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DNA-based association of adults and larvae in Baetidae (Ephemeroptera) with the description of a new genus *Adnoptilum* in Madagascar

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Abstract. The mayfly fauna of Madagascar is highly diverse and largely endemic. Many species remain undescribed, and many species are known from only the larval or adult life stage. The high biodiversity in Madagascar and in other areas has led to an increasing reliance on DNA-based approaches to taxonomy, i.e., to define species boundaries and to associate different developmental stages. We used the general mixed Yule-coalescent (GMYC) model to combine population- and species-level sequence variation of mitochondrial deoxyribonucleic acid (mtDNA) to detect species boundaries in Baetidae mayflies (Ephemeroptera). Starting with a database of 240 sequences (57 species), significant clustering of newly sequenced larvae allowed us to establish 1 new species and 1 new combination and to associate adult and larval stages for both. A molecular phylogeny using additional nuclear (18S) and mtDNA (*rrnS*, *rrnL*) gene regions recovered the new species and new combination as a monophyletic group, distinct from other Afrotropical lineages. Therefore, we established a new genus, *Adnoptilum*, endemic to Madagascar. *Adnoptilum* gen. n. can be distinguished from other species in both the imaginal and adult stages and appears to belong to the *Bugilliesia* complex. We conclude that routine sampling of population- and species-level genetic diversity, combined with coalescent-based methods of species delineation, has great potential to become a standard procedure for the study of poorly known taxonomic groups.

Key words: DNA taxonomy, GMYC model, integrative taxonomy, Madagascar, new genus, new species, new combination.

Madagascar is home to a high diversity of mayflies (Ephemeroptera), with $\geq$110 described species and a large number that remain undescribed (Elouard et al. 2008). Nearly ½ of the species diversity occurs within the Baetidae and 50 new species in 22 genera have been described in the last 10 y (Gattolliat and Sartori 2003, Gattolliat et al. 2008). This taxonomic richness is broadly representative of the high biodiversity of other freshwater organisms on Madagascar, which remains poorly known even in the face of population declines and extinctions (Elouard and Gibon 2001, Benstead et al. 2003). Deoxyribonucleic acid (DNA) sequence data have long been used to better understand the biogeographical origin and degree of endemism of this biodiversity (e.g., Monaghan et al. 2005, Daniels et al. 2006, Cumberlidge et al. 2008). Sequence data are used increasingly to accelerate the rate of species discovery and to increase our understanding of the spatial scale of local endemism on the island itself (e.g., Smith et al. 2005, Monaghan et al. 2009, Vieites et al. 2009).

The use of genetic data in mayfly taxonomy has a long history, but defining the units of interest (i.e., species) in a meaningful and repeatable way remains a significant challenge (reviewed by Monaghan and Sartori 2009). One technique is to use a threshold value of sequence divergence for species discrimination (i.e., DNA barcoding), and this method has been used for mayflies (Ball et al. 2005, Alexander et al. 2009). The success of this approach has been mixed (Hebert et al. 2004, Brower 2006), and possible explanations for its failure include nonuniform rates of sequence divergence and use of inaccurate a priori

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groups to calibrate a threshold of sequence divergence between species (Vogler and Monaghan 2007). Reliance on a single gene marker, usually mitochondrial DNA (mtDNA), also can lead to inaccurate estimates of diversity because of introgression or incomplete lineage sorting (Pollard et al. 2006, Alexander et al. 2009). A 2nd technique is character-based and uses diagnostic genetic markers rather than similarity measures (Sites and Marshall 2003). This approach has been used successfully for mayflies (Zloty et al. 1993, Funk et al. 2008), but a limitation of diagnostic techniques is that they require some initial criteria for grouping individuals, e.g., morphology, ecology, or geography (DeSalle et al. 2005). These data might not be available for specimens coming from unknown samples or from broad biodiversity surveys.

A 3rd technique uses a coalescent approach to identify the species boundary from large samples of DNA sequences. Instead of using a priori thresholds, the general mixed Yule-coalescent (GMYC) model (Pons et al. 2006, Fontaneto et al. 2007) identifies a shift in branching rates on a phylogenetic tree of sequences from multiple species and populations. Sequences are identified from the tree based on differences in branching rates at the level of species and populations (Pons et al. 2006). The GMYC model assesses the point of transition from coalescent (i.e., population) to speciation branching in a maximum likelihood framework, and species are defined as independently evolving lineages. Authors of a number of studies have used the GMYC approach successfully to delineate species based on single-locus data with nuclear DNA (nDNA), morphology, and symbiont cospeciation as independent evidence for species status (e.g., Fontaneto et al. 2009, Jousselin et al. 2009, Monaghan et al. 2009, Papadopoulou et al. 2009).

An important application of any DNA-based approach to taxonomy is association of different developmental stages of species. Unlike most other taxonomic characters, sequence polymorphisms are maintained throughout the life cycle. Many benthic invertebrates are described in either the adult stage or the late-instar larval stage, but not both. In some cases, the 2 stages have been described as 2 different species. After rearing and examining a number of large mayfly species from Madagascar, Gattolliat and Sartori (1999) concluded that the larva described as *Nesoptiloides intermedia* Demoulin (1973) was the immature stage of what had already been described as *Centroptilum electropterum* Demoulin (1966). Successful linkage of adults and larvae using DNA has been reported in several recent studies of aquatic invertebrates (see Holzenthal et al. 2010). Ahrens et al. (2007) used the GMYC model to link adult and larval tropical beetles, even though many of the species were undescribed. Thus, the GMYC model provides a means to establish species boundaries independent of other criteria and can be used for adults and larvae where no shared characters might otherwise exist.

Beyond delineation of species, genetic characters also provide important information about the evolution of mayfly diversity that can be applied to a higher classification of species and genera. A number of the recently described Malagasy Baetidae species were included in existing Afrotropical genera (e.g., *Afroptilum* Gillies, *Demoulinia* Gillies, and *Securiops* Jacobus, McCafferty and Gattolliat) or in more widely distributed genera (e.g., *Cloeon* Leach, *Cloeodes* Traver, *Cheleocloeon* Wuillot and Gillies) (Lugo-Ortiz and McCafferty 1997, 1999, Gattolliat 2003, Jacobus et al. 2006). This morphological classification was not well supported by a subsequent study of the origin of the Malagasy fauna, where many Malagasy species belonged to a single endemic lineage (Monaghan et al. 2005, Gattolliat et al. 2008). Many mouthpart characteristics are subject to convergence among independent lineages. Thus, new genera, endemic to Madagascar, must be established.

Other taxonomic and systematic difficulties remain that hinder our understanding of the evolutionary depth of endemism on Madagascar. The *Bugilliesia* complex (Lugo-Ortiz and McCafferty 1996) includes 4 Afrotropical genera with 2-segmented gonopods in the male imago (*Bugilliesia*, *Kivua*, *Mutelocloeon*, and *Rhithrocloeon*). A single species with 2-segmented gonopods, *Mutelocloeon thomasorum* Lugo-Ortiz and McCafferty, was described from Madagascar (Lugo-Ortiz and McCafferty 1997). The peculiar gonopods suggest the species belongs to the *Bugilliesia* complex, but its placement in *Mutelocloeon* is questionable because African species of *Mutelocloeon* are symbiotic with mussels (Gillies and Elouard 1990), and mussels or similar bivalves are not present in Madagascar (Elouard and Gibon 2001). *Mutelocloeon thomasorum* was subsequently considered as incertae sedis (Gattolliat 2006). Last, an undescribed larva was included in the tree of Afrotropical Baetidae. The larva had some characteristics of *Cheleocloeon*, *Delouardus*, and *Bugilliesia* (Gattolliat et al. 2009), but clearly was different and appeared distantly related to the other lineages (Monaghan et al. 2005, Gattolliat et al. 2008).

