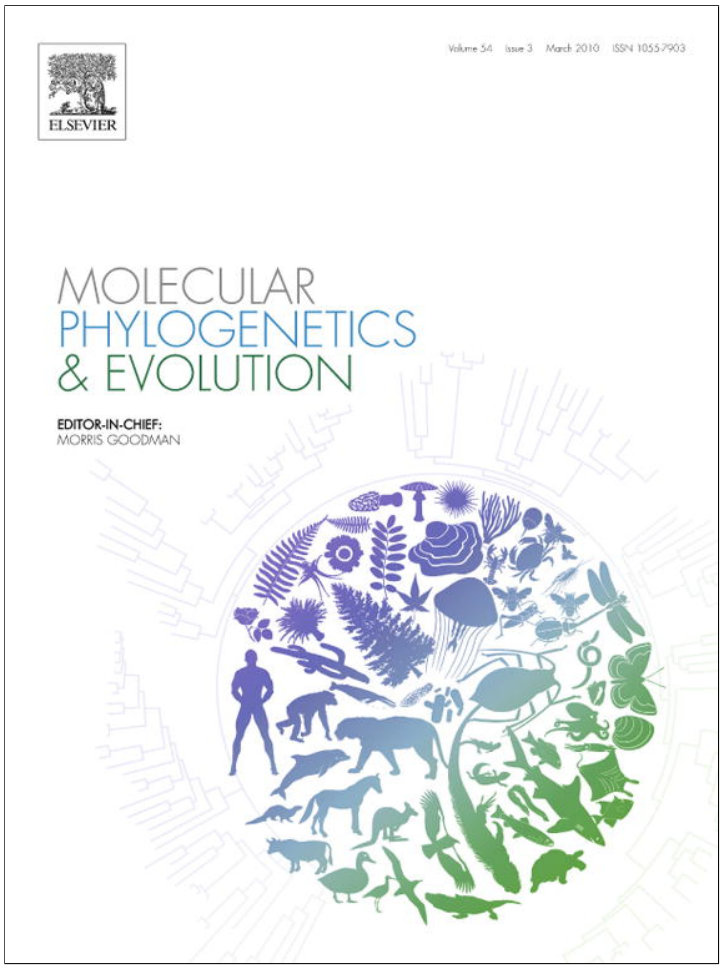

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Red drifters and dark residents: The phylogeny and ecology of a Plio-Pleistocene dragonfly radiation reflects Africa's changing environment (Odonata, Libellulidae, *Trithemis*)

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ABSTRACT

In the last few million years, tropical Africa has experienced pronounced climatic shifts with progressive aridification. Such changes must have had a great impact on freshwater biota, such as Odonata. With about forty species, *Trithemis* dominates dragonfly communities across Africa, from rain-pools to streams, deserts to rainforests, and lowlands to highlands. Red-bodied species tend to favor exposed, standing and often temporary waters, have strong dispersal capacities, and some of the largest geographic ranges in the genus. Those in cooler habitats, like forest streams, are generally dark-bodied and more sedentary. We combined molecular analyses of ND1, 16S, and ITS (ITSI, 5.8S, and ITSII) with morphological, ecological, and geographical data for 81% of known *Trithemis* species, including three Asian and two Madagascan endemics. Using molecular clock analyses, the genus's origin was estimated 6–9 Mya, with multiple lineages arising suddenly around 4 Mya. Open stagnant habitats were inferred to be ancestral and the rise of *Trithemis* may have coincided with savannah-expansion in the late Miocene. The adaptation of red species to more ephemeral conditions leads to large ranges and limited radiation within those lineages. By contrast, three clades of dark species radiated in the Plio-Pleistocene, each within distinct ecological confines: (1) lowland streams, (2) highland streams, and (3) swampy habitats on alternating sides of the Congo-Zambezi watershed divide; together giving rise to the majority of species diversity in the genus. During *Trithemis* evolution, multiple shifts from open to more forested habitats and from standing to running waters occurred. Allopatry by habitat fragmentation may be the dominant force in speciation, but possibly genetic divergence across habitat gradients was also involved. The study demonstrates the importance of combining ecological and phylogenetic data to understand the origin of biological diversity under great environmental change.

1. Introduction

Comparative phylogenetic and phylogeographic studies provide insights into the evolutionary consequences of environmental change during the Pliocene and Pleistocene, but our understanding of these processes largely relies on studies from the Northern Hemisphere. The recurrent formation of perennial ice over vast areas during glacial maxima caused the contraction of entire biotas into southern refugia, with subsequent expansion at each interglacial (Avise and Walker, 1998; Avise, 2000; Hewitt, 2004). These cycles are expected to be promoters for speciation. Molecular clock estimates roughly place the origin of extant species in many groups in this period (Brower, 1994; Klicka and Zink, 1997; Avise, 2000; Knowles, 2000; Ribera et al., 2004). In tropical regions most species divergence was also estimated to have taken place in the Pliocene (e.g. Hewitt, 2000; Moritz et al., 2000; Bell et al., 2007; de Paula et al., 2007).

The African continent experienced pronounced climatic shifts with the tendency to aridification especially in the last 5 million years. Alternating drier and wetter periods from the beginning of the Miocene resulted in major changes in the distribution and composition of the vegetation (Morley, 2000). The rainforest belt, which covered central Africa almost entirely 30 Mya (million years ago), decreased dramatically as savannahs expanded (Morley, 2000; Jacobs, 2004; Sepulchre et al., 2006). Different speciation models are proposed to explain the high diversification during these periods (reviewed in Moritz et al., 2000). The refugia model suggests speciation in allopatry, with forest species restricted to refuges separated by dry habitat, or vice versa. The riverine model

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suggests that large rivers are barriers for gene flow. In the gradient model, abrupt environmental transitions, e.g. between forest and savannah, force adaptive divergence and consequent speciation. Although the world's highest level of biodiversity resides in the tropics, especially in rainforests, and rainforest fragments and their borders have been discussed as centers of speciation (Fjeldsa and Lovett, 1997; Moritz et al., 2000; Schilthuizen, 2000), we have only begun to understand the evolution of this diversity in its historical complexity. One question, for example, is how often speciation coincided with certain habitat shifts, e.g. from forest to open habitat or vice versa.

While several studies deal with the radiation of terrestrial animals like squirrels, guenons, cobras, frogs, and birds (Fjeldsa and Lovett, 1997; Steppan et al., 2004; Tosi et al., 2005; Wuester et al., 2007; Blackburn, 2008), far less is known about the consequences of the climatic shifts for the freshwater fauna. Aridification should directly affect the aquatic fauna, leading to isolation in less and extinction in more affected areas (Daniels et al., 2006; Seehausen, 2006; Katongo et al., 2007; Koch et al., 2007). Moreover, it changes the character of aquatic habitats and their abundance, with permanent and flowing water becoming relatively scarce. Because more ephemeral habitats require better dispersal and faster development of their inhabitants, this affects species ecology, inducing larger ranges and more gene flow, which may diminish speciation potentials. Predominantly lentic groups (i.e. mainly occurring in standing water) have species with larger ranges, more dispersal, and possibly lower speciation rates than lotic (flowing) ones (Ribera et al., 2001; Monaghan et al., 2005; Hof et al., 2006; Suhling et al., 2009).

Amphibious insects like Odonata, Ephemeroptera, and Plecoptera require aquatic larval and terrestrial adult habitats. Thus climatic change affects them both above and below the surface. All odonates (dragonflies and damselflies) are associated with freshwater, although their habitat requirements range from opportunistic to highly specialized. Vulnerability to alterations of both aquatic and terrestrial habitats makes them a suitable model to study the effects of the changing environment and increasing aridity during the Plio-Pleistocene. With about 850 extant species, the Afrotropical odonate fauna is poor compared to the American and Asian tropics (Dijkstra and Clausnitzer, 2006). Africa's unstable climatic history is suggested to have lead to the demise of much of the original fauna, with rather few relicts remaining in some isolated stable areas, but also to the recent rise of a speciose but rather homogeneous fauna (Clausnitzer, 2003; Dijkstra, 2007; Kalkman et al., 2008). Indeed, libellulid dragonflies and coenagrionid damselflies, the two odonate families best adapted to unstable habitats, are notably dominant in tropical Africa (Dijkstra and Clausnitzer, 2006).

To learn more about the possible impact of climatic shifts on the evolution and diversity of freshwater organisms in Africa, we analyzed the phylogeny of the libellulid genus *Trithemis*, which dominates present-day odonate communities across Africa. Aside from about 40 continental African species (a few classified in probably synonymous genera), the genus includes five Asian and two Madagascan endemic species (Pinhey, 1970; Dijkstra, 2007). The species occupy most freshwater habitats in tropical Africa and Asia, from cool permanent streams to warm temporary pools, from desert to rainforest, and from lowlands to highlands. In association with such different habitat preferences, they differ in their dispersal capacities and coloration: species of open, often temporary, habitats are often bright red and disperse well, while those of more sheltered permanent conditions tend to be dark-bodied and probably more sedentary (pers. obs. K.-D.B. Dijkstra).

