between the prostheca and mola of the right mandible and by two rows of denticles on the tarsal claws. This subfamily comprises clades B to H (Figs. 1 and 2). Clade A is recovered in all three reconstructions, showing that the Baetinae subfamily represents a monophyletic group. The monophyly of the Cloeoninae cannot be rejected by the reconstructions stemming from direct optimisation and parsimony because of the poor support for most of the basal nodes; however, the reconstructions seem to indicate that the Cloeoninae is paraphyletic. In contrast, the likelihood reconstruction groups clades B to H in a monophyletic lineage. Therefore, the ML reconstruction supports the division of the African Baetidae in two subfamilies made by Gillies (1991), while DO and MP do not allow us to reject or confirm Gillies' classification. Additional sampling and sequencing is required to resolve more fully the basal nodes of the phylogeny and determine the higher-level relationships within and among the subfamilies.

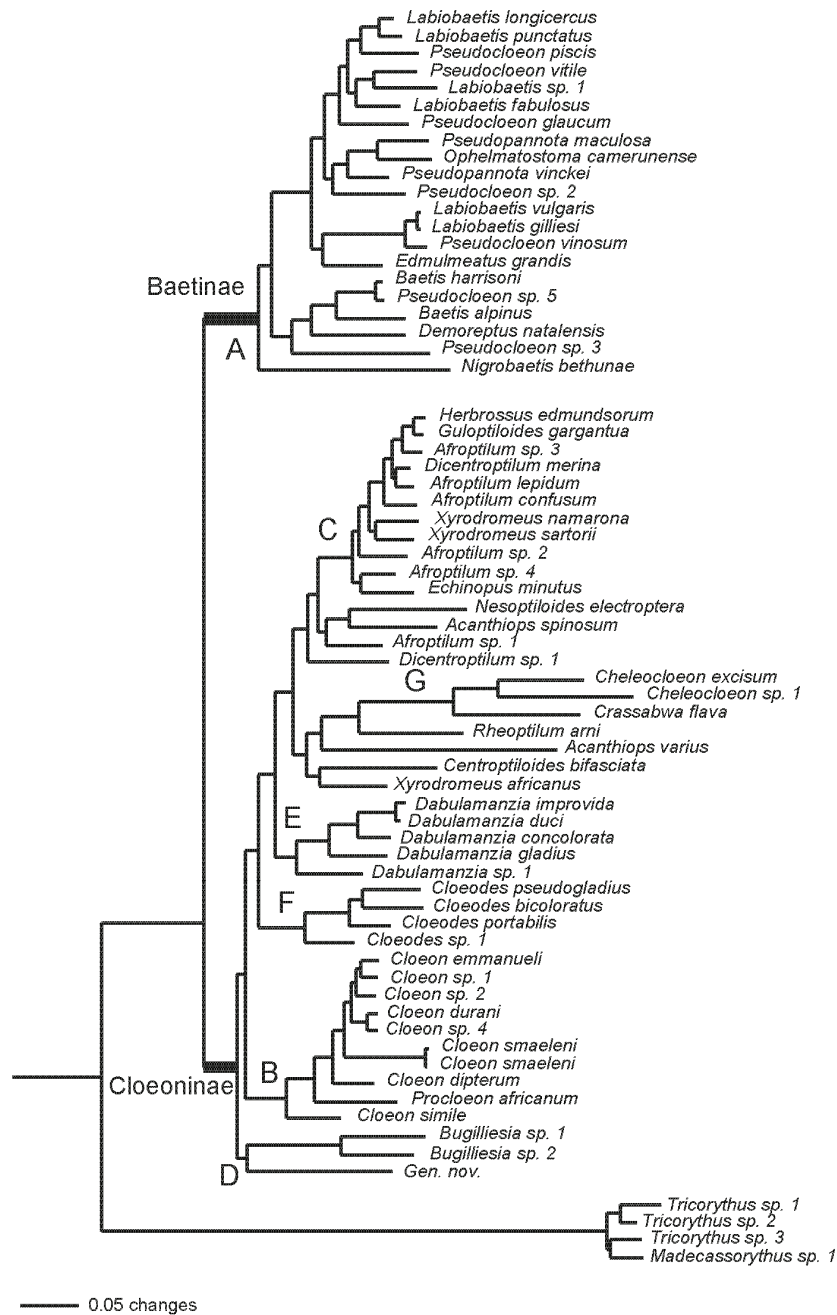


Figure 2. Phylogenetic reconstruction of Afrotropical Baetidae based on the single resulting tree from maximum likelihood reconstruction of 12S, 16S and two 18S rRNA gene regions. Branches are thickened for subfamilies.

Table 1. Distribution and status of the Afrotropical Baetidae genera included in our molecular analysis.

	Distribution	Origin of sequenced material	Status of the genus
Acanthiops	Madagascar + Africa	Madagascar + Africa	polyphyletic
Afroptilum	Madagascar + Africa	Madagascar + Africa	polyphyletic
Baetis	Africa + Palearctic	Africa + Palearctic	polyphyletic
Bugilliesia	Madagascar + Africa	Madagascar + Africa	monophyletic
Centroptiloides	Africa	Africa	nontestable
Cheleocloeon	Madagascar + Africa	Madagascar + Africa	monophyletic
Cloeodes	Pantropical	Madagascar + Africa	monophyletic