Here we analyze DNA sequence data using the GMYC model to establish species groups within Malagasy Baetidae and to determine whether undescribed adults and larvae belong to the same species. We examined larval specimens that share some

morphological characteristics with *Bugilliesia* and *Cheleocloeon* but are different from all described species. We also examined adults with 2-segmented, blade-shaped gonopods from museum and field collections. We used the GMYC model to delimit species by combining newly sequenced individuals with a larger, existing database. Newly established species were then incorporated into a molecular phylogenetic analysis using additional nuclear and mitochondrial DNA gene regions. Taken together, the results allowed us to establish 1 new species and 1 new combination, each with adult and larval stages, and to determine the evolutionary relationship to other Afrotropical lineages, and thereby, to erect a new genus, endemic to Madagascar. The holotype and some of the paratypes are housed in the Museum of Zoology, Lausanne (MZL). Other paratypes are deposited in the Muséum national d'Histoire naturelle, Paris.

Material and Methods

DNA extraction and morphological analysis

Forty larvae and 60 adults superficially resembling *Bugilliesia*, *Cheleocloeon*, or *Delouardus* but clearly different from all described species were selected from material deposited in the MZL collections by the Laboratoire de Recherche sur les Systèmes Aquatiques et leur Environnement (LRSAE). Mayflies and other aquatic insects had been collected by the LRSAE from ~650 localities throughout Madagascar over a 10-y period (Elouard and Gibon 2001, Elouard et al. 2008). No specimens belonging to the groups under study here were reared successfully. Thus, it has not yet been possible to associate the different life stages. More recent field sampling by the authors (2003–2007) provided additional material for comparison, which was suitably preserved in the field (>95% ethanol) for genetic analysis in the laboratory.

Total genomic DNA was extracted using nondestructive methods to enable taxonomic identification after genetic analysis. Whole specimens were placed directly in a mix of proteinase K (Sigma, Dorset, UK) and lysis buffer supplied as part of the DNeasy Blood and Tissue Kit (Qiagen, Crawley, UK) or the Wizard SV Kit (Promega, Southampton, UK) and incubated 8 to 12 h at 55°C. Specimens were then removed and placed in 80% ethanol while DNA was isolated from the lysis mixture according to the manufacturers' protocols. DNA was eluted into 0.1 Tris-ethylenediaminetetraacetic acid (EDTA) (TE) buffer and used for subsequent polymerase chain reaction (PCR) or deposited to the frozen collection of the Entomology Department, Natural History Museum, London. Mouthparts, legs, abdomens, and male imago genitalia were undamaged by the extraction procedure and were slide-mounted in Canada balsam for microscopic observation. The remaining body parts were kept in 80% ethanol.

Species assignment

Species limits for newly sequenced larvae and adults were established using the GMYC model (Pons et al. 2006, Fontaneto et al. 2007). First, we sequenced a 357-base pair (bp) region of mitochondrial cytochrome oxidase B (*cob*) for 5 individual larvae. This gene was used to incorporate new data into an existing database of Baetidae *cob* sequences from Madagascar (240 sequences comprising 57 GMYC species; Monaghan et al. 2009). This combined data set (245 sequences) was then subjected to multiple alignment using ClustalW (Kyoto University Bioinformatics Center, Kyoto, Japan; available from: align.genome.jp). No insertion/deletion events or stop codons existed in the protein-coding *cob*. Redundant haplotypes were removed from the matrix using a perl script (D. Chesters, Natural History Museum, London), but all haplotypes for newly sequenced individuals were retained to visualize each individual on the phylogenetic tree of *cob* (Fig. 1). Ultrametric branch lengths were estimated using a general time reversible (GTR) model of sequence evolution, a coalescent (constant size) prior, and an uncorrelated log-normal relaxed molecular clock (Drummond et al. 2006) on an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) starting tree as implemented in BEAST v1.4.7 (Drummond and Rambaut 2007, see Monaghan et al. 2009 for details of the analysis). The analysis ran for 30 M generations on a remote computing cluster (vital-it.ch), and all parameters had an estimated sample size >100 after a burn-in of 5 M generations. We analyzed the Markov Chain Monte Carlo (MCMC) output from BEAST using TreeAnnotater v1.4.7, keeping all trees after the burn-in, a posterior probability limit of 0.5, targeting the maximum clade credibility tree, and keeping the target node heights.

The GMYC model was optimized to the resulting tree using the *gmyc* tool (available as part of the 'splits' package at http://r-forge.r-project.org/projects/splits/) in R v 2.8.1 (R Development Core Team, Vienna, Austria). The *gmyc* function was used to do single-threshold (Pons et al. 2006, Fontaneto et al. 2007) analyses using functions from the ape library (Paradis et al. 2004). Model outputs include the threshold time of transition from coalescent to speciation branching (T), and the number of species, with confidence intervals as

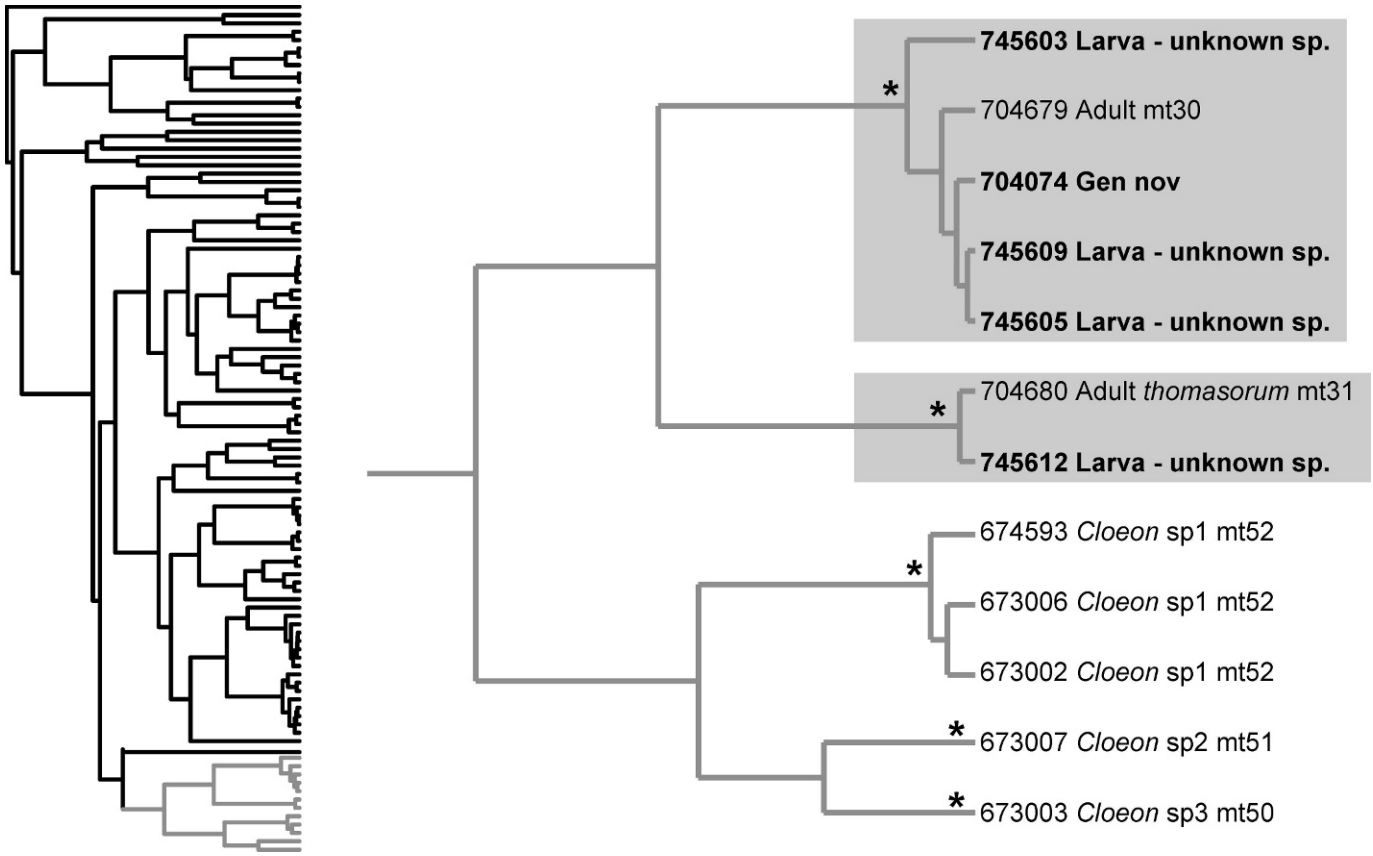

FIG. 1. Phylogenetic tree of Afrotropical Baetidae *cob* haplotypes (left) that includes newly added sequences from unknown larvae and published sequences (Monaghan et al. 2009). The general mixed Yule-coalescent (GMYC) model was optimized to branch lengths from this tree to establish species limits (see methods). The highlighted subtree (gray branches, enlarged) shows the grouping of unknown larvae (bold) into species (* = coalescent group). All unknown larvae were grouped into either species mt30 or mt31 of the database (grey boxes). Terminal labels include the 6-digit specimen identification number (BMNH number) in the frozen collection of the Entomology Department, Natural History Museum, London (Table 1), and genus (e.g., *Cloeon*) or species (e.g., *thomasorum*) membership. In this paper, we formally described mt30 as *Adnoptilum ottkei* n. sp. and renamed mt31 as *Adnoptilum thomasorum* n. comb.

solutions within 2 log-likelihood units of the maximum. Other outputs included branching rates and scaling parameters used to fit the model (described by Pons et al. 2006) and the likelihood ratio (LR) between null (neutral coalescent) and mixed (GMYC) model fits. Significance of the LR was evaluated with a χ^2 analysis with 3 degrees of freedom to compare null and GMYC models.