To understand this diversification we combine phylogenetic information with morphological, ecological and geographical data. By means of molecular clock analyses we intend to estimate the origin of the genus and timing of its main radiation. We investigate (1) how speciation is associated with past environmental change, (2) the direction, frequency, and relative importance of changes in the ecology of the species, such as shifts from forest to non-forest and lotic to lentic habitats (or vice versa), and (3) how these ecological characteristics are related.

2. Material and methods

2.1. Specimens examined

A total of 164 individuals of 38 species (81% of those thought to belong to *Trithemis*) were analyzed and 92 individuals covering all species were selected for the final alignment. *Porpacithemis trithemoides* (= *Anectothemis apicalis*) was included as an ingroup taxon because it is suspected to belong in *Trithemis*. Two individuals of *Pantala flavescens* were used as the outgroup, because phylogenetic studies of the Libellulidae showed that it is closely related to *Trithemis* (Ware et al., 2007; Pilgrim and Von Dohlen, 2008). In Table 1 the country and number of analyzed individuals per species are listed.

2.2. Choice of the sequence markers

To date the emergence of species, the applied genetic markers have to provide high parsimony-informative phylogenetic signals but the misleading effects of homoplasy or convergence have to be low (Collins et al., 2005). Three molecular markers were chosen: (1 and 2) Two mitochondrial genes; the NADH-dehydrogenase subunit 1 (ND1) and 16S rDNA, which show different evolutionary rates. Mitochondrial protein coding genes (like ND1) evolve up to three times faster than 12S and 16S (Knowlton and Weigt, 1998) and provide a good resolution for recently diverged species. In contrast, 16S is more appropriate for analyzing earlier speciation processes. (3) The nuclear internal transcribed spacer regions I and II including the 5.8S region in between (here simply named ITS). This fragment was successfully used for phylogenetic analyses in Libellulidae before (Hovmoller and Johansson, 2004). The three regions have different substitution rates: ITS I is highly variable, ITS II variable, and 5.8S highly conserved due to the typical proofreading mechanisms of nuclear genes. With ND1, 16S, and ITS a range of different substitution rates was covered to overcome difficulties with resolution and polytomy.

2.3. DNA extraction, amplification, and sequencing

DNA was extracted from single legs using a modified phenol–chloroform extraction (Hadrys et al., 1992) and stored at −20 °C. The ND1 fragment was amplified and sequenced with the primer pair P 850 fw and P 851 rev described in Abraham et al. (2001). The PCR product contained 610 bp and included a 5′ partial fragment of the 16S rDNA fragment, the $tRNA^{Leu}$ and a 3′ partial fragment the ND1 gene region. The PCR regime consisted of 30 cycles 95 °C for 30 s, 48 °C for 30 s, 72 °C for 1 min, an initial denaturation for 2 min at 95 °C and a final extension of 6 min at 72 °C. The reaction mixtures contained 2.5 mM $MgCl_2$, 1× Buffer (Invitrogen), 10 pmol of each primer, 0.1 mM dNTP, 0.75 U Taq DNA polymerase (Invitrogen), and 1–10 ng DNA template in a final volume of 25 μl. For 16S a 570 bp fragment was amplified with primers described in Simon et al. (1994). The PCR thermal regime was as follows: 5 min initial denaturation at 93 °C, followed by 35 cycles of 93 °C for 20 s, 52 °C for 30 s, 72 °C for 40 s, and 2 min extension at 72 °C. PCR was carried out in a total volume of 25 μl, containing 1× amplification buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.1 mM dNTPs, 5 pmol each primer, and 0.75 U *Taq* DNA polymerase (Invitrogen). For the nuclear

Table 1
Country and number of the sampled individuals as well as specific character states of each species. Abbreviations are as follows: *water body:* (a) strong flow, especially streams and rapids; (b) weak or variable flow, like calm rivers or stagnant section in streams; (c) standing waters, typically permanent; (d) standing waters with tolerance for temporary conditions; *landscape:* (a) forest shade; (b) sunny sites within forest, like openings; (c) half-open or patchy habitats, i.e. shaded in rather open and exposed in more closed environments; (d) open, exposed habitats; (e) open habitats in cooler climes, like highlands or Cape region; *range:* (>am, >m) species widespread in Africa and extending to Eurasia and/or Madagascar; (A) confined to Asia; (C) centered on central African forest, especially Congo Basin; (E) eastern and southern Africa; (Eth) endemic to Ethiopian highlands; (M) endemic to Madagascar; (N) centered on northern savannahs; (Ph) restricted to Philippines; (W) centered on western African forest; (Z) centered on 'Zambezian' swamps from Katanga to Botswana.

Species	Country	*n*	Color	Water body	Landscape	Range
T. aconita	Liberia	5	Dark	(b) Weak flow	(c) Half-open	N + E
T. adelpha	Philippines	2	Red	(d) Temporary	(d) Open	Ph
T. aenea	Cameroon	2	Dark	(b) Weak flow	(b) Openings	W + C
T. aequalis	Botswana	3	Dark	(b) Weak flow	(d) Open	Z
T. africana	Liberia	2	Dark	(a) Strong flow	(a) Shade	W
T. annulata	Namibia	10	Red	(d) Temporary	(d) Open	>am
T. arteriosa	Namibia	10	Red	(d) Temporary	(d) Open	>am
T. aurora	China	2	Red	(d) Temporary	(d) Open	A
T. basitincta	Liberia	2	Dark	(a) Strong flow	(a) Shade	W
T. bifida	Ghana	2	Dark	(a) Strong flow	(c) Half-open	N + E
T. bredoi	Ghana	2	Red	(b) Weak flow	(d) Open	N
T. brydeni	Botswana	1	Dark	(c) Standing	(d) Open	Z
T. dejouxi	Ghana	5	Dark	(a) Strong flow	(d) Open	N
T. dichroa	Congo-Kinshasa / Ghana	5	Dark	(a) Strong flow	(c) Half-open	W + C
T. donaldsoni	Namibia	10	Dark	(a) Strong flow	(d) Open	E
T. dorsalis	South Africa	3	Dark	(b) Weak flow	(e) Cooler	E
T. ellenbeckii	Ethiopia	2	Dark	(a) Strong flow	(e) Cooler	Eth
T. festiva	China	2	Dark	(a) Strong flow	(d) Open	A
T. furva	South Africa / Ethiopia	10	Dark	(a) Strong flow	(e) Cooler	>m
T. grouti	Liberia	8	Dark	(b) Weak flow	(c) Half-open	W + C
T. hartwigi	Cameroon	2	Red	(d) Temporary	(b) Openings	C
T. hecate	Namibia	3	Dark	(d) Temporary	(d) Open	>m
T. imitata	Liberia / Ghana	5	Red	(d) Temporary	(d) Open	N
T. kalula	Nigeria	1	Red	(b) Weak flow	(d) Open	N
T. kirbyi	Namibia	10	Red	(d) Temporary	(d) Open	>am
T. monardi	Botswana	3	Red	(d) Temporary	(d) Open	Z
T. morrisoni	Namibia	10	Dark	(a) Strong flow	(d) Open	Z
T. nuptialis	Congo-Kinshasa	3	Dark	(b) Weak flow	(b) Openings	C
T. palustris	Namibia	10	Dark	(c) Standing	(d) Open	Z
T. persephone	Madagascar	3	Red	(a) Strong flow	(b) Openings	M
T. pluvialis	South Africa	3	Red	(a) Strong flow	(e) Cooler	E
T. pruinata	Ghana	2	Dark	(a) Strong flow	(c) Half-open	W + C
T. selika	Madagascar	3	Red	(d) Temporary	(d) Open	M
T. sp. nov. nr *bifida*	Cameroon	2	Dark	(a) Strong flow	(a) Shade	C
T. stictica	Kenya	10	Dark	(b) Weak flow	(e) Cooler	>m
P. trithemoides	Congo-Kinshasa	1	Dark	(?) Unknown	(b) Openings	C
T. tropicana	Cameroon	3	Dark	(b) Weak flow	(b) Openings	C
T. werneri	Namibia	2	Red	(b) Weak flow	(d) Open	E
Pantala flavescens	Namibia	2	Red	(d) Temporary	(d) Open	>am

ITS region, primers were designed based on known insect sequences from GenBank. The forward primer (ITS-Odo fw: 5′CGT AGG TGA ACC TGC AGA AG3′) is located within the 18S rDNA and the reverse primer (ITS-Odo rev: 5′CTC ACC TGC TCT GAG GTC G3′) within the 28S rDNA region. Amplification was successful under the following conditions: Initial denaturation for 3 min by 95 °C, 35 cycles of 95 °C for 30 s, 60 °C for 40 s and 30 s at 72 °C, and a final extension at 72 °C for 3 min. The final volume of 25 μl contained 1× amplification buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.1 mM dNTPs, 5 pmol each primer, and 0.75 U *Taq* DNA polymerase (Invitrogen).