Cloeon	World wide except Neotropics	Madagascar + Africa + Palearctic	polyphyletic
Crassabwa	Africa	Africa	nontestable
Dabulamanzia	Madagascar + Africa	Madagascar + Africa	monophyletic
Delouardus	Madagascar	Madagascar	nontestable
Demoreptus	Africa	Africa	nontestable
Dicentroptilum	Madagascar + Africa	Madagascar + Africa	polyphyletic
Echinopus	Madagascar	Madagascar	nontestable
Edmulmeatus	Madagascar	Madagascar	nontestable
Gen nov	Madagascar	Madagascar	nontestable
Guloptiloides	Madagascar	Madagascar	nontestable
Herbrossus	Madagascar	Madagascar	nontestable
Labiobaetis / Pseudocloeon	World wide except Neotropics	Madagascar + Africa + Palearctic	polyphyletic
Nesoptiloides	Madagascar	Madagascar	nontestable
Nigrobaetis	Madagascar + Africa + Palearctic	Africa	nontestable
Ophelmatostoma	Africa	Africa	nontestable
Procloeon	Africa + Palearctic + Neartic	Africa	nontestable
Pseudopannota	Madagascar + Africa	Madagascar + Africa	polyphyletic
Rheoptilum	Madagascar	Madagascar	nontestable
Xyrodromeus	Madagascar + Africa	Madagascar + Africa	polyphyletic

*Genus Complexes.* We were able to test the monophyly of three complexes recognised by Lugo-Ortiz and McCafferty (1998a, 1998b, 1998c): the *Baetis* complex, the *Centroptiloides* complex and the *Cloeodes* complex. All were either paraphyletic or polyphyletic, revealing a number of inconsistencies in the classification as proposed by Lugo-Ortiz and McCafferty. Some can be easily corrected by the inclusion or exclusion of some taxa, e.g., by including *Crassabwa* in the *Centroptiloides* complex. The monophyly of other complexes seems much more difficult to establish (e.g., *Baetis* complex). Several characters used for the definition of the complexes are either plesiomorphic or homoplastic. Single characters are often misleading when trying to involve phylogenetic placement and combinations of characters offer more useful criteria for resolving the suprageneric classification.