Phylogenetic analysis

We used phylogenetic analysis to determine the evolutionary relatedness of the newly established species to other Afrotropical lineages. Single representatives of each were incorporated into a larger phylogeny of Afrotropical lineages. Phylogenetic analysis used the *cob* sequences (described above), mtDNA *rrnS* (12S) and *rrnL* (16S), and 2 fragments of nuclear ribosomal DNA (rDNA) (18S). Sequence data were newly generated with PCR and sequencing methods described by Monaghan et al. (2005) or were downloaded from GenBank (Table 1). *cob* data from a 2nd specimen from the same locality (BMNH 745680) were used to form a chimeric sequence of *Cheleocloeon madagascariense*, and 18S data from a GenBank accession from the same locality (BMNH 704116) were used to form a chimeric sequence for *Pseudocloeon glaucum*. A dragonfly (Odonata, Anisoptera) was used as the outgroup. This sequence was a chimeric sequence of 2 Libellulidae, with *cob* from *Ladona depressa* and all other gene fragments from *Libellula saturata* (Table 1).

The potential uncertainty in tree topology arising from length variation in ingroup *rrnS* (ingroup length variation 351–359 bp) and *rrnL* (510–514) was addressed by evaluating multiple alignments under a

TABLE 1. Source data for general mixed Yule-coalescent (GMYC) and phylogenetic analyses. Genus and species names are presented according to the new description (n. sp.) or new combination (n. comb.) in the present manuscript. Alternative names/codes by which specimens were presented in previous studies are given in parentheses, with superscripts used to denote publication sources. Life stage, entry record in the frozen tissue collection of the Natural History Museum, London (BMNH number) and GenBank accessions for each gene fragment are provided. Blanks indicate gene fragments were not sequenced. *Ladona depressa* and *Libellula saturata* sequences were combined to form a chimeric outgroup sequence for the phylogenetic analysis. (same as 5′) = full-length 18S (~1800 bp) is a single GenBank accession, given in the 5′ column.

Species	Life stage	BMNH number	*cob*	*rrnS*	*rrnL*	18S 5′	18S 3′
Adnoptilum ottkei n. sp. (Gen. nov)[a]	Larva	704074	HM185093	AJ969682	AJ971730	AM042606	(same as 5′)
Adnoptilum ottkei n. sp. (mt30)[b]	Adult	704679	FJ818601				
Adnoptilum ottkei n. sp.	Larva	745603	HM185094				
Adnoptilum ottkei n. sp.	Larva	745605	HM185095				
Adnoptilum ottkei n. sp.	Larva	745609	HM185096				
Adnoptilum thomasorum n. comb. (mt31)[b]	Adult	704680	FJ818602	HM185087	HM185090		HM185092
Adnoptilum thomasorum n. comb.	Larva	745612	HM185097				
Afroptilum lepidum	Larva	704115	HM185098	AJ969713	AJ971761	AM042645	AM042646
Bugilliesia margarete (*Bugilliesia* sp.)[a]	Larva	704124	HM185099	AJ969721	AJ971769	AM042654	AM042655
Bugilliesia mirandei[c]	Larva	704677	HM185100	AJ969740	AJ971788	AM042684	AM042685
Cheleocloeon excisum	Larva	704123	HM185101	AJ969720	AJ971768	AM042653	(same as 5′)
Cheleocloeon madagascariense	Larva	704676		AJ969739	AJ971787		AM042683
	Larva	704680	HM185102				
Cloeon durani	Larva	704070	HM185103	AJ969679	AJ971727	AM042602	(same as 5′)
Cloeon sp. 2	Larva	704127	HM185104	AJ969722	AJ971770	AM042656	(same as 5′)
Delouardus djabala	Larva	704678	HM185105	HM185088	HM185091		
Herbrossus edmundsorum	Larva	704082	FJ818763	AJ969690	AJ971738	AM042614	(same as 5′)
Labiobaetis longicercus	Larva	704060	FJ818806	AJ969675	AJ971724	AM042598	(same as 5′)
Pseudocloeon glaucum	Larva	704086	HM185106	HM185089			(same as 5′)
	Larva	704116			AJ971762	AM042647	
Libellula saturata				AY282562	AF037181	AY338717	(same as 5′)
Ladona depressa			AY960596				

[a] Monaghan et al. (2005), Gattolliat et al. (2008)
[b] Monaghan et al. (2009)
[c] previously *Cheleocloeon mirandei* see Gattolliat et al. (2009)

range of gap-opening penalties as follows. Each gene fragment was subjected to 5 multiple alignments, using gap-open penalties of 1, 5, 15, 20, and 25 (align.genome.jp). All possible combinations were evaluated for their incongruence length difference (ILD) using Parsimony searches with TNT v 1.1 (Goloboff et al. 2004) with 20 ratchet iterations, 10 cycles of tree drifting, and 3 rounds of tree fusing for each of 200 random addition sequences. The 2 alignments most highly congruent to one another were used for subsequent phylogenetic analysis.

Phylogenetic reconstruction was done with a partitioned model of sequence evolution in a Bayesian analysis. This model was used to account for the different evolutionary dynamics of functionally different components of the genetic matrix (e.g., Brandley et al. 2005). We used 3 different partitioning schemes in an effort to explore the data and chose the most appropriate settings. One search used a single general time reversible + invariant + gamma (GTR+I+Γ) model for the entire data set (6 parameters). A 2nd search used a separate GTR+I+Γ model for each of 6 data partitions with all parameters unlinked (27 parameters). Partitions included the 3 codon positions of *cob* and the 3 other gene regions *rrnL*, *rrnS*, and 18S, where the 2 sequenced fragments of 18S were combined. In an effort to reduce the number of parameters, a 3rd search was implemented based on results from the 27-parameter search. For this 3rd search, the Γ shape parameter was partitioned between *cob* codon position 1 (*alpha* >1) and all other data (*alpha* <1) and the proportion of invariable sites was partitioned between *cob* codon position 3 (pinvar = 0.01) and all other sites (pinvar = 0.1–0.6). This step resulted in a 19-parameter model. Each search consisted of 2 runs of 3 cold and 1 heated chain. After 1 M generations the likelihood of each search had stabilized, and the average standard deviation of split frequencies was <0.015. The optimal search was chosen from comparisons of the harmonic mean of the