The amplified products were purified by ethanol precipitation. The sequencing reactions were carried out using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit and subsequently purified using Sephadex columns (Sigma). Bidirectional sequencing was conducted with PCR primers on an ABI PRISM 310 Genetic Analyzer according to manufacturers' protocol (Applied Biosystems).

2.4. Phylogenetic analyses

Sequences were assembled and edited using Seqman II (vers. 5.03; DNAStar, Inc.). Multiple sequence alignments were done with MUSCLE vers. 3.6 (Edgar, 2004) and manually edited using Quickalign (Müller and Müller, 2003). Because of its high nucleotide and length variation, the final ITS sequence alignment was obtained in two steps. First, with the help of an interim alignment done with CLUSTAL_X (Thompson et al., 1997), the software Pfold (Knudsen and Hein, 2003) inferred a consensus secondary structure based on the KH-99 algorithm (Knudsen and Hein, 1999). Second, the consensus structure was used as input constraint for a secondary structure analysis in RNAsalsa (Stocsits et al., 2008). Here an alignment was obtained by searching for potential nucleotide interactions in the sequences while taking into account thermodynamic interactions and compensatory/consistent substitutions.

Phylogenetic reconstructions were conducted using Maximum Parsimony (MP) and Bayesian analysis (BA) for each single gene and for a combined dataset. Parsimony analyses were performed in PAUP vers. 4.0b10* (Swofford, 2002) using heuristic searches (10,000 stepwise random additions with TBR branch-swapping) and clade support was estimated via 1000 bootstrap (BS) pseudoreplicates with ten random additions (Felsenstein, 1985). All characters were unordered and weighted equally and gaps were treated as fifth state. For BA, the best fitting nucleotide substitution model was selected for each data partition according to the Akaike Information Criterion (AIC) in Modeltest 3.7 (Posada and Crandall,

1998). BA was performed in MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003) and was run with 3,000,000 generations each and four Markov chains with default heating values. Two independent runs were performed and trees were sampled every 1000 generations. At completion, the runs were checked for convergence between each run and for the initial burn-in period determined by examining each of the run parameters for convergence and plotting log-likelihood scores against generation. The first 750 trees were discarded as the "burn-in" and the 2251 remaining trees of each independent run were used to calculate the consensus topology and the posterior probabilities (PP) for nodal support. In the combined analyses, the data of the three markers were partitioned and parameters unlinked to allow the assignment of the appropriate model for each gene partition.

2.5. Molecular clock analyses

In order to test the applicability of a molecular clock to evaluate the time of divergence between the species, Maximum Likelihood (ML) analyses with the appropriate evolution model were performed for ND1 and 16S with and without clock enforced. The Shimodaira–Hasegawa (Shimodaira and Hasegawa, 1999; Goldman et al., 2000) and the Kishino–Hasegawa tests (Kishino and Hasegawa, 1989) were used to investigate whether the topologies of the two ML trees were significantly different. The genetic distances of ND1 and 16S were then used for comparisons and for molecular divergence time estimates. The dating calculations were based on the mutation rates of 2.3% for ND1 and 1.4% for 16S as proposed for insect mitochondria (Brower, 1994) and applied in several other odonate studies (e.g. Turgeon et al., 2005; Stoks and McPeek, 2006).

In addition we performed ML for the combined dataset of ND1 and 16S with and without molecular clock enforced. The tree obtained with clock enforced including branch length was used as a fixed input tree for divergence time estimation using *r8s* vers. 1.7 (Sanderson, 2003). The absolute age of the basal *Trithemis* node was set to 10, according to the approximate age of the only possible *Trithemis* fossil: *T. pseudodistanti* thought to be 11.2–7.1 Myr old (Nel, 1991).

2.6. Morphological, ecological, and distributional data

With the purpose of investigating their development in *Trithemis* evolution, characters of adult male coloration, aquatic/larval habitat (i.e. water body), terrestrial/adult habitat (i.e. landscape), and distribution were recorded, based principally on the extensive field experience of the second author. Definitions and states per species are provided in Table 1. Habitat selection depends on various interrelated factors: the preferred water body is principally determined by permanence and degree of flow, the preferred landscape by micro- (degree of shading by vegetation) and macro-climate (altitude and latitude). This complexity impedes meaningful comparative analyses, therefore binary variables were prepared for that purpose as follows: the species have preference for (1) flowing (= Water body a + b) vs. standing water bodies (= c + d), and permanent (= a + b + c) vs. temporary conditions (= d); their main occurrence in forest (= Landscape a + b) vs. non-forest habitats (= c + d + e), some cover (= a + b + c) vs. entirely open habitats (= d + e), and warmer (= a + b + c + d) vs. cooler climes (= e); and have intercontinental (Range >am, >m) vs. regional ranges (other categories).

2.7. Ancestral state reconstruction and character correlation

For each binary character the transformations of their states (including reconstruction of ancestral states), as well as the correlation of characters, were analyzed with the program SIMMAP (Bollback, 2006). For the estimation of transformations and the correlation of characters the final 1000 sampled post-burn-in trees were used, for the ancestral state reconstruction the consensus tree of the BA (Fig. 1). The estimation of transformations was based on 100 realizations sampled from the priors for ten realizations of each tree. The ancestral state reconstructions were performed using a Bayesian mutational mapping approach (Huelsenbeck et al., 2003). Here random realizations of character histories consistent with the character states observed at the tips of the tree are used to estimate posterior probabilities for specific ancestral state reconstructions. Bias parameters for each binary character follow a discrete beta-distribution with $k = 19$ and shape parameter $\alpha = 1.0$. The overall evolutionary rate prior had the parameters $\alpha = 3.0$, $\beta = 2.0$, and $k = 60$. The trees were rooted with the outgroup *P. flavescens* and the total tree lengths were rescaled to one before placing the morphological prior.

The *D* statistics in SIMMAP were used to calculate the correlation between the states of each character. Here d_{ij} (association among specific states) represents the divergence of the observed association between states from the expected association if the characters evolved independently given all trees, branch lengths and character mappings. The posterior predictive *P* value was based on ten null realizations for each observed realization.

3. Results

3.1. Molecular analyses

A final alignment of 1565 bp fragment was obtained containing the following three gene regions: a 425 bp fragment of ND1, a 475 bp portion of the 16S rDNA and the ITS I and II with their intermediate 5.8S (665 bp). 93 sequences of ND1 were analyzed covering the 39 species and showed 196 variable and 186 parsimony-informative sites with two gaps in the $tRNA^{Leu}$ fragment. The HKY + I + G model was chosen as the best fitting evolutionary model as suggested by Modeltest. The 16S fragment, which was analyzed for the same 93 individuals, revealed 125 variable sites with 118 parsimony-informative characters. Here the TVM + I + G model was applied. The amplification of the ITS region failed for one species, *T. africana*, and thus the final alignment contained sequences of 91 individuals of 36 species. The alignment consisted of 292 bp of ITS I, 140 bp of 5.8S and 232 bp of ITS II. In total, the length of the sequences varied between 544 bp and 604 bp with at most 121 gaps (73 gaps in ITS I, 48 in ITS II). No gap was found in the 5.8S region. The alignment showed 295 variable positions with 277 parsimony-informative sites.

Pairwise genetic distances (corrected by the respective evolutionary models determined by Modeltest) were found to be highest in ND1 (ranging from 0.5% to 20.1%), followed by the ITS region (ranging from 0.7% to 14.5%) and lowest in 16S (0.4–9.3%).

3.2. Phylogenetic relationships

MP and BA were performed separately for ND1, 16S, and the ITS regions. Here phylogenetic relationships between some species or species groups could not be resolved clearly. Therefore the single locus analyses are not presented here. Two combined datasets were used for comparative phylogenetic analyses: (1) the two mitochondrial markers, ND1 and 16S, and (2) all three markers, with ITS data lacking for *T. africana* only.