Genera of the *Baetis* complex share larval characters such as the presence of the femoral villopore and the absence of setae between the prostheca and mola of both mandibles. In the imaginal stage, *Baetis* complex genera possess forewings with double intercalaries (Waltz and McCafferty 1987). This complex is globally present where Baetidae are found, except for South America. In Africa, only the genera *Baetis*, *Demoreptus*, *Glossidion*, *Labiobaetis/Pseudocloeon* and *Tanzaniella* are included in this complex (Lugo-Ortiz and McCafferty 1997c, 1998b, Lugo-Ortiz et al. 1999). In the molecular reconstruction, this complex is represented by *Baetis*, *Demoreptus* and *Labiobaetis/Pseudocloeon*. They all belong to the clade A, but do not constitute a monophyletic group due to the inclusion of other genera such as *Ophelmatostoma*, *Pseudopannota* and *Edmulmeatus* (Figs. 1 and 2).

The *Centroptiloides* complex contains 15 genera and is the most diversified complex in the Afrotropical area, particularly in Madagascar. This complex was defined by Lugo-Ortiz and McCafferty (1998a) by larvae possessing two subparallel rows of denticles on the tarsal claws and adults with a single marginal intercalary in the forewings. In our molecular reconstruction, it encompasses the genera *Acanthiops*, *Afroptilum*, *Centroptiloides*, *Cheleocloeon*, *Dicentroptilum*, *Echinopus*, *Guloptiloides*, *Herbrossus*, *Nesoptiloides* and *Xyrodromeus* (Figs. 1 and 2). Consequently, it includes the whole of clade C (called the Malagasy clade as all the taxa included occur in Madagascar), members of clade G as well as genera with uncertain relationships throughout the tree. This complex appears paraphyletic in this reconstruction mainly due to the inclusion of the genus *Crassabwa* in the clade G and the presence of a clade of Malagasy endemics (see below).

The *Cloeodes* complex is a grouping of genera of Gondwanan origin possessing a more or less developed subproximal arc of setae on the tibiae. In the Afrotropical area, this complex encompasses *Cloeodes*, *Crassabwa*, *Dabulamanzia*, *Maliqua* and *Nesydemius* (Lugo-Ortiz and McCafferty 1998c). This complex is polyphyletic in our reconstruction, consisting of clade E (*Dabulamanzia*), Clade F (*Cloeodes*) and the genus *Crassabwa* (included in the Clade G as a sister group of *Cheleocloeon*) (Figs. 1 and 2). According to the molecular reconstruction, it is not possible to determine whether *Dabulamanzia* is the sister group of *Cloeodes*, but it is clearly



demonstrated that *Crassabwa* is not related to these two genera. Consequently, the *Cloeodes* complex is at least diphyletic.

*Generic Polyphyly.* The reconstruction allowed us to test the monophyly of 12 genera and only four were found to be monophyletic (Table 1). The remaining 14 genera were only represented by a single species and therefore it was not possible to test their monophyly. Several different reasons explain the polyphyly of the 8 remaining genera. For the Afrotropical genera *Afroptilum*, *Dicentropilum* and *Xyrodromeus*, it is clear that the African and Malagasy species do not belong to the same clade. The Malagasy species are included in the Malagasy clade (Clade C), while the African species are either sisters to clade C or have unclear relationships. The two Malagasy species of *Xyrodromeus* are sister species, but were not closely related to *X. africanus*. The inclusion of the Malagasy species in this African genus is mainly due to convergences observed in the mouthparts, especially the modified mandibles adapted for scraping epilithic algae. Several unrelated Malagasy genera present this kind of adaptation in one or more species; thus it seems to be a relatively common characteristic that has evolved independently in several different Malagasy lineages (Gattolliat and Sartori 2000, Gattolliat 2001a, 2001b, 2002). The inclusion by Lugo-Ortiz and McCafferty (1998a) of Malagasy *Dicentropilum merina* in the African genus *Dicentropilum* required a revision of the generic diagnosis with the deletion of several characters from the original description. The imaginal stage of *D. merina* was not known at that time and is still undescribed. The observation of imagos from material reared by the LRSAE team (Laboratoire de Recherche sur les Systèmes Aquatiques et leur Environnement, Antananarivo, Madagascar) clearly indicate that *D. merina* differs from African species; the Malagasy species does not possess the specialised characters of the type species such as hindwing with three longitudinal veins and two widely separated costal spurs (Wuillot and Gillies 1994). Consequently, it is necessary to erect new genera to include the Malagasy species of *Xyrodromeus* and *Dicentropilum*.