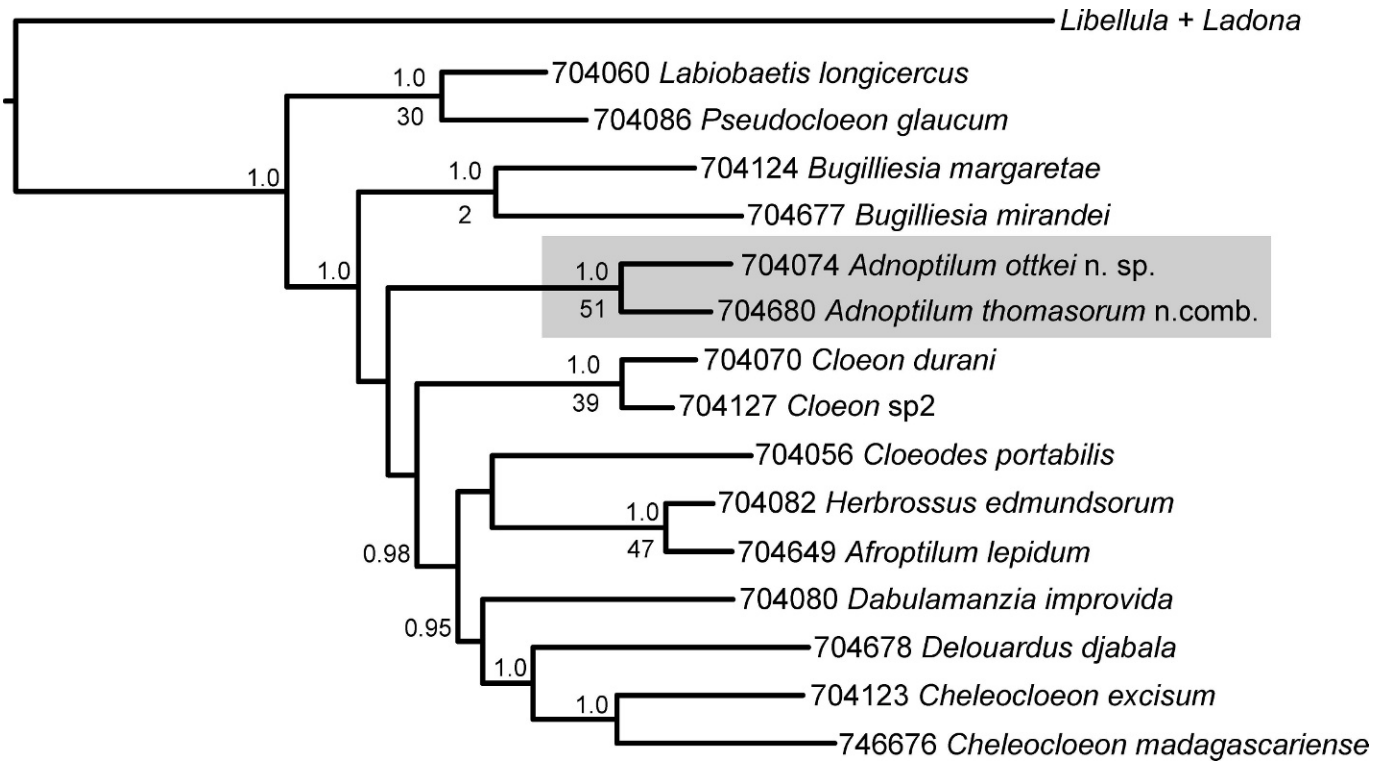

FIG. 2. Consensus tree of representative species of Afrotropical Baetidae using a 6-partition (27-parameter) Bayesian phylogenetic analysis of the gene regions *rrnS*, *rrnL*, *cob*, and 18S. The outgroup (Odonata) consisted of chimeric sequences from *Libellula* (*rrnS*, *rrnL*,18S) and *Ladona* (*cob*). Node support was calculated using Bayesian posterior probabilities (above branches) and Bremer support under maximum parsimony (below branches). Species of the new genus, *Adnoptilum*, are highlighted in grey. Six-digit numbers in the terminal labels are as in Fig. 1.

estimated marginal likelihoods (Brandley et al. 2005) after a burn-in of 0.7 M generations. Analyses were conducted using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003).

Results

Species diagnosis using cob *sequence data*

The single-threshold GMYC model was a significantly better fit to the branching patterns on the linearized phylogenetic tree of Baetidae *cob* than a null model of uniform (neutral coalescent) branching (likelihood ratio test, $L_{gmyc} = 576.0331$, $L_0 = 550.8837$, LR = 50.29897, $p < 0.001$). Using the model-output transition time ($T = -0.0178$ substitutions/site) as a coalescent boundary, the model assigned the sequences from the unknown larvae to 2 separate *cob* groups (Fig. 1). The earlier analysis of the large database had delineated these species from single adult specimens (mt30 and mt31; Monaghan et al. 2009). In the present study, 4 of the nymphs were grouped with mt30 and 1 was grouped with mt31 (Fig. 1). One of the nymphs placed into mt30 was the undescribed specimen with characteristics somewhat similar to *Cheleocloeon*, *Delouardus*, and *Bugilliesia* but labelled as "Gen. nov." in the molecular phylogenetic study of Monaghan et al. (2005).

Molecular phylogeny

Lowest incongruence between mtDNA *rrnS* and *rrnL* was found using gap-open penalties of 15 and 1, respectively. These settings produced an increase of 3% in the maximum parsimony tree length when both gene fragments were combined (ILD = 3%). This alignment also showed the lowest degree of incongruence with *cob* data (data not shown). All 4 gene fragments combined resulted in a total alignment length of 2734 bp. For the 3 subsequent Bayesian searches on the total alignment, comparison of the harmonic mean of the marginal likelihoods suggested that the 27-parameter model ($L = -14814.91$) was statistically superior to the 19-paramater ($L = -14868.92$) and 6-parameter ($L = -16599.63$) models.

The resulting phylogenetic tree exhibited a high degree of support for terminal nodes, including support for monophyly of the 2 new species (Fig. 2).

The 2 taxa constitute a monophyletic group that is distinct from other previously recognized lineages of Afrotropical Baetidae. Therefore, we consider them to belong to a new genus, *Adnoptilum* gen. n. Other well-supported nodes included *Labiobaetis*, *Cloeon*, and the very diversified Malagasy endemic lineage represented here by *Herbrossus edmundsorum* and a species originally classified as *Afroptilum lepidum* but whose generic attribution needs to be reassessed (see Monaghan et al. 2005 and Gattolliat et al. 2008 for discussion) (Fig. 2). *Bugilliesia* and *Cheleocloeon* were monophyletic with high Bayesian posterior probabilities, but parsimony Bremer support was low (*Bugilliesia*) or 0 (*Cheleocloeon*) (Fig. 2). As in previous analyses of the Afrotropical Baetidae, node support for deeper relationships was relatively low compared to tip nodes, with exceptions being that *Labiobaetis* was well-supported as a sister group to remaining ingroup taxa and that a well-supported node joins *Bugilliesia* to the remaining groups, including *Adnoptilum* gen. n. (Fig. 2).

Discussion

The application of DNA to taxonomy remains controversial (e.g., Will et al. 2005), but the idea of using genetic data for associating different developmental stages of organisms is generally accepted and fairly well established (Miller et al. 2005, Ahrens et al. 2007, see Holzenthal et al. 2010). Nonetheless, most studies that attempt to match query sequences to a database (e.g., Rao et al. 2006, Levkanicova and Bocak 2009) require validated reference sequences and analytical methods to discriminate between multiple possible matches in the face of intraspecific genetic variation. The result is a reliance on a priori reference taxa and thresholds of sequence divergence. Empirically, the similarity-based approach often confirms expectations (Hebert et al. 2003), but morphological taxonomy used for calibration can be subjective. Moreover, why all species in a clade should have equal pairwise genetic similarity remains to be established. Selective genetic sweeps might normalize the depth of subdivision at some genetic loci (Bazin et al. 2006), and further research should be done on this important topic.

The GMYC model was applied here to establish species boundaries that included both adults and larvae, using membership in a single coalescent group as a criterion. The approach does not rely on calibrated thresholds of sequence divergence and could establish species membership, if not identity, even if the database were incomplete. Nondestructive extraction of DNA allowed further morphological examination, and analysis of the 5 newly sequenced larvae and 2 unidentified adults already in the database allowed us to designate them as a new species and a new combination (see Taxonomy below). The use of an existing database is a strength of sequence-based approaches to taxonomy, in that any new gene sequence can be aligned and compared with an existing database. The creation of such species databases is well under way, either through deliberate creation (e.g., Ball et al. 2005) or through an accumulation of existing studies (e.g., Williams et al. 2006, Savolainen et al. 2007, Alexander et al. 2009). Our study provides evidence that the GMYC approach to species delineation is appropriate even in the absence of known reference specimens.