MP for the mtDNA dataset revealed 496 most parsimonious trees [length, 1105; consistency index (CI) 0.422; retention index (RI) 0.819]. For the combined dataset 64 most parsimonious trees were found [length, 2678; CI 0.438; RI 0.805]. BA of the mtDNA

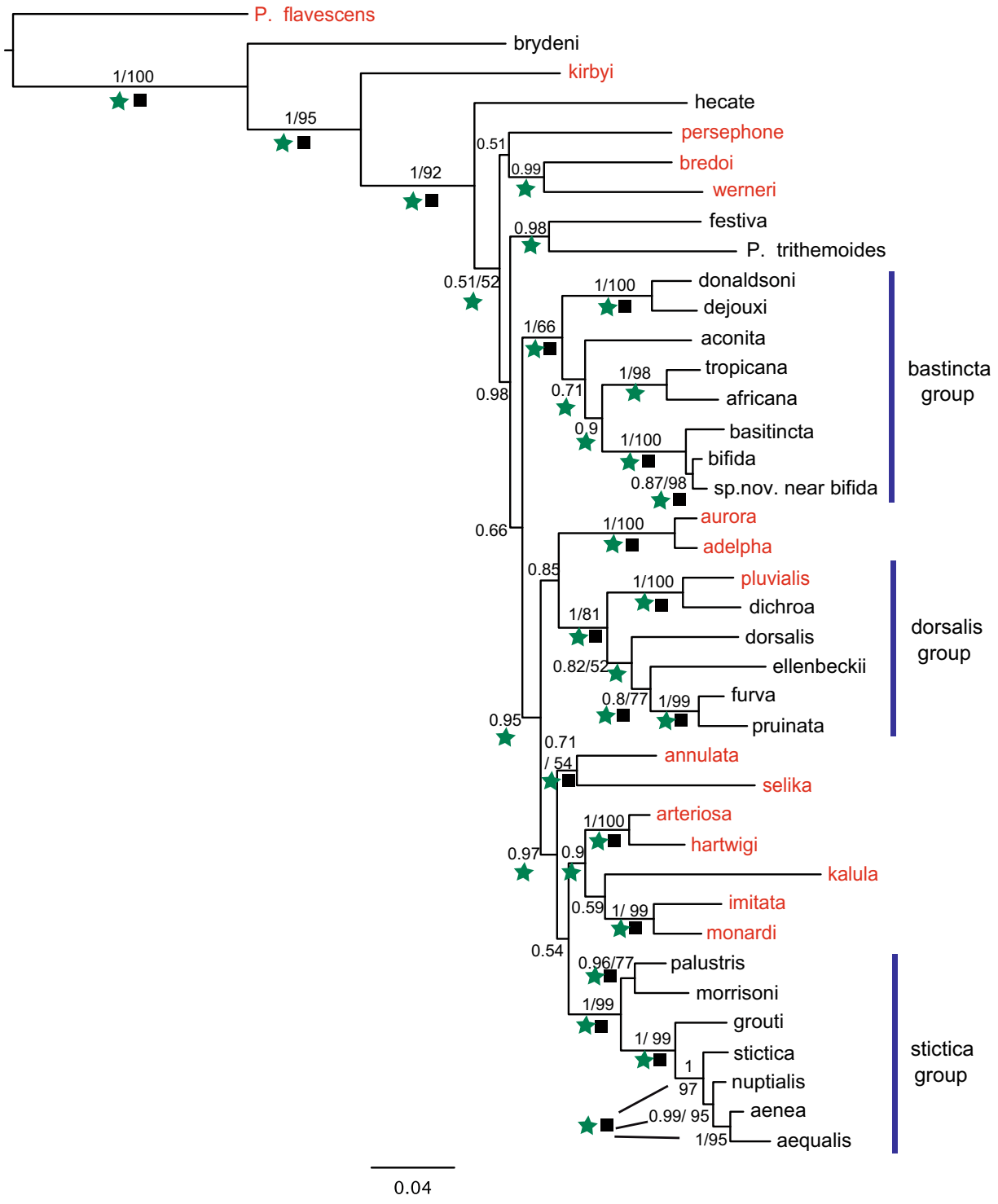

Fig. 1. Bayesian 50% majority-rule consensus phylogram obtained from the combined dataset of ND1, 16S and the ITS regions (including 5.8S). Posterior probabilities based on 4502 post-burn-in trees are shown for each node. Bootstrap supports after 1000 bootstrap replicates (shown values above 50) of the Maximum Parsimony analyses are included. Node support for each of the two data partitions is indicated by symbols; green star ★: mitochondrial data, black square ■: nuclear data. Nodes without symbols are only supported above 0.5 PP or 50% BS by the combined dataset. Red species are marked red. (For interpretation of color mentioned in this figure, the reader is referred to the web version of this article.)

dataset reached a final average standard deviation of split frequencies at 0.007 after 3,000,000 generations suggesting that the chains had reached convergence. For the combined dataset convergence was reached at a value of 0.008 after the same generation time.

Fig. 1 shows a consensus phylogram of the BA for the combined dataset. Tree topologies of MP and BA were highly similar for both datasets: the nodal support of the mitochondrial (900 bp) and nuclear data partitions (665 bp) are indicated for comparison. The addition of the ITS region did not change tree topologies, but

strengthened the support at some weaker nodes in the mtDNA dataset. Slight differences between MP and BA were mainly found at a few nodes resulting from a lower resolution in MP (BS < 50% as shown by the integrated BS values in Fig. 1).

Three species (*brydeni*, *kirbyi*, and *hecate*) were consistently placed as sister-species to all the remaining *Trithemis*. Furthermore, four species (*werneri*, *bredoi*, *persephone*, *festiva*) were placed between these three and the remaining species, but relationships could not be resolved clearly. They appeared in different clades in the different datasets and support values were low (<50% BS and low PP) in MP and BA. *Porpacithemis trithemoides* appeared most closely related to *T. festiva* and was placed in all analyses within the genus, suggesting it belongs to *Trithemis*.

Three monophyletic clades were found in all analyses with the same relationships between their species. All species of these clades, except the red *pluvialis*, are dark. The *basitincta*-group was placed as a sister group to the *dorsalis*-group, the *stictica*-group and a group of red species supported by 66% BS and 1.0 PP. It consists of eight species (*aconita*, *donaldsoni*, *dejouxi*, *basitincta*, *bifida*, sp. nov. near *bifida*, *africana*, and *tropicana*). Within this clade, four groups were found (*tropicana*/*africana; bifida*/sp. nov./*basitincta; aconita*; *donaldsoni*/*dejouxi*). The *dorsalis*-group formed a clade of six species (*dorsalis*, *ellenbeckii*, *pruinata*, *furva*, *pluvialis*, and *dichroa*) supported by 81% BS and 1.0 PP. *Pluvialis* and *dichroa* were closely related, while the other four species formed a separate group. The *stictica*-group contained seven species (*nuptialis*, *aequalis*, *aenea*, *stictica*, *grouti*, *palustris*, and *morrisoni*) supported by 99% BS and 1.0 PP in the combined dataset. Two recently described species, *T. palustris* and *T. morrisoni* (Damm and Hadrys, 2009) formed a separate group within this clade.

A group of red species was identified by the mtDNA and combined dataset in the BA including four species (*imitata*, *monardi*, *arteriosa*, *hartwigi*). The species-pair *annulata* and *selika*, also colored red, were the sister group of these four (and *kalula*) and the *stictica*-group. The exact position of the red *kalula* remained unclear, but MP and BA of the combined datasets indicated a close relation to the other red species. Neither the Asian endemics (*adelpha*, *aurora*, *festiva*) nor the Madagascan ones (*selika* and *persephone*) formed monophyletic clades, although the sister-species status of *aurora* and *adelpha* was confirmed. While *selika* was placed near the other red African species, the position of *persephone* remained unresolved.