The phylogeny of *Afroptilum* species seems more complicated than that of the Madagascar-Africa dichotomy observed above. For this study, we provisionally assigned undescribed or unnamed species to this genus. The Malagasy clade probably still includes several undescribed genera that have a low degree of adaptation to unusual feeding behaviour or environmental pressure. Nevertheless, the African *Afroptilum* is not the sister group of any Malagasy species. As the genus *Afroptilum* has been used for lumping different taxa *incertae cedis*, a complete revision of this genus is necessary.

With five genera (*Guloptiloides*, *Herbrossus* and *Nesoptiloides* from Madagascar; *Centroptiloides* and *Barnumus* from South Africa), carnivorous Baetidae are quite abundant and diverse in the Afrotropical area (Gattolliat and Sartori 2001). Two of them (*Herbrossus* and *Guloptiloides*) are sister-taxa and members of the Malagasy clade, but the two others included in the molecular

reconstruction (*Nesoptiloides* and *Centroptiloides*) are not closely related (Figs. 1 and 2). It implies that this unusual adaptation has appeared at least twice.

*Labiobaetis/Pseudocloeon*, even in its most restricted definition, remains polyphyletic. It is clear from the data that the Afrotropical taxa belong to two different lineages, both of which include taxa shared with southeastern Asia. This wide distribution suggests strong dispersal abilities in evolutionary time. Moreover, other non-Afrotropical species of *Labiobaetis/Pseudocloeon* were included in another group of taxa among the clade A. This shows very clearly that this genus greatly needs a complete revision that can only be made by including taxa from all the different biogeographic areas. It also means that the *Pseudocloeon* concept is not sufficiently defined (Lugo-Ortiz et al. 1999) and that new morphological characters need to be added. Because of the number of taxa and the wide geographical range involved, this revision constitutes a great challenge. Adults of the type species of *Pseudocloeon* need to be reexamined and nymphs correlated.

The eight species of *Cloeon* do not constitute a monophyletic group because of the inclusion a single species assigned to *Procloeon* among them. This conflicts the validity of the genus *Cloeon*. It implies that either the genus *Cloeon* must be restricted to a limited number of species and some of the species assigned to other genera, or the generic characterization of *Procloeon africanum* must be rectified. Gillies (1997) considered that there are no reliable characters for distinguishing adults of *Cloeon* and *Procloeon*. The only difference lies in the degree of development of the gills. According to this character, he attributed the different African species to one of the two genera. These attributes remain rather questionable and the concept of the genus *Procloeon* needs a global revision.

*Cloeon smaeleni* possesses a wide distribution including the whole Afrotropical area as well as the Arabian Peninsula (Gillies 1985); it was recently collected in Madagascar and is therefore the only Malagasy species of Ephemeroptera which is not endemic to Madagascar (Gattolliat and Rabeantoandro 2002). For this species, material from both Madagascar and South Africa was sequenced. The low molecular divergence confirms the specimens from Madagascar and South Africa belong to the same species (Fig. 2).

We conclude that our molecular reconstruction has greatly helped to clarify the Afrotropical Baetidae classification by revealing both the accuracy and inaccuracy of recent systematics research and by highlighting the groups in which taxonomic revisions are necessary. It appears the grouping of genera in different complexes is not satisfactory and the division of the African Baetidae in two subfamilies is probably too simplified. At the generic level despite recent improvements, many genera still require revision and several new genera must be erected, especially for Malagasy species previously assigned to African genera.

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