One important consideration is that our species delineation was based on a single mtDNA marker. The process of lineage sorting is stochastic and divergence at a single marker can be much slower than the divergence of lineages into species (Pollard et al. 2006). Introgression of mtDNA across species boundaries can also be problematic (Funk and Omland 2003). Nonetheless, a recent broad-scale comparison of mtDNA and nuclear rRNA found 98% congruence in >350 species of insect, with 100% congruence observed between *cob* mtDNA and 28S rRNA for the Baetidae sequences used here as a database (Monaghan et al. 2009). Therefore, we have a high degree of confidence that mtDNA sequence polymorphism here represents lineage diversification. Nonetheless, the GMYC species are a hypothesis to be tested with other criteria (sensu DeSalle et al. 2005). Authors of other studies of mayflies have used mtDNA as a proxy for species boundaries, with subsequent comparison to ecological (Savolainen et al. 2007) or morphological (Tojo and Matsukawa 2003, Funk et al. 2006, Webb et al. 2007) criteria.

Our results suggest that future association of egg, larva, subimago, and imago stages will be accomplished easily with sequence databases for mayflies. In the Baetidae, larvae and adults have very few shared morphological characters. In some species, the abdominal pattern can provide some evidence for species membership in both life stages. The hindwings and forewings also can be extracted from the wing pads of late-instar larvae. Nonetheless, the association of different life stages using only these characters can be unreliable (e.g., Jacobus et al. 2006). Eggs often are used in other families of Ephemeroptera for specific identification and association of the different stages (Elouard and Sartori 1997). However, Baetidae eggs normally are covered by mucus and any structures are difficult to observe and often not species-specific. One exception is the carnivorous

genus *Guloptiloides* in Madagascar, in which nymphs and female imagos can be associated using egg structure (Gattolliat and Sartori 2000).

The molecular phylogenetic reconstruction clearly showed that the 2 *Adnoptilum* gen. n. species constitute a single clade that is distinct from other Afrotropical lineages, in particular *Cheleocloeon*, *Delouardus*, and *Bugilliesia* with which the nymphs shared some morphological similarities. For this reason, we establish a new genus (below) to accommodate these species. Unfortunately, the data provide no further clarification of the evolutionary relatedness of Afrotropical groups than was made by previous studies (Monaghan et al. 2005, Gattolliat et al. 2008) despite the addition of a protein-coding mtDNA gene region (*cob*) and the Bayesian analysis used here. Resolution of deeper nodes is a general problem in mayfly phylogenetics, as is the dependence of topology on alignment parameters of ribosomal genes (see Ogden and Whiting 2005) such as *rrnS*, *rrnL*, and 18S used here. The phylogenetic position of *Adnoptilum* gen. n. in the Bayesian reconstruction contrasts with its position in earlier parsimony-based analyses (maximum parsimony and direct optimization), where *Adnoptilum* gen. n. (labeled ''Gen. nov.'' by Monaghan et al. 2005) was recovered as basal to the remaining Baetidae. The alternative placement could result from multiple substitutions (saturation of polymorphisms) or from the information content of gaps, both of which would affect parsimony and likelihood searches differently.

Adnoptilum gen. n.

Type species: *Adnoptilum ottkei* Gattolliat and Monaghan sp. n., original combination.

Other included species: *Adnoptilum thomasorum* (Lugo-Ortiz and McCafferty 1997), in *Mutelocloeon* Gillies and Elouard 1990, new combination. The specimens identified by Gattolliat (2006:295) as *Mutelocloeon thomasorum* Lugo-Ortiz and McCafferty, incertae sedis, are here referred to as *Adnoptilum thomasorum*.

Diagnostic characters

Larva.—Labrum (Fig. 3A) subrounded; subproximal arc of setae on dorsal surface with only a restricted number of long setae. Right and left mandibles (Fig. 3C, D) with abundant tuft of setae between prostheca and mola. Maxilla (Fig. 3G) with a long 3-segmented palp. Labium (Fig. 3H) with glossae shorter and more slender than paraglossae; paraglossae broad and tapered; labial palp 3-segmented, segment II with a broad rounded thumb-like disto-medial projection; segment III conical and elongated. Legs (Fig. 4A) slender and elongated; tarsal claw (Fig. 4B) moderately elongated with 2 rows of abundant small denticles. Median caudal filament $< \frac{2}{3}$ of length of cerci.

Male imago.—Forewing (Figs 5A, 6A) hyaline, single intercalary veins reduced in size, cross-veins without colored margination. Hindwing absent. Gonopods (Figs 5B, 6B) 2-segmented, moderately elongated; segment I broad with a bulbous projection; segment II triangular. Abdomen without any pattern or peculiar coloration.

Etymology.—The genus name is a combination of the French abbreviation for deoxyribonucleic acid (ADN), the analysis of which enabled us to link adults and larvae of the species, and the suffix *ptilum*, which is used for several other genera of Afrotropical Baetidae.

Description of larva

Head (Fig. 3A–H).—Antennae very long. Labrum (Fig. 3A) subrounded with subproximal arc of setae formed only by 1 central and 2 lateral long setae: Right mandible (Fig. 3C): canine with 2 sets of stout incisors partially fused, stout prostheca with minute denticles apically, abundant tuft of setae between prostheca and mola. Left mandible (Fig. 3D): canine with 1 set of stout incisors; stout prostheca with minute denticles and a comb-shaped structure apically; abundant tuft of setae between prostheca and mola; mola with a restricted number of stout denticles. Maxilla (Fig. 3G): apically with 4 elongated teeth none of them opposed to others, with at their base a row of about 5 long setae; long 3-segmented palp, segment II and III partially fused, segment III short and conical. Labium (Fig. 3H): glossae triangular, shorter than paraglossae; paraglossae broad and tapered, inner margin concave apical margin with 3 rows of long setae; labial palp 3-segmented, segment I slender, segment II with a broad apically rounded thumb-like disto-medial projection, segment III conical and slender with inner margin concave.

Thorax (Fig. 4A, B).—Hindwing pads absent in both male and female larvae in all known species. Legs (Fig. 4A) long and slender; femora with dorsal and ventral margins parallel, dorsal margin with reduced number of short setae, femoral patch completely absent; dorsal margins of tibiae and tarsi almost bare, ventral margins of tibiae and tarsi with abundant stout and medium setae; subproximal arc of setae completely absent on tibiae and tarsi; tibio-patellar suture absent on foretibia; tarsal claws (Fig. 4B) moderately elongated with 2 rows of abundant minute denticles; subapical setae absent.