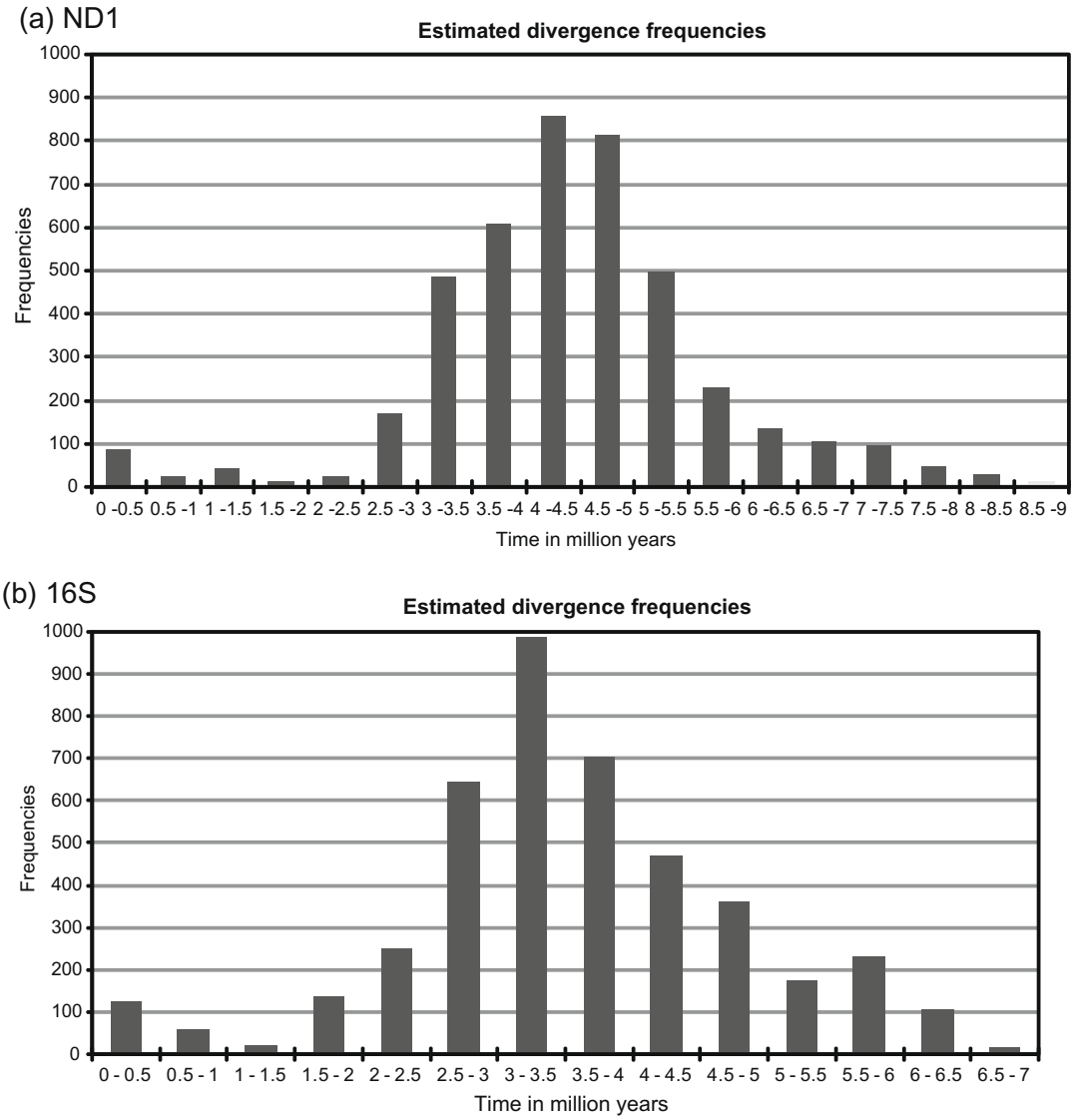

Fig. 2. Frequencies of the estimated divergence time in a species pairwise comparison based on sequence distances for the two mitochondrial sequence markers (a) ND1 and (b) 16S. All 4278 comparisons were assigned to time ranges of 0.5 million years and their frequencies calculated.

3.3. Molecular clock analyses

The Kishino–Hasegawa and Shimodaira–Hasegawa tests were conducted to compare ML trees reconstructed with and without molecular clock enforced. No significant difference in each mitochondrial marker (ND1: $p = 0.63$ and $p = 0.32$, respectively; 16S: $p = 0.66$ and $p = 0.33$, respectively) or in the combined dataset ($p = 0.764$ and $p = 0.388$, respectively) was found. Therefore the molecular clock was not rejected and divergence times were estimated. Using the mutation rate of 2.3% per million years for ND1 a wide time range of speciation events was found. The lowest sequence divergence between two species, 0.5–1.2%, corresponds with a Pleistocene age, 0.2–0.5 Mya. The great majority of observed pairwise sequence divergences ranged between 7% and 13%, suggesting a concentration of speciation events in the Pliocene, 3.0–5.6 Mya (Fig. 2a). The genus's origin might be in late Miocene 8.7 Mya, as indicated by the highest sequence divergence found, between *brydeni* and *kalula* (20.1%).

Genetic distances of 0.4–1.1% between nearest sister-species in the 16S region corresponded with their divergence 0.28–0.78 Mya, which is concordant with ND1. Also comparable were the most frequent pairwise genetic distances in 16S: these were found in the middle of the range (3.5–6%), i.e. with most divergences between 2.5 and 4.3 Mya (Fig. 2b). The highest sequence divergence was found between *kirbyi* and *persephone* (9.3%), which again suggests an origin in the late Miocene, 6.6 Mya.

Tree topology of ML analysis of the combined dataset of ND1 and 16S showed the same topology as the BA tree (Fig. 1) and the likelihood ratio test between molecular clocks enforced vs. not enforced showed no significant differences. Therefore the tree including branch lengths was used to obtain an ultrametric tree with absolute calibration of the basal *Trithemis* node set to 10 Mya and which showed similar divergence dates between species and clades as the calculated divergence date estimates according to the mutation rates used above (Fig. 3).

3.4. Morphology, ecology, and distribution

Fig. 4 provides the most conservative estimate of the evolutionary history of the genus: the nodes that were not supported by all gene fragments and analyses were collapsed. Mapped on this tree, adult and larval habitat showed a complex history of shifts and reversals. The estimation of these transformations and ancestral state reconstructions, however, revealed some clear trends (Table 2, Fig. 5a and b): the transformation from red to dark males occurred about as often as the reverse. Transformations in adult habitat (landscape) were more frequent than in larval habitat (water body), but both demonstrated dominant directions, most notably from standing to flowing and temporary to permanent water bodies, and from open landscapes to those with more cover. Indeed standing, probably temporary, water bodies in an open landscape were inferred to be the ancestral habitat of the genus. Adaptation to forest and/or flowing water subsequently evolved multiple times, dominating some clades, e.g. in the *basitincta*-, *dorsalis*-, and *stictica*-groups (Figs. 4 and 5a, b). Furthermore there were multiple transformations from regional to intercontinental ranges, whereas the reverse seldom occurred, indicating an origin and main radiation in continental Afrotropics and multiple subsequent invasions of Eurasia and Madagascar. Character association statistics showed strong associations of dark species with flowing and permanent water bodies, habitats with some cover, and a regional distribution (Table 3). Conversely the red species were correlated with standing and temporary waters, open habitats, and intercontinental distributions.

4. Discussion

4.1. Diversity and phylogeny of the genus Trithemis

The position of *T. brydeni* and *T. kirbyi* and the monophyly of three terminal clades were consistent in all analyses. The latter three are also well-defined morphologically (Pinhey 1970 and unpublished data) and conform to the *basitincta*-, *dorsalis*- and *stictica*-groups. They radiated in parallel within Africa, each within notably distinct ecological and geographic contexts, together giving rise to an estimated 55% of *Trithemis* species. The three not only contributed most to the genus's present diversity, but also independently invaded forest habitats. In contrast to these consistent results, the placement of three dark species and 12 red species was problematic, probably owing to rapid initial radiation. This possibility is discussed below, followed by separate discussions of the earliest branching events, the red species, and the three monophyletic radiations.

4.1.1. Rapid radiation in the Pliocene

Molecular clock estimates calculated with insect mitochondria mutation rates (Brower, 1994) dated the origin of the genus *Trithemis* in the late Miocene, approximately 6–9 Mya. This is congruent with the *Trithemis* fossil record (Nel 1991) which was dated 7.1–11.2 Mya. The main radiation is thought to have occurred in the Pliocene, 2.5–5.6 Mya, with ongoing speciation up to the Pleistocene. Pairwise comparisons of estimated divergence times demonstrate clear concentrations of divergences 3.0–5.6 Mya in ND1 and 2.5–4.3 Mya in 16S (Fig. 2). Also in the ultrametric tree the major clades separate in a relatively short period around 4 Mya (Fig. 3). The short branch lengths where the major clades diverged suggest a fast diversification. Short basal branches are a frequent problem in phylogenetic reconstructions, especially in ancient radiations (Whitfield and Lockhart, 2007). Because 81% of the recognized *Trithemis* species were studied and the missing species are regionally restricted, insufficient sampling of extant taxa is unlikely to account for the short branches. Another explanation is the choice of genetic markers, but the three used have previously resolved the phylogenies of odonate genera successfully (Misof et al., 2000; Hovmoller and Johansson, 2004; Hadrys et al., 2006; Groeneveld et al., 2007). All three, moreover, reveal similar topologies and the inclusion of the nuclear ITS region did not change the topology but strengthened the resolution of some weakly supported nodes. Thus we conclude that the difficulty in resolving the deeper nodes was caused by rapid radiation, possibly in response to sudden environmental change.

4.1.2. Early branching events

T. kirbyi and *T. brydeni* are neither close to each other nor to other *Trithemis* species. The dark *T. brydeni* is local in the open Okavango and Bangweulu swamps. Genetic distances between it and other *Trithemis* species are mostly greater than between *P. flavescens* and the others. With the more distantly related libellulid *Crocothemis erythraea* as outgroup, *T. brydeni* came out as the sister-species to *P. flavescens* and the remaining *Trithemis* species. A generic reassessment is needed to clarify the status of *T. brydeni* as a *Trithemis* species.