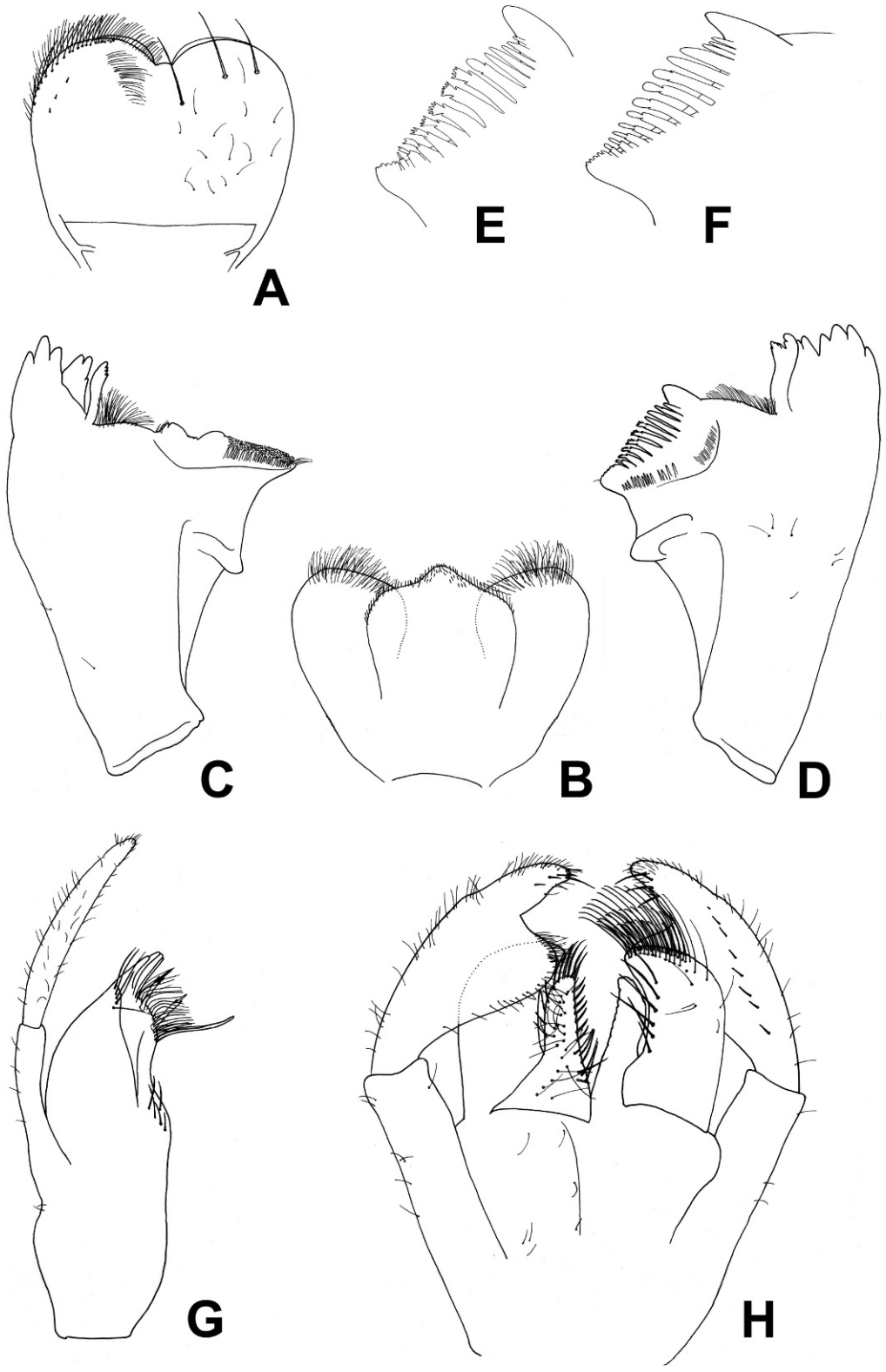

FIG. 3. A–E, G, H.—*Adnoptilum ottkei* sp. n., larva. F.—*Adnoptilum thomasorum*, larva. A.—Labrum (left: ventral, right: dorsal). B.—Hypopharynx. C.—Right mandible. D.—Left mandible. E.—Left mola. F.—Left mola. G.—Right maxilla. H.—Labium.

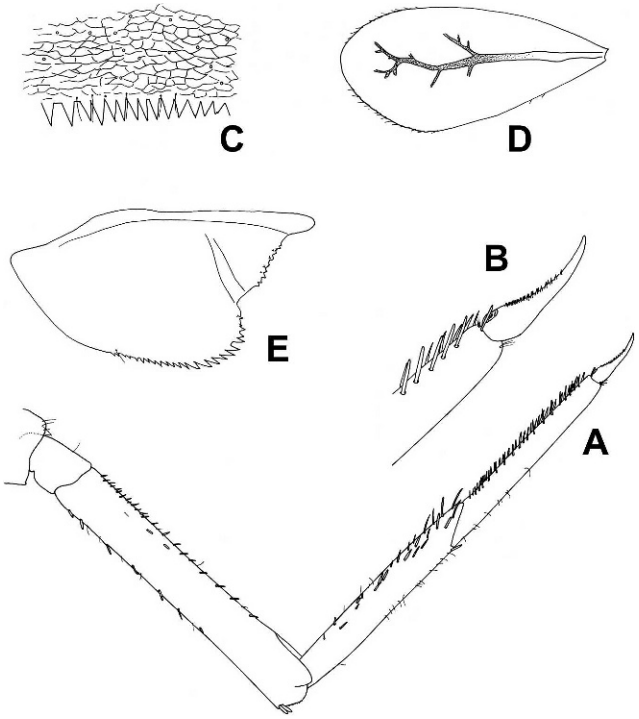

FIG. 4. *Adnoptilum ottkei* sp. n., larva. A.—Foreleg. B.—Tarsal claw. C.—Distal margin of tergum IV. D.—Gill IV. E.—Paraproct.

Abdomen (Fig. 4C–E).—Distal margins of terga (Fig. 4C) with well developed triangular spines. No spines on lateral margins of terga. 7 pairs of single gills (Fig. 4D), elongated and relatively symmetrical.

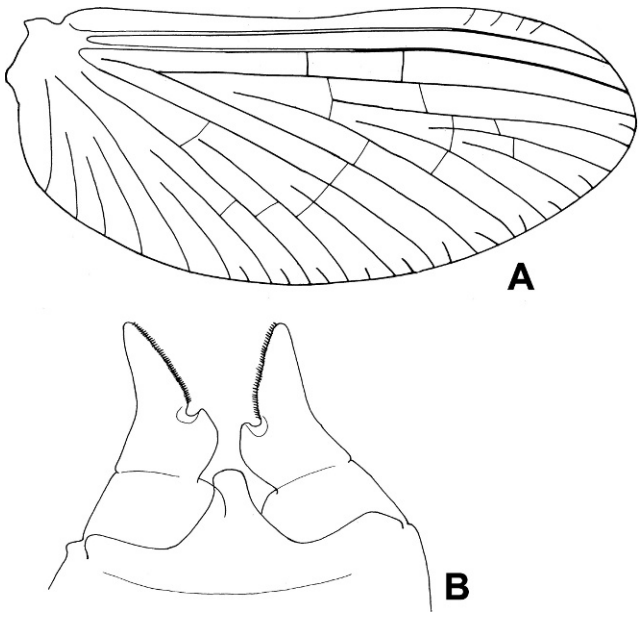

FIG. 5. *Adnoptilum ottkei* sp. n., male imago. A.—Forewing. B.—Male genitalia.

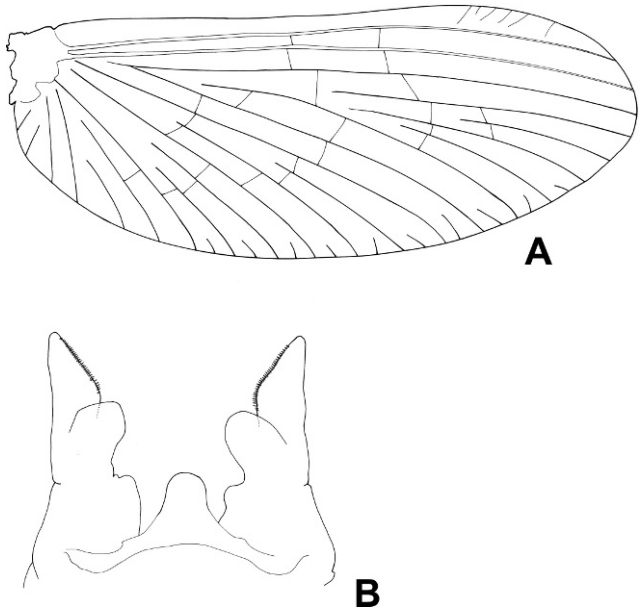

FIG. 6. *Adnoptilum thomasorum*, male imago. A.—Forewing. B.—Male genitalia.

Paraproct (Fig. 4E) with abundant medium spines along margin. Cerci long, median caudal filament $< \frac{2}{3}$ of length of cerci.

Description of imago

Turbinate eyes brown or purple. Forewing (Figs 5A, 6A) hyaline, single intercalary veins reduced in size or absent, cross-veins without colored margination. Hindwing absent. Gonopods (Figs 5B, 6B) 2-segmented, compact, moderately elongated; segment I broad with a bulbous projection, without additional triangular lobe dorsally; segment II triangular, inner margin covered with stout setae. Abdomen without any pattern or coloration.

Discussion

Larvae of *Adnoptilum* can be separated easily from other Afrotropical genera by the general aspect of the larvae. The body is tubular and extremely slender with only the mesothorax presenting a dorsal hump. The mouthparts are well separated from the head. The mouthparts of *Adnoptilum*, especially the mandibles (Fig. 3C, D), maxilla (Fig. 3G), and hypopharynx (Fig. 3B) are quite similar to those of *Cheleocloeon*, *Delouardus*, and *Bugilliesia* (Lugo-Ortiz and McCafferty 1999, Gattolliat et al. 2009). The labrum of *Adnoptilum* (Fig. 3A) has a setation and general shape that is similar to *Cheleocloeon* and *Delouardus* but differs significantly from *Bugilliesia* by the degree of development of the subproximal arc of setae (Gattol-

liat et al. 2009). In these 4 genera, the 2nd segment of the labial palp (Fig. 3H) presents a thumb-like disto-medial process. The shape and the degree of development of this process present important variations. This character is very important for specific identification among the genera *Bugilliesia* and *Cheleocloeon* but does not constitute a generic character (Gattolliat and Sartori 2008, Gattolliat et al. 2009). The shape of the 3rd segment of the labial palp is more stable among genera. *Adnoptilum* possesses a long and slender 3rd segment with a concave inner margin (Fig. 3H), whereas this segment is shorter and much broader in *Cheleocloeon*, *Delouardus*, and especially *Bugilliesia*. The legs of *Adnoptilum* (Fig. 4A) also are unusually long and slender. This character is the clearest way to separate *Adnoptilum* from *Delouardus* (Lugo-Ortiz and McCafferty 1999), and, to a lesser extent, from *Bugilliesia* and *Cheleocloeon* (Gattolliat et al. 2009). The legs of *Adnoptilum* appear more similar to those of *Demoulinia* and *Securiops*, but other morphological characters (e.g., mouthparts) are completely different between these 3 genera (Gattolliat 2003, Jacobus et al. 2006). *Bugilliesia*, *Cheleocloeon*, *Delouardus*, *Demoulinia*, and *Securiops* all possess relatively short cerci and median caudal filament subequal to cerci, whereas *Adnoptilum* possesses long cerci and median caudal filament much shorter than cerci. *Adnoptilum* can be easily separated from *Cloeon* and related genera by the presence/absence of spines on the lateral margins of the abdomen, the abdominal gills with simple or double lamellae, the shape of the labial palp, and the shape and setation of the labrum (Kluge and Novikova 1992).

Adnoptilum ottkei Gattolliat and Monaghan sp. n.

Figs 3A–E, G, H, 4A–E, 5A, B

Type material

Holotype, 1 larva; P0865: Madagascar, Rianila Bas, Loc Andasibe, Affl de Sahatandra Riv; 48°24′54″E/18°55′53″S; Alt 975m; 10.04.1999; J-L Gattolliat and N Raberiaka. Paratypes, 1 female larva on slide (865d), 1 male larva on slide (865e), 6 larvae; P0865: same data as holotype. 2 larvae (1 larva on slide), 12 male subimagos; P0373: same locality as holotype; 12.04.1995; M Sartori and L Ruffieux. 1 female larva on slide (380b) and 4 larvae; P0380: Madagascar, Mangoro Bas, Loc 1 km de Sahafitahana, Affl de Mangoro Riv; 48°13′39″E/18°59′42″S; Alt 880m; 26.04.1995; M Sartori and L Ruffieux. 14 male subimagos (1 male subimago on slide); P0557: Madagascar, Rianila Bas, Loc Réserve d'Andasibe, Petite rivière de Forêt; 48°25′14″E/18°54′37″S; 15.01.1996; J-M Elouard and R Oliarinony. 1 larva on slide; P0766: Madagascar, Mangoro Bas, Loc PK 20,4 route Anosibe an'ala, Affluent non nommé; 48°14′00″E/19°05′53″S; Alt 960m; 23.10.1998; JP Benstead and J Legrand. 2 larvae; P0768: Madagascar, Mangoro Bas, Loc PK 13 route Anosibe an'ala, Affluent non nommé; 48°13′57″E/19°03′00″S; Alt 895m; 24.10.1998; JM Elouard and J Legrand. 9 larvae (1 female larva on slide); P2005: Madagascar, Mangoro Bas, Loc PK 20,4 route Anosibe an'ala, Affluent non nommé; 48°14′00″E/19°05′53″S; Alt 960m; 03.04.2003; mision MZL and Monaghan. 2 male larvae on slide (2006A and 2006B) and 3 larvae; P2006: Madagascar, Mangoro Bas, Loc PK 20,4 route Anosibe an'ala, Affluent non nommé; 48°14′00″E/19°05′53″S; 03.04.2003; mision MZL and Monaghan.

Other material

2 larvae (1 larva on slide); P0047: Madagascar, Betsiboka Bas, Loc Andakana, Andranolava Riv; 47°09′59″E/18°16′27″S; Alt 1300m; 18.04.1991; ORSTOM Antananarivo. 2 male imagos on slides (405-19 and 405-20) and 3 male imagos; P0405: Madagascar, Bassin côtier Fort Dauphin/Manampanihy, Loc Belavenoka, Antorendrika Riv; 47°05′02″E/24°50′18″S; Alt 20m; 23.04.1995; JM Elouard and T Pilaka. 1 female larva on slide (427b) and 4 larvae; P0427: Madagascar, Onilahy Bas, Loc Ampandrabe, Sahavatoy Affl de l'Ilanana Riv; 45°43′45″E/22°38′24″S; Alt 910m; 26.05.1995; JM Elouard and R Oliarinony. 1 female larva on slide (488b) and 1 larva; P0488: Madagascar, Betsiboka Bas, Loc Amboasary, Amberomanga Riv; 47°56′17″E/18°28′03″S; Alt 1270m; 19.10.1995; FM Gibon. 2 male imagos (1 male imago on slide); P0573: Madagascar, Bassin côtier Fort Dauphin/Manampanihy, Anandrano Riv; 46°58′53″E/24°56′43″S; Alt 10m; 04.02.1996; FM Gibon and D Randriamasimanana. 2 male imagos (1 male imago on slide); P0661: Madagascar, Betsiboka Bas, Loc Anjozorobe, Mananara Riv; 47°52′53″E/18°24′47″S; Alt 1220m; 16.11.1996; R Oliarinony. 2 larvae (1 larva on slide); MD 048: Madagascar, Betroka (Tulear), right Affl of Riv Mangoky about 1 km NE village; Alt. 830m; 22.3°C, 0.139 mS/cm; 25.08.2001; Gerecke & Goldschmidt. 2 larvae (1 larva on slide); MD 051: Madagascar, Betroka (Tulear), aquaeduct crossing the spring stream of right Affl of Riv Mangoky NE village (MD 049); 830m; 21.2°C, 0.146 mS/cm; 26.08.2001; Gerecke & Goldschmidt.

Abbreviations.—Bas = basin, Riv = river or stream, Affl = tributary, Loc = locality, Alt = altitude.

Etymology

Named after Douglas P. Ottke, who has provided invaluable help collecting mayflies while a member of 2 research expeditions to Madagascar.

Description of larva

Maximal length.—Body 5.9 mm. Cerci 4.5 mm. Median caudal filament 2.7 mm.

Color.—Head uniformly medium amber brown, with vermiform marking on vertex and frons; antennae yellow cream; turbinate eyes light honey brown. Thorax amber brown with yellow pattern. Legs yellow except femora with brown transverse stripe in the distal quarter. Terga II, III, V, VI, VIII, and IX amber brown with yellow transversal stripe proximally; tergum I laterally amber brown and proximally and medially yellow; terga IV and VII yellow with an amber brown stripe distally; tergum X yellow. Sterna yellow, laterally and distally amber brown; sternum VII generally totally yellow; sternum generally totally amber brown. Cerci and median caudal filament uniformly yellow with a dark brown stripe at ⅓ of length of cerci.