The red *T. kirbyi* is best adapted to temporary pools, with rapid larval development and strong adult dispersal (Suhling et al., 2005, 2009). *T. kirbyi* consequently ranges throughout Africa, also deep into deserts, and to Madagascar, southern Europe, Arabia and India. The outgroup and nearest known relative of *Trithemis*, *P. flavescens*, is also highly adapted to ephemeral conditions, having the largest range and fastest development of all odonates (Corbet, 1999). Although the phylogeny of libellulids is insufficiently resolved

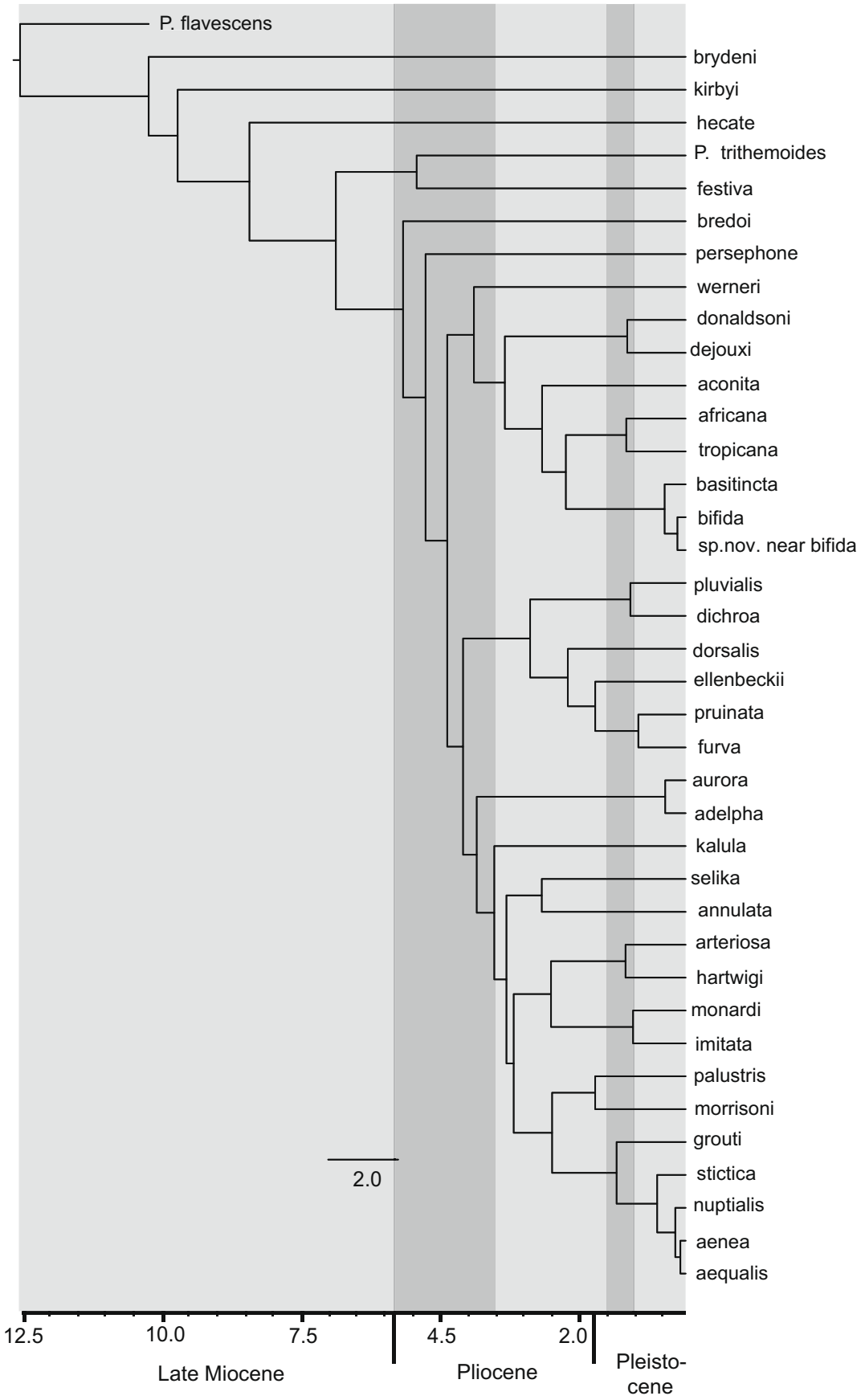

Fig. 3. Ultrametric tree obtained from ND1 and 16S sequences based on Maximum likelihood branch lengths. The time was calibrated in r8s using a fixed node age of 10 Mya for the basal *Trithemis* node according to a fossil record. The grey fields indicate relatively drier (pale) and wetter (dark) periods relevant to *Trithemis* evolution (see Section 4).

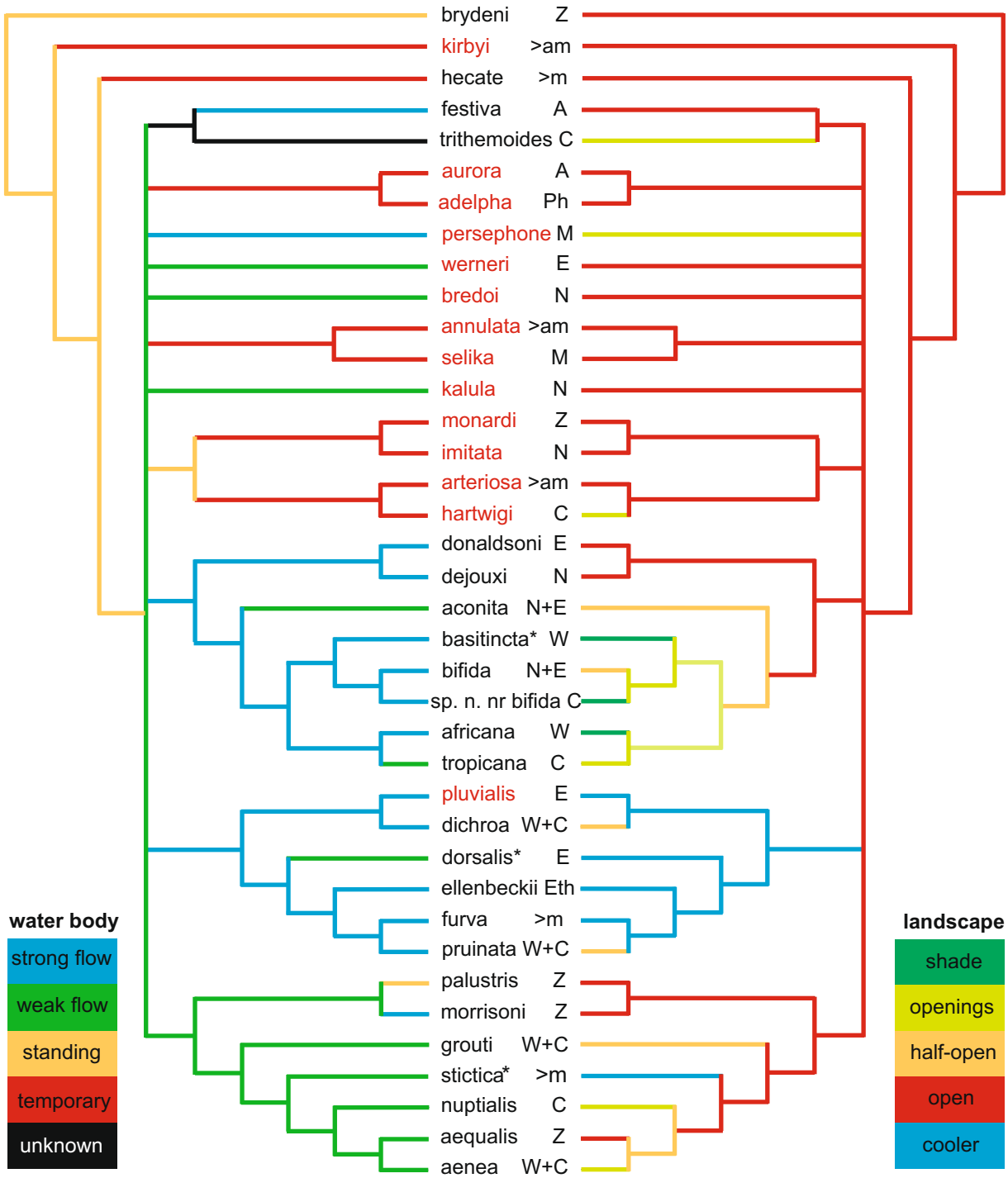

Fig. 4. Strict consensus of MP and BA trees, showing ecological characters on branches (ordered, optimized manually), as well as range and adult male coloration (see Table 1 for explanations). Internal nodes with either low BS (<50) or PP (<0.5) support have been collapsed. The name-giver of each group asterisked.

(Ware et al., 2007; Pilgrim and Von Dohlen, 2008), this supports the probability that the genus's ancestor bred in temporary pools.

While *T. kirbyi* and *T. brydeni* seem to date from before the main *Trithemis* radiation, around 5.0–7.5 Mya, three dark species without close affinities are also placed as sister groups to the remaining *Trithemis* species. *T. hecate* is local in open, possibly ephemeral, swamps throughout Africa and Madagascar. *T. festiva* is restricted to open streams from Turkey to Indonesia. *P. trithemoides* was the only sampled member of a complex of three or four diminutive species found mainly in central Africa, possibly in rainforest streams, variably placed in *Anectothemis*, *Congothemis*, *Porpacithemis*, and/or *Lokithemis* on account of their simplified wing venation. Finding the species firmly inside the *Trithemis* radiation offers another demonstration of the fallibility of venation to define libellulid genera (Dijkstra and Vick, 2006; Pilgrim and Von Dohlen, 2008) and all four genera must probably be subsumed in *Trithemis*.

4.1.3. Red lineages

Owing to the variable support of most clades containing red species, the exact number and position of independent red lineages

Table 2
The total estimated number of state transformations (character changes along the tree) for each character based on simulations of character histories using SIMMAP. Analyses are based on final 1000 post-burn-in sampled Bayesian trees.