Head (Fig. 3A–E, G, H).—Dorsal surface of the labrum (Fig. 3A) with thin setae scattered over surface; distal margin bordered with 2 rows of simple setae; ventral surface with 3 minute pointed setae apico-laterally. Hypopharynx (Fig. 3B) with superlingua with a small triangular and apically rounded projection covered with minute setae apically; lingua subequal in length to superlingua, apically with long thin setae. Right mandible (Fig. 3C) with 2 sets of incisors, outer and inner sets of 3 denticles; prostheca with 4 minute denticles apically; tuft of setae at the apex of the mola present; basal ½ almost without setae dorsally. Left mandible (Fig. 3D) with 1 set of incisors, with 6 denticles; mola (Fig. 3E) with 12 to 16 denticles, at least the medium ones, pectinate; tuft of setae at the apex of the mola absent or reduced to a single seta; basal ½ with a few thin setae dorsally. Maxillae (Fig. 3G): 1 row of setae slightly shorter than teeth, with 2 multipointed dentisetae and single long and very stout setae distally; row of 5 setae at base of galea; 1 long seta perpendicular to margin of galea; maxillary palp segment I ~0.8× length of segments II and III combined. Labium (Fig. 3H) with glossae shorter and much more slender than paraglossae; margin of glossae with medium stout setae, ventral surfaces with numerous long thin setae; paraglossae subrectangular; distal margin with 3 rows of long simple setae, inner margin with a row of 6 long stout setae; labial palp segment I with a few thin setae laterally, subequal in length to segments II and III combined; segment II with disto-medial projection with apical margin slightly concave, inner margin with numerous small thin setae, a longitudinal row of 8 setae dorsally decreasing in length toward the apex; segment III subconical, longer than broad, inner margin slightly concave, with a few medium pointed stout setae and several small thin setae.

Thorax (Fig. 4A, B).—Forelegs (Fig. 4A), coxae without setae. Femora: dorsal margin with a row of ~7 short pointed setae, absent in the distal ⅓; without row of setae subparallel to dorsal margin; dorso-apical setal patch formed by 2 small blunt setae; anterior face without setae; ventral margin with short pointed setae. Dorsal margin of tibiae with only a few small thin setae; ventral margin with short to medium stout setae. Dorsal margin of tarsi with only a few thin setae proximally; ventral margin with a row of ~20 blunt simple setae and one row of ~25 pointed simple setae; tarsal claws (Fig. 4B) ~0.3× length of tarsi, with 2 rows of ~15 minute pointed teeth.

Abdomen (Fig. 4C–E).—Terga (Fig. 4C) shagreen, with traces of insertion of setae, without scale bases; posterior margin with regular, pointed spination. Gills (Fig. 4D), poorly tracheated, serrated apically with short thin setae. Paraproct (Fig. 4E) without scale bases, margin with ~25 pointed spines increasing in length apically; posterolateral extension without scale bases, margin with small spination. Cerci with numerous extremely thin setae on inner margin.

Description of male imago

Maximal length.—Body 3.7–4.7 mm. Forewing 3.8–4.7 mm.

Head.—Uniformly light brown. Turbinate eyes broad apically, honey to yellow brown, darker laterally. Antennae light brown.

Thorax (Fig. 5A).—Light brown. Forewing with single intercalary veins at least shorter than intervein space, absent from 2 or 3 apical and 5 proximal interspaces; pterostigma with about 4 crossveins not reaching subcostal vein. Legs without visible markings.

Abdomen.—Terga colorless, without marking. Sterna colorless.

Genitalia (Fig. 5B).—Basal lobe well developed and apically flat; small concavity at junction of segment I and II.

Distribution and habitat

Adnoptilum ottkei is endemic to Madagascar and was mainly collected in the region of Moramanga and Andasibe, ~100 km east of Antananarivo in the Mangoro and Rianila basins, but it also occurs in the western, northern, and southern regions. The species is widely distributed in Madagascar, but based on >1000 sampling events throughout Madagascar (Elouard et al. 2008), the species is only present in a restricted number of localities. Nonetheless, it can be locally abundant in small-to-medium streams with sand or gravel beds.

Adnoptilum thomasorum (Lugo-Ortiz and McCafferty, 1997) n. comb.

Figs 3F, 6A, B

Material examined

1 male imago on slide; P0148: Madagascar, Rianila Bas, Loc Ambodirina, Sahatandra Riv; 48°20′28″E/19°01′32″S; 04.04.1992; ORSTOM Antananarivo. 11 male subimagos; P0373: Madagascar, Rianila Bas, Loc Andasibe, Affl de Sahatandra Riv; 48°24′54″E/18°55′53″S; Alt 975m; 12.04.1995; M Sartori and L Ruffieux. 2 male subimagos; P0529: Madagascar, Manampanihy Bas, Loc Bevoay, Andranohela Riv; 46°49′25″E/24°40′00″S; Alt 100m; 22.11.1995; J-M Elouard. 2 male subimagos (1 male subimago on slide); P0557: Madagascar, Rianila Bas, Loc Réserve d'Andasibe, Petite rivière de Forêt; 48°25′14″E/18°54′37″S; 15.01.1996; J-M Elouard and R Oliarinony. 9 male imagos and 2 male subimagos (1 male imago on slide); P0766: Madagascar, Mangoro Bas, Loc PK 20,4 route Anosibe an'ala, Affluent non nommé; 48°14′00″E/19°05′53″S; Alt 960m; 23.10.1998; JP Benstead and J Legrand. 1 male larva on slide; P2004: Madagascar, Rianila Bas, Loc Réserve d'Andasibe, Petite rivière de Forêt; 48°25′14″E/18°54′37″S; 03.04.2003; mision MZL and Monaghan. 1 male larva on slide (2006C); P2006: Madagascar, Mangoro Bas, Loc PK 20,4 route Anosibe an'ala, Affluent non nommé; 48°14′00″E/19°05′53″S; 03.04.2003; mision MZL and Monaghan.

Description of larva

Maximal length.—Body 3.5 mm. Cerci and median caudal filament broken.

Color.—Head uniformly medium amber brown, without vermiform marking; turbinate eyes dark brown. Prothorax amber brown with 2 lateral dark brown spots; mesothorax yellow with a central dark brown spot and dark brown lateral markings. Legs yellow except femora with a brown transverse stripe in the distal quarter. Terga dark brown with 2 yellow central spots near proximal margin; terga IV, VII, and X lighter uniformly amber brown; tergum X yellow. Sterna dark brown except proximally and laterally yellow; sternum VII much lighter. Cerci and median caudal filament yellow with dark brown band. Morphology similar to *Adnoptilum ottkei* except mola region of the left mandible (Fig. 3F) with 10 to 15 denticles all of them apically rounded and undivided.

Description of male imago

Maximal length.—Body 4.0–5.2 mm. Forewing 3.8–4.8 mm.

Head.—Uniformly light brown. Turbinate eyes broad apically, dark purple brown. Antennae light brown.

Thorax (Fig. 6A).—Medium yellowish brown. Forewing with single intercalary veins at least shorter than intervein space, absent from 2 or 5 apical and 5 proximal interspaces; pterostigma with about 6 crossveins not reaching subcostal vein. Legs without visible marking.

Abdomen.—Terga colorless, without marking. Sterna colorless.

Genitalia (Fig. 6B).—Basal lobe well developed and apically rounded, no visible concavity between segments I and II.

Discussion

The 2 species of *Adnoptilum* are quite similar. They can be distinguished at all stages by the coloration of male turbinate eye, honey brown in *A. ottkei*, whereas dark brown in alcohol and purple brown in living specimens in *A. thomasorum*; at the larval stage by the general coloration of the body, darker and more contrasted in *A. thomasorum*, lighter and almost homogenous in *A. ottkei*. Mouthparts, legs, and other parts generally used to separate the different species of Baetidae do not present any reliable difference for specific identification; only the apex of the denticles of the mola of the left mandible constitutes a useful character to separate the 2 species: the apex of these denticles is pectinate in *A. ottkei* (Fig. 3E), whereas it is rounded and undivided in *A. thomasorum* (Fig. 3F). In the imaginal stage, the hindwing presents variation between specimens, but it is difficult to use these as reliable characters for specific identification. The male gonopods are also slightly different; the basal lobe of the gonopods is less developed in *A. ottkei* (Fig. 5B) than in *A. thomasorum* (Fig. 6B). *Adnoptilum ottkei* is generally larger than *A. thomasorum*. However, only the male imago can be identified with certainty except when the 2 species co-occur in the same locality.

Distribution and habitat

Adnoptilum thomasorum was originally described from a single male imago collected at Andasibe. Most of the material we examined came from the Moramanga–Andasibe region, where *Adnoptilum thomasorum* co-occurs with *A. ottkei*. Additional specimens were collected in the extreme South of Madagascar in the Manampanihy basin. However, only male subimagos are known from this area, consequently the specific attribution remains provisional and is only based on the turbinate eye coloration. *Adnoptilum thomasorum* seems to live in the same habitat as *A. ottkei*, but is generally less abundant.

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