Character states (0 vs. 1)	Transformations	0=>1	1=>0
Dark vs. red	8.29	4.03	4.26
Flowing vs. standing	8.73	2.09	6.64
Permanent vs. temporary	8.56	1.88	6.68
Forest vs. non-forest	12.75	5.90	6.85
Some cover vs. open	11.91	3.41	8.50
Warmer vs. cooler climes	6.14	2.57	3.57
Regional vs. intercontinental	9.11	7.36	1.75

remain unclear, but between four and nine appear to have given rise to the 12 red species studied. In the latter extreme case, each lineage evolved just a single species, with the exception of three pairs of species well-separated by range or ecology. Eight out of 12 species inhabit temporary water bodies. Together with *T. kirbyi* and *T. hecate* these are all *Trithemis* species tolerant to such conditions. Among them are two of Africa's most widespread and numerous odonates, *T. annulata* and *T. arteriosa*, which dominate open freshwater in Africa, Madagascar, and adjacent Eurasia. Two sister-pairs originated within Africa 1.0–1.5 Mya: *T. imitata* and *T. monardi* inhabit open habitats north and south of the central forest belt. *T. hartwigi*, known from only six sites in central Africa, uniquely favors open pools within rainforest. Its sister-species *T. arteriosa* rarely penetrates dense forest and *T. hartwigi* may have diverged in open enclaves within the forest matrix. *T. adelpha* is the Philippine counterpart of *T. aurora*, one of Asia's most ubiquitous dragonflies, but their separation was estimated at only 0.3–0.4 Mya. The two differ little and are often treated as synonymous, but occur together locally in the Philippines (pers. obs. R.J.T. Villanueva). The four remaining species (*T. bredoi*, *T. kalula*, *T. persephone*, and *T. werneri*) have no clear affinities within the genus and inhabit flowing water, mostly calm and open, like savannah rivers. The Madagascar endemic *T. persephone* diverged 3–4 Mya and, atypically for a red species, inhabits forested streams. Perhaps it was pushed into this habitat by the arrival of another endemic, *T. selika*, that diverged from its probable sister-species *T. annulata* 2.6–2.9 Mya.

4.1.4. Lowland radiation (basitincta-group)

All species inhabit running waters, mainly in lowlands. The largest lineage contains species that inhabit forest streams with varying degrees of shading. Its sister group, *T. aconita*, favors half-open streams on the forest-savannah transition. *Trithemis dejouxi* and *T. donaldsoni* form the sister group to all other species in the group and inhabit exposed savannah rivers. This sequence confirms the hypothesis of stepwise occupation of, adaptation to, and speciation in increasingly closed habitats (Dijkstra and Clausnitzer 2006). Each lineage is divided into geographically separated species, suggesting speciation in allopatry. The distribution of, and genetic distance between, *T. dejouxi* and *T. donaldsoni* is similar to those of *T. imitata* and *T. monardi* (see above). Judging from their slight genetic difference, the split of *T. africana* and *T. tropicana* in forest west and east of the Dahomey Gap respectively, only occurred in the past 0.7 Mya. By morphology, *T. congolica* from the Congo Basin and *T. nigra* from the volcanic island of Príncipe are sister-species of *T. aconita*. Neither was sampled, but their separa-

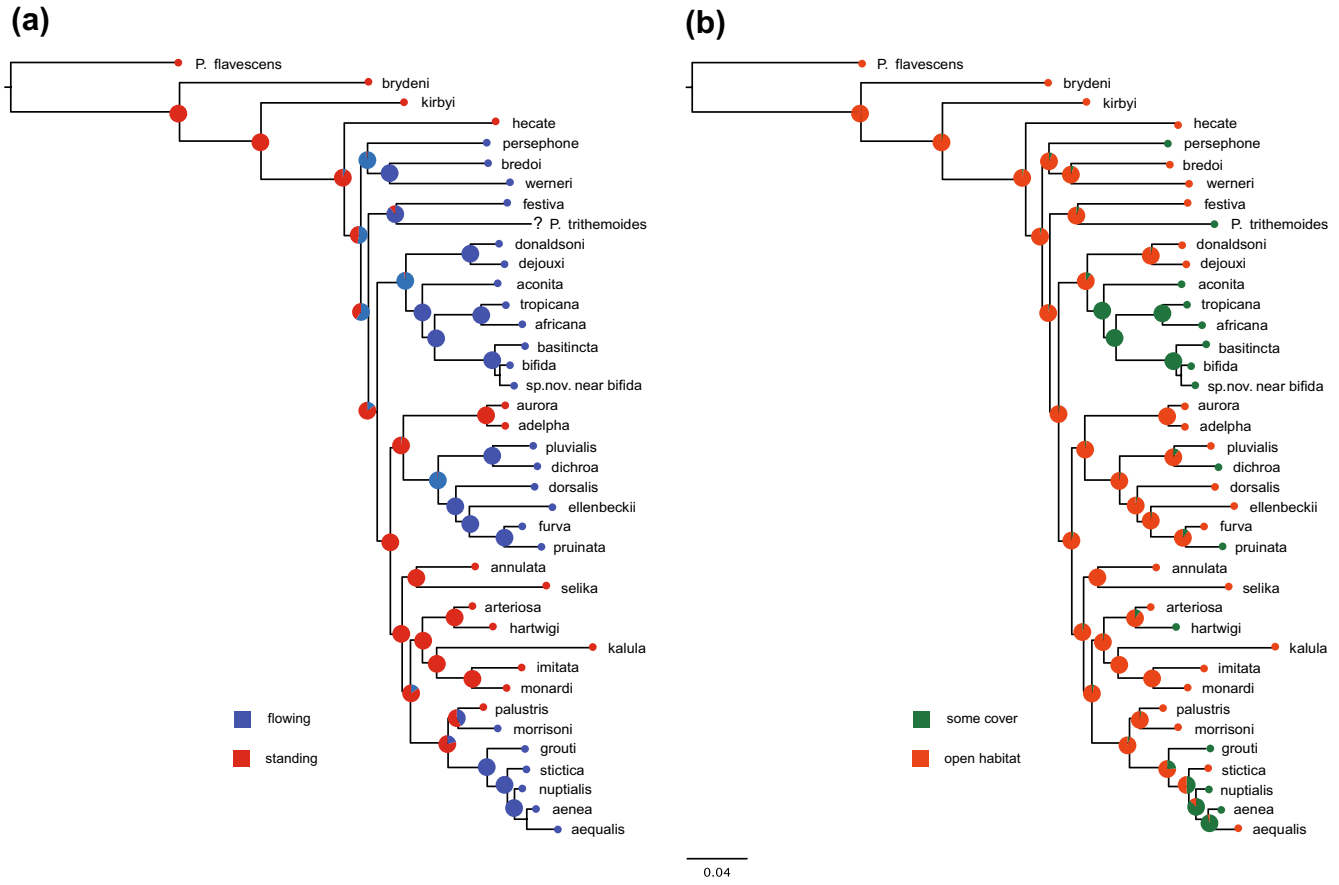

Fig. 5. Two examples of ancestral state reconstruction for (a) Water body: flowing vs. standing and (b) Landscape: some cover vs. open habitats. Shown is the BA tree of Fig. 1 including pie charts at each node indicating the posterior probabilities from the stochastic analyses of ancestral state reconstruction.

Table 3
Character association statistics for specific state associations, d_{ij}. Bold numbers indicate associations between specific states that occurred more frequently (positive with $p > 0.95$) or less frequently (negative with $p < 0.05$) than expected if the characters are evolving independently.

		Color		Range	
		Dark	Red	Regional	Inter-continental
Water body	Flowing	**0.076**	**−0.076**	**0.051**	**−0.051**
	Standing	**−0.076**	**0.076**	**−0.051**	**0.051**
	Permanent	**0.084**	**−0.084**	**0.048**	**−0.048**
	Temporary	**−0.084**	**0.084**	**−0.048**	**0.048**
Landscape	Forest	0.006	−0.006	0.007	−0.007
	Non-forest	−0.006	0.006	−0.007	0.007
	Some cover	**0.040**	**−0.040**	0.033	−0.033
	Open	**−0.040**	**0.040**	−0.033	0.033
	Warmer	−0.024	0.024	−0.007	0.007
	Cooler	0.024	−0.024	0.007	−0.007
Range	Regional	**0.016**	**−0.016**		
	Inter-continental	**−0.016**	**0.016**		
		Water body			
		Flowing	Standing	Permanent	Temporary
Landscape	Forest	0.015	−0.015	0.008	−0.008
	Non-forest	−0.015	0.015	−0.008	0.008
	Some cover	**0.042**	**−0.042**	0.033	−0.033
	Open	**−0.042**	**0.042**	−0.033	0.033
	Warmer	**−0.037**	**0.037**	−0.029	0.029
	Cooler	**0.037**	**−0.037**	0.029	−0.029

tion would have occurred after *T. aconita* separated from the other *basitincta*-group species, about 3 Mya.

4.1.5. Highland radiation (dorsalis-group)

This group includes two clades, one with two species (*T. dichroa*, *T. pluvialis*) and the other with the remaining four: *T. dorsalis*, *T. ellenbeckii*, *T. furva*, and *T. pruinata*. While most species favor open streams at higher altitude, *T. dichroa* and *T. pruinata* mainly inhabit shaded lowland streams, often in forest. The highland species show broadly overlapping ranges, mainly in the uplands from the Cape to Kenya. Most widespread, *T. furva* extends to Madagascar, Cameroon and Ethiopia; its relative *T. ellenbeckii* is restricted to the Ethiopian highlands. The habitat shift of *T. dichroa* and *T. pruinata* may result from an adaptation to cooler microhabitats in a highland area of origin, which allowed them to occupy shaded lowland streams. Both species diverged from their highland sister-species *T. pluvialis* and *T. furva* about 0.6–1.4 Mya and now occur throughout the central and western African forest. Morphologically these sister-pairs are almost identical, but *T. pluvialis* is unique within a clade of dark species to have reversed to (or retained) red coloration.

4.1.6. Swamp radiation (stictica-group)

The species occupy rather open habitats of 'mixed' flow, like channels in swamps and calm stretches and by-waters of streams, although they may prefer stronger current (*T. morrisoni*), a cooler microclimate (*T. stictica*) or more cover (*T. aenea*, *T. nuptialis*). With the exception of the widespread *T. stictica*, the distribution of the lineages alternates across the Congo-Zambezi watershed divide. While *T. aenea*, *T. grouti*, and *T. nuptialis* occur mainly in the Guineo-Congolian forests, three other species are concentrated in the 'Zambezian' swamps to the south: *T. aequalis* is confined to the Okavango and Bangweulu swamps, while the species-pair *T. palustris–morrisoni* is sympatric in the Okavango and adjacent Zambezi system. The latter species were only separated from *T. stictica* after a marked genetic distance was found, and may differ subtly in habitat (Damm and Hadrys, 2009). Neither is proven to overlap with *T. stictica*, which ranges in open and often elevated habitats from the Cape to Madagascar, Ethiopia and across western Africa. Judging from their morphology, two localized species, *T. anomala* (Zambia–Katanga border region) and *T. fumosa* (Congo), belong in this group too. Although the group's radiation started around 3.3 (16S) or 3.9 Mya (ND1), the genetic distance in the most recent split (*T. aenea-aequalis*) is nil (16S) or equivalent to only 0.3 Mya (ND1).

4.2. Evolutionary implications

4.2.1. Ecology and coloration

Summarizing, species of open habitats predominate, especially earlier in the *Trithemis* radiation, and a shift towards more shaded habitats occurred on numerous occasions (Table 2, Fig. 5b). Similarly, several shifts occurred from standing or slow-flowing habitats to stronger currents (Fig. 5a). Depending on the phylogenetic reconstruction and the assumed ancestral state, red vs. dark coloration developed or disappeared at least three times, but probably more often (Table 2). This evolutionary flexibility in ecology and coloration may be related, as the exposure of dark pigmentation to sunlight raises the body temperature (Corbet, 1999). Indeed of 14 studied red species, nine favor standing (often temporary) water and 12 inhabit open habitats, also indicated by a positive correlation of these characters (Table 3). In contrast, only three of 24 dark species favor such waters, while 13 dark species prefer half-open or closed habitats, such as forest. Moreover, while the three dark lineages each produced between six and 11 species, the red lineages each gave rise to only one or two, indicating different 'evolutionary potentials' (see Section 4.2.3.).

4.2.2. Distribution and speciation

The mode and location of speciation events can only be inferred from current distributions. However, most *Trithemis* species have large ranges and presumably good dispersal capacities. For example, eight species invaded Madagascar independently, while there were between five and seven dispersal events to Asia (two species not sampled). Nonetheless, of the well-supported sister-species relationships found, (1) four involve pairs of allopatric species (*aenea-aequalis*, *africana-tropicana*, *donaldsoni-dejouxi*, and *monardi-imitata*), (2) four show limited geographic overlap (*arteriosa-hartwigi*, *aurora-adelpha*, *dichroa-pluvialis*, and *furva-pruinata*), and (3) only one pair (*morrisoni-palustris*) is broadly sympatric within different habitats. Allopatry in regions of suitable habitat, separated by uninhabitable regions, may be the primary mode of speciation in these examples (followed by some secondary overlap) and the genus in general. Nonetheless, divergent selection across ecological gradients (e.g. on the forest-savannah transition) is also a potential force for speciation (Smith et al., 1997; Moritz et al., 2000; Schilthuizen, 2000). This gradient model may have operated in the invasion of increasingly shaded habitats in the *basitincta*-group, the two shifts from high- to lowland in the *dorsalis*-group, and the alternation between open swamp and forest in the *stictica*-group.

4.2.3. Biogeographical hypothesis

The African Neogene (<23 Mya) was characterized by climatic vicissitudes with a trend towards increasing aridity. During the early Miocene rainforest stretched between coasts and to northern Ethiopia, but savannah began expanding 16 Mya and became widespread 8 Mya (Lovett, 1993; Vbra, 1993; Morley, 2000; Jacobs, 2004; Sepulchre et al., 2006). At the end of the Miocene (5 Mya) rainforest was limited and much of Africa's Paleogene diversity was eliminated (Plana, 2004). While the evolution of *Trithemis* appears to have begun in this period of savannah bloom, the major

lineages originated afterwards, in the relatively wet early Pliocene (3.5–5 Mya). Aridification generally disadvantages water-dependent species, but it favors adaptations to exposed and temporary conditions, as seen in most red *Trithemis* species. While the genus may have arisen in the savannah-expansion of the late Miocene, populations in open areas possibly became isolated by forest-expansion in the early Pliocene, with subsequent allopatric speciation giving rise to the many poorly-resolved lineages. Adaptation to temporary conditions dictates good dispersal ability (see Suhling et al., 2009) and as forests shrank again and open habitats coalesced after 3.5 Mya, the species expanded to establish largely overlapping ranges (e.g. *T. annulata* and *T. arteriosa*). Without isolating mechanisms, these lineages did not radiate further, with the exception of a few allopatric species-pairs. Similar observations were made with water beetles and mayflies of stagnant habitats (Ribera et al., 2001; Monaghan et al., 2005).

By contrast, the three dark lineages were ecologically more constrained and therefore could radiate excessively under pressure of the changes in the next 3.5 Mya. Pronounced drying occurred 3.5, 3.2, and 3.0 Mya and especially 2.5–2.8 Mya with the onset of the first northern hemisphere glaciation (Morley, 2000), with further step-like increases in aridity 1.7–1.8 and 1.0 Mya (deMenocal, 1995). The highland radiation (*dorsalis*-group) coincided with the major Pliocene and early Pleistocene uplift that created the Great Rift Valley and the Congo Basin (Plana, 2004). The lowland shift of *T. dichroa* and *T. pruinata* may have been triggered by the expansion of forest in a wetter interlude 1.0–1.5 Mya, offering access to suitable new habitat in the form of shaded streams. At the same time the retreat of open habitats could separate the pairs *T. arteriosa–hartwigi*, *T. monardi–imitata*, and *T. donaldsoni–dejouxi*. There was a strong increase of climatic variability 0.8 Mya and perhaps the separation of forest species like *T. africana* and *T. tropicana* occurred at this time.

5. Conclusions and outlook

The present-day diversity and dominance of *Trithemis* result from its species' flexible responses to the climatic fluctuations in Africa since the late Miocene. Today the genus occupies a great variety of habitats, displaying its high adaptation potential. Its success seems to be related to the origin of extensive savannah, which favored opportunistic species and their dispersal ability. Less mobile species of more stable habitats (e.g. permanent water, rainforest) either became extinct under these conditions or were restricted to pockets of optimal habitat (e.g. Fjeldsa and Lovett, 1997; Hadrys et al., 2006; Burgess et al., 2007; Fjeldsa and Bowie, 2008). It has been suggested that the ecological constraints of ancestral adaptations dictate the direction of radiations (McPeek, 1995; Richardson, 2001). In this genus too, groups with more restrictive adaptations radiated within distinct ecological confines. Nonetheless, *Trithemis* straddled ecological barriers in different directions multiple times. Most shifts occurred from open to more closed habitats and from standing to running waters. Generally the ancestral habitat in Odonata is thought to be forest streams, but the Libellulidae are dominant in more open and stagnant habitats (Kalkman et al., 2008). The repeated re-invasion of these habitats via different ecological routes in *Trithemis* is exemplary of the rise of a 'modern' freshwater fauna in, and under influence of, Africa's changing environment (Dijkstra, 2007). This is one of several recent studies revealing explosive African radiations in the Plio-Pleistocene (e.g. Gaubert and Begg, 2007; Van Daele et al., 2007; Dubey et al., 2008; Koblmueller et al., 2008). It demonstrates the importance of combining ecological and phylogenetic data to understand the origin of biological diversity under great environmental change. Such studies may guide conservation efforts by anticipating ecological and evolutionary responses to future change.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.12.006.

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